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EVALUATION OF ANTIULCER AND PHYTOCHEMICAL ACTIVITIES OF LEAF EXTRACTS FROM *Tapinanthus dodoneifolius* DC. *(LORANTHACEAE)* GROWN ON *Tamarindus indica* TREE

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ABSTRACT
Present study was carried out to determine the phytochemical constituents and Antiulcer activities of aqueous ethanolic leaf extract from Tapinanthus dodoneifolius grown on Tamarindus indica tree. Results obtained for phytochemical revealed the presence of flavonoids, tannins, saponins, glycosides and alkaloids. Antiulcer activity of aqueous ethanolic leaf extract from Tapinanthus dodoneifolius grown on Tamarindus indica tree was tested on wistar rats' stomach which was compared with standard antiulcer drug Omeparazole. Oral administration of aqueous ethanolic leaf extract from Tapinanthus dodoneifolius (500-1500mg/cm^3) to Aspirin induced ulcerated wistar rats for one week reduced the incident of ulceration in a dose dependent manner. Acute toxicity study of the extract did not manifest any toxicological signs in rats. Thus the plant was considered relatively safe and it has potential antiulcer activity.
Keywords: Tapinanthus dodoneifolius, Antiulcer, phytochemical, Tamarindus indica, Omeparazole, toxicity.

INTRODUCTION

Current trends in drug development process are focused on natural sources especially sources of plant origin due to lower cost, availability, fewer adverse effect and perceived effectiveness. Medicine of plant origin contains natural substances that can promote health and alleviate illness. The most important of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenol compounds (Karanayil *et al.*, 2011).

Peptic ulcer is a sore on the lining of the gastro intestinal tract caused by mucosal erosions due to bacterial products of *Helicobacter pylori and* imbalance between the gastric aggressive factors (such as acid and pepsin) and the mucosal defensive factors (such as mucus and bicarbonate) (Abdulla *et al.*, 2010), other causes are the use of non steroidal anti-inflammatory drugs (NSAIDs) such as Aspirin, and ibuprofen, which can damage the lining of the stomach and duodenum. Factors associated with lifestyle such as smoking, alcoholism, intake of spicy foods and stress are also associated with peptic ulcer formation. The sign and symptom of peptic ulcer were numerous among which includes loss of weight, poor appetite, bloating, burning, nausea and vomiting Patients with ulcer bleeding may have bloody or black stools, weakness and persistent stomach pain. There could be signs of a serious problem, such as perforation where the ulcer burrows through the stomach or duodenal wall. Bleeding could be initiated when acid or the ulcer breaks blood vessels (Roy *et al.*, 2001).

Tapinanthus dodoneifolius DC. Danser *(Loranthaceae)* known as Kauchi in "Hausa", "Afomo onisana" in Yoruba and Mistletoe or lightning matches in English. It is a parasitic plant growing on a large number of tree species such as *Parkia biglobosa, Tamarindus indica,* kola, citrus, acacia, orange and many other trees as a host plants. It is found in North/Central Namibia, West Africa, North America and Europe. It is a green shrub, the leaves are ovate, round at the apex about 7cm long and 3cm broad with irregular pinnately arranged lateral nerves and small purple flowers with white sticky barriers which are considered poisonous (Ayorinde *et al.*, 2008). It grows on the branches, roots and twigs of host plant. They have chlorophyll but are parasites by invading the host plant xylem or phloem using a special structure, the haustorium, to obtain water and mineral (Robert *et al.*, 2011). It twigs and leaves are used in the treatment of various ailments in different areas around the world. It is used in Northern Nigeria in the treatment of stomach ache, diarrhea, dysentery, wound and cancer (Deeni and Sadiq, 2002). In Burkina Faso it is used for the treatment of cardiovascular and respiratory diseases (Ouedraogo *et al.*, 2005).

The leaves and young twigs of the plant is used to treat malaria, diabetes, hypertension and sterility in cow (Efuntoye *et al.,* 2010). Ekhaise *et al*. (2010) also reported that it is used in the treatment of diabetes, blood pressure, asthma, epilepsy and cancer.

Presence of tannins, flavonoids, alkaloids and saponins were revealed in the methanol and chloroform extracts of the plant (Efuntoye *et al.,* 2010). Deeni and Sadiq, 2002 revealed the presence of anthraquinones, saponins, tannins and alkaloids from *Tapinanthus dodoneifolius*. It has been investigated and found to have antimicrobial, anti-diabetic and antiviral properties (Robert *et al.*, 2011). Aqueous extract of *Tapinanthus dodoneifolius* (AETD) was investigated for cardiovascular activities on rat aorta and heart. It was observed that the AETD did not affect heart rate but significantly enhanced heart contraction force and relaxation capacity (Ouedraogo *et al.,* 2005). In an antimicrobial report, it was found that methanol extract of *Tapinanthus* grown on *Phyllanthus muellerianus* showed activity against *Escherichia coli* and *Staphylococcus aureus* while chloroform extract of *Tapinanthus* grown on *Parkia biglobosa* and *Citrus aurantifolia* respectively, showed activity against *Escherichia coli* and *Staphylococcus aureus* (Efuntoye *et al.,* 2010). Extracts obtained from *Tapinanthus* inhibit growth of *Bacillus* spp., *Proteus* spp. and *Pseudomonas* spp., a bacterial spp. known to be associated with gastrointestinal tract and wound infection (Deeni and Sadiq, 2002).

Very little pharmacokinetic and pharmacodynamic studies have been done on *Tapinanthus dodoneifolius* on its toxicity, curative effect, side and adverse effects particularly in its used as an antiulcer drug, therefore investigations are needed in this aspect. The present study was to investigate the phytochemical constituents and anti-ulcer activity from ethanolic leaves extract of *Tapinanthus dodoneifolius* grown on *Tamarindus indica* on Aspirin induced gastric ulcer in wistar rats.

MATERIALS AND METHODS

Plant Materials: Fresh leaves of *Tapinanthus dodoneifolius* DC (Loranthaceae) were collected from *Tamarindus indica* tree at Dan-Maliki village near Kafin-chiri Dam in Garko local government, Kano State, Nigeria. Plant was identified and authenticated by Prof. B.S. Aliyu of Biological Sciences Department, Bayero University Kano. Fresh leaves were air dried and powdered using mortar and pestle. Powdered leaves were extracted using ethanol and macerated into different fractions using different solvents as described by Kongkathip, 2003. The various fractions obtained were used for phytochemical analysis using standard method described by Sofowora (1993) and Trease and Evans (1989).

Experimental Animals: Adult healthy wistar rats of either sex weighing 100-150g were obtained from Pharmaceutical Sciences Department, Ahmadu Bello University (ABU) Zaria, Nigeria. The animals were housed in an animal room of the Biological Sciences Department, Bayero University Kano. These rats were provided with a free access to a standard feed and water ad-libitum. Acute toxicity studies of the ethanol extract from leaves of *Tapinanthus dodoneifolius* was carried out on wistar rats, conducted according to the method of Lorke (1983).

Aspirin induced Gastric Ulcer: Thirty (30) wistar rats (100-150g) were randomly divided five groups of six rats each and labeled as groups 1-5 thus: Group 1: received normal saline (2 ml/kg) and served as the negative control of the experiment Group 2: received omeprazole (20mg/kg) as a standard drug and served as a positive control. Group 3: This is the first test group, they were administered with 500mg/kg of test extract. Group 4: administered with 1000mg/kg of test extract. Group 5: This is the third test group; they were administered with 1500mg/kg of test extract. Oral administration of drugs or saline was achieved at 9:00am daily for 8 days. After 8 days of treatment, the rats to be experimented were kept fasting for 36 hours in separate cages with raised wide meshed wire bottom to ensure complete emptying of the stomach and to prevent coprophagia (eating their faeces) before subjecting them to ulcerogen, but allowed water ad-libitum. Ulcer was produced by administration of aqueous suspension of aspirin (200mg/kg) orally to rats. In treatment group drugs were administered orally 1hour before administration of Aspirin (Rajkapoor *et al.,* 2002). Animals were sacrificed 4 hours later by an over dose of chloroform inhalation. Stomachs were removed and afterwards incised along the greater curvature. It was washed gently in running tap water and gastric mucosa spread on a filter paper for gastric lesions assessment. A 2x hand lens was used to locate the ulcers. Stomach ulceration was expressed in terms of: ulcer score, ulcer index, preventive index and percentage of ulceration using the method of O`Hara *et al*. (1995).

Two (2) rat stomachs were selected from each group and preserved in 10% Formalin. They were taken to histology department of Aminu Kano Teaching Hospital for histopathological analysis.

Statistical Analysis: The results were treated using MacAnova and Anova statistical software. Quantitative data were summarized using mean and standard error of mean. Means were compared using student`s t-test and P value ≤ 0.05 was considered statistically significant.

RESULTS

Acute toxicity: No death were recorded in the rats treated orally with varying doses (10; 100; 1000; 1600 and 5000 mg/kg) *of Tapinanthus dodoneifolius* leaf extract. The extract did not manifest any toxicological signs in rats. Thus the plant was considered relatively safe.

Table 1: Result of Phytochemical constituents of *Tapinanthus dodoneifolius* grown on *Tamarindus indica* tree (TD_1)

	Phytochemicals					
Fraction	Alkaloids	Flavonoids	Tannins	R. sugar	Glycosides	Saponins
Ethanol	+	+	+	_	+	+
Pet. Ether	+	_	+	_	+	_
Chloroform	+	+	+	_	_	_
Ethyl acetate	+	+	+	_	+	_
Methanol	+	+	+	_	+	+

Key: + = Presence - = Absence

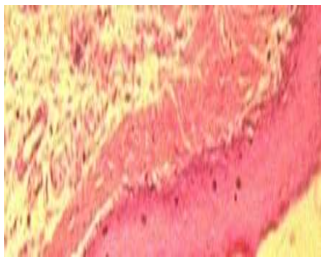
Table 2: Analysis of anti-ulcer activity of aqueous ethanol extract of leaves of *Tapinanthus dodoneifolius* grown on *Tamarindus indica* (TD_1) tree and Omeprazole on aspirin induced ulcers in wistar rats.

Groups	Drugs/Fractions	Dosage (mg/kg)	Mean ulcer score	Ulcer index	Preventive index
1	Normal saline	$2cm^3/kg$	6.17 ± 0.53	617	0%
2	Omeprazole suspension	20	1.00 ± 0.36*	100	84%
3	Aqueous ethanol extract of TD_1	500	2.83 ± 0.39*	283	54%
4	Aqueous ethanol extract of TD_1	1000	2.50 ± 0.34*	250	60%
5	Aqueous ethanol extract of TD_1	1500	1.17 ± 0.39*	117	81%

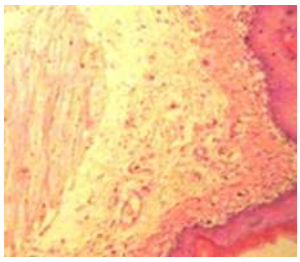
Values are means ± S.E of the mean; n = 6 rats in each group.
*Superscript indicates significant difference at P ≤ 0.05 when compared with the control

Histological findings

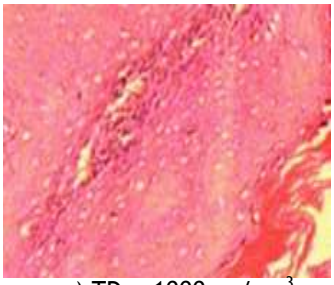
Histological observation of aspirin induced gastric lesions in ulcer control group pre-treated with only Normal saline showed comparatively an extensive damage to the gastric mucosa, oedema and leucocytes infiltration of the sub mucosal layer (inflammation or breakage of cells). Rats which received pre-treatment with *Tapinanthus dodoneifolius* ethanol leaf extract had comparatively better prevention of the gastric mucosa as seen by the reduction in the number of ulcer spots and reduced sub mucosal oedema and leucocytes infiltration. The TD_1 exerted cytoprotective effects in a dose dependent manner.



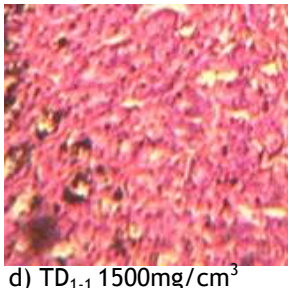
a) Negative control (n-saline)



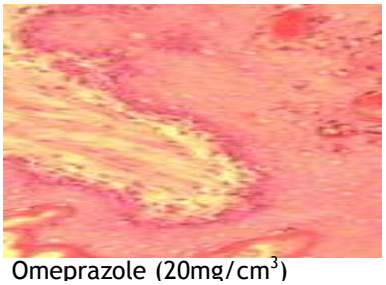
b) TD_1 500mg/cm^3



c) TD_{1-1} 1000mg/cm^3



d) TD_{1-1} 1500mg/cm^3



e) Omeprazole (20mg/cm^3)

Figure1: Histological section of gastric mucosa in rats pre-treated with

DISCUSSION

The synthetic drugs used in the treatment of ulcer include receptor blockers, proton pump inhibitors, drugs affecting the mucosal barrier, drugs which can reduce ulcer pain, drugs which can eradicate *Helicobacter pylori* and those agents which reduce acid secretion. In an effort to further search for curative and safe agents for the treatment of peptic ulcer in our indigenous medicinal plants, *Tapinanthus dodoneifolius* was selected for preliminary screening of its *in vivo* anti-ulcer activities in rats.. The preliminary studies in this research showed that *Tapinanthus dodoneifolius* ethanol extract significantly decreased the gastric ulcers incidence. The exact phytochemical constituent(s) responsible for this anti ulcer activity is not precisely known, but it may be due to the presence of flavonoids, tannins or alkaloids because it was demonstrated in some previous studies that tannins, flavonoids and alkaloids posses anti ulcer properties, antibacterial, anti-oedema, anti-pyretic and spasmolytic activities (Metowogo *et al*., 2008; Evans and Trease 1995). Many alkaloids such as hyoscine butyl bromide (Buscopan) have been used to suppress acid secretion (British National Formulary, 2000).

The reference drug (Omeprazole) used acts as proton pump inhibitor that suppresses gastric acid secretion by blocking the final step in acid production. The implication of *Helicobacter pylori* in the pathogenesis of ulcer and the use of antibiotics in the management of the disease stimulated the study of antimicrobial activity of *Tapinanthus dodoneifolius*. *Helicobacter pylori*, the enteric organism implicated in peptic ulcer was not used in this screening test because of the difficulty in culturing the organism (Anil *et al*., 2011). However other enteropathogenic gram positive bacteria (such as E. *coli*, and *Staphylococcus aureus*) to which it belonged was studied by many researchers and the extract of *Tapinanthus* were sensitive to those organisms. Therefore it could be inferred that leaves of *Tapinanthus dodoneifolius* have effect on *Helicobacter pylori* growth, since it has been established that eradication of *Helicobacter pylori* led to the cure of the disease and prevention of complication (Chiba *et al*., 1998).

CONCLUSION

This study has been a contribution to the assessment of possible anti-ulcer compounds from the leaves of *Tapinanthus dodoneifolius*. The various fractions posses' significant phytochemicals and the crude ethanol extract of the leaves demonstrated a potent anti-ulcer activity on animals (wistar rats). The evidence obtained from the research supported the use of the plant for the treatment of various ailments in various areas by the natives, especially Africans.

Recommendation

On the basis of this study, it is recommended that further detailed pharmacognostic studies should be intensified on the plant fractions in general, so that its potential chemotherapeutic and other economic values could be harnessed for man`s benefits.

Suggestion

It was suggested that further study need to be done to elucidate the mechanism of action involved in the anti-ulcer activity and identify the phytochemical constituents responsible for this pharmacological actions of *Tapinanthus dodoneifolius* leaves.

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