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EVALUATION OF THE ANTI-ULCER PROPERTIES OF THE METHANOL EXTRACT OF PROSOPIS AFRICANA TAUBERT (FAM: FABACEAE) LEAF.

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ABSTRACT

The anti-ulcer properties of the methanol extract of the leaf of Prosopis africana Taubert (Fabaceae) was studied in rats and the extract was also subjected to phytochemical, acute and lethality (LD_{50}) tests. Fresh dried leaves of Prosopis africana Taubert (Fabaceae) were extracted by cold maceration method using 90% methanol. Anti-ulcer effects of the methanol extract at 100, 200 and 400 mg/kg were evaluated in rats using aspirin-induced and indomethacin-induced ulcer models. Phytochemical analysis and lethality tests (LD_{50}) were carried out using standard methods. Results showed that the methanol extract exhibited significant ($P<0.05$) and dose-dependent anti-ulcer activity in all the models. Percentage ulcer inhibitions of extract at 400 mg/kg for aspirin and indomethacin-induced ulcers were 91.0 and 60.5 % respectively. Ulcer protections in all the models used by the extract were dose-dependent. The ulcer inhibitory effects of the extract were comparable to the standard drug omeprazole at 20 mg/kg. Oral LD_{50} recorded a death at the dose level of 5000 mg/kg indicating that the plant is toxic at dose level of 5000 mg/kg. Phytochemcial analysis showed the presence of saponins, tannins, glycosides, steroids, terpenoids, proteins, resins, flavonoids, alkaloids, carbohydrates and reducing sugars. Therefore, results of this study suggest that the methanol extract of P. africana possesses anti-ulcer activity as claimed by its ethanomedicinal use.

KEYWORDS : *Prosopis africana, Anti-ulcer activity, Phytochemical analysis, Omeprazole, Leaves.*

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INTRODUCTION

From time immemorial, plants have served as the primary source of medicine and food for man and they have continued to provide mankind with new, novel therapeutic remedies to date. Natural plants (cheaper accessibility and with fewer or no side effects) have emerged as a potential candidate [1]. The revival of interest in plant derived drug is mainly due to the wide spread belief that "natural medicines" are safe and more dependable than the costly, synthetic drugs, many of which are toxic and possess adverse effects. Plants are being used in the traditional systems of medicine in many parts of the world, especially in rural communities, for the control, management and/or treatment of a variety of human and animal ailments. The current world wide trend towards utilization of plant derived natural remedies has, therefore, created a dire need for accurate and up to date information on the properties and uses, efficacy, safety and quality of medicinal plant products [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 anti-ulcer activity with examples as [4-15].

Prosopis africana Taubert (Fabaceae) (Okpei in Igbo) Okpeye in Nsukka, Ayan in Yoruba, Okpeghe in Idoma and Tiv and Kiriya or kiriaya in Hausa) is a tree with a very hard wood and easily distinguishable by its dark rough bark, pale drooping foliage with small pointed leaf lets and sausage-shaped fruit [16-18]. The plant has been reported in literature to possess wound-healing, anti-microbial, anti-oxidant and hemolytic properties [18, 19].

In traditional medicine, the poultice of the boiled seeds is usually applied externally to relieve sore throat, while the fermented seed is used as a seasoning agent in food [20]. Juice expressed from the stem bark is applied on open wounds as an astringent and to cleanse the wound surface. The bark and the leaves were also crushed to a pulp and place on the wound surface as a dressing. The leaves of this plant is claimed to be effective in the treatment of stomach ache and bleeding gastric ulcers when chewed like that or taken as an aqueous decoction. Due to its use in ulcer treatment in traditional medicine, we designed to evaluate the anti-ulcer potentials of the plant and to identify the phytochemical constituents of the extract responsible for the observed activity.

MATERIALS AND METHODS

Collection and Identification of Plant Materials: The leaves of *P. africana* were collected from Nsukka in Enugu-State on December 2010 and was identified by Mr. A.O. Ozioko of the Bioresources Development Conservation Project (BDCP) in Nsukka. A voucher specimen has been deposited in the herbarium of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka with voucher number UNN/PCG/10/036 for future reference.

Preparation of extracts: A weighed quantity (500 g) of shade-dried coarse powdered leaves was macerated in 90 % methanol for 48 hrs. The mixture was filtered and the solution was subjected to evaporation using rotary evaporator (Stone, Staffordshire, ST15 OSA, U.K.) situated at Department of Pharmacognosy and Traditional Medicine Laboratory, Nnamdi Azikiwe University, Awka, Anambra-State, Nigeria. The dried extract was stored in a refrigerator till its use.

Phytochemical analysis: The methanol extract was screened for the presence of secondary metabolites using standard phytochemical procedures [21].

Animals: Mice of both sex (25 – 35 g) and rats of both sex (105 – 170 g) supplied by the staff of the Department of zoology of the University of Nigeria, Nsukka were used. They were housed in steel cages, placed on standard pellet feed (Niger feed, Nigeria) and were given free access to clean water. They were kept in well ventilated rooms with a 12/12 h light/dark conditions and ambient room temperature. Animals were procured two weeks before the experiments to acclimatize with the

laboratory environment. Animal experiments were done in compliance with the National Institute of Health Guide for care and use of Laboratory Animals (Pub. No. 85 – 23, revised 1985).

Acute toxicity and Lethality tests: The acute toxicity of the methanol extract of *P. africana* was ascertained using standard method [22]. Three groups of 3 mice each were administered 10, 100, and 1000 mg/kg of the methanol extract orally. The mice were observed for 24 hours for effects of toxicity and the number dying in each group within the period noted. When no deaths were recorded, another three groups of 3 mice each were administered 1600, 2900 and 5000 mg/kg of the extract orally. The animals were observed for 48 hrs for effects of toxicity and the number dying in each group within the period was recorded.

Anti-ulcer activity: Two models (Aspirin and Indomethacin) with effective induction of ulcer experimentally in rats were employed to evaluate the anti-ulcer activity of the methanol extract of *P. africana.* All the rats used were fasted for eighteen hours but were given water freely till the start of the experiment.

Aspirin-induced ulcer: Thirty fasted animals were used in five groups of six animals each. Groups A and B received 2 ml/kg 3 % Tween 80 (negative control) and 20 mg/kg p. o. omeprazole (Omezole®, Hovid) while rats in groups C, D and E were given 100, 200, and 400 mg/kg of the extract p. o. After one hr, 200 mg/kg p. o. of aspirin (Juhel) was given to each rat orally. The rats were sacrificed with chloroform (Sigma-Aldrich, Germany) anaesthesia after one hour. The stomachs were isolated, washed gently under clean flowing water and cut open along the greater curvature. The stomachs were fixed in 10% formalin and craters observed and ulcer scores were recorded using standard method [15].

Indomethacin-induced ulcer: Thirty fasted rats were also used in this model as five groups of six rats each. Groups A and B of this model received 3 % Tween 80 (2 ml/kg) and omeprazole 20 mg/kg p. o. (Omezole®, Hovid) respectively. After 30 mins, indomethacin 40 mg/kg (Methacin®, Hovid) p. o was administered to each rat. After 8 hrs of drug treatment [15], stomach were isolated, cut and ulcers counted and recorded as in above.

Statistical analysis: Ulcer indices were shown as the mean ± standard error of mean and level of ulcer protection presented as percentage inhibition. The significance of the differences in control was calculated at 95 % confidence interval using student's t-test.

RESULTS

Phytochemical analysis showed that the extract contains glycosides, saponins, tannins, reducing sugars, steroids, terpenoids, proteins, resins, alkaloids, carbohydrates and flavonoids. Acute toxicity result showed that the extract was toxic at dose level of 5000 mg/kg.

Table 1: Effects of Methanol Leaf Extract of *P. africana* on Aspirin-Induced Ulcers in Rats (n = 6).

Treatments	Dose mg/kg p.o.	Quantal Ulcer incidence	Ulcer index	Ulcer inhibition (%)
3%Tween 80	2 ml/kg	6/6	2.00 ± 0.14	
Omeprazole	20	5/6	0.14 ± 0.07	93.00
Extract	100	6/6	0.26 ± 0.06*	61.80
Extract	200	6/6	0.60 ± 0.40*	70.00
Extract	400	5/6	0.18 ± 0.14*	91.00

Values are Mean ± SEM; n=number of animals in each group; *: P<0.05 Vs negative control (student's t-test).

Table 2: Effects of Methanol Leaf Extract of *P. africana* on Indomethacin- Induced Ulcers in Rats (n = 6).

Treatments	Dose mg/kg p.o.	Quantal Ulcer incidence	Ulcer index	Ulcer inhibition (%)
3%Tween 80	2 ml/kg	6/6	2.00 ± 0.29	
Omeprazole	20	5/6	0.24 ± 0.17	88.00
Extract	100	6/6	1.30 ± 0.23	32.40
Extract	200	6/6	0.96 ± 0.16*	52.00
Extract	400	6/6	1.33 ± 0.17*	60.50

Values are Mean ± SEM; n=number of animals in each group; *: P<0.05 Vs negative control (student's t-test).

Aspirin-induced ulcer: The methanol extract at all the doses provided protection from ulcer and the protection was dose-dependent. The methanol extract at doses of 200 mg/kg and 400 mg/kg provided statistically significant protection (70.00 % and 91.00 %, P< 0.05) when compared with the negative control (Table I).

Indomethacin –induced ulcer: The methanol extract protected the rats from experimentally-induced ulcers at all dose-levels with greater severe lesions than in the aspirin-model. However, the protection was slightly dose-dependent; the percentage ulcer inhibition was the least when compared to values obtained in the aspirin model. Therefore, the doses of 200 mg/kg and 400 mg/kg also proved to be the doses with statistically significant protection (52.00 % and 60.50 %, P< 0.05) when compared with the negative control (Table 2).

DISCUSSION

The anti-ulcer activity of the methanol leaf extract of *P. africana* against aspirin- and indomethacin-induced ulcers was established in this study. Results of acute toxicity showed that the plant is toxic at the dose of 5000 mg/kg. The extract protected the stomach against necrotic damage of NSAIDS (aspirin and indomethacin). The protections in the two models (aspirin- and indomethancin-induced ulcer) were dose-dependent and the effect of the extract was comparable to omeprazole, a cytoprotective agent. The protection by the extract of this type may suggest a possible cytoprotective mechanism of action. The extract might also have an anti secretary effect as it protected the stomach mucosa from NSAIDS induced damage. This damage is elicited by the inhibition of prostaglandin synthesis, which is essential for mucosal integrity and regeneration [23]. This results to a sustained reduction in mucosal blood flow and a subsequent generation of ulcer. Ompeprazole was employed in this study for its cytoprotective but not anti-secretary effect but earlier study have reported that omperazole exhibits an anti-secretary and protective effects against ulcers and agents providing ulcer healing against NSAID induced ulcers may provide similar effect [24].

The presence of alkaloids, glycosides, saponins, flavonoids, tannins, steroids, reducing sugars, terpenoids and carbohydrates in this extract as seen in this study has also been reported by earlier studies [18, 25]. Ulcer protection may be attributed to any of these phytochemical constituents as flavonoids, tannins and saponins which have been shown to produce anti-ulcerogenic and anti-gastric activity [26, 27]. Until specific constituents are isolated and characterized, exact mechanism of action cannot be ascertained.

We have demonstrated in this study that the methanol leaf extract of *P. africana* has an ulcer healing property against experimentally induced ulcers in rats and this study confirms the traditional claims of the benefits of *P. africana* in treatment of ulcer.

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