Trop J Nat Prod Res, July 2020; 4(7):299-303 ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Tropical Journal of Natural Product Research

Available online at https://www.tjnpr.org

Original Research Article

Gastro-protective Properties of *Acalypha torta* Leaf against HCl/Ethanol-Induced Ulceration

Ugochi O. Njoku, Bonaventure Uzowuru, Martins O. Ogugofor

Department of Biochemistry, University of Nigeria Nsukka, Enugu State, Nigeria.

ARTICLE INFO

Article history:
Received 25 June 2020
Revised 08 July 2020
Accepted 23 July 2020
Published online 27 July 2020

ABSTRACT

Acalypha torta is one of the plants used in the treatment of various diseases in traditional medicine. The plant is known to possess antidiarrhoeal, immunomodulatory and antimicrobial activities. Despite the ethnomedicinal uses of this plant, there is a paucity of studies on its gastroprotective activity. However, this study was designed to investigate the gastro-protective properties of *Acalypha torta* leaf extract. The gastro-protective effect of *Acalypha torta* leaf methanol extract was evaluated using HCl/ethanol ulcer-induction model. A total of twenty-four (24) animals were randomly grouped into six (6) treatment groups labelled groups 1-6. Groups 1 and 2 received 1% carboxymethyl cellulose (CMC). Group 3 received 30 mg/kg b.w lansoprazole while groups 4-6 received 200, 400 and 600 mg/kg b.w extract, respectively. The present findings revealed that methanol extract of *Acalypha torta* leaf exerted gastro-protective activity, as shown by its consistent and significant dose-dependent increase in mean ulcer index (MUI) inhibition. Histological investigation of gastric lesions showed that the plant stimulates the scarring cicatrizing process. The gastro protection observed could be attributed to the phytoconstituents of the plant extract which exerted gastro-protective and anti-ulcerogenic effects, possibly by increasing antioxidant enzymes thereby reducing oxidative stress. The gastro-protective properties of the extract could also be due to the ability of the extract to modulate the activities of H^+/K^+ATPase thereby normalizing the pH of the gastric juice. The extract possesses a dose dependent gastro protective activity.

Keywords: *Acalypha torta*, Lansoprazole, HCl-ethanol ulcer-induction, Ulcer index, Histology.

Introduction

Peptic ulcer disease (PUD) has been referred to as the collapse of the stomach and/or duodenum epithelial mucosal barrier, characterized by inflammation and ulcer formation.[1] *Helicobacter pylori* infection, use of non-steroidal anti-inflammatory drugs (NSAIDs), lifestyle factors such as diet, smoking, and alcohol use are some of the causes of peptic ulcer disease.[2] NSAID-induced ulcer is regarded as the second major cause of ulcer after *H. pylori*. NSAIDs such as aspirin, indomethacin and ibuprofen are known to inhibit prostaglandin synthesis through cyclooxygenase pathway.[3] Several plants have been reported to be effective in the treatment of peptic ulcer. Some of these plants include *Hibiscus* asper,[4] *Eremurus* spectabilis,[5] *Cnidoscolus* aconitifolius.[6] Serafino *et al.*[7] reported that many plant extracts are as effective as the synthetic drugs with no or insignificant adverse effect. *Acalypha torta* is one of the several useful plants in folk medicine. According to Ezekwesili and Nwodo.[8] *Acalypha torta* leaves have been useful in the treatment of high blood pressure in Nigeria folklore medicine. Irobi and Banso[9] reported the antimicrobial activity of *Acalypha torta* leaves against *Staphylococcus aureus, Serratia marcesens, Yersinia spp, Bacillus spp, Trichophyton rubrum, and T. mentagrophytes* while Ezekwesili and Nwodo[8] opined that the ethanolic leaf extract of the plant has demonstrated antidiarrhoeal, antithrombotic and immunosuppressive activities.

*Corresponding author. E mail: obinna.ogugofor.pg76810@unn.edu.ng

Tel: +2348105504783

Citation: Njoku UO, Uzowuru B, Ogugofor MO. Gastro-protective Properties of *Acalypha torta* Leaf against HCl/Ethanol-Induced Ulceration. Trop J Nat Prod Res. 2020; 4(7):299-303. doi.org/10.26538/tjnpr/v4i7.8

Official Journal of Natural Product Research Group, Faculty of Pharmacy, University of Benin, Benin City, Nigeria.

In addition, Ezekwesili *et al.*[10] reported the antihypertensive and relaxant effect of *Acalypha torta* leaves on vascular smooth muscle. *Acalypha torta* has also been used medicinally in the treatment of fungal infections.[11] Despite the medicinal importance of *Acalypha torta*, there is a paucity of studies on its possible gastro-protective effect. In this present study, the gastro-protective properties of *Acalypha torta* leaves methanol extract against HCl/ethanol-induced ulceration was investigated.

Materials and Methods

Collection of plant material

The plant sample was collected from Ozom Mgbagbu-Owa, Ezeagu local government area of Enugu State, Nigeria on 27th November 2019. The plant was authenticated by Mr. Alfred Ozioko of Bioresources Development and Conservation Programme (BDCP), Nsukka, Enugu State, Nigeria. The plant was assigned the voucher number: Intercedd/2964: *Acalypha torta*.

Extraction of Acalypha torta leaf

The leaves of the plant obtained were air-dried and pulverized into fine powder. The powder (1000 g) was macerated in ethanol (7 L) for 48 h. The mixture was filtered using muslin cloth and Whatman No. 1 filter paper. The filtrate was concentrated under pressure using a rotary evaporator at a temperature of 30^0C, to obtain a semi-solid extract.

Phytochemical evaluation

The screening for phytoconstituents of the plant leaf extract was carried out according to the methods of Harbone[12] and Trease and Evans.[13] Quantitative analysis was carried out as described by Harbone[12] and Soni and Sosa.[14]

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Animals
Male Wistar rats of weight range 150 – 180 g, used for this study were obtained from the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka.

Experimental design
After acclimatization, the animals were randomly distributed into six (6) groups having four (4) animals each. The extract and standard drug were administered for one week. The route of administration of the extract and standard drug was oral using oral intubation tube. The rats were handled according to the guidelines of the National Institute of Health on the case and use of laboratory animals (NIH, 1985). The research was approved by the Ethical Committee of Faculty of Biological Sciences, University of Nigeria Nsukka.
Group 1 served as the normal Control (1% CMC only) while Group 2 was the untreated group. Group 3 received 30 mg/kg b.w. lansoprazole while Groups 4, 5 and 6 received the graded doses of the extract (200, 400 and 600 mg/kg b.w, respectively).

Induction of ulcer using HCl/Ethanol model
After 24 hours of fasting, the graded doses of the extract of *Acalypha torta* leaf (200, 400 and 600 mg/kg b.w.) and 30 mg/kg b.w. lansoprazole were administered. One hour later, the ulcer induction was done according to a modified method of LiraMota *et al.*[15] using 0.8 mL of 5% 0.3 M HCl/60% ethanol in groups 2-6. The rats were monitored for one hour and sacrificed, and the stomachs removed and opened along the greater curvature to remove gastric content. The gastric contents were weighed using a graduated cylinder while the pH was determined by the aid of a digital pH meter (HI 9021). The ulcers were viewed and counted with the aid of a magnifying hand lens (×10) and the ulcerative lesion index was calculated based on the following keys: Mildly ulcered = 1, moderately ulcered = 2, severely ulcered = 3.
The ulcer preventive/inhibition index was calculated using the formula prescribed by Onwukwe *et al.*[16]

$$\text{Ulcer protective index (\%)} = \frac{\text{Ulcer index of untreated} - \text{Ulcer index of treated}}{\text{Ulcer index of untreated}} \times 100$$

Determination of gastric parameters
Gastric juice volume was determined according to the method of Kiranmai *et al.*[17] The collected gastric content was centrifuged at 3000 rpm for 10 min, then separated and the volume measured using a graduated cylinder.

Histological studies
The stomachs of the sacrificed rats were excised and immersed in 10% formalin solution. The fixed specimens were trimmed, washed and dehydrated in ascending grades of alcohol. The Specimens were further cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness and stained with heamtoxylin and eosin for examination as described by Drury *et al.*[18]

Statistical analysis
The results obtained from the study were statistically analysed using One-way ANOVA, and the Duncan multiple test range was used to compare means. The results were expressed as mean ± SD. The analysis was carried out using the IBM SPSS statistical package version 20.

Results and Discussion

Phytochemical evaluation of ethanol extract of Acalypha torta leaf
The phytochemical profiling of the extract indicated a relative abundance of steroids, flavonoids, alkaloids, saponins, cardiac glycosides and tannins as shown in Table 1. This agrees with the report of Irobi and Banso[9] on the phytochemical composition of the leaf of *Acalypha torta* where steroids, tannins, saponins and cardiac glycosides were detected. However, alkaloids and flavonoids were absent in the reports of Irobi and Banso.[9] According to Sumbul *et al.*,[19] flavonoids act in the gastrointestinal tract exerting anti-spasmodic, anti-secretory, anti-diarrheal, anti-ulcer and antioxidant activities. In addition, flavonoids offer protection against a variety of ulcerogenic agents through several mechanisms of action which include free radical scavenging and antioxidant properties, increased mucus secretion, anti-secretory action, and inhibition of *Helicobacter pylori* proliferation in the gastric mucosa.[19] Also, de Jesus *et al.*[20] reported that tannins act as ulcer preventive agents resulting from their protein precipitating effects. The astringent action of tannins has also been reported to precipitate microproteins on the ulcer site, thus forming an impervious layer over the lining, which inhibits ulceration.[19]

Effect of ethanol extract of Acalypha torta leaf on ulcer parameters
The results of the effect of *Acalypha torta* leaf extract on gastric juice volume, pH, ulcer index and ulcer protective index are shown in Table 2. Administration of HCl/Ethanol to experimental rats produced a significant increase ($p < 0.05$) in gastric juice volume as observed in the group treated with HCl/Ethanol only (2.83 ± 0.06 mL), when compared to the normal control (1.17 ± 0.13 mL). Pre-treatment with graded doses of the extract exerted a significant ($p < 0.05$) dose-dependent decrease in gastric volume from 1.77 ± 0.04 to 1.35 ± 0.03 mL as the doses increased from 200 to 600 mg/kg b.w. of extract when compared to the ulcer group. Also, there was an improvement in the pH of the intestinal content of the groups that received the graded doses of the extract compared to the untreated group. The results of ulcer index revealed a significant ($p < 0.05$) decrease when the groups that received graded doses of the extract and standard drug (lansoprazole) were compared to the untreated group. There was also a significant ($p < 0.05$) increase in the ulcer protective index of normal control and groups that received the graded doses of the extract and standard drug were compared to the untreated group. Scanlon and Sanders[21] reported that the increase in gastric volume of treatment groups compared to the normal control could be due to the HCl used in ulcer induction. According to Ezeasiliji *et al.*,[22] ethanol induces ulceration by releasing superoxide anions and hydroperoxyl free radicals which leads to an increased lipid peroxidation of lipids in the membranes of stomach mucosa as a result of ethanol metabolism. The result of this study is consistent with the findings of LiraMota *et al.*,[15] who reported that oral administration of HCl/ethanol causes necrotizing lesions in the gastric mucosa.
The lesions which are described by the ulcer index are caused by loss of mucous layer and increase of acid secretion, hence an increase in gastric volume leading to ulcer occurrence.[24,25] The gastro-protective effect of the extract could be attributed to its phytoconstituents such as flavonoids, which have anti-secretory effect,[19] inhibiting secretion of HCl by the parietal cells of the gastric mucosa and hence reducing the volume of gastric content and increasing the pH of the gastric content.
The standard drug, lansoprazole is a proton-pump inhibitor and acts by inhibiting gastric acid secretion by blocking the H^+/K^+-adenosine triphosphate enzyme system (the proton pump) of the gastric parietal cells.[25] It is effective in alleviating ulcer, but in this study, the graded doses of the extract indicated a better ulcer protective effect as shown in Table 2.

Table 1: Phytochemical Constituents of ethanol extract of *Acalypha torta* leaf

Phytochemical constituents	Leaf Extract	Amount (mg/g)
Steroids	+	3.06 ± 0.17
Flavonoids	+	1.20 ± 0.10
Alkaloids	+	1.19 ± 0.09
Saponins	+	1.10 ± 0.13
Cardiac glycosides	+	0.23 ± 0.04
Tannins	+	0.21 ± 0.03

Values are mean ± SD, (n = 3). Key: + indicates present; ND = Not detected.

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

Histological examination of gastric tissues

There was no mucosal damage in the normal control as shown in Figure 1A. The histology revealed an intact gastric wall (muscularis mucosa) (green colour), submucosa (red colour) and serosa (black colour) with no lesions to the gastric mucosa observed. The histological examination of the gastric tissues of the animals indicated severe and extensive mucosal damage in the animals that were induced but not treated (Figure 1B) while there was a progressive ameliorative effect in the groups that received 200 and 400 mg/kg b.w extract as shown in Figures 2A-B and 3A. The animals that received 600 mg/kg b.w extract exerted the most ulcer protective effect characterized by an intact muscularis mucosa, granulated tissue, hyperplasia of epithelial cells (black arrow) and large aggregates of lymphocytes and fibrous scar (blue arrow) (H&E ×100) as shown in Figure 3B.

Conclusion

The present findings have shown that *Acalypha torta* leaf ethanol extract possesses gastro-protective activity, as shown by its significant and dose-dependent increase in ulcer protective index against gastric mucosa damage induced by HCl/ethanol induction. The anti-ulcerogenic effects of the extract could be attributed to its phytoconstituents.

Conflict of interest

The authors declare no conflicting interest

Table 2: Effect of ethanol extract of *Acalypha torta* leaf on Ulcer parameters

Groups	Gastric Juice volume (mL)	pH	Ulcer index	Ulcer protective index (%)
Normal control	1.17 ± 0.13^{a}	7.81 ± 0.22^{d}	0.00 ± 0.00	100
Untreated group	2.83 ± 0.06^{e}	3.71 ± 0.34^{a}	3.48 ± 0.67^{b}	0
Lansoprazole	1.77 ± 0.06^{d}	7.07 ± 0.22^{b}	1.73 ± 1.04^{a}	50.29
200mg/kg b.w. extract	$1.72 \pm 0.04^{c,d}$	$7.25 \pm 0.25^{b,c}$	1.65 ± 0.95^{a}	52.59
400 mg/kg b.w. extract	1.61 ± 0.05^{c}	$7.48 \pm 0.21^{b,c,d}$	1.28 ± 0.34^{a}	63.22
600 mg/kg b.w. extract	1.35 ± 0.03^{b}	$7.65 \pm 0.19^{c,d}$	0.75 ± 0.30^{a}	78.45

Values are mean ± SD, (n = 4). Values in the same column having different superscripts differ significantly ($p < 0.05$).

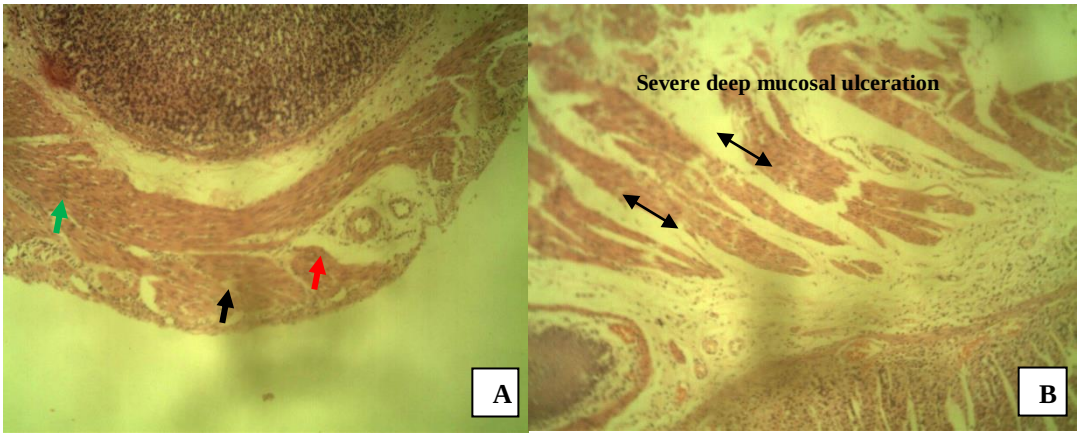

Figure 1: (A) Photomicrograph of normal control group (H&E ×100). The histological examination indicated an intact gastric wall (muscularis mucosa), submucosa and serosa with no lesions to the gastric mucosa observed. **(B)** Photomicrograph of untreated group characterized by an extensive mucosal damage, severe loss of surface epithelium and spotty haemorragic lesion (H&E ×100).

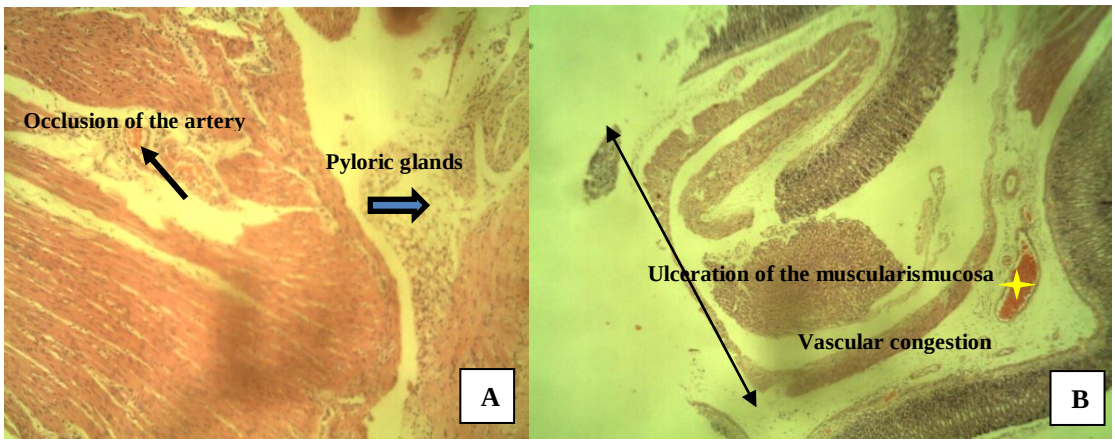

Figure 2: (A) Photomicrograph of rat pre-treated with 30 mg/kg b.w lanosprazole (H&E ×100). The histological examination revealed a mild mucosal damage characterized by occluded arteries while the gastric wall appearance similar to normal. **(B)** Photomicrograph of rat pre-treated with 200 mg/kg b.w of extract. Gastric mucosal lesions, loss of epithelium and inflammatory infilterates and vascular congestion are seen (H&E ×100).

ISSN 2616-0684 (Print)
ISSN 2616-0692 (Electronic)

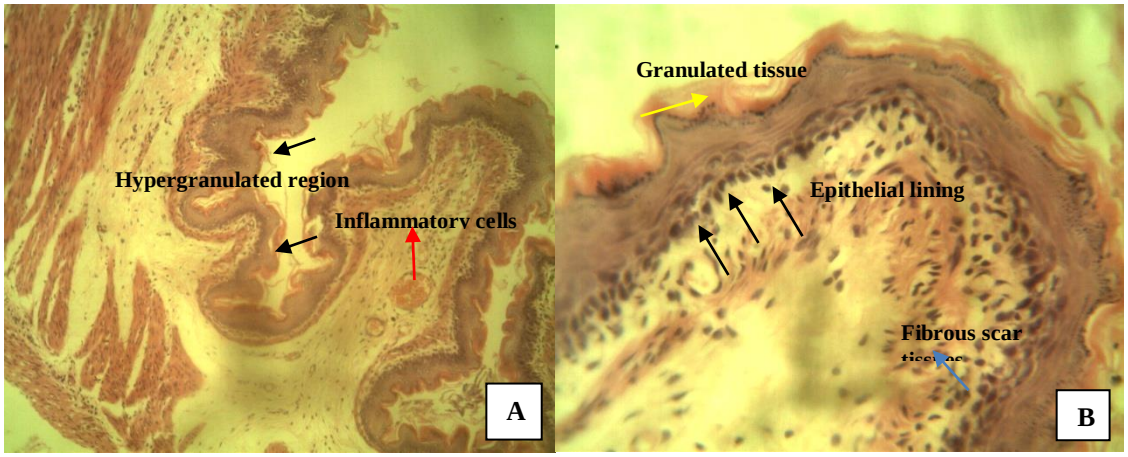

Figure 3: (A) Photomicrograph of rat pre-treated with 400 mg/kg b.w of extract (H&E ×100). The photomicrograph revealed a typical stomach region with hypergranulation of tissue, mild gastric mucosal lesions and mild inflammatory infiltrate. The gastric pits are intact with good formation similar to the normal. **(B)** Photomicrograph of rat pre-treated with 600 mg/kg b.w of extract characterized by an intact muscularis mucosa, granulated tissue, hyperplasia of epithelial cells and large aggregates of lymphocytes (H&E ×100).

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

Acknowledgements

The authors humbly acknowledge Mr. Mbaoji of the Department of Pure and Industrial Chemistry, University of Nigeria Nsukka and Mr. Alfred Ozioko for their assistance.

References

1. Proctor MJ and Deans C. Complications of peptic ulcer. Surg. 2014; 32(11):599-607.
2. Amandeep K, Robin S, Ramica S, Sunil K. Peptic ulcer: A review on aetiology and pathogenesis. Int Res J Pharm. 2012; 3(6):34-38.
3. Mayes PA and Botham KM. Metabolism of unsaturated fatty acids and eicosanoids. In: Murray RK, Granner DK, Mayes PA, Rodwell VW (Eds.). Harper's illustrated Biochemistry. (26th ed.); New York: McGraw-Hill Companies Inc; 2003. 192 p.
4. Njoku UO, Umeh CG, Ogugofor MO. Anti-ulcerogenic activity of methanol fraction of *Hibiscus asper* leaves in albino rats. Afr J Biomed Res. 2020; 23:267-272.
5. Karaoglan ES, Albayrak A, Kutulu Z, Bayir Y. Gastroprotective and antioxidant effects of *Eremurus spectabilis* Bieb. methanol extract and its isolated component isoorientin on indomethacin-induced gastric ulcers in rats. Acta Cir Bras. 2018; 33(7):609-618.
6. Njoku UO, Uzoagulu CH, Ogugofor MO. Protective effect of *Cnidoscolus aconitifoilus* leaves against diclofenac-induced gastric mucosal damage. Pak J Pharm Sci. 2020; 33(2):651-657.
7. Serafino A, Vallebona PS, Andreola F, Zonfrillo M, Mercuri L, Federici M, Rasi G, Garaci E, Pierimarchi P. Stimulatory effect of Eucalyptus essential oil on innate cell mediated immune response. BMC Immunol. 2008; 9:17.
8. Ezekwesili CN and Nwodo OFC. Evaluation of the pharmacological activity of ethanol leaf extract of *Acalypha torta* (Muell). The Bioscientist. 2013; 1(1):67-72.
9. Irobi ON and Banso A. Effect of crude leaf extract of *Acalypha torta* against some anaerobic bacteria. J Ethnopharmacol. 1994; 43:63-65.
10. Ezekwesili CN, Ghasi S, Nwodo OFC. Evaluation of the blood pressure lowering activity of leaf extract of *Acalypha torta* Muell. Afr J Pharm Pharmacol. 2012; 6(37):3235-3241.
11. Onocha PA, Oloyede GK, Owoye FF. Phytochemical, cytotoxicty and free radical scavenging activities of *Acalypha torta* leaf extracts (Euphorbiaceae). Arch Appl Sci Res. 2011; 3(6):413-422.
12. Harborne JB. Phytochemical methods: A Guide to modern techniques of plant analysis. London: Chapman and Hall Ltd; 1973. 279 p.
13. Trease GE and Evans WC. Pharmacognosy. (15th Ed.). London: Saunder Publishers; 2002. 42 - 44, 221 -229, 246 – 249, 404 -306, 331-332, 391-393 p.
14. Soni A and Sosa S. Phytochemical analysis and free radical scavenging potential of herbal and medicinal plant extracts. J Pharmacogn Phytochem. 2013; 2(4):22-24.
15. LiraMota KS, Pita JCLR, Estevam EC, Medeiros VM, Tavares JF, Agra MF, Diniz MFFM, da Silva MS, Batista LM. Evaluation of the toxicity and anti-ulcerogenic effect of the ethanol extract of *Maytenus obtusifolia* Mart. leaves. Rev Bras Farmacogn. 2008; 18(3):441-446.
16. Onwukwe OS, Achukwu PU, Azubike NC, Nwachukwu DC, Onyemelukwe AO, Uchendu IK, Eluke BC. Preliminary studies on the anti-ulcer potential of *Vitex doniana* crude extracts on experimental rat model of ethanol-induced gastric ulcer. Asian Pac J Trop Dis. 2016; 6(9):736-140.
17. Kiranmai M, Sri BU, Reddy DS, Kumar CBM, Ibrahim M. Evaluation of total phenolic contents and antiulcerogenic activity of root bark of *Azadirachta indica*. Glob J Med Res. 2012; 12 (6):23-29.
18. Drury R, Wallington A, Cameroun SR. In: Carleton's histological techniques. New York: Oxford University Press; 1967. 1-420 p.
19. Sumbul S, Ahmad MA, Asif M, Akhtar M. Role of phenolic compounds in peptic ulcer: An overview. J Pharm Bioallied Sci. 2011; 3(3):361-367.

20. de Jesus NZT, de Souza Falcão H, Gomes IF, de Almeida Leite TJ, de Morais Lima GR, Barbosa-Filho JM, Tavares JF, da Silva MS, de Athayde-Filho PF, Batista LM. Tannins, peptic ulcers and related mechanisms. Int J Mol Sci. 2012; 13(3):3203–3228.
21. Scanlon VC and Sanders T. Essentials of anatomy and physiology. Philadelphia: F.A. Davis and Company; 2007. 376-377 p.
22. Ezealisiji KM, Ijeomah SC, Agbo MO. Anti-ulcer activity of African walnut *Tetracarpidium conophorum* nuts against gastric ulcers in rats. Asian Pac J Trop Dis. 2014; 4(2):998-1001.
23. Mohammad KHH, Siddiqui RK, Dixit M, Salman KDIM, Azizur R. Amelioration of gastric ulcer using a hydro-alcholic extract of Triphala in indomethacin-induced Wistar rats. Eur J Integr Med. 2016; 8(4):546-551.
24. Zhu Y, Li X, Mo H, Zhang L, Zhang L, Zhou S, Ma X, Zheng B. Gastroprotective effects of ascaridole on gastric ulcer. Chin. Herb. Med. 2012; 4(1):58-62.
25. Amin D. Alimentary system and liver. In: Ritters JM, Lewis LD, Mant TGK, Ferro A (Eds). A Textbook of Clinical Pharmacology and Therapeutics. (5th ed.). London: Hodder Arnold; 2007. 251 p.