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Evaluation of the antiulcer activity of *Olax subscorpioidea* Oliv. roots in rats

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ABSTRACT

Objective: To evaluate the antiulcer activity of the methanolic root extract of the plant *Olax subscorpioidea* in experimental rats. **Methods:** Phytochemical tests and acute toxicity tests were carried out on its methanolic root extract. Pre-treatments with three doses of the extract (200, 400 and 600 mg/kg body weight orally) and Sucralfate at 100 mg/kg orally were used for the various groups of rats. The indomethacin and ethanol models for experimental induction of ulcers in rats were used. Mean ulcer indices were measured and percentage inhibition was derived. **Results**: Phytochemistry revealed presence of alkaloids, steroids, glycosides and terpenoids and the extract showed an LD_{50} of 2 154 mg/kg in mice. Ulcer was produced in all the rats in both models with the extract showing potent antiulcer activity of ethanol model. There was no significant ulcer inhibition by any of the treatments compared to control group in the indomethacin model but the extract´s antiulcer effect was dose-dependent (11.8%, 19.2%, 32.7%, $P>0.05$). The ulcer reduction in the ethanol model was significant ulcer reduction in the highest dose group and Sucralfate group compared to control group (79.3% and 82.9%, $P<0.05$). However, the extract at all dosage showed a dose-dependent ulcer inhibition in this model. **Conclusion**: The above results suggest that the roots of *Olax subscorpioidea* possess antiulcer activity in experimental rats as claimed by traditional users.

1.Introduction

Olax subscorpioidea Oliv. (Olacaeae), is a shrubby plant which practically confined in the tropics especially Africa. It is known as "Mtungapwezi" in Swahili, "Ukpakon" in Edo, "Ifon" in Yorubaland[1] and "Aziza" in Nsukka (Nigeria). Pharmacological reports show that saline extract of the root possesses membrane stabilizing activities and the sodium hydroxide extract possesses antiprotease activity[2]. Its ethanolic stem extract has been reported to possess antimicrobial activity[3]. Decoction of the roots is being used for treating asthma and constipation[4] as an antidiarrhoel and dressing skin ulcers by locals of Nsukka. The pathogenesis of peptic ulcer has been traced to a destruction of the stomach mucosal lining either by an excessive production of acid or direct destruction of the stomach mucosal linings by necrotizing agents [5]. The disease continues to be prevalent worldwide and treatment options available are still far from available to developing regions of Africa. A search for ulcer treatment that is affordable, effective and devoid of side effects seen in currently available drugs[6] is being intensified and current research has turn to natural resources[7]. This study was aimed at establishing the antiulcer activity of the methanolic extract of the roots of the plant *Olax subscorpioidea* in experimental animals.

2. Materials and methods

2.1. Plant material

The roots were collected in large quantities from the forests of Orba in Nsukka Local Government Area of Enugu State, Nigeria in mid-May. The plant was then identified by Mr A.O. Ozioko of the Bioresources and Development Center Programme (BDCP) in Nsukka. A voucher specimen of the roots was deposited at the hebarium of the Department of Pharmacognosy, University of Nigeria, Nsukka for future

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reference.

2.2. Preparation of the extract

The roots were washed and dried under the sun for 7 days and pulverized into coarse powders. The coarse powders were subjected to cold maceration extraction using 2 L analytical methanol (Sigma Aldrich, Germany) with intermittent shaking for 48 hours. The mixture was filtered and dried of solvent with a rotary evaporator (RV 05 Basic IB, IKA, Staufen, Germany) and the methanolic root extract of *Olax subscorpioidea* (MEOS) kept in a refrigerator.

2.3. Animals

Healthy adult wistar albino rats and mice of male sex weighing between 110-150 g and 25-35 g, respectively were selected for this study. The animals were obtained from the animal house Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. The animals were housed under standard conditions of light/dark at 12/12 hrs cycle in metallic cages. They were fed with standard animal feed (Nigerfeed, Nigeria) and allowed free access to clean drinking water. The animal studies were conducted in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No. 85-23, revised 1985).

2.4. Preliminary tests

Phytochemical tests were carried out according to methods described by Harbourne[8]. The acute toxicity (and lethality) of the extracts MEOS was determined by the Lorkes method[9].

2.5. Antiulcer activity

2.5.1. Ethanol induced gastric ulcer [10]

Thirty overnight fasted rats were divided into five groups with six rats each. Pre-treatment of animals (groups 1-5) were as follows: saline 5 mL/kg, Sucralfate (Antepsin®, Chugai Pharma, UK) 100 mg/kg, MEOS 200 mg/kg, MEOS 400 mg/kg and MEOS 600 mg/kg. Thirty minutes later, ulcers were induced by administering 1 mL absolute ethanol (99%) (Sigma Aldrich, Germany) to each rat. All administrations were by oral route. One hour later all the rats were sacrificed with chloroform (Sigma Aldrich, Germany). The stomach were excised, cut along the greater curvature and gently rinsed under tap water. The stomachs were stretched on a corkboard and a magnifying glass (×10 magnification) was used to spot and count the craters using a severity scale described by Tan PV *et al*[11]. The ulcer index was obtained by the sum of a group's crater score and divided by magnification. Percentage ulcer inhibition (%UI) was calculated using the formula below:

$$UI = \frac{\text{mean ulcer index (control group)} - \text{mean ulcer index (test group)}}{\text{mean ulcer index (control group)}} \times 100$$

2.5.2. Indomethacin induced gastric ulcer [12]

Thirty overnight fasted rats were divided into five groups with six rats in each group. All rats received treatments as described above in the ethanol model via the oral route. Thirty minutes later, ulcer was induced with indomethacin (40 mg/kg per oral) (Temido, Greenfield Pharma, China) in all the rats. Eight hours later, the rats were sacrificed as described above and their stomachs were isolated and cut along the greater curvature. The excised stomachs were rinsed under tap water and viewed for ulcer craters as above.

2.6. Statistical analysis

The results of ulcer indices were expressed as mean± SEM while ulcer inhibition expressed as a percentage. Differences in mean ulcer index in comparison with control was done using the one way ANOVA followed by the Dunnett's post hoc multiple comparison with statistical significance considered at $P>0.05$.

3. Results

3.1. Preliminary tests

The methanolic extract of *Olax subscorpioidea* revealed presence of glycosides, terpenoids, alkaloids and steroids. In the acute toxicity test, death was observed in the second stage of the test in mice with dosage at 2 900 mg/kg and 5 000 mg/kg. The lethality dose was calculated to give an LD_{50} of 2 154 mg/kg in mice.

3.2. Effect of MEOS on ethanol induced ulcers

Ulcers produced in this model were seen as reddish streaks of sores. Pre-treatment of rats with doses of MEOS (200-600 mg/kg) produced a dose-dependent protection from ethanol induced ulceration. However, only Sucralfate at 100 mg/kg and MEOS at 600 mg/kg produced a significant ulcer protection (82.90% and 79.27%, $P<0.05$) as compared to control group (Table 1).

3.3. Effect of MEOS on indomethacin induced ulcers

The ulcers observed under this model presented as large clotted black sores and was produced in nearly all the rats in this model. Pre-treatment of rats with doses of MEOS (200 mg/kg, 400 mg/kg and 600 mg/kg) produced ulcer protection (11.76%, 19.12% and 32.72%, respectively) which was dose-dependent. However, none of the treatments produced a statistically significant ulcer inhibition when compared to the control group (Table 2).

4. Discussion

This study establishes the ulcer healing property of *Olax subscorpioidea* against ethanol and indomethacin induced gastric ulcers. The exact mechanism of the pathogenesis of ulcer remains to be fully elucidated but there is a general acceptance that the imbalance between the protective (mucus, bicarbonate and prostaglandins) and aggressive factors (i.e. acid, *Helicobacter pylori* and pepsin) in the stomach is responsible for the destruction of the stomach mucosal linings[13]. However, some treatment protocol (including herbal preparations) may offer an inhibitory action against acid release or protect the mucosal linings from further acid damage. Ethanol has been shown to increase the risk of ulcer in humans [14] but produces potent

Table 1
Effect of methanolic root extract of *Olax subscorpioides* (MEOS) on ethanol induced ulcer in rats.

Treatment group (*n*=6 per group)	Dose	Percentage of animals with ulcers (%)	Mean ulcer index ±SEM(%)	Percentage ulcer inhibition
Distlled water	5 mL	100.0	1.93±0.31	–
Sucralfate	100 mg/kg	66.6	0.33±0.13	82.90[a]
MEOS	100 mg/kg	83.3	1.25±0.43	35.23
MEOS	400 mg/kg	100.0	1.12±0.47	41.97
MEOS	600 mg/kg	100.0	0.40±0.09	79.27[a]

Ulcer Indices are in mean±SEM, inhibition is compared to control (distilled water), [a] : significance at $P<0.05$ for Dunnett´s test vs.control.

Table 2
Effect of methanolic root extract of *Olax subscorpioides* (MEOS) on indomethacin induced ulcer in rats.

Treatment group (*n*=6 per group)	Dose	Percentage of animals with ulcers (%)	Mean ulcer index±SEM(%)	Percentage ulcer inhibition
Distlled water	5 mL	100.0	2.72±0.72	–
Sucralfate	100 mg/kg	83.3	2.12±0.29	22.06
MEOS	100 mg/kg	100.0	2.40±0.48	11.76
MEOS	400 mg/kg	100.0	2.20±0.40	19.12
MEOS	600 mg/kg	100.0	1.83±0.37	32.72

Ulcer Indices are in mean±SEM, inhibition is compared to control (distilled water), no siginificant inhibition produced.

ulceration in rats[15]. It is said to produce reactive species responsible for mucosal injury[16] and lipid peroxidation, a free radical mediated process that ultimately destroys lipid membrane[17]. Such injuries are often associated with extensive lesions of mucosal capillaries, increased vascular permeability and reduction of mucosal blood flow[18]. The protection from ulcer produced by the extract may likely suggest the ability of extracts to inhibit any of the above mechanisms. The dose-dependent effect of the extract further authenticates its ulcer protective effect. The different doses of MEOS produced lower ulcer protection than Sucralfate implying that only increased doses (i.e. greater than 600 mg/kg) above sub-lethal dose levels would produce higher antiulcer activity than the standard drug. The use of sucralfate in this study was prompted by its increasing prescription and its cytoprotective rather than antisecretory effect[5] which suites its use in this model. Ulcer inhibitions in the indomethacin model produced by the extracts were very low and statistically insignificant. Though there was large production of ulcers in this model, the different treatments produced poor protection possibly suggesting their ineffectiveness in this form of ulcers. Non steroidal antiinflamatory drugs (e.g. indomethacin) produce gastric ulceration by inhibiting the enzymes that promote synthesis of prostaglandins[19]. Prostaglandins protect the gastric mucosa by various mechanisms which may include (but not limited to) stimulation of bicarbonate and mucus output[20] and stimulation of cellular growth and repair[21]. Sucralfate offers a physical barrier to the mucosal linings but does not promote synthesis of protective factors and this may suggest its little efficacy in non-steroidal anti-inflammatory drugs (NSAID) induced ulcers. However, we believe that increasing the doses of the extracts to sub-lethal doses may offer better protection against ulcers produced by such drugs. Secondary metabolites present in this plant have been implicated in the antiulcer activity of this plant. Metabolites such as terpenoids and saponins are known to form colloidal solutions in water upon agitation and may offer protective over the gastric mucosa[22]. Other metabolites such as alkaloids have also been shown to possess antiulcer activity and it is suggestive that these phytochemical constituents are responsible for ulcer healing action of this plant.

In conclusion we have established in this study that the methanolic extract of the roots of *Olax subscorpioidea* possess antiulcer properties against ulcers produced by necrotizing agents, suggesting a possible cytoprotective action. Though the exact mechanism of action of its antiulcer action was not examined, there is ongoing research on that area.

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