The Journal of Phytopharmacology 2014; 3(6): 389-394
Online at: www.phytopharmajournal.com

Research Article

ISSN 2230-480X
JPHYTO 2014; 3(6): 389-394
November- December

Gastric acid anti-secretory effects of aqueous leaf extract of *Nauclea latifolia* (Rubiaceae) in rats

Morufu E. Balogun, Daniel Nwachukwu, Peter E. Onwe, Moshood A. Folawiyo*

Morufu E. Balogun
Department of Physiology, Faculty of Medicine, College of Health Sciences, Ebonyi State University, Abakaliki, Nigeria

Daniel Nwachukwu
Department of Physiology, Faculty of Medical Sciences, College of Medicine, University of Nigeria, Enugu Campus, Nigeria

Peter E. Onwe
Department of Physiology, Faculty of Medicine, College of Health Sciences, Ebonyi State University, Abakaliki, Nigeria

Moshood A. Folawiyo
Department of Physiology, Faculty of Medicine, College of Health Sciences, Ebonyi State University, Abakaliki, Nigeria

Correspondence:
Morufu E. Balogun
Department of Physiology, Faculty of Medicine, College of Health Sciences, Ebonyi State University, Abakaliki, Nigeria
Email: marufbalogun@yahoo.com

Abstract

The aqueous leaf extract of *Nauclea latifolia* has been shown to have anti-ulcer effect. **Objectives:** The present study was undertaken to investigate the effects of aqueous leaf extract of *N. latifolia* on gastric acid secretion as the possible mechanism of its anti-ulcer actions in male albino rats. **Materials and Methods:** A total of sixty (60) male albino rats were divided into two experimental studies of thirty (30) rats each. Each of the experimental studies was further divided into groups according to study design. The extract was administered orally at the doses of 100, 200 and 400 mg/kg for the experimental groups while the control and reference groups received distilled water (2 ml/kg, p.o) and cimetidine (32 mg/kg, p.o) respectively, 60 minutes prior to oral administration of indomethacin (30 mg/kg) to induce gastric mucosal injury. In the second study, gastric acid output was measured by the continuous perfusion of rat's stomach under anesthesia with normal saline at the rate of 1 ml/min. **Results:** Phytochemical analysis revealed the presence of saponins, tannins, flavonoids, alkaloids and cardiac glycosides. Acute toxicity studies showed there were no deaths within 24 h after the application of the extract up to 1600 mg/kg B.W (i.p). Rats pre-treated with *N. latifolia* exhibited significant ($P<0.05$), and dose-dependent inhibition of indomethacin-induced gastric ulceration. A significant decrease in gastric acid secretion was produced by the extract at all doses studied. **Conclusion:** Findings of this study suggest that, aqueous extract of *N. latifolia* significantly reduced gastric acid secretion in indomethacin-induced gastric ulceration by inhibiting histamine-stimulated gastric acid secretion probably by occupying H_2-receptors in rats.

Keywords: *Nauclea latifolia* leaf, Mucosal injury, Cimetidine, Indomethacin, Gastric acid secretion, Albino rats.

Introduction

Nauclea latifolia (smith) belongs to the family Rubiaceae. It is commonly known as a pin cushion tree being a straggling shrub or small tree, native to the tropical Africa and Asia.[1] It bears an interesting flower, large red ball fruit with long projecting stamens. It grows up to an altitude of 200 meters. It is widespread in the humid tropical rainforest zones or in the savannah woodland of West and Central Africa.[2] *N. latifolia* is commonly known as "Ubulu inu" among the Igbo in the Eastern part of Nigeria; as "Tafashiya" among the Hausas in the Northern part of Nigeria; as "Egbesi" among the Yoruba in the Western part of Nigeria and as "Itu" among the Itsekiri.[3] *N. latifolia* herbal remedies have been commonly seen in various cultures throughout recorded history and still serve as the main means of therapeutic medical treatment. It is used in the treatment of fever, diarrhea and even as an anti-parasitic drug. [4] The sticks are used as chewing sticks and a remedy against tuberculosis. [2, 5] Scientific studies have established hypolipidemic and hypoglycemic effects like most other plant extracts. [6-9] In West and South Africa, infusions and decoctions of the stem bark and leaves of *N. latifolia* are used for treatment of stomach pain, fever, diarrhea and constipation.[9] Abbiw[10] stated that root infusion of *N. latifolia* uses in Sudan for the treatment of gonorrhea, its roots and leaves are used in Ghana for treating sores. In Nigerian folklores the fruit is sometimes used in the treatment of piles and dysentery. [11] In addition, the plant is used in the treatment of sleeping sickness and to prolong menstrual blood flow. [12] Gidado *et al.* [10] reported anti-diabetic properties for the root and leaf extracts while Taiwe *et al.* [13] reported the anti-depressant and anti-anxiety effects of the root extract of the plant. Flavonoids in the plants are among the cytoprotective materials for which anti-ulcerogenic efficacy have been extensively confirmed.[14-16] We had earlier reported in our previous study that the aqueous leaf extract of

N. latifolia exhibits a dose-dependent anti-ulcer activity against indomethacin-induced ulcers in rats.[17] Nevertheless, the effect of *N. latifolia* on gastric acid secretion as a probable mechanism by which its anti-ulcer property occurs was neither examined nor explored. Therefore, the aim of the present study is to investigate the effects of aqueous extract of *N. latifolia* on gastric acid secretion, and the possible mechanism(s) of action by which the extract produced its anti-ulcer effects in male albino rats. This is with a view of providing a scientific justification for the ethnomedical uses of the plants leaf in the management, control and treatment of gastric lesions.

Materials and Methods

Chemicals and drugs

All chemicals and drugs used in this investigation were of analytical grade and were obtained from Sigma, Saint Louis, USA. Cimetidine (H_2-receptor antagonist) was used as the reference anti-ulcer drug. In this study, cimetidine was administered orally to reference control group of rats in a dose of 32 mg/kg suspended in distilled water (2 ml/kg).[18]

Experimental animals

Male albino rats of Wistar strain weighing between 180 to 240 g were obtained from the Central Animal House, Faculty of Medicine, Preclinical Section, Ebonyi State University, Abakaliki, Nigeria. They were housed in netted cages under standard laboratory conditions and were fed with standard rat pellets (Pfizer Feeds LTD, Enugu, Nigeria) and tap water was given *ad-libitum*. Excess feeds and water was removed and replaced daily. The rats were allowed to stabilize for 2 weeks before commencement of the experiment. The experimental procedures and techniques used in the study were in accordance with accepted principles for laboratory animal use and care by the National Institute of Health (NIH, 1985); all protocols and procedure were approved by Animal Ethics Committee of the University with reference number (EBSU/REC/BM14/021).

Plant material and preparation of aqueous extract

The fresh leaves of *N. latifolia* were collected within the campus of the Ebonyi State University, Abakaliki, Nigeria, identified and authenticated by Mr. P.O. Ugwuozo in the herbarium of the Plant Science and Biotechnology Department of University of Nigeria, Nsukka, with deposition of authenticated voucher specimen (UNH - 303i). The leaves were air-dried and blended to a fine powder. To 200 g of the powdered leaves in a container with lid, 1 liter of boiling water was added and covered. It was allowed to stand for 24 hours with intermittent shaking. The mixture was then filtered with NO.1 Whatman qualitative filter paper to obtain a pure filtrate. The collected extracts were concentrated and dried *in vacuo* and the percentage yield of the extract was 23.1%. The concentrate was later reconstituted in sterile distilled water to give the required doses of 100, 200 and 400 mg/kg/ body weight in 2 ml of the vehicle respectively. It was stored in the refrigerator throughout the period of the experiment to preserve the prepared extract.

Preliminary phytochemical screening

The aqueous leaf extract of the plant was subjected to various qualitative phytochemical tests, to identify the secondary metabolites; saponins, tannins, terpenoids, steroids, alkaloids, flavonoids, cardiac glycosides and anthraquinones present in the leaves. The methods of analysis employed were those described by Trease and Evans[19] and Sofowora[20].

Acute toxicity study

The lethal dose (LD_{50}) of the aqueous leaf extract of *N. latifolia* was determined by the method of Lorke[21] and Sandow[22] using thirteen[13] rats. In the first phase rats were divided into three groups of three (3) rats each and were treated with the aqueous leaf extract of *N. latifolia* at doses of 10, 100 and 1000 mg/kg body weight intraperitoneally. They were observed for 24h for signs of toxicity. In the second phase four rats were divided into four (4) groups of one rat each and were also treated with the aqueous extract *N. latifolia* at doses of 1000, 1600, 2900 and 5000 mg/kg body weight (i.p.). The median lethal dose (LD_{50}) was calculated using the second phase.

Experimental design

A total of sixty (60) rats were used for the study. The rats were divided into two groups, each of thirty (30) rats, each group being in a different study. The first study involved rats that underwent experimental Indomethacin-induced gastric ulceration. These were used to assess the degree of ulceration and total gastric acid content in control and pretreatment test groups. Rats in the second study were assessed for both basal and maximal (histamine-induced) gastric acid secretion.

Gastric ulceration

This was carried out as described by Ukwe and Nwafor.[23] Food was withdrawn 24 hours and water one hour before drug treatment. Thirty (30) male albino rats were randomly divided into 5 groups (n=6) rats each. Animals in groups 1 and 2 received distilled water and cimetidine, respectively, while those in group 3, 4 and 5 were pre-treated with 100, 200 and 400mg/kg of the extract respectively. After one hour, indomethacin 30 mg/kg/2 ml were administered orally to all the rats. Seven hours later the rats were killed by cervical dislocation. The rats' stomachs were removed and each opened along the greater curvature. After fixing the tissues by immersing in 10% formalin for 24 hours, it was rinsed under a stream of water and examined for ulcers. The ulcers were counted by the aid of a hand lens (X- magnification) and ulcer score was calculated for each animal according to the arbitrary scale used by Singh *et al.* [24], where 0 = no lesion, 1 = hyperemia, 2 = one or two slight lesions, 3 = very severe and 4 = mucosal full of the lesion.

Ulcer index was calculated as mean ulcer scores.[25]

Determination of gastric acid content

Before scoring the ulcer, the gastric content was drained into a centrifuge tube and 8 ml of freshly prepared normal saline was added and centrifuged at 3000 rpm for 10 min. The total gastric acidity was determined by titrating 5 ml of the supernatant against M/400 NaOH to an endpoint using 1–2 drops of phenolphthalein as an indicator according to Lai.[26]

Gastric acid secretion *in situ*

The effects of *N. latifolia* (200 mg/kg) on basal and histamine-induced gastric acid secretion in albino rats were studied as described by Ghosh and Schild[27], modified by Amure and Ginsburg[28]. This was used together with the titration method described by Olowokorun.[29] Thirty (30) male albino rats were randomly divided into 6 groups (n=5) rats each. Adult male rats (180–250 g) fasted for 24h were anesthetized with an i.p injection of 0.6 ml/100 g of 25% urethane (ethyl carbamate). The femoral vein, esophagus and pyloro-duodenal junction were cannulated. The stomach was perfused with normal saline (37°C) and gastric effluent was collected at a constant rate of 10 ml/10 min. The effluent was titrated against M/400 (NaOH) solution with phenolphthalein as indicator. The effects of *N. latifolia* extract (200 mg/kg) alone and in combination with histamine and/or cimetidine, on gastric acid secretion were studied. Titrable acidity was expressed in μEq/L/10mins. The histamine-induced gastric acid was collected 30 minutes post-surgery at which time a steady (basal) acid secretion had been obtained.

Statistical analysis

Results were expressed as mean ± S.E.M. The data were statistically evaluated by one way ANOVA. Comparison between treatment and control group were made by Student's t- test, then followed by Fisher's exact. Significance of difference was accepted at $P < 0.05$ using Graph-Pad Prism version 5.00 for Windows (Graph Pad Software, San Diego, California, USA).

Results

Phytochemical screening

The results of phytochemical analysis indicate that the extract contains saponins, tannins, alkaloids, flavonoids and cardiac glycosides.

Acute toxicity test

The intraperitoneal administration of the aqueous leaf extract of *N. latifolia* to albino rats up to the dose of 1600 mg/kg B.W did not record any mortality. However, a mild clinical sign of writhing, an increase in motor activity and tremors in rats treated with the dosage of 1600 mg/kg B.W was observed. Thus, the LD_{50} was determined to be above 1600 mg/kg B.W (i.p.).

Gross evaluation of gastric lesions

As shown in table 1, indomethacin induced ulcers in 100% of the animals in the negative control (distilled water; 2 ml/kg) group. The ulcer index was 4.87±0.25, which was characterized with severe disruption of surface epithelium of gastric mucosa. Pre-treatment with cimetidine significantly ($P< 0.05$) reduced the severity of indomethacin-induced ulcers compared to rats pre-treated with distilled water (ulcer control). The *N. latifolia* leaf extract were also shown to exert cytoprotective effects in a dose-dependent manner (Table 1).

Table 1: Effects *Nauclea latifolia* on gastric ulceration induced by indomethacin

Groups	Pre-treatment	Dosage (p.o)	Mean Ulcer Index ± SEM	Percentage Protection
1	Distilled water	2 ml/kg	4.87 ± 0.25	0.00
2	Cimetidine	32 mg/kg	1.56 ± 0.28*	67.97
3	Extract	100 mg/kg	1.92 ± 0.36*	60.57
4	Extract	200 mg/kg	1.50 ± 0.14*	69.20
5	Extract	400 mg/kg	0.54± 0.52*	88.91

*Significant. All values are expressed as mean ± SEM, n=6 in each group.*P<0.05 as compared with the negative control animal.

Percentage inhibition to ulcer formation in rats by the extract was calculated as follows:

$$\% \text{ Inhibition of Ulceration} = [(\text{Ulcer index}_{\text{Control}} - \text{Ulcer index}_{\text{Test}}) / \text{Ulcer index}_{\text{Control}}] \times 100\%$$

Gastric content acidity

The extract produced a significant ($p<0.05$) and dose dependent decrease in mean total gastric acidity in indomethacin induced gastric ulcers in pre-treatment groups compared to control (Table 2). Mean total gastric acidity decreases with increasing doses of the extract. Cimetidine produced a lower gastric acidity ($P < 0.05$) than any of the doses of the extract studied (Table 2). In order to determine the probable mechanism by which *N. latifolia* extract reduced total gastric acidity, the effect of 200 mg/kg B.W of the extract separately and in combination with histamine and/or cimetidine on acid secretion *in situ* was studied. As shown in Table 3, this extract produced a significant decrease in basal and histamine induced gastric acid secretion in rats. Moreover, the extract appears to augment cimetidine inhibition of gastric acid secretion.

Table 2: Effect of *Nauclea latifolia* on total gastric acid content induced by indomethacin

Groups	Pre-treatment	Dosage (p.o)	Total gastric acid content (μEq HCl/100g B.W)
1	Distilled water	2 ml/kg	10.92 ± 1.18
2	Cimetidine	32 mg/kg	5.52 ± 0.15*
3	Extract	100 mg/kg	7.25 ± 0.17*
4	Extract	200 mg/kg	6.85 ± 0.23*
5	Extract	400 mg/kg	6.14 ± 0.05*

*Significant. All values are expressed as mean ± SEM, n=6 in each group.*P<0.05 as compared with the negative control animal.

Table 3: Effects of *Nauclea latifolia* extract on gastric acid secretion in rats

Groups	Pre-treatment	Basal acid Output (µEq/L/10mins)	Gastric acid secretion (μEq/L/10 mins)
1	Normal saline (1 ml/kg)	1.42 ± 0.01	1.45 ± 0.15
2	Extract (200 mg/kg)	1.54 ± 0.03	1.15 ± 0.04*
3	Histamine (100 mg/kg)	1.50 ± 0.0	5.60 ± 0.28*
4	Histamine + Extract	1.55 ± 0.07	2.05 ± 2.52*
5	Cimetidine (32 mg/kg)	1.45 ± 0.02	1.05 ± 0.10*
6	Cimetidine + Extract	1.40 ± 0.03	0.86 ± 0.25*

* Significant. All values are expressed as mean ± SEM, n=5 in each group.*P<0.05 as compared with the negative control animal.

Discussion

This research work was designed to investigate the gastric acid anti-secretory effects of aqueous leaf extract of *N. latifolia* against indomethacin-induced gastric ulceration in albino rats. The results of the toxicity study suggest that the extract has a wide margin of safety and thus administration as done in folk medicine may not have any immediate deleterious effects. The findings of the present study demonstrated that aqueous extract of *N. latifolia* leaves significantly protected against mucosal damage induced by indomethacin and curative ratios of plant extracts 100, 200 and 400 mg/kg were 60.57%, 69.20% and 88.91% respectively. It is remarkable that the leaf extract at the tested doses of 200 and 400 mg/kg produced a greater protection than cimetidine (32 mg/kg) against the indomethacin. The effect of the extract compared favorably to cimetidine 32 mg/kg (positive control). As shown in Table 1 cimetidine produced a weaker anti-ulcer effect than the extract at the tested doses of 200 and 400 mg/kg body weight. It is not known which of the components of *N. latifolia* extract possesses this property. It has been established that indomethacin is an ulcerogenic agent especially when administered on an empty stomach.[30] The ulcerogenic activity of indomethacin and other non-steroidal anti-inflammatory agents as postulated might be due to their ability to inhibit prostaglandin synthesis.[31] Several lines of evidence suggest that prostaglandins inhibit gastric secretion and are important to normal gastric physiology and mucosal integrity.[32, 33] Some of the mechanisms suggested for their effect include tightening of the gastric mucosal barrier[34] and stimulation of the gastric sodium pump[35]. The protective effect of the extract on indomethacin induced-ulcers in rats might be related to any of the mechanism suggested.

Phytochemical analysis identifies saponins, tannins, flavonoids, alkaloids and cardiac glycosides as the major components. It has also been reported that, the flavonoids like flavones, glycosides, tannins and isoflavonoid (Indicanine B and Indicanine C) have been isolated from its leaves.[36, 15] Flavonoids are among the cytoprotective materials for which anti-ulcerogenic efficacy have been extensively confirmed.[14-16] It is suggested that, these active compounds would be able to stimulate mucous, bicarbonate, and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen.[37-39]

The significant reduction in total gastric acidity observed in this study strongly suggests that *N. latifolia* may act by inhibiting gastric acid secretion. Moreover, this extract inhibited basal and histamine-induced acid secretion and seems to augment the inhibitory action of cimetidine (an H_2-receptor blocker) on gastric acid secretion. These findings indicate that the extract probably acts by inhibiting H_2-receptor leading to blockade of histamine release whose stimulatory action on gastric acid secretion via H_2-receptor, has been well reported.[40-42] There is, however, the possibility of the involvement of other receptors, which are yet to be investigated.

Conclusion

The present study showed that pre-treatment with the leaf extract of *N. latifolia* caused a beneficial effect on indomethacin-induced gastric lesions in rats as evidenced by the reduction in the ulcer index and gastric acid secretion. The gastric acid anti-secretory effect of the extract is dose-dependent and this may justify its use as an anti-ulcerogenic agent. However, further studies are necessary to isolate the responsible active compound(s) and elucidate its mechanism of action especially with respect to mechanism of gastric mucous secreting activity and prostaglandin synthesis. If these findings are extrapolated to man, aqueous extract of *N. latifolia* may therefore be beneficial to peptic ulcer-prone individuals. Since the extract inhibits gastric acid released by histamine a potent secretagogue that acts via H_2 receptor.

Acknowledgements

The authors are grateful to Dr. (Mrs.) Besong Elizabeth of Physiology Department of Ebonyi State University, Abakaliki for the technical assistance received.

Ethical approval

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

Declaration of interests

Authors have declared that no competing interests exist.

References

1. Gidado A., Ameh D.A., Atawodi S.E. Effect of *Nauclea latifolia* leaves of aqueous extract on the blood glucose levels of normal and alloxane-induced diabetic rats. Afr J Biotech. 2005; 4(1): 91-93.

2. Burkil H.M. The useful plants of West Africa- Whiferrers Press Limited, London 1985; 401- 415.

3. Arise R.O., Akintola A.A., Olarinoye J.B., Balogun E.A. Effects of *Nauclea latifolia* stem on lipid profile and some Enzymes of Rat Liver and Kidney. Int J Pharmacol. 2012; 10(3): 23-39.

4. Deeni Y.Y., Hussain H.S.N. Screening for antimicrobial activity and for alkaloids of *Nauclea latifolia*. J Ethnopharmacol. 1991; 35:91-6.

5. Esimore C.O., Ebebe I.M., Chan K.F. Comparative antibacterial effect of Psidiu guajava aqueous extract. J Trop Med Plants. 2003; 4: 185-189.

6. Schiff P.L. Thalictrum alkaloids Lloydia 1979; 33: 403-452.

7. Chong Y.H. Effects of palm oil on cardiovascular risk. Med J Malaysia. 1991; 46: 41-50.

8. Udoh F.V. Effects of leaf and root extract of *Nauclea latifolia* on cardiovascular antagonist on radiation induced system. Fitoterap. 1998; 69:141-145.

9. Eno A.E., Owo O.I. Cardiovascular effects of extract from the roots of a shrub *Elaeophorbia drupifera*. Phytother Res. 1999; 13: 549-554

10. Abbiw K.D. Useful plants of Ghana, West Africa; Uses of Wild and cultivated plants, Intermediate Technology publication, London 1990; 98-212.

11. Reitman S., FrankelS. A colorimetricmethod for determination of Serumglutamate oxaloacete and pyruvate transaminase. Am J Clin Pathol. 1957; 28: 56-63.

12. Elujoba A.A.A. Female infertility in the Hands of traditional Birth Attendants in South West Nigeria. Fitoterap. 1995; 66 (3): 239-248

13. Taiwe G.S., Bum E.N., Dimo T., Talla C., Weiss N. The Effects of aqueous extract of *Nauclea latifolia* leaf in glucose concentration in rat. A paper on biochemistry and molecular biology (Calabar). 2010; 1-5.

14. Di Carlo G., Mascolo N., Izzo A.A., Capasso F. Flavonoid: Old and new aspects of a class of natural therapeutic drugs. Life Sci. 1999; 64:337-57.

15. Borrelli F., Izzo A.A. The Plant kingdom as a source of Anti-ulcer remedies. Phytother Res. 2001; 53:82-8.

16. Galati E.M., Monforte M.T., Tripodo M.M. Anti ulcer activity of *Opuntia ficus indica* L. (Cactaceae): Ultrastructural study. J Ethnopharcol. 2001; 76:19.

17. Balogun M.E., Oji J.O., Besong E.E., Ajah A.A., Michael E.M. Anti-ulcer activity of aqueous leaf extract of *Nauclea latifolia* (Rubiaceae) on indomethacin-induced gastric ulcer in rats. Afr J Biotech. 2013; 12 (32): 5080-5086.

18. Pendernera A.M., Guardian T., Caleron C.G. Anti-ulcerogenic and anti-inflammatory activity of the methanolic extract of *Larrea divaricata* Cav. in rat. J Ethnopharmacol. 2006; 105: 415-420.

19. Trease G., Evans W.C. Textbook of Pharmacognosy. 12th ed., Balliere, Tindall, London 1983; 343-383.

20. Sofowora A. Medicinal Plants and Traditional Medicine in Africa. Spectrum Books Limited, Ibadan, Nigeria 1993; 151-153.

21. Lorke D. A new approach to practical acute toxicity testing. Arch Toxicol 1983; 54: 275-287.

22. Sandow J. Toxicological evaluation of drugs affecting the hypothalamic pituitary system. Pharm Therap. 1979; 5: 297-303.

23. Ukwe C.V., Nwafor S.V. Anti-ulcer activity of leaf extract of *Persea americana*. Nig J Pharm Res. 2004; 3 (1): 91-95.

24. Singh S., Bani S., Singh G.B. Anti-inflammatory activity of Lupeol. Fitotererap. 1997; 68: 9-16.

25. Tan P.V., Nditafon N.G.,Yewah M.P., Dimo T., Ayafor F.I. *Eremomoatax speciosa*: effect of leaf aqueous extract on ulcer formationand gastric secretion in rats. J Ethnopharmacol. 1996; 54: 139-142.

26. Lai K.S. Studies on gastrin. Gut. 1964; 5: 327–341.

27. Ghosh M.N., Schild H.O. Continuous recording of acid gastric secretion in the rat. Brit J Pharmacol. 1958; 21: 1393–1396

28. Amure B.O., Ginsburg M. Inhibitors of histamine Catabolism and the action of gastrin in the rat. Brit J Pharmacol. 1964; 23: 476-85.

29. Olowokorun M.O. Influence of sex hormones and Atropine sulphate on gastric acid secretion in the rat. J. N. G. Vet. Med. Assoc. 1975; 4(2):72-74.

30. Blaargawa K.P., Gupta M.B., Tangri K.K. Mechanism of ulcerogenic activity of indomethacin and oxyphenbutazone. Eur J Pharmacol. 1993; 22: 191-195.

31. Vane J.R. L inhibition of prostaglandin synthesis as a mechanism of action for Aspirin like drugs. Nat New Biol. 1971; 231-232.

32. Jacobson E.P. Comparison of prostaglandins EE2 and nor epinephrine on the gastric mucosal circulation. Proceedings of the society of Experimental Biology and Medicine. 1970; 133: 516.

33. Main I.H.M, White B.W.R. The effects of E and A prostaglandins on the gastric mucosal blood flow and the acid secretion in the rat. Brit J Pharmacol. 1976; 49: 428

34. Bolton J.P, Cohen MM. Effect of 16, 16 dimethyl prostaglandin E2 on the gastric mucosal barrier. Gut. 1979; 20.

35. Roberts A. Cytoprotection by prostaglandins. Gastroenterol. 1979; 77: 761-767.

36. Yadava R.N., Reddy K.I.S. A novel prenylated flavones glycosides from the seeds of Erythrina indica. Fitoterap. 1999; 70: 357-360.

37. Salvayre R., Braquet P., Perochot L., Douste-Blazy L. Comparison of the scavenger effect of bilberry anthocyanosides with various flavonoids. Flavonoids Bioflavonoid. 1982; 11: 437-442.

38. Asuzu I.U., Onu O.U. Antiulcer activity of the ethanolic extract of *Combretum dolichopetalum* root. Int J Crude Drug Res. 1990; 28: 27-32.

39. Suja P.R., Anuradha C.V, Viswanathan P. Gastro protective effect of fenugreek seeds (*Trigonella foenum graecum*) on experimental gastric ulcer in rats. J Ethnopharmacol. 2002; 8: 393-397.

40. Berglindh T. Effects of common inhibitors of gastric acid secretion on secretagouges-induced respiration and aminopyrine accumulation in isolated gastric glands. Biochem Biophys Acta. 1977; 464: 217–233.

41. Dial E, Thompson J, Rosenfeld G. Isolated parietal cells histamine response and pharmacology. J Pharmacol Exp Therap. 1981; 219: 586–590.

42. Bottcher G., Hakanson G., Nilson R.S., Sundler F. Effects of long term hypergastrinaemia on the ultra structure of enterochromafin-like cells in the stomach of the rat, hamster, and guinea pig. Cell Tissue Resp. 1989; 256: 247–257.