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Protective Effect Of Indole-3-carbinol Against Aspirin Induced Duodenal Damage In Rats.

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Abstract:

The aim of this study was to evaluate the protective effect of indole-3-carbinol on aspirin induced duodenal damage in male albino rats. Animals were divided into four groups. The first group was received distilled water and served as normal control group, the second group was received the aspirin (ASP) at a dose of (500 mg/kg/body weight), third group was received indole-3-carbinol (I3C) at a dose of (20 mg/kg/body weight), and the fourth group was received ASP (500 mg/kg/body weight) + I3C (20 mg/kg/body weight) for seven consecutive days. Histopathological study of duodenum tissue of rats administrating aspirin for 7 days showed desquamation in the lining mucosal epithelium associated with inflammatory cells infiltration in the underlying lamina propria of the mucosa, submucosa and muscularis. Sections of duodenum from rats treated with I3C or ASP+I3C showed the normal mucosa with no histopathological alteration. The present study provides a strong evidence of indole-3-carbinol produced a deep permanent protection from aspirin induced duodenal damage.

Key words: Protective; Histopathological; Indole-3-carbinol; Duodenal damage; Aspirin.

Introduction:

Indole-3-carbinol is a major derivative of glucobrassicin (3-indolylmethyl glucosinolate), a plant product common to vegetables of the class Cruciferae [1]. Indole-3-carbinol (I3C) is both an anti-initiator and a promoter of carcinogenesis depending on the timing and dose of administration. It has been found to inhibit the development of tumours in forestomach, glandular stomach, mammary gland, prostate, uterus, tongue, and liver of rodents, as well as in the trout liver, when administered prior to or during carcinogen exposure by gavage or in the diet [2]. 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 [3]. Aspirin is a widely used non-steroidal anti-inflammatory drug, but it can damage the gastrointestinal mucosa and may reduce the incidence of thrombotic occlusive events in myocardial infarction and stroke. Aspirin is known to be rapidly hydrolyzed to salicylate by esterases in the gastrointestinal tract and liver and to a lesser extent in plasma. There is also evidence showing antioxidant effects of aspirin. Salicylate has been used as a trapping agent for detecting •OH. On the other hand, some reports indicate that aspirin induces free radical formation [4,5].

Aim of study:

The objective of the present study was to investigate the gastroprotective and antioxidant activities of indole-3-carbinol on aspirin induced duodenal damage in rats.

MATERIALS AND METHODS

Drugs:

Aspirin (ASP) tablets (Bayer AG, Germany) were given to animals in this study at a dose of 500 mg/kg/body weight dissolved in distilled water [6] by stomach tube after a fasting period of 24 hours. Indole-3-carbinol (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 [7] by stomach tube after a fasting period of 24 hours.

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 [8] and water ad-libitum with fresh

daily supplies. They were allowed 10 days pre-experimental period to adapt to the laboratory conditions.

Experimental groups:

Animals were divided into four experimental groups of six animals in each group as follows: normal control group (received distilled water), ASP group (received ASP at a dose of 500 mg/kg/body weight), I3C group (received I3C at a dose of 20 mg/kg/body weight), and ASP+I3C group (received ASP at a dose of 500 mg/kg/body weight with I3C at a dose of 20 mg/kg/body weight). All chemicals were administered to rats by stomach tube. At the end of the experimental period, the animals were fasted for 24 h. Animals from all groups were dissected after an experimental period of seven consecutive days.

Histopathological studies:

Tissue samples from each group were fixed in 10 per cent formalin for 24 h. The formalin fixed specimens were embedded in paraffin, sectioned (5µm) and stained with haematoxylin and eosin stains [9]. Sections were evaluated by light microscopy.

Results:

There was no histopathological alteration and the normal histological structure of the vili with covering lining mucosal epithelium were recorded in normal control group (Fig.1), throughout the whole experimental period, which was showed by hematoxylin &eosin technique (Figures 2,3,4,5 and 6).

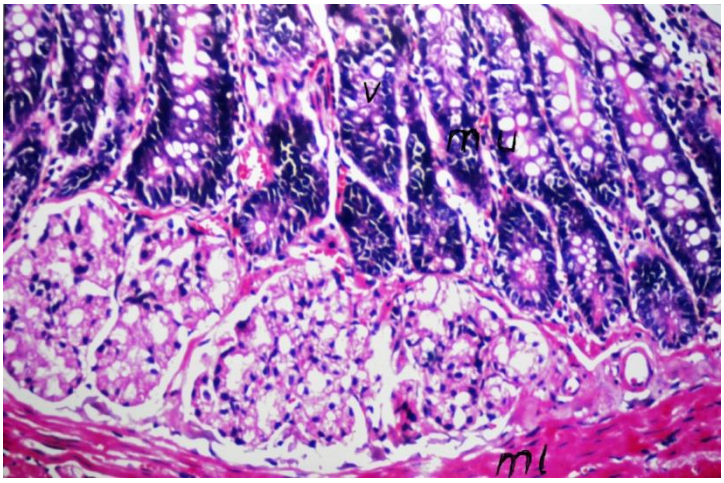

Figure 1:

Duodenum of rat in control group showing normal histological structure of the vili (v) with covering mucosa (mu) and underlying muscular layer (ml) (H&E, x400) .

In comparison with control, histopathogical examination of duodenal mucosal tissue of rats administrating aspirin for 7 days showed desquamation in the lining mucosal epithelium associated with inflammatory cells infiltration in the underlying lamina propria of the mucosa (Fig. 2&3), as well as the submucosa and muscularis (Fig.4). On the other hand, the sections of duodenum from animals treated with I3C manifested similar features as in normal control group during the experimental duration (Fig.5).

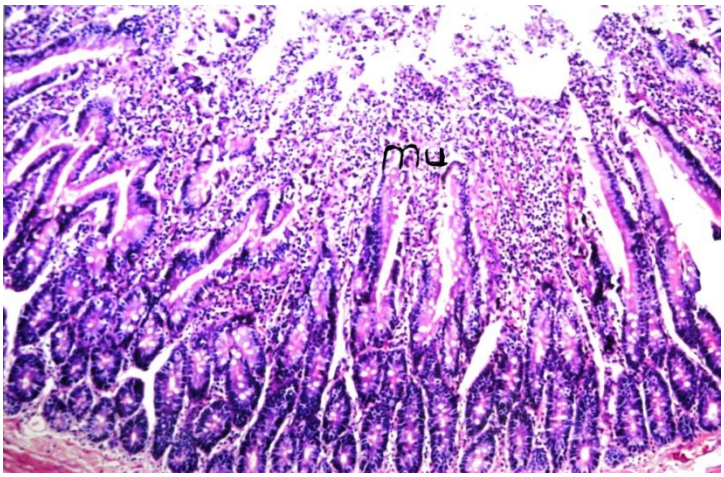

Figure 2:

Duodenum of rat treated with aspirin at a dose of 500 mg/kg/body weight for seven days showing mucosal desquamation with inflammatory cells infiltration in mucosa (mu) (H&E, x160).

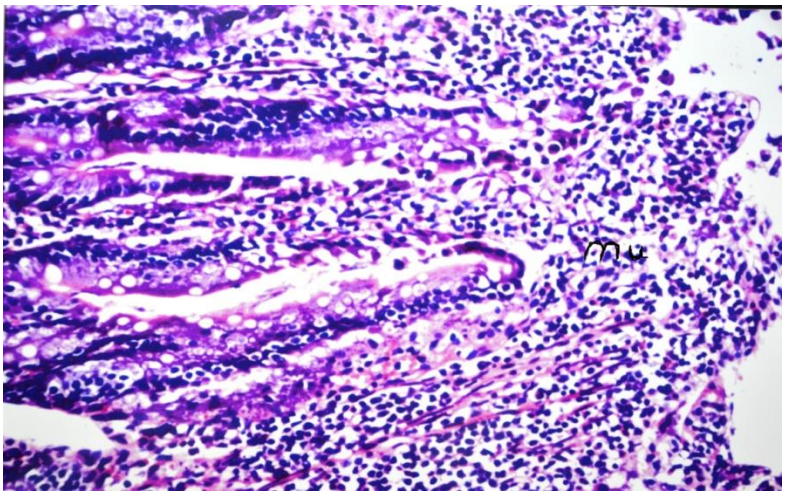

Figure 3:

Duodenum of rat treated with aspirin at a dose of 500 mg/kg/body weight for seven days showing the magnification of (fig. 2) to identify the inflammatory cells infiltration in mucosa (mu) (H&E, x400)

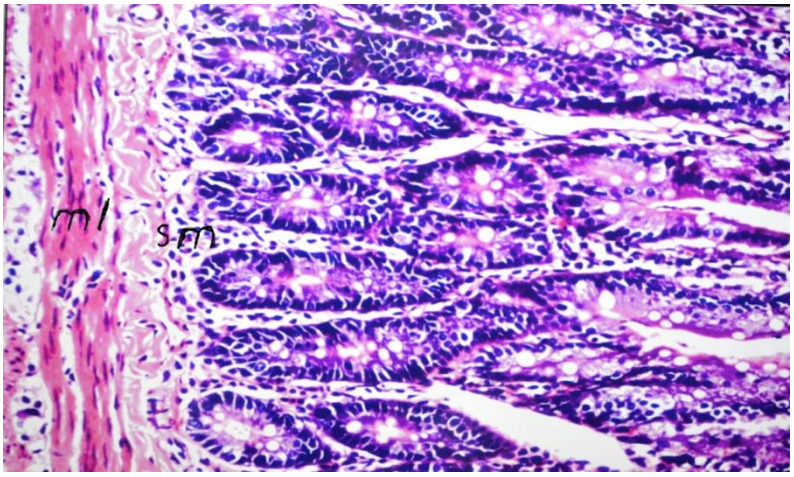

Figure 4:

Duodenum of rat treated with aspirin at a dose of 500 mg/kg/body weight for seven days showing inflammatory cells infiltration in submucosa (sm) and muscularis (ml) (H&E, x400) .

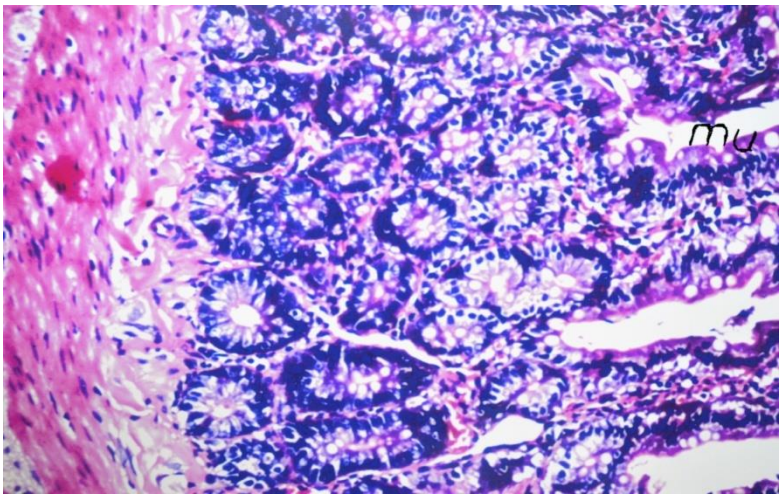

Figure 5:

Duodenum of rat treated with I3C at a dose of 20 mg/kg/body weight for seven days showing normal histological structure (H&E, x400).

Animals in ASP+I3C group were orally given ASP at a dose of 500 mg/kg/body weight and I3C at a dose of 20 mg/kg/body for 7 days noticed normal histological structure and no inflammatory cells infiltration in mucosa compared to the ASP group (Fig.6).

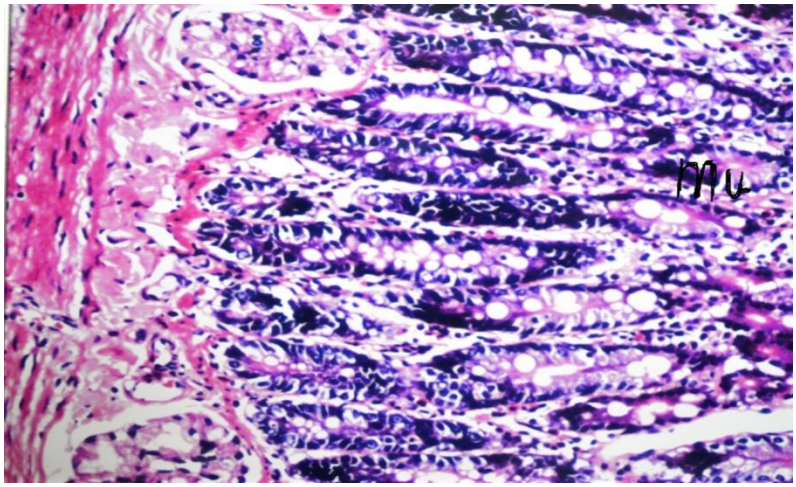

Figure 6:

Duodenum of rat treated with ASP at a dose of (500 mg/kg/body weight) + I3C at a dose of (20 mg/kg/body weight) for seven days showing normal histological structure and no inflammatory cells infiltration in mucosa (mu) (H&E, x400).

Discussion:

The present study indicated that deficiency of I3C could potentiate duodenal mucosal damage induced by aspirin in rats. It has been proposed that active oxygen species and lipid peroxidation are important in the pathogenesis of duodenal mucosal damage induced by NSAIDs [10]. Lipid peroxidation mediated by oxygen radicals is considered an important cause of cell membrane damage and destruction, because a single initiating event can result in the conversion of hundreds of fatty acid side chains into lipid peroxides, altering the structural integrity and biochemical functions of the membrane. Many factors and mechanisms are implicated in the ulcerogenesis and duodenal mucosal damage induced by different models employed in the present study involving, depletion of duodenal wall, mucin mucosal damage

induced by non-steroidal anti-inflammatory drugs and free radical production [11]. NSAIDs like aspirin causes duodenal mucosal damage by decreasing prostaglandin levels through inhibition of prostaglandin synthesis [12].

The histopathological examination of duodenal mucosal tissue of ASP group in the present study revealed desquamation in the lining mucosal epithelium associated with inflammatory cells infiltration in the underlying lamina propria of the mucosa, submucosa and muscularis. 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 duodenal wall [13]. Inhibitions of prostaglandin synthesis by aspirin coincide with the earlier stages of damage to the cell membrane of mucosal, parietal and endothelial cells [14]. Also and supporting this findings, [15,16] demonstrated that, Inflammatory cells infiltration into tissues is thought to be preceded by adherence to the endothelium via adhesion molecules expressed on cells. Furthermore, inflammation in duodenal mucosa by aspirin is accompanied by increased production of tumor necrosis factor α (TNFα), which augments neutrophil-derived superoxide generation and stimulates production of interleukin-1 (IL-1) leading to neutrophil accumulations [17]. On the other hand, one of the mechanisms by which aspirin damages the duodenal mucosa is the increased production of nitric oxide (NO) due to the overexpression of nitric oxide synthase (NOS) [18].

The results of this study indicated that the effect of ASP with I3C for seven days showed normal histological structure and no inflammatory cells infiltration in mucosa. These findings strongly support the hypothesis that I3C attenuates aspirin-induced neutrophil accumulation by inhibiting production of proinflammatory cytokines. I3C decreased the mucosal NOS activity, which suggest that gastroprotective effect of I3C may be due to the reduction of reactive oxygen species and NO toxicity [13]. The anti-inflammatory activity of I3C was associated with its ability to inhibit the production of pro-inflammatory cytokines such as IL-1 or TNFα and inducible NO synthase. I3C preserves erythrocytes against oxidative stress and exhibit strong antioxidant activity in vitro and in vivo model. The ability of I3C to enhance antioxidant enzymes demonstrates its possible preventative value in the inhibition of ulcerogenesis involving free radical reactions. This may be

possible by blocking oxidative damage through lipid peroxidation. In addition, I3C prevents loss of membrane permeability and dysfunction of cellular proteins, leading to survival of the functionally active cells [19].

On the other hand, [20] suggested that the cytoprotective action of I3C on the duodenal mucosa may be related to an increase in prostaglandin E2 production in mucosal of duodenum 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.

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

Conclusions:

In conclusion, it was found that indole-3-carbinol produced a deep permanent protection from aspirin induced duodenal damage. Combining I3C as gastroprotective and antioxidant phytochemical substance with either ASP in the treatment of duodenal damage is potentially a new approach for decreasing gastrointestinal injury caused by aspirin and other NSAIDs.

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