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ANTIULCEROGENIC ACTIVITY OF METHANOL EXTRACT FROM *Ipomoea asarifolia* LEAVES IN WISTAR RATS

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ABSTRACT
The phytochemical constituents and the antiulcerogenic activity of methanolic extract from Ipomoea asarifolia leaf were investigated to ascertain its medicinal potentials. The study revealed that the leaf contained saponins, tannins, alkaloids, terpenoids and eugenols. The methanolic extract was used as a protective agent against gastric ulcer in Wistar rats, using the experimental model of gastric ulcer (lesion) induced by ethanol. The ulcer count for the methanol extract ranged from 0.00 to 2.00, while the ulcer index ranged between 0.33 to 2.00 for all concentrations (100, 200 and 400mg/kg). Percentage inhibition at 100, 200 and 400mg/kg were respectively 25.09%, 12.73% and 87.64%. The extract prevented acute gastric mucosal injury induced by absolute ethanol in a non dose-dependent manner. This is because the protective action was observed at the lowest and highest doses, but not an intermediate dose of the extract. The results obtained indicate that the leaf extract has antiulcerogenic importance and may be utilized for the treatment of gastric ulcers.
Key words: Antiulcerogenic, extract, phytochemical, Ipomoea asarifolia, stomach

INTRODUCTION

The use of plants as medicine has become the mainstay of traditional health care system amongst rural communities worldwide (Sarker and Nahar, 2007). The therapeutic use of plants certainly dates back to the beginning of civilisation, as old as mankind. Over the years, these plants have assumed a central stage in modern civilisation as a natural source of medicine as well as in scientific studies amongst scientists in search for alternative sources of drugs. About 80% of plants selected for analysis on the basis of ethno-medicinal information have demonstrated significant pharmacological activity (Fatope, 2001).

According to the World Health Organisation (WHO, 2013), a medicinal plant is any plant which, in one or more of its organs, contains substances that can be used for therapeutic purposes, or which are precursors for chemo-pharmaceutical semi-synthesis. Such a plant will have its parts including leaves, roots, rhizomes, stems barks, flowers, fruits, grains or seeds, employed in the control or treatment of a disease condition which maybe associated with chemically active constituents. These non-nutrient plant compounds or bioactive components are often referred to as phytochemicals or phytoconstituents and are responsible for protecting the plant against microbial infections or infestations by pest (Nweze *et al.,* 2004; Doughari *et al.,* 2009). The science and application of these indigenous or local medicinal remedies including plants for treatment of diseases is called ethno-pharmacology but the practice dates back as far as the early years of mankind (Doughari and Obidah, 2008).

These plants are applied in various forms such as poultices, concoctions of different plant mixtures, infusions as teas or as component mixtures in porridges and soups, administered in different ways including oral, nasal (smoking, snuffing or steaming), topical (lotions, oils or creams), bathing or rectal (enemas). Different plant parts and components (roots, leaves, stem barks, flowers, essential oils or their combinations) have been employed in the treatment of infectious pathologies in the respiratory system, urinary tract and gastrointestinal systems, as well as on the skin (R´ıos and Recio, 2005; Adekunle and Adekunle, 2009).

Ipomoea asarifolia (Convolvulaceae) is a glabrous succulent perennial plant trailing on the ground. It reproduces from the seeds and shoots of the stem. It is a creeping or trailing plant that grows on sandy soils or waste lands.

It is found throughout West Africa from Nigeria to Senegal, Mali, the Cape Verde Island, and tropical Asia (Jegede *et. al.*, 2009). It is used for the treatment of disease conditions such as ulcers by various rural dwellers and traditional medicinal practitioners in Africa. In some parts of Africa, like Senegal, it is used traditionally for various gynaecological purposes such as haemorrhage; urinary problems during pregnancy; as an abortifacient and for the treatment of headache, arthritic pains and stomach ache. In Ivory Coast, a leaf decoction is usually taken internally as a wash for feverish chills and rheumatic pains. In northern Nigerian, the leaf poultice (a moist substance spread on cloth and placed on the skin) is applied to guinea worm sores while the face is steamed over a hot decoction of the plant along with husks of bulrush millet. The flowers are also boiled with beans and eaten as a remedy for syphilis The leaves are also used to treat dysmenorrhoea (painful menstruation) in the middle belt of Nigeria (Atawodi and Onaolapo, 2010).

A sub-chronic (90-day) toxicity trial in rats has also shown that an aqueous-ethanolic extract of *Ipomoea asarifolia* plant, when administered intraperitoneally, is relatively safe up to a dose of 1000 mg/kg (Akindele *et al*., 2015).

This study was therefore conducted to provide a scientific basis for the use of *Ipomoea asarifolia* leaves in the treatment and/or management of gastric ulcers by reviewing and analyzing the protective and therapeutic agents present and provide evidence for its effectiveness and potency in modern investigations for treatment of ulcers. There are a number of models that are available to test substances for their antiulcerogenic effects. Here, we report on the effect of methanolic extract of *Ipomoea asarifolia* leaves on gastric lesion (ulcer) induced in Wistar rats by employing absolute ethanol.

MATERIALS AND METHODS

Plant material

Leaves of *Ipomoea asarifolia* were collected in Ekosodin community, Benin City, Edo State, Nigeria at the location $6^0 24' 26.8'' N$ $5^0 36' 41.8'' E$. The plant was identified and authenticated at the Department of Plant Biology and Biotechnology, University of Benin, Nigeria by Prof. Emmanuel Isaka Aigbokhan.

The leaves were washed and dried under shade for two weeks (14 days) in the labouratory at the University of Benin, Chemistry Annex building. The dried leaves were pulverized to fine powder by using an electric grinder.

Extract preparation

Extraction of the plant material was done according to the method described by Aliyu *et al*., (2011), with some modifications. 85 g of the powdered plant material was soaked in 1 litre (1000 ml) of 70% methanol, in a stoppered container. The mixture was then intermittently agitated every 20minutes for I hour and left for twenty-four (24) hours to allow soluble matter dissolve. Thereafter, the mixture was filtered using No.1 Whatman filter paper (18cm in diameter) and the resulting extract was concentrated using a water bath, set at 60^0C to obtain the crude extract. The extract was collected in a sample bottle and kept in a refrigerator for phytochemical analysis and bioassay.

Phytochemical screening

The phytochemicals present in *I. asarifolia* leaves were qualitatively analyzed using the methods described by Soforowa (1993) as well as Trease and Evans (2002).

Animals

After obtaining clearance from the Ethics Committee on the use of animals for studies, fifteen (15) male Wistar rats, weighing between 137g and 194g, were obtained from the Faculty of Pharmacy, and kept in the Animal House, Department of Pharmacology and Toxicity, University of Benin. The rats were kept in metal-walled cages in groups of three (3). They were fed daily with formulated dual mash feed and allowed free access to water. The rats were maintained under normal environmental conditions for one week (7 days) in order for them to acclimatize.

Treatments

Doses of 100, 200 and 400 mg/kg of *Ipomoea asarifolia* extract were prepared as aqueous suspensions. Cimetidine (100 mg/kg) and 10ml/kg deionised water were used as the reference drug and control vehicle respectively. All treatments were administered orally (gavage), 30 minutes before the procedures for inducing gastric ulcer.

Ethanol-induced gastric ulcer

The rats were divided into five groups of three each. The rats were starved of food but allowed access to water 24 hours before the study. Rats in Group I were pre-treated with water (10ml/kg), to serve as the control. Rats in Group II were administered Cimetidine, standard drug, at a dose of 100 mg/kg. Rats in Groups III, IV and V received the extract at doses of 100, 200 and 400 mg/kg respectively. 1ml of absolute ethanol (99%) was then administered to all the animals 30 minutes after the pre-treatment.

Each group was placed in a transparent observation box. Sixty (60) minutes after the ethanol administration, the rats were sacrificed. The stomachs were then removed, examined, the number of lesions counted and the degree of severity recorded (Shirisha and Subash, 2012). The ulcer count and ulcer index were calculated in relation to the control group. The activity was also expressed as percentage ulcer inhibition. The stomach tissue from each group was then taken, placed in a sample bottle filled with formalin (as a preservative) for further histological studies.

Scoring of the ulcer was done as follows: Normal stomach = 0.00; Red coloration = 0.50; Spot ulcer = 1.00; Hemorrhagic streak = 1.50; Ulcers = 2.00 and Perforation = 3.00.

Mean ulcer score for each animal is expressed as ulcer index.

The percentage of ulcer protection was determined using the formular:

$$\% \text{ Ulcer Inhibition} = \frac{Control\ mean\ index - mean\ index}{control\ mean\ index} \ x\ \frac{100}{1}$$ (Nwafor *et al.*,2000)

HISTOPATHOLOGY

The stomach tissue samples harvested from the rats were fixed in buffered formalin for 24 hours. Sections of tissue from stomachs were examined histopathologically to study the ulcerogenic and/ or anti-ulcerogenic activity of *Ipomoea asarifolia leave extract*. The tissues were fixed in 10% buffered formalin and were processed using a tissue processor. The processed tissues were embedded in paraffin blocks and about 5-μm thick sections were cut using a rotary microtome. These sections were stained with hematoxylin and eosin using routine procedures. The slides were examined microscopically for patho-morphological changes such as congestion, haemorrhage, oedema and erosions using an arbitrary scale for the assessment of severity of these changes (Slaoui and Fiette, 2011).

RESULTS AND DISCUSSION

The results for the phytochemical screening of *Ipomoea asarifolia* leaf extract are presented in Table 1 below. The extract was found to contain saponins, tannins, alkaloids, terpenoids and eugenols. However, carbohydrates, glycosides, steroids, reducing sugars and flavonoids were not detected. The results compare favourably with those obtained for the leaves of *I. asarifolia* by Jegede *et al.,* (2009) and Aliyu *et al.,* (2011) who reported the presence of alkaloids, tannins and saponins in the plant leaves

Table 1. Results of phytochemical screening of *Ipomoea asarifolia* leaf extract.

Phytochemicals	**Results**
Carbohydrate	**--**
Phenolic compounds	**--**
Saponins	**++**
Terpenoids	**++**
Alkaloid	**++**
Tannins	**++**
Steroids	**--**
Reducing sugar	**--**
Flavonoids	**--**
Eugenols	**++**
Glycosides	**--**

Key: ++ = present -- = absent

The presence of these secondary plant metabolites shows a great potential for the application of *I. asarifolia* as a useful source of plant medicine. Several studies have shown the potentials of these phytochemicals in ulcer gastric treatment. This is supported by the report that tannins act by inhibiting the secretion of gastric acids (Jhansirani *et al.*, 2010), while alkaloids protect the gastric mucosa from damage caused by absolute ethanol through the scavenging of free radicals (Konturek *et al.*, 1997). Also, saponins have been shown to have significant effect in lowering ulcer index, while significantly increasing percentage protection against ethanol and restrained stress induced ulcer model in rats. Saponins aid increased levels of defensive mucin secretion in terms of total carbohydrate to protein ratio, thus being active in alleviating ulcers (Mohammad *et al.,* 2012).

Table 2. Effect of methanolic extract of *Ipomoea asarifolia* on ethanol-induced gastric ulcer in wistar rats.

Treatment	Mean ulcer index	Percentage inhibition (%)
Water (10ml/kg)	2.67	-
I. asarifolia		
100mg/kg	2.00	25.09
200mg/kg	2.33	12.73
400mg/kg	0.33	87.64
Cimetidine (100mg/kg)	1.33	50.18

The results of the effect of methanol extract of *I. asarifolia* on ethanol-induced gastric ulcers in Wistar rats are shown in Table 2 and also displayed pictorially on Plates 1,2,3,4 and 5. Ethanol-induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. This is very evident in Plate 1 for animals treated with deionised water at 10 ml/kg.

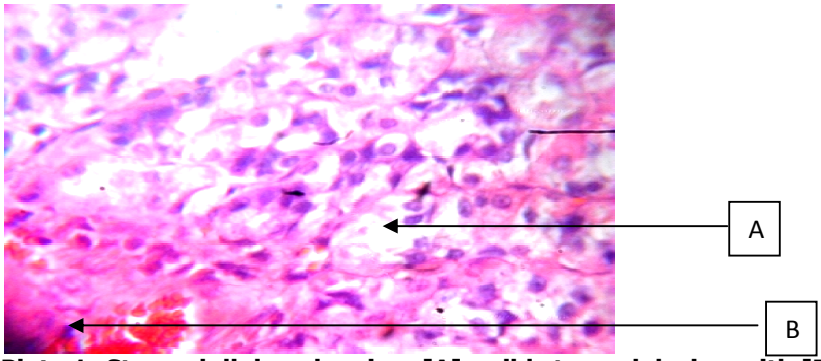

Plate 1: Stomach lining showing [A]: mild stomach lesion with [B]: severe haemorrhage. Treatment: Deionised water (10 ml/kg).

Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra-cellular membrane permeability to sodium and water. The resultant massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation on the surface (Raju *et al.,* 2009).

The extract showed protection against characteristic lesions produced by ethanol administration. The protective action against lesions was offered by the plant extract at varying degrees depending on the dosage.
Plate 2 shows the highest percentage inhibition (87.64%) which was observed at a dose of 400 mg/kg of the extract. There was no observable haemorrhage and the stomach lining is seen to be covered with mucosa lining.

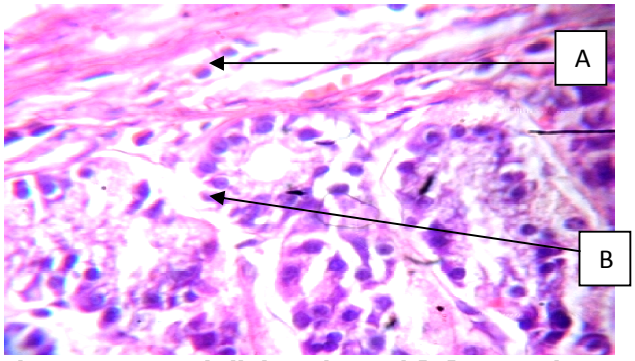

Plate 2: Stomach lining showed [A]: coated stomach with mucosa lining [B]: little or no lesion with absence of haemorrhage.
Treatment: *I. asarifolia* extract (400 mg/kg).

This antiulcer effect of the plant extract may have reduced gastric acid secretion leading to gastric cytoprotection. This is highly comparable to the effect produced by the standard drug, Cimetidine, which gave a percentage inhibition of 50.18% when administered at a dose of 100 mg/kg as shown in Plate 3 below.

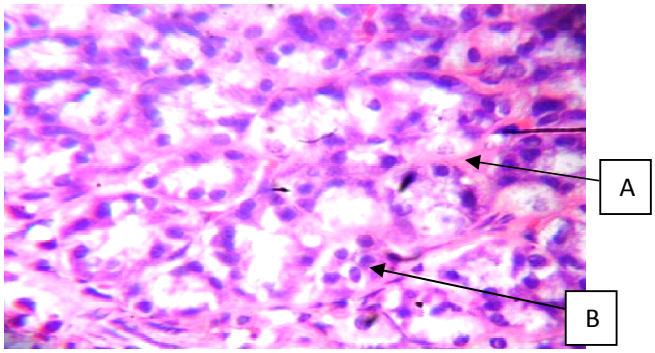

Plate 3: Stomach lining showing [A]: mucosa membrane with a well-defined and healthy stomach
[B]: thick coating of the stomach lining.
Treatment: Cimetidine (100mg/kg).

Since the result of the phytochemical screening of *I. asarifolia* leaf extract shows that it contains saponins, tannins, alkaloids, terpenoids and eugenols, it allows us to suggest that the antiulcerogenic effect of *I. asarifolia* leaf extract may be associated with the presence of these phytochemicals in the plant (Jhansirani *et al.*, 2010).

However, the intermediate dose of 200 mg/kg of the extract of *I. asarifolia* leaf gave the least percentage inhibition (12.73%) whereas the lowest dose administered (100 mg/kg) gave a better protection of 25.09%. These effects are respectively shown in Plates 4 and 5 below.

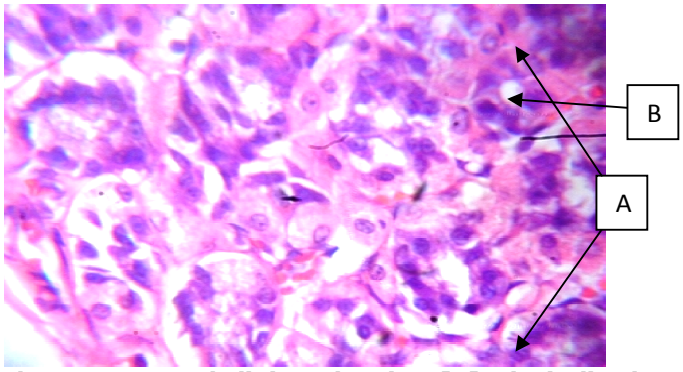

Plate 4: Stomach lining showing [A]: devitalised stomach and [B] lesion with spotted haemorrhage indicating ulceration.
Treatment: *I. asarifolia* extract (200mg/kg).

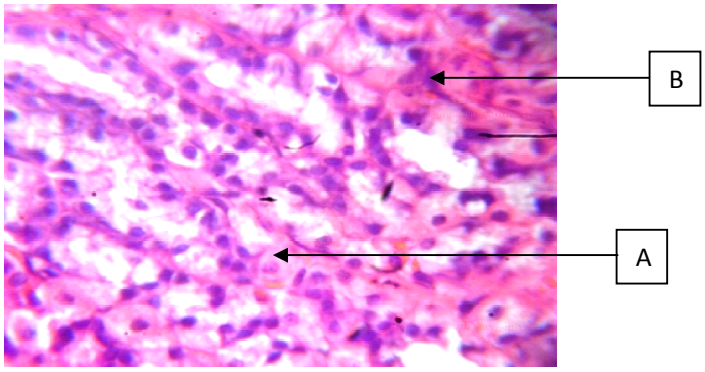

Plate 5: Stomach lining showing [A]: mucosa lining of the stomach and [B] lesion with mild haemorrhage indicating ulceration.
Treatment: *I. asarifolia* extract (100mg/kg).

In this experimental model using ethanol to induce gastric ulcer, the protective action of *Ipomoea asarifolia* leaf extract was produced at the lowest and at the highest dose, but not at intermediate doses of the extract. This shows that its antiulcer activity is not dose dependent. The specific mechanism underlying this action is however unknown.

CONCLUSION

The qualitative evaluation of *Ipomoea asarifolia* leaf extract has shown that it contains various secondary plant metabolites such as saponins, tannins, alkaloids, terpenoids and eugenols, hence making the plant a viable source of plant medicine.

This study has also shown that the extract of *I. asarifolia* has significant antiulcerogenic activity on Wistar rats. Therefore, the plant could be exploited for use in the formulation of relatively cheap and effective drug for the treatment of gastric ulcers. However, the mechanisms underlying the protective action of the extract against ethanol-induced gastric lesions are unclear. Further studies using more specific methods are required to explore the compounds responsible for the protective effect, and the mechanism of this activity.

Conclusively, this study supports the traditional use of *I. asarifolia* leaves in the treatment of gastric ulcers.

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