EFFECTS OF VINEGAR ACID ON THE DUODENUM TISSUES IN RATS. HISTOPATHOLOGICAL AND HISTOCHEMICAL STUDIES

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Abstract

The study aimed to investigate the histological and histochemical changes on the duodenum tissues induced by vinegar acid (VA) at a dose of 1 ml/kg/day (5 % and 10 %) in female rats for two weeks. Thirty rats were used, they were divided into three groups, the first group was given distilled water as the control group, the second group was given VA with a dose [1 ml/kg (5 %)] and the third group was given VA with a dose [1 ml/kg (10 %)] for two weeks. Histopathological statement of the duodenum tissues showed many vacuolations in epithelial cells, decrease in the number of goblet cells, erosion of mucosal epithelium, inflammatory cells infiltration in lamina propria, and submucosal oedema in all treated groups. Although that the histochemical study with PAS-reaction and bromophenol blue technique showed a decrease in the carbohydrate content and protein content in treated rats as compared with the control group. In conclusion, this study showed that vinegar acid was a harmful agent associated with histopathological changes that caused acute duodenum lesions and ulcers.

Keywords: Vinegar; Duodenum; Histochemical; Histopathological; Rats.

Introduction

The ulcer is an injury in the coating of the stomach or duodenum and is created by the deterioration of the gastric mucosa (Parimelazhagan, 2015). The peptic ulcer has initiated as open craters or sores in the inner lining of the mucosa of the stomach and duodenum (Gopinathan and Nija, 2014). A coating of mucus and biochemical substances normally shields the duodenum from digesting itself. When these protective mechanisms are disturbed, powerful digestive acids can erode into the lining of these organs and cause ulcers (Gopinathan and Naveenraj, 2013 and El-Shinnawy *et al.*, 2014). The ulcer occurs due to an imbalance between endogenous aggressive factors and cytoprotective factors of the gastric mucosa (Araujo *et al.*, 2011 and AlRashdi *et al.*, 2012).

Vinegar acid (VA) is the volatile organic acid that identifies the product as vinegar consists of about 3 to 10 % of vinegar content and is responsible for the tart flavor and pungent, biting odor of kinds of vinegar (Mahmoodi *et al.,* 2013). Moreover, the etiology of vinegar acid-induced ulcers mimics human gastric and duodenal ulcers in location, chronicity, and severity (Okabe and Amagase, 2005 and Alshailabi *et al.,* 2019). VA is absorbed from the gastrointestinal tract and through the lungs and almost completely oxidized by tissues. The toxic effects of VA are due to irritant properties as well as its effect on the central nervous system and kidneys (Chibishev *et al.,* 2013). Additionally, the genesis of VA-induced gastric lesions is a multifactorial process that starts mainly with the depletion of gastric wall mucous content. Such depletion is often associated with the significant production of free radicals, causing damage to the cell and cellular membrane due to excessive oxidative stress (Alshailabi *et al.,* 2019). The generation of reactive oxygen species, for example, superoxide anion, hydrogen peroxide, and hydroxyl radicals may cause lipid peroxidation, especially in membranes, and results in tissue injury (Almasaudi et al., 2017). Local effects of VA ingestion represent corrosive injury to the upper gastrointestinal tract (Kamijo *et al.,* 2000 and Alshailabi *et al.,* 2019). The VA produces round, deep ulcers in the stomach and duodenum, resembling a great extent human ulcer in terms of both pathological features and healing drugs (Kang *et al.,* 2010). The present study was aimed to evaluate the histopathological and histochemical changes by the vinegar acid on duodenum tissues in rats.

Material and Methods

Experimental Chemicals

Vinegar acid was obtained from Omar Al-Mokhtar University. Animals were given VA orally by gavage at a dose of 1 ml/ kg/ body weight/ day (Pastrelo *et al.,* 2017) for two weeks. VA was given to animals in this study at two concentrations:

- Rats were given (5 % of VA) according to Soykan *et al.,* (2015).
- Rats were given (10 % of VA) according to Souza *et al.,* (2007).

Experimental Animals

The present study was conducted using healthy female albino rats (*Rattus norvegicus*) with an average weight of 180-225 g. Animals were obtained from the animal house of the Zoology Department, Faculty of Science, University of Omar Al-Mokhtar, El-Beida, Libya. All animals were allowed 3 weeks per-experimentation period to acclimatize to laboratory conditions in order to avoid any complications along the course of the experiment. They were housed in cages at room temperature. Rats were fed with a laboratory diet and water ad libitum with

fresh daily supplies. All rats were weighed weekly and the weight was recorded before the experimental procedures and at the end of the experiment.

Experimental Design

In the present study, a total number of 30 female albino rats were used. All rats were abstained from food for 24 hours with given the water *ad libitum* prior to the experimental procedures then they were randomized into three groups 10 rats in each:

- Normal control group (NC): Rats were given orally distilled water for two weeks
- Treated group (TG1): Rats were given orally VA (5 %) by gavage at a dose of 1 ml/ kg/ b. w./ day for two weeks.
- Treated group (TG2): Rats were given orally VA (10 %) by gavage at a dose of 1 ml/ kg/ b. w./ day for two weeks.

After the completion of the treatment period, all rats fasted for 24 hours. Animals were sacrificed then the duodenum was removed.

Histopathological and Histochemical Examinations

Small pieces of the duodenum were taken from two distinct areas, washed in normal saline, dried, and placed in 10 % buffered formalin for histological and histochemical examinations. Dehydration of fixed tissues was carried out using ascending grades of ethyl alcohol (70 %, 90 %, and 100 %), then cleared with xylene. Infiltration with paraffin wax at 60 ºC was followed by embedding. Paraffin blocks were cut at 5 microns thick from all specimens, using a Cambridge Rocking Microtome, affixed to slides (Lillie, 1954), and stained with:

- Haematoxylin and Eosin (H and E) for general histological examination (Harris, 1990).
- Periodic Acid Schiff (PAS) technique (Drury and Wallington, 1980) for carbohydrates.
- Bromophinol blue (BPhb) technique (Mazia *et al.*, 1953) for total proteins.

Results

Histopathological Evaluation

The control female rats section showed the normal histological structure of the villi with covering lining mucosal epithelium, normal mucosal layers, normal ducts, crypts of Lieberkuhn, and goblet cells Figures (1) and (2). Microscopically, the

duodenum of female rats treated with 5% of VA revealed loss of normal architecture, many vacuolations in epithelial cells, decrease in the number of goblet cells, erosion of mucosal epithelium, inflammatory cells infiltration in lamina propria, and submucosal oedema Figures (3) and (4). VA treated groups with VA-10% showed, lost tips of villi with desquamated of mucosal epithelium and degenerative areas containing cellular debris "surface deeper ulceration" were revealed in Figure (5), severe vacuolations in epithelial cells decrease in the number of goblet cells, erosion of mucosal epithelium, oedematous of the lamina propria and many mononuclear cells infiltrate cells in lamina propria Figures (6) and (7).

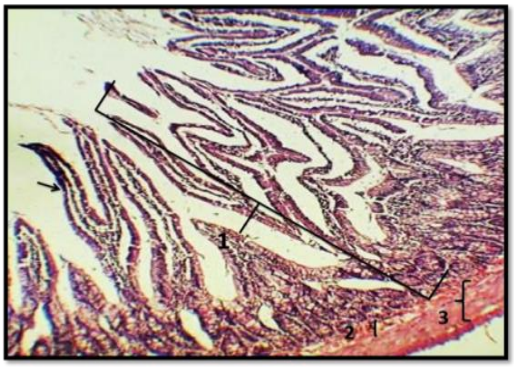

Figure (1): Photomicrograph of the Duodenum Section of Control Female Rats Showing, Normal Histological Structure of the Villi (Arrow) with Covering Lining Mucosal Epithelium and Normal Mucosal Layers (Mucosa (1), Submucosa (2) and Muscularis Externa (3)) (H & E stain, X100).

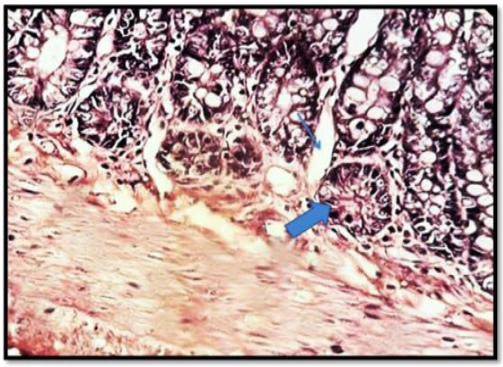

Figure (2): Photomicrograph of the Duodenum Section of Control Female Rats Showing, Normal Histological Structure of Ducts (Thin Arrow), Crypts of Lieberkuhn (Thick Arrow) and Some Goblet Cells (H & E Stain, X400).

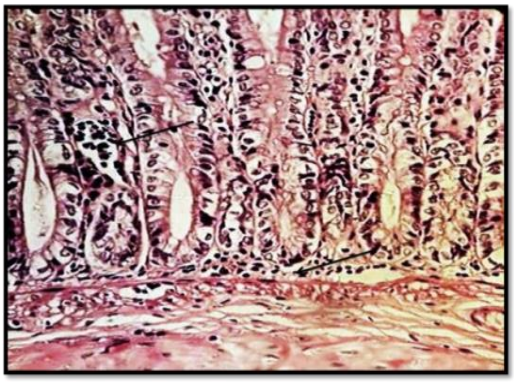

Figure (3): Photomicrograph of the Duodenum Section of Female Rats Treated with Vinegar Acid 5 % Showing, Inflammatory Cells Infiltration in Lamina Propria (Thin Arrows) and Submucosal Oedema (Thick Arrows) (H & E Stain, X400).

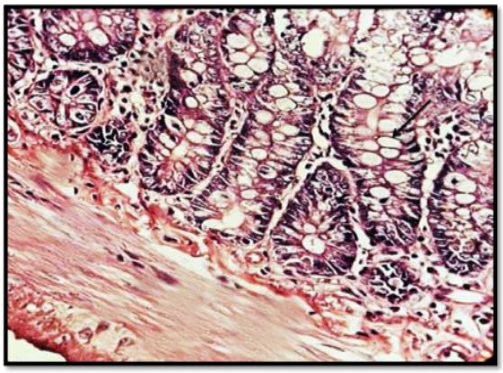

Figure (4): Photomicrograph of the Duodenum Section of Female Rats Treated with Vinegar Acid 5 % Showing, Many Vacuolations in Epithelial Cells (Arrow) (H & E Stain, X400).

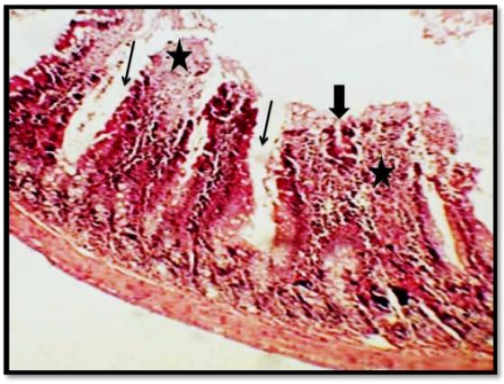

Figure (5): Photomicrograph of the Duodenum Section of Female Rats Treated with Vinegar Acid 10 % Showing, Lost Tips and Desquamated of Villi in Mucosal Epithelium "Surface Deeper Ulceration" (Thick Arrow), Degenerative Areas Containing Cellular Debris (Stars) and Inter Villous Space (Thin Arrows) (H & E Stain, X100).

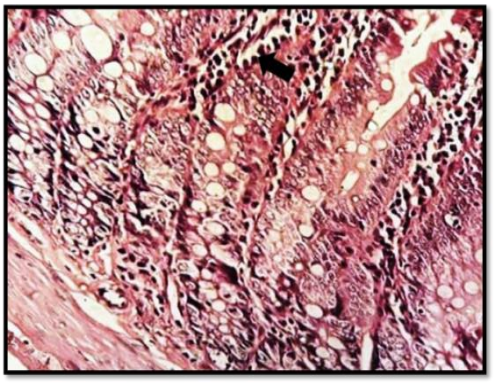

Figure (6): Photomicrograph of the Duodenum Section of Female Rats Treated with Vinegar Acid 10 % Showing, Severe Vacuolations in Epithelial Cells (Thin Arrow), Many Mononuclear Cell Infiltrates Cells in Lamina Propria (Thick Arrow) (H & E Stain, X400).

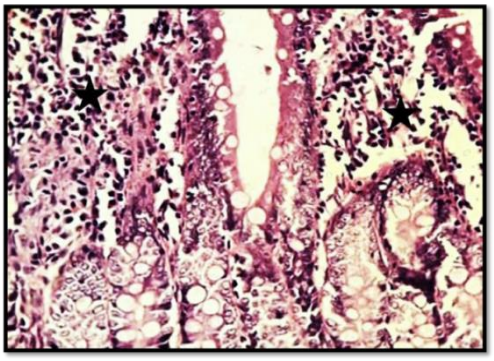

Figure (7): Photomicrograph of the Duodenum Section of Female Rats Treated with Vinegar Acid 10 % Showing, Severe Vacuolations in Epithelial Cells (Arrow), Degenerative Areas Containing Cellular Debris and Oedematous of the Lamina Propria (Stars) with Increase in Mononuclear Cell Infiltrates Cells (H & E Stain, X400).

Histochemical Investigation

The Periodic Acid Schiff's Reaction (PAS-Reaction)

The periodic acid Schiff reaction (PAS) showed a high positive reaction in the surface mucous cells and a high increase in PAS reactive in goblet cells, crypts, and muscularis mucosa among control duodenum sections Figures (8) and (9). Female rats treated with 5 % VA showed a marked decrease in PAS reactive mucosal carbohydrate content of surface cells and goblet cells, crypts, and muscularis mucosa Figures (10) and (11). In addition, female rats treated with 10 % of VA showed a

marked decrease or loss in PAS reactive mucosal carbohydrate content of surface cells and goblet cells, crypts, and muscularis mucosa Figures (12) and (13), that compared with the control group.

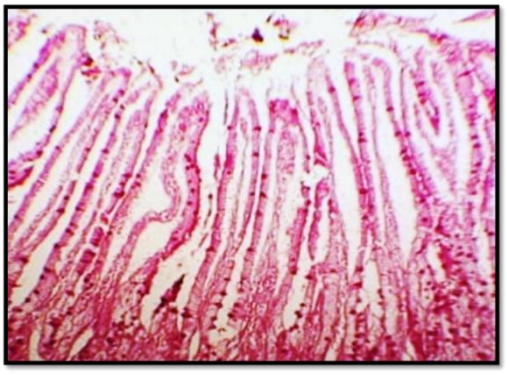

Figure (8): Photomicrographs of PAS-Reacted Duodenum Section of Control Female Rats Showing, a High Positive Reaction in the Surface Mucous Cells and High Increase in PAS Reactive in Goblet Cells (PAS Stain, X100).

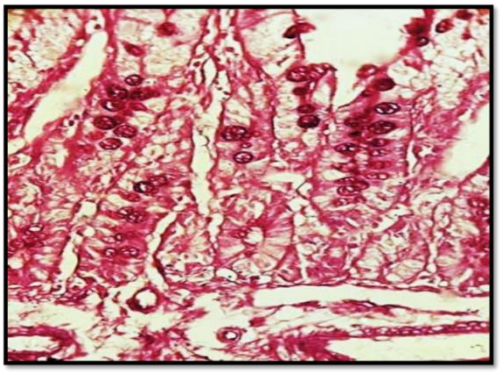

Figure (9): Photomicrographs of PAS-Reacted Duodenum Section of Control Female Rats Showing, a High Positive Reaction in Goblet Cells, Crypts and Muscularis Mucosa (PAS Stain, X400).

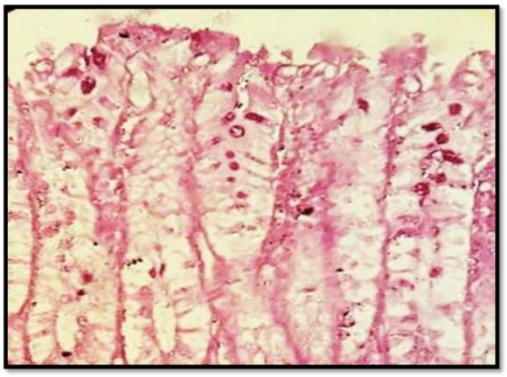

Figure (10): Photomicrographs of PAS-Reacted Duodenum Section of Female Rats Treated with Vinegar Acid 5 % Showing, Marked Decrease in PAS Reactive Mucosal Carbohydrate Content of Surface Cells and Goblet Cells (PAS Stain, X400).

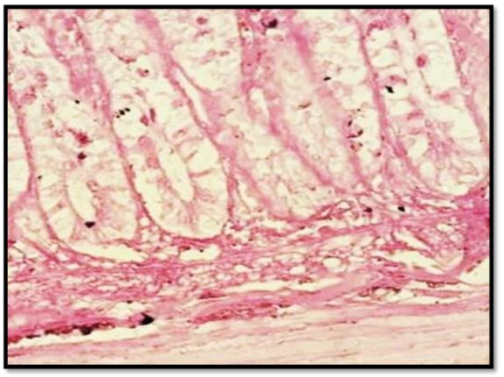

Figure (11): Photomicrographs of PAS-Reacted Duodenum Section of Female Rats Treated with Vinegar Acid 5 % Showing, Marked Decrease in PAS Reactive Mucosal Carbohydrate Content in Goblet Cells, Crypts and Muscularis Mucosa (PAS Stain, X400).

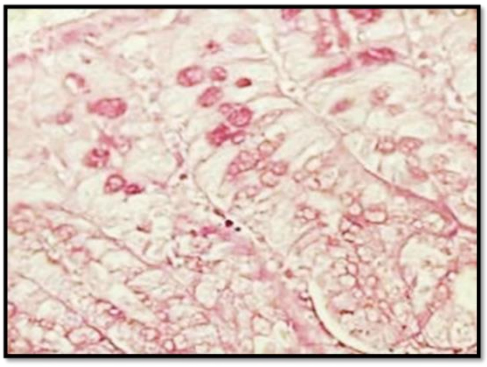

Figure (12): Photomicrographs of PAS-Reacted Duodenum Section of Female Rats Treated with Vinegar Acid 10 % Showing, Marked Decrease or Loss in PAS Reactive Mucosal Carbohydrate Content of Surface Cells and Goblet Cells (PAS Stain, X400).

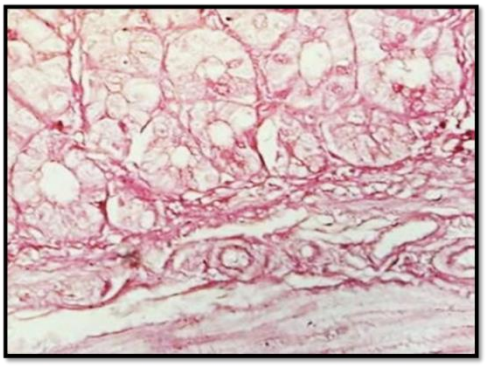

Figure (13): Photomicrographs of PAS-Reacted Duodenum Section of Female Rats Treated with Vinegar Acid 10 % Showing, Marked Decrease or Loss in PAS Reactive Mucosal Carbohydrate Content in Goblet Cells, Crypts and Muscularis Mucosa (PAS Stain, X400).

The Bromophenol Blue Reaction (Bphb-Reaction)

A strong reactivity in protein contents is located mainly in a mildly reactive ground cytoplasm. The nuclei of cells exhibited a strong reactivity with the bromophenol blue technique as seen in Figure (14). The cytoplasm and nuclei of the surface mucous cells and the mucous neck cells showed a strong reactivity in protein contents. Decreased protein content in the cytoplasm and nuclei were noticed in a group of female rats treated with 5 % of VA Figure (15). Moreover, the duodenum section of female rats treated with 10 % of VA showed a rather weak or feeble stainability with bromophenol blue was quite clear in the constituent cells Figure (16).

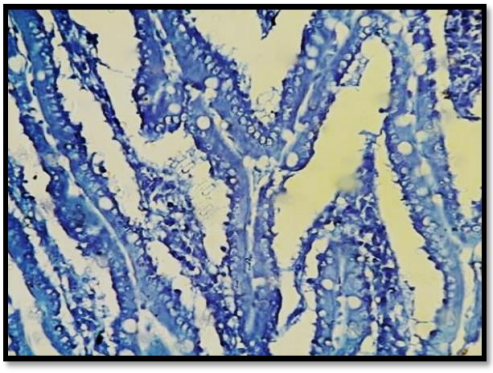

Figure (14): Photomicrographs of Bphb-Reacted Duodenum Section of Control Female Rats Showing, a Strong Reactivity in Protein Contents (Bphb Stain, X400).

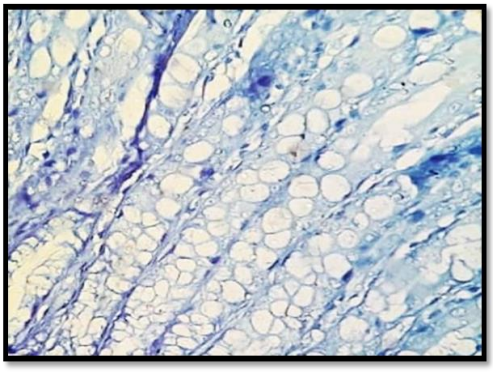

Figure (15): Photomicrographs of Bphb-Reacted Duodenum Section of Female Rats Treated with Vinegar Acid 5 % Showing, Decrease Reactivity in Protein Content (Bphb Stain, X400).

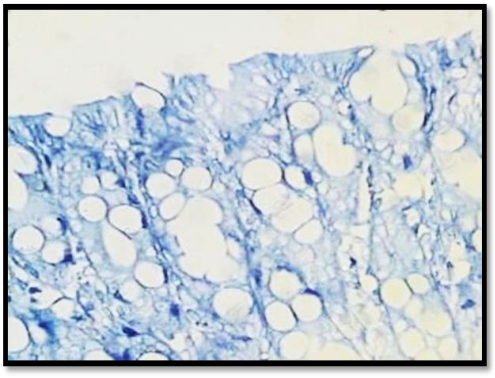

Figure (16): Photomicrographs of Bphb-Reacted Duodenum Section of Female Rats Treated with Vinegar Acid 10 % Showing, Weak or Feeble Stainability in the Protein Content (Bphb Stain, X400).

Discussion

Histopathological investigation of the duodenum section of female rats treated with 5% of VA in the present study showed loss of normal architecture, many vacuolations in epithelial cells, decrease in the number of goblet cells, erosion of mucosal epithelium, inflammatory cells infiltration in lamina propria, and submucosal oedema. Moreover, results in VA-treated groups with VA-10% presented lost tips of villi with desquamated of mucosal epithelium and degenerative areas containing cellular debris, severe vacuolations in epithelial cells decrease in the number of goblet cells, erosion of mucosal epithelium, oedematous of the lamina propria, and many mononuclear cells infiltrate cells in lamina propria. Similar results have been reported by Al-Rejaie *et al.* (2013), they revealed many inflammatory cells infiltration in the treated colon with VA. They suggested that the VA-induced colitis model is known to cause vascular dilatation and white blood cells accumulation, as well as an increase in blood flow, leading to increased production of oxygen and hence the excessive generation of free radical and ROS. Inflammatory cytokines are known to play a crucial role in modulating the mucosal immune system where the neutrophils and macrophages are responsible for disrupting epithelial integrity and causing colon injury. The pathogenesis of ulcer is characterized by migration of granulocytes and other leukocytes to the inflamed mucosa and superficial ulcers leading to increased levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) (Grisham, 1994 and Pavlick *et al.,* 2002). Oxidative stress causes the production of free radicals that elevate inflammatory mediators and damage intestinal mucosa (Tanideh *et al.,* 2014). Similar results have been reported by Abd El Maguid (2006), who found that colchicine administration revealed mononuclear cells infiltration and edema in lamina propria with large degenerative areas containing cellular debris and small multi-hemorrhagic areas in the examined intestine of rats. This may be due to the intestinal pathology after large doses of colchicine are probably related to the

cessation of cellular proliferation and has been compared to that observed in acute radiation injury of the bowl. The vacuolations of enterocytes observed in this work were confirmed by Hummadi (2012), who concluded that these cytoplasmic vacuoles are responsible for collecting the injurious elements and preventing them from interfering with the biological activities of cells. Furthermore, he suggested that the cellular degeneration might be attributed to the liberation of acid hydrolysis released from the destructed lysosomes to facilitate the process of autolysis. It was evident that the degenerative changes appeared earlier in the cytoplasm than in the nuclei, which explained that the nuclear damage is a sequence of cytoplasmic damage.

On the other hand, the results in this study showed a marked decrease in PAS reactive mucosal carbohydrate content of surface cells and goblet cells, crypts, and muscularis mucosa in rats treated with 5 % of VA. In addition, female rats treated with 10 % of VA showed a marked decrease or loss in PAS reactive mucosal carbohydrate content of surface cells and goblet cells, crypts, and muscularis mucosa as compared with the control group that showed a high positive reaction in the surface mucous cells and high increase in PAS reactive in goblet cells, crypts, and muscularis mucosa. Similar results resembled those of Schumacher *et al.* (2004) who stated that carbohydrate content histochemistry by PAS-Reaction in control rats demonstrated variation in the staining properties of the different mucus-producing cell types at the duodenum section in of rats investigated, where the results showed that the surface cells and goblet cells contained strong carbohydrate or glycogen content. A decrease in PAS reactive mucosal carbohydrate content in duodenum sections in the present work in treated groups may be due to a decrease in goblet cells in VA groups. So, most of the cells contained little mucus or were depleted. The present findings are similar to those in mammals, in that the goblet cells are the source of acid mucopolysaccharides (Hamdi *et al.,* 2014). They also found that the goblet cell in the alimentary tract contains mucoid secretions of an acid mucoprotein nature. VA showed a marked decrease or loss in PAS reactive mucosal carbohydrate content of surface cells and goblet cells, crypts, and muscularis mucosa as compared with the control group. Similar these results were in agreement and confirming the findings of some researchers by (El-Azab *et al.* 2018), they reported that ethanol-induced ulcer was associated with a decrease of PAS-positive reaction in fundic mucosal cells as a result of ethanol damaging effect on the mucus cells or excessive oxidative stress, this cause may be due to decrease in the collagen fibers deposition and expression TNF-α at the place of injury.

Moreover, the results of the current study showed a strong reactivity in protein contents located mainly in a mildly reactive ground cytoplasm, the nuclei of the surface mucous cells and the mucous neck cells showed strong reactivity with the bromophenol blue technique. While decreased the protein content in the cytoplasm and nuclei showed in rats treated with 5 % of VA. Moreover, the duodenum section

of rats treated with 10 % of VA showed a rather weak or feeble stainability with bromophenol blue. Proteins constitute a major part of the living protoplasm of animal cells. The function of protein is not only to supply energy but also to maintain nitrogen balance and furnish certain essential components of the living tissue to the organism, the formation of enzymes, certain hormones, and other physiologically important compounds (Mahmoud, 2006). Decreased total protein observed in the present study in duodenum tissues may be due to the degenerative changes that were noticed in the tissue or maybe also due to increased reactive oxygen species production which harms the mitochondria (Cogger *et al.,* 2004). Sakr *et al.* (2012) showed that the administration of the drug caused a significant decrease in the proliferation, nucleic acids, protein synthesis, and protein kinase C activity in the membrane of cells from the damaged tissues. VA rats showed a decrease in protein content in tissues of the duodenum. This decrease may be due to a decrease in ribosomal granules of the rough endoplasmic reticulum or due to a decrease in DNA content. The decrease of DNA content was associated with a decrease in protein content in the gastrointestinal (Shaffie *et al.,* 2010).

Conclusion

The present findings demonstrate that the massive histopathological and histochemical changes in the mucosae of the duodenum were the results of the consumption of vinegar acid for two weeks in female albino rats that, caused acute duodenum lesions and ulcers.

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