

Original Research Article

Anti-Ulcer Effect of Methanol Extract of the Root Bark of *Entandrophragma Angolense* (Meliaceae) in Rodents

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Abstract

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The anti ulcer activity of methanol extract (ME) of the root bark of *Entandrophragma angolense*, as acclaimed by herbalists was studied. The ME obtained by soxhlet extraction was subjected to anti-ulcer screening at different dose levels (200, 400, 800 mg/kg b.w.) using indomethacin and ethanol induced ulcer models. Cimetidine 100 mg/kg b.w. and 5 ml/kg b.w. solvent (Distilled water) served as positive and negative controls respectively. Acute toxicity and phytochemical screening were also carried out. The results revealed that ME exhibited a non significant ($p > 0.05$) dose-dependent anti-ulcer effect against both ulcer-induction models, compared to the negative control. This effect was not comparable to that shown by the positive control (cimetidine). The qualitative phytochemical analysis showed the presence of saponin, protein, tannin, glycosides, reducing sugar, resins, steroids and terpenoids, while the acute toxicity test showed no obvious sign of toxicity up to $>5,000$ mg/kg b.w. These findings demonstrated the potentials for methanol extract of *Entandrophragma angolense* as an anti-ulcer agent, hence substantiating its ethnomedical use in ulcer treatment by herbalist.

Keyword: Anti-ulcer, *Entandrophragma angolense*, Ethanol, Indomethacin, Rodent

INTRODUCTION

Peptic ulcer is one of the commonest diseases affecting man with prevalence rate of approximately 3-5 % and has a life time incidence rate of 15-20 % (Ojewole, 2004). Peptic ulcer is recurrent and approximately 50 % of ulcer patient will have a recurrence within one year of diagnosis (Ojewole, 2004). Increased gastric acid secretion, pepsin secretion, inhibition of prostaglandin synthesis, cell proliferation, diminished gastric blood flow and gastric motility have been implicated in the pathogenesis of gastric ulcer (Toma et al., 2005). The normal stomach maintains a balance between protective factors and aggressive factors (Rifat-uz et al., 2005). Gastric ulcers therefore develops when aggressive forces (increased hydrochloric acid and pepsin, parietal cell mass and gastrin production) overcome the protective factors (prostaglandins and increased mucous cells) (Wallace 2001 ; Zhu et al., 2008). Treatment is usually geared towards reduction of gastric acid production and

enhancing gastric mucosa production (Valle, 2005).

However, with the discovery of plant as medicines, many drugs have been obtained from natural sources (Williamson et al., 1996). The plant kingdom harbors an inexhaustible source of active substances invaluable in the management of many intractable diseases including peptic ulcer. Several plants and herbs are used in folkloric medicine to treat gastrointestinal disorders, including peptic ulcers (Akah et al., 1998a, 1998b). There is therefore a growing interest in identifying new anti-ulcer agents from plant sources (Njar et al., 1995; Raji et al., 2000, 2004).

Entandrophragma angolense (Meliaceae) is a tree distributed in West, Central and East Africa called mahogany tree (Huchinson and Dalziel, 1958). It grows in rain forest, deciduous forest and transitional formations. The root bark is boiled for more than 1 h and allowed to cool and filtered and the aqueous extract is used by herbalist

in treatment of heart burn and stomach pain and malaria (Njar et al, 1995). Njar et al (1995) revealed that the stem bark has a dose dependent anti-ulcer activity while Oluwole et al., (2007) reported that the stem bark significantly increase gastric mucus secretion thereby protecting against ulcer. Previous chemical investigations of this plant species yielded the following constituents: gedunin, methyl angolensate, B-sitosterol and entadrolide (Akinsanya et al., 1960; Okorie and Taylor 1997). Other studies reported the isolation of some tricyclic type triterpenoids, gallic acid, methylgallate and pentagalloyl glucose from the leaves of the plant (Orisadipe et al., 2005; Orishadipe et al., 2008). Further investigations on the isolates from the plant showed that methyl angolensate has activity against ulcer (Njar et al., 1995; Orishadipe et al., 2008), leukemia cell lines (Chiruvella et al., 2008; Chiruvella and Raghavan, 2011; Kishore et al 2010) while gedunin has activity against *Plasmodium falciparum* (Bray et al., 1990), and antineoplastic activity. Recently, the seed oil was shown to have activity against *Salmonella gallinallum* and *Klebsiella pneumonia* (Orishadipe et al., 2012).

This study therefore, was designed to investigate the constituents and anti-ulcer activity of the methanolic extract of the root bark of *Entandrophragma angolense*.

MATERIALS AND METHODS

Materials

Animals

Mice (19 - 31 g) and male albino rats of (110 - 220 g) were used. All the animals were obtained from animal house, Faculty of Veterinary Medicine, University of Nigeria, Nsukka. They were housed in clean wire mesh cage and fed well except during fasting periods for 3 weeks to acclimatize to laboratory conditions. All animals used were handled in accordance with the recommendations from the Declaration of Helsinki as recorded by the institute for Laboratory Animal Research, Washington DC (1996).

Plant material and extract preparation

Fresh root barks of *Entandrophragma angolense* were collected in April from Orba Enugu state and identified by Mr Ozioko a herbarium staff of Botany Department University of Nigeria Nsukka. About 1 kg of the plant was sun dried, pulverized and subjected to Soxhlet extracted using methanol. The methanol extract was collected in the round bottom flask with the solvent evaporated and condensed. The methanol extract (500 g) obtained was stored in the refrigerator at 4°C.

Methods

Phytochemical analysis

The presence of carbohydrate, glycosides, saponin, tannin, protein, flavonoids alkaloids, resins, steroids, terpenoids, fats and oil were determined as described by Orisadipe et al., (2005)

Acute toxicity test

The Miller and Tainter (1994) procedure of LD₅₀ determination was used. Briefly, 9 mice were divided into 3 groups (n=3), received oral administration of 200, 500 and 1000 mg/kg b.w. The animals were fasted for 16 h prior to administration of extracts and monitored for 24 h for death. Since no death was recorded, further doses were administered. Twenty four mice were divided into 4 different groups (n=6). The animals were fasted for 16 h before dose ranges of 1500, 2500, 5000 and 8,000 mg/kg b.w. of the extract were administered orally. The animals were constantly observed for the first 2 h, then intermittently for the next 4 h and over night, number of death was observed and recorded.

Induction of ulcer

Indomethacin-induced ulcer model

This experiment was carried out as described by Adesanwo et al., (2007). Twenty male rats were randomly assigned into 5 experimental groups (n = 4). Food and water were withdrawn for 16 h before the commencement of experiment. The drugs were administered orally to the various groups. Group 1 (negative control) received 5 ml distilled water; group 2 (positive control) received 100 mg/kg b.w. of cimetidine; group 3, 4, and 5 received methanol extract (ME) at 200, 400 and 800 mg/kg b. w. Two hours later 40 mg/kg b.w. indomethacin suspension was administered to the entire animals. The animals were sacrificed 4 h later by cervical dislocation. The stomach were removed and each opened along the greater curvature, rinsed under a stream of water and examined for ulcer. Ulceration in the stomach was accessed by means of a scoring technique using the method and criteria of Elegbe and Bamgbose (1976). Normal gastric mucosa was scored as 0, punctuate hemorrhage or pinpoint ulcer was scored as 0.5, one or two small hemorrhage ulcer was scored as 1.0 while ulcer greater than 3 mm was scored 2.0.

Ethanol-induced ulcer model

The ethanol-induced model was adopted from Morimoto

Table 1. Phytochemical analysis of methanolic extract of root bark of *Entandrophragma angolense* compared to the stem bark

Constituents	Relative Presence
Saponin	+++
Protein	+++
Acidity	-
Flavonoids	-
Alkaloids	-
Tannins	+++
Glycosides	+++
Reducing sugar	++
Resins	+++
Steroids	++
Terpenoids	++
Fats and oil	-

- = Absent; + = Present in little concentration; ++ = Present in moderately high concentration; +++ = Present in very high concentration; ++++ = Abundantly present

Table 2. Acute toxicity study of root bark of *Entandrophragma angolense* extract on mice

Dose (mg/kg b.w.)	No of animal	No dead	No alive	Mortality (%)
8000	6	0	6	0
5000	6	0	6	0
2500	6	0	6	0
1500	6	0	6	0

No – number

et al (1991). Twenty male rats were randomly assigned into 5 groups (n = 4); Group 1 (negative control) received 5 ml distilled water; group 2 (positive control) received 100 mg/kg b.w. of cimetidine; group 3, 4, and 5 received methanol extract (ME) at 200, 400 and 800 mg/kg b. w. One hour after treatment, 1 ml of 80 % ethanol (80 ml of absolute ethanol in 20 ml of water) was administered to each animal orally. After 4 h the animals were killed, and the stomach was removed and each opened along the greater curvature and washed. The ulcers were viewed and scored using the method and criteria of Elegbe and Bamgbose (1976).

Statistical analysis

The results obtained were expressed as mean \pm SEM. The data were analyzed using SPSS and one way analysis of variance (ANOVA) followed by Dunnett Post Hoc test were done. Differences between mean were accepted to be significant at $P < 0.05$.

RESULTS

Phytochemical Screening

Phytochemical analysis of the methanol extract of the root bark of *Entandrophragma angolense* tested positive

for saponin, protein, tannin, glycosides, reducing sugar, resins, steroids and terpenoids and negative for alkaloid, flavonoids, fats and oil. (Table 1)

Acute toxicity (LD₅₀) test

Oral administration of methanol extract of the root bark of *Entandrophragma angolense* up to 8000 mg/kg b.w. did not cause any death in mice. There were no signs of obvious behavioral and physical adverse effects (Table 2).

Effect of the extract on indomethacin-induced ulcer

The methanol extract produced a dose dependent and non-significant ($P > 0.05$) anti-ulcer effect compared to negative control. The percentage ulcer inhibitions of ME at 200, 400 and 800 mg/kg b.w. were 23.3, 36.5 and 69.0 % respectively, while that of cimetidine (100 mg/kg b.w.) and water (negative control) were 92.7 and 0 % respectively (Table 3).

Effect of the extract on ethanol-induced ulcer

The results showed that methanol extract exhibited dose dependent and non-significant ($P > 0.05$) anti-ulcer effect

Table 3. Effect of methanolic extract of root bark of *Entandrophragma angolense* on indomethacin induced ulcer in rats

Treatment	Dose (mg/kg b.w.)	Protection (%)	No of rats having ulcer (%)	Ulcer index \pm SEM
Distilled water	5 ml/kg	0	4 (100)	1.37 \pm .080
ME	200	23.3	3 (75)	1.05 \pm .530
ME	400	36.5	3 (75)	0.47 \pm .125
ME	800	69.0	2 (50)	0.29 \pm .129
Cimetidine	100	78.2	2 (50)	0.15 \pm .040

n = 4; $P < 0.05$ compared to control, ME = methanol extract

Table 4. Effect of methanolic extract of root bark of *Entandrophragma angolense* on ethanol induced ulcer

Drug	Dose (mg/kg b.w.)	Protection (%)	No of rats having ulcer (%)	Ulcer index \pm SEM
Distilled water	5 ml/kg	0	4 (100)	0.69 \pm .040
ME	200	18.8	3 (75)	0.56 \pm .350
ME	400	31.6	3 (75)	0.38 \pm .129
ME	800	66.6	2 (50)	0.26 \pm .125
Cimetidine	100	92.7	1 (25)	0.10 \pm .220

n = 4; $P < 0.05$ compared to control

compared to negative control. The percentage ulcer inhibition due to extract at 200, 400 and 800 mg/kg b.w. were 18.8, 31.6 and 66.6 % respectively, while that of cimetidine (100 mg/kg b.w.) and water (negative control) were 78.2 and 0 % (Table 4).

DISCUSSION

The antiulcer activity of the plant *Entandrophragma angolense* was evaluated using indomethacin and ethanol models which represent some of the common cause of gastric ulcer in human. Indomethacin is a known Non Steroidal Anti Inflammatory Drug (NSAID) used in the treatment of fever, pain, stiffness and swelling is a known inhibitor of prostaglandin synthesis (Koji, 2012). Indomethacin became the first choice drug to produce an experimental ulcer model as a result of having a higher ulcerogenic potential than other NSAIDs (Suleyman et al., 2010). It has been suggested that indomethacin induced gastric damage via inhibiting the release of protective factors like cyclooxygenase-1 (Cox-1) prostaglandin E₂ (PGE₂) (Koji, 2012). Prostaglandins on the other hand have significant roles in cyto-protection by exerting a positive influence on mucus and bicarbonate secretion on surface epithelial cells, mucosal circulation, prevention of hemorrhagic lesion and by aggregating platelets when required thus having protective effect on the gastric mucosa (Vane and Botting, 2003; Dey et al., 2006; Wallace, 2008). Free carboxyl group present in all NSAIDs form a strong electrostatic bond with positively charged head group of zwitterionic phospholipids of mucus layer. The increase in the solubility of the phospholipids thereby neutralizes its surface activity.

Thus NSAIDs topically act on tissue to disrupt the hydrophobic protective lining of the mucus gel layer (Jainu et al., 2006).

The anti-ulcer screening against indomethacin produced massive ulceration in the experimental animals. The methanol extract exhibited a dose dependent gastro-protective effect against indomethacin induced ulcer. The percentage protection by 800 mg/kg b.w. was greater than that shown by 400 and 200 mg/kg b.w. but less than that exhibited by 100 mg/kg b.w of cimetidine. This dose dependent effect of ME is similar to the effect observed with the stem bark recorded by Oluwole et al., (2007). Ulcer index is an established indication of ulceration in experimental animals (Ezike et al., 2009). It has been observed that plant with lower ulcer index has higher anti-ulcer activity (Eluka et al., 2015). The ME exhibited lesser ulcer index compared to the negative control. There was a dose dependent reduction in the mean ulcer index of animal treated with ME, which was not statistically significant ($p > 0.05$) when compared to the negative control. Cimetidine 100 mg/kg b.w. (positive control) showed a significant ($p < 0.05$) reduction in ulcer index and percentage protection.

Ethanol is well known as a damaging agent to gastric mucosa in animals. At concentrations greater than 400 mL/L, it causes marked mucosal hyperemia, necrosis, edema and mucosal or sub mucosal hemorrhage (AurelieAchie et al., 2014). The mechanism of ethanol induced gastric lesions may be attributed to these possible mechanism: (1) increase oxygen-derived free radicals (Terano et al., 1989; Mutoh et al., 1990), (2) decrease the concentration of non proteinsulphydiyls (NP-SH) contents in gastric mucosa (Gardes-Albert et al., 1993), (3) direct damage to the mucin layer or mucin synthesis (Slomiany et al., 1997), and (4) causing

gastric cell's apoptosis (Hoshino et al., 2002).

In the ethanol induced model, the entire animal in the negative control (distilled water) group developed ulcer with the highest mean ulcer index compared to treatment and positive control group. There was decrease in ulcer index and increase in percentage protection with increase in dose in the treatment group but cimetidine showed the least ulcer index and the highest percentage protection. The effect of the extract here was similar to that observed in the indomethacin model with ME showing a dose dependent anti-ulcer effect which was not statistically different ($p > 0.05$) compared to the negative control. This dose dependent anti-ulcer effect of the ME is similar to that observed with stem bark of *Entandrophragma angolense* by Njar et al. (1995) which revealed that it has a dose dependent anti-ulcer effect.

Even though the reduction in ulcer index and percentage protection presented by ME against both models were not statistically different compared to the negative control, ME exhibited a high degree of protection on the gastric mucosal lining against the ulcerative effect of indomethacin and ethanol. This is evident by the fact that the mucous layers of all the animals in the negative control group were massively eroded by the presence of indomethacin and ethanol. This is similar to the reports of Zhu and Kaunitz, (2008) and Lanza et al., (2009) in which prostaglandin and mucus protective function were inhibited by NSAIDs exposing animal to massive ulcerations. The effect of ME could also be seen in the (200 and 400 mg/kg b.w) pretreated group, where the indomethacin and ethanol administration resulted in some hemorrhage but not as erosive as in the negative group. In the group treated with 800 mg/kg b.w. ME only 2 animal had ulcer which were moderate, while in the positive control group (cimetidine), mild infiltration of the mucosa by inflammatory cells were observed in 2 animals.

Based on the facts that animal treated with methanol extract of *Entandrophragma angolense* inhibited in a dose dependent manner the formation of gastric ulcer in the stomach and protected the integrity of the stomach lining; that reduction of ulcer index by ME indicates its potent cyto-protective effect and that gastric ulcer has been known to result from an imbalance between aggressive factors and the maintenance of the mucosal integrity through endogenous defense mechanism (Wallace 2001; Zhu et al., 2008), it is suggested that its mechanism of action could be attributed to cyto-protective effect and possession of some degree of gastric acid anti secretory properties as its mechanism of action. However, further studies are required to confirm this claim.

Acute toxicity of medicinal plants is essentially studied to determine the lethality of the plant. Generally, human toxicity is estimated based on the test results on rats and other animal models (Raj et al., 2013). The lethal dose (LD_{50}) is the statistically derived single dose of a substance that produces death in 50 % of a population of

test animals to which it is administered by any of the methods like oral, dermal, inhalation and intravenous. Our study recorded 0 % mortality even at a dose of 8,000 mg/kg b.w. suggesting that the root bark of *Entandrophragma angolense* has sufficient margin of safety.

Phytochemical screening identifies different constituents and their degree of abundance in a plant. A comparison of our result on phytochemical screening of the root bark and that of the stem bark by other studies (Yenon et al., 2014) has demonstrated that the root bark has similar constituents with the stem bark but with more abundance of saponin, tannin and terpenoid in the stem than in the root bark. Our study also revealed an absence of alkaloid in the root bark which is present, though in small concentration in the stem bark. Since plants store different constituents at different part, it explains the slight difference observed between the stem and root bark. Studies have shown that alkaloids have anti ulcer activity (Falcão et al., 2008, Toma et al., 2004); hence its absence in the root bark may have contributed to the low anti-ulcer activity observed in this study. However, the results indicate that the extract possessed some biologically active compounds which could serve as potential sources of drugs.

CONCLUSION

The findings of this study has shown that methanolic extract of *Entandrophragma angolense* possesses dose-dependent anti-ulcer activity which could be attributed to its ability to protect gastric mucosal lining, substantiating its ethnomedical use in ulcer treatment by herbalist.

Conflicts of Interest

The authors declare no conflict of interest as the study was funded by the authors

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