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Research Article

Anti-ulcer Activity of *Combretum obanense* Stem Bark (Bak. F) Hutch and Dalz

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ABSTRACT
The study was designed to investigate the antiulcer activity of methanol/methylene chloride (1:1) of *Combretum obanense* using different models of gastric ulceration in rats. Gastric ulcers were induced by oral administration of aspirin, ethanol and water immersion resistant stress. The extract was administered at a dose of 200, 400 and 800mg/kg orally one hour prior to ulcer induction. Cimetidine (50 mg/kg) was used as a reference standard. The antiulcer activity was accessed by determining and comparing the ulcer index in the test group with that of the standard drug treated group. The ulcer index in the *C. obanense* treated animals was found to be significantly less in all the models compared to standard drug treated cases. The results suggest that *C. obanense* possesses significant antiulcer property which could be due to cytoprotective action of the drug or strengthening of gastric mucosa with the enhancement of mucosal defense or due to the presence of secondary metabolites found in the plant.

Key words: *Combretum obanense*, Antiulcer

INTRODUCTION

The exact pathogenesis of ulcer continues to elude scientists and medical researchers, but a common ground has been proposed. Ulcers are produced when any factor causes an imbalance between the protective factors (mucus and bicarbonate) and aggressive factors (acid and pepsin) in the stomach[1] such factors could range from natural causes (gastric cancer), infections, lifestyle (drugs, non-steroidal anti-inflammatory agents, alcohol, stress and cigarette smoking)[2,3] current treatment of ulcers in developing countries has been largely suppression of pain, with little or no strategy aimed at a cure. Herbal medicine is fast emerging as an alternative treatment to available synthetic drugs for treatment of ulcer possibly due to lower costs, availability, fewer adverse effects and perceived effectiveness. Many tropical herbs have been scientifically reported to possess potent antiulcer activity [4,5,6,7] so the present study has been focused on anti -ulcer activity of *combretum obanense* stem.

MATERIALS AND METHODS

Plant material and Preparation of extract

Combretum obanense stem was collected within the surrounding of Okutu in Nsukka Local Government Area of Enugu State, Nigeria in March 2013, Nigeria, and was identified and authenticated by Mr. Alfred Ozioko of International Centre for Ethnomedicine and Drug Development. The voucher specimen (INTERCEDD 022013) is deposited at the same center.

The air-dried and powdered plant material (5Kg) was macerated in a mixture of CH_2Cl_2-MeOH (1:1) for 24h. Removal of the solvent *in vacuo* in a rotary evaporator provided an organic extract (200g).

Phytochemical screening

The preliminary phytochemical test was carried out using methods described by (Trease and Evans). [8]

Animal

Wistar albino rats (56 - 247 g) of both sexes were used. They were housed in clean polypropylene cages under standard conditions of humidity (50 ± 5 %), temperature (25±2°C) and light (12 h light/12 h dark cycle) and fed with a standard diet (Amrut laboratory animal feed) and water *ad libitum*. All animals were handled with humane care.

Acute Toxicity Test (Median Dose Ld_{50})

The Lorke's (1983) procedure of LD_{50} determination was used. This method employs 3 albino mice with the following assumption made[9].

Substances more toxic than 1 mg/kg are so highly toxic that it is not important to calculate the LD_{50} exactly.

LD_{50} values greater than 5000 mg/kg are of no practical interest

An approximate figure for the LD_{50} is usually adequate to estimate the risk of acute intoxication.

The experiment involved first, a preliminary trial using three different doses of the plant extract. The mice were placed in three groups of three animals per group.

The first group (A) received 10 mg/kg of the extract, group (B) received 100 mg/kg of the extract while group (C) received 1000 mg/kg of the extract. The animals were

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Table 1: Phytochemical constituents of *Combretum obanense* stem

S. no	Constituents	Presence
1	Carbohydrate	+++
2	Alkaloids	+++
3	Flavonoids	+++
4	Tannins	+++
5	Proteins	+++
6	Terpenoids	+++
7	Reducing sugar	++
8	Resins	++
9	Saponins	++
10	Oil	++
11	Steroids	++
12	Glycosides	++
13	Acidic compound	+
14	Anthraquinone	-

Key
+++ High in concentration
++ Medium in concentration
+ Low in concentration
- Absent

constantly observed for the first 2 hours, intermittently for the next 4 hours and the overnight. There was no dead animal recorded at the end of 24 hours. From the result obtained from first phase, the second phase of the acute toxicity test was performed using doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg, administered to three groups of one animal per group.

There was no mortality recorded in the mice upon oral administration even at doses as high as 5000 mg/kg. This indicates that the experimental doses used are relatively safe.

Tests For Anti-Ulcer Activity

Adult Wistar albino rats of either sex weighing 180-200 g were used for the study. The effects of the *Combretum obanense* were evaluated on aspirin induced, ethanol induced, and water immersion resistant stress ulcer models in rats. Cimetidine was used as a standard drug for all the models, for comparing the anti-ulcer potential of the extract.

Aspirin Induced Gastric Ulceration

Albino Wistar rats of either sex were divided into five groups with six animals in each group as follows:

Group I: Control (untreated) group (normal saline 2 ml/kg)

Group II: Standard treatment group (cimetidine 50 mg/kg)

Group III: Test treatment group (*C obanense* 200 mg/kg)

Group IV: Test treatment group (*C obanense* 400 mg/kg)

Group V: Test treatment group (*C obanense* 800 mg/kg)

All rats were fasted for 24 hours but excess water was allowed. The standard drug (cimetidine 50 mg/kg) and the test drugs (*C. obanense* 200, 400 and 800 mg/kg) were administered orally to the respective groups. One hour after their pretreatment, all animals were gavaged with aspirin (200 mg/kg). After 4 hours, they were humanely sacrificed by using chloroform. The numbers of ulcer spots in the glandular portion of the stomach were counted in both toxicant and drug treated animals and the ulcer index was calculated [10, 11].

Ethanol Induced Gastric Ulceration

Albino rats were fasted for 24 hours prior to the experiment but allowed free access to water. The rats were randomized into 5 groups of 6 rats each. 1 ml 96 % ethanol was administered to all the animals intra-gastrically via orogastric cannula. The standard drug (cimetidine 50 mg/kg) and the test drugs (*C. obanense* 200, 400 and 800 mg/kg) were administered orally to the respective groups 30 minutes prior to ethanol administration. The animals were sacrificed under chloroform vapour and the stomach was removed and opened along the greater curvature and subjected to measurement of ulcer index [12].

Water Immersion Resistant Stress Induced Gastric Ulceration

Rats were fasted 48 hours with only free access to water before performance of the experiment. Each rat was orally administered with the standard drug (cimetidine 50 mg/kg) and the test drugs (*C. obanense*, 200400 and 800 mg/kg) to the groups:

Group I: Control (untreated) group (normal saline 2 ml/kg)

Group II: Standard treatment group (cimetidine 50 mg/kg)

Group III: Test treatment group (*C. obanense* 200 mg/kg)

Group IV: Test treatment group (*C. obanense* 400 mg/kg)

Group V: Test treatment group (*C. obanense* 800 mg/kg)

Thirty min later, the rats were placed in a restraint device and immersed up to their xiphoid process in a 22^0 C water bath for 4 hours for the induction of gastric ulcer. After sacrifice of the animals, their stomachs were removed

Table 2: Ulcer Index and Percentage Inhibition in Aspirin induced ulcer in rats

Group	Dose (mg/kg)	Total Score	Ulcer Index (UI)	% PI
I	2 ml/kg	60	10.1 ± 2.3	-
II	50	4	0.67 ± 0.33*	93.37*
III	200	27	4.5 ± 1.4*	55.45*
IV	400	20	3.2 ± 0.7*	68.32*
V	800	14	2.3 ± 0.3*	77.23*

Results are UI mean count ± S.E.M. (n = 6). * Significantly different from control at $p < 0.05$.

Key:
I is the negative control where normal saline was administered.
II is the positive control treated with cimetidine
III, IV and V are treatment groups treated with *C. obanense*
% PI is percentage inhibition.

Table 3: Ulcer Index and Percentage Inhibition Ethanol Induced Ulcer in Rats

Group	Dose (mg/kg)	Total Score	Ulcer index (UI)	% PI
I	2 ml/kg	191.5	38.0 ± 1.8	-
II	50	24	4.8 ± 0.96*	87.37*
III	200	100	20 ± 1.9*	47.37*
IV	400	63.5	13.2 ± 3.0*	65.26*
V	800	47.5	9.5 ± 1.6*	75.00*

Results are UI mean count ± S.E.M. (n = 6). * Significantly different from control at $p < 0.05$.
Key:
I is the negative control where normal saline was administered.
II is the positive control treated with cimetidine
III, IV and V are treatment groups treated with *C. obanense*
% PI is percentage inhibition.

Table 4: Ulcer Index and Percentage Inhibition Stress Induced Ulcer in Rat

Group	Dose (mg/kg)	Total Score	Ulcer Index (UI)	% PI
A	2 ml/kg	163	32.0 ± 4.88	-
B	50	30.5	6.1 ± 1.72*	80.94*
C	200	142	28.4 ± 5.13	11.25
D	400	76.5	15.2 ± 6.56*	52.50*
E	800	25	5.0 ± 1.69*	84.38*

Results are UI mean count ± S.E.M. (n = 6). * Significantly different from control at $p < 0.05$.
Key:
I is the negative control where normal saline was administered.
II is the positive control treated with cimetidine
III, IV and V are treatment groups treated with *C. obanense*
% PI is percentage inhibition.

longitudinally excised[13].

Ulcer Assessment

The stomachs were harvested, opened along the greater curvature and the mucosa was exposed for macroscopic evaluation. The ulcerated area was assessed and the ulcer index (UI, mm^2) was calculated as the arithmetic mean for each treatment. Following the analysis, the mucosal layer was blotted dry and scraped off the underlying muscularis externa and serosa[14].

Mean Scoring

A yardstick for ulceration was made as follows:

00: Normal colouration
0.5: Red colouration
1: Spot ulcers
1.5: Haemorrhagic streaks
2: Ulcers >3mm but <5mm
3: Perforation along the great curvature [15]

Ulcer Index and % Protection

Mean ulcer score in each group was calculated and was designated as ulcer index and percentage was calculated [16, 17].

$$\% \text{ Protection} = \frac{\text{control mean ulcer index - test mean ulcer index}}{\text{control mean ulcer index}} \times 100$$

STATISTICAL ANALYSIS

All values were reported as mean ± S.E.M. The statistical significance of differences between groups was assessed using one-way ANOVA. A probability value of $p < 0.05$ was considered to be statistically significant.

RESULTS

Phytochemical Analysis

Table 1 shows the phytochemical constituents of *C. obanense* stem bark. It indicates the presence of the following component; carbohydrates, alkaloids, flavonoids, terpenoids, tannins, proteins, > reducing sugar, glycosides, saponins, resins, oils, steroids, > acidic compounds respectively while anthraquinone was absent.

Acute Toxicity Test (LD_{50})

The acute toxicity indicated that per oral administration was safe as 5000 mg/kg.

Effect of C. obanense Stem Bark in Aspirin Induced Ulcer in Rats

From the result in Table 2, *Combretum obanense* extract showed anti-ulcer activity against aspirin induced ulcer at doses of 200, 400, and 800 mg/kg with percentage % PI of 55.45, 68.32, and 77.23 respectively. The anti-ulcer effect was observed to be dose dependent with 800 mg/kg having the highest percentage PI of 77.23. The statistical analysis of the result was shown to be significant with $p < 0.05$ when compared with positive and negative controls.

Effect of C. obanense Stem Bark in Ethanol Induced Ulcer in Rats

In Table 3 anti-ulcer activities was observed against ethanol induce ulcer at doses of 200, 400, and 800 mg/kg with % PI of 47.37, 65.26, and 75.00 respectively which was dose dependent also. It showed statistical significance at those doses when compared with positive and negative control with $p < 0.05$.

Effect of C. obanense Stem Bark in stress Induced Ulcer in Rats

Table 4 showed anti-ulcer activity against stress induced ulcer only at doses of 400 and 800 mg/kg when compared

with positive and negative controls, having % PI of 52.50 and 84.38 respectively. The statistical significance at these dose groups is $p < 0.05$.

DISCUSSION

Phytochemical analysis of the extract showed that the stem bark contains carbohydrate, alkaloids, flavonoids, proteins, terpenoids, saponins, glycosides, reducing sugar, resins, oil, steroids and acidic compounds. The anti-ulcer effect could be attributed to the flavonoids and tannins present in the plant extract.

In this study, exposure of the animals to aspirin may have caused severe ulcerogenic effects as apirin is known to increase gastric acid secretion which is involved in the formation of aspirin-induced mucosal lesions, suppression of prostaglandin synthesis which results in increased susceptibility to mucosal injury and gastro duodenal ulceration[18]. However, gastro-protection was observed by *C. obanense* at 200, 400 and 800 mg/kg which were statistically significant at $p < 0.05$ as shown in Table 2. The gastro-protective activity of *C. obanense* seems to be related to a reduction in the damage of the mucosa induced by free radicals and this activity may be due to its antioxidant action.

In ethanol induced ulcer, the administration of the crude of the *C. obanense* resin protected the rat gastric mucosa against hemorrhagic lesions produced by absolute ethanol. Disturbances in gastric secretion, damage to gastric mucosa, alterations in permeability, gastric mucus depletion and free-radical protection are reported to be the pathogenic effects of ethanol[18], which may be due to stasis in gastric blood flow[19]. However, the result of the current study indicated that pre-treated rats with the *C. obanense* resin (200, 400, and 800 mg/kg) and cimetidine 50 mg/kg significantly reduced the formation of gastric ulcer induced by absolute ethanol compare to animals pre-treated with normal saline

Water immersion stress is one of the best models of stress in rats to induce ulcer. This model provides both emotional stress as well as physiological stress to the animals. It elicits the purest form of psychological frustration accompanied by vigorous struggle which means muscular exercise[20]. Stress-induced ulcer is probably mediated by the release of histamine [21, 22.] It not only increases gastric secretion, often called the 'aggressive factor', but also causes disturbances of the gastric mucosal microcirculation and an abnormal motility, and reduces mucus production, known as the 'defensive factor'. Pre-treatment with test drugs (*C obanense* 400 and 800 mg/kg) produced a significant decrease in the intensity of gastric mucosal damage induced by the stress as compared with control. The reduction in the ulcer index and percentage protection may be attributed to the anti-ulcer activity of the stem bark of *C obanense* due to presence of antioxidants like flavonoids and tannins.

CONCLUSION

The present study provided time that the leaves of *Combretum obanense* possess significant anti-ulcer activity in animal models. The ant-ulcer activity is probably due to the presence of bioactive compounds like flavonoids and tannins. Further studies are required to confirm the exact mechanism underlining the ulcer healing and protecting property of extract and to identify the chemical constituents responsible for it.

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REFERENCE

1. Ojewole E.B.; Peptic ulcer Disease. In: Aguwa C.N, editors, Therapeutic Basis of Clinical pharmacy in the Tropics. 3rd ed, Enugu; SNAAP Press; 2004. pp.541-564.
2. Berardi R.R.S, Welage; Ulcer disease, In; Dipiro T.J., R. L.Talbert, G, Yes, G. Matzke G. Wells and M. Posey, editors. Pharmacotherapy: A Pathophysiologie Approach, 6th ed. McGraw-Hill; 2005. 629-648.
3. Suerbaun, S. and P. Michetti: Helicobacter pylort infection. N Engl. Med 2002; 347: 1175-1186.
4. Vela, S.M.C., Souccar, M, lima-Landman and A. Lapa: Inhibition of gastric acid secretion by the aqueous extract and purified extract of Stachtarpheta cayennensis. Plant Med. 1997; 63 (1): 36.
5. Goulart, Y.C.F., Sela, V., Evalaution of gastric antiulcer activity in a hydro-ethanolic extract from Kielmeyera coriacea. Braz. Arch. Bio Tech.,2005; 48(1): 211-216
6. Singh, R.J., Madam and Rao S., Antiulcer activity of black pepper against absolute ethanol induced gastric mucosal damage in mice. Pharmacog. Mag. 2008; 4(15); 232-235.
7. Aguwa, C.N. and Ukwe C.: Gastrointestinal activities of *Sterculia tragacantha* leaf extracts, Fiterapia 1997, 68 (2); 127-131.
8. Trease, G. E. and Evans, W. T., Text book of pharmacology. 15th edition WB Sanuders Company limited, London, 2002. p168, p249-251.
9. Lorke, D., A new approach to practical acute toxicity testing. Arch Toxicol. 1983 54: 275-287.
10. Singh, S., Majumdar, D.K. Evaluation of the gastric antiulcer activity of fixed oil of Ocimum sanctum (Holy Basil). J. Ethnopharmacol 1999.65:13–19.
11. Sairam, K., Rao, C.V., Dora, B.M., Vijay, K.K., Agrawal, V.K., Goel, R.K. Antiulcerogenic effect of methanolic extract of Emblica officinalis: an experimental study. J. of Ethnopharmacology 2002. 82: 1-9.
12. Robert, D.K., Bertram, G.K., Susan, B.M., Anthony, J.T. Basic and Clinical Pharmacology, 14th edition, Mc Graw-Hill Companies USA, 2007. pp. 982
13. Byun, S.K., Lee, Y.E., Shin, S.H., Jang, J.Y., Choi, B.I., Park, D.S., Jeon, J.H., Nahm, S.S., Hwang, S.Y., Kim, Y.B. The role of corticosteroids in stress-induced gastric ulceration in rats. Lab Anim Res. 2007. 23:127–131.
14. Malairajan, P., Gopalkrishnan, G., Nrasimhan, S., Jessi, K.V., Kavimani, S. Anti-ulcer activity of crude

alcoholic extract of Toona ciliata Roemer (heart wood). J. Ethanopharmacol. 2007. 110: 348-357.
15. Patil, P.H. and Surana, S.J. Gastroprotective effect of eran-themum roseum R. Br. Linn Root extracts in albino rats. IJPBS. 2009. 3 (1): 81-93.
16. Paunikar, G., Kadam, P., Dongare, S., Nakkawar, M., Sutar, R., Tarte, P.FArnottiana Leaf extract offer Anti-ulcer activity against absolute ethanol induced gastric ulcer in rats. IJPBS. 2009. 3 (3): 161-166.
17. Bandyopadhyay, S.K., Pakrashi, S.C., and Pakrashi, AThe role of antioxidant activity of Phyllanthus emblica fruits on prevention from indomethacin induced gastric ulcer. J. Ethnopharmacol. . 2000. 70: 171-176.
18. Salim, A.S. Removing oxygen-derived free radicals stimulates healing of ethanol induced erosive gastritis in rats. Digestion. 1990. 47:24-28.
19. Guth, P.H., Paulsen, G., and Nagata, H. Histologic and microcirculatory changes in alcohol induced gastric lesions in the rat: effect of prostaglandin cytoprotection. Gastroenterology. 1984. 87:1083-1090.
20. Bhattacharya, A., Ghosal, S., Bhattacharya, S.K. Anti-oxidant effect of Withania somnifera in chronic foot shock stress-induced perturbations of oxidative free radical scavenging enzymes and lipid peroxidation in rat frontal cortex and striatum. J. Ethnopharmacol. 2001. 74: 1–6.
21. Michael, N.P., Charles, T.R. Stressful life events, acid hypersecretion and ulcer disease. Gastroenterology. 1983. 84:114–119.
22. Brodie, D.A., Hanson, H.M. A study of the factors involved in the production of gastric ulcer by the restraint technique. Gastroenterology. 1960. 38:353–360.