IJPSR (2014), Vol. 5, Issue 8 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 08 February, 2014; received in revised form, 08 May, 2014; accepted, 28 June, 2014; published 01 August, 2014

ANTI-SECRETORY, GASTROPROTECTIVE AND ANTI-ULCER ACTIVITIES OF AQUEOUS EXTRACT OF *PHRAGMANTHERA CAPITATA* S. BALLE IN RATS

L. P. Takem [*1], P. M. Udia [1] and C. F. Poh [2]

Department of Pharmacology, Faculty of Basic Medical Sciences [1], University of Calabar, Cross River State, Nigeria.
Department of Pharmacology, Faculty of Basic Medical Sciences [2], College of Health Sciences, Niger Delta University, Bayelsa State, Nigeria.

Keywords:

Phragmanthera capitata, anti-secretory, anti-ulcer, gastroprotective, loranthaceae

Correspondence to Author:
L. P. Takem

Department of Pharmacology, Faculty of Basic Medical Sciences, University of Calabar, Cross River State, Nigeria.

Email: lapietak@yahoo.com

ABSTRACT: *Phragmanthera capitata* is mistletoe (parasitic plant) that grows on other plants including avocado trees. Leaves infusion or decoction is claimed to treat amongst other ailments abdominal pains by herbalists in Cameroon. Our goal was to evaluate possible secretory, gastro and ulcer activities of aqueous extract of *P. capitata* (AEPC) in indomathacin induced gastric ulcer and pylorus ligation induced gastric ulcer (PLIGU) in albino rats. Preliminary phytochemical and toxicity assays were performed following standard procedures. To evaluate indomethacin induced gastric ulcer, control group received 10 ml/kg normal saline, standard group received 100 mg/kg cimetidine, test groups received AEPC (100, 200 and 300 mg/kg). To evaluate PLIGU, treatment was same as indomethacin induced ulcer. Data obtained were analyzed using analysis of variance (ANOVA) with Tukey test used as post hoc. In preliminary phytochemical assays AEPC revealed the presence of terpenoids, tannins, saponins, glycosides, anthraquinones, flavonoids, alkaloids and phenols. In acute oral toxicity assay, AEPC was safe up to a concentration of 3000 mg/kg in mice. In anti-ulcer evaluation AEPC decreased significantly ($P < 0.05$) the number of ulcers, ulcer scores and ulcer index in both indomathacin and PLIGU. PLIGU rats also showed significant ($P < 0.05$) decrease in gastric volume, total acidity, free acid and an increase in pH of gastric fluid. These results, undoubtedly, suggest that AEPC possesses significant anti-secretory, gastroprotective and anti-ulcer activities in a dose-dependent manner. These findings thus corroborate the use of the extract in folkloric practice.

INTRODUCTION: Mucosal surface of gastrointestinal tract is covered by hydrated gel made of mucin and secreted by specialized cells such as Globlet cells to create barrier that prevents pathogens from contacting the epithelium directly [1]. Bulk flow of fluid across the mucosal layer is, thus, limited.

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.5(8).3560-65

Article can be accessed online on: www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.5(8).3560-65

In the stomach, bicarbonate ions (HCO_3^-) are secreted to maintain alkalinity at the mucosal surface and prevent acid erosion of the cells. The mucus layer also provides pathogen colonisation resistance by allowing adhesion of commensal bacteria and excluding intestinal pathogens [2]. The plasma membrane of enterocytes is further made impermeable by the apical junction complex made of tight and subjacent adherens junctions [3] to seal paracellular spaces. Enteric pathogen and endotoxin translocations are reported to increase paracellular permeability via tight junction alterations [4]. This causes imbalance between mucosal defensive factors like prostaglandin,

bicarbonate, peptides, nitric oxide, growth factors and injurious factors like pepsin and acid, thus, causing ulcer [5-7]. Polymorphism of multidrug resistance protein-1 also was reported to influence *Helicobacter pylori*-induced gastric inflammation [8]. Ulcers can also be caused or worsened by non-steroidal anti-inflammatory drugs (NSAIDs) [9].

Drugs currently used in the treatment of ulcers include antacids [10], anticholinergics [11], proton pump inhibitors [12] and H_2-receptor antagonists [10]. That notwithstanding, adverse reactions like arrhythmia, hypersensitivity, impotence, gynecomastia and hematopoietic changes, abound [11]. The use of plants for medicinal purposes is very commonplace and widespread in Africa. Plants and phytoconstituents are better choice to treat diseases than the allopathic drugs [13]. The use of natural medicine in the treatment of various diseases like peptic/duodenal ulcers is an absolute requirement of our time [14].

Phragmanthera capitata S. Balle is mistletoe plant of loranthaceae family [15] with woody shrub and pendent branches, often found with ants' nests [16]. It can be found in West Tropical Africa like Nigeria; West-Central Africa like Cameroon and Angola in South Tropical Africa [17]. Aqueous extract of *P. capitata* has anti-diarrheal properties [18], anti-pyretic and analgesic potentials [19] while infusion of leaves cures diabetes [20], Chlamydia infection and a variety of other diseases including fever and abdomnal pains in Cameroon herbal practices [21, 22]. Our objective was then to investigate scientifically, the anti-ulcerogenic property of AEPC in albino rats to corroborate herbal claims.

MATERIALS AND METHODS:

Drugs: Cimetidine and Indomethacin (Sigma Chemical Co., USA)

Plant material and preparation of extract: *P. capitata* or "ntsalar" as it is called in Babadjou vernacular was harvested from avocado trees in Konka in Baligham village in North Western region of Cameroon in July, 2013. Authentication of the plant was done in Cameroon National Herbarium (CNH), Yaounde, with Voucher No. 24673/SRF/CAM.

The whole plant was washed with tap and distilled water and shade dried at room temperature (25 ± 2^{o}C) for 3 weeks. The dried sample was pulverized using manual grinding machine and preserved in air tight container. 1 kg of the sample was macerated in 7.5 L of distilled water for 3 days. The filtrate was concentrated in sunlight for 3 days at 43±3^{o}C to give 15.5% yield.

Experimental animals: Healthy albino rats (120-140 g) of either sex were randomly selected and used for the experiment. The rats were housed in polyvinyl cages of 4 animals per cage and maintained under standard laboratory conditions of relative humidity (50 ± 5%), temperature (28 ± 2^{0}C), a 12 hour dark and light cycle and received standard pellet diet (Agro Feed, Calabar) and tap water *ad libitum*. These experimental rats were taken care of following the guidelines of the CPCSEA [23].

Phytochemical screening: Qualitative screening of AEPC had been done in our earlier study to detect the presence of phytoconstituents such as flavonoids, alkaloids, glycosides, tannins, saponins, terpenoids and anthraquinones following standard tests procedures [18].

Acute toxicity study: Acute toxicity study of AEPC had been in our earlier study[18] in female albino mice in accordance with the Organization for Economic Co-operation and Development guidelines no. 425 Up and Down Procedure [24, 25].

Induction of ulcer:

Indomathacin-Induced Gastric ulcer: Albino rats were randomized into 5 groups of 6 rats each and were fasted for 24 h though with free access to tap water *ad libitum* before the start of experiment. Group I (control) received distilled water (10 ml/kg), Group II (standard) received Cimetidine (100 mg/kg), Groups III-V (tests) received AEPC (100, 200, 300 mg/kg respectively) by oral gavage. One hour post treatment, IMC (40 mg/kg, per oral) was administered to all rats. Rats were sacrificed 5 h post IMC treatment and stomach was cut open in the greater curvature, rinsed and examined by a ×5 magnifier lens. The number of ulcers formed were recorded and ulcer scores were recorded as 0 = no ulcer, 1 = superficial ulcer, 2 = deep ulcer, 3 =

perforation. Ulcer index was measured by the formula [11]:

$$U_I = U_N + U_S + U_P \times 10^{-1}$$

Where;
U_I = ulcer index
U_N = average number of ulcers per animal
U_S = average number of severity score and
U_P = percentage of animals with ulcers.

The percentage inhibition of ulceration was calculated as:

$$\% \text{ Inhibition} = \frac{\text{U1 Control} - \text{U1 Test}}{\text{U1 Control}} \times 100$$

Pylorus ligation induced gastric ulceration: Albino rats (180-200 g) of either sex were randomized into 5 groups of 6 animals each. The rats were fasted for 24 h but with free access to tap water *ad libitum* before the start of experimentation. Group I (control) received distilled water (10 ml/kg), Group II (standard) received Cimetidine (100 mg/kg), Groups III-V (tests) received AEPC (100, 200, 300 mg/kg respectively) by oral gavage. 1 h post treatment, each rat was anesthetized with ether intraperitoneally. The abdomen was opened by a small midline incision below the xiphoid process while avoiding damage to blood supply. Pyloric portion of the stomach was slightly lifted out and ligated [26].

Then, the stomach was replaced carefully and the abdominal wall was sutured. The rats were sacrificed by an over dose of ether after 4 h of ligation [11]. The abdomen was opened, eosophageal sphincter end was cut and stomach contents were drained into a glass tube. The volume of the gastric juice was measured and centrifuged (Superspin, Plasto-crafts, India) at 2000 rpm for 10 min. 1 ml aliquot each was taken from the supernatant to determine the pH, total and free acidity. The inner surface of free stomach was rinse in distilled water to examine for gastric lesions.

Determination of pH: Aliquot of 1 ml gastric juice was diluted with 1 ml of distilled water and pH of the solution was read using pH meter [27].

Determination of total acidity: In a 50 ml conical flask, an aliquot of 1 ml gastric juice was diluted with 1 ml of distilled water. Two drops of phenolphthalein indicator was added to the flask and titrated with 0.01N NaOH until a permanent pink colour was observed. The volume of 0.01N NaOH consumed was noted. The total acidity was expressed as meq/L by the formula:

$$AT = n \times 0.01 \times 36.45 \times 1000$$

Where;
n = volume of NaOH consumed
36.45 = molecular weight of NaOH
0.01 = normality of NaOH
1000 = the factor (to be represented in litre) [28].

Determination of free acidity: Topfer's reagent was used in lieu of phenolphthalein indicator. Aliquot of gastric juice was titrated with 0.01 N NaOH until canary yellow colour was observed. The volume of 0.01 N NaOH consumed was noted. The free acidity was calculated by the same formula for the determination of total acidity [27].

Statistical analysis: Values were expressed as means ± SEM (n = 7). Differences between means were compared statistically by one-way analysis of variance (ANOVA) followed by Tukey test as post hoc. P <0.05 were considered statistically significant [29].

RESULTS:

Preliminary phytochemical screening of AEPC had revealed in our previous study high presence of terpenoids and tannins; moderate presence of saponins, glycosides, anthraquinones and flavonoids; and low presence of alkaloids and phenols. Acute toxicity evaluation from our previous study revealed no toxic reaction, no behavioral changes and no mortality up to a concentration of 3000mg/kg in mice during the limit test.

Effect of AEPC on indomethacin induced gastric ulcer in albino rats is shown in **Table 1**. Pretreatment of the rats at a concentration of 100, 200, and 300 mg/kg reduced the number of ulcers, ulcer scores and ulcer index. Significant reduction (P <0.05) occurred at a concentration of 300 mg/kg

which corresponded to 40.33% ulcer index reduction as compared to 69.19% for cimetidine (standard). **Table 2** shows effect of AEPC on PLIGU in rats. Treatment of gastric ulcer with, 200, 300 mg/kg also reduced number of ulcers, ulcer scores and ulcer index with significant reduction (P <0.05) occurring at a concentration of 300 mg/kg corresponding to 42.30% ulcer index reduction as compared to 72.85% for cimetidine (standard).

Table 3 shows effect of AEPC on gastric content, pH, total acidity and free acid in PLIGU in rats. Significant decrease (P <0.05) in gastric volume, total acidity, free acid and increase in pH occurred at a concentration of 300 mg/kg. Significant decrease (P <0.05) in total acidity and free acid also occurred at a concentration of 200 mg/kg.

TABLE 1: EFFECT OF AQUEOUS EXTRACT OF *PHRAGMANTHERA CAPITATA* ON INDOMETHACIN-INDUCED GASTRIC ULCER IN RATS.

Treatment	dose (mg/kg,	Average No. Of Ulcers	Average Ulcer Scores	Ulcer Index	% inhibition Ulcer Index
Control (D. water)	10ml/kg	15.12±3.12	3.10±0.13	12.53±2.11	00.00
Cimetidine	100	2.33±0.12	0.50±0.02	3.86±0.11	69.19
AEPC	100	13.50±0.14	2.10±0.11	10.45±0.42	16.60
AEPC	200	9.50±0.34	1.51±0.32	9.03±0.31	27.93
AEPC	300	3.31±0.21*	0.62±0.41*	7.47±0.16*	40.33*

The data were presented as mean ± SEM (7 animals in each group). *P <0.05

TABLE 2: EFFECT OF AQUEOUS EXTRACT OF *PHRAGMANTHERA CAPITATA* ON PYLORUS LIGATION INDUCED GASTRIC ULCER IN RATS.

Treatment	dose (mg/kg, p.o.)	Average No. of Ulcers	Average Ulcer Scores	Ulcer Index	% Inhibition of Ulcer Index
Control (D. water)	10ml/kg	13.12±3.02	3.01±0.16	12.12±2.11	00.00
Cimetidine	100	1.73±0.12	0.53±0.01	3.29±0.15	72.85
AEPC	100	10.40±0.12	2.07±0.12	9.95±0.23	17.90
	200	7.76±0.31	1.12±0.12	8.19±0.12	32.43
	300	2.11±0.41*	0.53±0.21*	6.99±0.03*	42.30*

The data were presented as mean ± SEM (7 animals in each group). *P <0.05

TABLE 3: EFFECT OF AQUEOUS EXTRACT OF *PHRAGMANTHERA CAPITATA* ON GASTRIC CONTENT, PH, TOTAL ACIDITY AND FREE ACID IN PYLORUS LIGATION INDUCED GASTRIC ULCER IN RATS.

Treatment	Dose (mg/kg)	Gastric contents (ml/100g)	pH	Total acidity (meq/L)	Free acid (meq/L
Control (D. water)	10 ml/kg	4.21±0.31	3.69±0.12	5963.83±654.42	4986.43±689.45
Cimetidine	100	2.31±0.11	5.31±0.10	1899.87±589.63	1698.64±473.62
AEPC	100	4.07±0.01	3.71±0.11	4897.66±387.75	3087.78±370.53
	200	3.75±0.21	4.64±0.21	3597.89±295.53*	2083.75±239.62*
	300	2.41±0.10*	4.98±0.11*	2487.84±278.74*	2004.53±211.14*

The Data were Presented as Mean ± Sem (7 Animals In Each Group). *P <0.05

DISCUSSION AND CONCLUSION: In the stomach, prostaglandins and nitric oxide play a crucial protective role of stimulating the secretion of bicarbonate and mucus [11], maintaining mucosal blood flow and regulating mucosal cells turnover and repairs [30]. Therefore, suppression of prostaglandin synthesis by non-steroidal anti-inflammatory drugs like IMC results in increased susceptibility to gastric mucosal lesions and mucosal injury [31, 32] which were observed in indomethacin control. AEPC at concentration of 300 mg/kg significantly protected the mucosa from being damaged by indomethacin suggesting that AEPC possesses bioactive ingredients mimicking prostaglandin and nitric oxide.

In PLIGU, it is known that gastric hydrochloric acid secretion and accumulation induce auto digestion [33] of gastric mucosa and subsequent breakdown of apical junction complex which is

made of tight and subjacent adherens junctions [3] to seal paracellular spaces of enterocysts. AEPC at concentration of 300 mg/kg also significantly protected the mucosa from this auto digestion by gastric acid accumulation. This is achieved through decreased gastric secretions, decreased total acidity, decreased free acidity and increased pH of the gastric juice as compared to PLIGU control. Parameters like number of ulcers, ulcer scores, and ulcer index were also significantly reduced by AEPC in a dose-dependent manner.

From the present study, AEPC appeared to exert an appreciable influence in anti-secretory, gastroprotective and anti-ulcer activities in albino rats. However, further research into identifying bioactive molecules involved in these activities is recommended.

ACKNOWLEDGEMENT: Authors wish to thank Mr. Takem Bertrand Mokom for the assistance rendered in sourcing and preparing extract following standard procedures.

REFERENCES:

1. Pluske J.: Accessed 23 December 2013. Available at:http://www.dsm.com/content/dam/dsm/anh/en_US/documents/GIT_Barrier_Function_JP.pdf
2. Collado MC, Grzeskowiak L, and Salminen S: Probiotic strains and their combination inhibit *in vitro* adhesion of pathogens to pig intestinal mucosa. Current Microbiology 2007; 55: 260-265.
3. Turner JR.: Intestinal mucosal barrier function in health and disease. Nature Reviews Immunology 2009; 9:799-809.
4. Glenn GM, Francis DH, and Danielson EM: Toxin-mediated effects on the innate mucosal defenses: implications for enteric vaccines. Infection and Immunity, 2009; 77: 5206-5215.
5. Hoogerwerf WA and Pasricha PJ: Pharmacotherapy of gastric acidity, peptic ulcers, and gastroesophageal reflux disease, In: L. L. Brunton, J. S. Lazo and K. L. Parker ed. Goodman & Gilman's The pharmacological basis of therapeutics. 11th ed. McGraw-Hill Medical Publishing Division, NewYork; 1005-020, 2006.
6. Goel RK and Sairam K: Anti-ulcer drugs from indigenous sources with emphasis on *Musa sapientum, Tamrabhasma, Asparagus racemosus and Zingiber ofcinale.* Indian Journal of Pharmacology 2002; 34:100-10.
7. Morsy M and El-Sheikh A: Prevention of Gastric Ulcers, Peptic Ulcer Disease. Accesed 30 October 2013. Available: http://www.intechopen.com/books/peptic-ulcerdisease/ prevention-of-gastric-ulcers
8. Tahara T, Shibata T, Yamashita H, Hirata I & Arisawa T: Influence of MDR1 polymorphism on H. pylori-related chronic gastritis. Digestive Diseases and Sciences 2011; 5*6:103-108.*
9. *Peptic Ulcer* Disease Causes, Diagnosis & Treatments - Clinical Key. Accessed 2*4* December 2013. Available at: http://www.clinicalkey.com/.../gastroenterology/peptic-ulcer-disease.htm...
10. Bighetti AE, Antonio MA, Kohn LK, Rehder VL, Foglio M, Possenti A et al: Anti-ulcerogenic activity of a crude hydroalcoholic extract and coumarin isolated from Mikania laevigata Schultz Bip. Phytomedicine 2005; 12:72-7.
11. Archana J, Sakat SS, Preeti T: Gastroprotective Effect of *Oxalis corniculata* (Whole Plant) on Experimentally Induced Gastric Ulceration in Wistar Rats. Indian Journal of Pharmaceutical Sciences 2012; 74(1):48–53.
12. Lakshimi V, Singh N, Shrivastva S, Mishra SK, Dharmani P, Palit G: Gedunian and photogedunin of Xilocarpus granatum show significant antisecretory effects and protect the gastric mucosa of peptic ulcer in rats. Phytomedicine 2009; 17:569-74.
13. Sen S, Chakraborty R, De B, Mazumder J.: Plants and phytochemicals for peptic ulcer. An overview, Pharmacognosy Review 2009; 3:270-9.
14. Sasmal D, Das S and Basu SP: Phytoconstituents and therapeutic potential of *Nyctanthes arbortristis* Linn. Pharmacology Review 2007; 1:344-49.
15. URL: Accessed 23 July 2013. Available: http://www.ars-grin.gov/cgi-bin/npgs/html/taxon.pl?435150
16. Mony R, Dibong SD, Ondoua JM, Boussim IJ, Amougou A and Bilong B: Ants and Phragmanthera capitata (Sprengel), Balle (Loranthaceae) impacts on considerable damages caused on fruit trees of the Ndogbong (Douala, Cameroon) chieftaincy's orchard. Journal of Agricultural Extension and Rural Development 2010; 2(3):048-053.
17. Dibong SD, Engone ON et al: An assessment on the uses of Loranthaceae in ethno pharmacology in Cameroon: A case study made in Logbessou, North of Douala. Journal of Medicinal Plants Research 2009; 3(8):592-595.
18. Takem LP, Lawal BAS and Lennox JA. Anti-diarrhoeagenic Properties of Aqueous Extract of *Phragmanthera capitata* S. Balle in Albino Rats. European Journal of Medicinal Plants 2014; 4(6):743-752.
19. Takem LP, Abe NP and Ogbonna OJ. Anti-Pyretic and Analgesic Potentials of Aqueous Extract of *Phragmanthera capitata* S. Balle in albino rats. American Journal of Pharmacy and Pharmaceutical Sciences 2014 In Press.
20. Din N, Dibong SD, Mpondo ME, Priso RJ, Kwin NF and Ngoye A: Inventory and Identification of Plants Used in the Treatment of Diabetes in Douala Town (Cameroon). European Journal of Medicinal Plants 2011; 1(3):60-73.
21. Engone ONL, Sallé G: Faut-il éradiquer Phragmanthera capitata, parasite des hévéas en Afrique? Conservation des Reserches Biologies 2006; 329:185-195. French.
22. Dibong SD, Mpondo ME, Ngoye A, Priso RJ: Modalities of exploitation of medicinal plants in Douala's region. American Journal of Food and Nutrition, 2011; 1(2):67-73.
23. CPCSEA Committee for the Purpose of Control and Supervision on Experiments on Animals. N. 13/1, 3rd Seaward Road, valmiki Nagar, Thiruvanmiyur, Chennai 600 041, Tamil Nadu, India, 2006.
24. OECD. OECD Guidelines for testing of chemicals. France: OECD Publishing Section 4, Health effects: Test No.425: Acute oral toxicity: Up and down procedure; 2006
25. Turner RA: Screening method in pharmacology. New York: Academic Press 152, 1965.
26. Wallace JL: Mechanisms of protection and healing: Current knowledge and future research. American Journal of Medicine 2001; 110:198-228.
27. Shay H, Komarov SA, Fels SS, Meranze D, Grunstein M, Siplet H: A Simple method for uniform production of gastric ulcers in rats. Gastroenterology 1945; 5:43-51.

28. Trease GE and Evans WC: Text Book of Pharmacognosy Bailliere Tindall Publication, Thirteenth edition 1992.
29. Duncan DB: Multiple ranges and multiple F tests. Biometrics1955; 11:1-42.
30. Hayllar J, Bjarnason I: NSAIDs, COX-2 inhibitors, and the gut. Lancet 1995; 346:521-522.
31. Malfertheiner P, Chan FKL, McColl KEL.: Peptic ulcer disease. Lancet 2009; 374:1149-1161.
32. Kushima H, Nishijima CM, Rodrigues CM, Rinaldo D, Sassa MF, Bauab TM et al: Gastroprotective, anti-inflammatory immunomodulatory and anti-*Helicobacter pylori* action. Journal of Ethnopharmacology 2009; 123:430-438.
33. Muriel PB, Marivane L, Edson LM, Mateus FL, João PB, Jairo KB et al: Evaluation of antiulcer activity of the main phenolic acids found in Brazilian Green Propolis. Journal of Ethnopharmacology 2008; 120:372-377.

How to cite this article:
Takem LP, Udi PM and Poh CF: Anti-Secretory, Gastroprotective and Anti-Ulcer Activities of Aqueous Extract of *Phragmanthera Capitata* S. Balle in Rats. Int J Pharm Sci Res 2014; 5(8): 3560-65.doi: 10.13040/IJPSR.0975-8232.5 (8).3560-65.