Available online at http://www.journalijdr.com

ISSN: 2230-9926

IJDR

International Journal of
DEVELOPMENT RESEARCH

International Journal of Development Research
Vol. 3, Issue, 10, pp.106-110, October, 2013

Full Length Research Article

EVALUATION OF THE ANTI-ULCER PROPERTIES OF AQUEOUS LEAF EXTRACT OF *DIALIUM GUINEENSE* (VELVET TAMARIND) ON EXPERIMENTALLY INDUCED ULCER MODELS IN RATS

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ARTICLE INFO

Article History:
Received 04th July, 2013
Received in revised form
30th August, 2013
Accepted 11th September, 2013
Published online 04th October, 2013

Key words:
Anti-ulcer property,
Dialium guineense leaf,
Aqueous extract,
Gastric mucus,
Rats.

ABSTRACT

The aqueous leaf extract of *Dialium guineense* was investigated for anti- ulcerogenic effects using ethanol/HCI and indomethacin as the ulcerogens. The effect of the extract on gastric mucus secretion was also investigated. The extract was administered orally at the doses of 100 and 200 mg/kg for the experimental groups while the control and reference groups received distilled water (5ml/kg, p.o) and cimetidine (32 mg/kg, p.o) respectively. The results show that the extract significantly ($p < 0.05$) reduced the ulcer index from 4.75 ± 0.17 to 0.20 ± 0.12 and from 3.95 ± 0.19 to 0.14 ± 0.09 in the ethanol/HCI and indomethacin induced ulceration respectively. The extract also significantly ($p < 0.05$) increased the gastric mucus secretion. Phytochemical studies revealed the presence of flavonoids, alkaloids, tannins and saponins. In conclusion this study has shown that the aqueous extract of *D. guineense* has anti-ulcer effects which might be due to its ability to increase gastric mucus secretion. The findings from this study also justify the folkloric uses of *D. guineense* for the treatment of gastric ulcer.

INTRODUCTION

Dialium guineense (Wild) belongs to the family of Fabaceae, commonly called black velvet or velvet tamarind. It is a tree of an average height of 30 m with densely leafy crown, smooth greyish bark. Leaves are hairy and the flowers are usually whitish while the fruits are less circular and flattened. The pulp of the fruit is edible and sweet, fairly low levels of ascorbic acid and tannins are present. It is a fairly good source of protein and minerals (Arogba *et al.*, 2006). *Dialium guineense* can also be found in West African countries such as Ghana where it is known as Yoyi, Sierra Leone, Senegal, and Nigeria. *Dialium guineense* is commonly known as "Awin" among the Yoruba in the Western part of Nigeria, as "Icheku" among the Igbo in the Eastern part of Nigeria, and as "Tsamiyar kurm" among the Hausas in the Northern part of Nigeria (Nwosu, 2000; Akinpelu *et al.*, 2011). The bark and leaves of *D. guineense* have medicinal properties and are used against several diseases. The fruits of the plant are chewed among some women in southeast Nigeria to improve lactation and check genital infection (Nwosu, 2000).

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D. guineense is used as chewing stick (indigenous tooth brush) among Nigerian populace (Akinpelu *et al.*, 2011). Okwu and Ekeke (2003) reported in their studies that the plant contains saponin which is presumed to add to the cleaning effect of teeth and at the same time prevent caries and plaques on the teeth of the users. *D. guineense* leaves and stem bark are used as folklore remedies for the treatment of infections such as diarrhoea, severe cough, bronchitis, wound, stomach aches, malaria fever, jaundice, antiulcer and haemorrhoids (Bero *et al.*, 2009). Some of the scientifically validated activities of the plant leaves and stem bark include its analgesic and antibacterial activities (Ezeja and Omeh, 2011; Orji *et al.*, 2012), antioxidant and antimicrobial activities (Gideon *et al.*, 2013). The current study was undertaken to evaluate the anti-ulcer effects of the aqueous extract of the leaves of this plant. This is based on the widespread practice of prescribing the aqueous extract of the plant for the treatment of gastric ulcer by traditional medical practitioners in the South Eastern part of Nigeria.

MATERIALS AND METHODS

Animals

Adult male albino rats (200 ± 15 g) were used for the study. The animals were bred and housed in the animal house of the

Faculty of Basic Medical Sciences, College of Health Sciences, Ebonyi State University, Abakaliki, Nigeria. Animals were kept in clean and standard cages with good ventilation and 12-h light/dark cycle. They were provided with standard rat's pellet (Pfizer Livestock Feeds PLC, Enugu, Nigeria) and water *ad libitum*. The rats were allowed to stabilize for 2 weeks before commencement of the experiment. The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care, as found in for example the European Community guidelines (EEC Directive of 1986; 86/609/ECC).

Drugs

Cimetidine was obtained from the Ebonyi State University Medical Centre (EBSUMC) Pharmacy and was used as the reference anti-ulcer drug. Cimetidine is H_2-receptor antagonist drug used for the treatment of peptic ulcers. Cimetidine blocks H_2-receptor channels in the wall of the stomach leading to reduction in acid production allowing the stomach to heal. In this study, the drug was administered orally to reference control group of rats in a dose of 32 mg/kg suspended in distilled water (5ml/kg) (Pedemera *et al*., 2006).

Plant material and Preparation of aqueous extract

The Fresh leaves of *D. guineense* were collected from Mgbabor-Achara in Ezza South Local Government Area of Ebonyi State, Nigeria. The leaves were identified and authenticated by Mr. P. O. Ugwuozo in the herbarium of Botany Department of University of Nigeria, Nsukka, with deposition of authenticated voucher specimen for reference. The harvested leaves were air dried and milled to fine powder. 1000 g of the powdered leaves was evaporated by cold maceration using 4 L of distilled water. The mixture was evaporated in a carefully regulated water bath (maintained at 65℃) to yield 50 g of a dark solid extract. The extract was stored in a refrigerator at 4℃ through the period of the study to preserve the prepared extract. The resulting residue was reconstituted in sterile distilled water to give the required doses of 100 and 200 mg/kg/ 5ml body weight, respectively (Gideon and Raphael, 2012).

Anti-ulcer Studies

Two models (ethanol-acid and indomethacin) for inducing acute experimental lesions in laboratory animals were used to evaluate the anti-ulcer activity of the extract. Twenty four rats were used for each model and they were divided randomly into four groups of six rats each and coded to prevent observer bias. Groups 1 and 2 had 6 animals each and served as negative and positive control as they received (5ml/kg) distilled water and cimetidine (32 mg/kg) respectively. Groups 3 and 4 had 6 animals each and received the extract at graded doses of 100 and 200 mg/kg body weight respectively. All the drugs were administered orally to all the rats.

Ethanol /HCl-induced ulcer

The method used by Mizui and Douteuchi, (1983) was also used in this study. Ulceration was induced in 24 h fasted rats by the oral administration of 1mL of ethanol/hydrochloric acid (0.3m HCl in 60% ethanol) 30 minutes after the extract of *D. guineense* (100 and 200mg/kg), cimetidine (32mg/kg) and distilled water (5ml/kg) were administered. The animals were sacrificed 1 h after the ethanol- acid administration. The abdominal cavities and subsequently the stomachs of the animals were dissected out. Gastric lesions were observed using a hand held lens (x 10) and an ulcer score was calculated for each animal according to the arbitrary scale used by Singh *et al*. (1997), where 0 = no lesion, 1 = hyperemia, 2 = one or two slight lesions, 3 = very severe lesions, 4 = mucosal full of lesions. Ulcer index was calculated as mean ulcer scores (Tan *et al*., 1996).

Indomethacin-induced ulcers

Indomethacin-induced ulcer was carried according to the method described by Parmar and Desai, (1993). Indomethacin (10mg/kg/5ml) was administered (orally) 30 min after the extract of *D. guineense* (100 and 200 mg/kg), cimetidine (32 mg/kg) and distilled water (5ml/kg) administration. Administration of indomethacin was repeated after 15 h. All the rats were sacrificed 1 h after the last dose of indomethacin and the stomachs were dissected in order to evaluate the level of mucosal damage. The ulcer index was determined as described earlier in ethanol-acid induced ulcers.

Determination of gastric mucus secretion

The adherent gastric mucus was determined by the method described by Ettarh and Okwari, (1999). The stomach was removed and washed in normal saline and then opened along the greater curvature. It was again rinsed in saline and pinned to a cork board with dissecting pins. Mucus was extracted using a spatula from the spread stomach into a known weight of beaker containing 4ml of water. The weight of mucus was derived from the difference in the initial and final weights of beaker + 4ml of water as follows:

Wt of beaker + 4ml of water = x
Wt of beaker + 4ml of water + mucus = y
Weight of Mucus = (y-x) gm
The procedure has also been described by Tan *et al*. (2002).

Preliminary phytochemical screening

The aqueous leaves extract was tested for presence or absence of alkaloids, flavonoids, glycosides, saponins, tannins, and fats and oils using standard phytochemical procedures and tests (Harbone, 1984).

Statistical analysis

Data are presented as mean ± standard error of the mean (SEM). Statistical significance was determined using the Student's t-test. Values with $p<0.05$ compared with the control group were considered significant.

RESULTS

Anti-ulcer activity

The results of the anti ulcer studies are shown in Table 1 and 2. Table 1 shows that the extract of *D. guineense* significantly ($p<0.05$) reduced the ulcer index from 4.75 ± 0.17 (control) to 0.20 ± 0.12 (200 mg/kg) in the ethanol/HCl induced ulceration group. Likewise in Table 2, the results show that the extract significantly ($p<0.05$) reduced the ulcer index from 3.95 ± 0.19 (control) to 0.14 ± 0.09 (200mg/kg) in the indomethacin-induced ulceration group. Pre-treatment with cimetidine and

the extract significantly ($p<0.05$) reduced the severity of ethanol/HCl and indomethacin-induced ulcers. The protective effects of the extract as shown in Table 1and 2 were dose-dependent.

Gastric mucus studies

The extract produced a significant ($p<0.05$) and dose dependent increase in gastric mucus production in the ethanol-acid and indomethacin induced gastric ulcers in rats compared to control (Table 1 and 2). The effect of the aqueous extract on gastric mucus secretion was more pronounced in indomethacin-induced gastric ulcers in rats (Table 2).

causes severe gastric mucosal ulceration either by acting directly on the gastric mucosal or indirectly by increasing the release of vasoactive products such as histamine from mast cells (Szabo, 1987; Oates and Hakkinen, 1987; Goulart *et al*., 2005). Histamine is a potent stimulator of acid secretion via the H_2 receptor and this forms the basis of using antacids and H_2 receptor antagonists (Mitra *et al*., 1996). The results obtained from ethanol-acid induced ulcer model show that the extract at the doses of 100 and 200mg/kg significantly ($p<0.05$) reduced the necrotizing effects of the ulcerogen and thus exhibits cytoprotective effects. The highest dose (200 mg/kg) produced better cytoprotection compared with the standard reference drug (cimetidine). The indomethacin-

Table 1. Effects *D. guineense* aqueous leaf extract on ethanol/HCl- induced ulcers in rats

Group	Pre-treatment	Dosage (p.o)	Mean Ulcer Index ± SEM	Percentage Protection	Mucus content (g)
1	Distilled water	5ml/kg	4.75 ± 0.17	0.00	0.31 ± 0.01
2	Cimetidine	32mg/kg	0.25 ± 0.10*	94.74	0.52 ± 0.03*
3	Extract	100mg/kg	1.30 ± 0.13*	72.63	0.50 ± 0.01*
4	Extract	200mg/kg	0.20 ± 0.12*	95.79	0.67± 0.07*

* Significant. All values are expressed as mean ± SEM, n=6 in each group.*P<0.05 as compared with the negative control animal.

Percentage inhibition to ulcer formation in rats by the extract was calculated as follows:
% Inhibition of Ulceration= [(Ulcer index $_{Control}$ - Ulcer index $_{Test}$) / Ulcer index $_{Control}$] x 100%

Table 2. Effects *D. guineense* aqueous leaves extract on indomethacin- induced ulcers in rats

Group	Pre-treatment	Dosage (p.o)	Mean Ulcer Index ± SEM	Percentage Protection	Mucus content (g)
1	Distilled water	5ml/kg	3.95 ± 0.19	0.00	0.25 ± 0.10
2	Cimetidine	32mg/kg	0.34 ± 0.12*	91.39	0.62 ± 0.04*
3	Extract	100mg/kg	0.25 ± 0.11*	93.67	0.60 ± 0.01*
4	Extract	200mg/kg	0.14 ± 0.09*	96.46	0.72 ± 0.03*

* Significant. All values are expressed as mean ± SEM, n=6 in each group.*P<0.05 as compared with the negative control animal.

Percentage inhibition to ulcer formation in rats by the extract was calculated as follows:
% Inhibition of Ulceration= [(Ulcer index $_{Control}$ - Ulcer index $_{Test}$) / Ulcer index $_{Control}$] x 100%

Phytochemical Screening

The results of the phytochemical analysis indicate that the extract contains alkaloids, flavonoids, saponins and tannins.

DISCUSSION AND CONCLUSION

In the present study, the cytoprotective effects of the aqueous extract of *D.guineense* was investigated using two important models (ethanol-acid and indomethacin induced ulcerogens). The finding of the present study demonstrated that aqueous extract of *D. guineense* significantly protected against mucosal damage induced by ethanol-acid and indomethacin. The curative ratios of plant extracts 100 and 200mg/kg were 72.63% and 95.79% respectively in ethanol-acid induced gastric ulceration, while in the indomethacin-induced ulceration the protective ratios of the extracts 100 and 200mg/kg were 93.67% and 96.46% respectively. It is remarkable that the leaf extract at 100mg/kg and 200mg/kg doses produced a greater protection than cimetidine (32mg/kg) against the indomethacin. The effect of the extract compared favorable to cimetidine 32mg/kg (positive control). However, the mechanism by which the aqueous leaf extract on *Dialium guineense* produced its gastroprotective effects in rats is not clear. It has been established that ethanol-acid ulcerogen induced ulcer model represents a form of gastric irritation resulting from the inhibition of prostaglandins synthesis. Indomethacin is an example of non-steroidal anti-inflammatory drugs which produces their effects by inhibiting prostaglandins synthesis (Vane, 1971; Steinmeyer, 2000). Acute toxic doses indomethacin (*e.g.* 20mg/kg) or lower chronic doses can produce gastric irritation (Steinmeyer, 2000; Goulart *et al*., 2005). Increase in prostaglandins especially PGE_2 and PGI_2 has been associated with cytoprotection (Neal, 1991; Deshpande *et al*., 2003). Therefore, agents that inhibit the effects of non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin will exhibit cytoprotection. The results obtained from using the indomethacin ulcer model showed that the extract can significantly inhibit the gastric effect of indomethacin (10mg/kg) and therefore further exhibiting cytoprotection. Generally, the activities of the extract may not be unconnected with the secretory effects of the extract. Since, gastric mucus production was significantly increased by the extract. Usually some substances like the NSAIDS produce gastric mucosal irritation in addition to various degrees of analgesic, anti-inflammatory and antipyretic effects (Neal, 1991; Steinmeyer, 2000). Leaves extract of *D. guineense* has been shown to contain phytochemical constituents like flavonoids which are capable of promoting gastric mucosal

formation, reduce gastric acid secretion and inhibit pepsinogen production thereby reduced gastric lesions and ulcers. Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed (Di Carlo *et al*., 1999; Borrelli *et al.*, 2001; Galati *et al*., 2001). It is suggested that, these active compounds would be able to stimulate mucus, bicarbonate and the Prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen (Salvayre *et al.*, 1982; Asuzu and Onu, 1990; Suja *et al*., 2002). Other components like alkaloids, saponins, and tannins are also present in the leaf extract of *D. guineense* which tend to exhibit some anti inflammatory and anti-oxidant properties (Ghosal *et al*., 1996; Nwaogu *et al*., 2007). It is obvious that the decrease in gastric ulceration, with concomitant in the gastric mucus secretion produced by the leaf extract of *D. guineense* in this study could be due to the presence of these components or some other mechanisms yet unidentified. This indicates that *D. guineense* can be subjected to further studies in order to isolate the active ingredients in the leaves as well as determine the mechanism of action of such active ingredients. These may ultimately yield better anti-ulcer agents than we have in the market today. In conclusion, the present study have established the anti-ulcer effects of aqueous extract of *D. guineense* leaves and further justifies the folkloric uses of the decoction of the leaves for the treatment of gastric ulcer.

Acknowledgements

The authors are thankful to the management of Ebonyi State University College of Health Sciences, Abakaliki, Nigeria for providing the required facilities to carry out the research work.

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