ANTIULCER ACTIVITY OF ETHANOL LEAF EXTRACT OF *Azadirachta indica* IN ALBINO RATS

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ABSTRACT

Azadirachta indica is used as a traditional remedy in ayurvedic medicine in India since antiquity and many parts of Africa including Nigeria to treat gastric ulcer. Current study was designed to investigate this plant leaves for potential antiulcer agent in aspirin induced ulcers in rats. Qualitative pytochemical screening and acute oral toxicity test of the ethanol leaf extract of *Azadirachta indica* was carried out according to standard methods previously described. Gastric ulcer was induced by oral administration of aspirin (300 mg/kg) to adult rats. The antiulcer activity was determined by calculating the mean decrease in number of epithelial shedding, frank haemorrhage, ulcers and perforations in the extract-treated rats at doses of 150, 300 and 600 mg/kg. Preliminary phytochemical screening of the ethanolextract of *Azadirachta indica leaf* revealed the presence of carbohydrates, reducing sugar, saponins, anthraquinones, flavonoids, tannins and alkaloids. The oral median lethal dose (LD_{50}) of the extract was estimated to be greater than 5000 mg/kg in rats. The extract was found to be an effective antiulcerogenic agent at different doses (150, 300 and 600 mg/kg). Oral administration of all the investigated doses of the extract reduced the severity of gastric mucosal damage. The extract showed a significant ($p<0.05$) protection against aspirin induced epithelium shedding at lower doses (150 mg/kg and 300 mg/kg) comparable to the standard drugs used misoprostol and omeprazole. Frank haemorrhage index was reduced by the extract at higher doses of 300 and 600 mg/kg with no statistically significant difference with omeprazole ($p>0.05$). The ethanol leaf extract of *Azadirachta indica* contains phytochemical constituents which may be responsible for its antiulcer activity and these amply justifies the traditional use of this plant in managing gastrointestinal disorders including peptic ulcer disease.

Keywords: *Azadirachta indica,* Aspirin, Omeprazole, Misoprostol, Ulcer

INTRODUCTION

Peptic ulcer disease is one of the most common gastrointestinal disorders characterized by mucosal damage secondary to pepsin and gastric acid secretion (Alkofahi and Atta, 1999). It usually occurs in the stomach and proximal duodenum; less commonly it occurs in the

lower esophagus, the distal duodenum or in the jejunum (Dharmani and Palit, 2006). It is one of the major gastrointestinal disorders that occur due to an imbalance between offensive and defensive factors along with weakness of the mucosal barrier (Alkofahi and Atta, 1999). The major offensive factors are acid, non-steroidal anti-inflammatory drugs (NSAIDs), pepsin, *Helicobacter pylori* (*H. pylori*), and bile salts, and defensive factors involve bicarbonate secretion and prostaglandins. Several field studies from different parts of our country suggest its occurrence is 4 to 10 per thousand populations (Dharmani and Palit, 2006). The estimated lifetime prevalence of PUD (gastric or duodenal ulceration) is 12% for men and 9% for women. Treatment options available are use of mucoprotective agents, antacids, alginates, motility stimulants and acid suppressants. Anti-reflux surgery is done in severe cases.

Numerous plants have been evaluated for the treatment of peptic ulcer (Devi *et al*., 2008). *Azadirachta indica* leaves (Annonaceae) is a tree commonly known as Neem. The leaf decoction is used for the treatment of peptic ulcers, leprosy, fever, asthma, epitaxis, intestinal worms, piles, diabetes, urinary tract infection, scabies, ringworm and spermatorrhoea (Biswas *et al*., 2002). The plant (mainly leaves) is rich of alkaloids, glycosides, terpenoids, steroids, flavonoids, tannins and reducing sugars. The alkaloid content favours antiulcer activity of *Azadirachta indica* (Bhat *et al.*, 2011). The *Azadirachta indica* leaves has been claimed to have antiulcer activity, but no detailed scientific investigations were carried out to define the antiulcer activities. Thus, the present investigation sets out to study the antiulcer activity of *Azadirachta indica* leaves extract.

MATERIALS AND METHODS

Collection and Preparation Extraction of Plant Material

Matured and fresh leaves of *Azadirachta indica* were collected in the month of January 2017 at the University of Maiduguri, Borno state, Nigeria. The plant was identified by Prof. S.S Sanusi, Department of Biological Sciences, University of Maiduguri, Borno state, Nigeria.

Freshly collected leaves were shade dried and size reduced using wooden pestle and mortar. The powdered plant materials were then extracted using cold maceration method for 48 hours with 70% ethanol and filtered. The filtrate was dried in flask evaporator under reduced pressure and controlled temperature (45- 50°C) over a water bath followed by air drying of aliquots on a large surface plate. The residue obtained was then powdered in a mortar and pestle, stored in an airtight container and then placed in a desiccator until used. The percentage yield was then calculated.

Preliminary Phytochemical Screening of Ethanol Leaf Extract of *Azadirachta indica*

The ethanol extract of *Azadirachta indica* was subjected to phytochemical screening for the detection of alkaloids, carbohydrates, tannins, saponins, anthraquinones, steroids and flavonoids as possible important constituents of the plant, according to standard method (Tona *et al*., 1998; Sofowora, 1993).

Animals and Housing Conditions

Adult albino rats of both sexes weighing between 140-180 g were used and kept at room temperature (25±2^{o}C) with 15 hrs dark and light cycle and fed with standard food (pelletized growers mesh) and water *ad libitum*. The experiments were performed by following the guideline of Institute of Laboratory Animal Resources, Commission on Life Sciences (National Research Council, 1996), approved by the Ethics Committee of University of Maiduguri, Borno state, Nigeria.

Acute Oral Toxicity Test of Ethanol Leaf Extract of *Azadirachta indica*

Acute oral toxicity test was carried out using modified Lorke's method (1983). The study was conducted in two phases; in phase one, three groups of one rat each was administered the doses of 10 mg/kg, 100 mg/kg and 1000 mg/kg body weight and observed for 24 hours for physical and behavioural changes as well as death. In phase two, three groups of two rats each were administered doses of 1600 mg/kg, 2900 mg/kg and 5000 mg/kg and was observed for the mortality within 24 hours.

Anti-Ulcerogenic Activity of Ethanol Leaf Extract of *Azadirachta indica*

Six groups of 5 rats each were used for the study. Group I served as negative control was pre-treated with distilled water (10 ml/kg) for three days. Group II and III were pre-treated with misoprostol 150 µg/kg and omeprazole 20 mg/kg respectively for three days. Groups IV, V and VI were also pre-treated with ethanol leaf extract of *Azadirachta indica* at 150, 300 and 600 mg/kg respectively for three days. Induction of peptic ulcer was carried out using acetylsalicylic acid-induced ulcer model described by Suleyman *et al* (2002). Assessment of gastric lesions was carried out according to Cho and Ogle (1979). Lesion scores were quantified by the scoring system 1 or 2 and >2 lesions with number of perforations according to Morris *et al* (1989).

Statistical Analysis

The results of the antiulcer activity of crude extract of *Azadirachta indica* were expressed as mean ± standard error of the mean (SEM). The data was analyzed statistically using SPSS version 20. The difference between means was determined by One Way ANOVA and independent student T-test with *p* values considered significant at < 0.05.

RESULTS

Preliminary Phytochemical Screening of Ethanol Leaf Extract of *Azadirachta indica*

Preliminary phytochemical screening of the ethanol leaf extract of *Azadirachta indica* revealed that carbohydrates, reducing sugar, saponins, anthraquinone, flavonoids, tannins and alkaloids were present, while cardiac glycosides and cyanogenic glycosides were not detected (Table 1).

Acute Toxicity Study of Ethanol Leaf Extract of *Azadirachta indica*

The results of acute toxicity study of ethanol leaf extract of *Azadirachta indica* showed no death after phase 1 in which three dose levels were used. There was also no death observed when three doses of the extract were used in phase II. Therefore, the

LD50 was found to be greater than 5000 mg/kg (Table 2).

Table 1: Preliminary phytochemical screening of *Azadirachta indica* ethanol leaf extract

Phytochemical constituents	Results
Carbohydrates	+
Reducing sugar	+
Cardiac glycosides	-
Salkwoski test for steroidal nucleus	+
Saponins glycosides	+
Anthraquinones glycosides	+
Cyanogenetic glycosides	–
Flavonoids	+
Tannins	+
Alkaloids	+

Note: (+) = present, (–) =absent

Table 2: Acute toxicity study of ethanol leaf extract of *Azadirachta indica* in albino rats

Phases	Ethanol extract (mg/kg)
Phase I	10mg/kg
	100mg/kg
	1000mg/kg
Phase II	1600mg/kg
	2900mg/kg
	5000mg/kg
LD_{50}	>5000mg/kg

LD_{50} = Lethal dose that can kill 50% of rats orally

Antiulcer screening of ethanol leaf extract of *Azadirachta indica*

In this study, ethanol leaf extract of *Azadirachta indica* at 150 mg/kg and 300 mg/kg showed protection against epithelium shedding caused by aspirin whereas, there was little or no activity of the extract at 600 mg/kg on the epithelium shedding. Misoprostol was found to protect the epithelial shedding significantly higher ($p<0.05$) than the highest tested dose of the plant extract (600mg/kg). The leaf extract at 300 mg/kg and 600 mg/kg were able to protect the gastrointestinal mucosa from haemorrhage that was found to be statistically insignificant ($p>0.05$) when compared with the activity of misoprostol and omeprazole. The antiulcer activity of the misoprostol and omeprazole was found to be significantly higher than the activity of the extract at 150 mg/kg ($p<0.05$). Misoprostol a cytoprotective agent was able to protect the rats from having 1 or 2 ulcers when compared with the extract at 150 and 600 mg/kg of the ethanol leaf extract ($p<0.05$). The standard drugs used (misoprostol and omeprazole) were able to completely protect the gastrointestinal tract of the rats from aspirin induced multiple ulcers, whereas the extract did not provide complete protection. However, the protection of multiple ulcers by the extract at all the tested doses was found to be statistically significantly higher than the negative control group ($p<0.05$). The extract did not protect the rats from acetylsalicylic acid induced perforation at the tested doses when compared with the controls (Table 3).

DISCUSSION

The presence of phytochemical compounds in the ethanol leaf extract of *Azadirachta indica* observed in this study agrees with Vibha and Divya (2014) in which glycosides, flavonoids, proteins, carbohydrate, phenolic compounds, tannins and saponins were found to be present. However, the presence of alkaloids in leaf

extract of *Azadirachta indica* in the present study which was not detected by Vibha and Divya (2014) could be as a result of differences in the geographical location of the plant samples used. The phytochemical constituents identified in the present study are well known for their curative activity against several human problems such as peptic ulcers, malaria, dysentery and diarrhoea (Ogbuewu, 2008). They may also responsible for most of the protective effect and other biological action such as hypoglycaemic, antioxidant, anti-inflammatory, anticancer agent, anticholinergic, anti-leprosy and antimicrobial activity (Kirtikar and Basu, 1987; Chathopadhyay *et al.,* 1993).

Table 3: Effect of ethanol leaf extract of *Azadirachta indica* in aspirin induced ulcer

Treatment	ES	FH	1 or 2 Ulcers	>2 Ulcers	PF
(I) *ASA (300mg/kg)*	10.00 ±0.00	14.00± 2.45	28.00± 2.00	36.00±4.00	32.00± 4.90
(II) *Misoprostol (150µg/kg) + ASA*	4.00 ± 0.61	3.00 ± 1.22	2.00 ±2.00	0.00 ±0.00	10.00 ± 6.12
(III) *Omeprazole (20mg/kg) + ASA*	6.00 ± 1.00	7.00 ± 1.22	7.00 ±3.00	0.00 ±0.00	15.00 ±6.12
(IV) *AIE (150mg/kg) + ASA*	6.00 ± 1.87	14.00 ± 1.87*º	13.00 ±2.00*	10.00±4.47©	25.00±0.00
(V) *AIE (300mg/kg) + ASA*	6.00 ± 1.00	7.00 ± 2.00	9.00 ±2.45	6.00±4.00©	25.00±0.00
(VI) *AIE (600mg/kg) + ASA*	9.00 ± 1.00*	8.00 ± 2.00	12.00±2.00*	4.00±4.00©	30.00±9.35

Ulcer index = mean ± standard error of the mean, n = 5, ASA = Acetylsalicylic acid, ES = Epithelium shedding, FH: Frank haemorrhage, PF = Perforation, * = $p<0.05$ (significant when extract is compared with misoprostol tablet), º =$p<0.05$ (significant when extract is compared to omeprazole), © = $p<0.05$ (significant when compared to acetylsalicylic acid only).

Neither morbidity nor mortality was recorded in rats treated with the extract during 24 h of observation. Doses up to 5000 mg/kg did not produce any symptom of acute toxicity which suggest that ethanol leaf extract of *Azadirachta indica* are hereby considered less or nontoxic acutely, since substances possessing LD_{50} greater than 5000 mg/kg are relatively non-toxic as previously reported by Abdel-Rahman *et al* (2015).

The ethanol leaf extract of *Azadirachta indica* at all doses used significantly decreased the aspirin induced ulcer index. Aspirin is a non-steroidal anti-inflammatory drug which induces ulcers by inhibiting prostaglandin synthesis in the stomach via blockade of cyclooxygenase enzymes (Wallace, 2012). Aspirin can also cause inflammatory response by increasing the reactive oxygen species in the gastric mucosa (Whittle, 2003) resulting in ulceration. Gastric damage induced by aspirin in the current study was characterized by epithelial shedding, Frank hemorrhage, ulcer lesions and perforations. The number of ulcers (36.00±4.00) and perforations (32.00± 4.90) in the control rats that received aspirin were significantly increased when compared with normal untreated animals. These results were in agreement with the results of Jainu *et al* (2006) in which similar observations were made. In the present work, omeprazole and misoprostol were selected as reference drugs for aspirin model because they provide much better protective effect on aspirin-induced gastric damage than other antiulcer drugs (Daneshmend *et al*., 1990; Graham *et al*., 1988).

In the present study, the extract was found to be an effective antiulcerogenic agent at

different doses which was observed to be dose independent. The perforation induced by aspirin was lower with lower doses (150 and 300 mg/kg) of the extract used which agrees with the report of Bhajoni and his colleagues (2016) in which aqueous extract of *Azadirachta indica* significantly reduced ulcer index when it was compared with misoprostol. Thus, indicating a possible involvement of the prostaglandin pathway. It is also consistent with Aziz (2011) that previously reported that ethanol extract of *Azadirachta indica* leaves has antiulcer activity.

CONCLUSION

Ethanol leaf extract of *Azadirachta indica* contains phytochemical constituent that may be responsible for its antiulcer activity against aspirin induced gastric ulceration in rats. The cytoprotective activity of this plant extract justifies the traditional use of this plant in gastrointestinal disorders. Further study is hereby encouraged that would determine the mechanism of action of the plant extract.

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