EVALUATION OF ANTIULCER ACTIVITY OF AQUEOUS ETHANOL EXTRACT OF Thesium viride ON ETHANOL AND ASPIRIN INDUCED MODELS IN RATS

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ABSTRACT
Thesium viride Hill (Santalaceae) is a sub-shrub hemiparasite that grows up to 45cm tall and widely distributed in Europe, Asia and Africa. It is used in treatment of ulcer and jaundice. Phytochemical screening was carried out on the aqueous ethanol extract of the whole plant by using standard phytochemical methods. Acute oral toxicity test was carried out and antiulcer activity was conducted using absolute ethanol and aspirin as the ulcerogenic agents on rats where the ulcer index was the parameter and percentage preventive index was determined. The aqueous ethanol extract of the plant was found to contain flavonoids, anthraquinones, glycosides and alkaloids. Acute toxicity test showed an oral LD₅₀ greater than 5000 mg/kg. In ethanol model, aqueous ethanol extract at doses of 250 and 500 mg/kg exhibited significant (P<0.0001) protection against ulcer with mean ulcer indices of 4.40 ± 0.60 and 1.80 ± 0.37 respectively as compared with negative (12.80 ± 0.97) and positive (4.00 ± 0.71) control mean ulcer indices. In aspirin model, aqueous ethanol extract at doses of 250 and 500 mg/kg demonstrated significant (P<0.05) protection against ulcer with mean ulcer indices of 3.20 ± 0.80 and 2.60 ± 0.24 respectively as compared with negative (5.60 ± 0.97) and positive (1.00 ± 0.44) control mean ulcer indices. The higher dose of the extract demonstrated greater protective ability with percentage preventive index 85.94% in ethanol induced model and 53.57% in aspirin induced model. The aqueous ethanol extract of the plant was found to be non-toxic and contains some phytochemicals that could be responsible for its antiulcer activity.

Keywords: Thesium viride, Phytochemical, LD₅₀, Ulcer index, Aqueous ethanol extract

INTRODUCTION
Ulcer is an inflamed break in the skin or mucus membrane lining the alimentary tract. Ulceration occurs when there is a disturbance of the normal equilibrium caused by either enhanced aggression or diminished mucosal resistance (Ukwuani et al., 2012). The pathogenesis of peptic ulcer disease includes a complex imbalance between gastric offensive factors like acid, pepsin secretion, Helicobacter pylori (H.pylori), bile salts, ethanol, some medications like NSAIDS, lipid peroxidation, nitric oxide and defensive mucosal factors like prostaglandins, gastric mucus, cellular renovation, blood flow, mucosal cell shedding, glycoproteins, mucin secretion, proliferation and antioxidant enzymes like catalase, superoxide dismutase and glutathione levels (Dilpreet et al., 2012).

Plants as source of phytomedicines are important choice to treat peptic ulcer. Most of the phytoconstituents showed better result than the modern medicine. Various phytochemicals like flavanoids, tannins, saponins, and terpenoids showed their antiulcer activity due to their cytoprotection, antsecretory and antioxidant property (Saikat et al., 2009).

Thesium viride Hill (Family: Santalaceae) is mainly distributed in Europe, Asia and Africa (Moore et al., 2010). It is a sub-shrub hemiparasite up to 45cm tall, tufted stems starting from a woody rootstock, branched stems, about 2mm thick, greyish green (Bosch, 2008). It is prescribed to cure ulcers (Polhill, 2005).

The objective of the present study was to carry out preliminary phytochemical screening and to investigate the antiulcer activities of the aqueous ethanol extract of Thesium viride using ethanol and aspirin induced models in rats.

MATERIALS AND METHODS

Plant Material
The Fresh whole plant of T. viride was collected from Karau-kaara village, Giwa Local Government Kaduna State, Nigeria and it was authenticated at the Department of Biological Sciences, Ahmadu Bello University, Zaria with a voucher number 415. The plant was air dried under shade.

Preparation of Extract
Dried plant of T. viride was powdered using mechanical grinder. Dried powder plant (400 g) was macerated with 1200 ml of aqueous ethanol (70% v/v) for 72 hours at room temperature and filtered. The filtrate obtained was concentrated on a water bath (75 °C) and allowed to dry at room temperature.
Phytochemical Screening
Aqueous ethanol extract of *T. viride* was screened for the presence of various secondary metabolites like, alkaloids, glycosides, terpenoids, flavonoids and anthraquinones using standard methods.

Acute Oral Toxicity Study of the Extract
Female wistar rats weighing 90-100 g maintained under standard laboratory conditions were used for the acute toxicity test according to the Organization for Economic Cooperation and Development (OECD) guidelines 423 (OECD guideline, 2002). Three rats received a single oral dose of 2000 mg/kg of the extract after 6 hours of fasting. After administration of the extract, food was withheld for further 4 hours. Animals were observed individually during the first 30 minutes after dosing and periodically during 24 hours for changes in skin fur, respiratory rate, salivation, urinary incontinence, defecation, tremor and death. After first 24 hours without any death of the first group, second group of rats were administered with 5000 mg/kg of the extract orally and were observed.

Antiulcer Studies
Wistar rats of both sexes were fasted for 48 hours before commencement of experiment with only free access to water (Weisher and Theiemer, 1983). This has been proved to be non-ulcerogenic and sufficient for absolute emptying of stomach (El-Sokkaryet al., 1991, Okashaet al., 2008 and Magajiet al., 2010).

Ethanol induced ulcer model
Ulcer was induced by administration of absolute ethanol orally. The animals were divided into four different groups each consisting of five rats. Rats in group I serves as a control group, receives distilled water (10 ml/kg) orally. Rats in group II receive Omeprazole (20mg/kg) as the standard reference drug. Rats in group III, and IV received extract at a dose of 250 and 500 mg/kg respectively. After 45 minutes of treatment with Omeprazole and extract, 1 ml of absolute ethanol was administered orally to induce gastric ulcers. The rats were sacrificed after 1 hour under chloroform. Their stomachs were removed and open along the greater curvature, rinsed slowly with normal saline and then stretched out on a Whatman's filter paper. The ulcerated surface in each stomach was measured with a transparent millimetre scale rule and the result in each group was expressed in mm as the Ulcer index (Scepovic and Radmanovic 1984).

Aspirin induced ulcer model
Ulcer was induced by administration of Aspirin (200 mg/kg) orally. The animals were divided into four different groups each consisting of five rats. Rats in group I serves as a control group, receives distilled water (10 ml/kg) orally. Rats in group II receive Omeprazole (20mg/kg) as the standard reference drug. Rats in group III and IV received extract at a dose of 250 and 500 mg/kg respectively. After 45 minute of treatment with Omeprazole and extract, 1 mg of the extract was administered orally to induce gastric ulcers. The rats were sacrificed after 4 hours under chloroform. Their stomachs were removed and open along the greater curvature, rinsed slowly with normal saline and then stretched out on a Whatman’s filter paper. The ulcerated surface in each stomach was measured with a transparent millimetre scale rule and the result in each group was expressed in mm as the Ulcer index (Scepovic and Radmanovic 1984).

Preventive index (P.I %) in both ulcer induced models were calculated according to the method of Hanoet al. (1976).

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P.I = \frac{\text{Ulcer Index of Ulcer control} - \text{Ulcer Index of Treated}}{\text{Ulcer Index of Ulcer control}} \times 100
\]

RESULTS

Phytochemical Screening
The results of preliminary phytochemical screening of plant extract showed presence of alkaloids, flavonoids, anthraquinones, and glycosides. The presence of these active phytoconstituents may be responsible for antiulcer activity.

Acute Toxicity Study
Acute toxicity study in which the animal treated with the aqueous ethanol extract at a higher dose of 5000 mg/kg did not manifest any abnormal signs, behaviour changes, body weight changes even after 24 hours of administration. Hence the dose of 250 and 500 mg/kg were used for the study.

Antiulcer Activity of extract in Ethanol Induced Ulcer
Oral administration of aqueous ethanol extract at different doses showed significant reduction in ulcer index as compared to the ulcer control group. The preventive index of 65.63% and 85.94% of the extract at the doses of 250 and 500 mg/kg were obtained respectively. Whereas the preventive index of the reference standard drug was 68.75% (Table 1).

<p>| Table 1: Effect of extract on ethanol-induced gastric ulcerations in rats |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Previous to Dose</th>
<th>Ultra index</th>
<th>Preventive index (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ulcer control</td>
<td>Distilled water</td>
<td>10 ml/kg</td>
<td>12.80 ± 0.97</td>
<td>-</td>
</tr>
<tr>
<td>Reference</td>
<td>Omeprazole</td>
<td>20 mg/kg</td>
<td>4.00 ± 0.71***</td>
<td>68.75</td>
</tr>
<tr>
<td>Experimental</td>
<td>Extract</td>
<td>250 mg/kg</td>
<td>4.40 ± 0.60***</td>
<td>65.63</td>
</tr>
<tr>
<td>Experimental</td>
<td>Extract</td>
<td>500 mg/kg</td>
<td>1.80 ± 0.37***</td>
<td>85.94</td>
</tr>
</tbody>
</table>

The percentage preventive index for each group was calculated by comparison with the ulcer control group considered as 100% of gastric damage. Values indicate mean ± S.E.M. significant variation against control at ***P < 0.0001 (Anova, with multiple comparison method by Dunnett).
Experimental Extract 250mg/kg 3.20 ± 0.80* 42.86

Reference
Ulcer control Distilled water 10 ml/kg 5.60 ± 0.97 -

Groups Treatment Previous to Dose Ulcer index Preventive index (%)

Ulcer control Distilled water 10 ml/kg 5.60 ± 0.97 -
Reference Omeprazole 20mg/kg 1.00 ± 0.44*** 82.14
Experimental Extract 250mg/kg 3.20 ± 0.80* 42.86
Experimental Extract 500mg/kg 2.60 ± 0.24** 53.57

The percentage preventive index for each group was calculated by comparison with the ulcer control group considered as 100% of gastric damage. Values indicate mean ± S.E.M. significant variation against control at *P < 0.05, **P < 0.01 and ***P < 0.0001 (Anova, with multiple comparison method by Dunnett).

**DISCUSSION**

The LD₅₀ of the aqueous ethanol extract was found to be greater than 5000 mg/kg when administered orally in rats. These studies showed the extract is practically non-toxic when administered using the oral route. This is based on Loomis and Hayes (1996) toxicity classification.

In ethanol induced ulcer model, the extract shows a significant (P<0.0001) reduction in ulcer index at all doses of the extract and a dose dependent preventive index. The high and the low dose gave a higher preventive index than that of the reference control group. In the aspirin induced ulcer model, the extract shows significant at (P<0.05) reduction in ulcer index at all doses of extract with a dose dependent preventive ulcer index. The high dose of the extract conferred a low preventive index as compared to the reference control group.

The two different ulcer models shows the significant reduction in ulcer index and a promising preventive index compared to reference standard drug. The extract shows a better preventive index in the ethanol induced model than the aspirin induced model. Adinortey et al., (2003) reported that the ethanol-induced ulcer model is useful for studying the efficacy of potential drugs or testing agents that have cytoprotective and/or antioxidant activities as it produce necrotic lesions in the gastric mucosa by its direct toxic effect reducing the secretion of bicarbonate and production of mucous. Whereas NSAIDS induced model is important in investigating the potential usefulness of antisecretory and cytoprotective agents since the underlying pathophysiology involves gastric acid secretion and mucosal prostaglandin synthesis.

Different constituents like flavonoids, tannins, saponins and gums and mucilages have been reported to have antiulcer activities (Borrelli and Izzo; 2000). Therefore the antiulcer activity of this extract may be attributed to this phytoconstituents of the plant which ameliorate the imbalance between the aggressive factors (i.e. acids, pepsin and H. Pylori) and maintaining the mucosal integrity. The significant antiulcerogenic property in ethanol induced is attributed to the antioxidant compounds present in the extract that exhibit the cytoprotective activity while that in the aspirin induced may be due to increased synthesis of mucous and prostaglandins.

**CONCLUSION**

The results have shown promising antiulcerogenic potential of aqueous ethanol extract of T. viride in the two different ulcer induced models probably due to the antioxidant and cytoprotective effects of its phytoconstituents.

**REFERENCES**


