**Ain Shams University**
**Women's College for Arts, Science and Education**
**Zoology Department**

# Effect of Antiulcer and Ulcer Healing Property of Some Antioxidants on Experimentally Induced Gastric Ulcer in Albino Rats.

***Thesis***

***Submitted for Fulfillment of the Degree of Doctor of Philosophy in Zoology***

**Department of Zoology**

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# QUALIFICATION

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# ***LIST OF ABBREVIATIONS***

| | |
|---|---|
| ADP | Adenosine diphosphate |
| ATPase | Adenosine triphosphatase |
| ASA | Aspirin |
| CA | Carbonic anhydrase |
| COX | Cyclooxygenase |
| COX-1 | Cyclooxygenase-1 |
| COX-2 | Cyclooxygenase-2 |
| DAB | Diaminobenzidine |
| DTNB | 5,5´ dithiobis ( 2- nitrobenzoic acid ) |
| G1 | Gap 1 phase |
| GPx | Glutathione peroxidase activity |
| GR | Glutathione reductase. |
| GST | Glutathione *S*-transferases |
| GSH | Glutathione |
| *H.pylori* | *Helicobacter pylori* |
| I3C | Indole-3-carbinol |
| iNOS | Inducible nitric oxide synthase |
| IL-1 | Interleukin-1 |
| IL1β | Interleukin-1β |
| IL-4 | Interleukin-4 |
| IL-6 | Interleukin-6 |
| NO | Nitric oxide |
| NOS | Nitric oxide synthase |
| NSAIDs | Non steroidal-anti-inflammatory drugs |
| $NF_K$-B | Nuclear factor-kappa-B |
| OMP | Omeprazole |
| CYPs | Phase I cytochrome P-450 |
| PBS | Phosphate-buffered saline |
| PDE | Phosphodiesterase |
| PCNA | Proliferating cell nuclear antigen |

| | |
|---|---|
| PGs | Prostaglandins |
| PGE 2 | Prostaglandin E2 |
| PKB | Protein kinase-B |
| PPIs | Proton pump inhibitors |
| ROS | Reactive oxygen species |
| SOD | Superoxide dismutase |
| S phase | Synthesis phase |
| TX | Thromboxane |
| TXA2 | Thromboxane A2 |
| TNF-α | Tumor necrosis factor-α |
| UI | Ulcer Index |
| U | Ulcer |
| vs | Versus |

# ***ABSTRACT***

The present work is an attempt to elucidate the anti-ulcer activity of indole-3-carbinol as antioxidant alone or in combination with omeprazole a proton pump inhibitor to diminish the effects of induced gastric ulcer by aspirin.

It also emphasizes on the role of omeprazole and indole-3-carbinol on the extent of healing gastric ulcer.

To attain this goal, a total number of 96 male albino rats were presently investigated. It involved different aspects of study namely physiological, biochemical, histological, histochemical and immunohistochemical changes on stomach tissues.

The present findings were then discussed in view of the relevant literature available in similar fields of study.

This study showed that the use of indole -3 - carbinol as antioxidant with omeprazole in the treatment of gastric ulcers in rats, may help in healing gastric ulcers and improvement of all different parameters.

Thus, suggestions for further studies using indole -3 - carbinol as anticancer and antioxidant agent either administrated directly from vegetables of the *Cruciferae* family or using this active ingredient indole-3 - carbinol in drug preparation of anti-inflammatory drugs.

# ***INTRODUCTION***

Gastric ulcer is a major drawback in modern days due to various factors, such as the impairment of the balance between aggressive (increased acid secretions) and protective factors, stress, trauma, sepsis, haemorrhagic shock, burns, pulmonary and liver diseases, *Helicobacter pylori,* use of cigarettes and alcohol. Steroidal and non-steroidal drugs also have been shown to play a role in gastric ulcerogenesis ***(Suleyman et al., 2009)***.

Non-steroidal anti-inflammatory drugs (NSAIDs) are one of the most widely prescribed medication in the world. Their main benefit derives from their anti-inflammatory and analgesic effect, but the use of these agents is not innocuous since they mainly increase the risk of gastrointestinal and cardiovascular complications ***(Sostres et al., 2010)***.

Aspirin is a common NSAIDs usually used to treat swelling, inflammation, to relieve pain and fever. Despite the cardiovascular benefits of aspirin, a potential gastrointestinal harm has been noted in several clinical and preclinical studies. The main undesirable side effects of aspirin are gastrointestinal ulcers, stomach bleeding, and tinnitus ***(Choi et al., 2010)***.

Hypersecretion of gastric acid due to the use of non- steroidal anti-inflammatory drug (NSAID) is a pathological condition, which occurs due to uncontrolled secretion of hydrochloric acid from the parietal cells of the gastric mucosa ***(Wallace and Muscara, 2001)***. Recent studies have shown that activation of neutrophils and oxygen free radicals may also play an important role in the etiology and pathophysiology of NSAID-induced gastric mucosal ulcers ***(Sugimoto et al., 2000 and Atawodi, 2005)***. Moreover, luminal acid interferes with the process of restitution, resulting in the conversion of superficial injury to deeper mucosal lesion and inactivates the acid-labile growth factors important for maintenance of mucosal integrity and repair of superficial injury ***(Wallace and Muscara, 2001)***.

Furthermore, the pharmacological activity of NSAIDs is related to the suppression of prostaglandin biosynthesis popularly known as cyclooxygenases. Prostaglandins synthesized by the gastric mucosa are one of the defensive factors known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate ***(Bhandari et al., 2008)***.

Proton pump inhibitors have been widely used as acid inhibitory agents for the treatment of disorders related to gastric acid secretion for about 15 years ***(Li et al., 2004)***.

Omeprazole, a substituted benzimidazole derivative, is a potent inhibitor of gastric acid secretion both in humans and in animals. The proton pump inhibitor is clinically used for the treatment of gastric ulcers. Many of these protective effects of omeprazole against gastric mucosal injuries have been thought to depend on its inhibitory action on gastric acid secretion ***(Kobayashi et al., 2002)***.

However, are some of the most attractive sources of new drugs and have been shown to produce promising results in the treatment of ulcers ***(Sunilson et al., 2008)***.

Ulcer is now mainly focused on limiting the deleterious effects of offensive acid secretion, but the search for new safer alternative drugs have rekindled the interest in cytoprotective drugs, which protect the gastric mucosa from damaging agents without influencing acid secretion or neutralising intragastric acidity ***(Sairam et al., 2003)***.

Diet composition influences both, oxidative damage and antioxidant mechanisms and this explains, at least in part, the relationship among diet and some chronic diseases ***(Pérez et al., 2002)***.

Indole-3-carbinol (I3C) is one promising anticancer agent, a naturally occurring compound found in vegetables of the *Brassica* genus, such as cabbage, broccoli and brussels sprouts, has been shown to reduce tumor occurrences in the colon, lung, skin, liver, cervix and mammary gland in mouse and rat models ***(Hsu et al., 2005)***.

One of the most important anticarcinogenic phytochemicals contained in cruciferous vegetables is indole-3-carbinol (I3C), an enzymatic breakdown product of indole glucosiniolates, sulfur-containing compounds contained in cruciferous vegetables. In the stomach, I3C undergoes condensation reactions to produce various products, the major one being 3,3-diindolylmethane ***(Anderton et al., 2004)***, to which most of the biological activities of I3C are attributed**.** Considerable evidence shows that I3C inhibits experimentally induced tumorigenesis in murine models at different sites such as colon, lung, skin, liver, cervix and mammary gland in mouse and rat models ***(Melkamu et al., 2010)***.

Omeprazole (OMP) accelerated ulcer healing but the administration of indole-3-carbinol either alone or in combination with OMP to aspirin-ulcerated rats produced a profound protection to the gastric mucosa from injury induced by aspirin ***(El-Shinnawy et al., 2012 a and 2014)***.

## ***AIM OF THE PRESENT STUDY***

The present study has been a trial to contemplate for the following goals:

- Assessment of the effects of indole-3-carbinol as a new safer cytoprotective alternative drugs that protect the gastric mucosa from ulcer without influencing acid secretion or neutralising intragastric acidity.

- The efficacy of combined effect of omeprazole and indole-3-carbinol to normalize the acidity, pH of stomach, ulcer index, platelets and antioxidant activity in aspirin induced ulcerated rats.

- Assessment and estimation of extent of histological damage in response to the different experimental regimens on stomach tissues.

- Histochemical and immunohistochemical alterations following the administration of indole-3-carbinol to ulcerated rats throughout the period of investigation.

- Discussion of the implied results in view of the relevant literature.

- Suggesting some recommendations on the modification of the supplemented omeprazole regimens for treatment ulcers.

# *LITERATURE REVIEW*

## 1-General Effect of Non-Steroidal Anti-Inflammatory Drugs :-

The non-steroidal anti-inflammatory drugs (NSAIDs) are chemically heterogenous compounds that have therapeutic and toxic effect in common. Non-steroidal anti-inflammatory drugs are used for the relief of pain and inflammation associated with arthritis and other musculoskeletal disorders. Relief from pain and stiffness is accompanied by the risk of developing a peptic ulcer and a serious, life-threatening ulcer complication ***(Graham et al., 2002)***.

Non-steroidal anti-inflammatory drugs are known to induce gastric mucosal damage including bleeding, ulceration and perforation in animals and humans. Over 30 million people worldwide use non-steroidal anti-inflammatory drugs daily ***(Nafeeza et al., 2002)***.

In addition, NSAIDs such as aspirin are widely used as anti-inflammatory, analgesic drugs and in the prevention of cardiovascular events ***(Weisman and Graham, 2002)***.

Non-steroidal anti-inflammatory drugs such as ibuprofen administration at a dose of 100 mg/kg for 7 and 14 days causes damage in the gastric mucosa and impairment of ulcer healing as unwanted side effects in rats ***(Sánchez-Fidalgo et al., 2005)***.

Moreover, ***Konturek et al., (2006)*** found that the major limitations of patients clinical application are serious gastrointestinal side effects of NSAIDs, especially peptic ulcerations and gastrointestinal bleeding.

***Laine, (2006)*** demonstrated that ulcers are found at endoscopy in 15-30% of patients using NSAIDs regularly. The annual incidence of upper gastrointestinal bleeding is approximately 1-1.5% in patients taking regularly NSAIDs. Important risk factors for these gastrointestinal events include old age, prior history of upper gastrointestinal events, use of corticosteroids or anticoagulants, and high-dose or multiple NSAIDs.

Non-steroidal anti-inflammatory drugs are widely used in the treatment of pain, fever and inflammation. However, these drugs have some side effects, especially on the gastrointestinal tract. Recently, reactive oxygen species (ROS) have also been shown to play a critical role in the development of pathogenesis in acute experimental gastric lesions induced by stress, ethanol and NSAIDs ***(Odabasoglu et al., 2006)***.

***Mizushima, (2007)*** reported that gastric mucosal cell death induced by non-steroidal anti-inflammatory drugs (indomethacin, diclofenac and ibuprofen) at different doses are suggested to be involved in NSAID-induced gastric lesions. Therefore, cellular factors that suppress this cell death are important for protection of the gastric mucosa from NSAIDs.

Nevertheless, some NSAIDs, particularly those of acidic nature, can directly kill epithelial cells. NSAIDs can also reduce mucus and bicarbonate secretion, thereby decreasing the effectiveness of the juxtamucosal pH gradient in protecting the epithelium ***(Wallace, 2008)***.

**2- <u>Aspirin and Gastric Ulcer</u>:-**

Aspirin often used as an analgesic to relieve minor aches and pains, as an antipyretic to reduce fever and as an anti-inflammatory medication. Aspirin also has an antiplatelet or anti-clotting effect and is used in long-term at low doses to prevent heart attacks, strokes and blood clot formation in people at high risk for developing blood clots. On the other hand, aspirin was the first-discovered member of the class of drugs known as non-steroidal anti-inflammatory drugs (NSAIDs), not all of which are salicylates, although they all have similar effects ***(Julian et al., 1996)***.

Aspirin (ASA), also known as acetylsalicylic acid, is a salicylate drug. Therefore, the most serious side effect of chronic low-doses of ASA (81 and 325 mg for 45 days) in human is gastric and duodenal ulcer formation , sometimes accompanied by ulcer bleeding or perforation in patients ***(Feldman et al., 2000)***.

***Ma et al., (2001)*** reported that aspirin at a dosage of 30 mg/kg on male Wistar rats from 3 days to 9 days, caused significant decrease in numbers of platelets count.

A study was made by ***Naito et al., (2001)*** demonstrated that male Sprague Dawley rats that received aspirin (200 mg/kg) for three hours, showed multiple erosions and bleeding developed in the glandular stomach, large areas of epithelial crypt loss, predominantly neutrophilic infiltrate throughout the mucosa and submucosa, erosion and mucosal bleeding.

***Sener et al., (2001)*** examined the gastric acidity and glutathione level on male albino rats, administrating 200 mg/kg of aspirin for one hour, where they found gastric acidity in the ASA group, which was significantly higher than that of the control group with the decrease of glutathione level and haemorrhagic lesions in the mucosa of the glandular stomach at the light microscopic level. ASA group mucosae indicated prominent cellular damage. Surface epithelium cells lining the gastric glands were also degenerated and possessed pyknotic nuclei. Distinct extravasated free erythrocytes were also localized, especially at the luminal region of the mucosae and indicated true ulcer formation.

***Galunska et al., (2002)*** conducted a study on male Wistar rats with ASA at a dose of 300mg/kg, then the animals were sacrificed after 4 hours. The authors found that ASA induced multiple gastric mucosal lesions, most often 1–2mm in size or petechial, bleeding at the moment of the observation. The area of involvement was confined to the glandular part of the stomach, where the mean ulcer area was 9.10 $mm^2$.

***Broome et al., (2003)*** suggested that the oral administration of dose of ASA (20 mg/kg) to horses for 36 hours, caused decreases the plasma thromboxane concentration by 68% to 93% and bleeding time.

***Souza et al., (2003)*** found that the oral administration of aspirin 50 and 100 mg/kg to normal rats caused haemorrhagic damage in the stomach which increased in severity in a dose-dependent after 3 hours of aspirin administration.

Gastric ulcer is a deep lesion penetrating through the entire thickness of the gastrointestinal mucosa and muscularis mucosa ***(Takeshi et al., 2003)***.

***Jainu and Devi, (2004 a and b)*** suggested that aspirin at a dosage of 400 mg/kg on male albino rats for 4 hours, caused a marked reduction in the levels of GSH, protein levels and gastric mucosal damage with severe haemorrhagic lesions in ulcerated rats.

According to, ***Merchant and Modi, (2004)*** chronic doses of aspirin (150 and 600 mg/kg) for 7 days and 25 weeks on mice, produced a reduction in the total red blood cell count (R.B.Cs), haemoglobin (Hb) and haematocrit (HCT). The mean cell haemoglobin (MCH) and the mean cell volume (MCV) were significantly increased. Total leucocyte counts (W.B.Cs), absolute neutrophil count, eosinophil count and lymphocyte count were higher in mice treated with acute or chronic doses of aspirin. The monocyte count was higher than the controls in the acutely treated animals while no significant difference was noted in the monocyte counts of chronically treated animals.

***Pilotto et al., (2004)*** studied acute and chronic users in older patients who had taken aspirin at a dose of 300 mg for 7, 30 days and two months, where the authors found that the prevalence of peptic ulcers was significantly higher in acute than chronic users.

***Nargund, (2005)*** said that gastric ulcer induced by aspirin is an ulcer between cardia and pylorus of the stomach. This is caused by increased acid secretion and decreased parietal cell mass and back-diffusion of acid. There may be increased concentration of bile acids, delayed gastric emptying and pancreatic juice in the stomach as a result of duodeno gastric reflux. The delayed gastric emptying accentuates the release of gastrin and the secretion of hydrochloric acid.

Aspirin causes the induction of gastric injury with large areas of epithelial crypt loss, predominantly neutrophilic infiltration, erosions, haemorrhagic mucosal erosions and inflammatory cell infiltration developed in the glandular stomach of rats three hours after intragastric administration of aspirin at a dose of 200 mg/kg ***(Odashima et al., 2005)***.

***Burke et al., (2006)*** demonstrated that marked decrease in thromboxane A2 (TXA2) production in male Sprague Dawley rats at a dose of ASA 65 mg/kg for 4 weeks.

The result of a study conducted by ***Fesharaki et al., (2006)*** pointed to the decreased level of total glutathione in Wistar rats at a dosage of (300 mg/kg) for 3, 6, 9 and 24 hours after the aspirin administration.

In a study made by ***Goldstein et al., (2006)*** suggested that patients requiring low-dose aspirin at doses of 81and 325mg for 12 weeks along with non-steroidal anti-inflammatory drugs (celecoxib 200 mg or naproxen 500 mg twice daily) are at increased risk for gastrointestinal injury.

***Jainu and Devi, (2006)*** said that aspirin at a dosage of 200 mg/kg on albino Wistar rats for 4 hours, caused a marked reduction in the levels of glutathione **(**GSH), superoxide dismutase (SOD), catalase (CAT) and a significant increase in lipid peroxide (LPO) level, the concentration of tumor necrosis factor-α (TNF-α), cytokines interleukin-1β (IL-1β) and nitric oxide synthase (NOS) activity in ulcerated rats. Also, they elucidated sharply defined ulcer crater at the site of exposure to aspirin, that almost reached the submucosal layer with a deep alteration of glandular epithelium, damaged mucosal epithelium, leukocyte infiltration and ulcerated area covered with inflammatory exudates in ulcerated rats.

A study was made by ***Odashima et al., (2007)*** to report that the administration of aspirin (200 mg/kg) resulted in the development of linear and dotted erosions in the gastric mucosa of rats.

***Wang et al., (2007)*** made a study on male Wistar rats weighing 180-200 g, animals were maintained on a low ASA (40 mg /kg). Then rats were killed on day 55, the results showed the gastric juice volume was significantly increased; while the pH value and mucus were significantly decreased in aspirin group.

***Al-dalain et al., (2008)*** demonstrated that marked increase in gastric volume, total acidity, ulcer index and protein concentration in both gastric mucus and in gastric juice were significantly increased in aspirin treated rats following a dose of 400 mg/kg for 4 hours. However, they found that carbohydrate levels in gastric mucus as well as in gastric juice and glutathione level were significantly lower in ulcerated group. Microscopical slides examination of stomach showed focal desquamation of liming epithelium of gastric mucosa, necrosis, sloughing of lamina epithelialis of gastric mucosa, inflammatory cells infiltration in lamina propria associated with marked submuscol edema.

According to ***Argenio et al., (2008)*** aspirin induced the appearance of multiple gastric erosions, ranging 2-10mm in length and about 1mm in width in male Wistar rats taking aspirin at a dose of 200mg/kg orally for four hours. Also, they noticed higher acidity and greater the damage in aspirin group.

***Das et al., (2008)*** pointed to the increase in ulcer index, pepsin activity, free acidity, total acidity and volume of gastric juice, accompanied by a decrease in gastric mucus in albino rats at a dosage of aspirin were orally tested 400 mg/kg for 24 hours.

***Malairajan et al., (2008)*** suggested that aspirin at a dosage of 200 mg/kg on Wistar albino rats for three days, caused a significant increase in ulcer index, ulcer score, free acidity, total acidity and volume of gastric juice.

***Sarkar and Guha, (2008)*** randomized adult albino rats into groups receiving aspirin at a dose of 500mg/kg for 4 hours, they noticed that opening the stomach of aspirin treated rats indicated a significant increase in ulcer index with a mucosal thickness.

Similarly, aspirin group (orally administrated 200 mg/kg for five days) demonstrated significant increase in the ulcer index and decrease in gastric wall mucus thickness. Also, total acidity was increased but no significant change occurred in volume of gastric juice ***(Khushtar et al., 2009 )***.

Moreover, as regard to gastric secretion parameters, there were significant increase in the volume of titratable acidity and total acid output in albino Wistar rats that were given ASA at a dose (150 mg/kg for 4 hours) than the group that received distilled water. Also, as regard to ulcer parameters there was significant increase in ulcer score and ulcer index in aspirin group ***(Mabrouk et al., 2009)***.

***Sancar et al., (2009)*** were able to investigate total protein, potassium, uric acid, creatinine, blood urea nitrogen and haematocrit values, where they suggested that no significant alteration in the prementioned parameters were found in aspirin rats following a dose of 200 mg/kg for three days or four weeks and control group, but they found that the ulcer score in aspirin group was (3.00 mm).

Moreover, ***Tuorkey and Abdul-Aziz, (2009)*** reported that aspirin dose was administered by oral gavage at the dose of 400 mg/kg to Wister albino male rat weighing 200-250 gm for nineteen hours, aspirin significantly increased ulcer index, total acidity, mucosal bleeding rate and gastric juice volume of gastric secretion with significant reduction in the pH value and gastric juice mucin content.

The results of a study conducted by ***Akilandeswari et al., (2010) and Angelo et al., (2010)*** pointed to the significant increase in both level of free and total gastric HCl, glutathione level and gastric lesion score with a significant decrease in gastric mucin level after the oral administration of ASA 300 mg/kg body weight for three days.

***Bharti et al., (2010)*** reported a decrease in the level of glutathione (GSH) in Wistar rats by the oral administrating aspirin (500mg/kg) thrice at an interval of 3 hours.

Moreover, ***Choi et al., (2010)*** demonstrated that no significant changes in body weight were found in albino rats administrating aspirin at a dose of 400 mg/kg during 14 days and 2 weeks, with significant decrease in pH value. Histopathological changes in rats were demonstrated by mucosal hyperemia and haemorrhagic lesions with edema covering the total glandular area of the stomach, and it was evident in indicating acute ulceration. In addition, gastric mucosal damage with dilation and exfoliation of gastric epithelial cells and disruption of mucosal layer were also observed. There was patchy decrease of mucin in the ulcerated area in the glandular, the loss of mucin layer was more pronounced in the necrosed areas.

***Giri et al., (2010)*** proved that aspirin at a dose of 20 mg/kg for four hours given to albino Wistar rats, caused irritant effect and mucosal damage, increasing acid secretion,

overproduction of leukotrienes, decreased mucin surface active phospholipids, bicarbonate secretion, mucosal proliferation.

***Jaikumar et al., (2010)*** pointed to the significant increase in total acidity, gastric volume, protein content and ulcer index with a significant decrease in pH level following administration of aspirin at the dose of 200 mg/Kg orally once daily for 5 days in rats. Stomach at the light microscopic level showed ulcerated mucosa with haemorrhage and discontinuity of lining epithelium.

A study was conducted on albino Wistar rats administrating aspirin at a dose of 80mg/kg body weight for 19, 22 and 25 days. The authors found that aspirin treated rats showed significant reduction in the level of glutathione. In addition, aspirin induced ulcer with a significant reduction in total protein, red blood cells, white blood cells and haemoglobin levels. Histopathological studies also showed an ulcer crater indicating gastric lesion with damaged mucosal epithelium and acute inflammation in the stomach in aspirin group ***(John et al., 2010)***.

***Khatib et al., (2010)*** elucidated that the aspirin group at the dose of 200 mg/kg for male Wistar rats, significantly increased gastric volume, free acidity, total acidity and ulcer index, while it decreased the pH and the mucus content.

Aspirin administration at a dose of 500 mg/kg body weight orally to male Wistar rats for five hours caused a significant increase in ulcer index, volume of gastric secretion and total acidity, with significant reduction of gastric pH and gastric mucus content ***(Nair et al., 2010)***.

Histopathological examination of stomach of male albino rats receiving aspirin at a dose of 200 mg/kg orally once daily for 10 days showed ulceration with haemorrhage and discontinuity in the mucosal epithelial lining, various changes, including depletion of gastric wall, mucous content, damaged gastric mucosa and blood vessels in the mucosal layer, venular constriction were also observed in the epithelial cells of mucous membrane ***(Prakash and Gunasekaran, 2010)***.

***Roy et al., (2010)*** revealed that ulcer index was increased significantly in albino rats that were given aspirin at a dose of 200 mg/kg body weight.

In addition, Swiss albino rats taking aspirin at the dose of 200 mg/kg showed significantly increased ulcer index, gastric volume, free acidity and total acidity, but it decreased the pH value and mucus content ***Sivaraman and Muralidharan, (2010)***.

Similar results were obtained by ***Thamotharan et al., (2010) and Ubaka et al., (2010)*** when they examined rats model having an induced ulcer by aspirin (200 mg/kg), ethanol (5 ml/kg) and cold stress, where they found that aspirin caused a significant increase in gastric juice volume, total acidity, free acidity and ulcer index with significant decrease in glutathione level (GSH).

***Vinothapooshan and Sundar, (2010)*** employed rats administrating aspirin at a dosage of 200 mg/kg for four hours as animal model to induce gastric ulcer. They indicated that many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage involving depletion of gastric wall mucin mucosal damage.

Similarly, ***Deoda et al., (2011)*** suggested that the total acidity and total acid output and lipid peroxidation were significantly increased with a decrease in superoxide dismutase (SOD), catalase, and reduced glutathione levels in Wistar rats that were given aspirin at a dose of 200mg/kg.

***Divakar and Devi, (2011)*** reported that oral aspirin administration at the dose of 200 mg/kg for 2 hours to Wistar rats, significantly increased ulcer index, free acidity, total acidity and gastric volume with decreased the pH value.

***Nagesh and Gokul, (2011)*** found that the oral administration of aspirin (100 mg/kg) to albino rats caused congetion, oedema, cellular debris and damaged mucosal epithelium in ulcerated stomach membrane after five hours of administration aspirin.

***Raghavendran et al., (2011)*** employed albino rats that received aspirin at a dose of 400 mg/kg body weight orally for eight hours, where they noticed induced lesions in stomach tissue, characterized by glandular erosions, blood spots in rugae, and severe degenerative changes in the stomach tissue, characterized by gastric pit damage and vacuolization of the glandular portion, particularly in mucus-secreting cells.

***Wang et al., (2011)*** noticed that the total acidity of the gastric juice in the stomach of male Wistar rats was not significantly influenced by aspirin (200 mg/kg/body weight). In addition, microscopical examination revealed haemorrhage and discontinuity in the mucosal epithelial lining, various changes, including depletion of gastric wall, mucous content, damaged gastric mucosa and blood vessels in the mucosal layer in ASA group.

Recently, ***Panda and Sonkamble, (2012) and Shenoy et al., (2012)*** demonstrated that albino Wistar rats of either sex which given aspirin at a dose of 200 mg/kg for 4 hours showed significant increase in ulcer index, ulcer score, percentage of ulceration and level of lipid peroxide (LPO) and reduction in the levels of glutathione **(**GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR).

In the same year, ***Singh and Guha, (2012)*** proved that aspirin at a dose of 500 mg/kg on albino rats (of either sex) for 4 hours, caused a significant increase in ulcer index, volume of gastric secretion, total acidity and total protein with significant reduction of gastric pH, gastric mucus content, total carbohydrate and gastric mucosal damage with reduction of surface mucus coat of the gastric mucosa in ulcerated rats.

**3- <u>Cyclooxygenases and Gastric Ulcer</u>:-**

Prostaglandins (PGs) are of the major groups of chemical mediators in the mammalian body, which are involved in numerous physiological reactions, such as

inflammation and cellular differentiation. PGs have strong cytoprotective effects on the gastric mucosa as a consequence of various indirect mechanisms that include increased epithelial mucus production and bicarbonate secretion, inhibition of gastric motility, inhibition of acid secretion, amelioration of mucosal blood flow, inhibition of free radical and enzyme release from neutrophils, and vascular, luminal and/or extrinsic and intrinsic neural mechanisms ***(Morris et al., 1998)***.

Prostaglandins play an important role in protecting the mucosa of the stomach, by acting on mucus and bicarbonate secretion as well as on blood flow, PGs contribute to maintenance gastric mucosal integrity. Cyclooxygenases (COX) have three distinct membrane anchored isoenzymes, cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and cyclooxygenase-3 (COX-3), also known as prostaglandin synthase, is the first rate-limiting enzyme in the biosynthesis of prostaglandins from arachidonic acid. NSAIDs causes the suppression of local PGs synthesis in the gastric mucosa ***(Tatsuguchi et al., 2000)***.

***Wallace et al., (2000)*** observed that the inhibition of both the COX isoforms, COX-1 and COX-2 in intestinal injury and gastric mucosa from observations in the rats by celecoxib at doses of (5, 15 and 45 mg/kg) and indomethacin at a dose of (5 mg/kg).

Several tissues such as kidney, small intestine and stomach have been shown to express COX-2 under normal conditions. ***Fiorucci and Antonelli, (2001)*** used non-steroidal anti-inflammatory drugs (naproxen (5mg/kg) and diclofenac (10 mg/kg) were received orally to Wistar albino rats for 7 days), where they showed that COX-2 is highly upregulated in the inflamed mucosa in the stomach and small intestine of rats.

***Gretzer et al., (2001)*** reported that the ulcerogenic properties of NSAIDs are not solely explained by the inhibition of COX-1 and require the inhibition of both COX-1 and COX-2, suggesting a role for COX-2 as well as COX-1 in maintaining the integrity of the gastrointestinal mucosa.

The etiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration, and endogenous protective agents (prostaglandins and epidermic growth factors) ***(Repetto and Llesuy, 2002)***.

***Tanaka et al., (2002)*** noticed a role for COX-2 as well as COX-1 in maintaining of the integrity of the small intestine, and strongly indicated that the inhibition of both COX-1 and COX-2 is required for NSAIDs (10 mg/kg of rofecoxib and 10 mg/kg of indomethacin orally for 24 hours) which induced small intestinal damage to male Sprague Dawley rats.

On the other hand, ***Souza et al., (2003)*** elucidated that the administration of aspirin at doses of 50 and 100 mg/kg for three hours to rats resulted in a marked increase in gastric COX-2 expression.

Prostaglandins is mediators of multiple aspects of mucosal barrier function. It causes an increase in mucosal blood flow, stimulate mucus secretion. Suppression of PGs synthesis increases mucosal susceptibility to damage ***(Björne et al., 2004)***.

***Whittle, (2004)*** reported the inhibition of both COX isoforms, COX-1 and COX-2 in intestinal injury and gastric mucosa from observations in the rats by NSAIDs.

Moreover, inhibition of cyclooxygenase (COX), leading to depletion of endogenous prostaglandins, is a major pathogenic factor as a result of 100 mg/kg administration of ibuprofen to male Wistar rats for 7 and 14 days, it is unlikely that prostaglandins deficiency alone is sufficient to initiate the process that ultimately results in gastric ulceration ***(Sánchez-Fidalgo et al., 2005)***.

***Hatazawa et al., (2007)*** determined the localization of the COX-2 in the stomachs with ulcers by immunohistochemical staining. Where they found strong COX-2-immunoreactivity was abundant in the upper portion of the ulcer base on rats and mice that were given NSAIDs (rofecoxib and indomethacin) at doses of 3, 5 and 10 mg/kg for 7 and 14 days. Also, they showed the COX-2 protein was expressed in fibroblasts, macrophages/ monocytes and granulocytes in the upper portion of the ulcer base.

Aspirin at a dose of 40 mg/kg caused gastrointestinal tract ulcer which was elucidated by a multifactorial process involving: inhibition of both COX-1and COX-2; topical injury to the mucosa; inhibition of local blood flow, leukocyte activation/adhesion to the endothelium and induction of the apoptotic pathway of epithelial cells in male Sprague Dawley rats ***(Lichtenberger et al., 2007)***.

***Torüner, (2007)*** suggested that older human that taken aspirin at different doses from 40 to 400 mg, this inhibited both COX-1 and COX-2 but is a more potent inhibitor of COX-2 and prostaglandins. Irreversible acetylation of COX-1 by aspirin inhibits formation of prostaglandins and thromboxane A2 from arachidonic acid which results in preventing platelet aggregation and thrombi formation.

A study made by ***(Iwakiri and Fujimoto, 2008)*** on the gastrointestinal damage caused by NSAIDs, they showed that NSAIDs inhibited the expression of COX-1, a constitutively expressed isozyme present in most organs including the stomach, and COX-2, an isozyme induced at sites of inflammation.

***Niv, (2010)*** reported that the inhibition of cyclooxygenase by NSAIDs (diclofenac (5 mg/kg) and indomethacin (3 mg/kg) on rats and mice for 30 min and one hour), prevented mucin secretion and exposed the mucosa to toxic effect of acid and enzymes in peptic ulcer disease.

NSAIDs like aspirin at a dose of 200 mg/kg to albino rats for 4 hours causes gastric mucosal damage by decreasing prostaglandin levels though inhibition of PGs synthesis ***(Vinothapooshan and Sundar, 2010)***.

Moreover, immunohistochemical analysis was performed to ascertain the localization of COX-1and COX-2 in gastric mucosa tissue. ***Raghavendran et al., 2011)*** confirmed that gastric mucosal tissue of rats treated with aspirin at a dose of 400 mg/kg body weight did not display significant immunoreactivity for COX-1 and COX-2.

**4-<u>Proliferating Cell Nuclear Antigen and Gastric Ulcer</u>**:-

The proliferating cell nuclear antigen (PCNA) is a 36 kDa molecular weight protein also known as cyclin. PCNA has a very granular distribution and is absent from the nucleoli. In cells fixed with organic solvents, PCNA is seen to be strongly associated in the nuclear regions where DNA synthesis is occurring, whereas in cells fixed with aldehydes the staining is more diffuse but intense and occurs throughout the cell cycle. This is due to the presence of two basic forms of the PCNA protein, a soluble form sensitive to organic fixation and not involved in replication, and a second form that is insoluble and is associated with ongoing DNA synthesis. PCNA is a very conserved protein present not only in animal but also in plant cells ***(Yu et al., 1991)***.

The proliferating cell nuclear antigen is a highly conserved protein that has been identified in all eukaryotes as well as in *Archaeobacteria*. It is a multifunctional protein that participates in a variety of essential cellular processes, including DNA replication, DNA repair, and cell-cycle control, by interacting with proteins involved in these processes ***(Balajee and Geard, 2001 and He et al., 2001)***.

On the other hand, the proliferating cell nuclear antigen (PCNA) technique is an accepted method for measurement of cell proliferation. PCNA is the co-factor of DNA-polymerase and can be detected mostly in the late gap 1 phase (G1) and synthesis phase (S phase), but it is also present in every phase of the cell cycle ***(Majka and Burgers, 2004)***.

To investigate the effect of ibuprofen and rofecoxib at a dose of one ml/kg orally for seven and fourteen days on cell proliferation in chronic gastric ulcers. ***Sánchez-Fidalgo et al., (2004)*** used male Wistar rats as an animal model, where they found that the number of PCNA-positive cells was significantly lower in rofecoxib-treated animals than in control animals, while ibuprofen did not decrease cell proliferation significantly.

***Sánchez-Fidalgo et al., (2005)*** made a similar study on male Wistar rats which received 100 mg/kg of ibuprofen for 7 and 14 days, where the immunoreaction for PCNA was observed as a dark reaction products in the nuclei of the middle and lower parts of the crypt in the normal gastric mucosa, while in the ulcerated group, PCNA-positive were augmented respect to distributed in epithelial cells of the ulcer margin and in fibroblastic cells of granulation tissue.

***Potrich et al., (2010)*** showed that PCNA expression in the rats treated orally with omeprazole (40 mg/kg), twice daily for 7 days, was not different from control group.

***Fornai et al., (2011)*** reported that in male Wistar albino rats with gastric damage induced by 14 days treatment with indomethacin at a dose of 6µmol/kg, the expression of PCNA was decreased. The continued indomethacin administration for additional 7 days did not affect further the expression of PCNA. Under these conditions, the concomitant

administration of esomeprazole (5µmol/kg) or lansoprazole (15µmol/kg) caused an increase in the expression of PCNA.

**5- <u>Omeprazole and Gastric Ulcer</u>:-**

Proton pump inhibitors (PPIs) inhibit selectively and irreversibly the gastric $H^+/K^+$ ATPase (the proton pump) that accomplishes the final step in acid secretion. All PPIs inhibit both basal and stimulated secretion of gastric acid, independent of the nature of parietal cell stimulation. In addition, Proton pump inhibitors (PPIs) inhibit release of hydrogen ion from parietal cells. It inhibits gastric acid secretion by blocking $H^+/K^+$ ATPase pump ***(Hatlebakk and Berstad, 1996)***.

Proton pump inhibitors are weak bases carried in the circulation and delivered to the parietal cell as pro drugs. PPIs blocks the enzymes in the wall of the stomach from producing acid and the production of stomach acid is decreased, thus allowing the stomach to heal ***(Richardson et al., 1998)***.

***Sener et al., (2001)*** studied omeprazole (20 mmol/kg/one hour) before administration of ASA (200 mg/kg/two hours), where they found that prevented gastric ulcerogenesis significantly and decreased ulcer index, accompanied by a significant decrease in gastric acidity and increase the level of glutathione. Also, surface epithelial cells were not usually desquamated, but epithelial cell degeneration still existed, glandular cells were vacuolated with regions of haemorrhage.

***Suleyman et al., (2001)*** demonstrated that male albino Wistar rats that were given omeprazole (20 mg/kg for 30 minutes) with the ethanol-induced ulcer, showed a decrease in glutathione level.

On the other hand, ***Berenguer et al., (2002)*** proved that COX-2 was detected in mucous surface cells and mucous cells lining the foveoles adjacent to the ulcer crater in stomach rats which were given 50 ml of 5% acetic acid by injection before administration omeprazole at a dose of 0.35 mg/kg/twice daily for eight and fifteen days. Also, they showed the thinnest granulation tissue and omeprazole group had a high density of microvessels in the ulcer base.

A study was made by ***(Deshpande et al., 2003)*** on albino rats that were given omeprazole (OMP) at a dose of 8 mg/kg for 30 minutes before administration of indomethacin in a dose of 10 mg/kg for one hour, OMP produced a significant gastric and duodenal ulcer protection which was demonstrated by decrease in gastric volume, total acid output, pepsin activity and ulcer index.

***Topaloglu et al., (2004)*** employed albino rats that were injected by omeprazole of 1.14 mg/kg for 12 hours after ulcer induction with 100 ml/kg intraperitoneal injection of ketamine hydrochloride. The authors found that OMP treatment was more effective for maintaining a high gastric pH and lowering total gastric acid output. In addition, OMP

treated rats had lower ulcer indexes, gastric mucosal erosions and better protected mucosal integrity.

In normal gastric mucosa, the greatest density of PCNA positive cells was found in the neck cell compartment. ***Hritz et al., (2005)*** found that inhibition of express PCNA in ulcerated patients by *Helicobacter pylori*. The greatest increment in cell proliferation in response to patients treated by omeprazole (20 mg/day) or esomeprazole (40 mg/day) for 6 months, occurred in the gland compartment of the gastric mucosa. The increase was limited to the deepest portions of the crypts. In both, prior to and after omeprazole or esomeprazole administration, parietal cells did not express PCNA.

Additionally, ***Robinson, (2005)*** said that the pharmacokinetic and pharmacodynamic differences between PPIs are reflected in their influence on both speed and degree of gastric acid suppression, which subsequently may affect their clinical efficacy.

Moreover, ***Hussain et al., (2008)*** reported that male Sprague Dawley rats that were gavaged with absolute ethanol (5 ml/ kg) for sixteen minutes for ulcer induction before receiving omeprazole with a dose of 20 mg/kg for an additional sixteen minutes, showed marked reduction of gastric mucosal damage, reduction of oedema, decrease in leucocyte infiltration of submucosal layer and reduced ulcer area.

Omeprazole (10 mg/kg) was administered orally to albino Wistar rats for 30 minutes before ulcer induction by aspirin treatment at a dose of 200 mg/kg for five days. OMP treatment caused a decrease in ulcer index, total acidity and increase in gastric wall mucus thickness but no significant changes were found in volume of gastric juice as compared with aspirin group ***(Khushtar et al., 2009)***.

***Giri et al., (2010)*** reported that omeprazole treatment at a dose of 20 mg/kg for five days before administrating of aspirin at a dose of 20 mg/kg for four hours, produced a significant reduction in the ulcer index, gastric volume, free and total acidity in albino rats that administrated.

Similarly, ***Nair et al., (2010)*** elucidated that male Wistar rats which received omeprazole in a dose of 1.8 mg/kg body weight for sixty minutes after administration aspirin in a dose of 500 mg/kg body weight, significantly decreased gastric volume, free acidity, total acidity and ulcer index, while it caused an elevation in the pH value and the mucus content.

***Potrich et al., (2010)*** investigated the glutathione level in female Wistar rats that administrated omeprazole (40 mg/kg) one hour before intragastric administration of ethanol (0.5 ml), where they found a decrease glutathione levels in omeprazole treated rat.

Consequently, ***Thippeswamy et al., (2010) and Ubaka et al., (2010)*** reported that omeprazole significantly reduced the gastric juice volume, total and free acidity, ulcer index, protein and pepsin content with an increase the pH of the gastric fluid and mucin

content in Wistar rats that were given a dose of 20 mg/kg body weight for 30 min of OMP after oral administration of dexamethasone (5 mg/kg).

***Borra et al., (2011)*** found that the oral administration of indomethacin (20 mg/kg) to Wister albino rats for 6 hours caused serosal surface of stomach showed marked indurations, dilated blood vessels, ecchymosis and haemorrhagic sites. Omeprazole treatment at a dose of 20 mg/kg for 5 days was given to ulcerated rats, showed that the serosal surface amber colour with few signs of dilated blood vessels and haemorrhagic suffusions, but mucosal surface retained the normal rugae pattern with minimal signs of mucosal injury.

Significant reduction in ulcer score, ulcer index, volume of gastric juice, free and total acidity along significant increase in pH and linear haemorrhagic in the glandular portion of stomach mucosa was found when omeprazole in a dose of 20 mg/kg body weight for 30 min was given to Wister rats after oral administration of absolute ethanol one ml/200 g body weight ***(Gulia and Choudhary, 2011)***.

The results of ***Kandhare et al., (2011)*** suggested that omeprazole treatment (20 mg/kg) after absolute ethanol (8 ml/kg) administration for 8 hours in adult male Swiss albino mice and male Wistar rats decreased infiltration and haemorrhages in the stomach compared to the ulcer group.

***Srinivas and Baboo, (2011)*** suggested that omeprazole produced a significant reduction in the ulcer index, gastric volume, free and total acidity in albino rats that administrated omeprazole (2mg/kg for three days) before thirty minutes of aspirin administrating with a dose of 200 mg/kg.

Recently, ***AlRashdi et al., (2012)*** elucidated that Sprague Dawley rats receiving omeprazole in a dose of 20 mg/kg body weight for one hour after HCl/ethanol solution (5 mL/kg) administration. Significantly decreased total acidity, ulcer index, superoxide dismutase (SOD) activity and malondialdehyde (MDA) with the increase the pH value and mucus content.

In the same year, ***Panda and Sonkamble, (2012) and Shenoy et al., (2012)*** reported similar results on omeprazole treatment by a dose of 20 mg/kg for one hour to ulcerated Wistar albino rats by aspirin in a dose of 200 mg/kg for 4 hours. They confirmed that OMP produced a significant reduction in ulcer index, total acidity and level of lipid peroxide (LPO) with an increase in the levels of glutathione **(**GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GR).

**6- <u>Antioxidants and Indole-3-Carbinol</u>:-**

**A- <u>Antioxidants</u>:-**

Antioxidants are molecules which can interact with free radicals and stop their chain reactions before important and essential molecules are damaged. As oxidative stress is an important part of many diseases, the use of antioxidants is intensively studied in medicinal chemistry, particularly as treatments for vital diseases such as stroke, cancer and

neurodegenerative diseases. Free radicals are produced basically during cellular metabolism and some functional activities and have essential roles in cell signaling, apoptosis and gene expression. On the other hand, excessive free radical attack can damage DNA, proteins and lipids, resulting very important diseases. Antioxidants can decrease the oxidative damage by reacting with free radicals or by inhibiting their activity ***(Tan et al., 1993)***.

Moreover, ***Lutnicki et al., (2001)*** several mucosal defined the stomach and duodenum from noxious agents such as antioxidants that act as radical scavengers, inhibit lipid peroxidation, and other free radical-mediated processes, and therefore they protect the human body from several diseases attributed to the reactions of radicals.

The reactive oxygen species generated by the metabolism of arachidonic acid, platelets, macrophages and smooth muscle cells may contribute to gastric mucosal damage. therefore, by scavenging free radicals, the reactive oxygen metabolites might be useful by protecting gastric mucosa from oxidative damage or by accelerating healing of gastric ulcer ***(Repetto and Llesuy, 2002)***.

***Vasconcelos et al., (2008)*** elucidated that male Wistar rats that were given methanolic fractions extracted from an antioxidant plant (*Mouriri pusa*) at a dose of 250 mg/kg for 14 days and 30 days by gavage to ulcerated rats demonstrated increased PCNA-positive stains in animals treated.

***Lima et al., (2011)*** said that, cell proliferation is known to play amajor role in the healing of gastric ulcers, and PCNA has been demonstrated to be a useful marker of cell proliferation. They showed results indicated that ethyl acetate fraction which acts as an antioxidant that was extracted from a medicinal plant (*Alchornea triplinervia*) at a dose of 100 mg/kg for 14 days to male Wistar albino rats stimulated gastric epithelial cell proliferation by enhancing the expression of PCNA and COX-2 at the base of the stomach glands compared to the ulcerated rats that showed the inhibition of PCNA and COX-2.

Recently, ***De-Faria et al., (2012)*** demonstrated that male Wistar rats that were given butanolic fractions extracted from a medicinal plant (*Rhizophora mangle*) at a dose of 0.5 mg/kg for 14 days by gavage to ulcerated rats demonstrated a great number of PCNA in stomachs of animals treated.

**B- <u>Indole-3-Carbinol</u>:-**

Indole-3-carbinol (I3C) is a naturally occurring hydrolysis product of glucobrassicin found in vegetables of the *Cruciferae* family such as broccoli, brussels sprouts, and cauliflower. Epidemiological studies suggest high dietary intake of cruciferous vegetables is associated with lower cancer risk, and it is possible that the chemopreventive properties are in part attributable to I3C ***(Verhoeven et al., 1997)***.

***Esther et al., (1998)*** elucidated that male Wistar rats that had gastrointestinal cancer by ibuprofen in a dose of 88mg/kg, received diets with 250 ppm of indole-3-carbinol for

two weeks. These diets caused a decrease in glutathione *S*-transferases (GST) and total glutathione peroxidase activity (GPx), with an increase in glutathione activity in oesophagus, stomach, intestine and liver.

A study made by ***Malejka-Giganti et al., (2000)*** confirmed the decrease in mammary tumorigenesis, cumulative mammary tumor incidences and multiplicities by I3C treatment at a dose of 250 mg/kg body weight to female Sprague Dawley rats with mammary tumors for 15 weeks.

Indole-3-carbinol (I3C), a common phytochemical in the human diet, is present in all members of the cruciferous vegetable family. It has become clear that I3C has the potential to prevent and even to treat a number of common cancers, especially those that are estrogenrelated. Pure I3C taken as a dietary supplement (the equivalent of one third of a head of cabbage per day) is rapidly converted in the stomach to a variety of condensation products ***(Chen et al., 2001)***.

Moreover, indole-3-carbinol is the aglycone of glucobrassicin, a microconstituent of cruciferous vegetables. I3C, which is generated by glycoside-hydrolyzing enzymes when plant cells are disrupted by processing, possesses chemopreventive properties against chemically induced tumors. I3C is a potent inducer of cytochrome P450 enzymes, including CYP3A ***(Donald et al., 2004)***.

***Garikapaty et al., (2005)*** suggested that I3C injected twice weekly at 20 mg/kg and 80 mg/kg inhibited prostate cancer cells and proliferating cells.

***Plate and Gallaher, (2006)*** evaluated a decrease in aberrant crypt foci, dysplasia and proliferating cell nuclear in rats' colon cancers by I3C at doses of 1.36 mmols/diet and 36.7 mmols/diet for 2 weeks.

Like other vegetables, cruciferous vegetables contain a number of components with cancer chemopreventative properties, including folate, fiber, carotenoids and minerals. However, cruciferous vegetables are unique in that they are rich sources of glucosinolates that may play a significant role in the association between cruciferous vegetable consumption reduced cancer rates. Each cruciferous vegetable contains a mixture of glucosinolates ***(Higdon et al., 2007)***.

***Souli et al., (2008)*** reported that I3C at a dose of 20 mg/kg to mice with prostate cancers for 14 days, caused reduction in proliferation rates, promoted apoptosis, tumor growth, tumor volume and microvessel density.

***Okulicz et al., (2009)*** noticed that the administration of I3C at a dose of 150 mg/kg for 7 day to male rats caused the inhibition of the development of tumors in forestomach, glandular stomach, mammary gland, liver and decreased the level of glycogen in the liver with a significant increase in the content of triglycerides, glucose, cholesterol and insulin.

***Kassie et al., (2010)*** demonstrated that I3C (30 µmol g/diet and 70 µmol g/diet) when administrated to female mice that had lung cancer for 6 weeks caused a reduction in

multiplicities of adenoma and size of pulmonary adenocarcinoma with cellular pleomorphism.

A cell culture by I3C at a dose of 50µm for 24 hours possessed anti-inflammatory effects by are suppression of nitric oxide (NO) production by inhibiting inducible nitric oxide synthase (iNOS) expression and decreasing tumor necrosis factor- α (TNF-α) production and interleukin-1β (IL-1β) mRNA expression, respectively, in Lipopolysaccharide-activated macrophages ***(Tsai et al., 2010)***.

Recently, ***Weng et al., (2012)*** demonstrated that oral squamous cell carcinoma were plated and treated with the concentration of 400µM I3C for 48 hours. I3C treatment caused a decrease in the number of cells cancer, nuclear factor kappa-B ($NF\text{-}_kB$) in cells carcinoma, phosphorylation of protein kinase-B (PKB), with increases in the phosphorylation of p53.

# *MATERIAL AND METHODS*

## I- MATERIAL:

**1-Experimental Animals:-**

The present study was conducted using male albino rats of the strain *Rattus norvegicus* weighing 140-160 gm. Animals were purchased from Center of Medical Researches and Bilharzias. Hospitals of Ain Shams University (Cairo. Egypt) and housed under standard laboratory conditions. Rats were fed with standard laboratory diet ***(Sunilson et al., 2008)*** and water *at-libitum* with fresh daily supplies. They were allowed 7 days pre-experimental period to adapt to the laboratory conditions.

**2-Experimental Drugs and Chemicals:-**

**A -Aspirin:-**

Aspirin (ASA) ($C_9H_8O_4$), also known as acetylsalicylic acid (Fig. 1), is a salicylate drug, often used as an analgesic to relieve minor aches, pains, antipyretic, reduce fever and anti-inflammatory medication ***(Choi et al., 2010)***. The most common adverse effects of aspirin (ASA) are gastric bleeding and formation of gastrointestinal ulcers.

Aspirin tablets (Bayer AG, Germany) were given to animals in this study at a dose of 500 mg/kg/body weight dissolved in distilled water ***(Sarkar and Guha, 2008)*** by stomach tube after a fasting period of 24 hours.

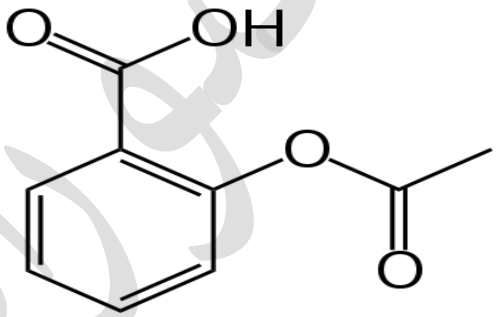



**Figure (1): The Chemical Formula Of Aspirin**
***(Wallace et al., 1995).***

**B-Omeprazole:-**

Omeprazole (OMP) ($C_{17}H_{19}N_3O_3S$) (Fig. 2), omeprazole is proton pump inhibitors (PPIs). Omeprazole is substituted benzimidazoles that inhibit acid secretion by gastric parietal cells through alteration of the $H^+/K^+$ ATPase. Animals were given omeprazole (European Egyptian Pharm. IND. Egypt) at a dose of 20 mg/kg/body weight dissolved in distilled water ***(Suleyman et al., 2001 and Giri et al., 2010)*** by stomach tube.

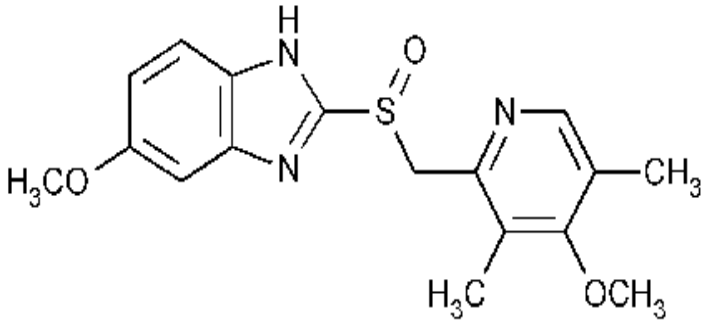



**Figure (2): The Chemical Formula Of Omeprazole** ***(Cheon et al., 1999)***.

**C-Indole-3-Carbinol:-**

Indole-3-Carbinol (I3C) ($C_9H_9NO$) (Fig. 3), is a naturally occurring antioxidant compound found in vegetables of the *Brassica* genus, such as cabbage, broccoli and brussels sprouts. Active component of indole-3-carbinol is thioglucoside conjugates, namely glucosinolates ***(Pappa et al., 2006)***, acts through selective beneficial alteration of Phase I and Phase II carcinogen-metabolising enzymes ***(Anderton et al., 2004)***.

I3C was purchased from Sigma-Aldrich Chemical Company U.S.A. (Cairo, Egypt). Animals were given (I3C) at a dose of 20 mg/kg/body weight dissolved in distilled water ***(Crowell et al., 2006)*** by stomach tube.

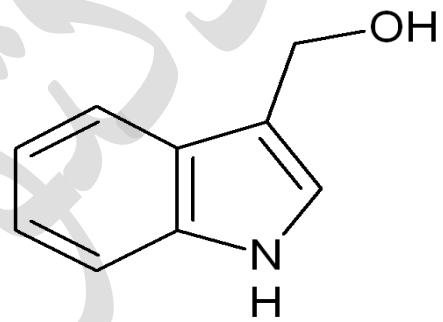



**Figure (3): The Chemical Formula Of Indole-3-carbinol** ***(Higdon et al., 2007).***

It is to be mentioned hereby that all drug doses were calculated according to fluctuations in rats body weights.

**3-<u>Fixative For Tissue Sampling</u>:-**

Dissected organ were washed in saline and fixed in neutral buffered formalin ***(Lillie, 1954)*** and processed for microtomy at 6 microns thick by a Cambridge Rocking Microtome.

**4-<u>Stains For Tissue Investigation</u>:-**

**a-**Haematoxylin and Eosin for general histological examination.

**b-**Bromophinol blue technique for total proteins.

**c-** Alcian blue-Periodic Acid Schiff (PAS) technique for acid and neutral mucin.

**d-**Cyclooxygenase-2 (COX-2) for demonstration of particular immunohistochemical features.

**e-**Proliferating cell nuclear antigen (PCNA) for demonstration of particular immunohistochemical features.

## II-METHODS:

### 1- Housing of Experimental Animals:-

Rats were housed in wire mesh laboratory animal cages. Each cage was provided with a mesh drawer for collecting and removing wastes which was done daily. Fresh supplies of food and water were subsequently offered. All animals were allowed to acclimatize for 7 days before the commencement of the experiment.

### 2- PilotTest for Ulcer Induction:-

For ulcer induction, different doses of aspirin were orally tested 100 mg/kg/body weight ***(Ubaka et al., 2010)***, 400 mg/kg/body weight ***(Kamsiah et al., 2005)***, 500 mg/kg/body weight ***(Bharti et al., 2010)*** and 750 mg/kg/body weight. The animals were sacrificed after 4 hours ***(Jainu et al., 2006)***, 24 hours, two days, four days and seven consecutive days.

### 3-Body Weight:-

Rats were individually weighted by means of a Meopta sensitive balance. Whole weights were recorded to the nearest one gram to determine weekly changes during the experimental duration.

### 4-Stomach Weight:-

After dissection, stomach of each rats was removed, then weighted and recorded to the nearest one milligram to determine changes during the experimental duration. In order to obtain a more precise measure the change in stomach weights were recorded relative to whole weights according to the following equation:

Relative Stomach Weight =

$$\frac{Absolute\ stomach\ weight\ (gm)}{Body\ weight\ of\ rat\ (gm)} \text{ X } 100$$

The relative stomach weight was measured according to ***Aniagu et al., (2005)***.

### 5-Mortality Rates:-

Number of dead individuals were recorded daily followed by calculation of percentage of mortality in weekly average for each group separately according to the following equation:

Mortality percentage = $\frac{No.of\ dead\ rats}{Total\ No.of\ rats} \times 100$

**6-<u>Biochemical Parameters</u>:-**

**A - Haematological Investigation:-**

Blood samples were collected on EDTA tubes. The samples were kept at room temperature for maximally five hours before they were run on haemocytometer (Medical Electronic machine. Scopus-micro, Egyptian Medical Centre). The levels of total red blood cells count (R.B.Cs), haemoglobin (Hb), haematocrit (HCT), total leucocytic counts (W.B.Cs), percentage of lymphocytes, percentage of monocytes and platelets count (PLTs) were investigated.

**B- Determination of Serum Total Protein Levels:-**

Blood samples were drawn from the heart of the rats and allowed to coagulate at room temperature then centrifuged at 3000 rpm for 15 minutes. Clear non haemolysed serum was used to investigate serum total protein which was determined by the colorimetric method according to ***Doumas, (1975)*** using spectrophotometer (Humalyzer, Junior, Semi-automated Bench top Chemistry Photometer. Human). Reagent kits was purchased from Biodiagnostic, Cairo. Egypt.

**Principle:**

In the presence of an alkaline cupric sulfate, the protein produces a violet colour, the intensity of which is proportional to their concentration.

**Reagents:**

1. Total protein reagent (solution of cupper sulphate-pentahydrate 0.3 g/dl in aqueous sodium hydroxide 0.8 g/dl + potassium iodide and potassium tartrate).
2. Total protein standard (aqueous solution of Bovine albumin, fraction v, with sodium azide as preservative).

**Procedure:**

Three series of tubes were assigned for unknown sera, blank and standard. 1000 µl of total protein reagent were added to each tube. 10 µl of standard were put in standard tube and 10 µl of serum were put in the sample tube before mixing and incubation for 5 minutes at 37ºC. The absorbance of the sample and the standard were measured against reagent blank at 550 nm within 60 minutes .

Calculation:

Serum total protein(µg/dl) =

$$\frac{Optical\ density\ of\ sample}{Optical\ density\ of\ standard} \times 5$$

**C-Determination of Serum Albumin Levels:-**

Clear non haemolysed serum was used to investigate serum albumin which was determined by the colorimetric method according to ***Doumas et al., (1971)*** using spectrophotometer (Humalyzer, Junior, Semi-automated Bench top Chemistry Photometer. Human). Reagent kits was purchased from Biodiagnostic, Cairo. Egypt.

**Principle:**

Albumin was bounded to bromocresol green dye to produce an increase in the blue green colour. The colour intensity was proportional to the concentration of the albumin present.

**Reagents:**

Bromocresol green 0.15 g/dl. Buffer solution pH 4.66 ± 0.1. Surfactant, non-reactive ingredients and stabilizers. Albumin Standard.

**Procedure:**

Three sets of test tubes blank, standard and sample tubes were labeled 1.0 ml of the reagent were pipette in the three series of tubes. 10 µl of standard were put in the standard tube and 10 µl of serum were put in the sample tube. All tubes were incubated at room temperature for one minute. The absorbance of the sample and the standard were read and recorded against reagent blank at 630 nm.

Calculation:

Albumin conc. (µg/dl) =

$$\frac{Optical\ density\ of\ sample}{Optical\ density\ of\ standard} \times 4$$

**D -Glutathione Activity (GSH)**:-

A known weight of the stomach was homogenized in a suitable aliquot of ice cold phosphate buffered saline solution (pH 7.4) to make 10% homogenate (w/v). The homogenate was centrifuged at 4000 rpm for 15 minutes and the supernatant was separated and used for assaying stomach tissue reduced glutathione (GSH) according to ***Villegas et al., (2000)***. The laboratory kit was purchased from Biodicgnostic, Cairo. Egypt.

**Principle:**

The method base on the reduction of 5,5´ dithiobis (2- nitrobenzoic acid) (DTNB) with glutathione (GSH) to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm.

**Reagents:**

1- Trichloroacetic acid (TCA) 500 mmol/L.
2- buffer 100 mmol/L .
3- DTNB 1.0 mmol/L .

**Sample preparation:**

1-Stomach tissue was perfused with a PBS (phosphate buffered saline) solution, pH 7.4 containing 0.16mg/ml heparin to remove any red blood cells and clots.
2-The stomach tissue was homogenized in 5-10 ml cold buffer (i. e, 50 Mm potassium phosphate, pH 7.5,1 Mm EDTA) per gram tissue, using tissue homogenizer, then Centrifuged at 4,000 rpm for 15 minutes at 4ºC.
3-The supernatant was removed, stored on ice and assayed.

**Proceduse:**

Two series of tubes were assigned for unknown samples and blank.

**A.** 0.5 ml of reagent 1 was added to each tube.
**B.** 0.5 ml of distilled water was added to blank tube .
**C.** 0.5 ml of tissue supernate was added to each sample tube.

All tubes were mixed well, allowed to stand for 5 min and centrifuged at 3000 rpm for 15 min.

**D.** 0.5 ml of the supernate was added to another series of tubes followed by 1 ml of reagent 2 and o.1 ml of reagent 3.

All tubes were mixed well. The absorbance was measured after 5-10 min at 405 nm of $A_{sample}$ against the blank.

Calculation:

GSH concentration in stomach tissues=

$$\frac{A\ sample \times 2.22}{g.tissue\ used}\ \text{mmol/g. tissue}$$

**E-Measurement of Gastric Total Acid and pH:-**

The rats' stomach were ligated from its two ends, the pylorus and the lower esophagus, and injected with 2 ml distilled water. A small incision were made on the forestomach, and the stomach contents were expelled ***(Liu et al., 2003 and Hsu et al., 2009)***.

The gastric contents were collected in tubes then measured. Gastric juice was centrifuged at 3500 rpm for 15 min and the supernatant was used for determining total acidity. One ml of the supernatant liquid was pipetted of out and diluted to 10 ml with distilled water. The solution was titrated against 0.01 N NaOH using phenolphthalein reagent as indicator, to the endpoint (the solution turned to pink colour). The volume of NaOH required was noted and was taken as corresponding to the total acidity ***(Al-dalain et al., 2008)***.

The pH of the supernatant and total acidity were measured according to ***Sadler and Murphy, (2010) and Vinothapooshan and Sundar, (2010)*** respectively.

$$pH = - \text{Log } [H^+].$$

Total acidity =

$$\frac{\text{Volume of NaOH x Normality x 100}}{0.1} \text{ m Eq/1}$$

**7- <u>Determination of Ulcer Index (UI)</u>:-**

Each stomach was opened along the greater curvature and washed with a distilled water and examined using magnifying lens to determine the ulcer index according to ***Parmar and Desai, (1993)***.

The ulcerative index was calculated by severity of gastric mucosal lesions which graded as follows:

Grade1 = less than1mm Erosions.
Grade2 = 1-2mm Erosions.
Grade3 = More than 2mm Erosions.

The UI was calculated for each ulcered rat using this formula:

UI= 1× (number of lesions of grade 1) + 2× (number of lesions of grade 2) + 3 × (number of lesions of grade 3).
Then the overall score was divided by a factor of 10, which was designated as ulcer index according to ***Al-dalain et al., (2008)***.

Evaluation of degree of ulceration was expressed in terms of ulcer score which is calculated by dividing the total number of ulcers in each group by number of rats in that group ***(Robert et al., 1968)***.

$$\text{Ulcer score} = \frac{\text{Total number of ulcers in group}}{\text{Number of rats in group}}$$

$$\text{Percentage of ulceration} = \frac{\text{Number of ulcerated rat} - \text{Number of non ulcerated rat}}{\text{Number of rat in group}} \times 100$$

The degree of ulceration was calculated according to the method of ***Radwan et al., (2003) and Mabrouk et al., (2009)***.

**8-Histological , Histochemical and Immunohistochemical Methods:-**

**Fixation:**

For histological, histochemical and immunohistochemical examination stomach were placed in 10% buffered formalin ***(Lillie, 1954)***.

**Processing:**

Dehydration of fixed tissues was carried out using ascending grades of ethyl alcohol, then cleared with xylene. Infitration with paraffin wax at 60ºC was followed by embedding. Paraffin blocks were cut at 6 microns from all specimens, using a Cambridge Rocking Microtome, and affixed to slides.

**Staining:**

For **general histological** examination; sections were stained as a routine in: Harris's alum haematoxylin and easin (H&E) ***(Bancroft and Cook, 1994)***.

For demonstration of particular **histochemical features**, the following staining techniques were employed:

**i- Bromophenol blue *(Mazai et al., 1953):-***

For the demonstration of sites of total protein content. Mercuric bromophenol blue has the ability to combine with proteins yielding a blue conlouration.

**ii- Alcian blue-Periodic Acid Schiff (PAS) method *(Mowry, 1956)*:-**

For the demonstration of the presence of mucins and clearly distinguishes between acid and neutral mucins. By first staining all acid mucins with alcian blue, those acid mucins which are also PAS positive will not react with subsequent PAS reaction, only the neutral mucins, where the result by this reagent were acid mucins a blue, neutral mucins a magenta and nuclei a pale blue.

For demonstration of particular **immunohistochemical features**, the following staining techniques were employed:

**i- Immunohistochemistry of Cyclooxygenase-2 (COX-2) *(Niijima et al., 2002)*:-**

From 10% formalin fixed paraffin embedded samples, 6-µm thick sections were prepared. The sections were deparaffinized in xylene, hydrated through standard graded ethanol solutions and treated with 0.2% saponin (CliniLab Cairo, Egypt. Aran B Chemical

Company U.K.) at room temperature for 30 minutes. After the sections were treated with methanol containing 3% hydrogen peroxide ($H_2O_2$) for 15 minutes to eliminate endogenous peroxidase, the sections were reacted with 10% normal rabbit serum for 10 minutes to block nonspecific reactions. As the primary antibody, cyclooxygenase-2 (COX-2) polyclonal antibodies were diluted 100 times in phosphate-buffered saline (PBS) and reacted with the sections at 4°C for 15 minutes. After the streptavidin–biotin complex method in kits of (CliniLab Cairo, Egypt. Aran B Chemical Company U.K.) biotin-labeled anti-rabbit immunoglobulin G antibody as the secondary antibody was reacted with the sections at room temperature for 15 minutes, and the peroxidase-labeled streptavidin was reacted at room temperature for 10 minutes, followed by color development using diaminobenzidine (DAB) reagent. After counterstaining with Mayer's hematoxylin the sections were observed.

**ii-Immunohistochemistry of Proliferating Cell Nuclear Antigen (PCNA)** ***(Niijima et al., 2002)*****:-**

From 10% formalin fixed paraffin embedded samples, 6-µm thick sections were prepared. The sections were deparaffinized in two changes of xylene, hydrated and autoclaved at 121°C for 15 minutes to increase antigenicity. The basic methodology used was the same as that for COX-2. As the primary antibody, anti-PCNA monoclonal antibody (CliniLab Cairo, Egypt. Aran B Chemical Company U.K.) was diluted 100 times in phosphate-buffered saline (PBS) before use.

Method of counting: The count of immunopositive cells in relation to the number of COX-2 and PCNA were carried out by of a cell Imaging Software on fine picture (×400) randomly selected ***(Sun et al., 2005)***.

Immunoreactivity was evaluated in a blinded fashion by two independent observers using a grading system where no staining was regarded as negative (-ve). Furthermore, positivity was rated as (+) when stained cells accounted for between 1% and 10%, (++) for between 11% and 30%, (+++) for between 31% and 50% and (++++) for 51% or more ***(Lajoie et al., 2002 and Niijima et al., 2002)***.

**9- <u>Statistical analysis</u>:-**

Results are expressed as mean ± standard error (SE). The macroscopic and microscopic lesion scores and other parameters were analyzed using significance by one way ANOVA.

All statistical procedures were performed with the SPSS for Windows, version (17.0) statistical analysis package. The significance of the difference between means of the normal control and all treated rats (a) and between the treated ulcerated rats with ASA/U alone (b).

The percentage change was calculated according to the following equation:

$$\text{Percetage of change } = \frac{\bar{X}_2 - \bar{X}_1}{\bar{X}_1} \times 100$$

Where : $\bar{X}_1$ : The mean of normal control value.

$\bar{X}_2$ : The mean of experimental groups.

## *Experimental Design:*

In the present investigation a total number of 96 male albino rats were used. They were divided into two main studies.

In the first, 48 rats were divided into eight experimental groups of six animals in each group. Normal control group, OMP group, I3C group and OMP+I3C group. Treated group was further divided into another four subgroups of six animals in each group which are ASA group, ASA+OMP group, ASA+I3C group, ASA+OMP+I3C group for a duration of seven days.

Second, 48 rats were divided into two main groups control groups and ulcerated groups (administrated aspirin for seven consecutive days). Aspirin administration was stopped after 7 days representing the initial duration (zero time) for the experiment and was followed by beginning of different experimental regimens for a total experimental duration of four weeks. Control group was further divided into four subgroups of six animals each which are normal control group, OMP group, I3C group and OMP+I3C group. Ulcerated group was further divided into another four subgroups of six animals in each group which are Ulcer group (U group), U+OMP group, U+I3C group and U+OMP+I3C group.

Animals were divided according to the following experimental design ***(Table 1)***.

**Table (1): Experimental design and group distribution:-**

| Group | Experimental condition | Individual No. |
|---|---|---|
| **1-** | **Normal control group.** For 7 days**.** | **6** |
| **2-** | **OMP group.** Animals were given orally omeprazole 20 mg /kg/b.w . for 7 days. | **6** |
| **3-** | **I3C group.** Animals were given orally indole-3-carbinol 20 mg/kg/b.w. for 7 days**.** | **6** |
| **4-** | **OMP+I3C group.** Animals were given orally omeprazole 20 mg /kg/b.w.+ indole-3-carbinol 20 mg/kg/b.w. for 7 days**.** | **6** |
| **5-** | **ASA group.** Animals were given orally aspirin 500 mg/kg/b.w. for 7 days**.** | **6** |
| **6-** | **ASA+OMP group.** Animals were given orally aspirin 500 mg/kg/b.w. + omeprazole 20 mg/kg/b.w. for 7 days. | **6** |
| **7-** | **ASA+I3C group.** Animals were given orally aspirin 500 mg/kg/b.w. + indole-3-carbinol 20 mg/kg/b.w. for 7 days**.** | **6** |
| **8-** | **ASA+OMP+I3C group.** Animals were given orally aspirin 500 mg/kg/bw + omeprazole 20 mg/kg/b.w. + indole-3-carbinol 20 mg/kg/b.w. for 7 days**.** | **6** |
| **1-** | **Normal control group.** For 4 weeks. | **6** |
| **2-** | **OMP group.** Animals were given orally omeprazole 20 mg /kg/b.w . for 4 weeks. | **6** |
| **3-** | **I3C group.** Animals were given orally indole-3-carbinol 20 mg/kg/b.w . for 4 weeks**.** | **6** |
| **4-** | **OMP+I3C group.** Animals were given orally omeprazole 20 mg /kg/b.w.+ indole-3-carbinol 20 mg/kg/b.w. for 4 weeks**.** | **6** |
| **5-** | **U group.** Animals were given orally distilled water for 4 weeks. | **6** |
| **6-** | **U+OMP group.** Animals were given orally omeprazole 20 mg/kg/b.w. for 4 weeks. | **6** |
| **7-** | **U+I3C group.** Animals were given orally indole-3-carbinol 20 mg/kg/b.w. for 4 weeks. | **6** |
| **8-** | **U+OMP+I3C group.** Animals were given orally omeprazole 20 mg/kg/b.w. + indole-3-carbinol 20 mg/kg/b.w. for 4 week. | **6** |
| **Total** | | **96** |

At the end of each experimental duration i.e. (seven days and four weeks), rats were fasted overnight, anesthetized under ether, and blood was collected by cardiac puncture

where a part of the blood was collected in empty, dry, clean tubes containing ethylene diethyl tetra acetic acid (EDTA) as the sodium salt.

Various haematological parameters were performed including :

a). Determination of Total Red Blood Cells Count (R.B.Cs).
b). Determination of Haemoglobin Content (Hb).
c). Determination of Percentage of Haematocrit (HCT).
d). Determination of Total Leucocytic Counts (W.B.Cs).
e). Determination of Lymphocytes Count (LYM)
f). Determination of Monocytes Count (MON).
g). Determination of Platelets Count (PLTs).

Other blood samples were collected and left to clot, then centrifuged at 3000 rpm for 10 minutes. Sera were divided into small aliquitots to avoid the repeating of thawing and freezing. Sera were used for the determination of biochemical analysis.

Rats were dissected, and the gastric contents were collected into centrifuge tubes and measured, then stomach was removed and opens along the greater curvature, washed and cleaned. The stomach tissues were cut into pieces and frozen for the determination of the glutathione (GSH).

All sera and stomach samples were stored at -20˚C until analysis.

The total number of lesions per stomach was counted and each lesion was scored to the experimental groups.

The stomach samples were fixed in 10% buffered formalin for subsequent light microscopic examination. Stomach tissues were processed by routine methods for subsequent histological, histochemical and immunohistochemical evaluation.

Sections were stained by Haematoxylin and Eosin for the assessment of general structure.

Bromophenol blue stain was used for demonstration of sited of total protein content .

Alcian blue-Periodic Acid Schiff (PAS) stain was used for demonstration of the presence of mucins and clearly distinguishes between acid and neutral mucins.

Immunohistochemical localization for the demonstration of cyclooxygenase-2 (COX-2) and proliferating cell nuclear antigen (PCNA).

# ***RESULTS***

## I . Pilot Test for Ulcer Induction:-:-

For ulcer induction different doses of aspirin were orally tested 100 mg/kg/body weight, 400 mg/kg/body weight, 500 mg/kg/body weight and 750 mg/kg/body weight. The animals were sacrificed after 4 hours, 24 hours, two days, four days and seven consecutive days.

The best confirmed histological and biochemical dose for acute ulcer induction was 500 mg of aspirin (ASA)/kg/body weight for seven consecutive days.

## II .Morphological Investigation:-

### 1-Growth Rates:-

Averages of body weights of rats belonging to the control and experimental groups are given in ***table (2)***. The data are graphically presented by ***figure (4)***, both the figure and table showed progressive increase in body with the lapse of time. Normal control rats designated a more or less constant value of initial body weights and body weights during the study period.

In relation to the control animals, a significant increase ($P \leq 0.05$) was denoted in the aspirin alone (ASA) or with omeprazole and/or indole-3-carbinol groups. The percentage of increase were (31.45%, 16.35% and 15.09%) respectively at seven days.

Omeprazole and indole-3-carbinol groups administration for 4 weeks showed slight more or less varying levels from the normal control rats, whereas a significant increase in the body weight was detected in the I3C group at four weeks compared to the normal control group.

OMP and I3C treatments to aspirin ulcerated rats were increased with a percentage of (23.71% and 18.19%) respectively in these groups.

### 2- Absolute Stomach Weight :-

Stomach weights of rats belonging to the control and experimental groups are given in ***table (3)*** and graphically represented by ***figure (5)***. Normal control animals designated a more or less constant value of stomach during the study period at 7 days and 4 weeks.

Compared to the aspirin, a decrease in stomach weight was recognized in aspirin with omeprazole and/or indole-3-carbinol groups respectively with a percentage of decrease of (29.19%, 23.78% and 24.86%) in these groups.

The mean value of stomach weight showed an increase after four weeks in ulcer (U) group was 1.75±0.05 compared to 1.50±0.15 in the normal control rats.

**3-Relative Stomach to Body Weight:-**

The data of relative stomach weights in rats following seven days and 4 weeks of aspirin administration are given in ***table (4)*** and graphically represented by ***figure (6)***. Normal control animals signed a more or less constant value of relative stomach weights.

No significant changes in relative stomach to body weight between groups in 7 days.

Statistically, in four weeks a significant decrease ($P < 0.01$) was showed in the ulcer rats treated with omeprazole (U+OMP) to the normal control animals.

**4-Mortality Rates:-**

The mortality rate was found in aspirin group that took 500 mg/kg body weight for seven days where two rats from 6 rats died after five days of aspirin administration with a percentage of 33.33% .The mortality rate was tabulated in ***table (5)*** graphically represented by ***figure (7)***.

**III- Biochemical Parameters :-**

**1-Haematological Investigation:-**

**A. Evaluation of Total Red Blood Cells Count (R.B.Cs):-**

The mean values of total red blood cells count (R.B.Cs) of control and experimental groups were presented in ***table (6)*** and graphically represented by ***figure (8)***. Normal control animals signified more or less constant levels of total red blood cells count within the experiment of period of 7 days and 4 weeks.

At seven days, aspirin treated with omeprazole and indole-3-carbinol showed a significant decrease ($P \leq 0.05$) in R.B.Cs, the mean values recording (5.37±0) as compared to normal control group (6.68±0.45).

Whereas, no significant changes found in total red blood cells values at four weeks.

**B. Evaluation of Haemoglobin Content (Hb):-**

From results tabulated in ***table (7)*** and graphically lined in ***figure (9)***, a non significant change in haemoglobin content was recorded for rats at seven days and four weeks. Normal control animals signified more or less constant levels of haemoglobin within the experiment of period of 7 days and 4 weeks.

**C. Determination of the Percentage of Haematocrit (HCT):-**

Percentage of haematocrit in normal and aspirin rats are given in ***table (8)*** and graphically represented by ***figure (10)*** showed that normal control animals had constant levels of haematocrit within the experiment of period of 7 days and 4 weeks.

After seven days, ASA treatment with OMP and I3C showed a significant decrease ($P \leq 0.05$) in HCT values, the mean values recording (38.55±0.26) as compared to normal control group (44.33± 3.39).

Whereas, no significant changes found in haematocrit values at four weeks.

**D. Evaluation of Leucocytic Counts (W.B.Cs):-**

From the inspection of the data recorded in ***table (9)*** and ***figure (11)***, it were shown that normal control animals designated more or less normal levels of leucocytic counts (W.B.Cs) during the course of study of seven days or four weeks.

There was a slight increase in the mean values of W.B.Cs in aspirin group at seven days to be (20.53±1.75) compared to normal control group of (17.90±0.17).

Nevertheless, at the four weeks in relation to normal control rats, ulcerated rats alone or with OMP and OMP+I3C showed a significant increase ($P < 0.05$) in their W.B.Cs mean values. The percentage of elevation was (23.42%, 20.84% and 21.57%) respectively compared to the normal control animals .

**E. Evaluation of Lymphocytes Count (%):-**

The data recorded in ***table (10)*** and ***figure (12)***, where normal control rats denoted more or less normal levels of percentage of lymphocytes during the course of study of seven days or four weeks.

A significant decrease ($P < 0.001$) in the percentage of lymphocytes was recorded in aspirin group treated with omeprazole or omeprazole and indole-3-carbinol groups with percentage of (14.78% and15.19%) respectively at seven days.

Ulcer treatment groups (U) at four weeks showed significant decrease ($P < 0.001$) in the percentage of lymphocytes, and a still higher refuse level was reached in the ulcer treatment with omeprazole (U+OMP) group and the percentage decrease were (8.33% and 3.93%) respectively.

**F . Evaluation of Monocytes Count (%):-**

From the results recorded in ***table (11)*** and ***figure (13)***, it were shown that normal control animals designated more or less normal levels of percentage of monocytes during the study period at 7 days and 4 weeks.

At seven days, a pronounced decrease in the percentage of monocytes was detected in the aspirin rats with a percentage decrease of 41.18% from normal control rats.

At the end of the study, a significant decrease ($P < 0.05$) in the percentage of monocytes was found in the ulcerated rats with omeprazole and indole-3-carbinol group with a percentage of (22.26%) as compared to normal control groups.

**G . Evaluation of Platelets Count (PLTs):-**

From the data recorded in ***table (12)*** and ***figure (14)***. Normal control animals designated more or less normal levels of platelets count during the experimental period.

In aspirin animals, alone or treated with omeprazole and omeprazole with indole-3-carbinol groups, there were a significant decline ($P < 0.001$) in the mean values of platelets levels to record (136±33.02, 177.50±1.44 and 339.50±0.2) respectively at seven days compared to normal control group of (570±35.22). There was a percentage of increase of (319.85% and 149.63%) in the platelets levels of ASA+I3C and ASA+OMP+I3C groups respectively compared to aspirin administrated animals.

Ulcerated rats at four weeks of treatment still a significant decline ($P < 0.05$) in their PLTs mean values recording (453±25.98) compared to normal control animals of (568.5± 35.51).

**2-Evaluation of Serum Total Protein Levels :-**

On measuring serum total protein levels, the data tabulated in ***table (13)*** and graphically represented by ***figure (15)*** were given. Normal control animals signified more or less constant values of serum total protein levels during the seven days and four weeks of experimental period.

An increase in the mean values of serum total protein levels was recorded in aspirin group alone or with omeprazole and/or indole-3-carbinol groups recording means of (9.63±0.82, 9.00±0.79, 10.57±0.42 and 10.06±0.60) respectively at seven days as compared to (7.55±0.02) of normal control rats.

In four weeks a slight increase in the mean values of ulcerated group and ulcerated rats with omeprazole and/or indole-3-carbinol groups recording to (8.93±0.84, 9.86±0.66, 9.13±1.54 and 8.50±0.29) compared to (7.59± 0.04) of normal control animals.

**3- Evaluation of Serum Albumin Levels:-**

The mean values of serum albumin levels of control and experimental groups were presented in ***table (14)*** and ***figure (16)***, where more or less normal levels of serum albumin were manifested in normal control animals.

A increase in the mean of serum albumin levels in rats from control omeprazole, indole-3-carbinol and omeprazole with indole-3-carbinol groups at throughout experimental period.

Statistically, a significant increase ($P < 0.001$) occurred in serum albumin levels in aspirin rats with a mean value of 4.16±0.24 at seven days compared to 2.76±0.31of normal control group. Compared to aspirin group the percentage of decrease in ASA plus OMP, ASA plus I3C and ASA plus OMP plus I3C groups was recorded to be (29.81%, 20.91% and 25.24 %) respectively.

Ulcerated rats alone or with omeprazole group showed significant increase ($P < 0.05$) at four weeks with the mean values of increase of (4.05±0.85 and 4.13±0.09) respectively against 2.80±0.38 of normal control group.

**4- Evaluation of Stomach Glutathione Activity (GSH)*:-***

On detecting stomach glutathione levels from the data tabulated in ***table (15)*** and graphically represented by ***figure (17)***, it is denoted that normal control animals had constant levels of stomach glutathione during the investigation.

Rats treated with aspirin alone or combined with different treatments showed a significant decrease ($P < 0.001$) in the mean value of aspirin group 0.18±0.08 compared to 0.41±0.03 of normal control animals in stomach glutathione levels throughout the experimental period, while ASA+I3C groups showed higher a significant ($P < 0.001$) at seven days with a mean values of increase (1.32±0.28). Significant increase ($P < 0.001$) found in ASA+OMP, ASA+I3C and ASA+OMP+I3C groups, the percentage of increase was recorded to be (311.11%, 633.33% and 300%) respectively as compared to 80.49% of aspirin group.

At four weeks ulcerated treatment with indole-3-carbinol or with omeprazole and indole-3-carbinol had a significant increase ($P < 0.01$). The mean values of (3.37±0.33 and 1.55±0.54) respectively as compared to 0.45±0.03 of normal control groups, while there was a significant decrease ($P < 0.001$) in the mean value of ulcer group 0.25±0.03 compared to normal control group. The percentage of increase in U plus OMP, U plus I3C and U plus OMP plus I3C groups was recorded to be (124%, 1248% and 520%) respectively as compared to 80% of ulcer group.

**5- Evaluation of Total Gastric Acid (Total Acidity):-**

Data recorded for total gastric acid (total acidity) were presented in ***table (16)*** and ***figure (18)***. Normal control animals signed more or less constant varying levels of total acidity throughout the experimental period.

Aspirin rats alone showed a significant increase ($P < 0.001$) in total acidity than normal control group rats at seven days. The percentage of increase were (112.61%). More pronounced significant decline ($P < 0.001$) in total acidity was established in aspirin with omeprazole and/or indole-3-carbinol groups after seven days or four weeks of treatment compared to the ulcer groups.

A significant increase ($P < 0.001$) was showed in ulcer rats alone in total acidity compared to normal control group rats at four weeks with a percentages increase was (44.77%). More pronounced significant decrease ($P < 0.001$) in total acidity was established in ulcer group with omeprazole, ulcer with indole-3-carbinol and ulcer with omeprazole and indole-3-carbinol groups after four weeks of treatment compared to the ulcer groups.

**6- Evaluation of pH Value of the Gastric Juice:-**

From the result recorded for pH value of the gastric juice were presented in ***table (17)*** and ***figure (19)***. Normal control animals signed more or less constant varying pH value of the gastric juice throughout the experimental period.

A significant decrease ($P \leq 0.05$) in the mean pH value occurred in the aspirin group (1.13±0.45) compared to normal control rats values of (2.93±0.66) at seven days, whereas significant increase ($P \leq 0.05$) in the mean values in aspirin with omeprazole, aspirin with indole-3-carbinol and aspirin with omeprazole and indole-3-carbinol groups was denoted to be (2.83±0.27, 2.67±0.19 and 3.73±0.19) respectively at seven days as compared to aspirin group (1.13±0.45 ).

Ulcer groups showed a decrease in the mean pH value (1.07±0.52) compared to normal control rats values of (2.82±0.56) after four weeks, whereas significant increase ($P \leq 0.05$) in the mean values in ulcer with omeprazole and/or indole-3-carbinol groups as compared to aspirin group recording (3.50±0.76, 3.20±0.10 and 4.30±0.55) respectively at four weeks.

**IV- <u>Ulcer index, Ulcer Score and Percentage of Ulceration:-</u>**

***Table (18)*** and ***figures (20 and 21)*** represent ulcer index, ulcer score and percentage of ulceration in normal control group and ulcerated groups at seven days and four weeks.

No ulcers were detected in the stomach control rats and ASA with omeprazole and/or indole-3-carbinol groups at 7 days and 4 weeks.

A significant increase ($P < 0.001$) with mean value of ulcer index, ulcer score and percentage of ulceration of stomach rats of administrated aspirin for seven days to be (2.40±0.87, 1.67±0.71 and 71.28±0.14) respectively as compared to normal control rats.

Moreover, a significant increase ($P < 0.001$) in ulcer index, ulcer score and percentage of ulceration of ulcerated rats after four weeks to be (0.53±0.44, 0.38±0.23 and 33.10±0.10) respectively as compared to normal control animals.

Table ( 2 ) : Averages Of Growth Rates In Control And Experimental Groups (gms) .

| uration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+OMP | ASA+I3C | ASA+OMP +I3C |
| Zero Time | Initial body weight | Mean±S.E. | 156±9.17 | 164±5.81 | 165.33±10.73 | 154.67 ±1.7 | 168±4.80 | 171.4±5.50 | 157±5.77 | 174.6±3.47 |
| days | Final body weight gms | Mean±S.E.. | 159±3.79 | 167±8.73 | 172.40±5.94 | 159.4 ±3.73 | 209±16.59 | 185 ±0.58 | 162±6.43 | 183±8.67 |
| | | % of change[a] | | 5.03 | 8.43 | 0.25 | 31.45[3a] | 16.35[1a] | 1.89 | 15.09[1a] |
| | | % of change[b] | | | | | | -11.48 | -22.49[3b] | -12.44[1b] |
| **uration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| Zero Time | Initial body weight | Mean±S.E. | 170±7.11 | 180±5.01 | 173.03±6.33 | 168.62 ±4.3 | 179±6.76 | 176.4±8.43 | 169±6.07 | 175±5.77 |
| weeks | Final body weight gms | Mean± S.E. | 194±7.02 | 201.67±39.22 | 238.50±2.02 | 188.67±6.96 | 218.33±16.95 | 240±2.89 | 218.33±2.40 | 229.33±10.27 |
| | | % of change[a] | | 3.95 | 22.94[2a] | -2.74 | 12.54 | 23.71[3a] | 12.54 | 18.19[2a] |
| | | % of change[b] | | | | | | 9.93 | 0.000 | 5.04 |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U=Ulcer.

a : Values vs. normal control group.

b : Values vs. aspirin group .

vs: Versus.

1= Significant i.e. ($P < 0.05$).

2= Highly Significant i.e. ($P < 0.01$).

3= Very Highly Significant i.e. ($P < 0.001$).

Fig. (4): Averages Of Growth Rates In Control and Experimental Groups (gms) .

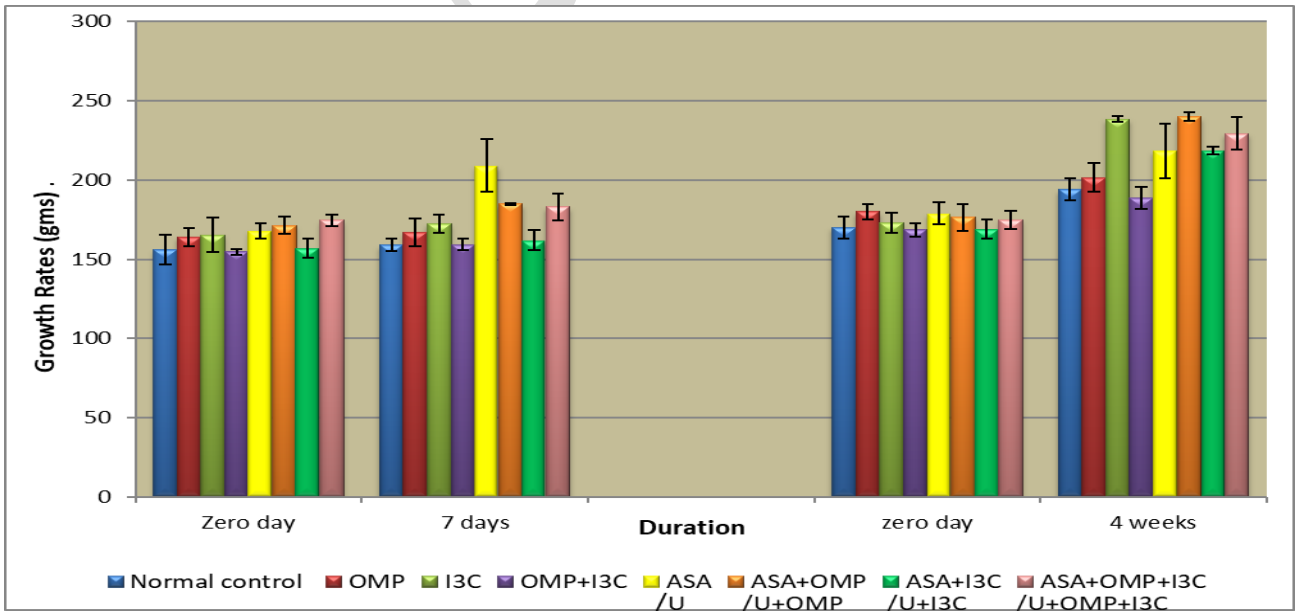


Table (3) : Averages Of Stomach Weight In Control And Experimental Groups (gms)

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+ OMP | ASA+I3C | ASA+OMP +I3C |
| 7 days | Stomach weight gms | Mean± S.E. | 1.48±0.16 | 1.61±0.06 | 1.56±0.08 | 1.67±0.28 | 1.85±0.12 | 1.31±0.09 | 1.41±0.04 | 1.39±0 .08 |
| | | % of change[a] | | 8.78 | 5.41 | 12.84 | 25 | -11.49 | -4.73 | -6.08 |
| | | % of change[b] | | | | | | -29.19[2b] | -23.78[1b] | -24.86[1b] |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| 4 weeks | Stomach weight gms | Mean± S.E. | 1.50±0.15 | 1.94±0.35 | 1.91±0.38 | 1.72±0.02 | 1.75± 0.05 | 1.58±0.13 | 1.65±0.05 | 1.85±0.12 |
| | | % of change[a] | | 32.88 | 30.82 | 17.81 | 19.86 | 8.22 | 13.01 | 26.71 |
| | | % of change[b] | | | | | | -9.71 | -5.71 | 5.71 |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U=Ulcer.

a : Values vs. normal control group.

b : Values vs. aspirin group .

vs: Versus.

1= Significant i.e. ($P < 0.05$).

2= Highly Significant i.e. ($P < 0.01$).

3= Very Highly Significant i.e. ($P < 0.001$).

Figure (5) : Averages Of Stomach Weight In Control And Experimental Groups (gms) .

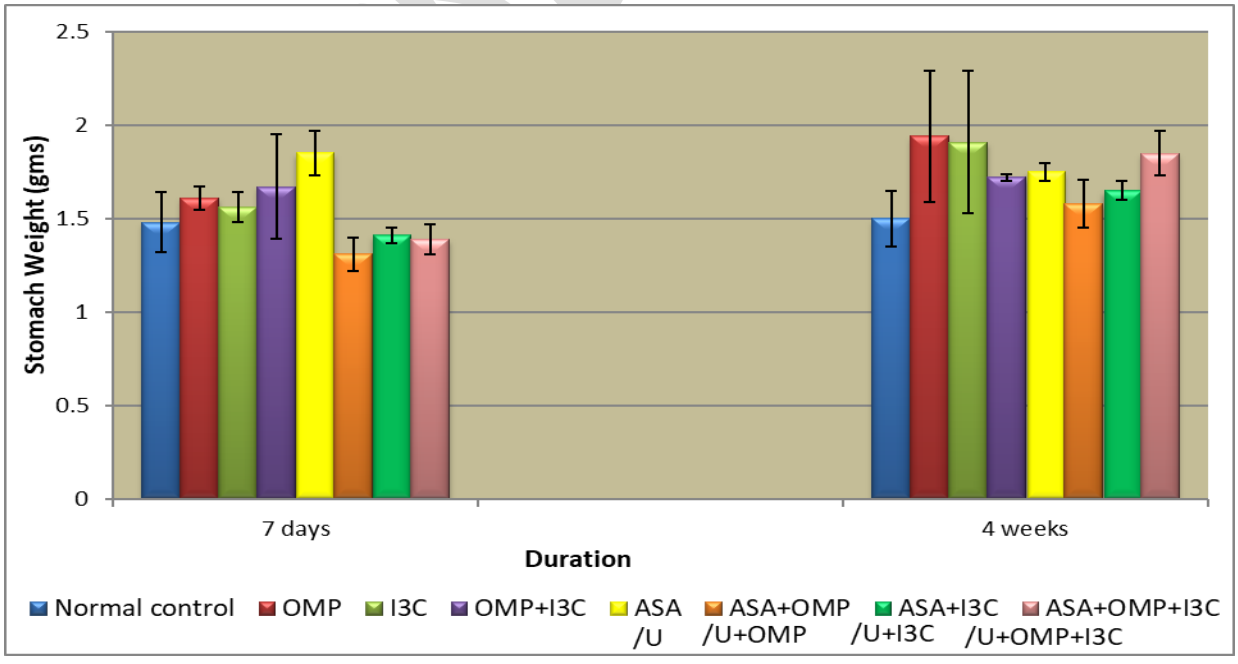


Table (4): Averages Of Relative stomach weight In Control And Experimental Groups (%).

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+OMP | ASA+I3C | ASA+OMP +I3C |
| 7 days | Relative stomach weight % | Mean± S.E. | 0.85±0.01 | 1.02±0.03 | 0.99±0.12 | 1.12±0.19 | 0.90±0.13 | 0.76±0.01 | 0.87±0.05 | 0.80±0.01 |
| | | % of change[a] | | 20 | 16.47 | 31.76 | 5.88 | -10.59 | 2.35 | -5.88 |
| | | % of change[b] | | | | | | -15.56 | -3.33 | -11.11 |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| 4 weeks | Relative stomach weight % | Mean± S.E. | 0.86±0.02 | 0.97±0.02 | 0.65±0.07 | 0.98±0.07 | 0.81±0.07 | 0.66±0.05 | 0.75±0.03 | 0.82±0.08 |
| | | % of change[a] | | 12.79 | -32.31[2a] | 13.95 | -5.81 | - 23.26[2a] | -12.79 | -4.65 |
| | | % of change[b] | | | | | | -18.52 | -7.41 | 1.23 |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U=Ulcer.

a : Values vs. normal control group.

b : Values vs. aspirin group .

vs: Versus.

1= Significant i.e. ($P < 0.05$).

2= Highly Significant i.e. ($P < 0.01$).

3= Very Highly Significant i.e. ($P < 0.001$).

Fig. (6): Averages Of Relative Stomach Weight In Control And Experimental Groups (%).

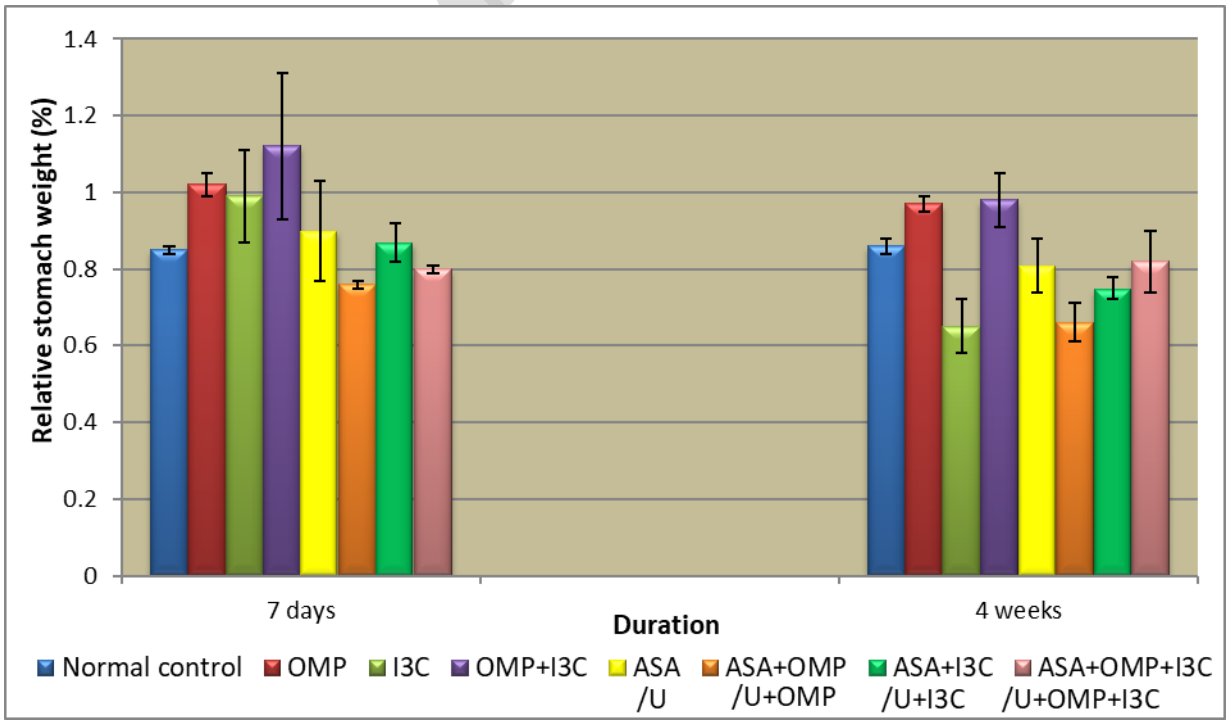


Table (5): Averages Of Percentage Of Mortality Rates In Control And Experimental Groups (%).

| Duration | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Normal control | OMP | I3C | OMP +I3C | ASA | ASA+ OMP | ASA+ I3C | ASA +OMP +I3C |
| Zero day | Number | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | N. Survival | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | N. Dead | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | % | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 days | Number | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | N. Survival | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | N. Dead | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 |
| | % | 0 | 0 | 0 | 0 | 33.33 | 0 | 0 | 0 |
| **Duration** | **Groups** | Normal control | OMP | I3C | OMP+ I3C | U | U+ OMP | U+ I3C | U+ OMP +I3C |
| Zero day | Number | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | N. Survival | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | N. Dead | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | % | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 4 weeks | Number | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | N. Survival | 6 | 6 | 6 | 6 | 6 | 6 | 6 | 6 |
| | N. Dead | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| | % | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

• OMP: Omeprazole. I3C: Indol-3-carbenol. ASA: Aspirin. U=Ulcer.

Fig. (7): Averages of Percentage of Mortality Rates In Control And Experimental Groups (%).

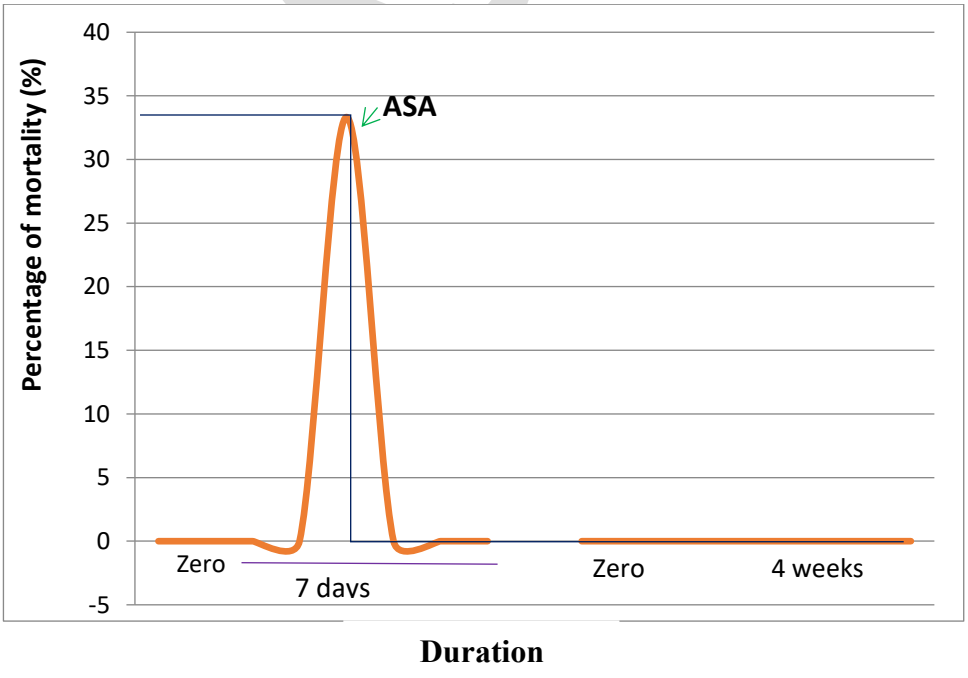


Table (6) : Averages Of Total Red Blood Cells Count (R.B.Cs) In Control And Experimental Groups ($10^6$/$mm^3$ blood).

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+OMP | ASA+I3C | ASA+OMP +I3C |
| 7 days | R.B.Cs $10^6$/$mm^3$ | Mean± S.E. | 6.68±0.45 | 6.61±0.35 | 6.52±0.47 | 7.10±0.17 | 5.88±0.22 | 5.77±0. 55 | 6.64±0.08 | 5.37±0.52 |
| | | % of change[a] | | -1.05 | -2.39 | 6.29 | -11.98 | -13.62 | -0.59 | -19.61[1a] |
| | | % of change[b] | | | | | | -1.87 | 12.93 | -8.67 |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| 4 weeks | R.B.Cs $10^6$/$mm^3$ | Mean± S.E. | 6.47±0.33 | 6.50±0.15 | 6.87±0.03 | 6.20±0.40 | 6.43±0.19 | 6.63±0.37 | 6.67±0.19 | 6.20±0.15 |
| | | % of change[a] | | 0.46 | 6.18 | -4.17 | -0.62 | 2.47 | 3.09 | -4.17 |
| | | % of change[b] | | | | | | 3.11 | 3.73 | -3.58 |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U=Ulcer.

a : Values vs. normal control group.

b : Values vs. aspirin group .

vs: Versus

1= Significant i.e. ($P < 0.05$).

2= Highly Significant i.e. ($P < 0.01$).

3= Very Highly Significant i.e. ($P < 0.001$).

Fig. (8): Averages Of Total Red Blood Cells Count (R.B.Cs) In Control And Experimental Groups ($10^6$/$mm^3$ blood).

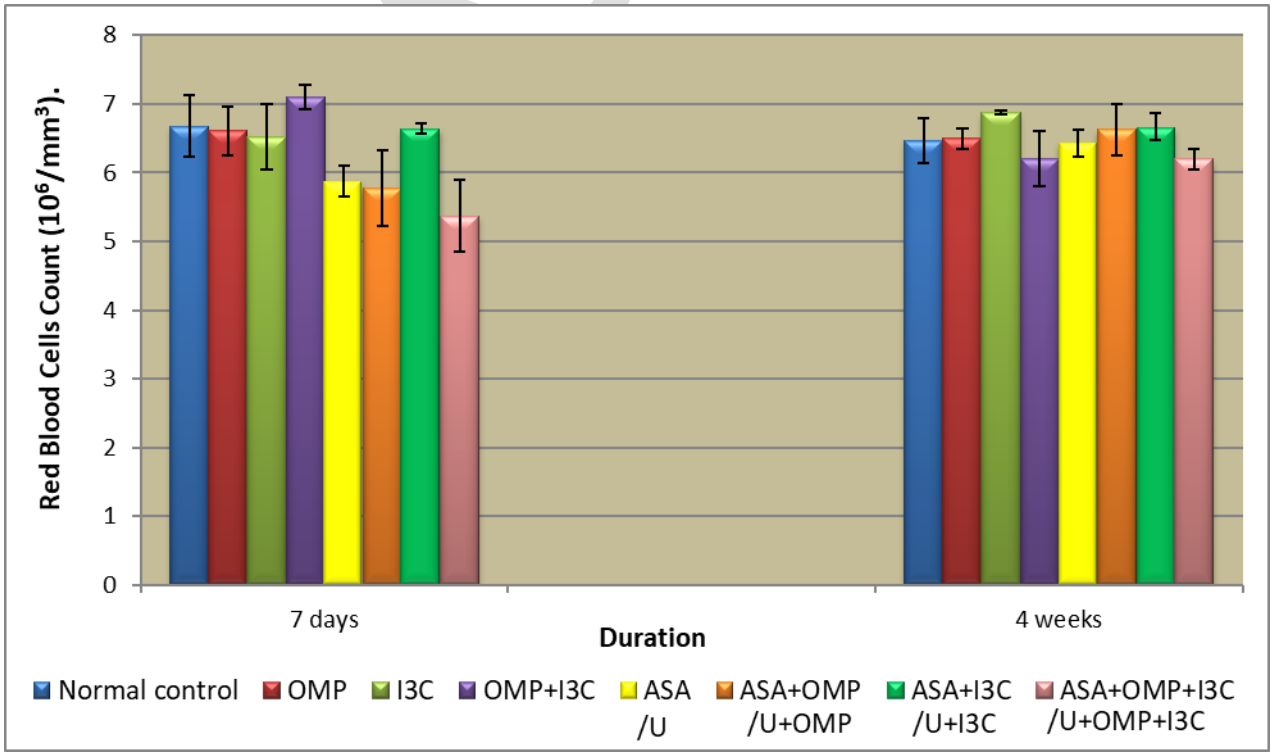


Table (7) : Averages Of Haemoglbin Content (Hb) In Control And Experimental Groups (gms/100 ml).

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+OMP | ASA+I3C | ASA+OMP +I3C |
| 7 days | Hb gms/100 ml | Mean± S.E. | 14.40±1.36 | 13.95±0.03 | 13.85±0.84 | 14.40±0.29 | 13.95±0.03 | 14.07±0.29 | 14.35±0.38 | 13.40±0.35 |
| | | % of change[a] | | -3.13 | -3.82 | 000 | -3.13 | -2.29 | -0.35 | -6.94 |
| | | % of change[a] | | | | | | 0.86 | 2.87 | -3.94 |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| 4 weeks | Hb gms/100 ml | Mean± S.E. | 14.15±1.39 | 15.20±0.29 | 14.97±0.43 | 13.93±0.15 | 14.87±0.26 | 14.97±0.13 | 15.13±0.03 | 14.60±0.31 |
| | | % of change[a] | | 7.42 | 5.79 | -1.55 | 5.09 | 5.79 | 6.93 | 3.18 |
| | | % of change[b] | | | | | | 0.67 | 1.75 | -1.82 |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U: Ulcer

a : Values vs. normal control group.

b : Values vs. aspirin group .

vs: Versus.

1= Significant i.e. ($P < 0.05$).

2= Highly Significant i.e. ($P < 0.01$).

3= Very Highly Significant i.e. ($P < 0.001$).

Fig. (9): Averages Of Haemoglbin Content (Hb) In Control And Experimental Groups (gms/100 ml).

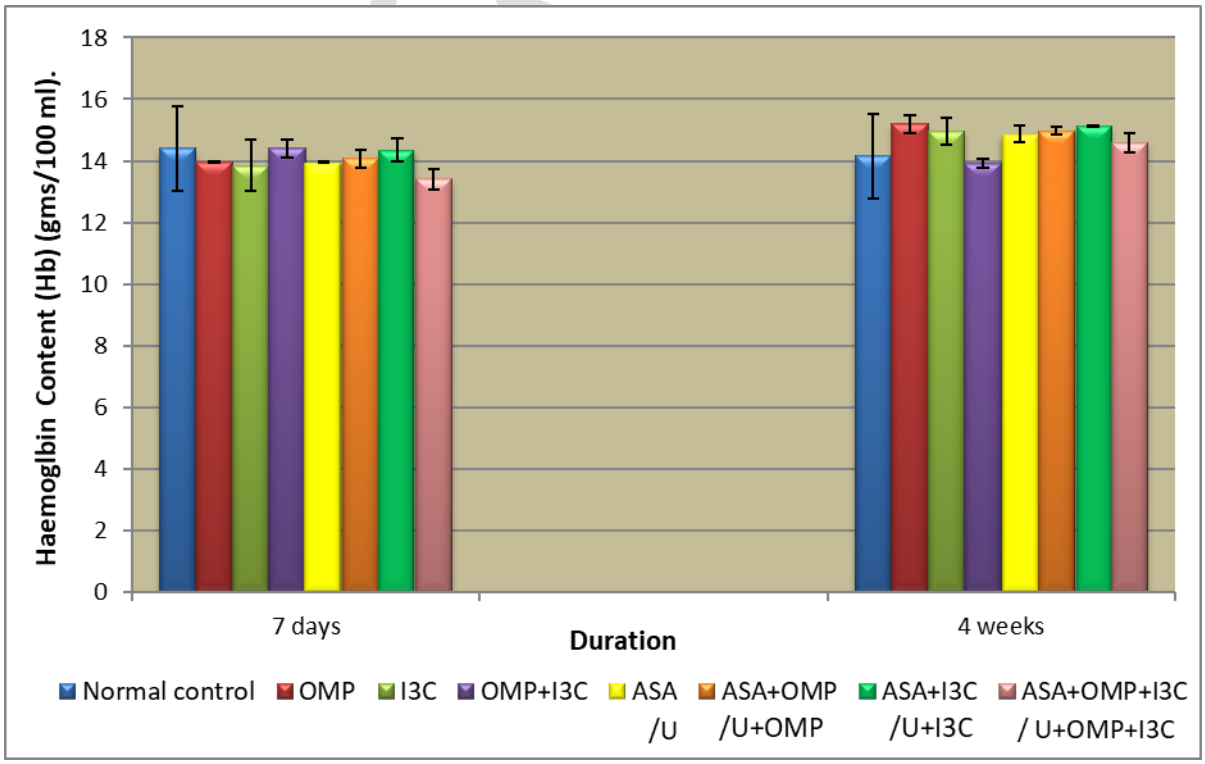


Table (8) : Averages Of Percentage Of Haematocrit (HCT) In Control And Experimental Groups (%).

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+OMP | ASA+I3C | ASA+ OMP +I3C |
| 7 days | HCT % | Mean± S.E. | 44.33± 3.39 | 45.63±1.89 | 45.73±3.09 | 43 ±1.82 | 47.93±0.97 | 41.70±0.58 | 42.20±0.87 | 38.55±0.26 |
| | | % of change[a] | | 2.93 | 3.16 | -3.00 | 8.12 | -5.93 | -4.80 | -13.04[1a] |
| | | % of change[b] | | | | | | -12.99[1b] | -11.95[1b] | -19.57[2b] |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| 4 weeks | HCT % | Mean± S.E. | 42.33±3.38 | 45.17±2.34 | 46.03±0.57 | 40.85±0.55 | 47.67±1.37 | 46.50±1.01 | 46.03±0.57 | 45.07±1.05 |
| | | % of change[a] | | 6.71 | 8.74 | -3.49 | 12.62 | 9.85 | 8.74 | 6.47 |
| | | % of change[b] | | | | | | -2.45 | -3.44 | -5.45 |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U=Ulcer.

a : Values vs. normal control group.

b : Values vs. aspirin group .

vs: Versus.

1= Significant i.e. ($P < 0.05$).

2= Highly Significant i.e. ($P < 0.01$).

3= Very Highly Significant i.e. ($P < 0.001$).

Fig. (10): Averages Of Percentage Of Haematocrit (HCT) In Control And Experimental Groups (%).

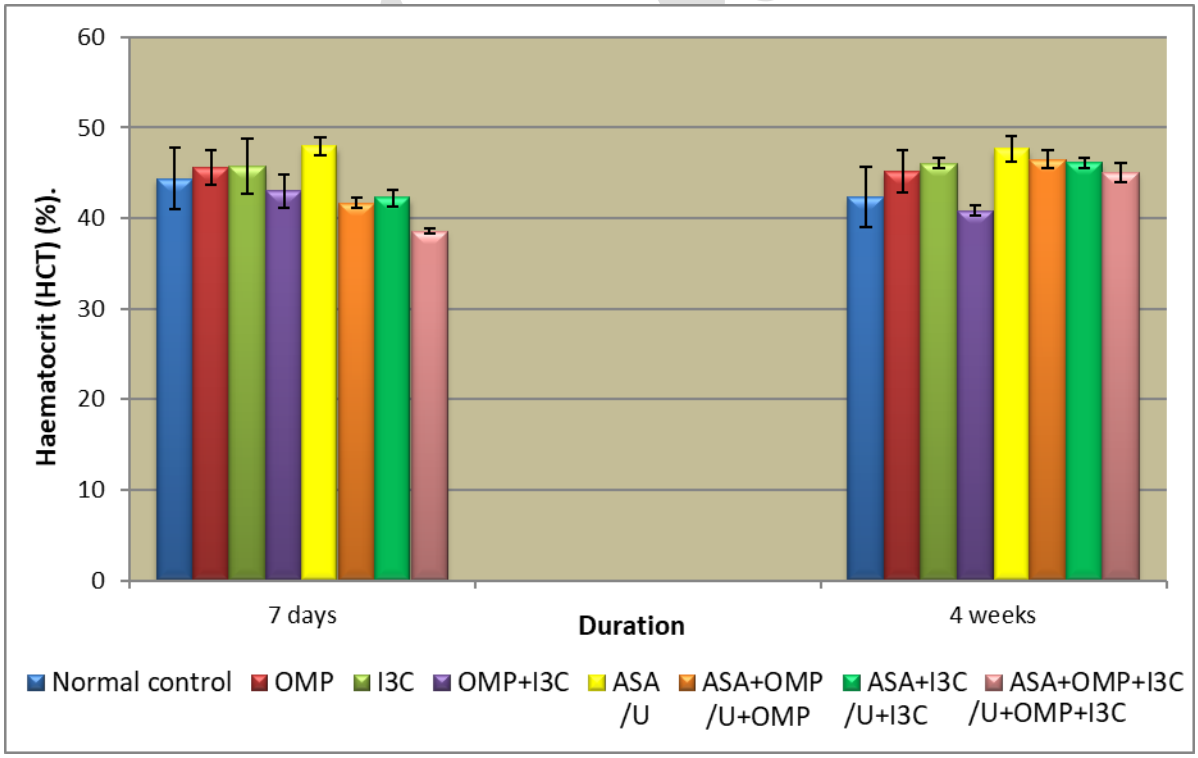


Table (9): Averages Of Leucocytic Counts (W.B.Cs) In Control And Experimental Groups ($10^3$/ $mm^3$ blood).

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+OMP | ASA+I3C | ASA+OMP +I3C |
| 7 days | W.B.Cs $10^3/mm^3$ | Mean± S.E. | 17.90± 0.17 | 18.77± 1.11 | 19.60± 0.78 | 18.87± 3.43 | 20.53± 1.75 | 16.25± 0.20 | 17.60±0.64 | 16.50±0.23 |
| | | % of change[a] | | 4.86 | 9.49 | 5.42 | 14.69 | -9.22 | -1.68 | -7.82 |
| | | % of change[b] | | | | | | -20.85 | -14.27 | -19.63 |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| 4 weeks | W.B.Cs $10^3/mm^3$ | Mean± S.E. | 17.85±0.20 | 19.33±0.88 | 20.83±1.01 | 18.97±2.59 | 22.03± 1.37 | 21.57±0.66 | 20.90±0.59 | 21.70±0.40 |
| | | % of change[a] | | 8.29 | 16.96 | 6.27 | 23.42[1a] | 20.84[1a] | 17.09 | 21.57[1a] |
| | | % of change[b] | | | | | | -2.09 | -5.13 | -1.49 |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U=Ulcer.

a : Values vs. normal control group.

b : Values vs. aspirin group .

vs: Versus.

1= Significant i.e. ($P < 0.05$).

2= Highly Significant i.e. ($P < 0.01$).

3= Very Highly Significant i.e. ($P < 0.001$).

Fig. (11): Averages Of Leucocytic Counts (W.B.Cs) In Control And Experimental Groups ($10^3$/ $mm^3$ blood ).

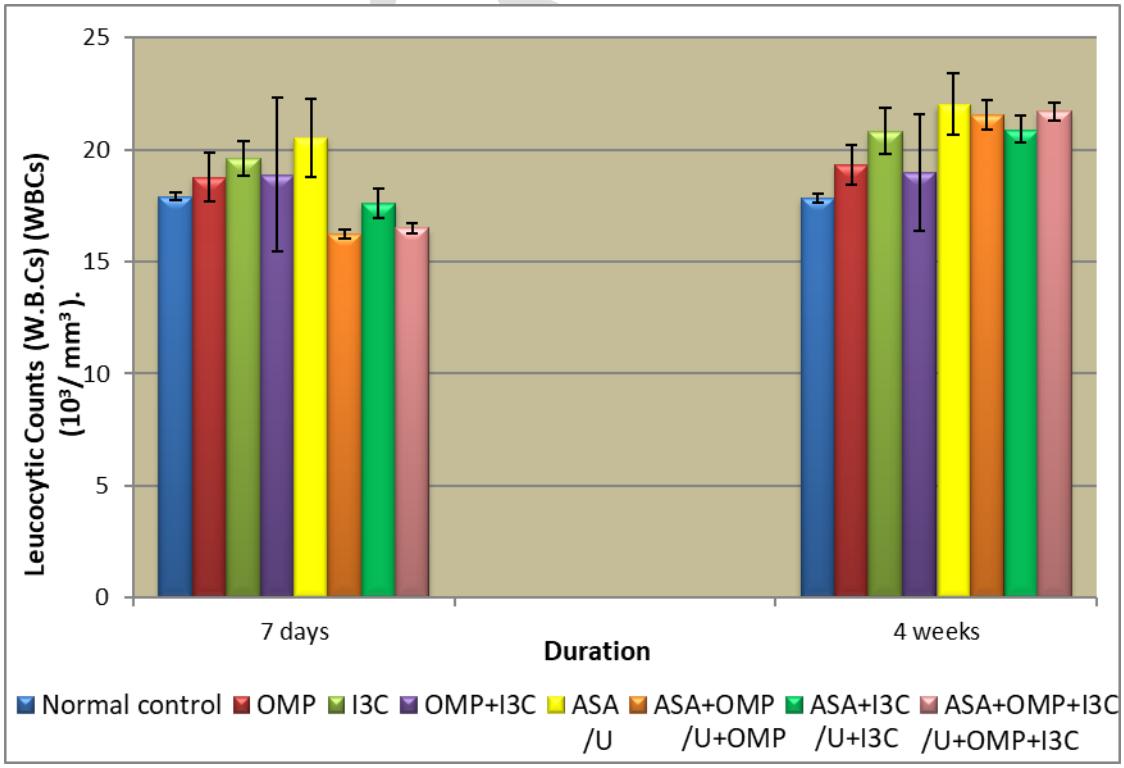


Table (10): Averages Of the Number Of Lymphocytes (LYM) as Percentage Of W.B.Cs In Control And Experimental Groups (%).

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+OMP | ASA+I3C | ASA+OMP +I3C |
| 7 days | LYM % | Mean± S.E. | 80.77± 0.65 | 75.43± 3.78 | 79.17± 2.13 | 76.33± 2.49 | 82.23± 1.23 | 68.83± 1.17 | 75.83±0.60 | 68.50±0.29 |
| | | % of change[a] | | -6.61 | -1.98 | -5.49 | 1.81 | $-14.78^{3a}$ | -6.12 | $-15.19^{3a}$ |
| | | % of change[b] | | | | | | $-16.29^{3b}$ | $-7.78^{2b}$ | $-16.69^{3b}$ |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| 4 weeks | LYM % | Mean± S.E. | 79.63± 0.68 | 75.13± 0.19 | 73.00± 0.58 | 77.00±1.73 | 73.00± 1.27 | 76.50±0.87 | 80.65±1.13 | 82.50±0.87 |
| | | % of change[a] | | $-5.65^{2a}$ | $-8.33^{3a}$ | $-3.30^{1a}$ | $-8.33^{3a}$ | $-3.93^{2a}$ | 1.28 | 3.60 |
| | | % of change[b] | | | | | | $4.79^{1b}$ | $10.48^{3b}$ | $13.01^{3b}$ |

- OMP: Omeprazole. a : Values vs. normal control group. 1= Significant i.e. ($P < 0.05$).
- I3C: Indol-3-carbenol. b : Values vs. aspirin group . 2= Highly Significant i.e. ($P < 0.01$).
- ASA: Aspirin. vs: Versus 3= Very Highly Significant i.e. ($P < 0.001$).
- U=Ulcer.

Fig. (12): Averages Of the Number Of Lymphocytes (LYM) as Percentage Of W.B.Cs In Control And Experimental Groups (%).

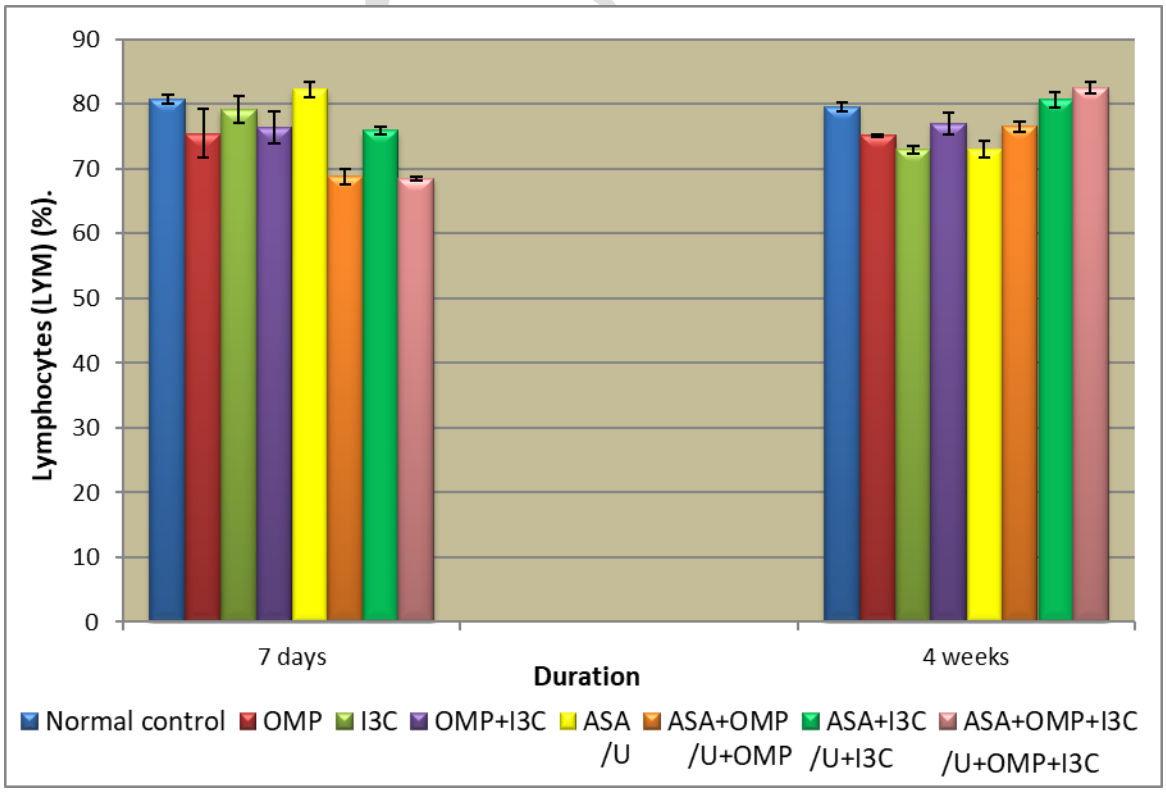


Table (11) : Averages Of Number Of Monocytes (MON) as Percentage Of W.B.Cs In Control And Experimental Groups (%).

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+OMP | ASA+I3C | ASA+OMP +I3C |
| 7 days | MON % | Mean± S.E. | 12.70± 1.37 | 15.17± 0.49 | 13.87± 2.31 | 14.10± 1.21 | 7.47± 0.52 | 10.33± 0.88 | 10.57±0.32 | 12.50±0.29 |
| | | % of change[a] | | 19.45 | 9.21 | 11.02 | -41.18[2a] | -18.66 | -16.77 | -1.57 |
| | | % of change[b] | | | | | | 38.29 | 41.49 | 67.33[2b] |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| 4 weeks | MON % | Mean± S.E. | 13.03± 0.78 | 13.65± 0.78 | 12.50± 0.29 | 12.00±0.58 | 10.87± 0.85 | 11.70±1.91 | 11.97±0.61 | 10.13±0.78 |
| | | % of change[a] | | 4.76 | -4.07 | 7.91- | -16.58 | -10.21 | -8.14 | -22.26[1a] |
| | | % of change[b] | | | | | | 7.64 | 10.12 | -6.81 |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U=Ulcer.

a : Values vs. normal control group.

b : Values vs. aspirin group .

vs: Versus.

1= Significant i.e. ($P < 0.05$).

2= Highly Significant i.e. ($P < 0.01$).

3= Very Highly Significant i.e. ($P < 0.001$).

Fig. (13): Averages Of Number Of Monocytes (MON) as Percentage Of W.B.Cs In Control And Experimental Groups (%).

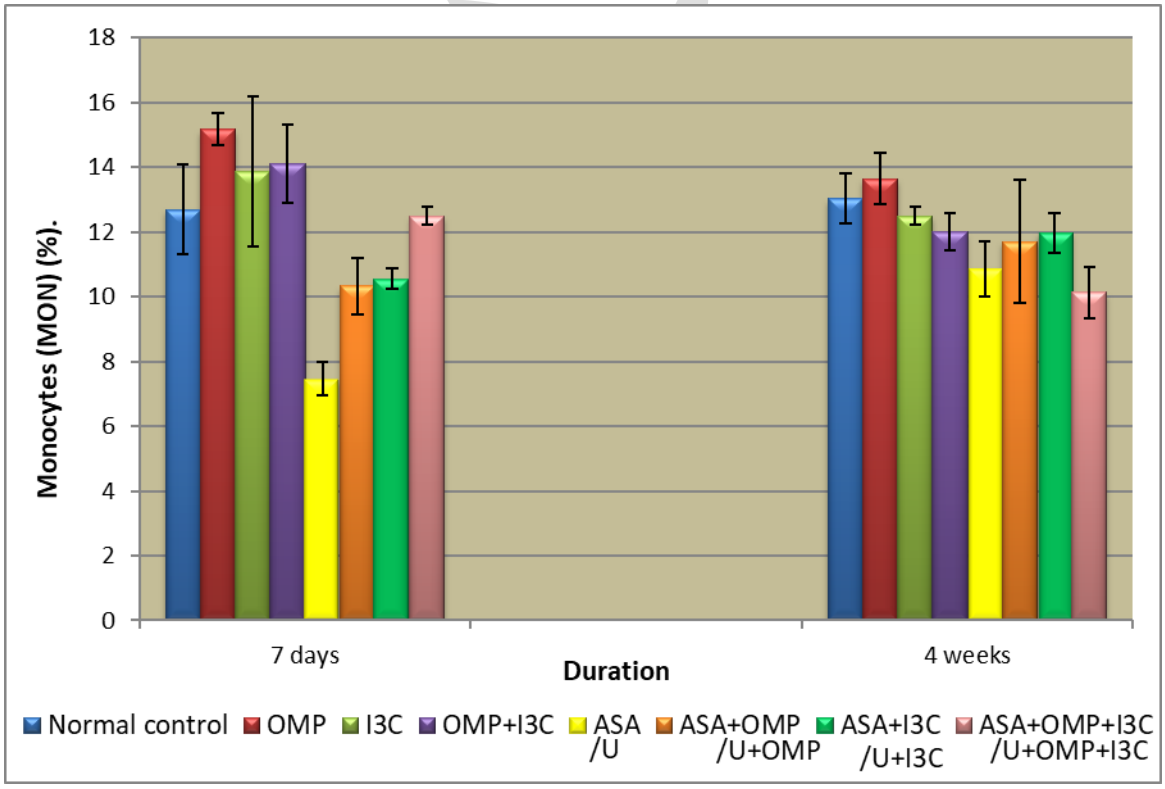


Table (12) :Averages Of Blood Platelets Count (PLTs) In Control And Experimental Groups ($10^3$/µl ).

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+OMP | ASA+I3C | ASA+OMP +I3C |
| 7 days | PLTs $10^3$/ µl | Mean± S.E. | 570±35.22 | 579±31.79 | 551.5±45.89 | 494±34.64 | 136±33.02 | 177.50±1.44 | 571.67±71.32 | 339.50±0.2 |
| | | % of change[a] | | 1.58 | -3.25 | -13.33 | -76.14[3a] | -68.95[3a] | 0.18 | -40.44[3a] |
| | | % of change[b] | | | | | | 30.51 | 319.85[3b] | 149.63[3b] |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| 4 weeks | PLTs $10^3$/ µl | Mean± S.E. | 568.50±35.51 | 525.67±43.01 | 550±28.87 | 530±40.41 | 453±25.98 | 583±19.05 | 564±22.52 | 547±37.24 |
| | | % of change[a] | | -7.53 | -3.17 | -6.77 | -20.32[1a] | 2.55 | -0.79 | -3.78 |
| | | % of change[b] | | | | | | 28.69[2b] | 24.50[1b] | 20.75 |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U=Ulcer.

a : Values vs. normal control group.

b : Values vs. aspirin group .

vs: Versus.

1= Significant i.e. ($P < 0.05$).

2= Highly Significant i.e. ($P < 0.01$).

3= Very Highly Significant i.e. ($P < 0.001$).

Fig. (14): Averages Of Blood Platelets Count (PLTs) In Control And Experimental Groups ($10^3$/µl ).

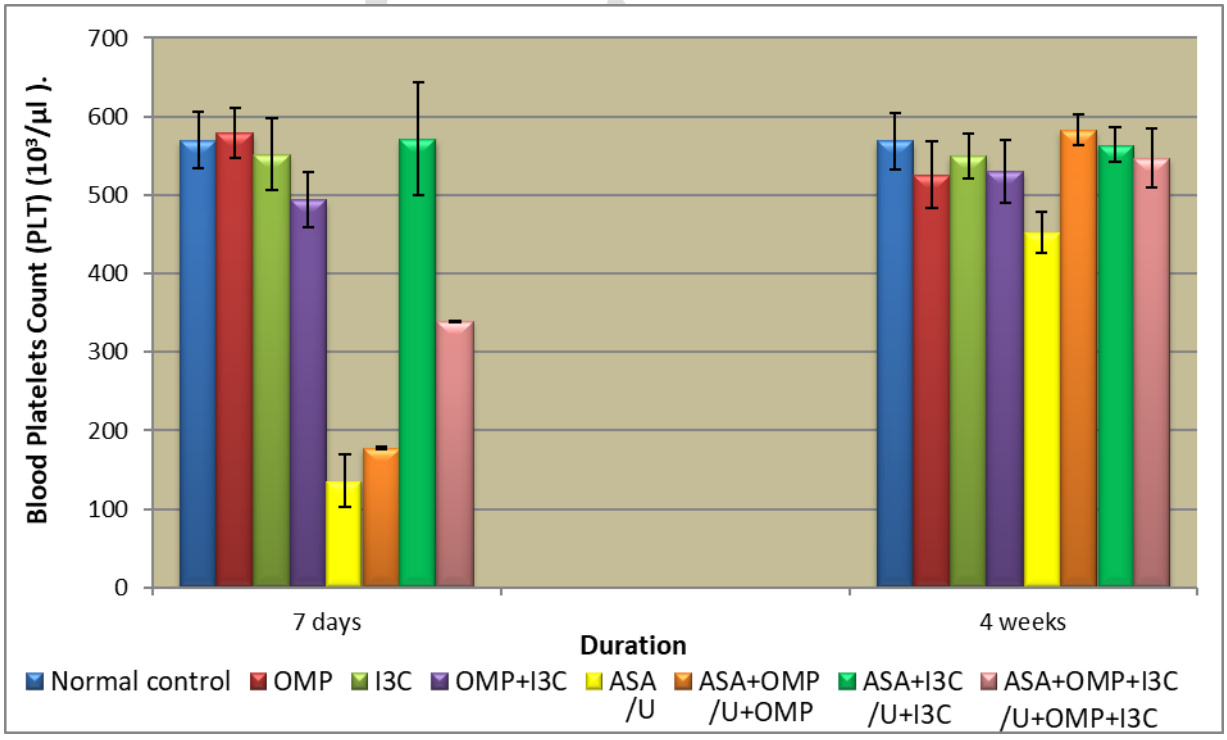


Table (13): The Mean Values Of Serum Total Protein Levels In Control And Experimental Groups (g/dl) .

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+OMP | ASA+I3C | ASA+OMP +I3C |
| 7 days | Total protein g/dl | Mean± S.E. | 7.55±0.02 | 9.81± 0.03 | 10.35± 0.36 | 8.51±1.16 | 9.63±0.82 | 9.00± 0.79 | 10.57±0.42 | 10.06±0.60 |
| | | % of change[a] | | 29.93[1a] | 37.09[2a] | 12.72 | 27.55[1a] | 19.21 | 40[2a] | 33.25[2a] |
| | | % of change[b] | | | | | | -6.54 | 9.76 | 4.47 |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| 4 weeks | Total protein g/dl | Mean± S.E. | 7.59± 0.04 | 9.64±0.93 | 10.26±0.75 | 8.29±0.48 | 8.93±0.84 | 9.86±0.66 | 9.13±1.54 | 8.50±0.29 |
| | | % of change[a] | | 27.01 | 35.18[1a] | 9.22 | 17.65 | 29.91 | 20.29 | 11.99 |
| | | % of change[b] | | | | | | 10.41 | 2.24 | -4.82 |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U=Ulcer.

a : Values vs. normal control group.

b : Values vs. aspirin group .

vs: Versus.

1= Significant i.e. ($P < 0.05$).

2= Highly Significant i.e. ($P < 0.01$).

3= Very Highly Significant i.e. ($P < 0.001$).

Fig. (15): The Mean Values Of Serum Total Protein Levels In Control And Experimental Groups (g/dl).

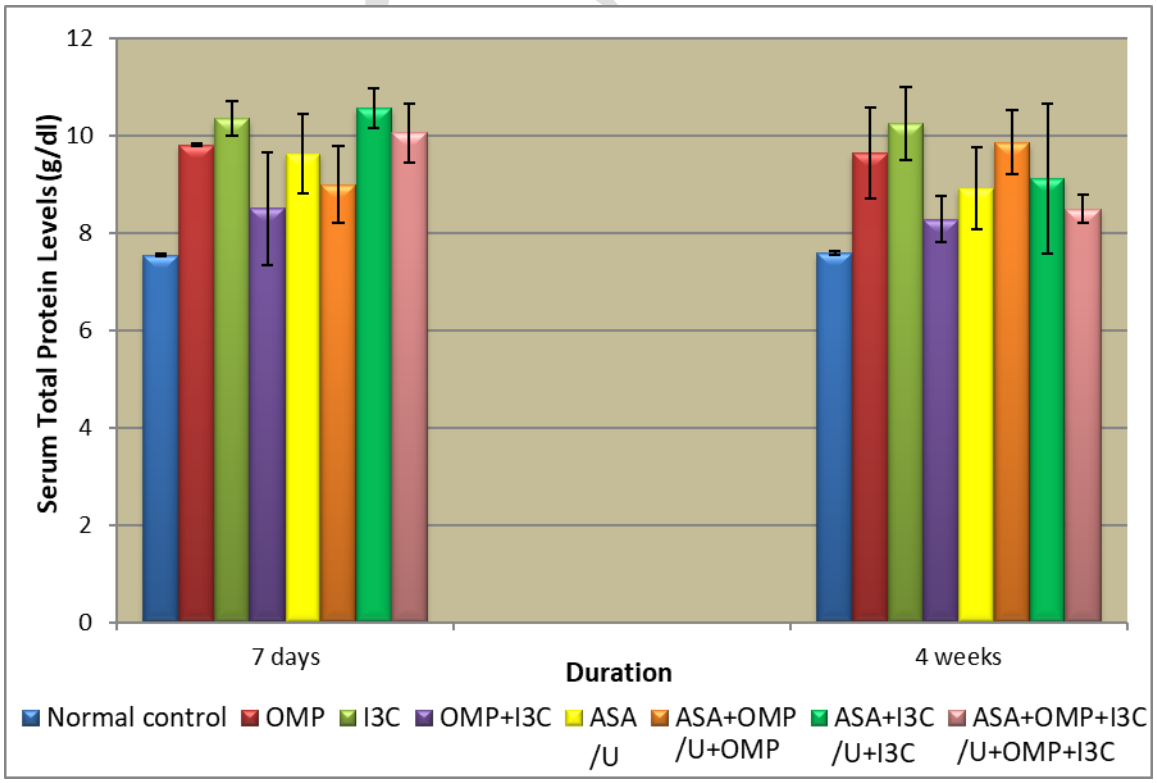


Table (14): The Mean Values Of Serum Albumin Levels In Control And Experimental Groups (g/dl).

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+OMP | ASA+I3C | ASA+OMP +I3C |
| 7 days | Albumin g/dl | Mean± S.E. | 2.76±0.31 | 4.57± 0.03 | 4.05± 0.24 | 3.82± 0.37 | 4.16±0.24 | 2.92± 0.09 | 3.29± 0.26 | 3.11±0.27 |
| | | % of change[a] | | 65.58[3a] | 46.74[3a] | 38.41[2a] | 50.72[3a] | 5.79 | 19.20 | 12.68 |
| | | % of change[b] | | | | | | -29.81[3b] | -20.91[2b] | -25.24[2b] |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| 4 weeks | Albumin g/dl | Mean± S.E. | 2.80±0.38 | 3.85± 0.13 | 3.90±0.05 | 3.43±0.14 | 4.05± 0.85 | 4.13±0.09 | 3.71±0.09 | 3.43±0.29 |
| | | % of change[a] | | 37.5[1a] | 39.29[1a] | 22.5 | 44.64[1a] | 47.5[1a] | 32.5 | 22.5 |
| | | % of change[b] | | | | | | 1.98 | -8.39 | -15.31 |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U=Ulcer.

a : Values vs. normal control group.

b : Values vs. aspirin group .

vs: Versus.

1= Significant i.e. ($P < 0.05$).

2= Highly Significant i.e. ($P < 0.01$).

3= Very Highly Significant i.e. ($P < 0.001$).

Fig. (16): The Mean Values Of Serum Albumin Levels In Control And Experimental Groups (g/dl).

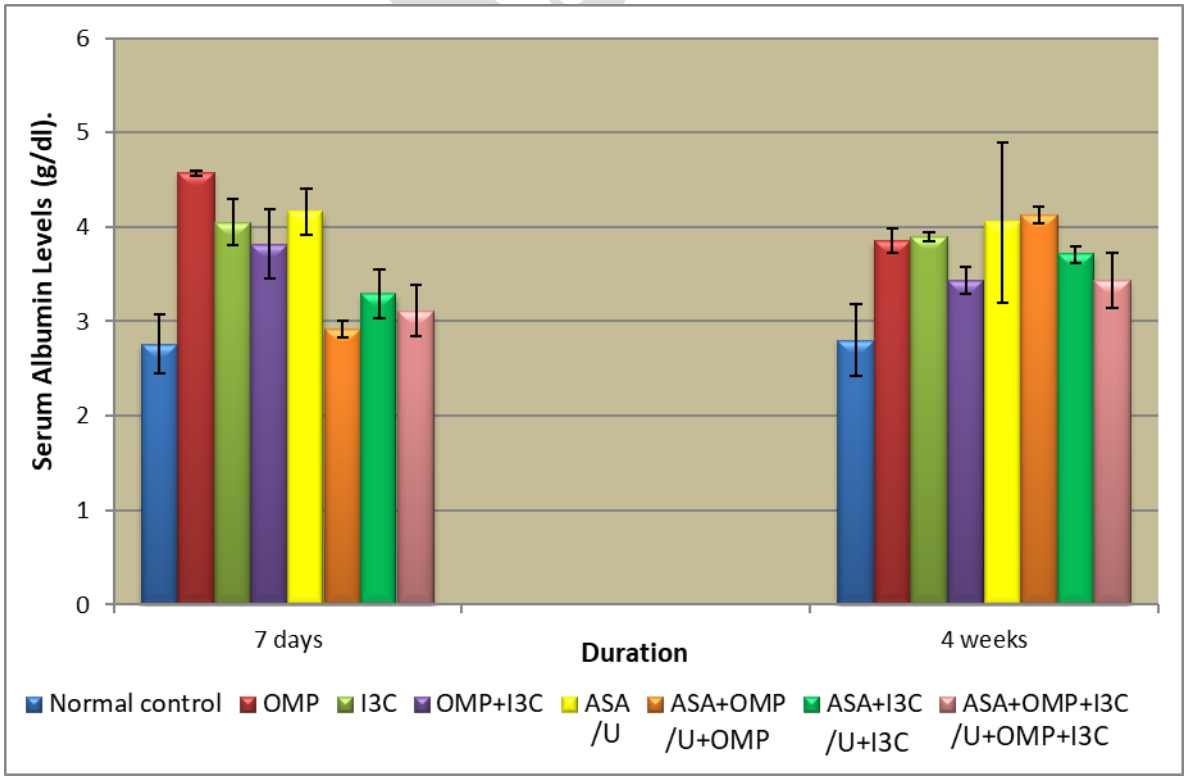


Table (15): The Mean Values Of Tissue Glutathione (GSH) Activity In Control And Experimental Groups (mg/g tissue) .

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+OMP | ASA+I3C | ASA+OMP +I3C |
| 7 days | GSH mg/g | Mean± S.E. | 0.41±0.03 | 0.51± 0.07 | 0.58± 0.07 | 0.38± 0.05 | 0.18± 0.08 | 0.74± 0.09 | 1.32± 0.28 | 0.72±0.19 |
| | | % of change[a] | | 24.39 | 41.46 | -7.32 | - 56.09[3a] | 80.49 | 221.95[3a] | 75.61 |
| | | % of change[b] | | | | | | 311.11[3b] | 633.33[3b] | 300[3b] |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| 4 weeks | GSH mg/g | Mean± S.E. | 0.45±0.03 | 0.50±0.05 | 0.56±0.02 | 0.53±0.02 | 0.25±0.03 | 0.56±0.08 | 3.37±0.33 | 1.55±0.54 |
| | | % of change[a] | | 11.11 | 24.44 | 17.78 | - 80[3a] | 24.44 | 648.89[3a] | 244.44[2a] |
| | | % of change[b] | | | | | | 124 | 1248[3b] | 520[3b] |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U=Ulcer.

a : Values vs. normal control group.

b : Values vs. aspirin group .

vs: Versus.

1= Significant i.e. ($P < 0.05$).

2= Highly Significant i.e. ($P < 0.01$).

3= Very Highly Significant i.e. ($P < 0.001$).

Fig. (17) : The Mean Values Of Tissue Glutathione ( GSH ) Activity In Control And Experimental Groups (mg/g tissue).

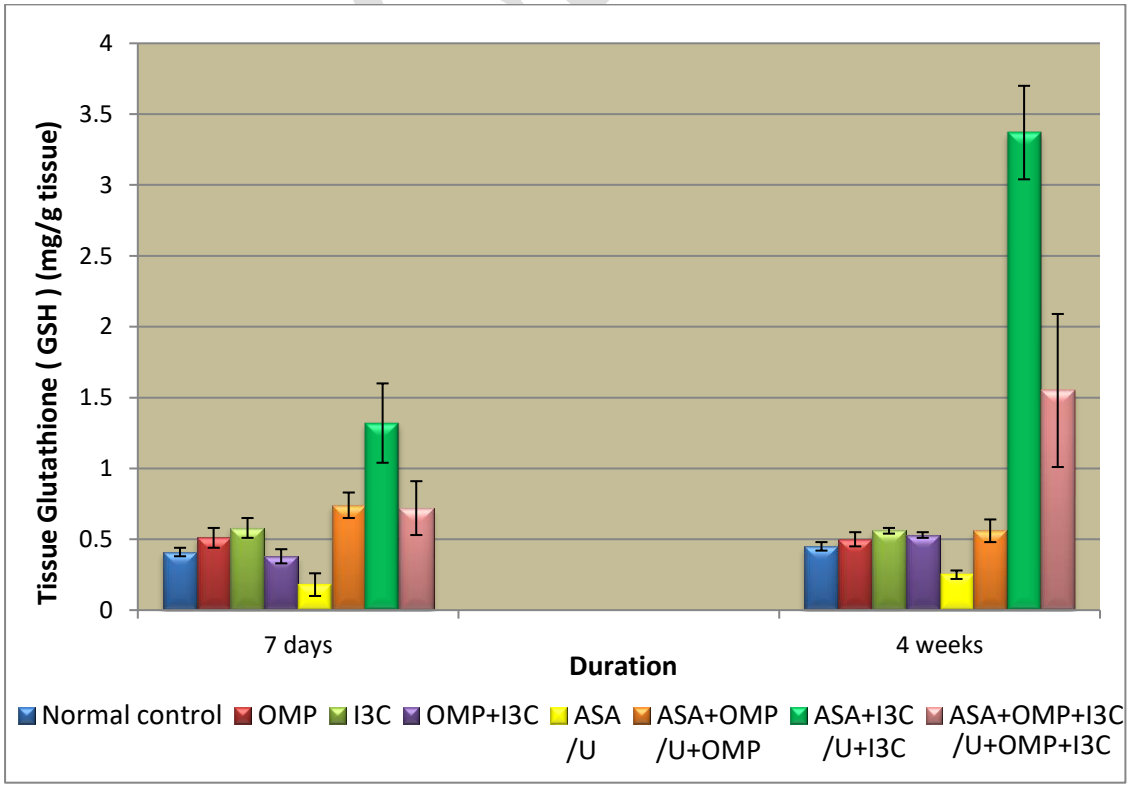


Table (16) : The Mean Values Of Total Gastric Acid (Total Acidity) In Control And Experimental Groups (m Eq/l).

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+OMP | ASA+I3C | ASA+OMP +I3C |
| 7 days | Total activity m Eq/l | Mean± S.E. | 18.00±1.15 | 19.33±0.33 | 18.80± 0.61 | 20.00±0.58 | 38.27±4.95 | 21.33±0.88 | 21.00±1.00 | 20.33±0.33 |
| | | % of change[a] | | 7.39 | 4.44 | 11.11 | 112.61[3a] | 18.5 | 16.67 | 12.94 |
| | | % of change[b] | | | | | | -44.26[3b] | -45.13[3b] | -46.87[3b] |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| 4 weeks | Total activity m Eq/l | Mean± S.E. | 17.96±1.13 | 17.66±0.67 | 17.33±0.33 | 18.67±1.33 | 26.00±3.21 | 19.33±0.65 | 18.67±0.67 | 19.63±0.32 |
| | | % of change[a] | | -1.67 | -3.51 | 3.95 | 44.77[3a] | 7.63 | 3.95 | 9.29 |
| | | % of change[b] | | | | | | -25.65[3b] | -28.19[3b] | -24.5[3b] |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U=Ulcer.

a : Values vs. normal control group.

b : Values vs. aspirin group .

vs: Versus.

1= Significant i.e. ($P < 0.05$).

2= Highly Significant i.e. ($P < 0.01$).

3= Very Highly Significant i.e. ($P < 0.001$).

Fig. (18): The Mean Values Of Total Gastric Acid (Total Acidity) In Control And Experimental Groups (m Eq/l).

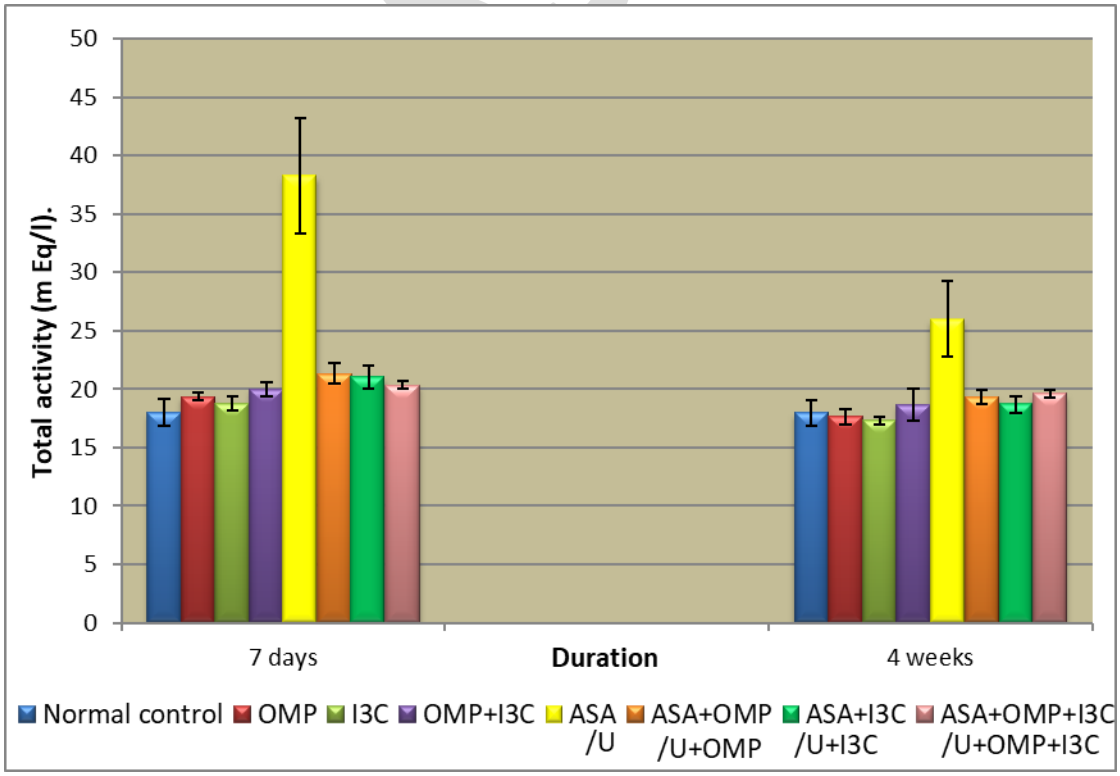


Table (17) : The Mean Values Of pH Level In Control And Experimental Groups .

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP+I3C | ASA | ASA+OMP | ASA+I3C | ASA+OMP +I3C |
| 7 days | pH | Mean± S.E. | 2.93±0.66 | 4.10± 0.86 | 4.40± 0.06 | 3.37±0.43 | 1.13±0.45 | 2.83±0.27 | 2.67± 0.19 | 3.73±0.19 |
| | | % of change[a] | | 39.93 | 50.17[1a] | 15.02 | -61.43[2a] | -3.41 | -8.87 | 27.30 |
| | | % of change[b] | | | | | | 150.44[2b] | 136.28[1b] | 230.09[3b] |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP+I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| 4 weeks | pH | Mean± S.E. | 2.82±0.56 | 5.17±0.82 | 5.50±0.29 | 4.80±0.70 | 1.07±0.52 | 3.50±0.76 | 3.20±0.10 | 4.30±0.55 |
| | | % of change[a] | | 83.33[2a] | 95.04[2a] | 70.21[1a] | -62.06[1a] | 24.11 | 11.88 | 13.48 |
| | | % of change[b] | | | | | | 227.10[2b] | 119.07[2b] | 301.87[3b] |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U=Ulcer.

a : Values vs. normal control group.

b : Values vs. aspirin group .

vs: Versus.

1= Significant i.e. ($P < 0.05$).

2= Highly Significant i.e. ($P < 0.01$).

3= Very Highly Significant i.e. ($P < 0.001$).

Fig. (19): The Mean Values Of pH Level In Control And Experimental Groups .

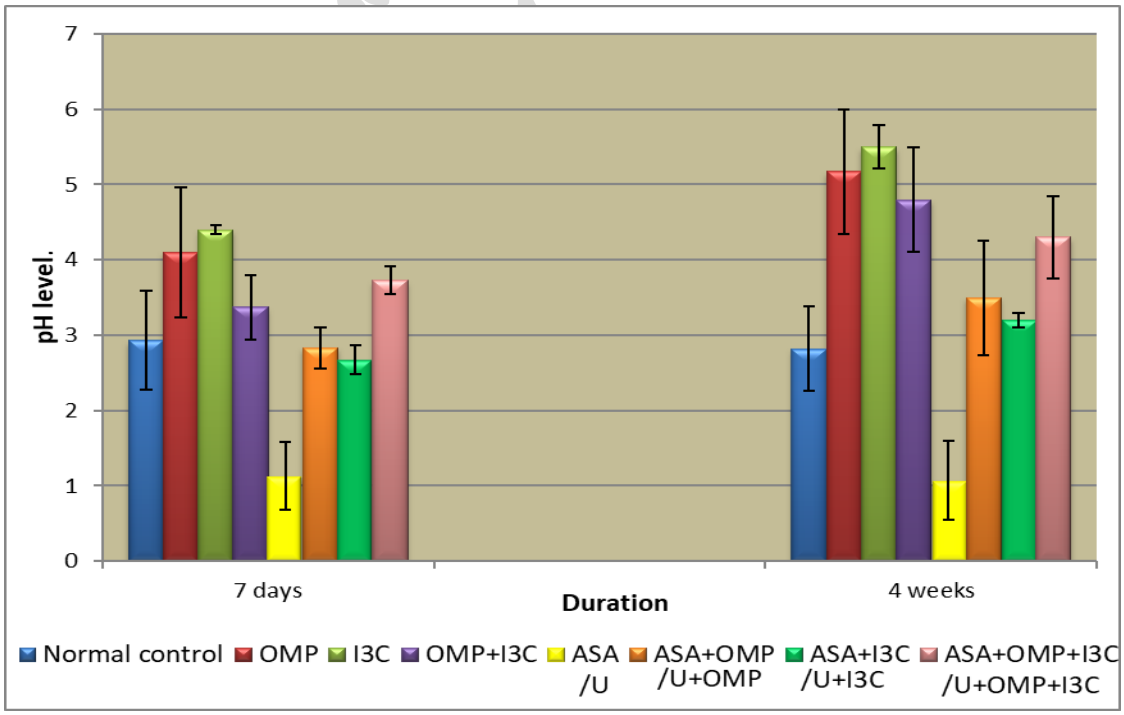


Table (18) : Averages Of Ulcer Index (mm), Ulcer Score (mm) And Percentage Of Ulceration (%) In Control And Experimental Groups.

| Duration | Parameters | Groups | Control groups | | | | Experimental groups | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Normal control | OMP | I3C | OMP +I3C | ASA | ASA +OMP | ASA +I3C | ASA +OMP +I3C |
| 7 days | Ulcer index mm | Mean± S.E. | 0.00 | 0.00 | 0.00 | 0.00 | 2.40±0.87*** | 0.00 | 0.00 | 0.00 |
| | Ulcer score mm | Mean± S.E | 0.00 | 0.00 | 0.00 | 0.00 | 1.67±0.71*** | 0.00 | 0.00 | 0.00 |
| | % of Ulceration | Mean± S.E | 0.00 | 0.00 | 0.00 | 0.00 | 71.28±0.14*** | 0.00 | 0.00 | 0.00 |
| **Duration** | **Parameters** | **Groups** | Normal control | OMP | I3C | OMP +I3C | U | U +OMP | U +I3C | U+ OMP +I3C |
| 4 weeks | Ulcer index mm | Mean± S.E. | 0.00 | 0.00 | 0.00 | 0.00 | 0.53±0.44*** | 0.00 | 0.00 | 0.00 |
| | Ulcer score mm | Mean± S.E | 0.00 | 0.00 | 0.00 | 0.00 | 0.38±0.23*** | 0.00 | 0.00 | 0.00 |
| | % of Ulceration | Mean± S.E | 0.00 | 0.00 | 0.00 | 0.00 | 33.10±0.10*** | 0.00 | 0.00 | 0.00 |

- OMP: Omeprazole.
- I3C: Indol-3-carbenol.
- ASA: Aspirin.
- U=Ulcer.

* : Values vs. normal control group.

vs: Versus.

***= Very Highly Significant i.e. ($P < 0.001$).

Fig. (20): Averages Of Ulcer Index And Ulcer Score In Control And Experimental Groups (mm).

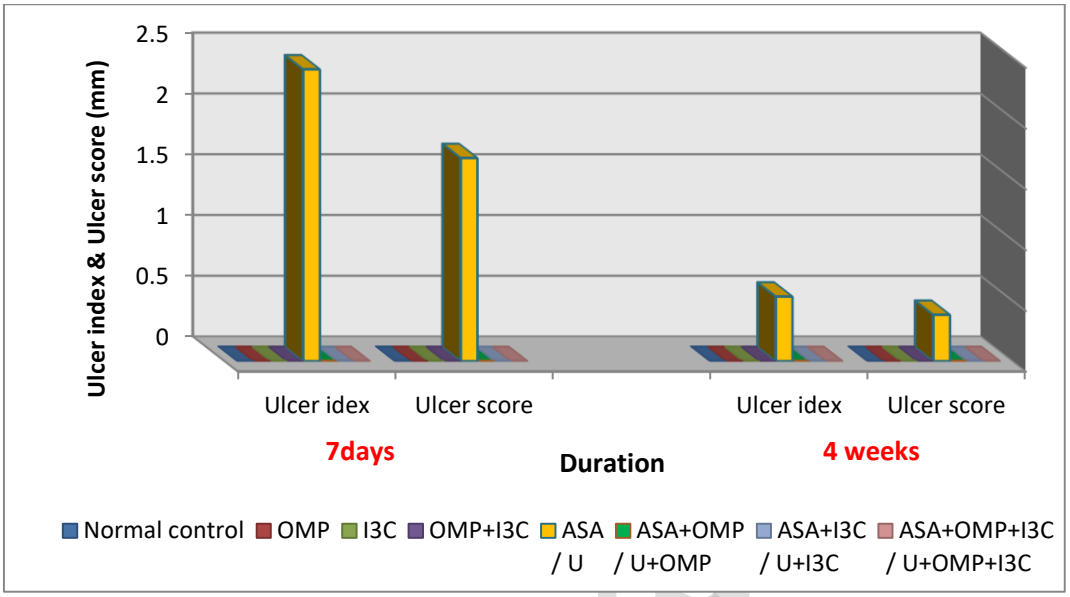
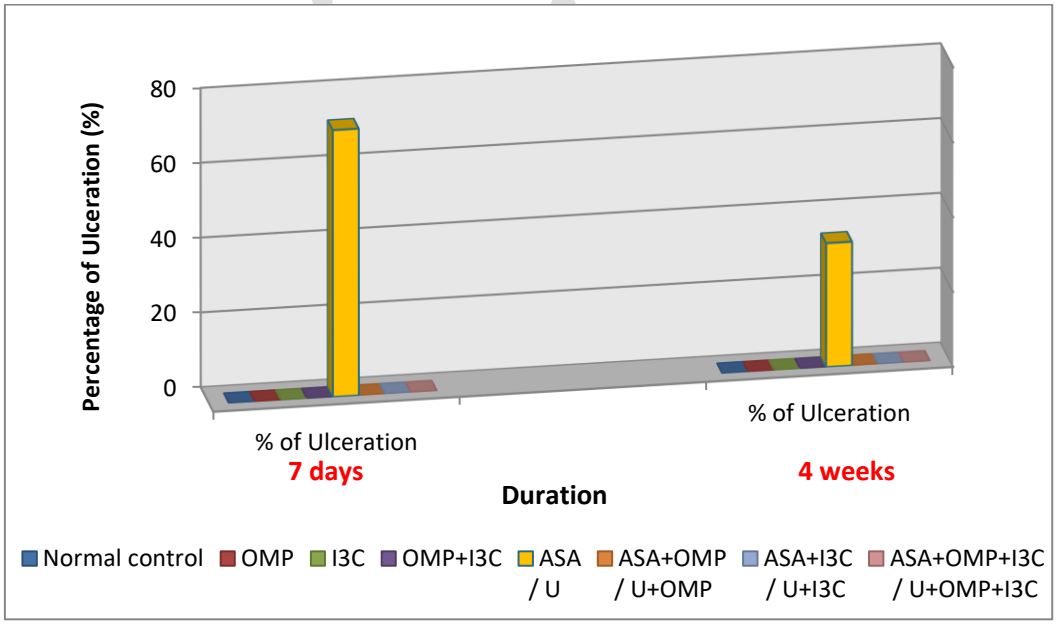



Fig. (21): Averages Of Percentage Of Ulceration In Control And Experimental Groups (%).



# V. Histological, Histochemical and Immunohistochemical Studies:-

## 1-Histological Investigation:-

### d. Normal Control Group:-

The stomach is the expanded part of the digestive tract. It acts mainly as a digestive organ. It's cells secrete; pepsin, lipase, rennin, mucous and HCl.

Macroscopically, stomach of control rats has two parts:-

- Forestomach: Thin-walled, non-glandular section that receives the esophagus and serves as a holding chamber for food. Its walls are similar to those of the esophagus.

- Corpus (Glandular stomach): Thick-walled, glandular section. Its walls have secretory glands that produce digestive enzymes and mucus. Digestion begins in the corpus. The pyloric sphincter controls the movement of food from the corpus to the intestines (specifically, the duodenum).

The forestomach and the corpus are separated by a low fold of tissue called the limiting ridge (*margo plicatus*). The limiting ridge extends circumferentially from the large curvature of the stomach to the small curvature, just below the esophagus. At the esophagus, the course of the limiting ridge bends into a U-shape and almost surrounds the esophageal opening ***(Fig. 22)***.

The stomach is subdivided into fundus, body and pylorus. It connects respectively. The wall of the stomach consists of 4 layers ***(Fig.23)***.

(I)- Mucosa:-

It is relatively thick, consisting 3 layers:

a). Surface epithelium:-

This is a tall, simple columnar epithelium formed of mucous secreting columnar cells, for lubrication and protection. The apical post of the cells is clear due to dissolved mucinogen granules.

b). Corium:-

This is a connective tissue layer containing reticular fibers, lymphocytes, plasma cells, blood vessels, nerves, lymphatics and gastric glands.

c). Muscularis mucosa:-

It consists of inner circular and outer longitudinal smooth muscle layers.

Gastric glands :-

They are simple branched tubular glands which are narrow, straight and perpendicular to the surface epithelium. The main cell types are:

**1-** Mucous neck cells :-

They secrete mucous, present in the necks of the gland. Their cytoplasm is paler because they contain numerous (dissolved) mucinogen granules. Their nuclei are flattened and basal in position.

**2-** Peptic (Zymogenic or chief ) cells:-

They secrete enzymes eg. pepsin and rennin.They are numerous, at the bottom and the bodies of the glands. The cells are columnar with a basal basophilic part and a pale apical part. Their nuclei are rounded and nearer to the base.

**3-** Parietal (Oxyntic) cells:-

They secrete free HCl and intrinsic factor which is essential for absorbing vitamin $B_{12}$ in the small intestine. The cells are triangular or ovoid with deeply acidophic cytoplasm and a rounded central nucleus. They lie on the basement membrane between the peptic and the neck cells, but never reach the lumen of the gland ***(Fig. 24)***.

**4-** Enterochromaffin cell (argentaffin or argyrophil):-

They are of several types: some secrete serotonin, others secrete gastrichermone. They are triangular or columnar with rounded nucleus and basal cytoplasmic granules.

(II). Submucosa :-

It is a loose connective tissue layer with blood vessels, lymphatics, nerves, mast cells and some fat cells.

(III). Musculosa :-

It is formed of an inner oblique a middle circular and an outer longitudinal smooth muscle layer.

(IV). Serosa:-

It consists of loose areolar tissue containing blood vessels and nerves and covered by a mesothelial layer.

## b. Control Groups:-

All animals administered of OMP, I3C and OMP+I3C at 7 days or 4 weeks there were no evident macroscopical change in stomach throughout the experimental time as compared to normal control group at the same period {***Figs. 25 (b, c &d) and 26 (b, c &d)***}.

Section of animals treated with OMP showed minimal histological changes throughout the whole experimental period, i.e 7 days and 4 weeks where stomach architecture seemed to be preserved with normal appearance ***(Fig. 27)***.

On the other hand, the sections of stomach from rats treated with I3C or I3C and OMP manifested more or less similar section as in control group during the experimental duration i.e 7 days and 4 weeks ***(Figs. 28 and 29)*** .

## c. Experimental Groups:-

Macroscopically, stomach of rats treated with aspirin (ASA) for 7 days, were severe red coloration spot ulcer and severe haemorrhagic streaks in the dissected stomach of rats ***(Fig. 25 e)***.

Microscopically, stomach sections from rats treated with ASA after 7 days, manifested many histological changes. This was established in the form of some eroded areas in the gastric mucosa. Oedematous connective tissues occurred in the mucosal layer resulting in the swelling of the mucosa and separation of the glands, large number of oxyntic cells which more acidophilic granules and with more vesicular nuclei were appeared ***(Fig.30)***. Reduction in chief cells was also observed where degeneration became common feature ***(Fig.31)***. On the other area, the mucosa layer was composed of irregular and tortuous glands limed by closely packed columnar epithelial cells. Among these cells, few number of mucous secreting cells was noticed. Both parietal and chief cells were considerably damaged ***(Fig.32)***. Moreover, this was accompanied by the presence of multiple erosions and gastric ulceration appeared as saucer-shaped areas of necrosis affecting the mucosa layer with destruction of the glandular tissue ***(Fig. 33)***. The connective tissues fibres of lamina propria was more abundant in between the fundic gland and gastric pits. Chronic inflammatory cells infiltrated the lamina propria where haemorrhage was also revealed. In addition, inflammatory cells mainly polymorphonuclear leucocytes, lymphocytes and plasma cells among the gland layer adjacent to the lumina muscularis mucous and dilated blood vessels were noticed ***(Fig.34)***.

In the gross level, the stomach was markedly severe red coloration at seven days in animals administered of aspirin and omeprazole ***(Fig. 25 f)***.

Microscopical changes of the stomach of rats administered ASA and OMP after 7 days was characterized as many eroded areas were noticed, involving luminal surface epithelial cells and gastric pit cells ***(Fig. 35)***.

Evidence of damage in the form of vacuolated or lightly stain cytoplasm with pyknotic or fragmented nuclei. Some mucous neck cells showed the same lesions. Most of

parietal cells among glands region, appeared faintly stained cytoplasm with pyknotic or karyolitic nuclei. Dilatation of the glandular luminae and haemorrhagic lesions in the mucosa of glandar stomach were also observed ***(Fig. 36)***. The connective tissues of the lamina propria between the gastric pits was demolished with aggregation of inflammatory cells near the bases of the gastric pit and the muscularis mucosa ***(Fig. 37)***.

No evident macroscopical changes were seen in stomach of rats treated with aspirin and indole-3-carbinol or aspirin with omeprazole and indole-3-carbinol at 7 days as compared to control group and ASA group ***(Fig. 25 g and h)***.

By the 7 days of aspirin and indole-3-carbinol treatment, many remarkable injury was stomach in the gastric mucosa. Heavy infiltration by chronic inflammatory cells, mainly lymphocytes and plasma cells was manifested. The surface epithelium of the gastric mucosa was denuded and separated from the under lying tissues. Hereby, the aggregation of these degerated parts were seen in the lumen of the stomach forming cellular debris. However, in areas exhibiting extensive damage, partial or complete necrosis of the mucosal lining cells including the mucous neck cells, parietal cells and peptic (zymogenic) cells was seen. The mucous neck cells designated obvious vacuolated cytoplasm and distinct pyknotic nuclei***(Fig. 38)***.

Microscopically, stomach sections from rats treated with ASA plus OMP plus I3C revealed few histological lesions. This was manifested in scattered areas invaded by polymorphnuclear and lymphatic cells being mainly of the mononuclear type. Moreover, haemorrhage in the superficial layer of mucosa and congestion of blood vessels in muscularis mucosa and submucosa layers were noticed ***(Fig. 39)***. The mucosa between gastric glands markedly infiltrated by lymphocytes and plasma cells ***(Fig.40)***, ulceration of the glandular mucosa was scanty.

Macroscopically, stomach of ulcerated rats receiving distilled water for 4 weeks, were slight of red coloration and slight of haemorrhagic streaks in the dissected stomach of rats. On the other hand, stomach from rats treated with I3C or I3C and OMP manifested more or less similar section as in control group ***(Fig. 26 e, f, g and h)***.

Histopathology of ulcerated stomach of male albino rats receiving distilled water for 4 weeks, stomach revealed many histological alteration. This was established in the form of many eroded areas in the gastric tissue. The surface epithelium manifested exfoliation and sloughing of the surface mucosal cells, which were aggregated in the lumen of the stomach forming debris of the damaged tissue ***(Fig. 41)***. The mucous neck at luminal parts of the gastric pits realized swelling of the cytoplasm with obvious nuclear karyolysis. However, in other parts of the gastric mucosa cells, some of these cells have illustrated remarkable features of necrosis ***(Fig. 42)***. The zymogenic or chief cells and the parietal cells were swollen in many areas of gastric pit and necrosis in another regions which notice with disrupted cells membrane, damaged cytoplasm and nucleus showed obvious features of karyolysis. The connective tissues localized between gastric pit and muscularis mucosa was damaged with marked aggregation of inflammatory cells mainly eosinophils, plasma

cells and lymphocytes ***(Fig. 43)***. The blood vessels localized in these regions were dilated (damaged) ***(Fig. 44)***, these signs of haemorrhages were observed as the result of extravasted blood vessels. Also, the picture of chronic superficial gastritis appeared in many areas of the mucosa where the glands were tortuous ***(Fig. 45)***, and lined with cells secreting little mucin.

Sections of stomach from ulcer group after 4 weeks post-treatment with OMP revealed some focal area of the surface epithelial cells exhibited obvious damage, erosion, flattening and the mucous neck cells were deteriorated with pyknotic nuclei. Evidence of fatty degeneration was frequently noticed in the peptic and parietal cells ***(Fig. 46)***. However, many cells appeared with vacuolated cytoplasm and pyknotic nuclei. The connective tissues forming the lamina propria showed congested blood vessels and chronic inflammatory cells were scattered between the gland cells ***(Fig. 47 )***.

Following 4 weeks, post-treatment with I3C, few pathological consequences in the gastric mucosal cells was revealed. The surface mucous cells have designated noticeable damage and erosion. Exfoliated cells and few cellular debris were observed on the mucosal surface obscuring the gastric pit openings. The mucous neck cells displayed prominent vacuolation of their cytoplasm with marked increase of mitotic figures in some areas ***(Fig. 48)***, while in other area they often show pyknotic nuclei.

Microscopically, stomach sections from ulcer rats group treated with omeprazole and indole-3-carbinol manifested minimal histological lesions in the gastric mucosal cells through the 4 weeks. This was apparent in the limit areas in the form of the divergence of parietal oxyntic cells at the gastric gland where chief peptic cells were paucity ***(Fig. 49)***. As displayed in figure ***(50)***, the connective tissues of the lamina propria suffered from inflammatory cellular infiltration. Also, haemorrhage was revealed. Nevertheless, it should be justified at this point that many areas of reviewed stomach sections still attained almost normal patterns ***(Fig. 51)***.

## 2-Histochemical Investigation:-

### iii. Protein Content:-

#### d. Normal Control Group:-

Total protein were demonstrated in the present material by applying the bromophenol blue technique. A strong reactivity was displayed by the peptic cells and oxyntic cells. Their protenic contents located mainly in a mildly reactive ground cytoplasm. The nuclei of these cells exhibited a strong reactivity with the same technique as seen in figure ***(52 a)***. The cytoplasm and nuclei of the surface mucous cells and the mucous neck cells showed moderate reactivity for their proteinic contents where as proteinic granules more concentrated in the luminal poles of surface mucosa cells.

#### b. Control Groups:-

Stomach sections stained with bromophenol blue for identification of protein content in OMP and/or I3C treated animals ratified no change in their distribution throughout the whole experimental duration (7 days and 4 weeks) ***(Fig.52 b, c, d, e, f and g)***.

### c. Experimental Groups:-

Histochemical staining of sections of stomach from treated animals for the identification of total protein content (with bromophenol blue stain) manifested decrease in the group administered with ASA within the 7 days post-treatment. The stainability of the cytoplasm and nuclei of the oxyntic, peptic and mucous neck cells was greatly reduced and the protein granules in the most cells were highly diminuted ***(Fig. 53)***. Furthermore, the group of rats treated with aspirin and omeprazole for 7 days, the protein staining illustrated diminution in stomach total protein content. Pyknotic cells showed dark nuclear staining while their cytoplasm was completely faint staining affinity ***(Fig. 54)***.

Decreased the proteinic content in the cytoplasm and nuclei of oxyntic, peptic cells, surface mucous cells and mucous neck cells was also noticed in group of animals treated with ASA and I3C for seven days. The stainability of such cells revealed moderately reduced ***(Fig.55)***. Other aspirin group treated with omeprazole and indole-3-carbinol were slightly diminuted in the nuclei and the cytoplasm of the peptic and parietal cells and in the apical parts of the surface mucous cells as well as in the mucous neck cells ***(Fig. 56)***. A slight decline also, in the proteinic reactivity of the nuclei of other cells were noticed.

On the other hand, the stomach section from ulcerated group treated with distilled water for 4 weeks, revealed a diminution in mucosal cells total protein content. In such case a rather weak or feeble stainability with bromophenol blue was quite clear in the constituent cells ***(Fig. 57)***.

On examining sections of stomach of ulcer rats group after 4 weeks post-treatment with OMP showed slightly decrease in staining quality for total proteins. Nevertheless, the areas of degenerative and necrotic cells were faintly stained and ill-differentiated while areas of regeneration were heavily stained ***(Fig. 58)***.

Also, sections from ulcer rat treated with I3C for 4 weeks, stomach showed the cytoplasm and nuclei of peptic, parietal, mucous neck cells, surface mucous neck cells and surface mucous cells had been restored a part of their reactivity with bromophenol blue. So, these cells appeared moderately stained with bluish colouation ***(Fig. 59)***.

Moreover, sections of stomach from ulcer animals treated with omeprazole and indole-3-carbinol, manifested slight decrease in total protein content within the 4 weeks post-treatment. This was confined to several cells that revealed faintly stained while the majority of stomach cells showed near to normal pattern of distribution ***(Fig. 60)***.

## ii. Alcian blue-P.A.S.:-

### a . Normal Control Group:-

Applying the alcian blue-P.A.S. technique, the mucin granules are distributed in the stomach tissue ***(Fig. 61 a)***.

## b .Control Groups:-

Histochemical study of stomach section of animals treated with OMP and/or I3C using alcian blue-P.A.S. technique revealed normal distribution of mucin granules (mucopolysaccharides) in the gastric tissue throughout the whole experimental duration ***(Fig. 61 b, c, d, e, f and g)***.

## c. Experimental Groups:-

Alcian blue-P.A.S. for mucin (neutral mucopolysaccharides and acid mucopolysaccharides) manifested severe decrease in mucin positive material in the group treated with ASA for 7 days. Attenuation of these carbohydrate content in both the surface mucous cells and mucous neck cells while degenerative cells ratified pale colouration of these areas ***(Fig. 62)***.

In the group of rats treated with ASA and OMP for 7 days, stomach sections showed decrease in mucin granules. This was demonstrated in the form of pale stain in stomach cells cytoplasm ***(Fig. 63)***.

In addition, a moderate decrease in mucin granules was observed in animals group treated with ASA and I3C during 7 days ***(Fig. 64)***.

Furthermore, in aspirin rats treated with omeprazole and indole-3-carbinol such decrease was evident from the 7 days post-administration ***(Fig. 65)***.

In the ulcer group of rats treated with distillate water for 4 weeks, the carbohydrates reaction (mucin) showed decreased, the surface mucous cells as well as the mucous neck cells appeared to be obviously attained rather faint maganita stainability with P.A.S. and faint blue with alcian blue procedure ***(Fig. 66)***. However, the parietal cells (oxyntic) cells and peptic cells showing decline stainability with alcian blue-P.A.S.

Marked diminution of alcian blue-P.A.S. reaction i.e. diminution mucopolysaccharides content has been observed in the animals from ulcer group treated with OMP for 4 weeks. The pattern of decrease was manifested in the mucous neck cells and other wall of gastric tissue ***(Fig.67)***.

Histochemical study of stomach sections of treated ulcer animals with I3C ratified mild diminution of (mucopolysaccharides) of the carbohydrate inclusions P.A.S. positive materials as reflected by moderate reactivity of both surface mucous cells and mucous neck cells ***(Fig. 68)***. On the other hand, degenerative cells and cells debris in the lumen were devoid of stainable alcian blue-P.A.S., while regenerative cells were richly loaded with mucopolysaccharides.

A moderated decrease in mucopolysaccharides in both the surface mucous cells and mucous neck cells was detected in stomach section of animals from ulcer group treated with omeprazole and indole-3-carbinol during 4 weeks ***(Fig. 69)***.

## 3-Immunohistochemical Investigation:-

- **Immunohistochemistry of Cyclooxygenase-2 (COX-2) and Proliferating Cell Nuclear Antigen (PCNA)**:-

The gastric tissues obtained from animals treated with aspirin, ASA+OMP, ASA+I3C and ASA+OMP+I3C for seven days were used for immunohistochemical localization of proliferating cell nuclear antigen (PCNA) antibodies and cyclooxgenase (COX-2). The analysis of immunohistochemical sections showed few number of proliferation cells in the stomach of animals treated with ASA plus I3C and ASA plus OMP and I3C ***(Figs. 73 and 74)***. The same profile was found to COX-2 ***(Figs. 82 and 83)***. Inhibition of proliferating cell nuclear antigen ***(Figs. 71 and 72)*** and cyclooxgenase-2 antibodies ***(Figs. 80 and 81)*** in gastric mucosa from observations in the animals by aspirin alone or with omeprazole compared with control group ***(Figs. 70 and 79)***.

Proliferating cell nuclear antigen (PCNA) was used to determine proliferating cells in aspirin-induced ulcers. Moreover, immunohistochemical analysis was performed to ascertain the localization of cyclooxygenase-2 in gastric mucosa tissue.

Histological analysis of the gastric ulcers revealed extensive deep damage induced by aspirin. Sections from ulcers treated with OMP, I3C and OMP plus I3C for 4 weeks demonstrated sign of regenerated mucosa. These results were confirmed by PCNA immunohistochemical analysis, where the OMP and/or I3C treated to ulcer animals for 4 weeks increased the proliferating cells when compared to control groups ***(Figs. 76, 77 and 78)***. Gastric mucosal tissues of ulcer rats group treated with distilled water for four weeks did not display significant immunoreactivity for PCNA ***(Fig. 75)***. The expression of COX-2 was increased in gastric tissue obtained from ulcer group ***(Figs. 85, 86 and 87)***, where rats given OMP and/or I3C for 4 weeks as compared to ulcerated rats given distilled water for four weeks that showed inhibition of COX-2 ***(Fig. 84)***.

Thus, the results indicate that their protein participated in the healing of the gastric ulcer treated OMP and/or I3C.

Table ***(19)*** and Figures ***(88 and 89)*** showed that the most pronounced statements of PCNA and COX-2 stains were in I3C treated ulcerated rats.

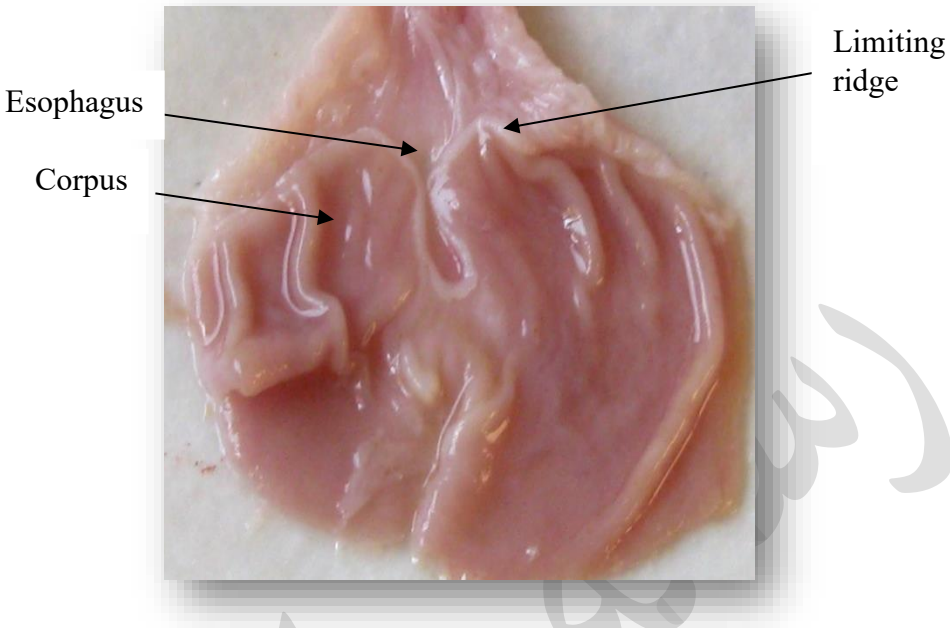



**Fig.( 22)** : Representative stomach of normal control rats.

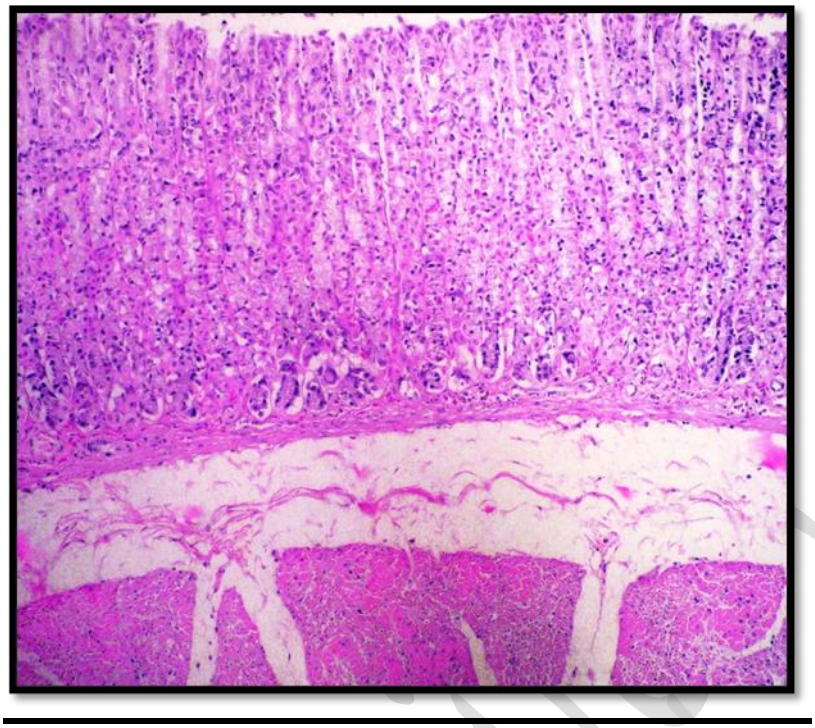


**Fig. (23):** Photomicrograph of stomach section from normal control rat showing, different gastric layers.

(Hx-E; x100).

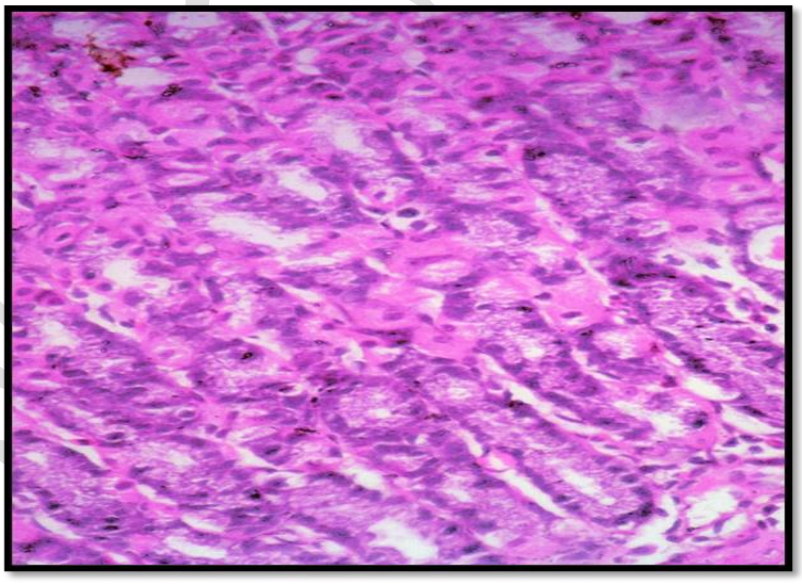


**Fig. (24):** Photomicrograph of stomach section from normal control rat showing, mucous, peptic and parietal oxyntic cells.

(Hx-E; x400).

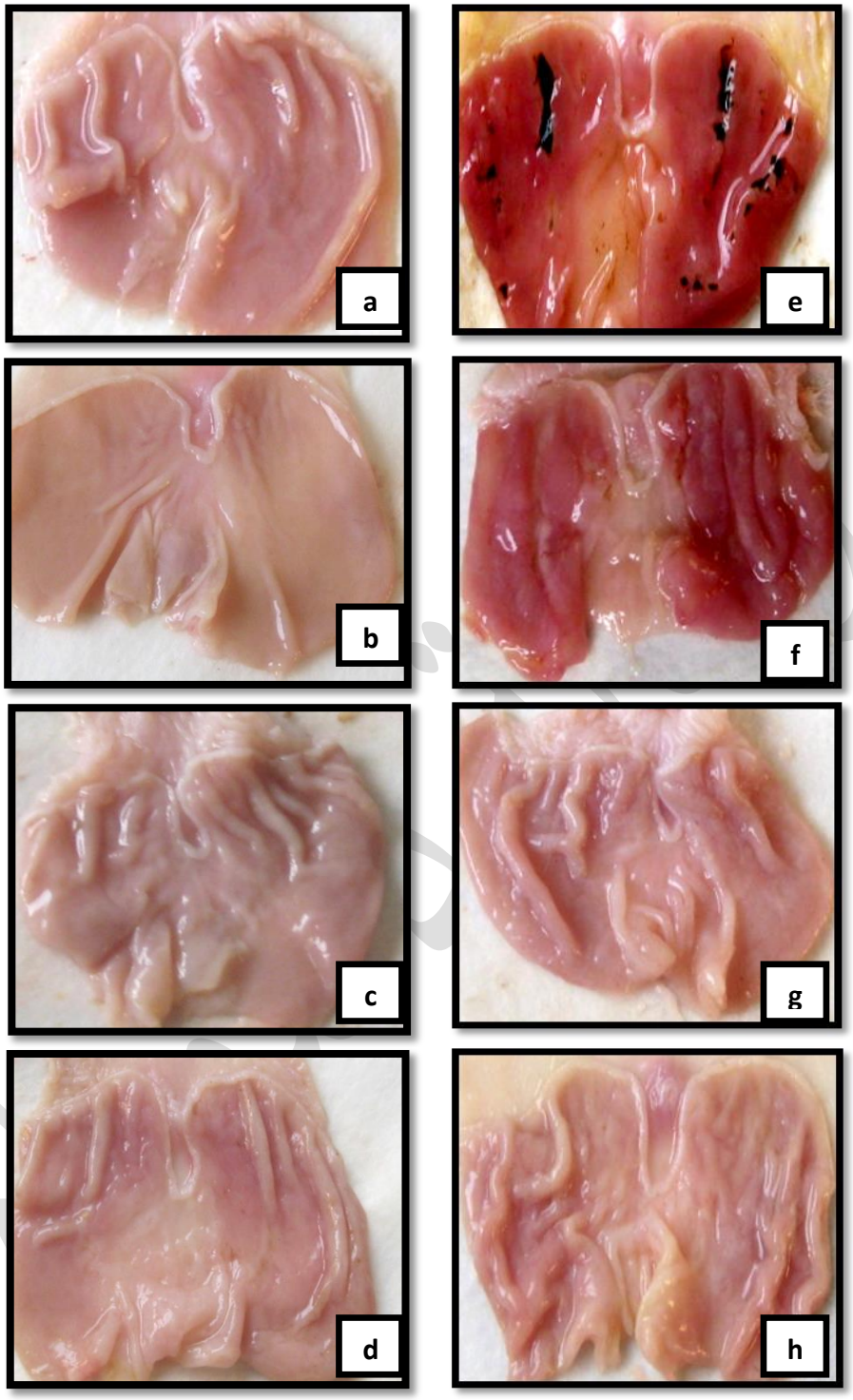



**Fig. (25):** Representative stomach from control groups and experimental groups for 7 days.

**(a):**Normal control group.
**(b):** OMP group.
**(c):** I3C group.
**(d):** OMP+I3C group.
**(e):** ASA group.
**(f):** ASA+OMP group.
**(g):** ASA+I3C group.
**(h):**ASA+OMP+I3C group.

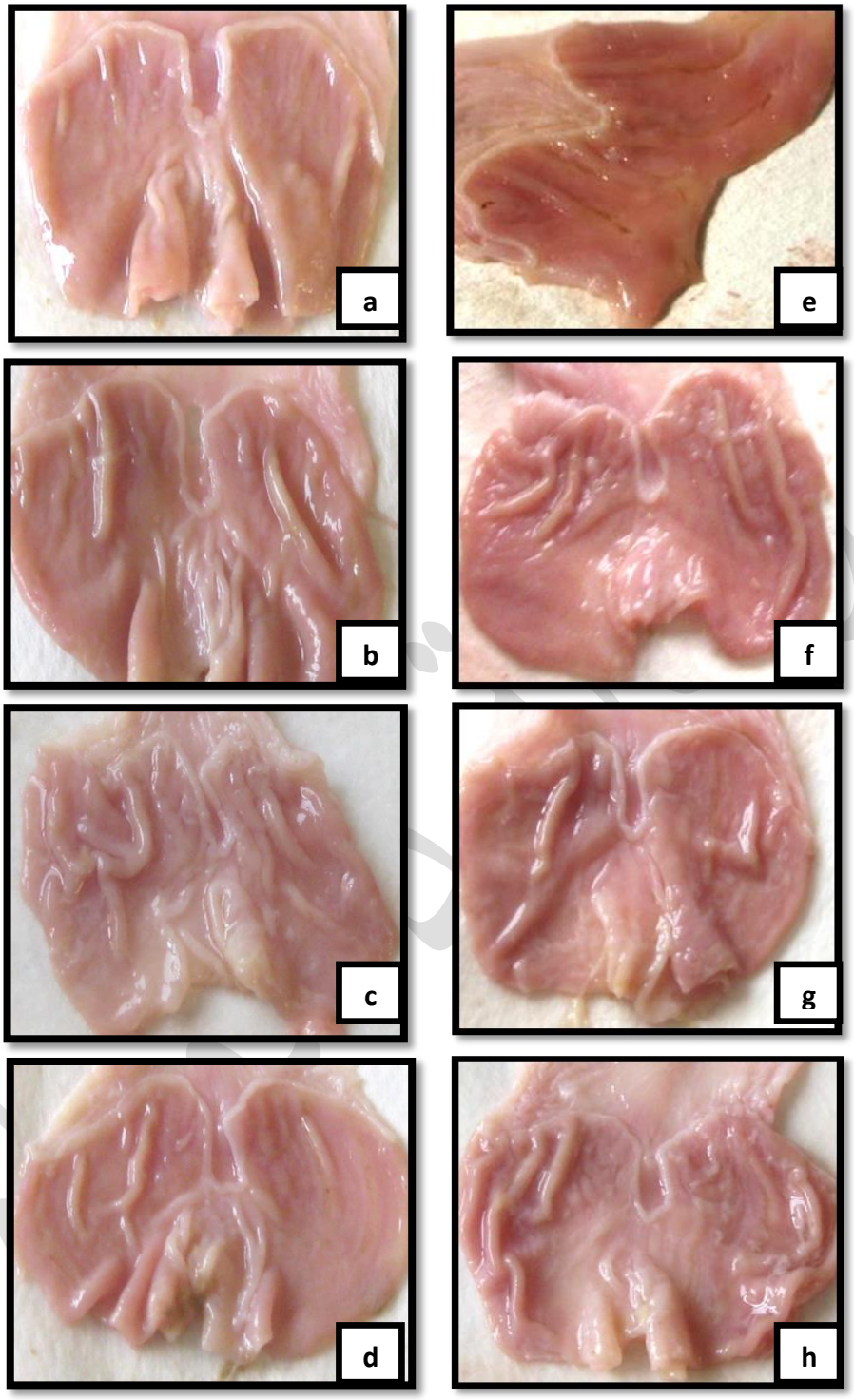



**Fig. (26):** Representative stomach from control groups and experimental groups for 4 weeks.

**(a):**Normal control group.
**(b):** OMP group.
**(c):** I3C group.
**(d):** OMP+I3C group.
**(e):** ASA group.
**(f):** ASA+OMP group.
**(g):** ASA+I3C group.
**(h):**ASA+OMP+I3C group.

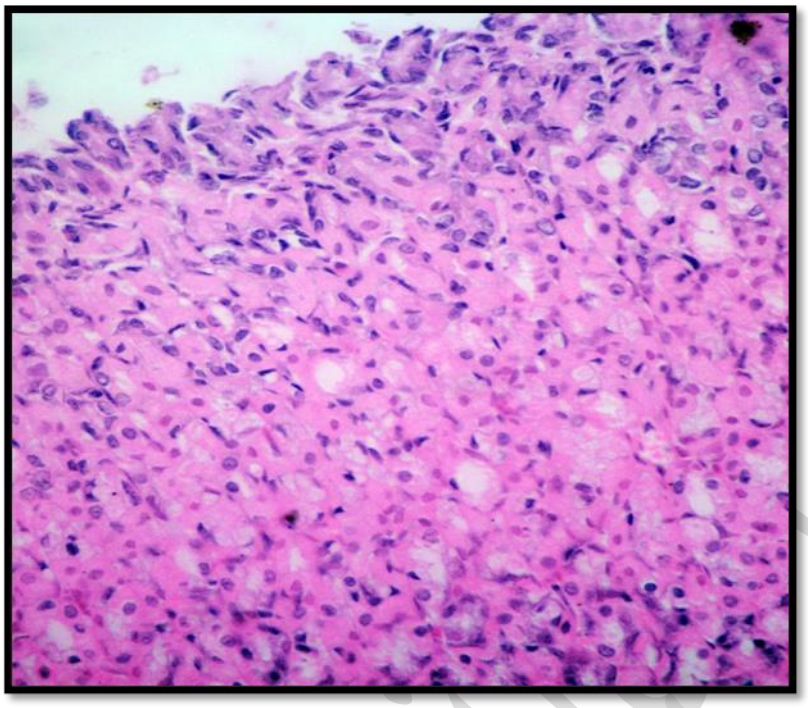


**Fig. (27):** Photomicrograph of stomach section from control a rat receiving omeprazole for 4 weeks showing, normal different stomach layers.

(Hx-E; x400).

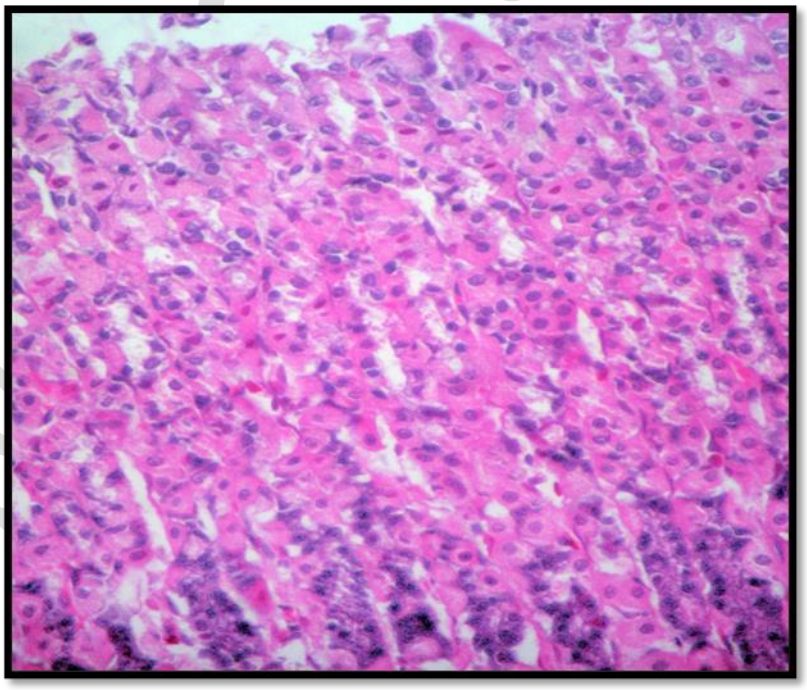


**Fig. (28):** Photomicrograph of stomach section from control a rat receiving indole-3-carbinol 4 weeks showing, the normal pattern of different stomach layers.

(Hx-E; x400).

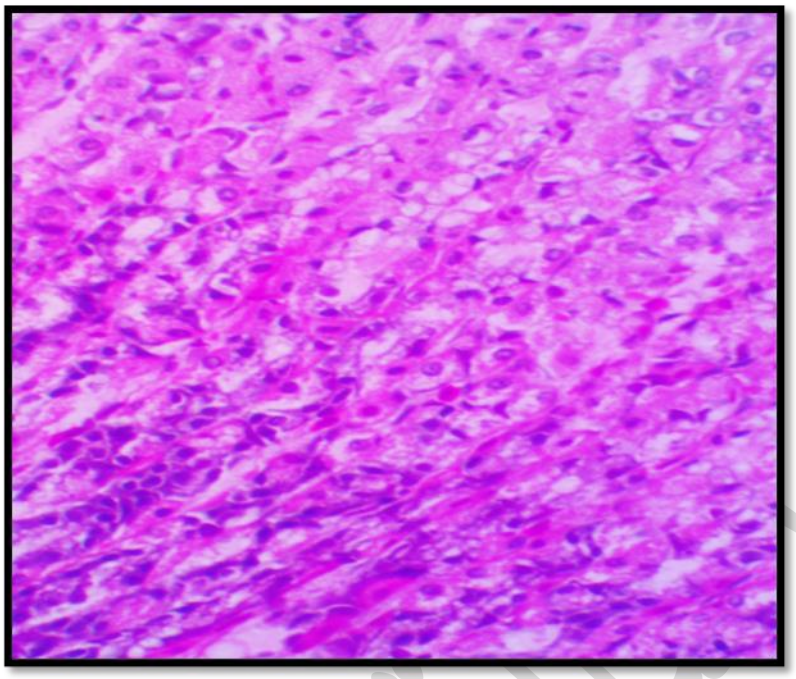


**Fig. (29):** Photomicrograph of stomach section from control a rat receiving omeprazole plus indole-3-carbinol for 4 weeks showing, normal pattern gastric tissue.

(Hx-E; x400).

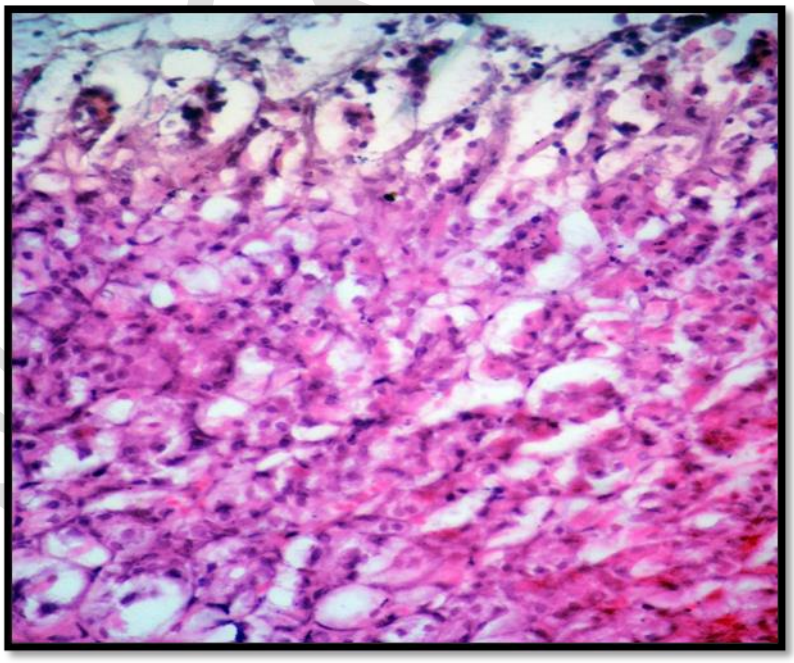


**Fig. (30):** Photomicrograph of stomach section from treated a rat receiving aspirin for 7 days showing, eroded areas and a large number of oxyntic cells acidophilic granules with vesicular nuclei (arrows).

(Hx-E; x400).

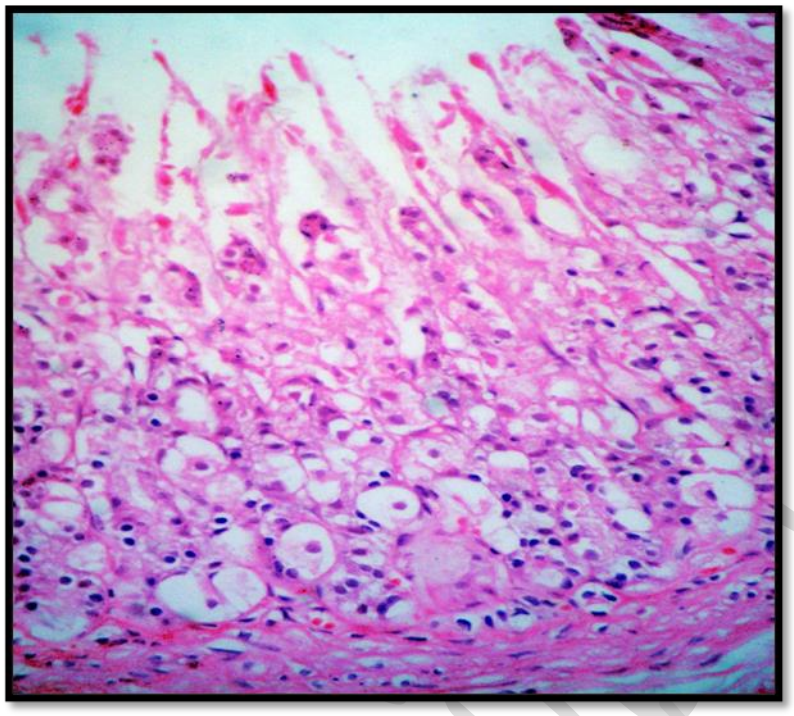


**Fig. (31):** Photomicrograph of stomach section from treated a rat receiving aspirin for 7 days showing, degeneration of mucosa cells.

(Hx-E; x400).

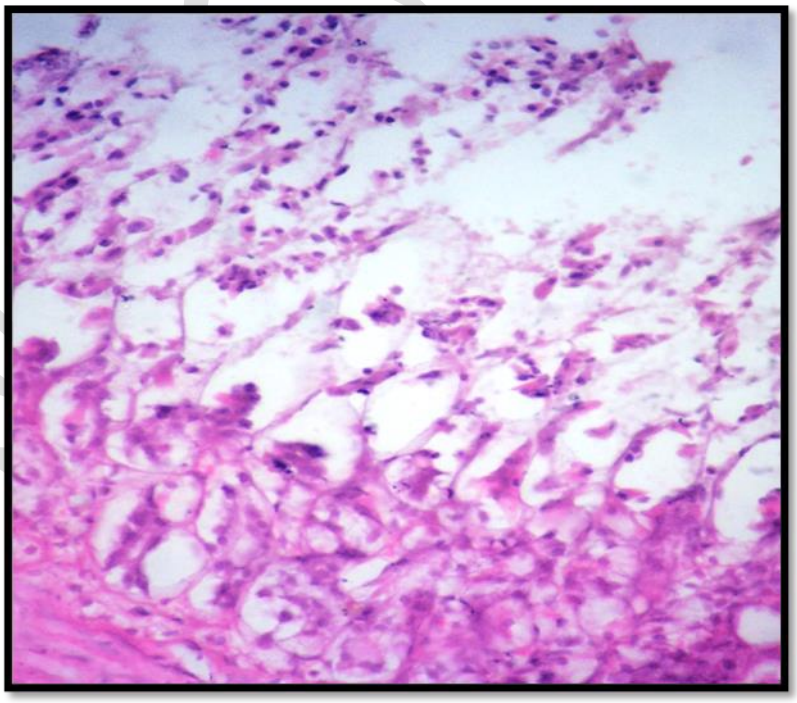


**Fig. (32):** Photomicrograph of stomach section from treated a rat receiving aspirin for 7 days showing, mucosal damage (arrows) and glandular disturbance (*****).

(Hx-E; x400).

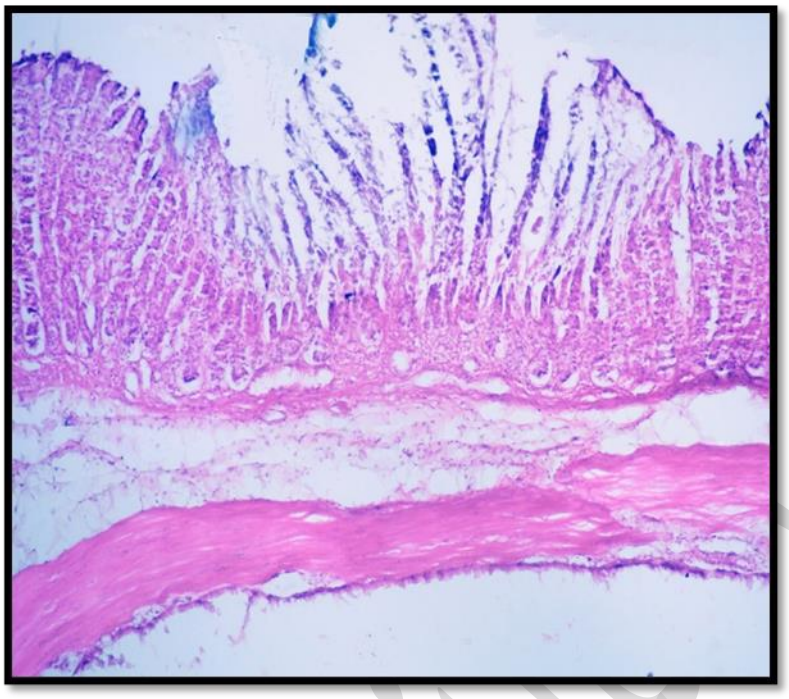


**Fig. (33):** Photomicrograph of stomach section from treated a rat receiving aspirin for 7 days showing, ulceration in glandular mucosa layer.
(Hx-E; x100).

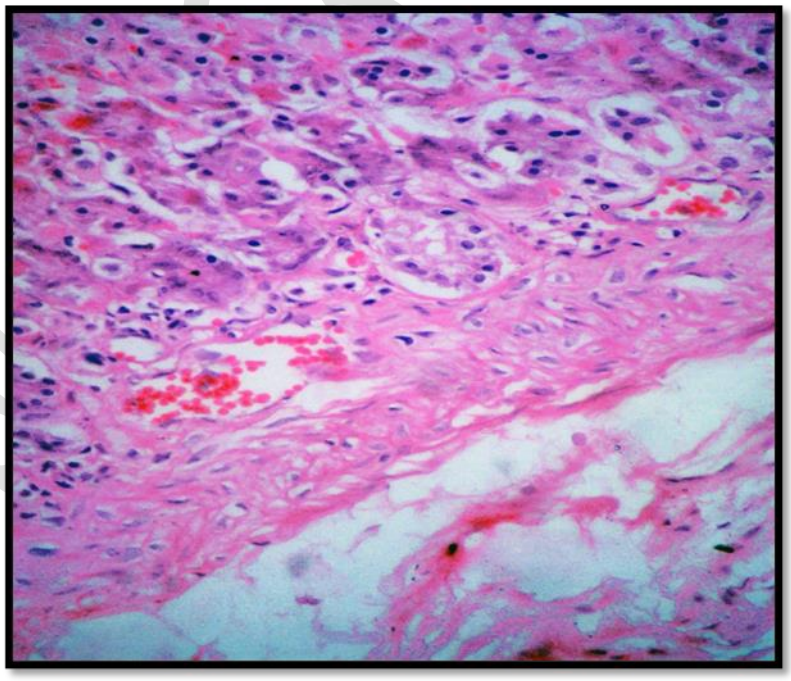


**Fig. (34):** Photomicrograph of stomach section from treated a rat receiving aspirin for 7 days showing, dilatation of blood vessels (*****), chronic inflammatory cells (arrows) and haemorrhage (******).
(Hx-E; x400).

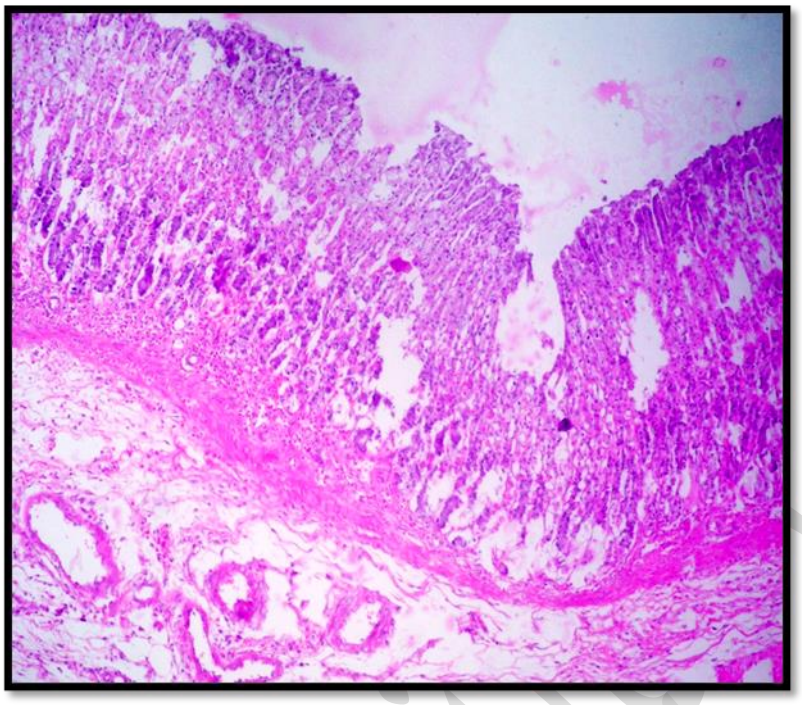


**Fig. (35):** Photomicrograph of stomach section from treated a rat receiving aspirin plus omeprazole for 7 days showing, many eroded areas in gastric mucosa.
(Hx-E; x100).

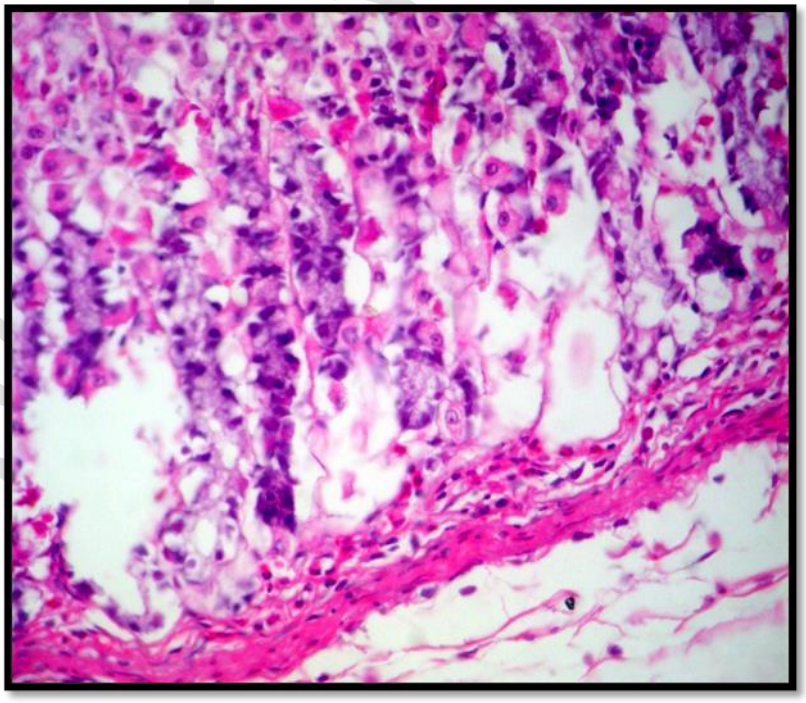


**Fig. (36):** Photomicrograph of stomach section from treated a rat receiving aspirin plus omeprazole for 7 days showing, inflammatory cells (*****), haemorrhage (******) and vacuolation of the cytoplasm of mucosal cells and disruption of their cells membrane with pyknotic nuclei (arrows).
(Hx-E; x400).

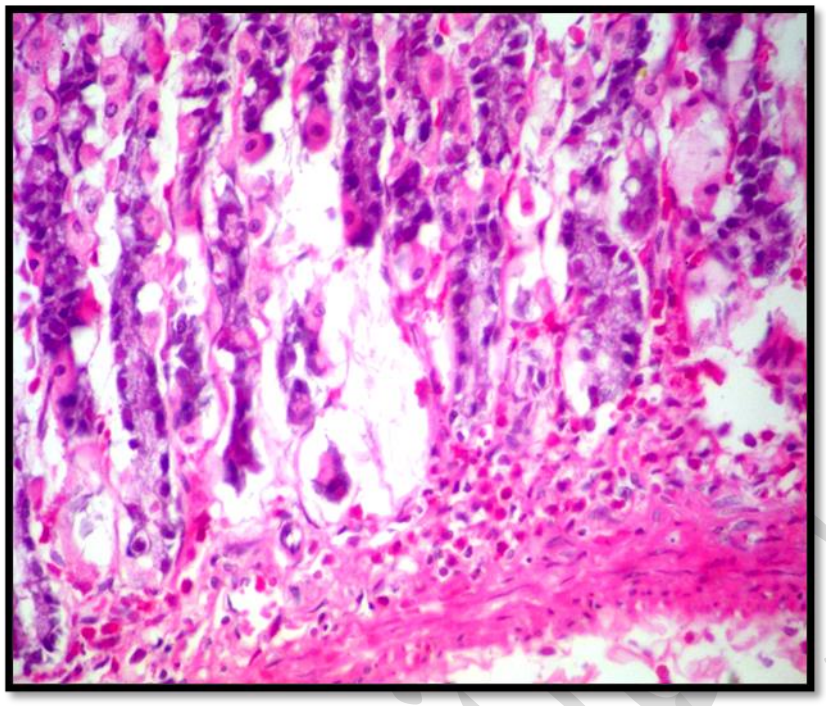


**Fig. (37):** Photomicrograph of stomach section from treated a rat receiving aspirin plus omeprazole for 7 days showing, aggregation of inflammatory cells near the bases of the gastric pits and muscularis mucosa (arrows).

(Hx-E; x400).

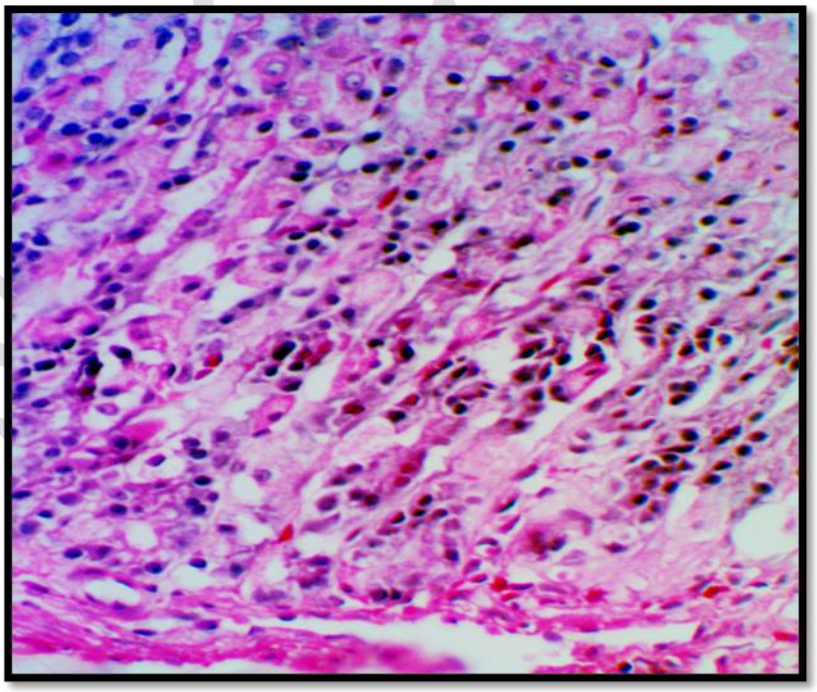


**Fig. (38):** Photomicrograph of stomach section from treated a rat receiving aspirin plus indole-3-cabinol for 7 days showing, the mucous neck cells designated obvious vacuolated cytoplasm and distinct pyknotic nuclei.

(Hx-E; x400).

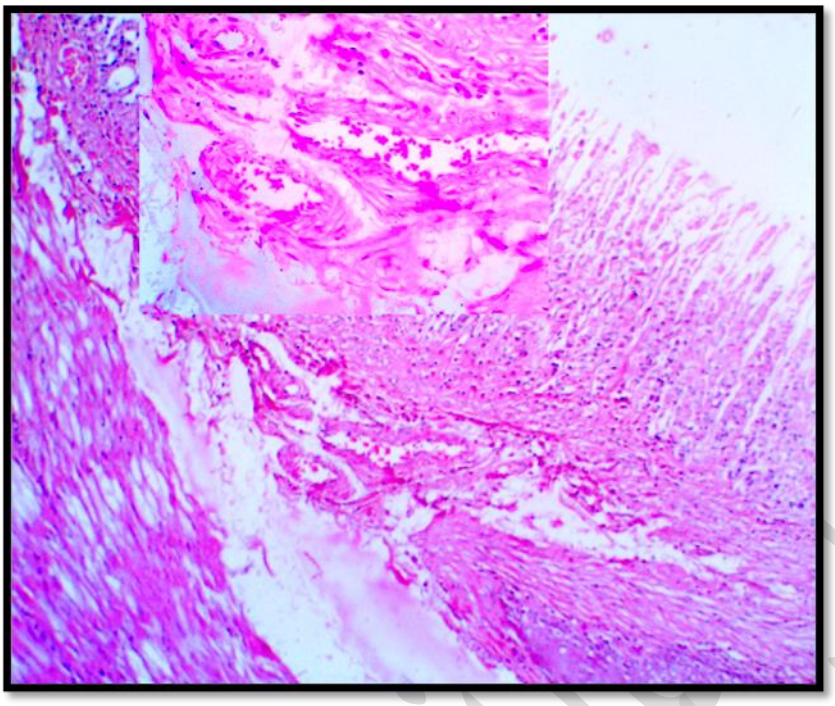


**Fig. (39):** Photomicrograph of stomach section from treated a rat receiving aspirin plus omrprazole plus indole-3-cabinol for 7 days showing, haemorrhage in the superficial layer of mucosa and congestion of blood vessels in muscularis mucosa and submucosa layers.
(Hx-E).

**a**-x100)
**b**-x400).

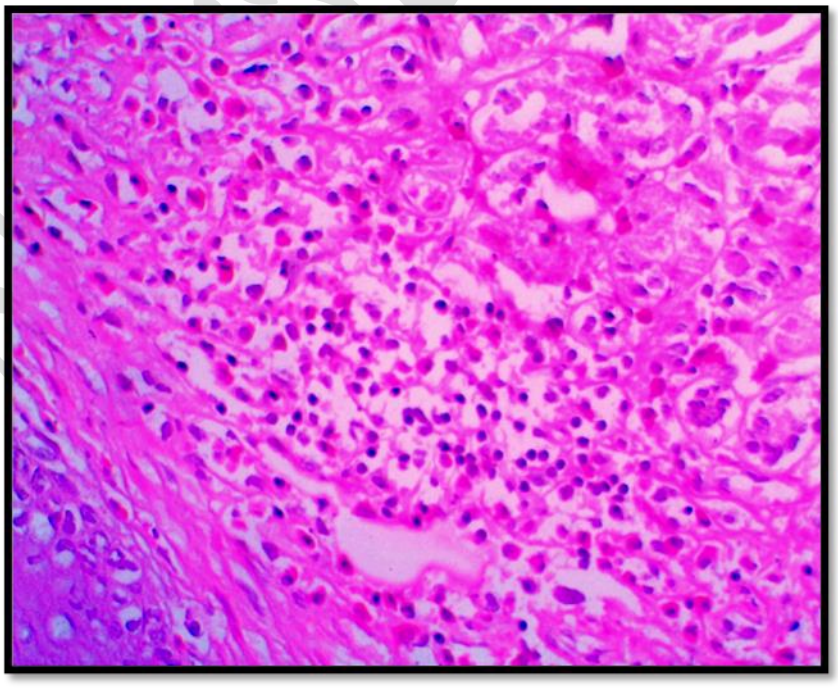


**Fig. (40):** Photomicrograph of stomach section from treated a rat receiving aspirin plus omrprazole plus indole-3-cabinol for 7 days showing, lymphocyte infiltration between gastric gland (arrows).
(Hx-E; x400).

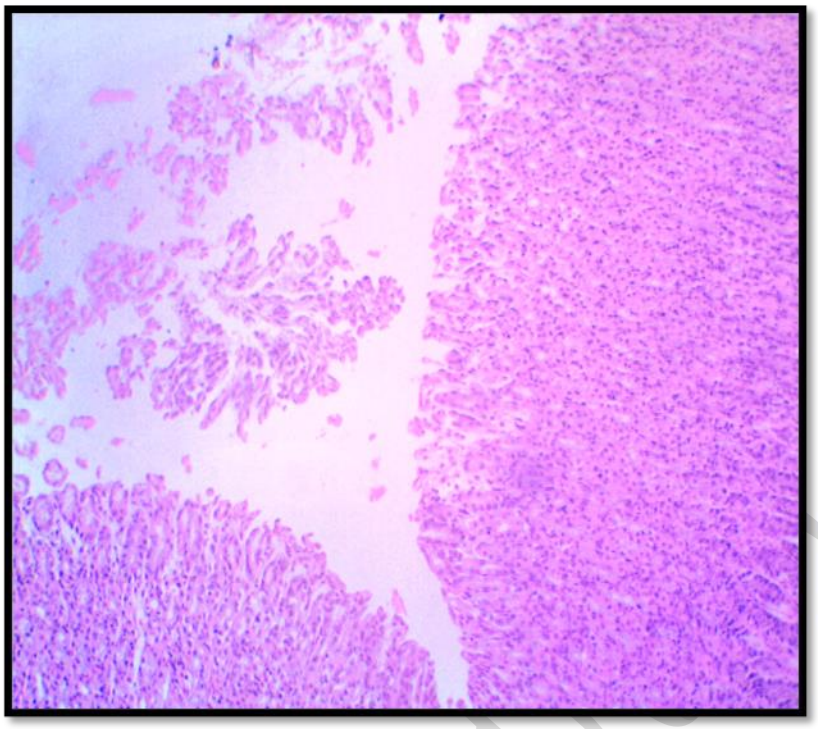


**Fig. (41):** Photomicrograph of ulcerated stomach section from a rat receiving distilled water for 4 weeks showing, cellular debris and fragment in gastric lumen.
(Hx-E; x400).

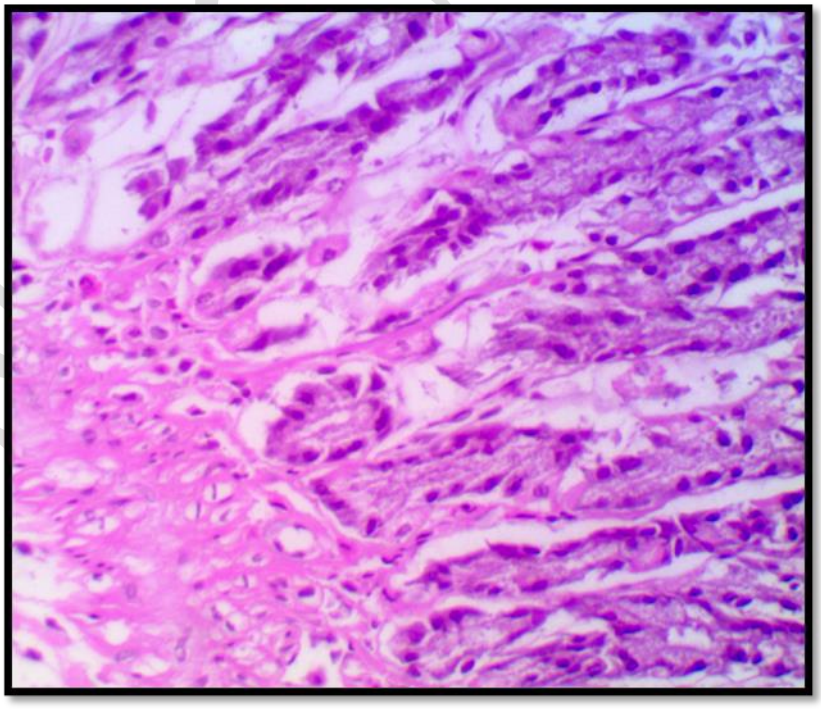


**Fig. (42):** Photomicrograph of ulcerated stomach section from a rat receiving distilled water for 4 weeks showing, damaged glandular epithelium.
(Hx-E; x400).

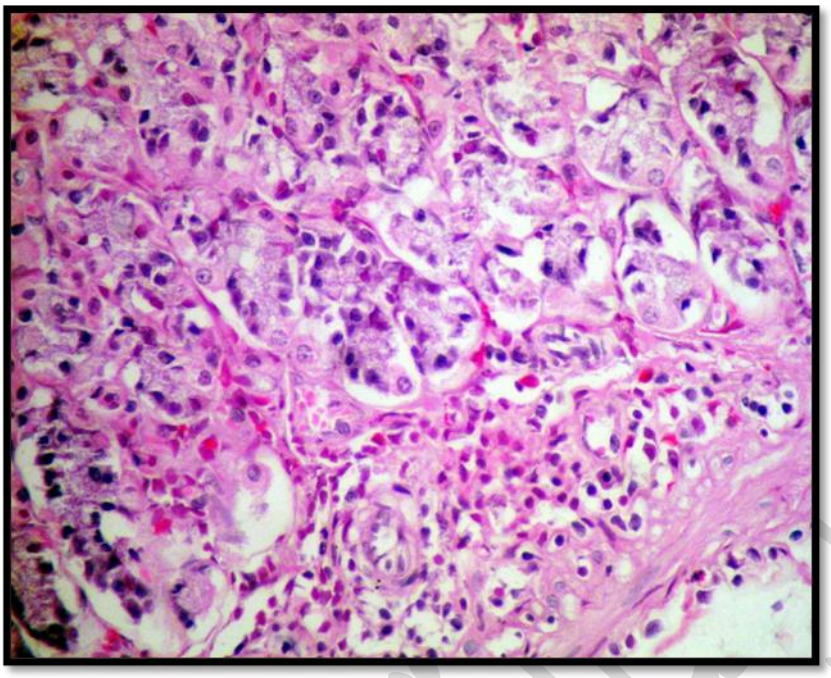


**Fig. (43):** Photomicrograph of ulcerated stomach section from a rat receiving distilled water for 4 weeks showing, aggregation of inflammatory cells between gastric gland (arrows).
(Hx-E; x400).

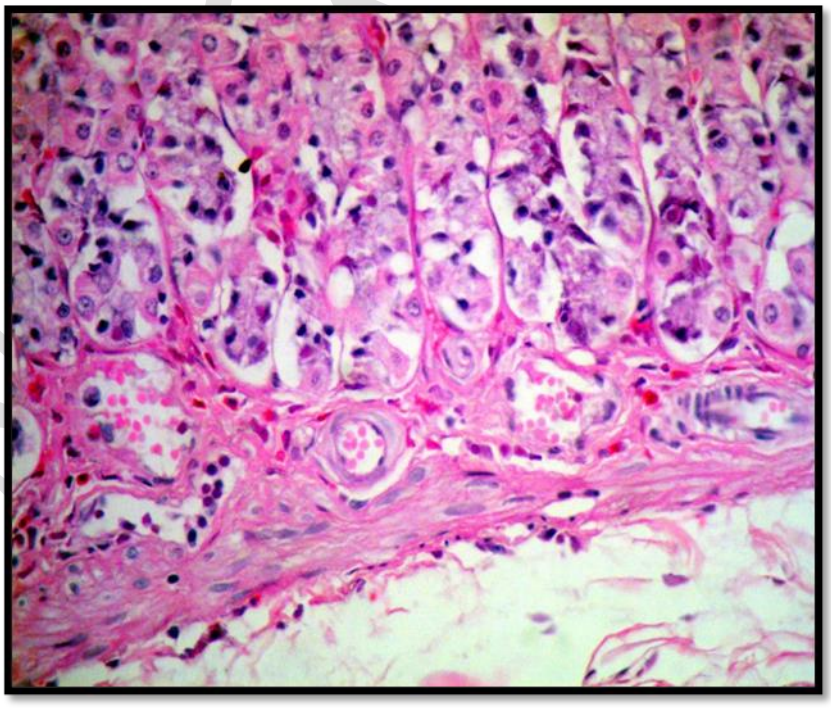


**Fig. (44):** Photomicrograph of ulcerated stomach section from a rat receiving distilled water for 4 weeks showing, dilated blood vessels (arrows).
(Hx-E; x400).

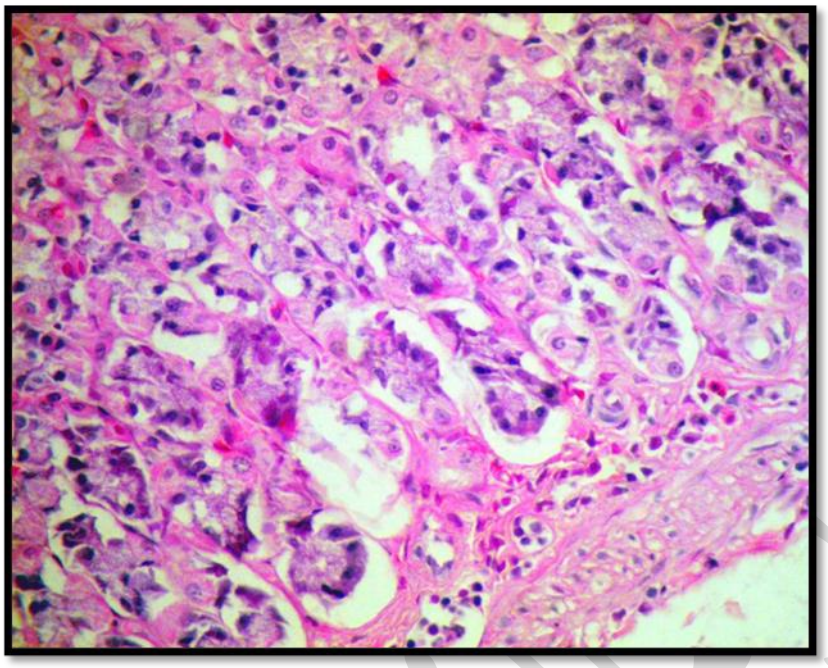


**Fig. (45):** Photomicrograph of ulcerated stomach section from a rat receiving distilled water for 4 weeks showing, glandular distortion (arrows).
(Hx-E; x400).

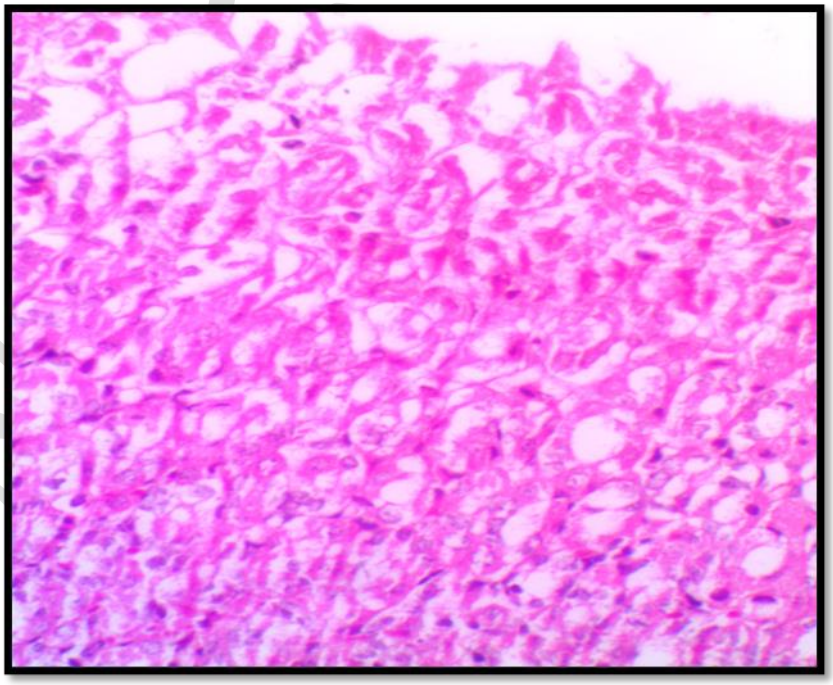


**Fig. (46):** Photomicrograph of ulcerated stomach section from a rat receiving omeprazole for 4 weeks showing, fatty degeneration in the mucosa cells (arrows).
(Hx-E; x400).

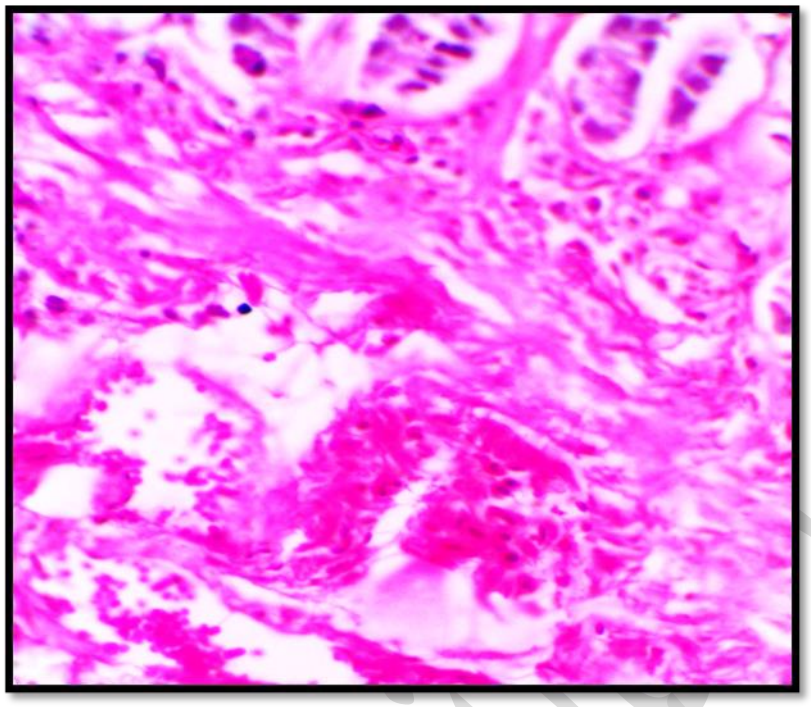


**Fig. (47):** Photomicrograph of ulcerated stomach section from a rat receiving omeprazole for 4 weeks showing, congested blood vessels and inflammatory cells between gland cells.
(Hx- E; x400).

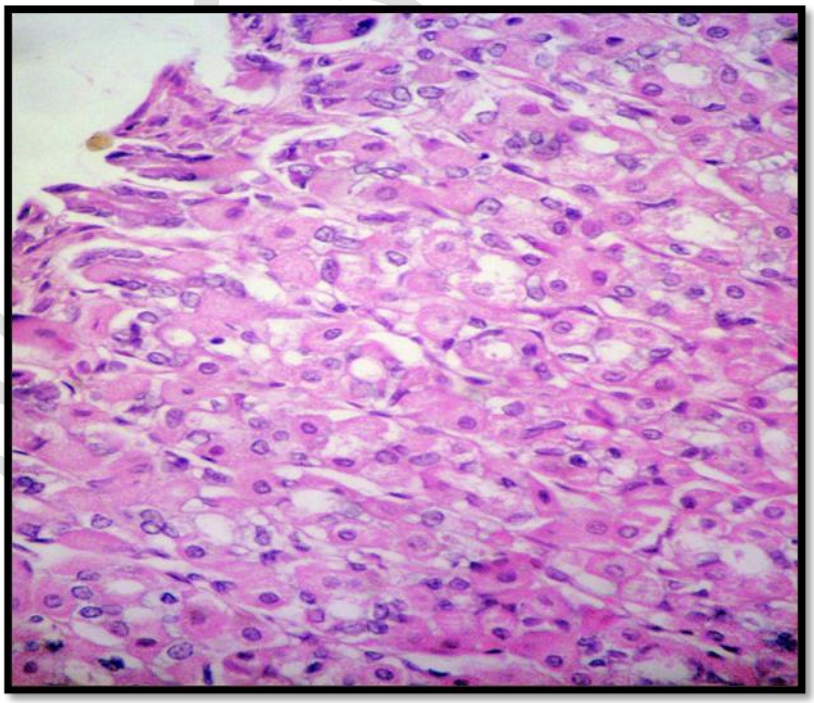


**Fig. (48):** Photomicrograph of ulcerated stomach section from a rat receiving indole-3-carbinol for 4 weeks showing, mitotic acting of many cells (arrows).
(Hx-E; x400).

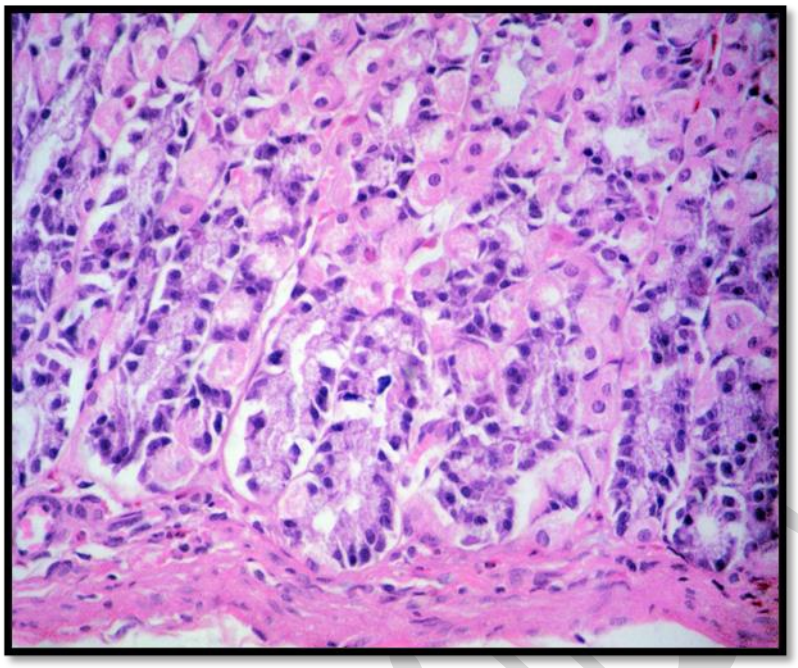


**Fig. (49):** Photomicrograph of ulcerated stomach section from a rat receiving omeprazole plus indole-3-carbinol for 4 weeks showing, degeneration in mucosa cells.
(Hx-E; x400).

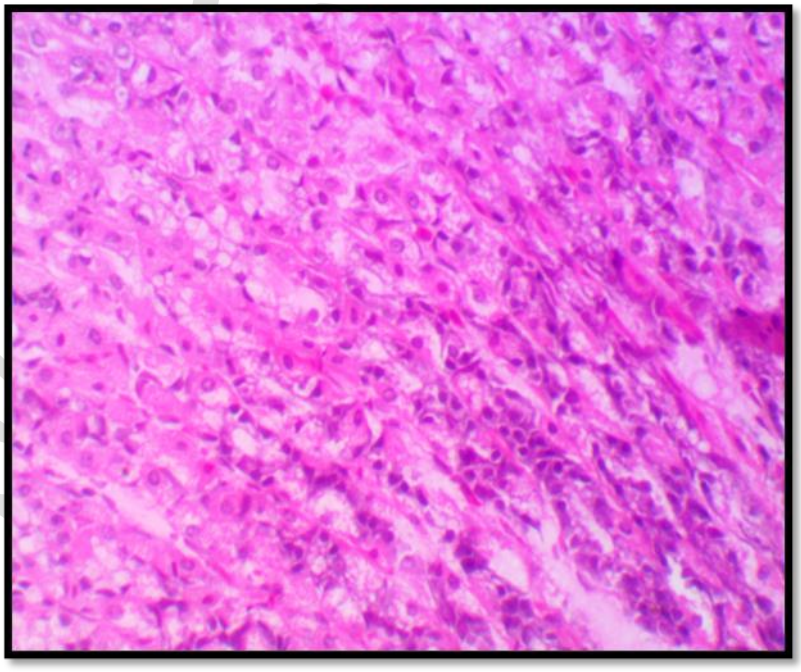


**Fig. (50):** Photomicrograph of ulcerated stomach section from a rat receiving omeprazole plus indole-3-carbinol for 4 weeks showing, inflammatory cellular infiltration.
(Hx-E; x400).

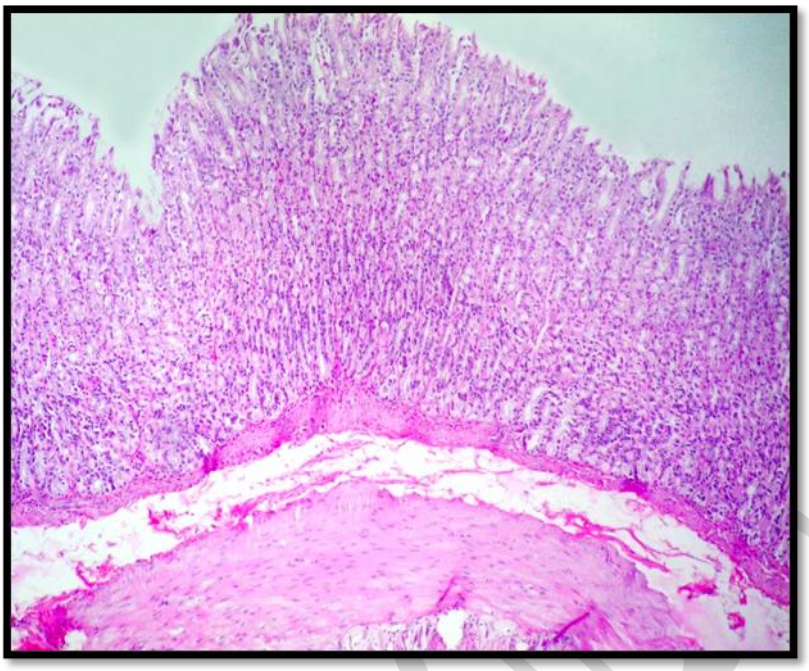


**Fig. (51):** Photomicrograph of ulcerated stomach section from a rat receiving omeprazole plus indole-3-carbinol for 4 weeks showing, a near to normal pattern of gastric tissue.
(Hx-E; x100).

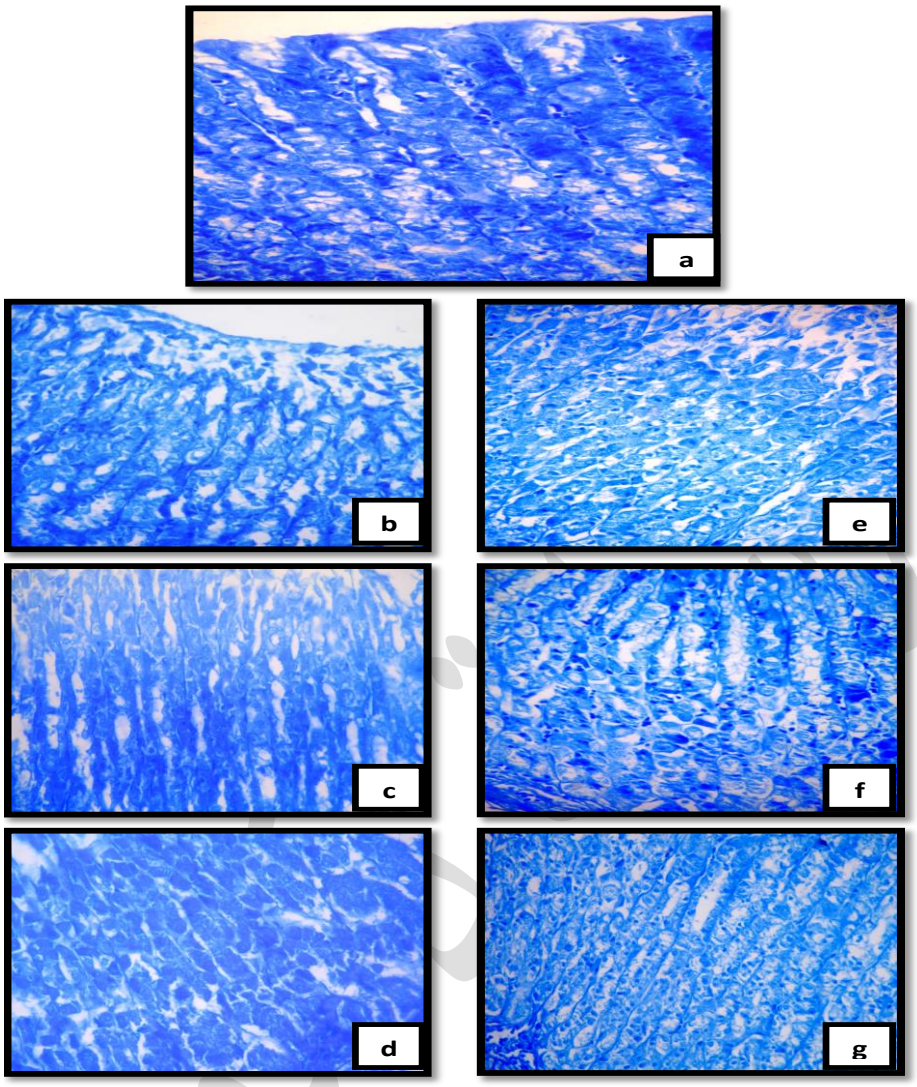



**Fig. (52):** Photomicrograph of stomach section from:

**(a):**Normal control rat showing, normal distribution of protein content.

**(b-g):**Control rat after administration of OMP and/or I3C showing, normal distribution of protein content as in control pattern.

**(b):** OMP group after 7 days.
**(c):** I3C group after 7 days.
**(d):** OMP+I3C group after 7 days.
**(e):** OMP group after 4 weeks.
**(f):** I3C group after 4 weeks.
**(g):** OMP+I3C group after 4 weeks.
(Bromophenol blue; x400).

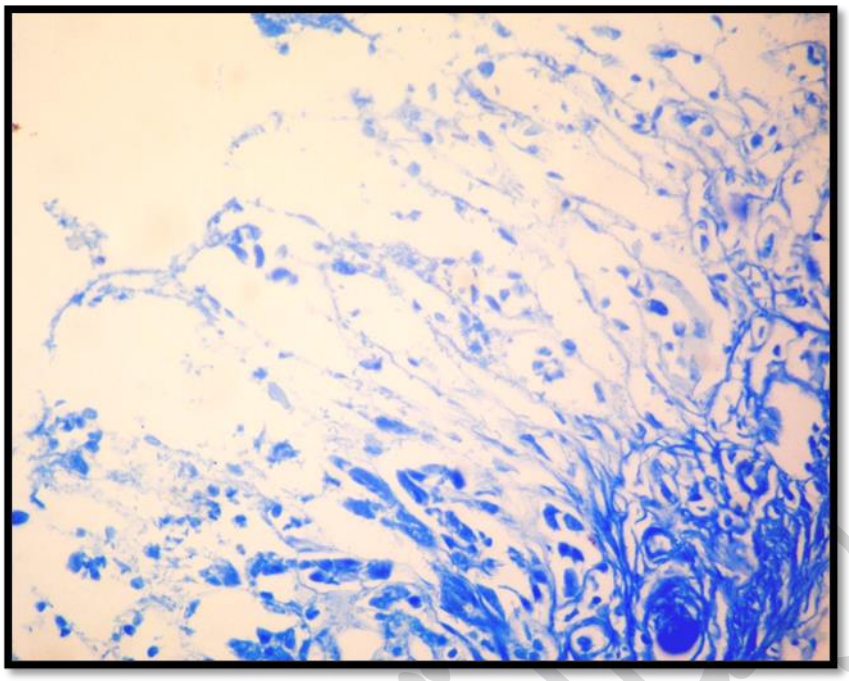


**Fig. (53):** Photomicrograph of stomach section from treated a rat receiving aspirin for 7 days showing, decrease in the protein content in gastric tissue.
(Bromophenol blue; x400).

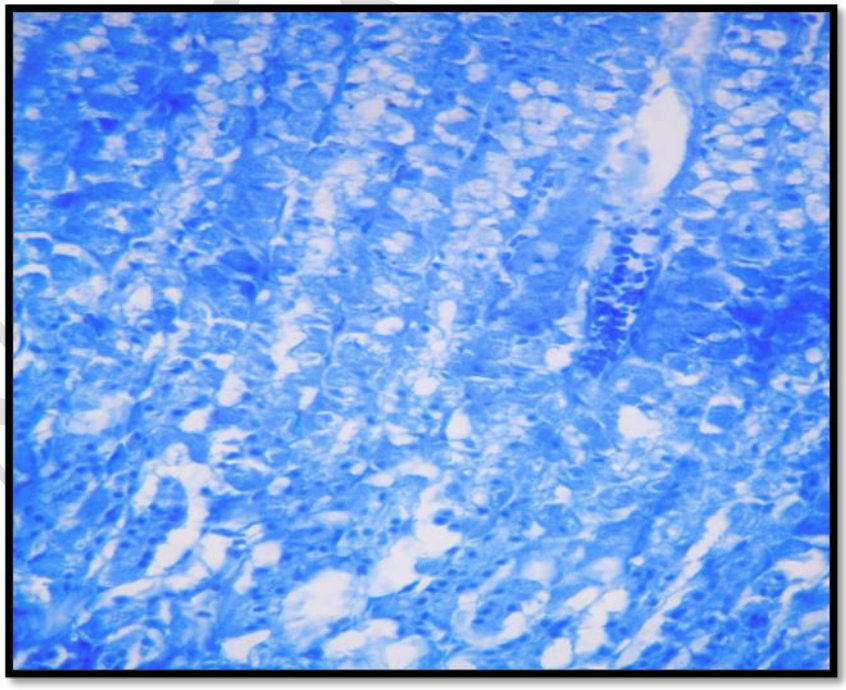


**Fig. (54):** Photomicrograph of stomach section from treated a rat receiving aspirin plus omeprazole for 7 days showing, decrease in the protein content and dark nuclear staining with faint staining in the cytoplasm in pyknotic cells.
(Bromophenol blue; x400).

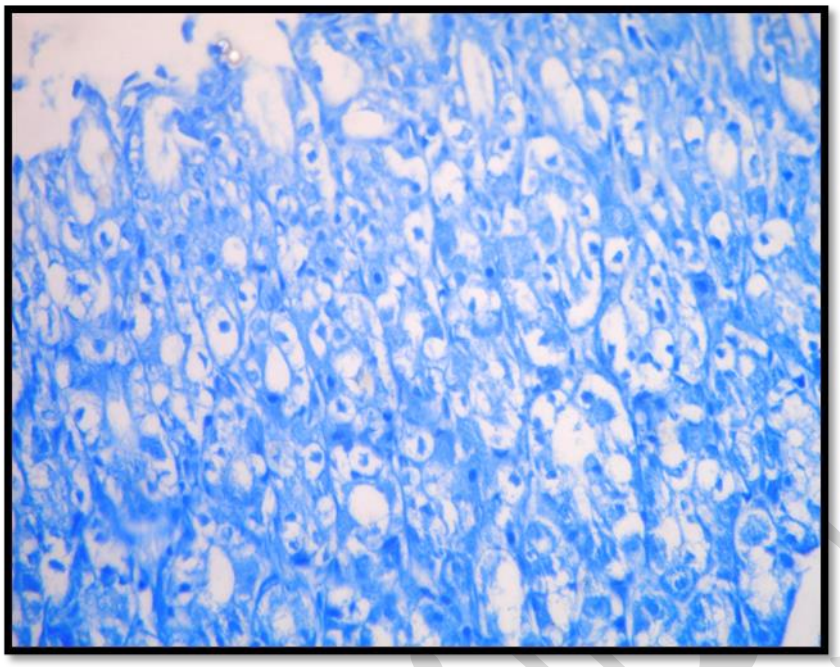


**Fig. (55):** Photomicrograph of stomach section from treated a rat receiving aspirin plus indole-3-carbinol for 7 days showing, reduced stainability in cytoplasm of mucous cells and oxyntic cells. (Bromophenol blue; x400).

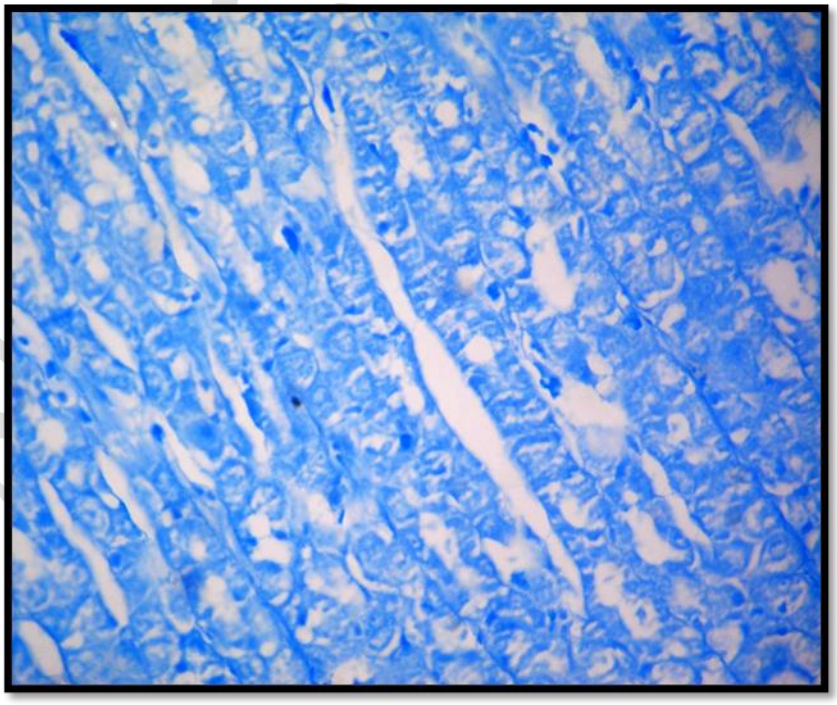


**Fig. (56):** Photomicrograph of stomach section from treated a rat receiving aspirin plus omeprazole plus indole-3-carbinol for 7 days showing, a mild decrease in protein content in gastric cells. (Bromophenol blue; x400).

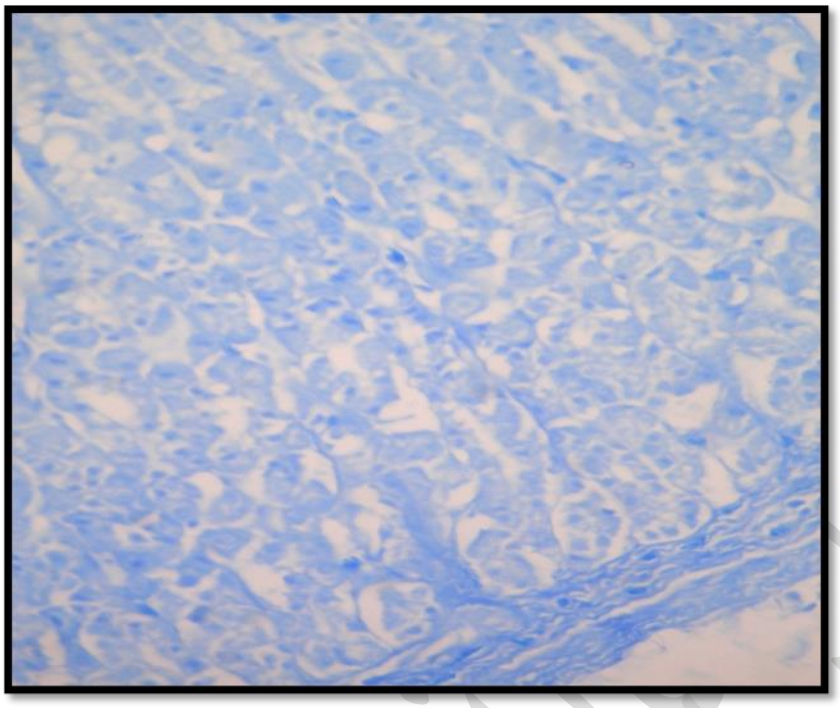


**Fig. (57):** Photomicrograph of ulcerated stomach section from a rat receiving distilled water for 4 weeks showing, decrease in protein content in the cytoplasm and nucleus of the gastric cells. (Bromophenol blue; x400).

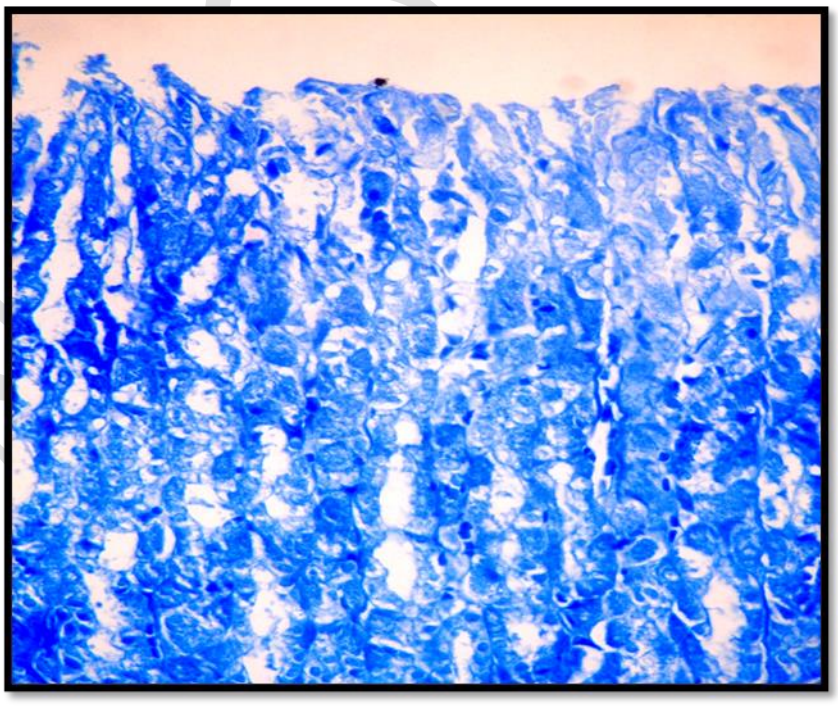


**Fig. (58):** Photomicrograph of ulcerated stomach section from a rat receiving omeprazole for 4 weeks showing, slightly decrease in protein content and heavily stain in regeneration area (arrow). (Bromophenol blue; x400).

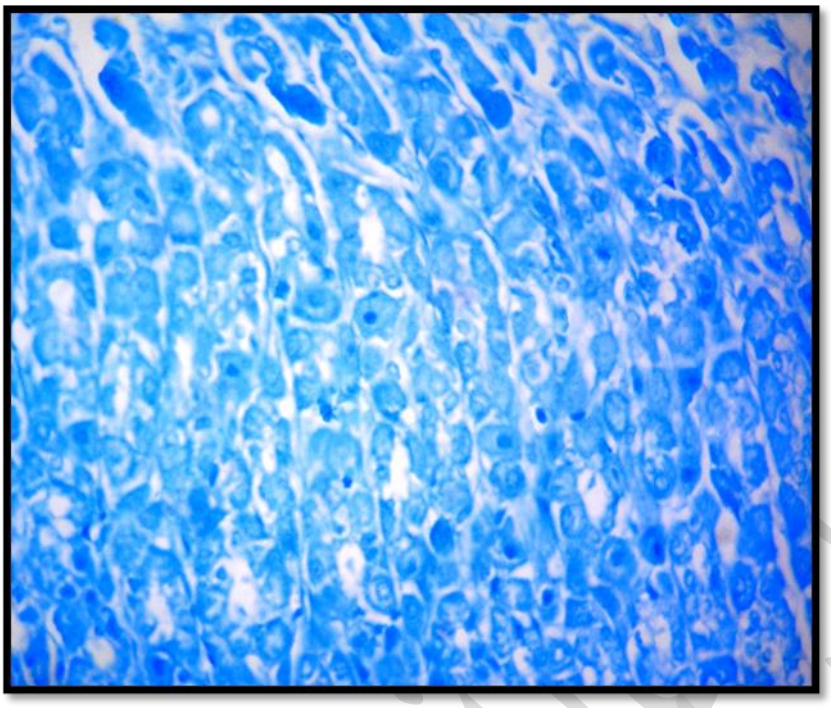


**Fig. (59):** Photomicrograph of ulcerated stomach section from a rat receiving indole-3-carbinol for 4 weeks showing, moderate reactivity of bromophenol blue in many gastric cells.
(Bromophenol blue; x400).

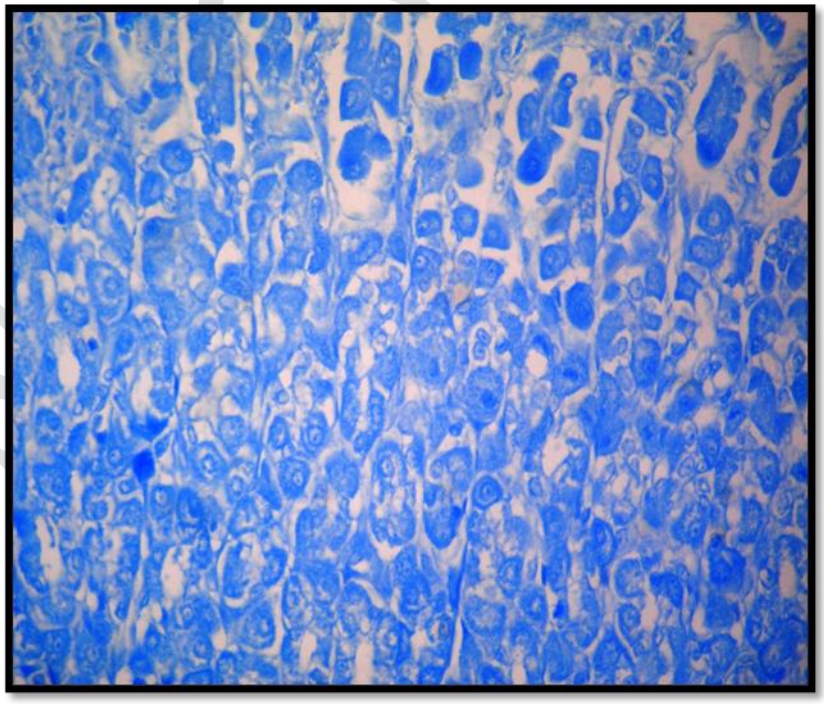


**Fig. (60):** Photomicrograph of ulcerated stomach section from a rat receiving omeprazole plus indole-3-carbinol for 4 weeks showing, slight decrease in protein content limited to the several cells while the majority of gastric tissue appeared near to normal pattern.
(Bromophenol blue; x400).

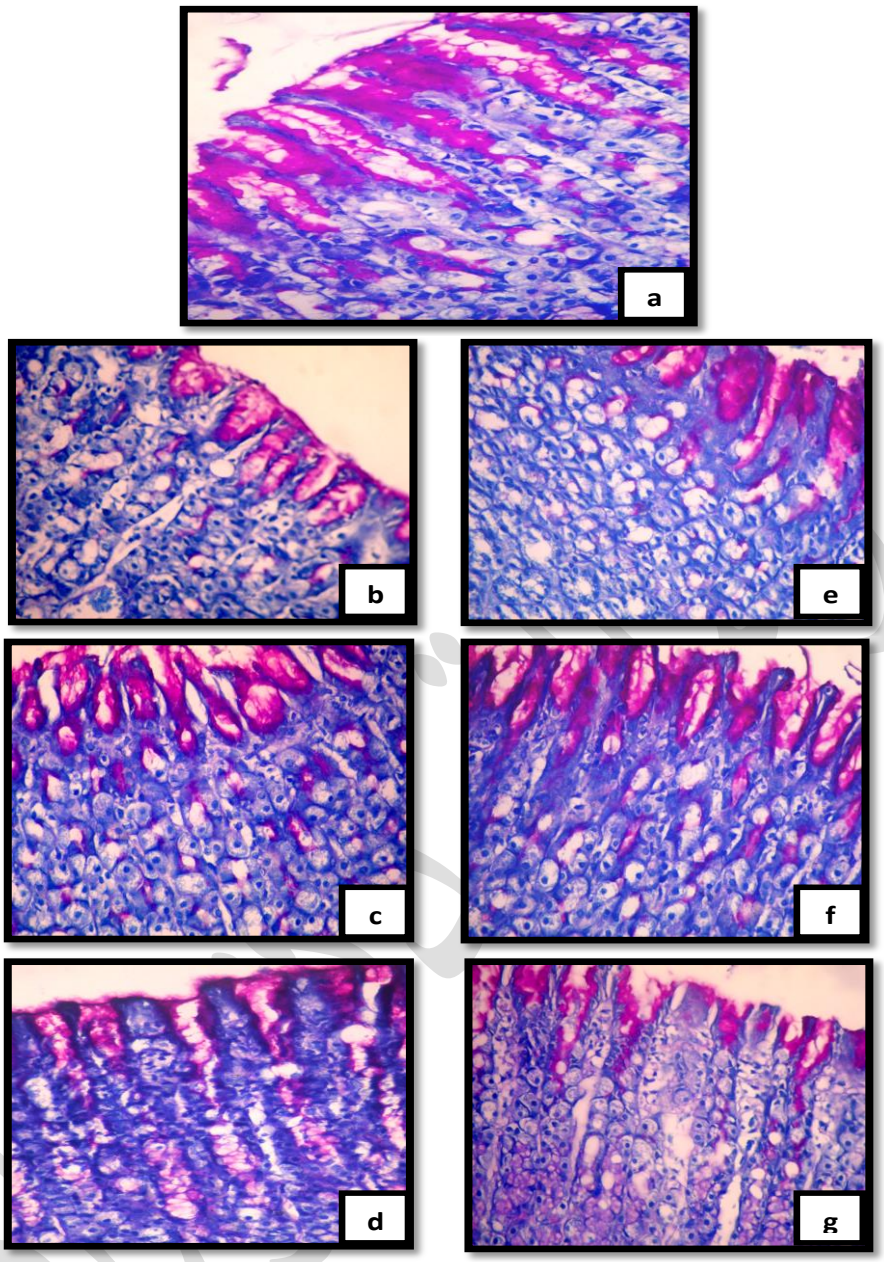



**Fig. (61):** Photomicrograph of stomach section from:

**(a):**Normal control rat showing normal distribution of mucin granules in gastric tissue.

**(b-g):**Control rat after administration of OMP and/or I3C showing, distribution of mucin granules near to normal pattern.

**(b):** OMP group after 7 days.
**(c):** I3C group after 7 days.
**(d):** OMP+I3C group after 7 days.
**(e):** OMP group after 4 weeks.
**(f):** I3C group after 4 weeks.
**(g):** OMP+I3C group after 4 weeks.

(Alcian blue-P.A.S.; x400).

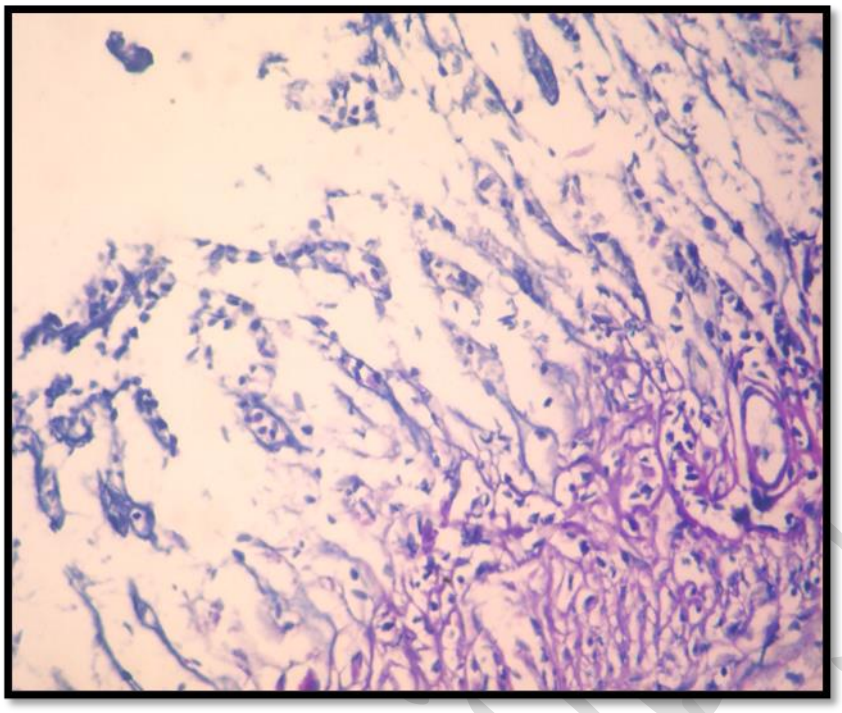


**Fig. (62):** Photomicrograph of stomach section from treated a rat receiving aspirin for 7 days showing, a marked decrease in mucin granules in the gastric cells and degenerated areas.
(Alcian blue-P.A.S.; x400).

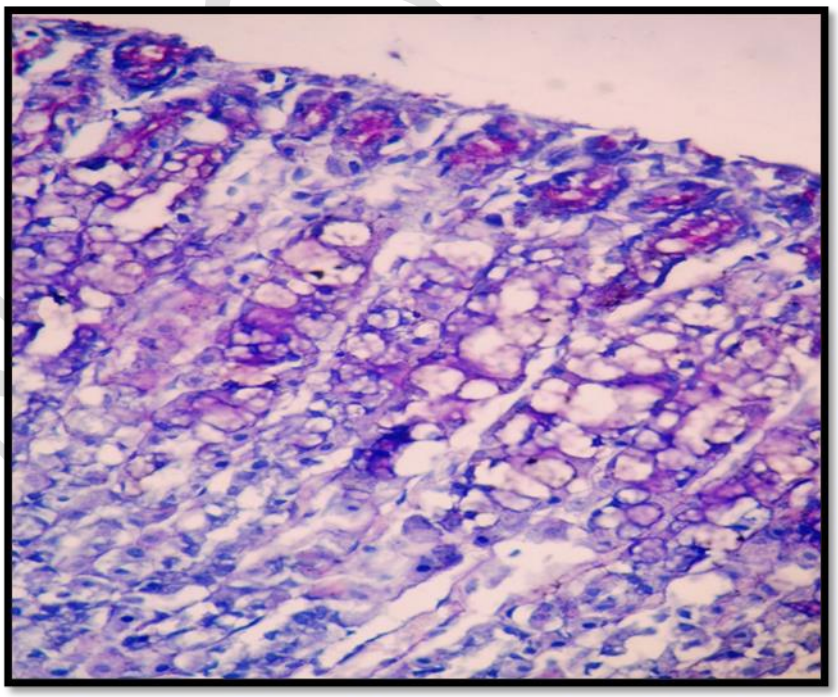


**Fig. (63):** Photomicrograph of stomach section from treated a rat receiving aspirin plus omeprazole for 7 days showing, decrease in mucin granules in the gastric tissue.
(Alcian blue-P.A.S.; x400).

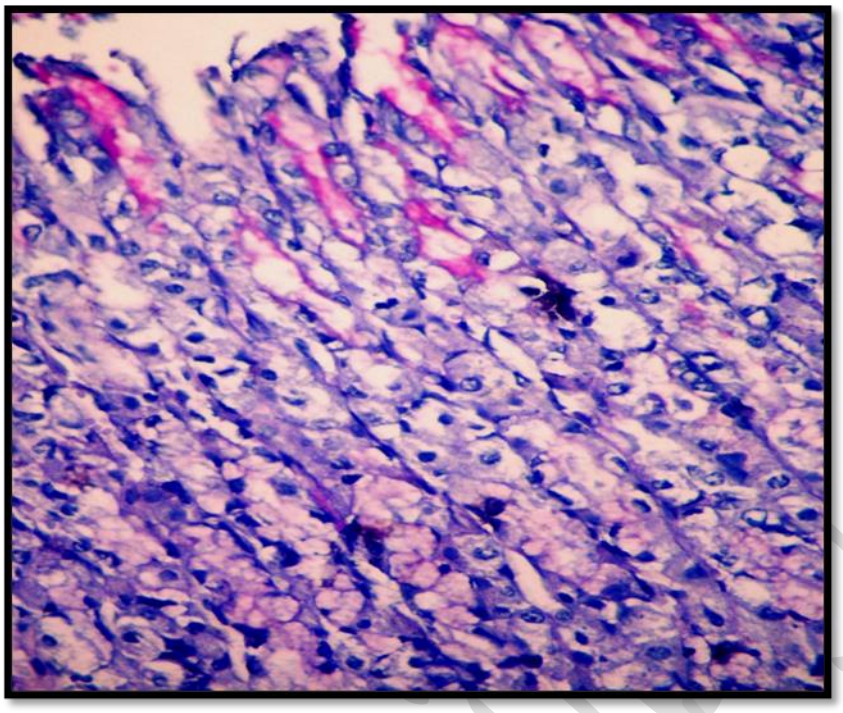


**Fig. (64):** Photomicrograph of stomach section from treated a rat receiving aspirin plus indole-3-carbinol for 7 days showing, a mild decrease in mucin granules in the stomach cells.
(Alcian blue-P.A.S.; x400).

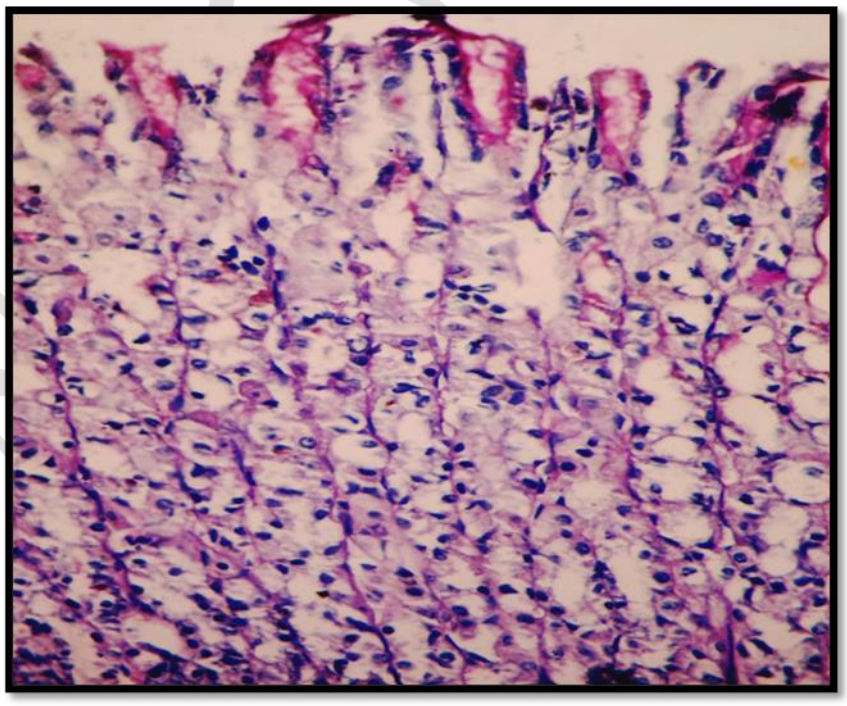


**Fig. (65):** Photomicrograph of stomach section from treated a rat receiving aspirin plus omeprazole plus indole-3-carbinol for 7 days showing, mucin positive granules in the gastric tissue.
(Alcian blue-P.A.S.; x400).

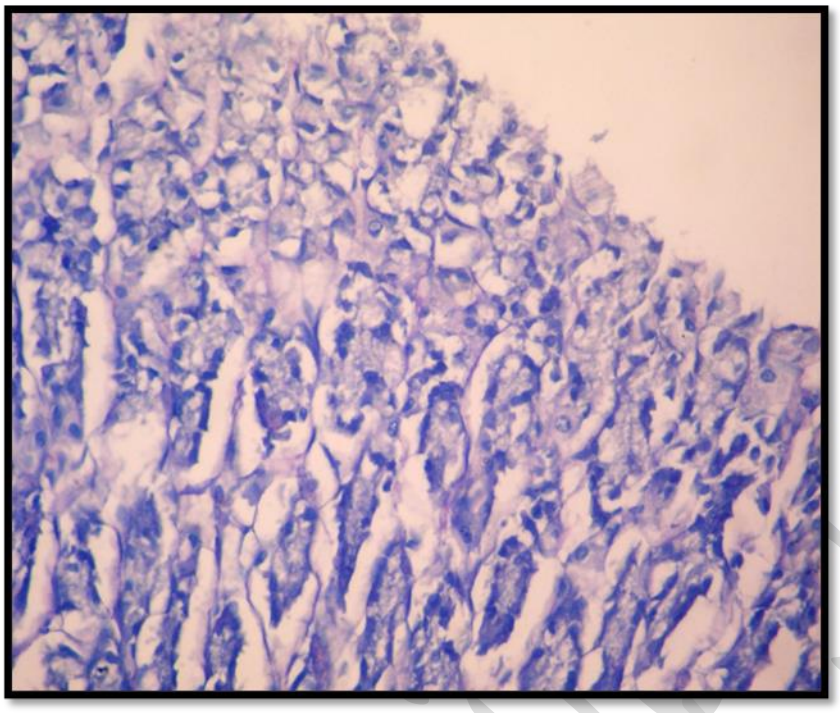


**Fig. (66):** Photomicrograph of ulcerated stomach section from a rat receiving distilled water for 4 weeks showing, a severe decrease in mucin granules in the gastric tissue.
(Alcian blue-P.A.S.; x400).

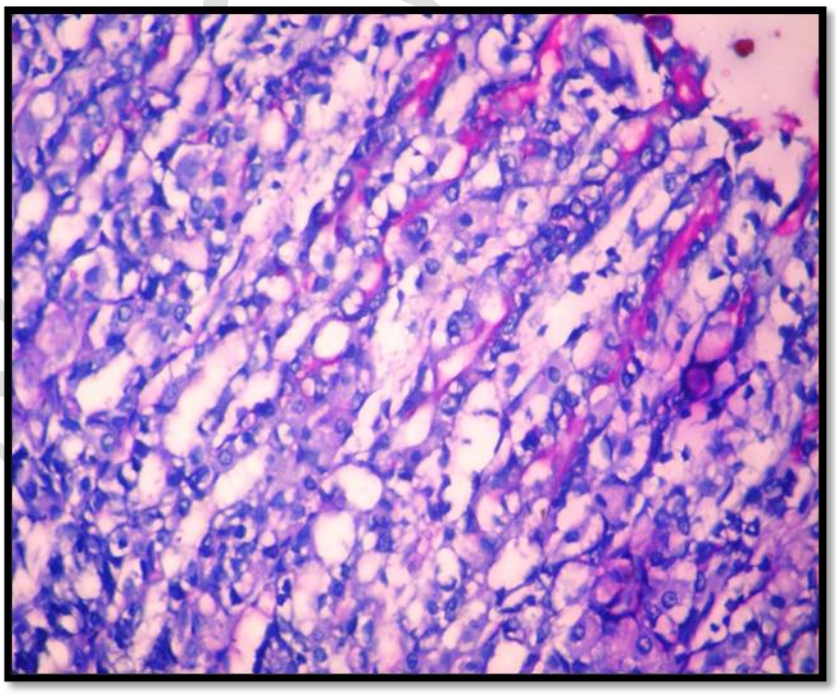


**Fig. (67):** Photomicrograph of ulcerated stomach section from a rat receiving omeprazole for 4 weeks showing, decreased in mucin granules in the gastric cells.
(Alcian blue-P.A.S.; x400).

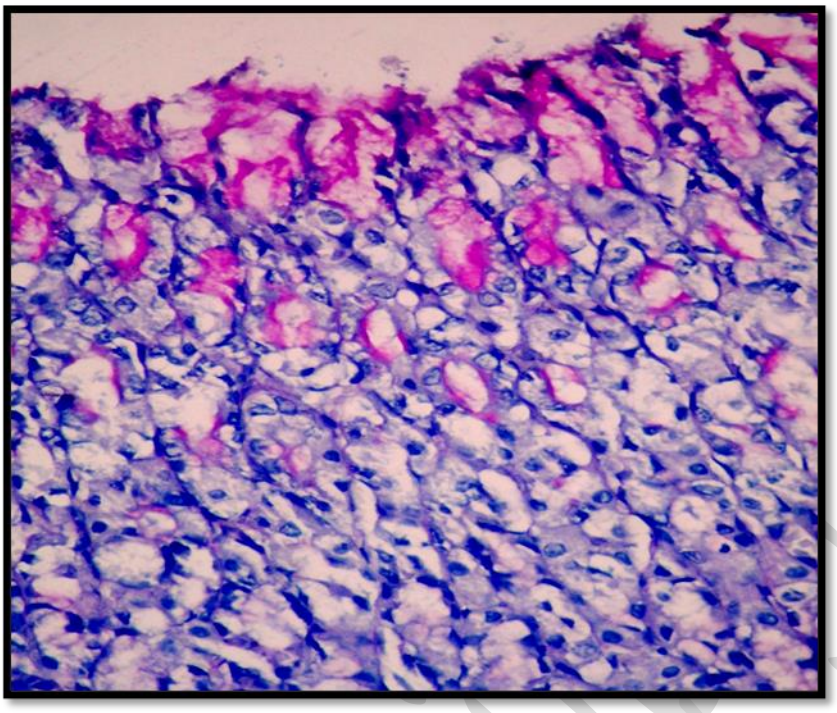


**Fig. (68):** Photomicrograph of ulcerated stomach section from a rat receiving indole-3-carbinol for 4 weeks showing, mild diminution in mucin granules in stomach cells and mucin granules scattered in the regenerative cells (arrows).
(Alcian blue-P.A.S.; x400).

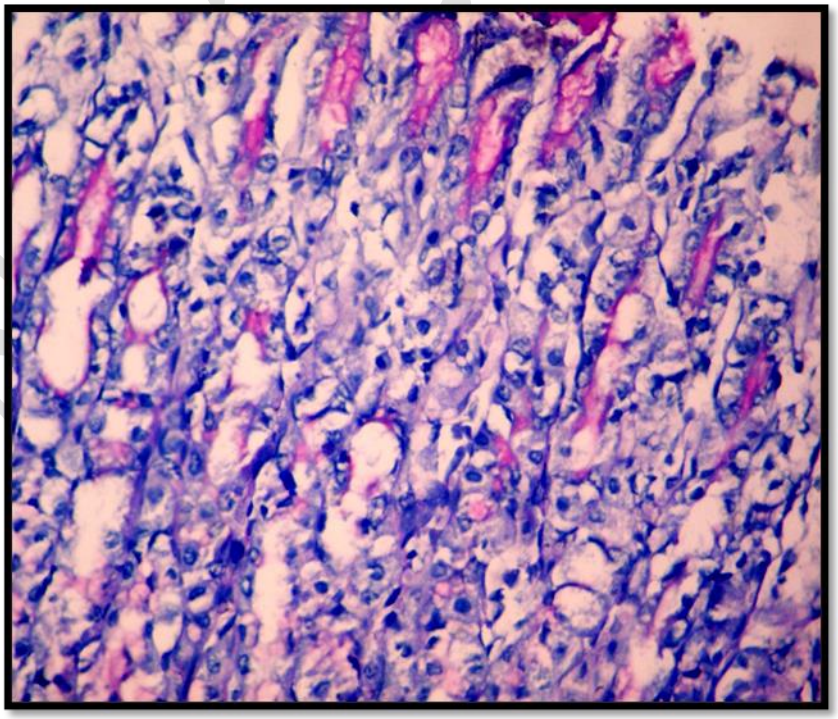


**Fig. (69):** Photomicrograph of ulcerated stomach section from a rat receiving omeprazole plus indole-3-carbinol for 4 weeks showing, a few mucin granules scattered in the gastric tissue.
(Alcian blue-P.A.S.; x400).

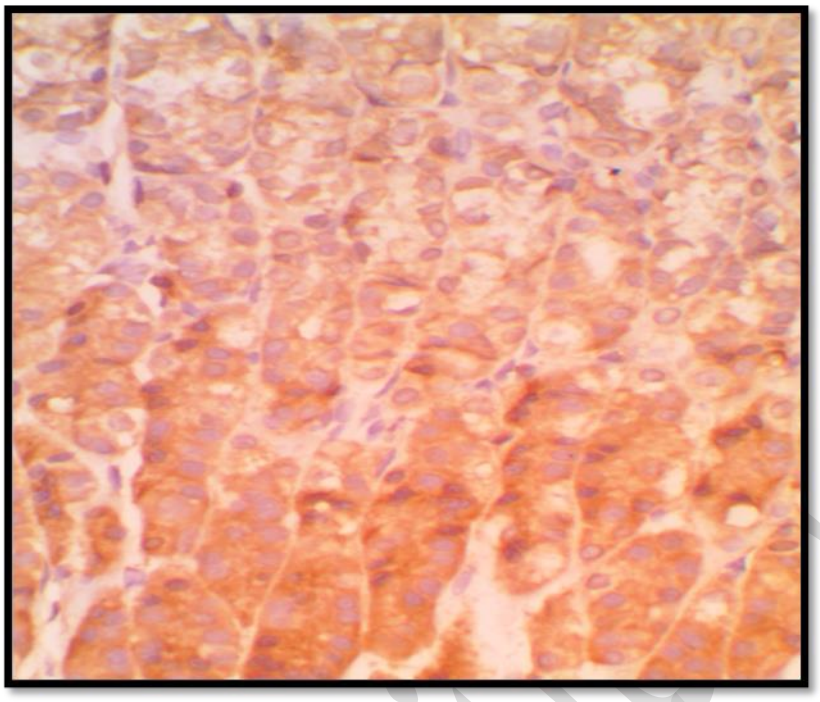


**Fig.(70):** Photomicrograph of stomach section from normal control rat showing, homogeneously colored light pink stain nuclei.

(PCNA; x400).

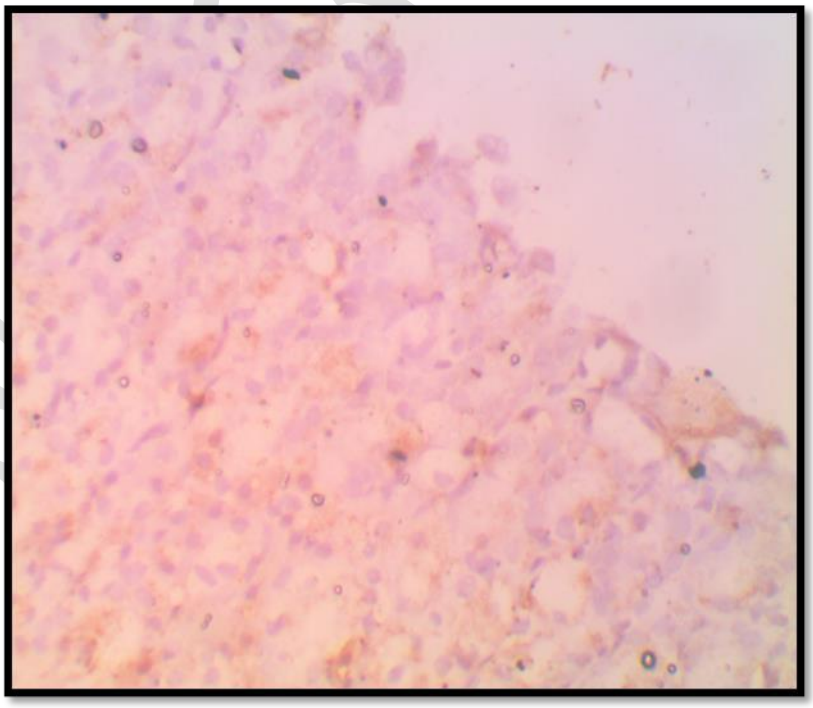


**Fig. (71):** Photomicrograph of stomach section from treated a rat receiving aspirin for 7 days showing, severe inhibition of PCNA antigen in gastric tissue.

(PCNA; x400).

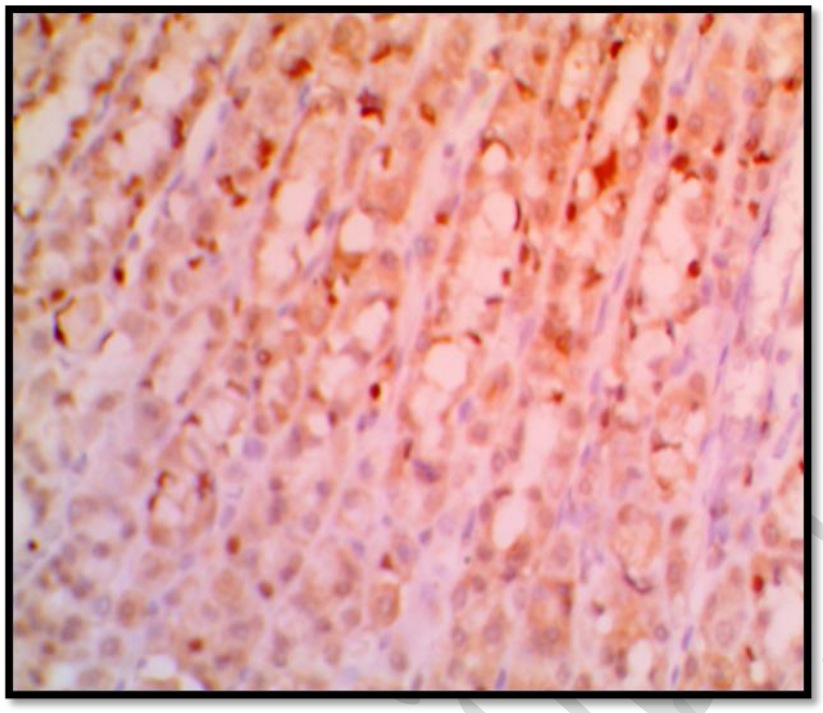


**Fig. (72):** Photomicrograph of stomach section from treated a rat receiving aspirin plus omeprazole for 7 days showing, inhibition of PCNA antigen in gastric mucosa.
(PCNA; x400).

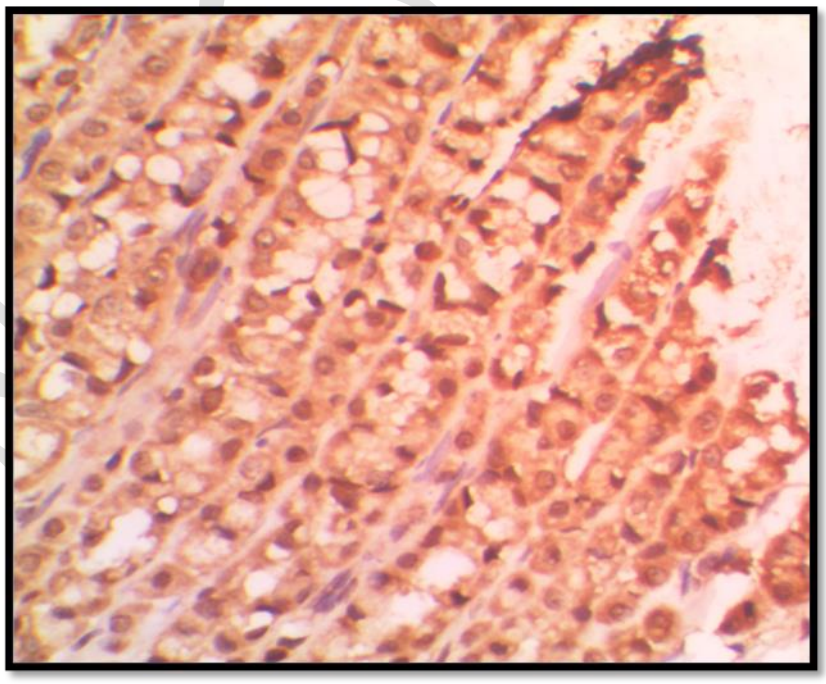


**Fig. (73):** Photomicrograph of stomach section from treated a rat receiving aspirin plus indole-3-carbinol for 7 days showing, a great number of dusky stains of immunohistochemistry of proliferating cell nuclear antigen.
(PCNA; x400).

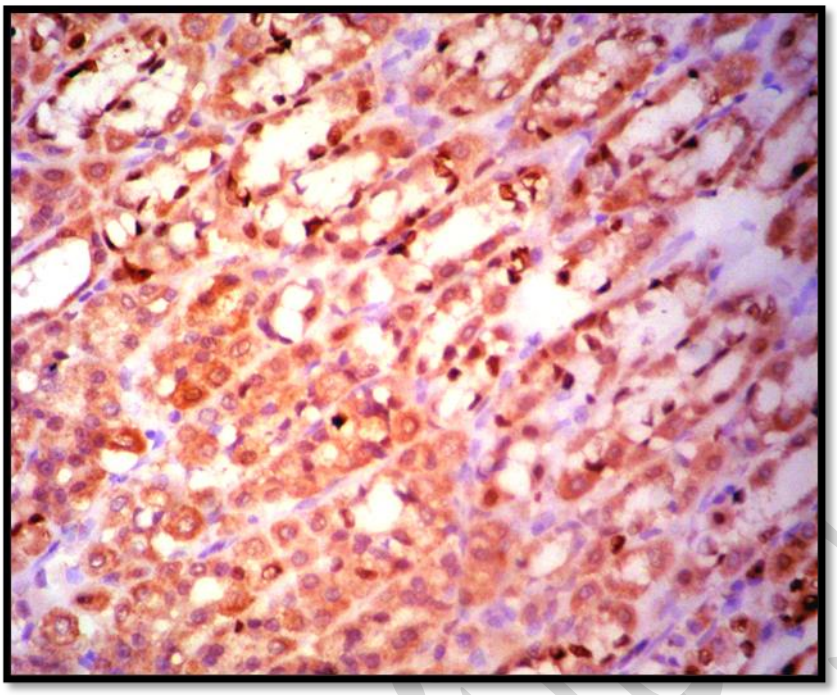


**Fig. (74):** Photomicrograph of stomach section from treated a rat receiving aspirin plus omeprazole plus indole-3-carbinol for 7 days showing, a mild decrease activity of the PCNA.
(PCNA; x400).

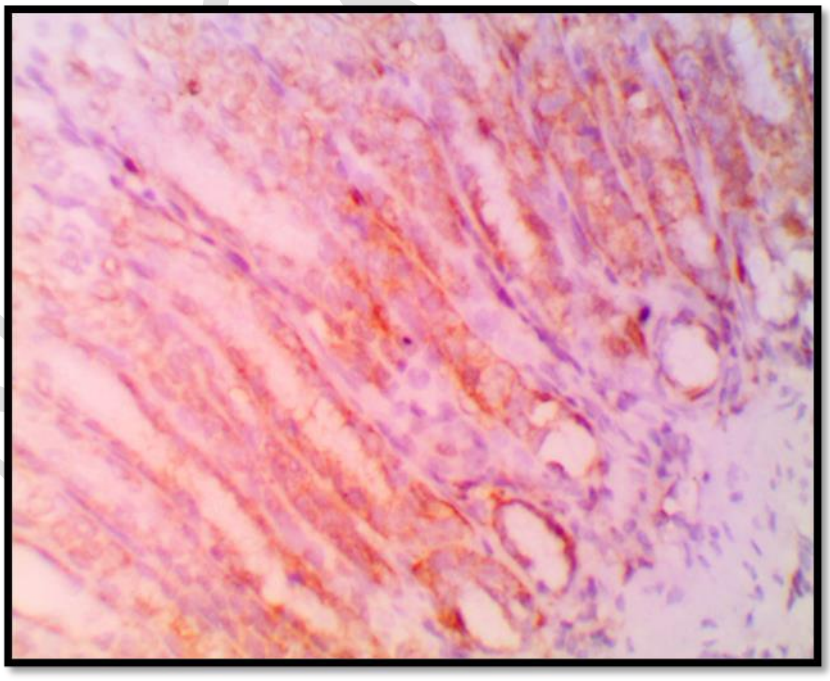


**Fig. (75):** Photomicrograph of ulcerated stomach section from a rat receiving distilled water for 4 weeks showing, decrease activity of the PCNA (+ve).
(PCNA; x400).

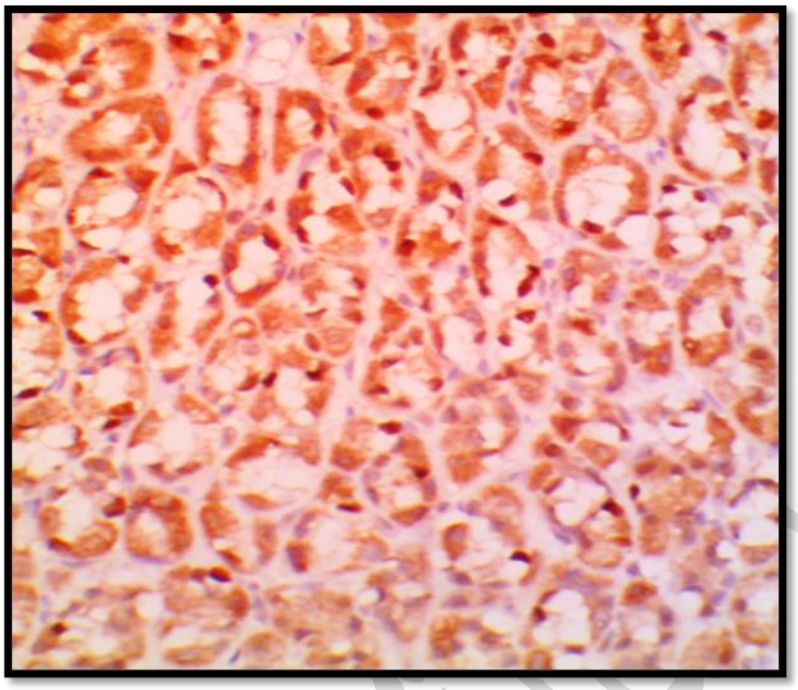


**Fig. (76):** Photomicrograph of ulcerated stomach section from a rat receiving omeprazole for 4 weeks showing, higher levels of widespread PCNA (+ve) cells.
(PCNA; x400).

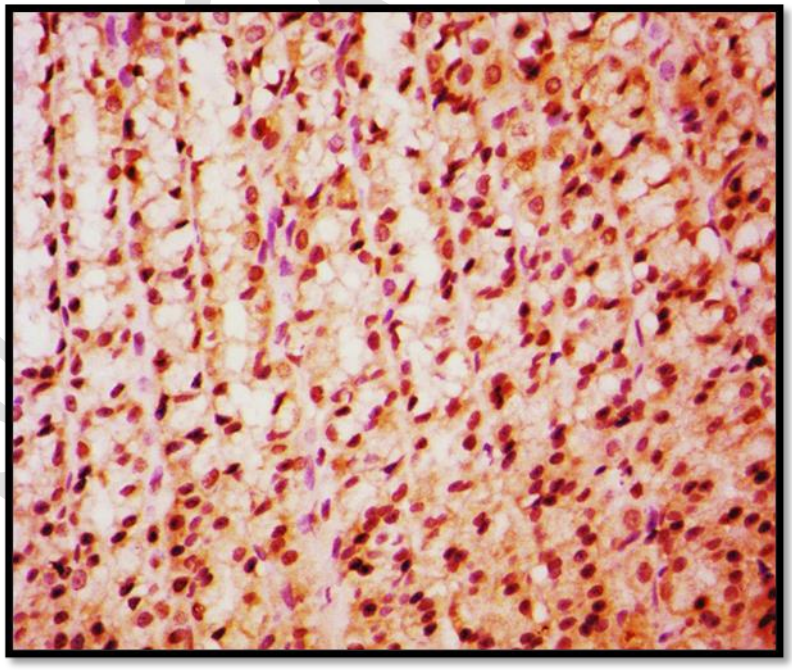


**Fig. (77):** Photomicrograph of ulcerated stomach section from a rat receiving indole-3-carbinol for 4 weeks showing, PCNA (+ve) cells increased in the gastric tissue.
(PCNA; x400).

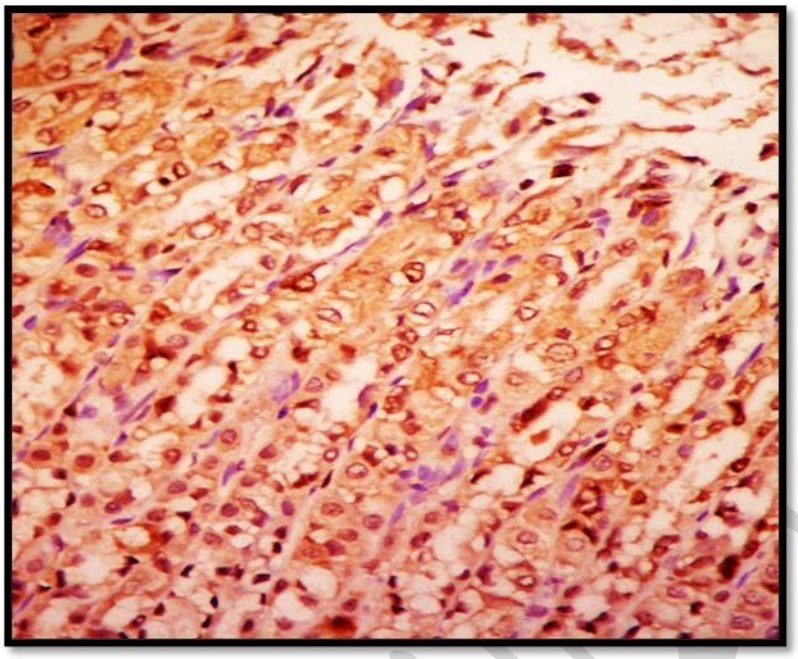


**Fig. (78):** Photomicrograph of ulcerated stomach section from a rat receiving omeprazole plus indole-3-carbinol for 4 weeks showing, increased activity of the PCNA (+ve) stainable materials manifesting as deeply stained pink, dispersed deeply intense nuclei scattered all over the gastric tissue.

(PCNA; x400).

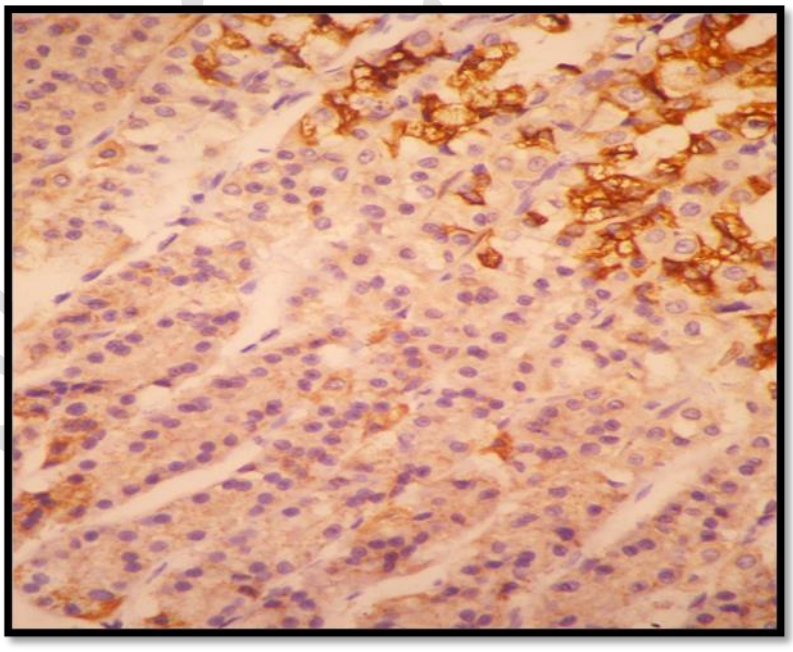


**Fig. (79):** Photomicrograph of stomach section from normal control rat showing, normal activity of accumulation of COX-2 in the form of deeply intense brownish granules in the cytoplasm of the gastric tissue.

(COX-2; x400).

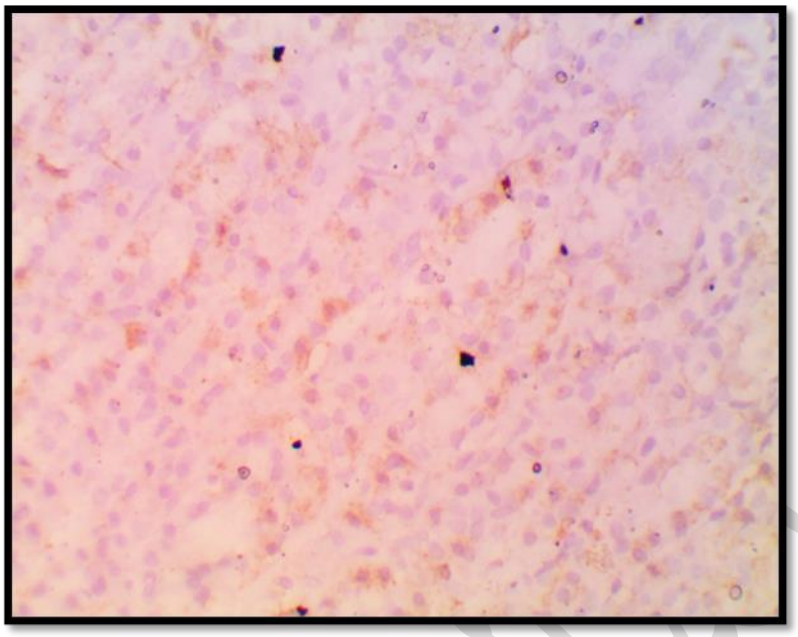


**Fig. (80):** Photomicrograph of stomach section from treated a rat receiving aspirin for 7 days showing, decreased activity of COX-2 in stomach tissue.
(COX-2; x400).

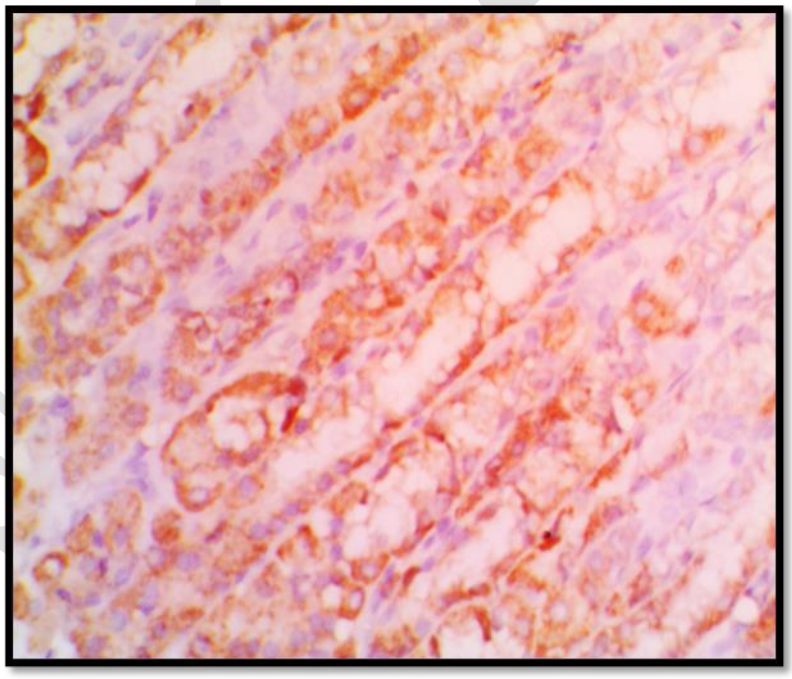


**Fig. (81):** Photomicrograph of stomach section from treated a rat receiving aspirin plus omeprazole for 7 days showing, inhibition activity of COX-2 in the cytoplasm of the gastric tissue.
(COX-2; x400).

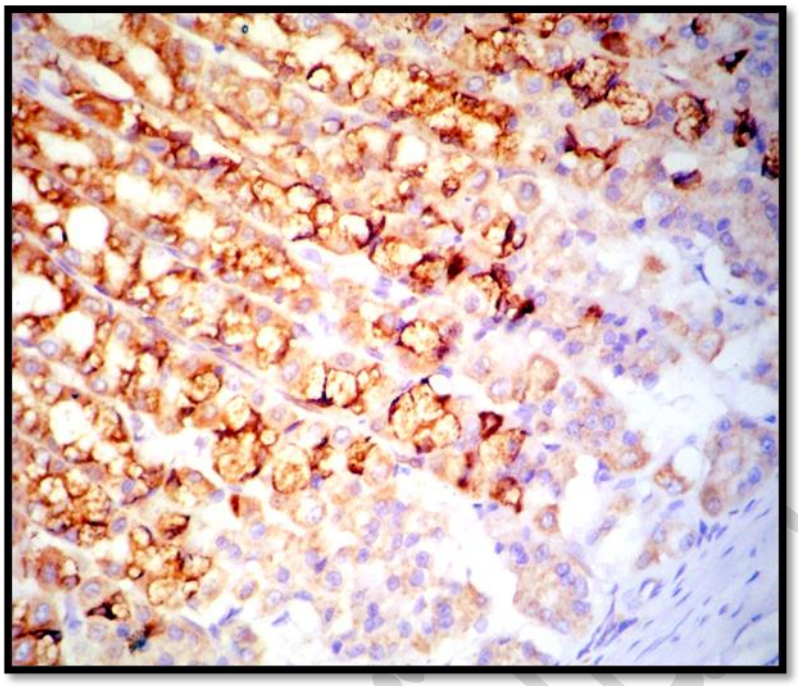


**Fig. (82):** Photomicrograph of stomach section from treated a rat receiving aspirin plus indole-3-carbinol for 7 days showing, increased activity of the COX-2 in the gastric cells.
(COX-2; x400).

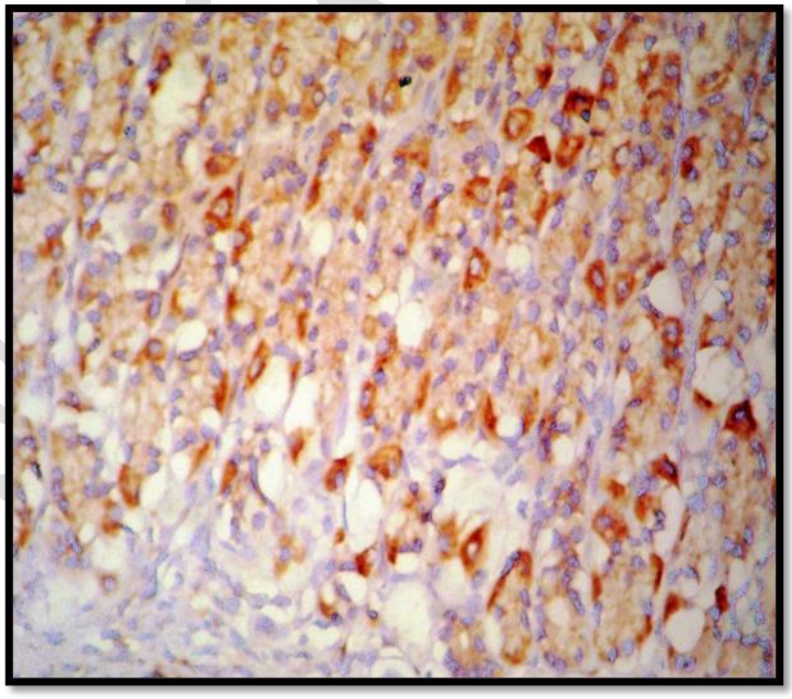


**Fig. (83):** Photomicrograph of stomach section from treated a rat receiving aspirin plus omeprazole plus indole-3-carbinol for 7 days showing, increased activity of the COX-2. Stainable materials appeared as randomly dispersed intense brownish-red granules scattered all over the gastric tissue.
(COX-2; x400).

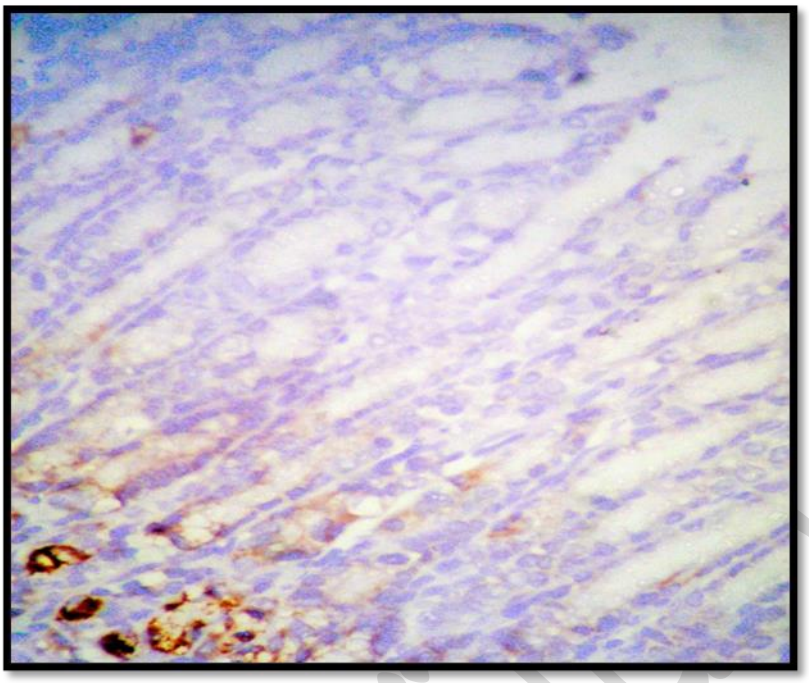


**Fig. (84):** Photomicrograph of ulcerated stomach section from a rat receiving distilled water after 4 weeks showing an extreme decrease in the activity of the COX-2 antigen.

(COX-2; x400).

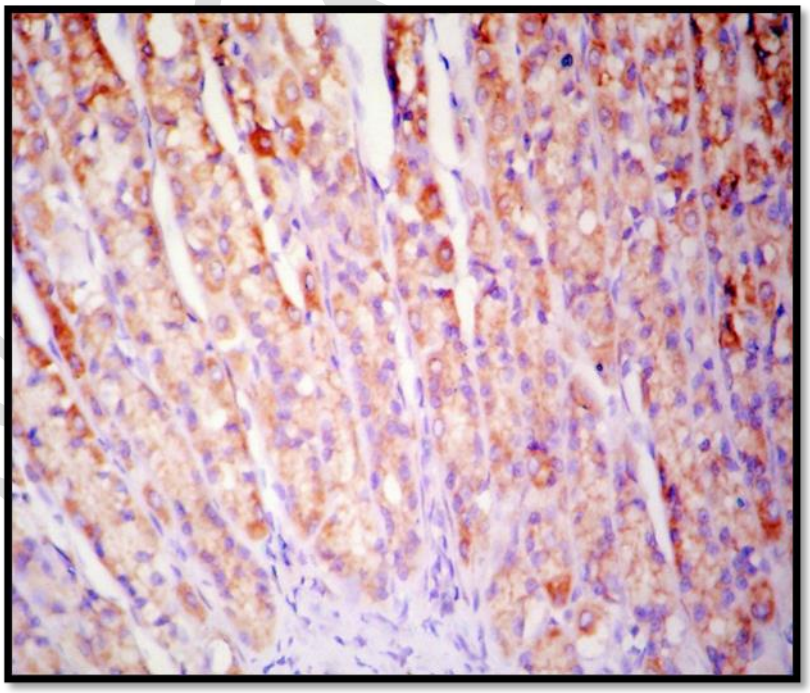


**Fig. (85):** Photomicrograph of ulcerated stomach section from a rat receiving omeprazole for 4 weeks showing, mild inhibition of the activity of the immunohistochemistry of cyclooxygenase-2 antigen in the gastric cells.

(COX-2; x400).

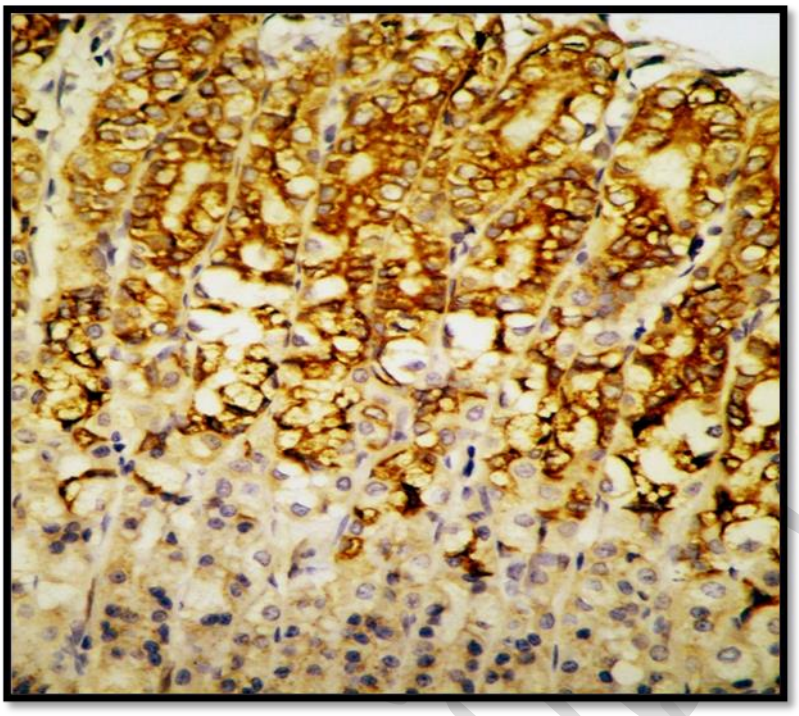


**Fig. (86):** Photomicrograph of ulcerated stomach section from a rat receiving indole-3-carbinol for 4 weeks showing, increased activity of the cyclooxgenase-2 antigen.
(COX-2; x400).

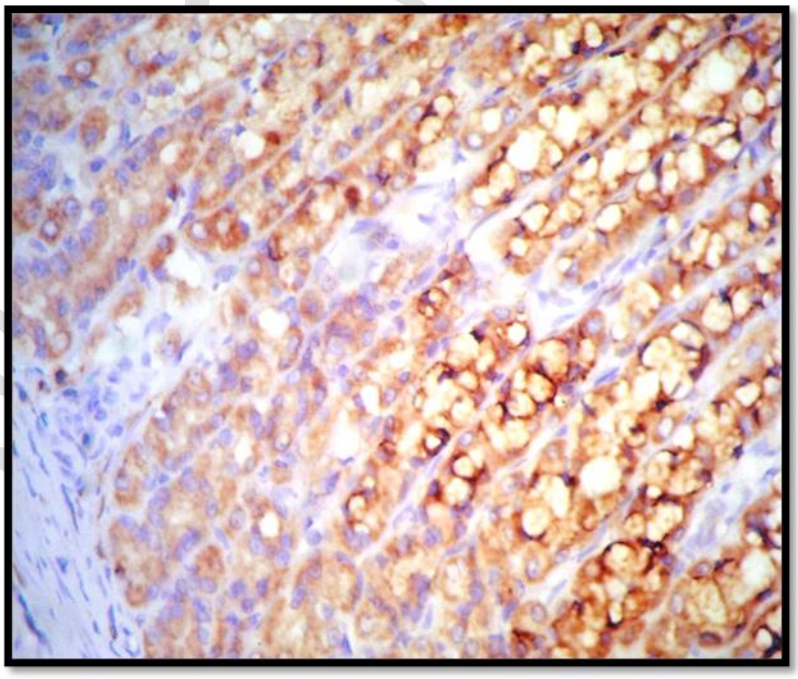


**Fig. (87):** Photomicrograph of ulcerated stomach section from a rat receiving omeprazole plus indole-3-carbinol for 4 weeks showing, increase activity of the COX-2 antigen.
(COX-2; x400).

Table (19): PCNA And COX-2 Expression In Control And Experimental Groups (%).

| Groups / Cells | | | Normal control | Experimental groups for seven days | | | | Experimental groups for four weeks | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | ASA | ASA +OMP | ASA +I3C | ASA+OMP +I3C | U | U+OMP | U+I3C | U+OMP +I3C |
| Peptic cells | | PCNA | + | -ve | ++ | ++++ | +++ | + | +++ | ++++ | +++ |
| | | COX-2 | + | -ve | ++ | ++++ | +++ | + | ++ | ++++ | +++ |
| Oxyntic cells | | PCNA | + | -ve | +++ | ++++ | +++ | + | +++ | ++++ | +++ |
| | | COX-2 | + | -ve | ++ | ++++ | +++ | + | +++ | ++++ | +++ |
| Gland cells | Peptic cells | PCNA | + | -ve | ++ | ++++ | +++ | -ve | ++ | +++ | ++ |
| | | COX-2 | + | -ve | ++ | +++ | +++ | -ve | ++ | +++ | ++ |
| | Oxyntic cells | PCNA | + | -ve | ++ | +++ | ++ | -ve | ++ | +++ | ++ |
| | | COX-2 | + | -ve | ++ | ++++ | +++ | -ve | ++ | +++ | ++ |

- positivity cells:- ( + ) = between 1% and 10%, ( ++ ) = between 11% and 30%, ( +++ ) =between 31% and 50% and (++++)= 51% or more.
- Negativity cells :- ( -ve )= no staining .
- OMP: Omeprazole …. I3C: Indol-3-carbenol ….. ASA: Aspirin.
- U=Ulcer.

Fig. (88): PCNA Expression Of Both Control And Experimental Groups.

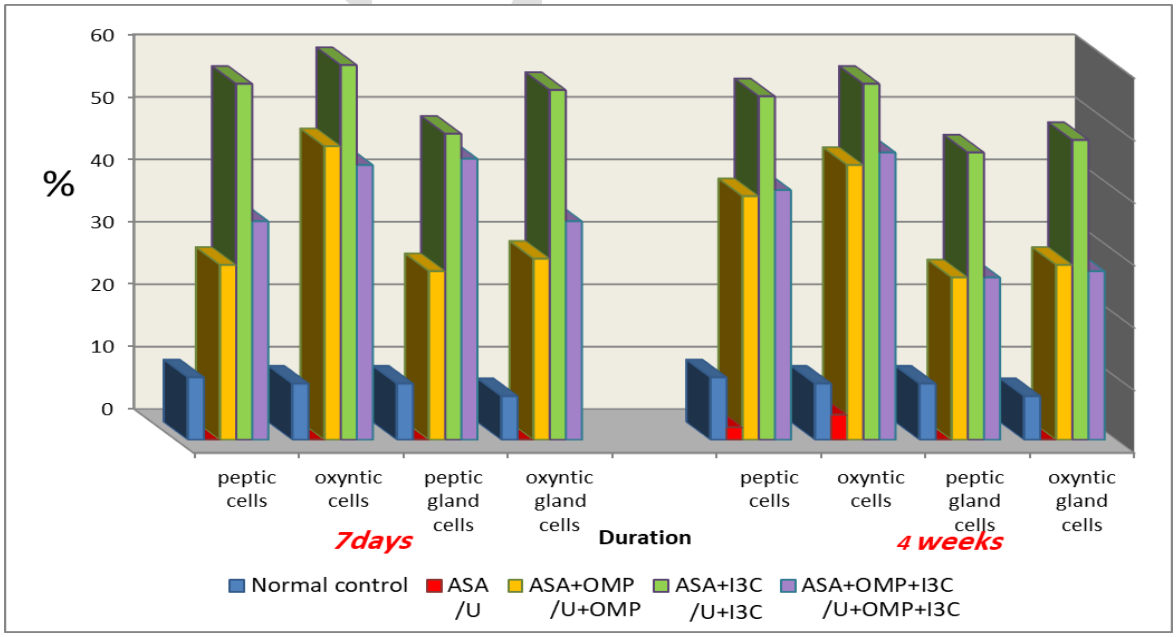
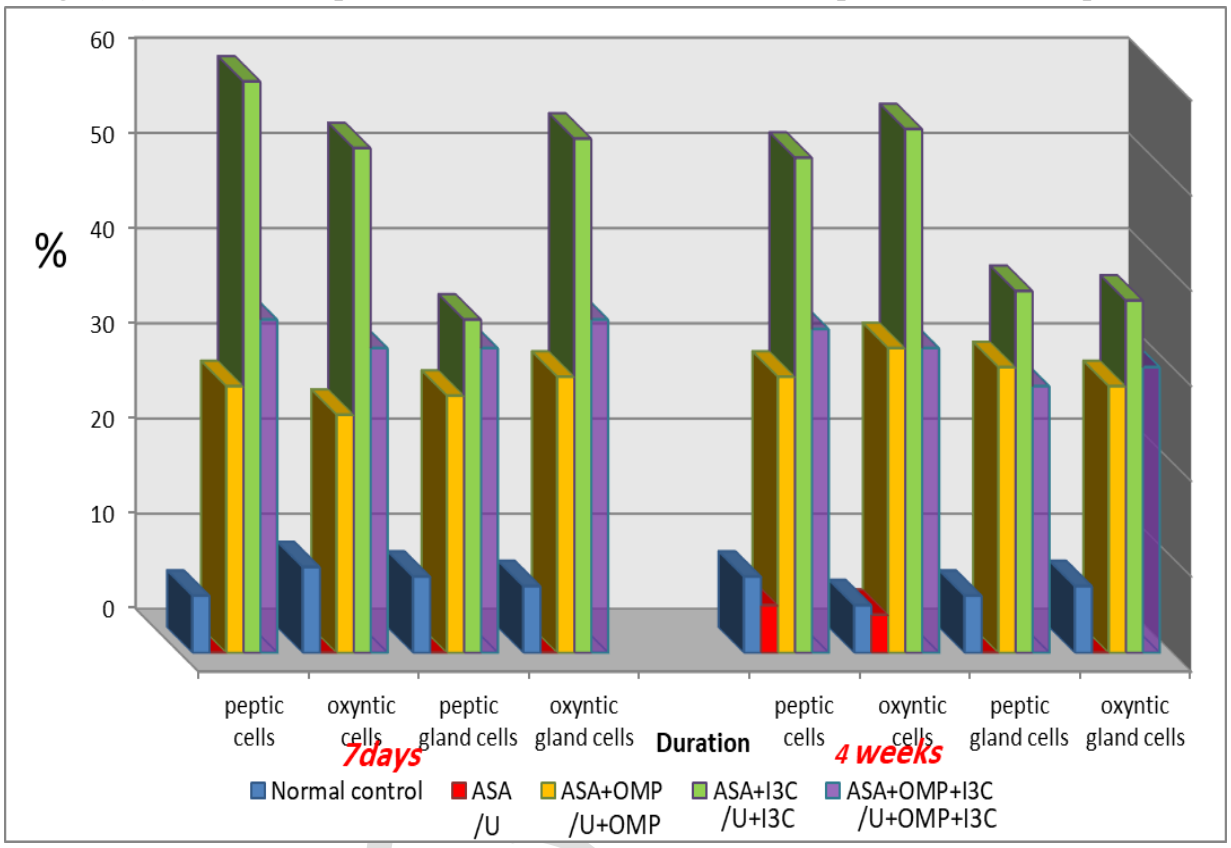


Fig. (89): COX-2 Expression Of Both Control And Experimental Groups.



# DISCUSSION

In a normal individual, the gastric mucosal layer is well protected against the corrosive effect of gastric acidity by a cytoprotective process. When this process is disturbed by any means, the gastric mucosa will be disrupted leading to an ulcer formation which if not properly treated will lead to chronic bleeding ***(Salim, 2009)***.

Gastric ulcer is a major drawback in modern days due to the unpredictable side effects of the grain long-term uses of commercially available drugs. ***(Debashis et al., 2002 and Al-dalain et al., 2008)***, the treatment of this painful disease and its prevention has become one of the challenging problems.

There are several factors that may induce ulcer in human beings, such as stress, chronic use of anti-inflammatory drugs and continuous alcohol ingestion, among others. It is accepted that it is result of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism. There are many products in the pharmacy for the treatment of gastric ulcer, including antacids, proton pump inhibitors, anticholinergics and histamine $H_2$-antagonists. Most of these drugs produce several adverse reactions ***(Khatib et al., 2010)***.

Aspirin, one of the widely used non-steroidal anti-inflammatory drugs (NSAIDs), is probably one of the most highly consumed pharmaceutical products in the world. It has gained greater importance not only as analgesic but also as a cardio-protective drug. However, the use of aspirin is also associated with significant morbidity and mortality due to its adverse effects on multiple organ systems ***(Matzke, 1996)***.

Previous studies showed that aspirin (ASA) as one of the commonly used NSAIDs induced gastric ulcer and delayed its healing ***(Hawkey, 2000)***. ASA damages the gastrointestinal tract by at least two mechanisms which are the suppression of prostaglandin synthesis through cyclooxigenase (COX) inhibition and direct irritant action causing alteration of mucosal permeability ***(Szabo et al., 1992)***. These mechanisms leads to oxidative tissue injury which seems to play a major role in the pathogenesis of NSAIDs induced damage ***(Forani et al., 2011)***. Proton pump inhibitors including omeprazole (OMP) have proven to be effective in promoting the healing of NSAIDs induced ulcers ***(Scheiman et al., 2006)***.

But most of the commonly used pharmaceutical treatments of gastric ulcer have some rational side effects or the incidence of returning the ulcer after treatment discontinuation ***(Szabo and Vincze, 2000)*** there must be a need for some bioactive natural alternatives combined with the perused treatments to subside the adverse effects of NSAIDs.

The world health organization in 1980 has recommended the evaluation of the effectiveness of the plants in conditions where there is lack of safe synthetic drugs ***(Upadhayay and Pandey, 1984)***. Thus, it is a need for more effective, less toxic and less expensive antiulcer agents ***(Foster et al., 1993)***.

Medicinal plants are amongst the most attractive sources of new therapeutic agents, and have been shown to give promising results in the treatment of gastric ulcers ***(Borrelli and Izzo, 2000)***.

Many research have proved that antioxidants may play an important role not only by protecting against gastric mucosal injury, but also by inhibiting progression of gastric ulcer ***(Jainu and Devi, 2004b)***.

Like other vegetables, cruciferous vegetables contain a number of components with cancer chemopreventative properties, including folate, fiber, carotenoids and minerals. However, cruciferous vegetables are unique in that they are rich sources of glucosinolates that may play a significant role in the association between cruciferous vegetable consumption reduced cancer rates. Each cruciferous vegetable contains a mixture of glucosinolates ***(Saati, 2009 and Wang et al., 2012)***.

For the above mentioned reasons, this work was conducted to evaluate the gastric ulcer induced by aspirin administration on some biochemical, hematological histological, histochemical and immunohistochemical changes in serum and stomach tissue. This is in addition of studying the ameliorative effect of interaction of omeprazole (OMP) as anti-ulcer and indole-3-carbinol (I3C) as antioxidant in either alone or in combination with omeprazole on the above mentioned parameters in aspirin induced ulcerated rat model.

In the present investigation rat was bused as an animal model for the induction of gastric ulcer by oral administration of aspirin (ASA) at a dosage of 500 mg/Kg/body weight for seven consecutive days. This in turn led to a significant increase in the acidic activity, ulcer index and percentage of ulceration with a decrease in pH value in ASA ulcerated rats. Macroscopic examination of the internal dissected stomach features revealed severe red coloration, with haemorrhagic streaks in the dissected stomach of ASA treated rats was also observed.

In general, the formation of gastric mucosal lesions may be due to the reduction of gastric blood flow, which results in a rapid decrease in the pH within the mucoid cap, causing the formation of haemorrhagic erosions ***(Wallace, 2005)***. This contributes to the development of necrosis, hemorrhage, and to the solubilization of mucus constituents in stomach. In addition, to the increased pepsin, acid secretion and flux of $Na^+$ and $K^+$ ***(Szabo, 1987)***. This is accompanied with decreased mucin activity by means of a decrease in histamine and back diffusion of $H^+$ ions which produced auto digestion of the gastric mucosa and breakdown of the gastric mucosal barrier ***(Jaikumar et al., 2010)***.

Prostaglandin E2 (PGE2) is one of the major protective factors in gastric tissue which inhibits gastric acid secretion. PGE2 is significantly declined with aspirin treatment. Thus, PGE2 might indirectly take part in ulcer relapse via acid secretion ***(Wang et al., 2007)***.

Moreover, Cyclooxygenase (COX), which exists in three isoforms cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and cyclooxygenase-3 (COX-3) is the key enzyme for synthesis of prostaglandins. Administration of aspirin was accompanied by the

suppression of COX-1 and COX-2 activity in the gastric mucosa so aspirin, is recognized an irreversible COX inhibitor that decreases prostaglandin synthesis ***(Brzozowski et al., 2001a)***. This may be due to a plethora of factors involved in ulcerogenesis by aspirin. Aspirin has been reported to produce ulcers both by local and systemic effects. Aspirin has direct irritant effect by increasing the $H^+$ ion transport.

On the mucosal epithelial factors, it decreases mucin surface-active phospholipids bicarbonate secretion mucosal proliferation and on the microvasculature produced damage by formation of free radicals ***(Sairam et al., 2003)***. These results were in accordance with the results of the immunohistochemical stain of COX-2 in the present study in ASA ulcerated rats which supports the suppression of COX-2 activity in ASA ulcerated gastric mucosa. This may be related to the inhibition of prostaglandin synthesis that probably weakened the function of the gastric mucosal defense ***(Wallace, 2005)***.

It is worth mentioning that NSAIDs including ASA have been categorized into cyclooxygenase-1 or cyclooxygenase-2 inhibitors which have antiproliferative and antiangiogenic activity according to their ability to inhibit these isoenzymes selectively ***(Furst, 1999)***.

Also, ASA may damage the gasterointestinal mucosa causing lesions ranging from trivial petechiae and superficial erosions to potentially serious deep peptic ulcers that will lead to gastric distress and gasterointestinal blood loss ***(Sener et al., 2001)***. Macroscopic as well as histological observations of this study are in agreement with this. ASA caused gastric haemorragic lesions in the glandular mucosa, which appeared as prominent cellular damage at the light microscopic level, demonstrating true ulcer formation.

The significant increase serum total protein and albumin in ASA ulcerated rats in this study was supported by ***Jaikumar et al., (2010) and Prakash and Gunasekaran, (2010)*** who stated that ASA causes leakage of plasma protein from the gastric tissue into the serum as a result of the damage to the gastric mucosa. This reflects the increased protein concentration in aspirin treated rats**.**

***Weberg et al., (1990)*** stated that the level of protein was significantly decreased in the gastric mucosal tissue. This indicated that acute stress caused the corrosion of gastric mucosa, resulting in the disruption of gastric mucosal cells. The net effect would be the loss of protein from gastric tissue leading to their leakage into the serum. Furthermore, plasma proteins largely consist of albumin and globulins such as immunoglobulins, carrier proteins, and acute phase reactants. So, elevated proteins may be due to an increase in multiple immunoglobulins due to choronic infection or stress caused by ulcer.

It has also been suggested that lipid peroxidation mediated by oxygen free radicals due to ASA administration is believed to be an important cause of destruction and damage to cell membranes. Membrane peroxidation can lead to exchanges in membrane fluidity and permeability and also to enhanced rates of cell lysis and protein degradation ***(Sant et al., 1995)***.

The results of this study demonstrated a non significant decrease in red blood cells and haematocrit in ASA ulcerated rats. ***Merchant and Modi, (2004)*** confirmed the association of anemia, reduced circulating red cell counts in mice treated with aspirin. These results may be due to the altered iron uptake from the gastrointestinal tract which was coupled with acute or chronic blood loss due to the erosion in the gastrointestinal tract induced by aspirin uptake.

***Langman et al., (1994)*** reported similar results to our study, that intravascular haemolysis may be a major contributing factor for the reduced red cell count observed. These results also support the red coloration and the blood streaks present in the macroscopic examination of the dissected stomachs of rats administrating aspirin. At the biochemical level aspirin alters cell membrane functions by altering the ion permeability across the cell wall. With these defects it is reasonable to assume that the cells may be more susceptible to intravascular haemolysis ***(Tomoda et al., 1994)***.

Contrary to that, ***Sunday et al., (2009)*** showed that ASA had no effect on HCT, Hb and R.B.Cs. On the other hand, ASA is used in the prevention of the formation of thrombosis in the myocardium coronary blood vessel by inhibiting platelet aggregation. This effect may also be one of the side effects of aspirin as preoperative aspirin administration increases blood loss during bleeding in sensitive operations ***(Douketis et al., 2008)***.

This was clearly demonstrated in the present study by the significant decline in the mean values of platelets and monocyte levels in ASA treated rats. These results were in accordance with ***(Maree et al., 2005)*** who suggested that aspirin causes inhibition platelets aggregation by irreversibly blocking the enzyme cyclooxygenase the enzyme that catalyzes conversion of arachidonic acid to thromboxane A2 (TXA2), essential for the synthesis of TXA2, a substance that causes both vasoconstriction and amplifies the platelet activation process leading to platelet aggregation.

Recently, ***Espinosa et al., (2012)*** aspirin belongs to the group of drugs that inhibit platelet activation. Platelet activation can be blocked by inhibited the thromboxane A2 (TXA2) pathway, adenosine diphosphate (ADP) pathway, thrombin pathway, and phosphodiesterase (PDE). Aspirin meets its effects by inhibiting the TXA2 pathway in a dose-dependent manner. Aspirin inhibits cycloxygenase-1 (COX-1) in such a way that only TXA2 production is inhibited. Gastrointestinal tract bleeding increase by inhibits platelet aggregation and thromboxane concentration by NSAIDs. Aspirin acetylates COX in platelets in the presystemic circulation, where there is a high concentration of aspirin in the portal vein before its metabolism by the liver.

Presently, in ulcer group there were significant increase in the W.B.Cs. Concomitant results and explanations were confirmed by ***(Neha et al., 2011)*** who suggested that aspirin administration for fifteen and thirty days caused increase in white blood cells. The increase in WBC may be due to the inhibition of prostaglandin synthesis through cyclooxygenase enzyme and enhances haematopoisis, because of prostaglandin E2 (PGE2) increases

intracellular cyclic adenosine monophosphate (AMP) levels in target cells ***(Daud et al., 2003)***. In addition to, cyclic AMP and PGE2 block neutrophils recruitment and aspirin enhanced by two fold neutrophils recruitment ***(Chignard et al., 1996)***.

Glutathione (GSH) is an important constituent of intracellular protective mechanisms against oxidative stress. The stomach is rich in glutathione where it acts its protective role in the removal of free radicals for the maintenance of mucosal integrity. Depletion of gastric glutathione by ASA produces ulceration since it is essential in protection of gastric epithelial and chief cells against oxidative stress ***(Hoppenkamps et al., 1984)***.

Moreover, GSH is an important tripeptide thiol which in addition to being the substrate for glutathione *S*-transferases (GST), maintains cellular oxidation–reduction balance and protects cells against free radical species. Thus, determination of tissue GSH levels in combination with GST activities was used to evaluate the detoxifying potential of anticarcinogens ***(Van Lieshout et al., 1997)***.

In parallel with ulcer formation lipid peroxidation was observed to be clearly increased in ASA group verified by the significant decline in glutathione level in the present study indicating that lipid peroxidation plays an important part in the pathogenesis of gastric mucosal damage produced by ASA.

Furthermore, the decrease in gastric tissue GSH in the present study was supported by ***(Tanaka et al., 2001)*** who explained that gastric mucosal oxidative stress is important in the pathogenesis of aspirin-induced gastro-toxicity. Aspirin ulceration involves damage by reactive oxygen species (ROS) apart from acid and pepsin related factors. During this, lipid peroxidase and superoxide dismutase increases and catalase level decreases due to increased ROS generation during mucosal damage. This led to increased generation of $H_2O_2$ and its accumulation due to decreased catalase level. Inactivation of gastric peroximes may also aggravate the mucosal damage. This evidently caused increased lipid peroxidation and mucosal damage as was clearly demonstrated from the increase in ulcer index in ASA ulcerated group in comparison to the control group ***(Sairam et al., 2003)***.

In the present study, there was a decrease in glutathione levels (GSH) in aspirin group compared to normal control animals after seven days and four weeks . Judging from these data, they are in harmony and confirmed by ***(Sener et al., 2001; Fesharaki et al., 2006 and Bharti et al., 2010)*** were reported that administration of ASA caused a decrease in GSH level in rats compared to the control levels, this result may due to oxidative tissue damage of gastric mucosa by decreasing PGE2 and increasing free radical metabolites upon neutrophil activation involving the myeloperoxidase pathway. Generated from derangements of the antioxidant enzymes OH to developing ulcer.

Oxidative stress plays an important role in the pathogenesis of ulcers. The radicals also promote mucosal damage by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism. The damage to the membrane proteins decreases membrane permeability, activities of enzyme and receptors and

activation of cells ***(Thamotharan et al., 2010)***. Aspirin caused reduced in the sulfhydryl groups (-SH) compounds in mitochondria and decrease the glutathione levels ***(Sánchez-Mendoza et al., 2011)***.

In addition, ***Potrich et al., (2010)*** proposed that reactive oxygen species (ROS) are involved in the development of gastric ulcers induced by non-steroidal anti-inflammatory drugs**.** Non enzymatic antioxidants like GSH, are the first line of defense against lipid peroxidation. They are highly specific in their catalytic mode of action and decrease the gastric mucosal damage against free radicals. Glutathione therefore, constitutes one of the most important cytoprotective mechanisms against lesion formation.

***Jainu and Devi, (2005)*** verified that gastric mucosal lipid peroxidation, mediated by oxygen free radicals, is an important cause of destruction and damage to mucosal cells and gastric mucosal integrity. Also, ***Bharti et al., (2010)*** confirmed that the exposure of gastric mucosa to aspirin has been shown to affect cellular integrity and such changes are associated with oxidative stress and mitochondrial damage with a decrease in gastric GSH.

Results obtained in this study showed that there was a significant increase in total acidity of gastric juice, ulcer index values and a significant decrease in pH level as a result of giving aspirin to the animals compared to normal groups. These results could be supported by the findings of ***(Wang et al., 2007; Al-dalain et al., 2008 and Das et al., 2008)*** who suggested that aspirin causes inhibiting prostaglandin synthesis, interferes with protective mechanisms, (i.e. mucus, bicarbonate secretion, surface epithelial hydrophobicity and mucosal blood flow). These changes permit back diffusion of acid through the breached surfaces to destroy the capillaries cells and vein causing hemorrhagic ulcer. Also, ***Al-dalain et al., (2008)*** they reported that some radicals such as OH- causes increase in lipid peroxidation and increases ulcer index induced by aspirin.

The increase in total acidity of gastric juice , ulcer index values and decrease in pH level by ASA may be due to increase the aggressive factors (acid and pepsin) but decreased of pH level because of so called back diffusion of HCl through the broken barrier, inhibition of mucosal blood flow, acute inflammation and a decrease in the synthesis of sulphated mucus glycoprotein has been implicated in the etiology of gastric ulcer ***(Jainu et al., 2006)***. Also, increasing acid secretion by increasing the $H^+$ ion transport/back diffusion of $H^+$ ions ***(Scheiman et al., 1996 and Giri et al., 2010)***.

So, the increase in total acidic activity and decrease in pH value of the ulcer group is undoubtedly due to the increased production of HCl as it is evident from the total acidity of the gastric juice. Also, it is well documented that aspirin administration causes inhibition of prostaglandins ***(Potrich et al., 2010)***. Prostaglandins are important cytoprotective agents in the gastrointestinal tract because they increase mucosal blood flow. Inhibition of prostaglandin synthesis by aspirin causes damage to the cell membrane of mucosal, parietal and endothelial cells ***(Agnelarul et al., 2010)***. Besides, ASA causes decreased pH value resulting in mucosal injury due to its direct irritant effect and by permitting back diffusion of hydrogen ion through the mucosa ***(Bharti et al., 2010)***.

In ulcer group the formation of gastric mucosal lesions persisted even after 4 weeks of stopping ASA administration. Ulcers are defined histologically as a breach in the mucosa of the alimentary tract that extends through the muscularis mucosa into the submucosa or deeper. Although they may occur anywhere in the alimentary tract, none are as prevalent as the peptic ulcers that occur in the stomach ***(Jaikumar et al., 2010)***. Ulcer formation as a result of aspirin administration may involve several mechanisms which are reducing gastric blood flow, there by contributing to the development of necrosis and haemorrhage and solubilization of mucus constituents in stomach. These actions result in an increased pepsin secretion and flux of $Na^+$ and $K^+$, with a decrease in histamine and $H^+$ ions into the lumen ***(Tour and Talele, 2011)***. Histamine is regarded as the critical regulator of gastric acid secretion ***(Laurence et al., 1997)***. In addition ASA penetrates the gastric mucosa, promoting membrane damage, erosion and ulcer formation through destruction of the mucus barrier, increases in vascular permeability and decreases in non-proteic sulphydrilic groups (NP-SH) of the gastric mucosa ***(Repetto and Llesuy, 2002 and Siegmund et al., 2003)***.

The histological examination of gastric mucosal tissue of ASA group in the present study revealed sharply damaged mucosal epithelium reaching the submucosal layer with haemorrhage, discontinuity of lining epithelium. These consequences may be related to the back-diffusion of acid into the mucosa which directly leads to vascular leakage and aggressive damaging effect in the basement membrane of both epithelial and mucosal cells in the gastric wall ***(Jainu et al., 2006)***.

Furthermore, inflammation in gastric mucosa by aspirin is accompanied by increased production of tumor necrosis factor-α (TNF-α), which augments neutrophil-derived superoxide generation and stimulates production of interleukin-1 (IL-1) leading to neutrophil accumulations ***(Kokura et al., 2000)***. Cytotoxic effects of ASA that affect the mucosal integrity of gastrointestinal tract are manifested by the disturbances of nitric oxide synthase (NOS), a key mediator of signaling events linked to apoptotic cell death ***(Slomiany et al., 1998)***. The enhanced expression of NOS upon aspirin administration results in the formation of nitric oxide (NO) related species, which exert a direct inhibitory effect on nuclear factor-kappa B ($NF_KB$), (a zinc-dependent transcription factor) which appears to play a key role in the process of ulcer healing, because its activation is upregulated in rat gastric ulcers and the blockade of $NF_kB$ activation resulted in impairment of ulcer repair ***(Mei et al., 2009)***. Inhibition of $NF_KB$ leads to upregulation of proinflammatory cytokine production, reactive oxygen species and enhanced rate of apoptosis ***(Slomiany and Slomiany, 2001)***.

From the inspection of the data represented in this study, it is clear that omeprazole (OMP), indole-3-carbinol (I3C) or both gathered treatments attenuated the damage caused by ASA represented by the significant decrease in stomach weight, acidity, followed by the disappearance of ulcers and haemorhagic streaks in the dissected treated stomachs. This was accompanied with increase in pH value, GSH levels, platelets count and monocyte percentage, these observations are in agreement with that obtained by ***(Khushtar et al., 2009 and Nair et al., 2010)***. These results were in accordance with ***Scheiman et al. (2006)***

who proved that, proton pump inhibitors (PPIs), including omeprazole, are effective in the prevention of NSAID-induced gastric injury as well as in promoting the healing of NSAID-induced ulcers by inhibition of acid secretion

The gastric acid pump is an ATPase present in cytoplasmic membranes of the resting parietal cell. On activation, the pump is translocated to the canalicular membrane, where it pumps out $H^+$ ions into the canalicular space in exchange for $K^+$ ions. Gastric acid secretion by the parietal cell is controlled through food-stimulated and neuroendocrine pathways involving the activity of gastrin, histamine, pituitary adenylate cyclase-activating peptide and acetylcholine. Therefore, several potential ways in which gastric acid secretion might be modified. The final effector in the secretion pathway is the gastric $H^+/K^+$ ATPase which is likely the most effective pharmacological approach ***(Sachs, 2003)***.

Omeprazole was the first clinically useful proton pump inhibitor. Proton pump inhibitors (PPIs) are weak bases carried in the circulation and delivered to the parietal cell as prodrugs. In this form, PPIs are capable of crossing cell membranes. The parietal cell is the only membrane- enclosed space in the body with a pH below 4.0. In this acidic environment of pH (1.0), omeprazole accumulates in the secretory canaliculus of the parietal cell at the luminal side of the gastric $H^+/K^+$ ATPase. Proton pump inhibitors inhibit the gastric $H^+/K^+$ ATPase via covalent binding to cysteine residues of the proton pump. proton pump inhibitors must undergo acid accumulation in the parietal cell through protonation, followed by activation mediated by a second protonation at the active secretory canaliculus of the parietal cell favouring a longer durationof gastric acid antisecretory effect ***(Sachs et al., 2006)***.

These results were in accordance with ***Scheiman et al., (2006)*** who proved that, proton pump inhibitors (PPIs), including omeprazole, are effective in the prevention of NSAIDs-induced gastric injury as well as in promoting the healing of NSAIDs-induced ulcers by inhibition of acid secretion.

Besides, PPIs can protect the gastric mucosa through mechanisms related to the reduction of tissue oxidative damage. Therefore, OMP counteracted tissue oxidation and produced reduction of mucosal cell proliferation associated with NSAIDs. But on the other hand, OMP does not influence mucosal PGE2 production ***(Fornai et al., 2011)***.

It is very important to bear that omeprazole efficiently scavenges hydroxyl radicals but not superoxide radical. Since, the main danger for $H_2O_2$ arises from its ability to cross cell membranes rapidly. Once inside the cells, it can probably react with $Fe^{++}$ and $Cu^{++}$ ions to form hydroxyl radicals, which may be the origin of many toxic insults ***(Simon et al., 2006)***.

On the other hand, ***Thippeswamy et al.,(2010)*** reported that inhibitor of gastric acid by omeprazole may be due to increased expression of COX-2 protein and elevating the levels of prostaglandin E2 (PGE 2). It also showed increased gastric pH and reduction in gastric acid secretion, which may be due to inhibition of gastric mucosa enzymes, carbonic

anhydrase (CA) II and CA IV, which are located in abundance in the gastric parietal cells and in the secretory canaliculi walls. This inhibition potentiates the inhibitory effect on the proton pump.

Additionally, ***Szabo and Vincze, (2000)*** elucidated that the healing rates of ulcer with the proton pump inhibitor decreased after treatment discontinuation, and that the percentage of the recurrence of ulcer is between 40% and 80% in most of the studies.

Generally, treatment animals with antiulcer drug omeprazole decreased ASA induced gastric damage and inhibited lipid peroxidation which was clearly verified by the decrease in ulcer index, ulcer score, percent of ulceration with the increase in pH value, GSH levels, platelets count and monocyte percentage in ASA ulcerated rats treated with OMP although OMP was not as efficient in gastric ulcer amelioration as I3C. Results of OMP treatment reflected a lower degree of cytoprotection than I3C.

The protection to the gastric tissue offered by OMP was evident by the desquamated surface epithelial cells and haemorragic areas in the lamina propria. Irregular gastric glands reflected degenerated glandular cells in this group. So, OMP is not alone the only drug of choice for gastric ulcer treatment.

Research of naturally occurring antioxidant compounds in edible plants reduces the risk of gastrointestinal cancer seems to have some potential source for ulcer-related problems, and it could make a substantial contribution to drug development by providing novel chemicals to these drugs ***(Brzozowski et al., 2001b)***.

Epidemiological studies indicate an inverse relationship between consumption of cruciferous vegetables and mortality from different types of cancers. The cancer preventive effect of cruciferous vegetables is attributed to their different phytochemical constituents. One of the most important anticarcinogenic phytochemicals contained in these vegetables is indole-3-carbinol (I3C) ***(Anderton et al., 2004)***.

Indole-3-carbinol (I3C) is a naturally occurring hydrolysis product of glucobrassicin found in vegetables of the *Cruciferae* family such as broccoli, brussels sprouts, and cauliflower ***(Verhoeven et al., 1997)***.

Presently, the significantly reduction in the ulcer index and gastric acid produced by indole-3-carbinol may be due to cytoprotective in nature either by stabilizing the integrity of the gastric mucus or by increasing mucus secretion ***(Onasanwo et al., 2010)***. He also reported that reactive oxygen species have been implicated in the aetiology and pathophysiology of gastrointestinal inflammation and gastric ulcers. There is a need for agents to minimize and repair free radical-induced damage.

The antioxidants play a key role in these defense mechanisms. An inhibitor potential of gastric acid by antioxidants which may be related to the conception that antioxidants has interaction with enzymes at the $H^+/K^+$ ATPase pump. Also, decreased of ulcer index and

gastric damage by indole-3-carbinol may be due to enhancement in antioxidant enzymes in gastric mucosal tissues ***(Jainu and Devi, 2006)***.

On the other hand, ***Luiz-Ferreira et al., (2010)*** suggested that the cytoprotective action of I3C on the gastric mucosa may be related to an increase in PGE2 production in mucosal of stomach for maintenance of mucosal integrity and protection against ulcerogenic and necrotizing agents. Where prostaglandins (PGs) inhibit acid secretion; stimulate mucus, bicarbonate and phospholipid secretion; increase mucosal blood flow; accelerate epithelial restitution and mucosal healing.

***Kumar and Rajani, (2011)*** showed that an antioxidant plant displays an antiulcerogenic effect related to cytoprotective activity. Indole-3-carbinol might have scavenged the free radicals produced by metabolism of aspirin and thereby healed the ulcer.

Nevertheless, significant increase was found in the platelets levels of ASA+I3C compared to ASA administrated animals. This result supported by the findings of ***(González-Correa et al., 2006)*** who showed that indole-3-carbinol (I3C) can increase prostacyclin levels and increased thromboxane production. I3C could help to protect cells from damage caused by oxidative stress.

On the other hand, rats treated with ASA+I3C showed significant increase in glutathione (GSH) level. Similar results were confirmed by ***(John et al., 2010)*** who reported that antioxidant plants causes increase GSH level to offers gastric protection against aspirin induced ulcer by significantly blocking lipid peroxidation which is proved by the reduced levels of lipid peroxide and increase mucin activity. The ability of the antioxidant plants to scavenge the free radicals contribute to the gastric cytoprotective activity ***(Thamotharan et al., 2010)***.

I3C protected gastric mucosa from aspirin injury through neutralization of released free radicals and inhibition of HCl secretion. These results concurs with ***Borek, (2001)*** who reported that I3C inhibits lipid peroxidation and inhibits the activation of oxidant induced transcriptation factor and nuclear factor Kappa B, thus protecting endothelial cells from injury by the oxidizing molecules.

In addition, I3C prevents loss of membrane permeability and dysfunction of cellular proteins, leading to survival of the functionally active cells ***(Chen et al., 2003)***.

Moreover, by the suppression of proinflammatory cytokine production and NOS activity that would greatly facilitate various healing mechanisms such as increased epithelial cell proliferation and decreased epithelial apoptosis in ASA ulcerated animals. Another possible protective mechanism of action of I3C as anti-inflammatory is by acting on first phase by inhibiting the mediator of inflammation, probably by inhibiting the platelets activating factor receptors present in the proinflammatory cells like mast cells and neutrophils ***(Tour and Talele, 2011)***. Histologically, revealed that I3C absolutely inhibited aspirin-induced lesions of rat gastric mucosa. I3C treatment was found to preserve the

functional cytoarchitecture of the entire gastric mucosa. These findings confirm the cytoprotective nature of I3C.

In the present investigation, ASA ulcerated rats showed a decrease in the mucin content represented by combined alcian blue periodic schiff technique and sites of protein content demonstrated by bromophenol blue stain which began to be attenuated by the I3C, or the combined treatments of I3C and OMP.

The etiology of gastric ulcer is influenced by various aggressive and defensive factors such as acid pepsin secretion, parietal cell, mucosal barrier, mucous secretion, blood flow, cellular regeneration and endogenous protective agents which are prostaglandins and epidermic growth factor ***(Repetto and Llesuy, 2002)***.

Gastric mucous is an important protective factor for the gastric mucosa. An improvement in mucus production guides the healing process by protecting the ulcer crater against the endogenous aggressors, like stomach secretions and oxidants as well as against exogenous damaging agents, such as NSAIDs. The ulcer prevention or healing by oral administration of 20 mg/Kg body weight of I3C to ulcerated ASA rats may be due to the increase in the mucus layer in the gastric mucosa offered by I3C treatment ***(Chang et al., 2005)***.

The mucin content staining method confirmed the role of I3C enhancing the mucus level and protecting the inflammatory cytokine-mediated oxidative damage to gastric mucosa. Thus, the anti-ulcerogenic activity of I3C may involve its beneficial effect on both offensive and defensive gastric mucosal factors ***(Choi et al., 2010)***.

***Manson et al.,(1997) and Zhang, (2004)*** confirmed that I3C is able to inhibit chemically induced neoplasia in forestomach by selective beneficial alteration of Phase I cytochrome P-450 (CYPs) and induction of phase II detoxification enzymes.

***Verhoeven et al., (1997)*** indicated that I3C in cruciferous vegetables induced gastric glutathione *S*-transferases production. These results were in accordance with our results which signified the increase in stomach GSH level after I3C administration to ulcerated rats more than those under OMP treatment.

In addition, I3C has also been shown to have protective effects due to its oligomerization under acidic conditions and thus, it has been suggested that the observed biological activity may be attributable mainly to these acid condensation products ***(Grose and Bjeldanes, 1992)***.

Precisely, after ingestion, in the stomach acidic medium indole-3-carbinol is converted into 3,3diindolylmethane as a major condensation product. 3,3diindolylmethane was found to inhibit the growth of human colon adenocarcinoma ***(Gamet-Payrastre et al., 1998)***.

Besides, inflammation intersects at COX-2 and inducible nitric oxide (iNOS) level ***(Lee et al., 2003)*** and is accompanied by activating neutrophils which results in the overproduction of proinflammatory mediators, including tumor necrosis factor-α (TNF-α), Interleukin-4 (IL-4) and Interleukin-6 (IL-6) ***(Raghavendran et al., 2011)***.

I3C also inhibits nitric oxide production through decreasing iNOS expression in activated macrophages ***(Chen et al., 2003)***, possess in vitro nitric oxide clearance activity ***(Wang et al., 2012)*** and suppresses nuclear factor-kappa B (NF-κB) activity which leads to the decreased production of reactive oxygen species ***(Kim and Milner, 2005)***. These results were clearly demonstrated by the immunohistochemical stain of COX-2 in this study showing that I3C treated ulcerated rats produced an increase in the COX-2 production in the stomach tissue verifying the healing process of I3C.

These results were in agreement with ***(Gilroy et al., 1999)*** who explained that cyclooxygenase-2 produces prostaglandins that exert anti-inflammatory actions and play an important role in the healing of gastric ulcers ***(Shigeta et al., 1998)***.

Cyclooxygenase-2 expression derived prostaglandins promote epithelial cell proliferation and granulation tissue formation by improving blood flow and stimulating the expression of growth factors ***(Ma et al., 2002)***.

***Berenguer et al., (2002)*** have demonstrated an up-regulation of cyclooxygenase-2 expression and activity in the ulcer base not only during the acute phase of inflammation but also in the ulcer healing stage and especially in areas of intense tissue repair. The present study corroborates previous data showing the deleterious effect of ASA as a classic and highly specific COX-2 inhibitors, and I3C as an essential antioxidant for tissue healing, stimulating the development of new vessels to supply oxygen and nutrients to the healing mucosa and also providing the matrix substrate for the proliferation and differentiation of epithelial structures ***(Sánchez-Fidalgo et al., 2004)***.

On the basis of the results made by ***Hatazawa et al., (2007)*** they brought about that endogenous prostaglandin subtype 2 derived from COX-2 plays an important part in the spontaneous healing of gastric ulcers and the up regulation of COX-2 appears to be a defensive and anti-inflammatory response aimed at enhancing mucosal defense.

Cell proliferation plays a predominant role in wound healing, and proliferating cell nuclear antigen (PCNA) has been demonstrated to be a useful marker of cell proliferation. PCNA is an auxiliary protein of DNA polymerase, an enzyme necessary for DNA synthesis and cell proliferation ***(Lima et al., 2011)***. Gastric ulcer healing is a process involving cell proliferation and migration at the gastric ulcer margin and angiogenesis in granulation tissue. When cells are exposed to gastric irritants, expression of heat shock proteins is induced, making the cells resistant to the irritants ***(De-Faria et al., 2012)***. Moreover, ***Ishihara et al., (2011)*** examined the role of PCNA in gastric ulcer healing, providing the

evidence that PCNA accelerates the process of healing by increasing the level of PGE2 and expression of growth factor, thereby stimulating cell proliferation at the gastric margin and angiogenesis in granulation tissue. Immunohistochemical localization showed a great quantity of PCNA in the gastric mucosa of animals treated with omeprazole or I3C ***(De-Faria et al., 2012)***.

In this respect, the present results showed that the injuring actions of ASA are associated with a significant reduction in the mucosal expression of PCNA as reliable markers of cell proliferation and, in this setting, I3C was able to reverse the inhibitory effects of NSAIDs. The effects of I3C on PCNA was mimicked, it can be proposed that its beneficial influence on mucosal repair depends on acid-independent mechanisms, which are likely related with its antioxidant properties. This view is supported by ***Jainu and Mohan, (2008)*** who demonstrated that both the I3C and omeprazole enhanced the expression of growth factors, including transforming growth factor-alpha, in the gastric mucosa of rats treated with aspirin. Moreover, I3C partly counteracted the ASA-induced delay in rat duodenal ulcer healing, without affecting mucosal PCNA expression ***(Pérez-Aisa et al., 2003)***.

In addition, I3C treatment resulted in re-epithelization of the gastric lesions induced by aspirin. Interestingly, histological analysis of gastric ulcers revealed the presence of extensive deep damage in the gastric mucosa after administration of aspirin. The treatment with I3C significantly regenerates the gastric mucosa.

Moreover, cell proliferation plays an important role in wound healing ***(Tarnawski, 2005)*** and it was shown that PCNA, a tissue marker of cell proliferation, was increased during healing of gastric mucosal injury ***(Sun et al., 2003)***. In this study observed that I3C administration increased the number of PCNA-positive cells, suggesting that I3C treatment could promote gastric cell regeneration and proliferation.

# Recommendations:-

It is obvious from the present physiological, biochemical, histological, histochemical and immunohistochemical studies that gastric ulcer produced from ASA administration has serious harmful effects that ended with stomach disorders, Thus it is presently well recommended that gastric ulcer individuals using aspirin as analgesic and also as a cardio protective drug must be under control treatment of both omeprazole and indole-3-carbinol to decrease the risk of aspirin-induced gastric mucosal injury.

Thus it would seem most convenient to conclude at this level the ability of I3C as one promising anticancer, cytoprotective, antioxidant agent naturally occurring in vegetables of the *Brassica* genus to act synergegistically with OMP to ameliorate most of the investigated analyses because of the difficulty of using OMP alone as antiulcer treatment as the incidence of ulcer recurrence after stopping OMP treatment.

Again, indole-3-carbinol and omeprazole increased the inhibition of aspirin-induced gastric haemorrhage, ulceration, gastric mucosal oxidative stress and inflammation in rats. Moreover, we concluded that combining I3C with OMP in the treatment of gastric ulcer is potentially a new approach for decreasing gastrointestinal injury caused by aspirin and other NSAIDs.

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# ***SUMMARY AND CONCLUSION***

The present study is a trial to investigate the anti-ulcer activity of indole-3-carbinol (I3C) as one of the anticarcinogenic phytochemicals naturally occurring compounds found in vegetables of the *Cruciferae* family.

Moreover, to assess the treatments of I3C either alone or in combination with omeprazole a proton pump inhibitor to diminish the effects of induced gastric ulcer by aspirin in male albino rats and evaluate the protective role.

To attain this goal, a total number of 96 male albino rats were presently investigated. Animals were divided into two studies. The first study for seven days, to evaluate the protective role of indole-3-carbinol (20 mg/kg) and/or omeprazole (20 mg/kg) with use of aspirin at a dose of 500 mg/kg of body weight. The other study for four weeks, to evaluate the protective and therapeutic role of indole-3-carbinol and omeprazole from gastric ulcer. The animals treated with aspirin at a dose of 500 mg/kg of body weight for seven consecutive days to cause gastric ulcers, and were divided into four subgroups experimental. The first group was treated with distilled water, second group has treatment with omeprazole, third group was treated with indole-3-carbinol and fourth group was treated with omeprazole and indole-3-carbinol for four weeks.

The study included the following:-

**I .Morphological Investigation:-**

A significant increase in the body weight was denoted in the aspirin alone (ASA) or with omeprazole and/or indole-3-carbinol groups at seven days and four weeks compared to the normal control group.

A decrease in stomach weight was recognized in aspirin with omeprazole and/or indole-3-carbinol groups at 7 days. Whereas stomach weight showed an increase after four weeks in ulcer group.

No significant changes in relative stomach to body weight between groups in seven days, but in four weeks a significant decrease was showed in the ulcer rats treated with omeprazole compared to the normal control animals.

The mortality rate was found in aspirin group that took 500 mg/kg body weight for seven days where two rats from 6 rats died after five days of aspirin administration with a percentage of 33.33.

**II- Biochemical Parameters :-**

**1-Haematological Investigation:-**

Aspirin treated with omeprazole and indole-3-carbinol for seven days showed a significant decrease in R.B.Cs. Whereas, no significant changes found in R.B.Cs at four weeks.

No significant changes found in haemoglobin values at seven days and four weeks compared to normal control.

A significant decrease in haematocrit values found in ASA+OMP+I3C at seven days, but no significant changes found in haematocrit values at four weeks.

There was a slight increase in W.B.Cs in aspirin group at seven days as compared to normal control group. At the four weeks ulcerated rats alone or with OMP and OMP+I3C showed a significant increase in their W.B.Cs values.

A significant decrease in the percentage of lymphocytes was recorded in aspirin group treated with omeprazole and/or indole-3-carbinol groups at seven days and in ulcer treatment groups at four weeks.

At seven days, a pronounced decrease in the percentage of monocytes was detected in the aspirin rats and in ulcerated rats with omeprazole and indole-3-carbinol group in 4 weeks as compared to normal control animals.

In aspirin alone or combined with different treatments showed a significant decline in the platelets levels at seven days compared to normal control group. While there was an increase in the platelets levels of ASA+I3C and ASA+OMP+I3C groups compared to aspirin administrated animals. Aspirin rats at four weeks of treatment recorded a significant decline in their platelets levels values compared to normal control animals.

**2-Serum Total Protein Levels:-**

An increase in serum total protein levels was recorded in aspirin group alone or with omeprazole and/or indole-3-carbinol groups at 7 days or 4 weeks as compared to normal control rats.

**3-Serum Albumin Levels:-**

Marked increase in serum albumin levels in aspirin rats at seven days and ulcer rats alone or with omeprazole group at four weeks against normal control group. Decrease in ASA plus OMP, ASA plus I3C and ASA plus OMP plus I3C groups as compared to aspirin group.

**4-Stomach Glutathione Activity (GSH)**:-

A significant decrease in aspirin group compared to normal control animals throughout the experimental period, while a significant increase in ASA+I3C groups at 7 days and 4 weeks compared to ASA group.

**5-Total Gastric Acid (Total Acidity):-**

Aspirin rats alone showed a significant increase in total acidity at seven days and in ulcer rats alone in total acidity compared to normal control group rats at four weeks. On the other hand, significant decline in total acidity was established in aspirin with omeprazole and/or indole-3-carbinol groups after seven days or four weeks of treatment compared to the ulcer groups.

**6- pH Value of the Gastric Juice:-**

A significant decrease in the pH value occurred in the aspirin group at seven days and in ulcer groups compared to normal control rats after four weeks. Whereas significant increase was recorded in aspirin combined with different treatments at seven days and four weeks as compared to aspirin group.

**III-Ulcer index, Ulcer Score and Percentage of Ulceration:-**

An increase in ulcer index, ulcer score and percentage of ulceration of stomach rats of administrated aspirin for seven days and ulcerated rats for 4 weeks.

**IV .Histological, Histochemical and Immunohistochemical Studies:-**

Stomach of rats in 7 days treated with aspirin, were severe red coloration spot ulcer and severe haemorrhagic streaks in the dissected stomach of rats. Stomach was markedly severe red coloration in animals administered of aspirin and omeprazole. Whereas, no evident macroscopical changes in stomach ulcerated rats treated with omeprazole and/or indole-3-carbinol.

Stomach sections from rats treated with ASA after 7 days showed inflammatory cellular infiltration on the connective tissues of lamina propria, congested and dilated blood vessels in the muscularis layer, large number of oxyntic cells which more acidophilic granules and with more vesicular nuclei and many gastric erosions were appeared. Aspirin rats with OMP was characterized as many eroded areas, involving luminal surface epithelial cells and gastric pit cells and haemorrhagic lesions. These alterations were ameliorated under treatment I3C and/or OMP.

Histopathology investigate (ulcer group) of ulcerated stomach of rats receiving distilled water for 4 weeks, stomach revealed many eroded areas in the gastric tissue and the surface epithelium manifested exfoliation and sloughing of the surface mucosal cells. Many histological alteration showed in ulcer group of rats treated with OMP including some eroded areas in superficial layer, aggregation of inflammatory cells and interstitial haemorrhages. The stomach sections from ulcerated rats with I3C and omeprazole plus indole-3-carbinol manifested limited histological changes.

Histochemically, stomach sections showed decrease in total protein content in ASA group within the 7 days and 4 weeks.

On the other hand, the group of ulcer rats post-treatment with OMP showed slightly decrease in staining quality for total proteins. These alterations were ameliorated under treatment OMP and/or I3C.

There are marked decreases in mucin granules in stomach sections from rats treated with ASA and ASA with OMP for 7 days as observed by alcian blue P.A.S. technique. Also, in aspirin rats treated with I3C and OMP+I3C showed slight decrease.

In the ulcer group of rats treated with distillate water for 4 weeks, the carbohydrates reaction showed decreased. Furthermore, group of ulcer rats treated with OMP and/or I3C confirmed moderate reactivity of carbohydrates reaction (mucin) on the surface mucosa cells and mucous neck cells, but strong reactivity of mucin P.A.S. +ve the materials was noticed in degenerative area and cells debris.

The gastric tissues obtained in the ASA model of gastric ulcer were used for immunohistochemical showed a great number of proliferation cells in the stomach of animals treated with OMP, I3C and OMP+I3C. Thus, the results indicate that this protein participated in the healing of the gastric ulcer treated with OMP, I3C and OMP+I3C. I3C shows that the most pronounced expression of Cyclooxygenase-2 (COX-2) and Proliferating Cell Nuclear Antigen (PCNA) stains.

**Thus it was concluded that:** Omeprazole alone accelerated ulcer healing by inhibiting gastric acid secretion but indole-3-carbinol produced a deep permanent protection from aspirin induced gastric ulcer without influencing acid secretion or neutralizing intragastric acidity.

# الملخص العربى

## تأثير بعض مضادات القرحة والأكسدة على الوقاية من قرحة المعدة المستحدثة والتئامها في الجرذان البيضاء.


استهدفت الدراسة إلقاء الضوء على تأثير الاندول-3- كربنول المستخلص من مواد كيميائية نباتية الموجودة في العائلة الصليبية كمضاد للأكسدة و الأوميبرازول كعلاج للجرذان البيضاء المصابة بقرحة المعدة المستحدثة بالأسبرين وتقييم الدور الوقائي.

وقد أجريت الدراسة على 96 من ذكور الجرذان البيضاء, تم تقسيمهم إلى دراستين. الدراسة الاولى لمدة سبعة أيام, لتقييم الدور الوقائي للاندول-3- كربنول (20 مجم/كجم ) و الأوميبرازول (20 مجم/كجم ) معا أو فرادى مع تعاطي الأسبرين بجرعة 500 مجم/كجم من وزن الجسم عن طريق الفم. أما الدراسة الثانية لمدة أربعة أسابيع, وذلك لتقييم الدورالوقائي العلاجي للاندول-3- كربنول و الأوميبرازول من قرحة المعدة. وعوملت الحيوانات بالأسبرين بجرعة 500 مجم/كجم من وزن الجسم لمدة سبعة أيام متتالية لإحداث تقرحات بالمعدة, و تم تقسيمها الى أربع مجاميع تجريبية مصابة بالقرحة المعدية. المجموعة الاولى عوملت بالماء المقطر, والثانية تمت المعالجة بالأوميبرازول, والثالثة تمت المعالجة بالاندول-3- كربنول والرابعة بالأوميبرازول و الاندول-3- كربنول معا لمدة أربعة أسابيع, وقد أجريت مقارنة بالمجاميع الضابطة.

وقد اشتملت الدراسة على المعايير التالية:-

**1- التغيرات المورفولوجية :**

أوضحت النتائج زيادة ملحوظة في وزن الجسم لمجموعة الأسبرين على حده أو مع الأوميبرازول أو مجموعات الاندول-3- كربنول خلال فترات التجربة ( سبعة أيام وأربعة أسابيع ) مقارنة بالمجموعة الضابطة.

كما أوضحت الدراسة انخفاض في وزن المعدة في مجموعة الأسبرين مع الأوميبرازول أو مجموعات الاندول-3- كربنول خلال سبعة أيام, في حين لوحظ زيادة الوزن بعد أربعة أسابيع في مجموعة الحيوانات المصابة بقرحة المعدة.

لم يلاحظ تغيرات كبيرة في نسبة وزن المعدة إلى وزن الجسم بين المجموعات المختلفة خلال سبعة أيام، بينما سجلت الدراسة انخفاضا ملحوظا في نسبة وزن المعدة إلى وزن الجسم للجرذان المصابة بقرحة المعدة مقارنة مع مجموعة الضابطة.

كذلك سجلت الدراسة عدم حدوث أي نسبة وفيات لجرذان المجموعات المعاملة بالأوميبرازول أو الاندول-3-كربنول أو كلاهما معا. بينما سجلت الدراسة حدوث نسبة وفيات طفيفة في مجموعة الحيوانات المعاملة بالأسبرين (500 مجم/كجم ) بنسبة 33.33٪.

**2- التغيرات البيوكيميائية : -**
**أ-الدراسات الهيماتولوجية:**

سجلت النتائج انخفاضا ملحوظا للعد الكلي لكريات الدم الحمراء للمجموعات التي عوملت بالأسبرين مع الأوميبرازول و الاندول-3- كربنول لمدة سبعة أيام, و لم يلاحظ أي تغيرات معنوية في العدد الكلي لكريات خلال فترة التجربة (4 أسابيع).

وأوضـــحت النتائج تغير غير ملحوظ في محتوى الهيموجلوبين في كل المجموعات التجريبية بالمقارنة بالمجموعة الضابطة.

كما سـجلت الدراســة انخفاضــا ملحوظا في نسـبة الهيماتوكريت لمدة سـبعة أيام للمجموعات التي عوملت بالأسبرين مع الأوميبرازول و الاندول-3- كربنول. بينما لم يلاحظ تغيرات معنوية خلال أربعة أسابيع.

كذلك سجلت النتائج زيادة طفيفة للعد الكلي لخلايا الدم البيضاء لمجموعة الحيوانات المعاملة بالأسبرين لمدة سبعة أيام بالمقارنة مع المجموعة الضابطة. بينما سجلت النتائج زيادة ملحوظة خلال أربعة أسابيع لمجموعة الجرذان المصابة بقرحة المعدة والمجموعات التي عولجت بالأوميبرازول منفردا أو الأوميبرازول و الاندول-3- كربنول معا.
سجلت النتائج انخفاضا ملحوظا في النسبة المئوية للخلايا اللمفاوية في مجموعات الحيوانات المعالجة بالأسبرين مع الأوميبرازول أو مع مجموعة الاندول-3-كربنول خلال سبعة أيام. كما سجلت الدراسة انخفاضا ملحوظا لمجموعة الجرذان المصابة بقرحة المعدة لمدة 4 أسابيع.

كما حدث انخفاضـــا ملحوظا خلال ســـبعة أيام في النســـبة المئوية للخلايا وحيدات النواة للمجموعة المعاملة بالأســـبرين ومجموعة الجرذان المصـــابة بقرحة المعدة مع مجموعة الأوميبرازول و مع الاندول-3- كربنول خلال أربعة أسابيع مقارنة بالمجموعة الضابطة.

أظهرت النتائج انخفاضا ملحوظا في مستويات الصفائح الدموية خلال سبعة أيام لجميع المجموعات التجريبية مقارنة بالمجموعة الضابطة. بينما سجلت النتائج زيادة في مستويات الصفائح الدموية في مجموعة الحيوانات المعالجة بالأسبرين مع الاندول-3- كربنول ومجموعة الحيوانات المعالجة بالأسبرين مع الأوميبرازول و الاندول-3- كربنول بالمقارنة مع الحيوانات المعاملة بالأسبرين. أظهرت الدراسة أيضا انخفاضا ملحوظا في قيم الصفائح الدموية في مجموعة الجرذان المعاملة بالأسبرين بالمقارنة مع المجموعة الضابطة.

**ب- المحتوى الكلي للبروتين في مصل الدم:**

ســـجلت الدراســـة زيادة كبيرة ذات دلالة احصـــائية في المحتوى الكلي للبروتين لمجموعة التجربة المعاملة بالأسبرين منفردا أو مع الأوميبرازول أو مع الاندول-3- كربنول خلال فترات التجربة (7 أيام - 4 أسابيع ) مقارنة مع المجموعة الضابطة.

**ج- مستويات الزلال في مصل الدم:**

سجلت زيادة ملحوظة في مستويات الزلال في الدم لمجموعة الجرذان المعاملة بالأسبرين خلال سبعة أيام و مجموعة الجرذان المصابة بقرحة المعدة و المجموعة المعالجة بالأوميبرازول بعد أربعة أسابيع, كما لوحظ انخفاضا في مستويات الزلال في مجموعة الأسبرين و الأوميبرازول، و مجموعة الأسبرين و الاندول-3- كربنول بالمقارنة مع مجموعة الأسبرين.

**د- نشاط الجلوتاثيون في المعدة:**

أظهرت النتائج انخفاضـا ذو دلالة احصـائية لنشـاط الجلوتاثيون في مجموعة الحيوانات المعاملة بالأسـبرين خلال فترات التجربة بالمقارنة مع المجموعة الضابطة. بينما أظهرت النتائج زيادة احصائية في مجموعات الأسبرين و الاندول-3- كربنول خلال فترات التجربة ( 7 أيام - 4 أسابيع ) مقارنة مع المجموعة المعاملة بالأسبرين.

**هـ- النشاط الحمضي للمعدة :**

سجل المعدل الحمضي للمعدة زيادة معنوية في مجموعة الحيوانات المعاملة بالأسبرين و الحيوانات المصابة بالقرحة خلال فترة التجربة مقارنة بالمجموعة الضابطة. كما سجلت الدراسة انخفاضا كبيرا في الحموضة الكلية في

مجموعة الأسبرين مع الأوميبرازول أو مع الاندول-3- كربنول خلال فترات التجربة مقارنة مع المجموعة المصابة بقرحة المعدة.

**و- قيمة الأس الهيدروجيني للعصير المعدي:**

لوحظ انخفاضا كبيرا في قيمة الأس الهيدروجيني لمجموعة الجرذان المعاملة بالأسبرين والمجموعة المصابة بقرحة المعدة مقارنة مع مجموعة الضابطة. بينما سجلت زيادة معنوية للأس الهيدروجيني لمجموعة الأسبرين المعالجة بالأوميبرازول أو مع الاندول-3- كربنول على حده أو معا خلال سبعة أيام أو أربعة أسابيع بالمقارنة مع مجموعة الأسبرين.

**3- مؤشر القرحة, و نقاط القرحة والنسبة المئوية للتقرح:**

لوحظ زيادة في مؤشر قرحة المعدة, ونقاط القرحة والنسبة المئوية للتقرح في الجرذان المعاملة بالأسبرين لمدة سبعة أيام و المجموعة المصابة بقرحة المعدة بالمقارنة مع المجموعة الضابطة.

**4- الدراسات الهستولوجية، والهستوكيميائية والهستوكيميائية المناعية:**

أظهرت الدراسة العديد من التقرحات النزفيه بمعدة الجرذان المعاملة بالأسبرين لمدة سبعة أيام, كما لوحظ أيضا احمرارا شديدا لمعدة الجرذان المعاملة بالأسبرين و الأوميبرازول. كما لم تظهر أي تغيرات واضحة في معدة الجرذان المعاملة بالأسبرين و الأندول-3- كربنول على حده أو مع الأوميبرازول. وتميزت التغيرات النسيجية المرضية لمعدة الجرذان المعاملة بالأسبرين لمدة سبعة أيام بظهور خلايا التهابية في النسيج الضام, وتحلل في بعض الخلايا الطلائية, وزيادة في نشاط الخلايا الحامضية واتساع في الاوعية الدموية وكثير من التقرحات المعدية. أما مجموعة الجرذان المعاملة بالأسبرين و الأوميبرازول أظهرت العديد من التقرحات السطحية و العميقة مع وجود نزف دموي. كما لوحظ تحسن ملحوظ في مجموعة الجرذان المعاملة بالاندول-3- كربنول على حده أو مع الأوميبرازول.

كما أظهرت مجموعة الحيوانات المصابة بالقرحة والمعطاة الماء المقطر لمدة أربعة أسابيع العديد من التقرحات المعدية مع تحلل وانفصال للخلايا الطلائية خصوصا الخلايا المخاطية. وقد ظهرت عدة تغيرات هستولوجية في معدة الجرذان المصابة بقرحة المعدة والمعالجة بالأوميبرازول وتآكل الخلايا المعدية السطحية في الغشاء المخاطي للمعدة مع وجود نزف دموي. وقد أظهرت مجموعة الجرذان المصابة بقرحة المعدة والمعالجة بالاندول-3-كربنول على حده أو مع الأوميبرازول تغيرات هستولوجية محدودة.

أوضحت الدراسة الهستوكيميائية نقصا في المحتوى الكلي للبروتين في جميع المجموعات التجريبية, حيث ظهر بوضوح في مجموعة الحيوانات المعاملة بالأسبرين خلال سبعة أيام. بينما ظهر تحسن واضح في المحتوى الكلي للبروتين في مجموعة الجرذان المصابة بقرحة المعدة بعد المعالجة بالاندول-3- كربنول على حده أو مع الأوميبرازول.

كما أوضحت الدراسة نقص في المواد المخاطية, حيث ظهر انخفاضا ملحوظا في محتوى المخاط في مجموعة الجرذان المعاملة بالأسبرين و مجموعة الجرذان المصابة بقرحة المعدة خلال فترة التجربة. كذلك ظهرت المواد المخاطية بوضوح في مجموعة الجرذان المصابة بقرحة المعدة و المعالجة بالاندول-3-كربنول على حده أو مع الأوميبرازول.

كما أظهرت الدراسة الهستوكيميائية المناعية للخلايا المعدية لمجموعة الجرذان المصابة بقرحة المعدة والمعالجة بالاندول-3- كربنول زيادة ملحوظة لنشاط انزيم الكوكس-2 (COX-2) مع ارتفاع نسبة (+ve PCNA) مقارنة بالمجموعة الضابطة و المجموعة المعاملة بالأسبرين خلال فترات التجربة.

**لذلك نوصي** بتناول الاندول -3- كربنول كإحدى مضادات الأكسدة عن طريق تناوله من النباتات الموجود بها أوعن طريق تحضيره كعقار و تناوله مع عقار الأوميبرازول في علاج قرحة المعدة.

# مستخلص


استهدفت الدراسة إلقاء الضوء على تأثير بعض مضادات القرحة و الأكسدة ( الاوميبرازول و الاندول -3- كربنول ) كلا على حده أو معا على قرحة المعدة المستحدثة بالأسبرين و مدى التئامها.

وقد أجريت التجربة على 96 من ذكور الجرذان البيضاء وقد اشتملت على تقييم بعض التغيرات الفسيولوجية، والبيوكيميائية, والهستولوجية، و الهستوكيميائية والهستوكيميائية المناعية لأنسجة المعدة.

و قد أوضحت الدراسة أن تعاطي الاندول -3- كربنول كمادة مضادة للأكسدة مع الاوميبرازول في علاج الجرذان المصابة بقرحة المعدة قد يساعد في التأم كثير من التقرحات المعدية و تحسن في جميع القياسات المختلفة.

وقد أوصت الدراسة بتناول الاندول -3- كربنول كإحدى مضادات السرطان و الأكسدة عن طريق تناوله مباشرة من النباتات الموجود بها أو عن طريق تحضيره كعقار.

Ain Shams University
Women's College for Arts, Science and Education
Zoology Department

# Effect of Antiulcer and Ulcer Healing Property of Some Antioxidants on Experimentally Induced Gastric Ulcer in Albino Rats.

*Thesis*

*Submitted for Fulfillment of the Degree of Doctor of Philosophy in Zoology*

**Department of Zoology**

**Women's College for Arts, Science and Education**

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جامعة عين شمس
كلية البنات للآداب والعلوم والتربية
قسم علم الحيوان

# تأثير بعض مضادات القرحة والأكسدة على الوقاية من قرحة المعدة المستحدثة والتئامها في الجرذان البيضاء

رسالة مقدمة

للحصول على درجة دكتوراه الفلسفة في العلوم ( علم الحيوان )
قسم علم الحيوان
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