PACIFIC JOURNAL OF MEDICAL SCIENCES

{Formerly: Medical Sciences Bulletin}

ISSN: 2072 – 1625

Pac. J. Med. Sci. (PJMS)

www.pacjmedsci.com. Email: pacjmedsci@gmail.com.

GASTROPROTECTIVE EFFECTS OF AQUEOUS EXTRACT OF UNRIPE CARICA PAPAYA FRUIT IN RATS

***Bamidele V. Owoyele, Atinuke F. Gbago and Olabode S. Ashaolu**

Department of Physiology, University of Ilorin, Ilorin, Nigeria

*Corresponding author: B. V. Owoyele: deleyele@yahoo.com or owoyele@unilorin.edu.ng

Running title: Pawpaw fruit and gastroprotection.

GASTROPROTECTIVE EFFECTS OF AQUEOUS EXTRACT OF UNRIPE CARICA PAPAYA FRUIT IN RATS

***Bamidele V. Owoyele, Atinuke F. Gbago and Olabode S. Ashaolu**

Department of Physiology, University of Ilorin, Ilorin, Nigeria

*Corresponding author: B. V. Owoyele: deleyele@yahoo.com or owoyele@unilorin.edu.ng

Running title: Pawpaw fruit and gastroprotection.

ABSTRACT:

Aqueous extract of unripe Carica papaya fruit (AEUCPF) was investigated for its anti-ulcer, mucus secretion, anti-acid secretory and pepsin binding effects in rats. Ethanol/HCl and Indomethacin were used to induce ulcers while acid and mucus secretion was measured in ulcerated and treated animals. The animals were divided into five groups for each of the anti-ulcer studies and each group was made up of five animals each. These groups included a control and reference groups administered saline and cimetidine (Kg/Kg b.w), while the remaining three groups were administered with 2.5, 3.5 and 4.5ml/Kg of the decoction of the unripe fruits. The extract, cimetidine and saline were all administered orally twice daily for ten days while necrotizing agents were administered (p.o) once daily from day 8 through day 10. The results showed that higher doses of the extract significantly ($p<0.05$) reduced the ulcer index from 3.6± 0.24 (control) – to 0.70 ± 0.37 (4.5ml/Kg) in the ethanol induced ulcer. The extract also produced similar effects in the indomethacin induced ulcer and in both cases the gastric acidity was significantly reduced. The extract did not increase mucus secretion but it bind substantially with pepsin. In conclusion this study has shown that AEUCPF has beneficial effects on the normal function of the stomach. It has the capacity to ameliorate gastric ulcer as suggested by local traditional medical practitioners.

KEYWORDS: Carica papaya; Extract; Gastric acid; Pepsin; Ulcer; Rats.

(Submitted March 2013; Accepted June 2013)

INTRODUCTION:

The stomach is the most distensible and one of the vital parts of the gastrointestinal tract [1]. It is involved in digestion of various foods which it receives from the oesophagus. In addition to food, the stomach is exposed to many potentially injurious agents such as acids, pepsin, bacterial products and drugs [2,3]. There is a continuous effort to find good synthetic or phytochemical agents that will offer gastroprotective effects. Thus many plants and plants derived products have been screened for their anti-ulcer effects. These include: Persea americana, Landolphia owarriensis, Ananas ananassoides, Garcinia kola seeds, a biflavonoid kolaviron isolated from G. Kola seeds, licorice, etc [3-7].

Carica papaya (L) commonly called pawpaw is a large tree like plant with stem growing from 5 to 10 metres tall. It has spirally arranged leaves which are confined to the top of the trunk. It produces fruits which are mainly oval in shape with light green colour in the unripe state but which may turn yellow when it ripens. The unripe fruit can be cooked as parts of salads, jellies and stews while the ripe fruits are usually eaten raw without the skin or seed [8-9]. The plant is employed in the treatment of several ailments by traditional medical practitioners with such uses including but not limited to the treatment of the following: sore throat, asthma, sickle cell anaemia, wound, ulcers, boils, malaria, fever, pain, tonsillitis, indigestion, dyspepsia, jaundice and cancer [8-11]. The unripe fruit have been reported to have anti-sickling, laxative, arbortifacient and diuretic properties [11] while the intake of the extract of unripe fruit of the plant has been linked with an anti-ulcer effect [12].

Ezike et al, [12] had investigated the probable beneficial effect of unripe papaya fruit on the treatment of gastric ulcer by administering extract of unripe papaya fruit however; the focus of the present study is to investigate the use of aqueous extract of the unripe fruit on gastric mucosa irritation, mucus secretion and gastric acidity. Therefore this study was designed to specifically mimic the exact practice of the traditional medical practitioners and to see if this practice is effective. Thus we investigated if the aqueous extract of unripe and mature fruit of C. papaya has therapeutic effects in animal models of ulcer and the probable mechanism for such effects.

MATERIALS AND METHODS:

Plant material and preparation of decoction:

The unripe fruit of C. papaya fruits were collected from fruit gardens in Ilorin metropolis and the mini campus of University of Ilorin. The plant had been previously [9] identified at Forestry Research Institute of Nigeria (FRIN) with a voucher specimen number FHI 106933. The unripe fruits (with total weight of 2.9 Kg) were washed with distil water and sliced into small cubed shaped pieces each weighing 50g each. The slices of each of the three fruits were soaked

in 2.5 litres of distil water for 96 hours after which the resulting solution was sieved and immediately used for pharmacological studies.

Animals

Male Wistar rats weighing 180±10.1g were used for these studies. The animals were bred in the animal house of the Faculty of Basic Medical Sciences, University of Ilorin and fed on standard mouse cubes (exotic Feeds, Ilorin, Nigeria). They were kept in clean cages with optimum temperature of about 250C, humidity of 60-65% and 12 hours light/dark cycle. Animals were provided with water ad libitum. The research was conducted in accordance with the ethical rule for animal experimentation, approved by Ethical Committee, College of Health Sciences University of Ilorin.

The animals were divided into five groups with each group comprising of five animals each. Group A (control) was administered saline (10ml/Kg), group E was administered 11.5g/Kg of cimetidine. The animals in groups B-D were administered 2.5, 3.5 and 4.5ml/Kg of the decoction of unripe papaya fruit extract twice daily for ten days. Ethical approval was obtained from the Ethic Committee of the Department of Physiology, University of Ilorin in accordance with the University of Ilorin guidelines on the care and use of laboratory animals.

Anti-ulcer studies

HCl/Ethanol induced ulcer: Ulceration was induced in experimental animals by the administration of 1 ml of necrotizing solution (150 mm of HCl in 60% ethanol) in accordance with the method used by Mizui and Douteuchi [13]. Animals were orally administered saline, cimetidine or decoction of unripe fruit (2.5, 3.5 or 4.5mg/Kg) of C. papaya twice daily for ten days. However the administration of the necrotizing agent started on the eighth day once daily for three days. The animals were sacrificed 2hrs after the administration of the test substances and saline. The stomachs of the animals were dissected out and an incision was made at the greater curvature in order to collect gastric contents and observation of gastric mucosa for the presence of gastric ulceration. Ulceration was confirmed by using a hand held lens (x10) and the ulcer scores were determined using the arbitrary scale used by Singh et al, [14] as in previous studies [9]. A score of 0 was assigned to no visible lesion; 0.5 for hyperaemia; 1 for one or two slight lesions, 2 for severe lesions; 3 for very severe lesions and 4 for mucosal that is full of many lesions. The ulcer index was also calculated as the means of ulcer scores.

Indomethacin induced ulcer: Ulceration was induced in these groups of animals by administration of 20 mg/Kg of indomethacin as necrotizing agent. Animal grouping and drug administration were as in the HCl-ethanol induced ulcer above. The same ulcer scoring method was also used.

Determination of gastric acidity

Samples of gastric contents from each rats used for antiulcer studies were collected and

centrifuged (2000 rpm)for 10 min. after which 1 ml of the supernatant was analysed for hydrogen ion concentration by titration against 0.1 M NaOH to a pH of 7.0 using phenolphthalein as an indicator.

Mucus secretion

Measurement of mucus production

Gastric mucus production was assessed in rats that were administered HCl/ethanol necrotizing agent immediately after the determination of the ulcer scores of the animals as described previously [7]. Briefly, the mucus layer of the stomach of each rat was scraped using a glass slide into a glass tube containing 1 ml of water whose weight was predetermined. The final weight of the container and the mucus was determined using a digital electronic balance and the difference between the final weight and the predetermined weight was taken as the weight of the mucus.

Pepsin binding activity

Pepsin binding activity of AEUCPF was determined as previously reported, [6,15] 50 ml of the aqueous extract was added to 1 mL of pepsin solution (2 mg/mL) in a test tube followed by the addition of 4 ml of 0.2 N HCl buffered with 1 ml of 0.2 N sodium citrate solution. Thereafter, 1 ml of bovine serum albumin (5 mg/mL) was added to treat the excess pepsin except the control test tubes. All reagents were kept at a temperature 37°C for 30 minutes prior to incubation and at the same temperature for 30 minutes after incubation. The remaining protein in each tube was treated with 1.0 ml of Biuret reagent and 5 ml 0.2 N NaOH solution. The absorbencies were read at 546 nm and the result was expressed as percentage binding of pepsin.

Phytochemical analysis

Preliminary phytochemical analysis of the extract was carried out using standard procedures for alkaloids, reducing sugars, tannins, flavonoids, saponins, steroids, and anthraquinones [16-18].

Statistical analysis

All values are expressed as mean ± standard error of the means (SEM). Statistical significance was determined using the Student's t-test. Values with $P < 0.05$ compared with the control group were considered as being significantly different.

RESULTS:

Anti-ulcer studies

The results of the anti-ulcer studies showed that gastric mucosa lesions were significantly ($p< 0.05$) reduced by all the doses of AEUCPF. The ulcer score was reduced by the 4.5ml/Kg from 3.6± 0.24 (control) – 0.70±0.37 in the HCl/Ethanol induced lesion (Table 1.). Likewise 4.5ml/Kg of AEUCPF significantly ($p< 0.05$) reduced the ulcer score from 3.8± 0.2 (control) - 0.9 ± 0.33 in the indomethacin induced gastric lesion (Table 2.). Fig. 1 shows the percentage protection of the mucosal by AEUCPF in the two models of ulcerogenesis.

Table 1: Effects of aqueous extract of unripe fruit of Carica papaya on HCl/Ethanol induced ulcer in rats

Groups	Dose (mL/Kg)	Ulcer index	Total Gastric acidity (µEq/mL)
Control (saline)	-	3.60 ± 0.24	62.2 ± 8.1
C. papaya	2.5	1.70 ± 0.44*	32.3 ± 7.8*
C. papaya	3.5	1.10 ± 0.51*	25.8 ± 1.2*
C. papaya	4.5	0.70 ± 0.37*	24.6 ± 2.5*
Cimetidine	11.5 (mg/Kg)	0.60 ± 0.19*	24.3 ± 7.2*

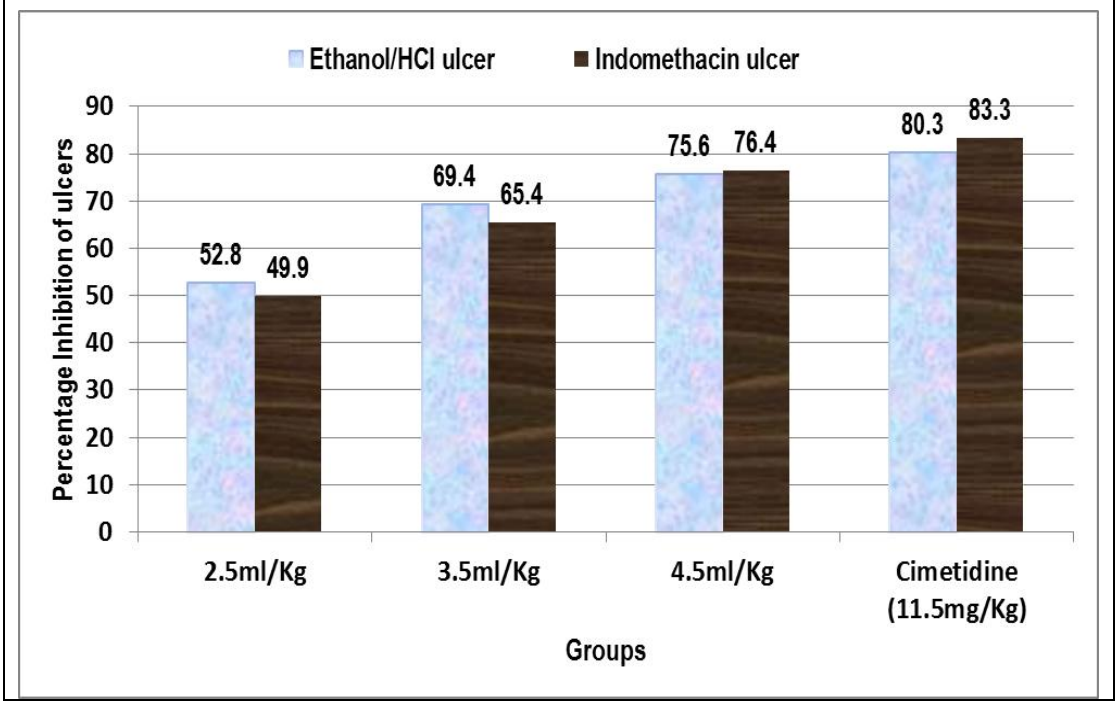
[a]Each value is mean ± S.E.M. for 5 rats. *P < 0.05 compared with control.

Table 2: Effects of aqueous extract of unripe fruit of Carica papaya on indomethacin induced ulcer in rats

Groups	Dose (ml/Kg)	Ulcer index	Mucus secretion	pH	Total Gastric acidity (µEq/mL)
Control (saline)	-	3.81 ± 0.20	11.10±5.51	2.28± 0.10	66.0 ± 2.9
C. papaya	2.5	1.91 ± 0.51*	12.51± 4.57	2.21± 0.19	24.2 ± 2.1*
C. papaya	3.5	1.32 ± 0.54*	9.69± 5.0	2.42± 0.19	12.4 ± 1.1*
C. papaya	4.5	0.90 ± 0.33*	11.3± 6.40	2.59± 0.09	22.1 ± 1.4*
Cimetidine	11.5 (mg/Kg)	0.75 ± 0.19*	54.21± 10.70*	2.68± 0.14	22.4 ± 0.7*

[a]Each value is mean ± S.E.M. for 5 rats.*P < 0.05 compared with control..

Fig 1: Percentage inhibition of ulcers by aqueous extract of unripe fruit of Carica papaya. Each value is the mean of 5 rats.



Gastric acidity, Pepsin binding and mucus production

Tables 1 and 2 show the results of the effects of administration of AEUCPF on gastric acid secretion in the HCl/ethanol and indomethacin induced lesions respectively. The acidity was significantly ($p < 0.05$) reduced by all the doses of AEUCPF.

AEUCPF produced 103.25% binding with pepsin. However, the decoction did not produce any significant changes in the mucus production in stomach of animals.

Phytochemical Analysis

The results of the phytochemical screening showed that the extract contains alkaloids, flavonoids, polyphenols, anthraquinones, reducing sugars, saponins and steroids

DISCUSSION:

It is indeed amazing why certain plants are used for the treatment of specific ailments. C. papaya is such a useful plant for traditional medical practitioners. The desire to unravel the usefulness of this plant has led us to previously investigate the anti-inflammatory, analgesic and anti-ulcer effects of the leaves based on ethnopharmacological information [9].

The present study investigated the gastroprotective effects of aqueous extract of unripe C. papaya fruit (AEUCPF) based on its use locally for the treatment of ulcer which included the administration of 96 hours soaked unripe fruit solution to treat ulcer patient for a period of ten days in the first instance. The doses chosen in the study were carefully calibrated to resemble the common doses used by the traditional practitioners where 3.5 ml/kg approximated the doses used twice per day and the 2.5 and 4.5ml/Kg were alternate doses used for comparison with the standard dosage of 3.5 ml/Kg. The two methods used for producing gastric lesion (HCl/Ethanol and indomethacin) are validated models [19-22].

The findings showed that administration of AEUPCF for ten days produced a dose dependent anti-ulcer effects in the HCl/ethanol induced ulcers with the minimum dose producing a percentage inhibition of 52.8% which is relatively high (Fig.1). The highest dose (4.5ml/Kg) of AEUPCF produced comparable anti-ulcer effects with the standard drug cimetidine (11.5mg/Kg). In the indomethacin induced gastric lesion AEUPCF also produced similar dose dependent pattern of anti-ulcer effects with highest dose of the extract producing effects that is comparable with that of cimetidine. The minimum dose also inhibited ulcerogenesis by 50% which is a value similar to what the equivalent dose produced in the HCl/Ethanol induced ulcer model. This showed that the results can probably be replicated in most models of ulcer caused by effects of diverse ulcerogens.

Prior treatment of the animals with AEUPCF before the administration of necrotizing agent was able to strengthen the gastric mucosa against the activities of necrotizing agents. Generally, necrotizing agents may produce gastric lesion by a combination of many factors which includes but not limited to the following; inhibition of prostaglandins (PGE2 and PGI2) synthesis especially with indomethacin a non-steroidal anti-inflammatory agents [23-25], promotion of acid-pepsin aggression on gastric mucosal [26-28] decrease in gastric mucosal barrier/resistance [26] and an increase in lipid peroxidation [26, 29], or the direct increase of gastric acid secretion. Observation in this study showed that AEUPCF use some of these mechanisms to inhibit gastric lesion hence its effectiveness in binding pepsin and reduction of gastric acidity. These help in strengthening mucosal barrier and reducing the direct effects of gastric acid on the mucosa. It can also counteract the effects of NSAIDS on prostaglandins synthesis. Mucus production seems not to be parts of AEUPCF mechanism of protecting gastric mucosal from insults of necrotizing agents as there was no significant changes in the mucus secretion in treated rats compared with the control.

The results of the phytochemical analysis also showed that the AEUPCF might be exerting it effects via its contents of flavonoids, alkaloids, anthocyanides or saponins. Flavonoids have been specifically linked with gastroprotective activities [4,30]. Likewise some alkaloids and saponins have been implicated as active principles responsible for gastroprotective activities of some plants such as Pyrenacantha staudii and Zizyphus sativa.[31-33]. The findings from the phytochemical analysis agree with that of Ezike et al,[12]. They used aqueous and methanol extract of unripe fruit of the plant but the dosages and preparation of the plant material was different from what was used in this study. While they used a single dose of 300 mg/Kg for both aqueous and methanol extract, we used three doses of aqueous decoction which was freshly prepared to simulate the practice by traditional medical practitioners and local users. Oduola et al, [8] also administered a decoction of the unripe fruit for the treatment of sickle cell disease. The ulcer models by Ezike et al, [12] and in this study were nearly the same with modifications in the dose of ulcerogen in indomethacin induced ulcer; however, they used absolute ethanol as the second ulcerogen while we employed HCl/Ethanol in our studies. Nevertheless our method of extraction is relatively easier for would be users of this plant product if it is to be consumed raw and Ezike et al, [12] recognized that water extraction is the preferred method by traditional practitioners. Therefore, the present study has thrown more light into the beneficial effect of the use of unripe and mature fruit of C. papaya.

CONCLUSION:

In conclusion, the overall finding of this study is that aqueous extract of unripe fruit of Carica papaya possess antiulcer properties which may be due to its ability to inhibit gastric acid secretion and reduction in pepsin activity and availability. It is a promising material for treatment of gastric mucosal injury and therefore further studies on this plant are encouraged.

ACKNOWLEDGEMENT:

Authors are grateful to Mrs F.E. Olawale-Bello for technical assistance.

REFERENCES:

1. Fox SI Human physiology 8ed The McGraw−Hill Companies, 2003, pp 564.
2. Peskar B M, Maricic N. Role of prostaglandins in gastroprotection, Digestive Diseases Sciences 1998; 43: 23S.
3. Onasanwo SA, Singh N, Olaleye SB, Palit G. Anti-ulcerogenic and proton pump (H+, K+ ATPase) inhibitory activity of Kolaviron from Garcinia kola Heckel in rodents. Indian Journal of Experimental Biology 2011; 49: 461-468.
4. Owoyele BV, Adebayo IK, Soladoye AO. Anti-ulcer effects of aqueous extract of Persea Americana Mill (Avocado) leaves in rats. Compendium of Bioactive Natural products 2010; 8: 183 - 188.
5. Ibironke, G. F., Olaleye, S. B., Balogun, O. And Aremu, D. A. Antiulcerogenic effect of diet containing seeds of Garcinia kola (Heckel). Phytotherapy Research 1997, 11: 312-313.
6. Olaleye SB, Owoyele BV, Odukanmi AO. Antiulcer and gastric antisecretory effects of Landolphia owariensis extracts in rats. Nigerian Journal of Physiological Sciences 2008; 23; 23-26.
7. Owu DU, Obembe AO, Nwokocha CR, Edoho IE, Osim EE. Gastric Ulceration in Diabetes Mellitus: Protective Role of Vitamin C. International Scholarly Research Network ISRN Gastroenterology 2012, 362805, doi:10.5402/2012/362805.
8. OduolaT,Adeniyi FAA, Ogunyemi EO, Idowu TO, Bello IS. Evaluation of the Effects of Intake of Extract of Unripe Pawpaw (Carica Papaya) on Liver Function in Sickle Cell Patients. World Journal of Medical Sciences 2007; 2: 28-32.
9. Owoyele BV, Olubori MA, Adeoye AF, Soladoye AO. Anti-inflammatory activities of ethanolic extract of Carica papaya Leaves. Inflammopharmacology 2006; 16: 1–6.
10. Morton, J.F., 1987. Major medical plants C.C Thomas, spring field, IL.
11. Oduola T, Adeniyi FAA, Ogunyemi EO, Bello IS, Idowu TO. Antisickling agent in an extract of unripe pawpaw (Carica papaya): Is it real? African Journal of Biotechnology 2006; 5: 1947-1949.
12. Ezike AC, Akah PA, Okoli CO, Ezeuchenne NA, Ezeugwu S. Carica papaya (paw-paw) unripe fruit may be beneficial in ulcer. Journal of Medicinal Food 2009; 12: 1268–1273.
13. Mizui T, Douteuchi M. Effect of polyamines on acidified ethanol induced gastric lesions in rats. Japan Journal of Pharmacology 1988; 33: 939-945.
14. Singh S, Bani S, Singh GB, Gupta BD, Banerjee SK, Singh B. Antiinflammatory activity of Lupeol. Fitotererapia, 1997; 68: 9-16.
15. Rifat-uz-Zaman M, Akhtar S, Khan MS. Evaluation of acid buffering and pepsin binding properties of Ispaghula (Plantago ovata, Forsk) and its extracts. Hamdard, 2002; XLV: 32-36.
16. Harborne JB. Phytochemical methods. In: A Guide to Modern Techniques of Plant Analysis (Harborne JB, ed.). Chapman and Hall, London, 1973, p. 279.

17. Trease GE, Evans WC. A Textbook of Pharmacognosy, 13th ed. Bailliere-Tindall Ltd., London, 1989, pp. 5–53.
18. Sofowora A. Medicinal Plants and Traditional Medicine in Africa, 2nd ed. Spectrum Books Ltd., Ibadan, Nigeria, 1993, pp.134–156.
19. Hara N, Okabe S. Effect of gefernate on acute lesions in rats.Folio Pharmacologia Japonica 1985; 85:443-448.
20. Oates PJ, Hakkinen JP. (1988). Studies on the mechanism of ethanol-induced gastric damage in rats. Gastroentereterology 1988; 94: 10-21.
21. Tan PV, Nditafon NG, Yewah MP, Dimo T, Ayafor FI. Eremomoastax speciosa: effect of leaf aqueous extract on ulcer formation and gastric secretion in rats. Journal of Ethnopharmacology 1996; 54: 139-142.
22. Goulart YCF, Sela VR, Obici S, Martins JVC, Otobone F, Cortez DA, Audi EA. Evaluation of gastric anti-ulcer activity in a hydroethanolic extract from Kilmeyera coracea. Brazilian Archives of Biology and Technology 2005; 48: 211-216.
23. Vane JR. Inhibition of prostaglandin synthesis as a mechanism of action for aspirin like drugs. Nature (New Biology), 1971; 231: 232-235.
24. Allen A, Garner A. Mucus and bicarbonate secretion in the stomach and their possible role in mucosal protection. Gut 1980; 21: 249-262.
25. Deshpande SS, Shah GB, Parmar NS. Anti-ulcer activity of Tephrosia purpurea in rats. Indian Journal of Pharmacology 2003; 35: 168-172.
26. Nafeeza MI, Fauzee AM, Kamsia J, Gapor MT. Comparative effects of a tocotrienol rich fraction and tocopherol in aspirin-induced gastric lesions in rats. Asia Pacific Journal of Clinical Nutrition 2002; 4: 309-313.
27. Goel, R.K., G. Saroj, R. Shankar and A.K. Sanya. 1986 Anti ulcerogenic effect of Banana powder (Musa sapientum var. paradisiaca) and its effects on mucosal resistance. Journal of Ethnopharmacology 1986; 18:33-44.
28. Njar VCO, Adesanwo JK, Raji Y. 1995. Methyl angolensate: anti-ulcer agent of the stem bark of Entandrophragma angolense. Planta Medica1995;61:91-92.
29. Pandit S, Sor TK, Jana O, Bhattaacharyya D, Debnath PK. 2000. Anti-ulcer effect of Shankar Bhasma in rats: A preliminary study. Indian Journal of Pharmacology 2000; 32: 378-380.
30. Zayachkivska OS, Konturek SJ, Drozdowicz D, Konturek PC, Brzozowski T, Ghegotsky MR. Gastroprotective effects of flavonoids in plant extracts. J of Physiology and Pharmacology, 2005; 56 (suppl1): 219-231.
31. Aguwa CN, Okunji CO. Gastrointestinal studies of Pyrenacantha staudii leaf extracts. Journal of Ethnopharmacology 1986;15: 45–55.
32. Shah AH, Khari ZA, Baig MZA, Qureshi S, Al-Bekairi AM. Gastro protective effects of pretreatment with Zizyphus sativa fruits against toxic damage in rats. Fitoterapia 1997; 68: 226- 234.
33. Maity S, Chaudhuri T, Vedasiromoni JR, Ganguly DK. Cytoprotection mediated anti-ulcer effect of tea root extract. India Journal of Pharmacology; 2003; 35: 213-219.