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The histopathological and histochemical alterations of the gastric ulcer induced by acetic acid in rats

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Abstract:

The pathophysiology of gastric ulcer has centered on an imbalance between aggressive and protective factors in the stomach. The aim of this study was to evaluate the histopathological and histochemical changes in the rat's gastric ulcer induced by acetic acid (AA). For this purpose, thirty albino female rats were divided into three groups, each group carried ten rats. Group I was given distilled water for 14 days and served as the control group, while group II was given 1 ml/ kg/ day (5 %) of AA and group III was given 1 ml/ kg/ day (10 %) of AA orally by gavage for 14 days. Results showed the appearance of some histopathological changes including exfoliated dead cells appear in the lumen in the stomach, deeper ulceration with the onset of necrosis and focal necrosis of epithelium with the disrupted cell membrane in AA-5 %. Furthermore, the AA-10 % showed dilatation of the glandular lumina and hemorrhagic lesions in the mucosa of the glandular stomach with the aggregation of inflammatory cells near the bases of the gastric pit and the muscular mucosa. Although the histochemical studies with PAS-reaction and bromophenol blue technique showed a decrease in the carbohydrate and protein content in treated rats. In conclusion, this study showed that acetic acid was a harmful agent associated with histopathological changes that caused acute gastric lesions and ulcers.

Keywords: Histopathological, Gastric ulcer, Histochemical, Acetic acid, Rat.

Introduction:

Gastric ulcer is caused by an imbalance between aggressive factors such as hydrochloric acid, pepsin, bile acids, food ingredients, *Helicobacter pylori*, non-steroidal inflammatory drugs, leukotrienes, smoking, alcohol, trauma, shock, reactive oxygen species (ROS) and protective mechanisms

such as mucous gastric mucosal barrier in the stomach tissues (*1-3*). Local mechanisms associated with mucosal defense are mucous alkaline secretion, mucosal hydrophobicity, rapid epithelial cell regeneration and rich mucosal blood flow (*4, 5*). Many factors such as gastric microcirculation, prostaglandin E2 (PGE2) content and pro-inflammatory cytokines interleukin (IL)-1 and tumor necrosis factor (TNF) play important roles in the genesis of gastric mucosal damage, and its subsequent development (*6, 7*). PGE2 and I2 are the predominant prostaglandins synthesized by the gastric mucosa and are known to inhibit the secretion of gastric acid and stimulate the secretion of mucus and bicarbonate (*1*). Gastric ulcer produced by acetic acid is due to the release of histamine, which increases the capillary permeability and back diffusion of hydrochloric acid (*6*). Moreover, Okabe and Amagase (*8*) indicated that the etiology of acetic acid-induced ulcers mimics human gastric and duodenal ulcers in location, chronicity, and severity. Acetic acid (AA) produced gastric ulcers by stimulating gastric acid hypersecretion and the statement of histamine, which increases the capillary permeability and back diffusion of Hydrochloric acid (*9*). Acetic acid is a widely used organic acid with corrosive properties that depend on its concentration. At concentrations between 5 % and 8 %, it is mainly found in vinegar and is safe for consumption as a vegetable preservative or condiment, and between 30 % and 90 %, it is used as an antiseptic or a household cleaning agent. In some countries 80 % acetic acid in the preparation of pickled food (*10*). Local effects of AA ingestion represent corrosive injury to the upper gastrointestinal tract (*11*). The acetic acid produces round, deep ulcers in the stomach, resembling a great extent human ulcer in terms of both pathological features and healing drugs (*12*). The aim of this study was to assess the histopathological and histochemical changes of the gastric ulcer induced by acetic acid in female albino rats.

Materials and Methods :

Chemicals:

Acetic acid (CH_3COOH) (99.8 %, Sigma-Aldrich). Acetic acid was obtained from Omar Al-Mukhtar University.

Experimental animals:

The present study was conducted using 30 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 Science, University of Omar Al-Mukhtar, El-Beida, Libya. All animals were allowed three 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 laboratory diet and water *ad libitum* with fresh daily supplies.

Experimental design:

Thirty female rats were abstained form 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:** Rats were given orally distilled water for 14 days.
- **Treated group 1:** Rats were given orally AA (5 %) by gavage at a dose of 1 ml/ kg/ b.w./ day according to (*13*) for 14 days.
- **Treated group 2:** Rats were given orally AA (10 %) by gavage at a dose of 1 ml/ kg/ b.w./ day according to (*13*) for 14 days.

After the completion of the treatment period, all rats were sacrificed then the stomach was removed and fixed in the suitable fixative for histopathological and histochemical studies (*14*).

Histopathological and histochemical studies:

Small pieces of the stomach were fixed in formalin 10 % and embedded in paraffin. All sections of 5µm thickness were stained with Harris's hematoxylin and eosin satin (H and E) for histopathological examination (*15*), the periodic acid Schiff's reaction (PAS), (*16*) as applied for carbohydrate demonstration for histochemical investigation, and the mercuric bromophenol blue method (BPhb) (*17*) as applied for total proteins demonstration for histochemical investigation.

Results:

Histopathological examination:

Microscopically, the stomach of control groups showed normal histological structure, normal mucosal layers and cells, normal gastric pits and gastric glands (Fig, 1), whereas the stomach of rats treated with 5 % of AA exposed some histopathological alteration. The mucous neck at luminal parts of the gastric pits showed exfoliated dead cells appear in the lumen, deeper

ulceration with the onset of necrosis, focal necrosis of epithelium with disrupted cells membrane. However, in other parts of the gastric mucosa cells noticed damaged cytoplasm and karyolitic nuclei with marked aggregation of lymphocytic inflammatory cells were showed in figure (2). Additionally, (fig. 3) showed focal necrosis of epithelium with the disrupted cell membrane, damaged cytoplasm, and karyolitic nuclei, deeper mucosal ulceration and marked aggregation of lymphocytic inflammatory cells. Acetic acid-treated groups with 10 % showed many eroded areas, involving evidence of damage in the form of vacuolated or lightly stained cytoplasm with pyknotic or fragmented nuclei, most of the parietal cells among glands region, appeared faintly stained cytoplasm with pyknotic or karyolitic nuclei. Dilatation of the glandular luminae and hemorrhagic lesions in the mucosa of glandular stomach "Ulceration at the mid part of the mucosa" were also observed (fig. 4). Moreover, the connective tissues of the lamina propria between the gastric pits were demolished with the aggregation of inflammatory cells near the bases of the gastric pit and the muscular mucosa. In these regions were noticed submucosal necrotic area (fig. 5).

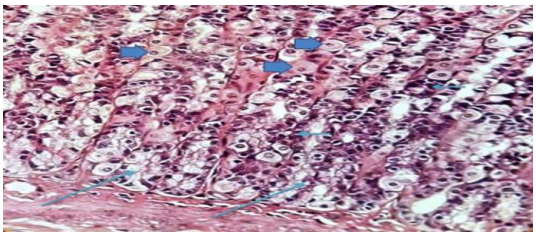

Figure 1: Photomicrograph of the stomach section of control female rats showing, normal histological structure, normal parietal cells (thick arrows), chief cells (small arrows) and gastric glands (thin arrows) (H & E stain, X400).

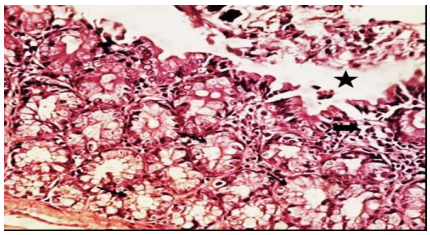

Figure 2: Photomicrograph of the stomach section of female rats treated with acetic acid 5 % showing, exfoliated dead cells appear in the lumen, deeper ulceration with onset of necrosis (star), focal necrosis of epithelium with disrupted cells membrane, damaged cytoplasm and karyolitic nuclei (arrows) with marked aggregation of lymphocytic inflammatory cells (thick arrows) (H & E stain, X400).

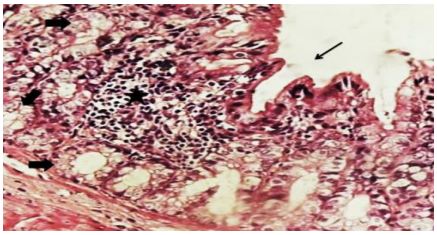

Figure 3: Photomicrograph of the stomach section of female rats treated with acetic acid 5 % showing, focal necrosis of epithelium with disrupted cells membrane, damaged cytoplasm and karyolitic nuclei (thick arrows), deeper mucosal ulceration (thin arrow) and marked aggregation of lymphocytic inflammatory cells (star) (H & E stain, X400).

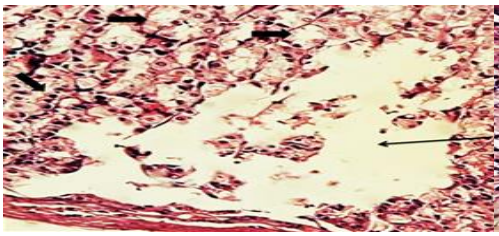

Figure 4: Photomicrograph of the stomach section of female rats treated with acetic acid10 % showing, vacuolated or lightly stain cytoplasm with pyknotic nuclei (thick arrows), dilatation of the glandular luminae and hemorrhagic lesions in the mucosa of glandular stomach "Ulceration at mid part of the mucosa"(thin arrow) (H & E stain, X400).

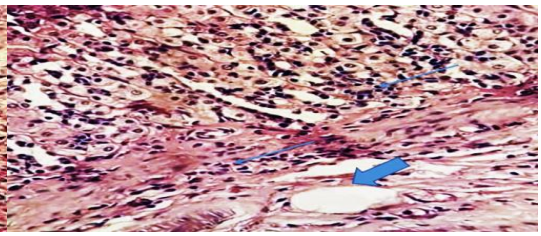

Figure 5: Photomicrograph of the stomach section of female rats treated with acetic acid 10 % showing, demolished of connective tissues with the aggregation of inflammatory cells in the lamina propria and the muscularis mucosa (thin arrows) and submucosal necrotic area (thick arrow) (H & E stain, X400).

Histochemical examination:

The periodic acid Schiff reaction (PAS) of control rats showed a high positive reaction in the surface mucous cells and a high increase in PAS reactive substance extending down along gastric glands, between the gastric pit and muscularis mucosa among control stomach sections (Figs. 6 and 7). Whereas, rats treated with 5 % of AA showed moderate-increase in PAS reactive mucosal carbohydrate content of surface cells and between gastric pit, also in muscularis mucosa. While was noticed decrease in PAS reactive substance extending down along gastric glands (Figs. 8 and 9). Also, rats treated with 10 % of AA showed the same reaction in PAS reactive mucosal carbohydrate content (Figs. 10 and 11), when compared with 5 % group.

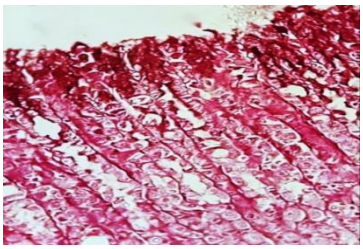

Figure 6: Photomicrographs of PAS-reacted stomach section of control female rats showing, a high positive reaction in the surface mucous cells and high increase in PAS reactive between gastric pit (PAS stain, X400).

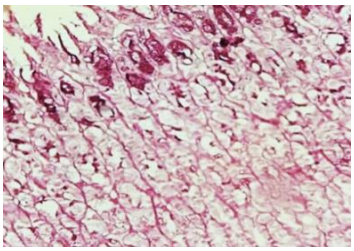

Figure 7: Photomicrographs of PAS-reacted stomach section of female rats treated with acetic acid 5 % showing, moderate-increase in PAS reactive mucosal carbohydrate content of surface cells and between gastric pit (PAS stain, X400).

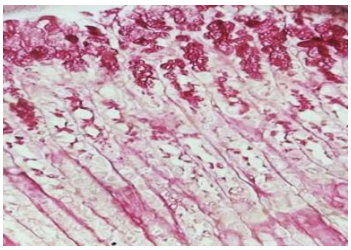

Figure 8: Photomicrographs of PAS-reacted stomach section of female rats treated with acetic acid 10 % showing, moderate-increase in PAS reactive mucosal carbohydrate content of surface cells and between gastric pit (PAS stain, X400).

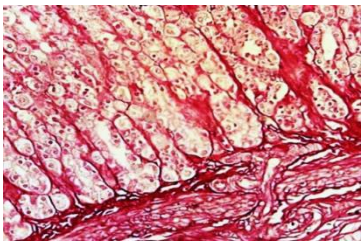

Figure 9: Photomicrographs of PAS-reacted stomach section of control female rats showing, a high positive reaction substance extending down along gastric glands and muscularis mucosa (PAS stain, X400).

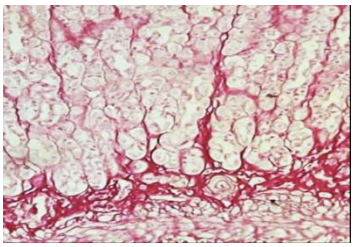

Figure 10: Photomicrographs of PAS-reacted stomach section of female rats treated with acetic acid 5 % showing, decrease in PAS reactive substance extending down along gastric glands and moderate-increase in muscularis mucosa (PAS stain, X400).

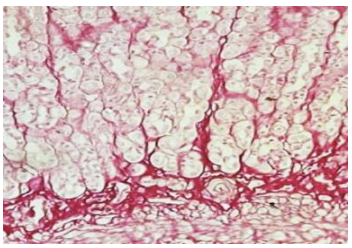

Figure 11: Photomicrographs of PAS-reacted stomach section of female rats treated with acetic acid 10 % showing, decrease in PAS reactive substance extending down along gastric glands and moderate-increase in muscularis mucosa (PAS stain, X400).

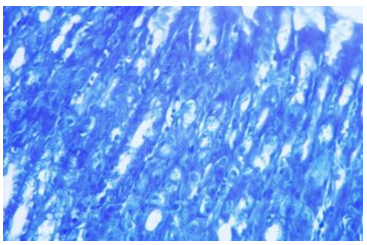

Figure 12: Photomicrographs of Bphb-reacted stomach section of control female rats showing, strong in the protein content in gastric tissue (Bphb stain, X400).

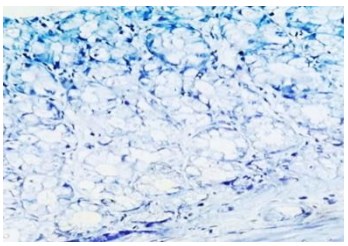

Figure 13: Photomicrographs of Bphb-reacted stomach section of female rats treated with acetic acid 5 % showing, decrease in the protein content in the cytoplasm and nucleus of the gastric cells (Bphb stain, X400).

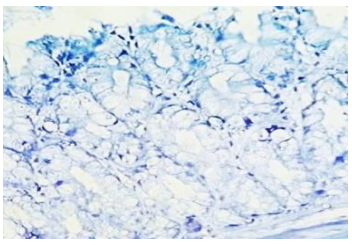

Figure 14: Photomicrographs of Bphb-reacted stomach section of female rats treated with acetic acid 10 % showing, weak or feeble stainability in the protein content in the cytoplasm and nucleus of the gastric cells (Bphb stain, X400).

Total protein was demonstrated in the present material by applying the bromophenol blue technique. Control groups showed a strong reactivity was displayed by the peptic cells and oxyntic cells. Their protein contents located mainly in a mildly reactive ground cytoplasm, the nuclei of these cells exhibited a strong reactivity as seen in figure (12). While, decreased the protein content in the cytoplasm and nuclei of oxyntic, peptic cells, surface mucous cells, and mucous neck cells were noticed in the group of rats treated with 5 % of AA (Fig. 13). Also, the stomach section from rats treated with 10

% of AA revealed a diminution in mucosal cells totals protein content. In such case a rather weak or feeble stainability with bromophenol blue was quite clear in the constituent cells (Fig. 14).

Discussion:

Gastric ulcers develop inside the stomach, affect many people worldwide, and represent a discontinuity in the gastric mucosal penetration through the muscularis mucosa (*18*). Neutrophils have a major cause of inflammatory mediators and can release potent ROS such as superoxide, hydrogen peroxide, and myeloperoxidase derived oxidants, as a result, they mediate lipid peroxidation, these reactive oxygen species are highly cytotoxic and can induce tissue damage. Also, oxygen-free radicals derived from infiltrated neutrophils in ulcerated gastric tissues and have an inhibitory effect on gastric ulcers healing in rats (*19*).

The present results indicate that treatment with acetic acid caused ulcer areas, mucosal erosions, submucosal oedema, inflammation cells, surface and part of deeper mucosal ulceration, necrosis focal areas of ulceration and mild mucosal ulceration, these marks were agreements with (*20*), they reported that the reduced bicarbonate secretion and mucosal cell proliferation may due to mucosal oedema, inflammation and ulceration seen in rats during ulcer suggests that rats' stomach have inherent higher inflammatory activities, a property of agents that have higher incidence of ulceration complications by selectively inhibiting cyclooxygenase-2 (COX-2) enzyme activity. These consequences in this work 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 (*21*).

Previous studies demonstrated that the inflammation in gastric mucosa by drugs is accompanied by increased production of tumor necrosis factor-α (TNF-α), which augments neutrophil-derived superoxide generation and stimulates the production of interleukin-1 (IL-1) leading to neutrophil accumulations (*22*). The genesis of acetic acid induced gastric lesions is a multifactorial process that starts mainly with the depletion of gastric wall mucous content (*23*). Such depletion is often associated with significant production of free radicals causing damage to the cell and cellular membrane due to excessive oxidative stress (*24*).The generation of ROS, for example,

superoxide anion, hydrogen peroxide, and hydroxyl radicals, may cause lipid peroxidation, especially in membranes, and results in tissue injury (*25*). In the current study, applying acetic acid to rats' gastric mucosa resulted in ulceration of the gastric mucosa that was associated with increased proliferation and apoptosis as indicated by DNA flow cytometry analysis. These observations are consistent with previous reports on the induction of apoptosis in ulcerative gastric mucosa (*18*).

In the current study, the acetic acid was able to decrease the mucin like glycoproteins in the stomach, as observed by staining with PAS that is critical cytoprotective glycoproteins due to their mucus secretion activities. Such an action may be caused by AA, which caused an increase the gastric acid secretion of the gastrointestinal tract injury by PGE2 (*18*). The decrease in PAS reactive mucosal carbohydrate content in stomach sections of the present work in treated groups may be due to a decrease in gastric cells in AA groups. So, most of the cells contained little mucus or were depleted. The present findings are similar to those in mammals, in that the gastric cells are the source of acid mucopolysaccharides (*26*). They also found that the gastric cell in the alimentary tract contains mucoid secretions of an acid mucoprotein nature. Moreover, results showed decreased the protein content in the cytoplasm and nuclei of oxyntic, peptic cells, surface mucous cells and mucous neck cells were showed in female rats treated with acetic acid. Moreover, decreased total protein observed in the present study in stomach tissues may be due to the degenerative changes that were noticed in the tissue or may be also due to increased ROS production which harms the mitochondria (*27*). This decrease may be due to a decrease in ribosomal granules of the rough endoplasmic reticulum or due to a decrease in DNA content (*28*).

Conclusions:

In conclusion, the present study indicated that the massive histopathological and histochemical changes in the stomach tissues were the results of the consumption of acetic acid for 14 days in female albino rats. Also, the acetic acid was a harmful agent associated with histopathological changes that caused acute gastric lesions and ulcers.

References:

1. Z. Bakaeva *et al.*, Glyprolines exert protective and repair-promoting effects in the rat stomach: potential role of the cytokine GRO/CINC-1. *J Physiol Pharmacol* **67**, 253 (2016).
2. S. Jaikumar, S. Ramaswamy, B. Asokan, T. Mohan, M. Gnanavel, Anti ulcer activity of methanolic extract of Jatropha curcas (Linn.) on Aspirin-induced gastric lesions in Wistar strain rats. *Research journal of pharmaceutical, Biological and chemical sciences* **1**, 886 (2010).
3. A. T. Salami *et al.*, Gastroprotective Properties of Manganese Chloride on Acetic Acid Induced Ulcer in Wistar Rats. *African Journal of Biomedical Research* **17**, 109 (2014).
4. M. Hajrezaie *et al.*, Biochanin a gastroprotective effects in ethanol-induced gastric mucosal ulceration in rats. *PloS one* **10**, (2015).
5. H. P. Rang, *Pharmacology*. (Churchill Livingstone, 2001).
6. M. Umamaheswari *et al.*, Antiulcer and in vitro antioxidant activities of Jasminum grandiflorum L. *Journal of ethnopharmacology* **110**, 464 (2007).
7. G.-Z. Wang *et al.*, Aspirin can elicit the recurrence of gastric ulcer induced with acetic acid in rats. *Cellular physiology and biochemistry* **20**, 205 (2007).
8. S. Okabe, K. Amagase, An Overview of Acetic Acid Ulcer Models—The History and State of the Art of Peptic Ulcer Research—. *Biological and Pharmaceutical Bulletin* **28**, 1321 (2005).
9. J. L. Wallace, Prostaglandins, NSAIDs, and gastric mucosal protection: why doesn't the stomach digest itself? *Physiological reviews* **88**, 1547 (2008).
10. Y. Kamijo, K. Soma, K. Iwabuchi, T. Ohwada, Massive noninflammatory periportal liver necrosis following concentrated acetic acid ingestion. *Archives of pathology & laboratory medicine* **124**, 127 (2000).
11. A. Chibishev *et al.*, Severe renal function impairment in adult patients acutely poisoned with concentrated acetic acid. *Archives of Industrial Hygiene and Toxicology* **64**, 153 (2013).

12. J. M. Kang *et al.*, Enhancement of gastric ulcer healing and angiogenesis by cochinchina Momordica seed extract in rats. *Journal of Korean medical science* **25**, 875 (2010).
13. M. M. Pastrelo *et al.*, Effect of concentrated apple extract on experimental colitis induced by acetic acid. *International journal of molecular and cellular medicine* **6**, 38 (2017).
14. R. Lillie. (Moscow, 1969).
15. H. Harris, After Bruce Casselman WC (1959). *Histochemical Technique, by Methuen and Co. Ltd*, (1900).
16. R. A. B. Drury, E. A. Wallington, S. R. Cameron, *Carleton's histological technique*. (London, 1967).
17. D. Mazia, P. A. Brewer, M. Alfert, The cytochemical staining and measurement of protein with mercuric bromphenol blue. *The Biological Bulletin* **104**, 57 (1953).
18. S. B. Almasaudi *et al.*, Manuka honey exerts antioxidant and anti-inflammatory activities that promote healing of acetic acid-induced gastric ulcer in rats. *Evidence-Based Complementary and Alternative Medicine* **2017**, (2017).
19. A. S. AlRashdi *et al.*, Mechanisms of gastroprotective effects of ethanolic leaf extract of Jasminum sambac against HCl/ethanol-induced gastric mucosal injury in rats. *Evidence-Based Complementary and Alternative Medicine* **2012**, (2012).
20. A. Ajayi, S. Olaleye, Age-Related Changes in the Response of the Rat Gastric Mucosa to Acetic acid-and Indomethacin-induced Ulceration. *Arch. Bas. App. Med*, 379 (2015).
21. M. Jainu, K. V. Mohan, C. S. Devi, Gastroprotective effect of Cissus quadrangularis extract in rats with experimentally induced ulcer. *Indian Journal of Medical Research* **123**, 799 (2006).
22. S. Kokura, R. E. Wolf, T. Yoshikawa, D. N. Granger, T. Y. Aw, T-Lymphocyte–Derived Tumor Necrosis Factor Exacerbates Anoxia-Reoxygenation–Induced Neutrophil–Endothelial Cell Adhesion. *Circulation research* **86**, 205 (2000).
23. K. Amagase, M. Yokota, Y. Tsukimi, S. Okabe, Characterization of" unhealed gastric ulcers" produced with chronic exposure of acetic acid ulcers to indomethacin in rats. *Journal of physiology and pharmacology* **54**, 349 (2003).
24. F. B. Potrich *et al.*, Antiulcerogenic activity of hydroalcoholic extract of Achillea millefolium L.: involvement of the

antioxidant system. *Journal of ethnopharmacology* **130**, 85 (2010).

25. L. M. da Silva *et al.*, Ethanolic extract of roots from Arctium lappa L. accelerates the healing of acetic acid-induced gastric ulcer in rats: Involvement of the antioxidant system. *Food and chemical toxicology* **51**, 179 (2013).
26. H. Hamdi, A. El-Ghareeb, M. Zaher, A. Essa, S. Lahsik, Anatomical, histological and histochemical adaptations of the reptilian alimentary canal to their food habits: II-Chamaeleon africanus. *World Appl. Sci. J* **30**, 1306 (2014).
27. V. C. Cogger *et al.*, The effects of oxidative stress on the liver sieve. *Journal of hepatology* **41**, 370 (2004).
28. N. M. Shaffie, F. A. Morsy, A. G. Ali, H. A. Sharaf, Effect of caraway, coriander and fennel on the structure of kidney and islets of Langerhan in alloxan-induced diabetic rats: Histological and histochemical study. *Journal of American Science* **6**, 405 (2010).