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## Methanolic Extract of *Entandrophragma angolense* Induces Gastric Mucus Cell Counts and Gastric Mucus Secretion

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**Abstract:** In this study, the effect of the methanolic extract of the stem bark of *Entandrophragma angolense* on gastric mucus secretion, gastric mucus cell count, malonaldehyde concentration were investigated in albino rats. Two doses of the extract (100 and 200 mg kg<sup>-1</sup>) significantly increased gastric mucus secretion in the pre-treated animals (p<0.05). Similarly, the reduction in gastric mucus secretion was found to be statistically significant at a dose of 400 mg kg<sup>-1</sup> (p<0.05). *Entandrophragma angolense* extract also increased gastric mucus cell count significantly in animals pre-treated with low dose (p<0.05). These results indicate that the mechanism of antiulcer activity of the extract may be due to increase gastric mucus secretion which is mediated via increased gastric mucus cell counts and activity of the gastric mucus cells.

**Key words:** *Entandrophragma angolense*, gastric mucus secretion, mucus cell count, malonaldehyde concentration, oxygen radicals, antioxidants

### INTRODUCTION

*Entandrophragma angolense* extracts are locally used for the treatment of ailments such as stomach pain. It had been reported to manifest antiulcer activity by decreasing gastric acid secretion.

Various pathogenetic factors have been suggested to explain the development of peptic ulceration, prominent among these is an imbalance between the protective mechanisms and aggressive factors (Soll, 1980).

Factors equally implicated in gastric ulcers are oxygen derived radicals, pepsinogen and gastric mucosal blood flow (Garg *et al.*, 1993; Desai *et al.*, 1997).

Depression of gastric blood flow reduces bicarbonate secretion and mucus production thus allowing back diffusion of hydrogen ions (Bjorne *et al.*, 2004). The role of antioxidant against gastric mucosal damage is emphasized in their ability to both prevent initiation of lipid peroxidation and by scavenging free radicals (Halliwell and Gutteridge, 1990). All known risk factors for erosive and ulcerative gastric disorders deplete the natural antioxidant defence of our body and cause a free radical overload. Some well established endogenous antioxidants are superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase (Kelly, 1998).

There is extensive and mounting evidence that free radicals are significantly involved in the pathogenesis of tissue damage (Freeman and Crapo, 1982). This study

intends to examine the influence of the methanolic extract of the stem bark of *Entandrophragma angolense* on gastric mucus secretion, gastric mucus cell count and lipid peroxidation (a biochemical marker for cell tissue damage).

### MATERIALS AND METHODS

**Extract preparation:** Fresh stem barks of *Entandrophragma angolense* were collected through Mr. T.K. Odewo, an Herbarium Staff of Federal Research Institute of Nigeria (FRIN) in October, 2006. The barks were dried under shade for 6 weeks during the harmattan season between November and December, 2006. Voucher Specimens of *Entandrophragma angolense* were deposited at FRIN herbarium. The dried barks were ground to powdery form and 1.43 kg of the powdery sample was soaked in 15 L of methanol for 72 h. The solvent was removed under reduced pressure in a rotatory evaporator at 52°C. The solid sample of the extract was stored in the refrigerator at 4°C until when needed. The extractive was prepared with different dilution of the extract in distilled water.

**Animals:** Male Wistar Albino rats weighing between 180-200 g raised on commercial stock diet obtained from Ladokun feeds, Ibadan were used in all the studies. The animals were procured from the Preclinical Animal House, Department of Physiology, College of Medicine, University of Ibadan.

**Experimental design:** Three studies were carried out namely; the effects of *Entandrophragma angolense* extract on gastric mucus secretion, gastric mucus cell count and lipid peroxidation.

In each study a total number of sixteen rats were used. They were divided into four groups with four rats in each group.

**Group 1(Control):** These were normal rats fed and given water for 30 days but no extract. Groups 2, 3 and 4 were treated with the extract at doses of 100, 200 and 400 mg kg<sup>-1</sup> b.wt., respectively for 30 days.

After 30 days, the rats were fasted for 24 h but allowed free access to water and sacrificed by cervical dislocation. Blood samples were collected from the jugular vein into tubes containing heparin, centrifuged at 3000 rpm for 15 min and the plasma was used for measuring lipid peroxidation using the method of Gutteridge and Wilkins (1982). The stomachs were removed and washed gently in normal saline, blotted and weighed in preparation for gastric mucus study.

**Gastric mucus secretory study:** This was done using the procedure described by Mojzis *et al.* (2000).

**Gastric mucus cell count:** The number of gastric mucus cells that stained for Haematoxylin and Eosin as indicated in red patches were counted. The counting was done using calibrated microscope in five randomly selected areas of the gastric mucosal tissue on each slide. Each selected area assessed five square boxes each with an area of 4 mm<sup>2</sup>. This was an improvement over the foremost blind manner approach for counting (Li *et al.*, 2002). The microscope was calibrated by inserting into its eyepiece a transparent nylon with drawn squares (2 by 2 mm each) on its surface. Squares were faintly drawn so that the gastric mucus cells can be seen properly. The nylon was cut out in such a way that it directly fixed into the round hole of the eyepiece of the microscope.

**Assessment of lipid peroxidation:** Lipid peroxidation was assessed by the method described by Gutteridge and Wilkins (1982). This method is based on the reaction between 2-thiobarbituric acid (TBA) and malonaldehyde (MDA) which is an end product of lipid peroxides during lipid peroxidation.

**Statistical analysis:** Results were expressed as Mean±SEM. Statistical analysis was performed using students t-test and significant difference was accepted at p<0.05.

## RESULTS AND DISCUSSION

The mean gastric mucus secretion in control animals was 5.93±0.49 as against 18.51±0.04 and 10.91±0.01 in animals treated with low and medium dose of *Entandrophragma angolense* respectively showing a significant increase in gastric mucus secretion (p<0.05) (Table 1). In contrast, there is a significant decrease in gastric mucus secretion in animals treated with high dose of *Entandrophragma angolense* when compared with values obtained for control animals (p<0.05).

A significant increase in the number of mucus cell count was observed in animals treated with low dose of the extract when compared with values obtained for control group (p<0.05) (Table 2).

Treatment with the low, medium and high doses of extract prevented accumulation of lipid peroxidation products; malonaldehyde concentration in the plasma as there was no significant change in the concentration when compared to the control animals (Table 3).

The methanolic extract of the stem bark of *Entandrophragma angolense* was earlier reported to be highly potent in inhibiting gastric ulceration and reducing

Table 1: The mean gastric mucus secretion in control and animals treated with the methanolic extract of *Entandrophragma angolense*

Animal treatment	No. of animals	Mean±SEM gastric mucus secretion
Normal	4	5.93±0.49
Low dose (100 mg kg <sup>-1</sup> b.wt.)	4	18.51±0.04*
Medium dose (200 mg kg <sup>-1</sup> b.wt.)	4	10.91±0.01*
High dose (400 mg b.wt.)	4	3.45±0.12*

p-value at p<0.05 \*: Significantly (S) different from control. Control animals were not given extract. Unit of gastric mucus secretion is mg g<sup>-1</sup> tissue ×10<sup>-2</sup>

Table 2: The mean gastric mucus cell count cm<sup>-2</sup> in control and animals treated with the methanolic extract of *Entandrophragma angolense*

Animal treatment	No. of animals	Mean±SEM gastric mucus cell count (cm <sup>-2</sup> )
Normal	2	262±25.47
Low dose (100 mg kg <sup>-1</sup> b.wt.)	2	458±12.45*
Medium dose (200 mg kg <sup>-1</sup> b.wt.)	2	270±35.67
High dose (400 mg b.wt.)	2	216±5.97

\*: Significantly different from control. p-value at p<0.05

Table 3: The mean malonaldehyde concentration (lipid peroxidation index) in control and animals treated with methanolic extract of *Entandrophragma angolense*

Animal treatment	No. of animals	Mean±SEM concentration (µmL/l×10 <sup>6</sup> )
Normal	4	1.82±0.16
Low dose (100 mg kg <sup>-1</sup> b.wt.)	4	1.67±0.09
Medium dose (200 mg kg <sup>-1</sup> b.wt.)	4	1.68±0.02
High dose (400 mg b.wt.)	4	1.61±0.10

\*Significantly (S) different from control at p<0.05

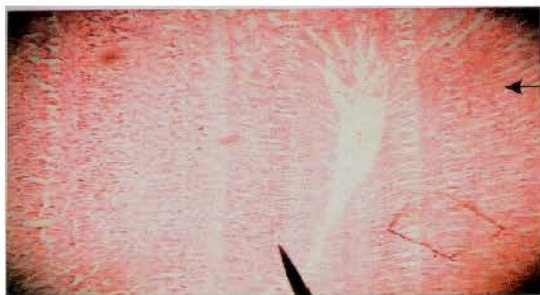


Fig. 1: A photomicrograph of stomach of a normal rat. The stomach appears to have an intact layer of mucus cells. Magnification x10



Fig. 3: A photomicrograph of stomach of a rat treated with medium dose of *Entandrophragma angolense*. The mucus cells appear intact. They are quite numerous and showing signs of mild degree of cellular activity. Magnification x10

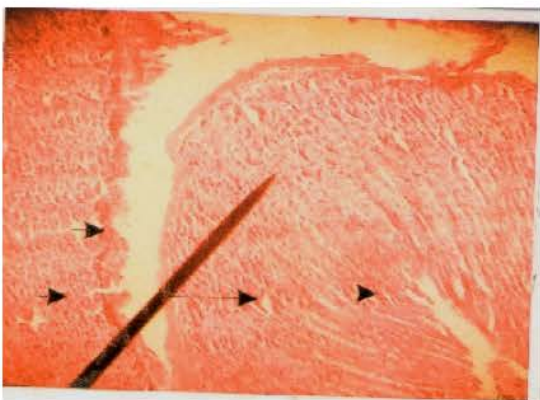


Fig. 2: A photomicrograph of stomach of a rat treated with low dose ( $100 \text{ mg kg}^{-1} \text{ b.wt.}$ ) of *Entandrophragma angolense*. The mucus cells are indicated as red patches. They are numerous as shown in the above. The mucus secreting cells are opened, prominent and active spitting their secretion into the surrounding tissue. There are signs of hypercellularity and hyperactivity. Magnification x10

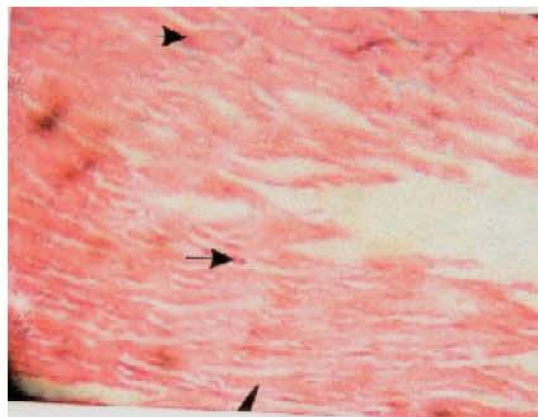


Fig. 4: A photomicrograph of stomach of a rat treated with high dose of *Entandrophragma angolense*. The mucus cells appear intact but are less numerous. Magnification x10

gastric acidity (Njar *et al.*, 1995). In this study, animals pre-treated with low and medium doses of the extract produced significant amount of gastric mucus ( $p < 0.05$ ) (Table 1). The results indicate that the anti-ulcer property of the extract is associated with its ability to cause rapid stimulation of gastric mucus. This is in addition to the fact that the drug has antisecretory property for gastric acid. This study supports the hypothesis that gastric mucus secretion remains the main factor protecting the gastric mucosa (Azzumi *et al.*, 1993).

The significant increase in mucus cell counts in animals pre-treated with low and medium doses of the

extract as shown in Fig. 2 and 3 compared with the control further (Fig. 1) support the observed increase in gastric mucus secretion. The observed reduction in gastric mucus secretion (Table 1) and cell count (Table 2) in animals treated with high dose of *Entandrophragma angolense* is at variance with the drug-dose response effect. It appears that long duration of treatment with high dose of extract initiates desensitization of gastric mucus cells involved in the gastric mucus secretion (Fig. 4).

*Entandrophragma angolense* caused increase in the secretion of malondialdehyde enzyme concentration over treatment period and was most pronounced at the low dose ( $p < 0.05$ ). The reduction in MDA concentration

which implies reduced lipid peroxidation may be due to the ability of the extract to increase antioxidant activity and also scavenge reactive oxygen radicals. This is justified by the intactness of the gastric mucosa in Fig. 2 and 3 and the hypercellularity and hyperactivity of mucus cells. The stimulation of gastric mucus by the extract thus play an important role in the anti-ulcer property of the drug. Therefore, the purified methanolic extract would definitely be a promising antiulcer agent when developed into drugs used in the treatment of stomach ulcer.

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