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METHANOLIC EXTRACT OF *TETRACERA POTATORIA*, AN ANTIULCER AGENT INCREASES GASTRIC MUCUS SECRETION AND ENDOGENOUS ANTIOXIDANTS

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Summary: In this study, the possible mechanism underlying the antiulcer activity of the methanolic extract of the root of *Tetracera potatoria* (MeTp) was studied in albino rats. Misoprostol and omeprazole were used as reference drugs. The animals had MeTp administered to them at varying doses of 100, 400 and 800 mg/kg for 15 days. MeTp significantly ($P<0.05$) increased gastric mucus secretion and gastric mucus cell counts when compared to control. MeTp treated animals also showed significant ($P<0.05$) increase in the activity of superoxide dismutase (SOD) with concurrent decrease in the level of malonialdehyde (MDA) with respect to control. These findings suggest that part of the gastroprotective property of MeTp is associated with the ability of the extract to cause stimulation of gastric mucus secretion through increased number of gastric mucus cells. Increased SOD-activity and decreased MDA-levels further lend support to its gastroprotective effect.
Key words: *Tetracera potatoria, gastroprotective, gastric mucus secretion, superoxide dismutase*

Introduction

Tetracera potatoria Afzel, family *Dillenniaceae* is known as *liane a eau* in France and water tree in Sieerra-leone (Burkil,1985).It is found in wooded areas of Senegal, Southern part of Nigeria, Central and Eastern Africa (Dalziel,1937).

The leaves of the plant boiled in its own sap are used for the treatment of gastrointestinal sores (Burkil, 1985). Adesanwo *et al* (2003) reported the antiulcer activity of the methanolic extract of the root of *Tetracera potatoria.* Two doses (400mg/kgBW and 800mg/kgBW) administered to albino rats completely inhibited gastric ulceration and significantly reduced gastric acidity. Previous antiulcer drugs were designed to inhibit gastric acid secretion. However, it is not in all cases of hyperacidity that ulceration develops. Gastric ulcer has been discovered to develop in patients with normal gastric acid output (Lawrence, 2000).

Some other factors implicated in gastric ulcers are oxygen derived radicals, pepsinogen and blood flow (Desai *et al,* 1997). These radicals are eliminated by the scavenging action of natural antioxidants (Halliwell and Gutteridge, 1990). Some well established endogenous antioxidants are superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase (Kelly, 1998). Flavonoids, another major phytochemical of *Tetracera potatoria* were reported as effective gastroprotective agents due to their antioxidant activity (Mirossay *et al,* 1996a, b; 1999).

Though the exact anti-ulcerogenic mechanism of *Tetracera potatoria* is not fully understood, mucus secretion is regarded as a crucial defensive factor in the protection of the gastric mucosa from gastric lesions (Mallika *et al*, 2005). In this study, we examined the influence of the methanolic extract of the root of *Tetracera potatoria* on gastric mucus secretion, gastric mucus cell counts, and the level of production of gastric anti-oxidant enzymes.

Materials and methods

Extract preparation:

Fresh roots of *Tetracera potatoria* were purchased from Ago-Iwoye, Ogun State, Nigeria and were authenticated by Mr. T.K. Odewo of Forestry Research Institute of Nigeria (FRIN), Ibadan. The roots were air-dried for six weeks, sawn into tiny pieces and later ground, weighing about 5.2kg. A large quantity (3.4kg) of the grinded material was soxhlet-extracted with methanol for about 72h. The MeTp was then dried in the Gallenhamp oven at 30°C for three days. The starting sample gave a mean yield of 7.1%. The extract was reconstituted in distilled water to make up the required concentrations and was stored at 4°C until use.

Animals:

Adult Wistar strain rats (180-220g) obtained from the Pre-Clinical Animal house, College of Medicine, University of Ibadan, were used for this study. There were four experimental study groups namely; gastric mucus secretion, gastric mucus cell count, superoxide

dismutase activity and malondialdehyde level. Control animals were not given any treatment but were fed normally and given water *ad libitum*. MeTp-treated rats were given MeTp at different doses (100mg/kg BW, 400mg/kg BW and 800mg/kg BW) for 15 days. Some rats pretreated with Omeprazole (0.67mg/kg) and Misoprostol (0.875µg/kg) served as positive controls.

Drugs used

Chemicals used were Adrenaline, Magnesium chloride (sigma), Carbonate buffer (sigma), Potassium chloride (sigma), Thiobarbituric acid (sigma), Trichloroacetic acid (sigma), Sodium acetate and Alcian blue. Stock solutions were prepared in distilled water.

Determination of gastric mucus secretion

Gastric mucus secretion was estimated using the earlier method described by Oluwole *et al*, (2007) after two weeks of pretreatment with the drugs.

Gastric mucus cell count

The gastric mucus cells were counted using an improvised calibrated microscope. This was an improvement over the foremost blind manner approach for counting (Li *et al*, 2002). Twenty-five squares, each measuring 2mm by 2mm, were drawn faintly on a transparent nylon. The nylon was then affixed onto the eyepiece of the microscope. The gastric mucus cells were counted as cells that stained for Haematoxylin and Eosin indicated as red patches. The mucus cells were counted in five squares during each view. The number of gastric mucus cells in each microscopic view was recorded and the mean number of gastric mucus cells in each square millimeter of gastric tissue was calculated.

Determination of superoxide dismutase activity

The assay method involves the inhibition of autooxidation of adrenaline to adrenochrome by SOD. The rate of autoxidation of adrenaline and the sensitivity of this inhibition of autooxidation by SOD were both augmented as the pH was raised from 7.8 – 10.2. The animals were bled through the eye and the blood samples were centrifuged in a cold centrifuge. Plasma samples were stored at 4°C until use. 0.2ml of the test sample was added to 2.5ml of 0.05M carbonate buffer. It was allowed to equilibrate in the spectrophotometer. 0.3ml of freshly prepared 0.3mM adrenaline was added to the buffer-supernatant mixture, which was quickly mixed by inversion. The reference cuvette contained 2.5ml of the buffer, 0.1ml of adrenaline and 0.2ml of water. The increase in absorbance at 480nm was monitored every 30 seconds for 150 seconds.

Calculation:

Change in absorbance/min ($\Delta A/min$) = A2-A1

Where A2 = Final absorbance after 150 seconds

A1 = Initial absorbance after 30 seconds

t = 2.5 min

$$\% \text{ inhibition} = \frac{1 - (\Delta A \text{ Sample/min}) \times 100}{\Delta A \text{ blank/min}}$$

ΔA blank/min = constant = 0.025/min

Using the above calculations, a standard curve for SOD activity was plotted and the percentage SOD activity of each experimental group was deduced from the curve.

Estimation of lipid peroxidation

Lipid peroxidation was assessed by the method described by Gutteridge and Wilkins(1982), This is based on the reaction between 2-thiobarbituric acid (TBA) and malonialdehyde(MDA) which is the end-product of lipid peroxidation.

Statistical analysis

Results were expressed as Mean ± SEM. Statistical analysis was performed using student's t-test and significant differences were accepted at $P<0.05$.

Results

Gastric mucus secretory activity

From Table 1, the mean gastric mucus secretion in control animals was 4.16±0.08 as against 4.55±0.09, 6.44±0.13, and 5.67±0.08 in low dose, medium dose and high dose pretreated animals respectively. The observed increase with each of the doses was significant ($P<0.05$). Similarly, Misoprostol significantly increased gastric mucus secretion compared with the control ($P<0.05$). However, omeprazole significantly reduced gastric mucus secretion comparable to control ($P<0.05$).

Effect of Tetracera potatoria on gastric mucus cell counts

Methanolic extract of *Tetracera potatoria* significantly increased the number of mucus cell count in animals pretreated with medium dose (MD) and High dose (HD) when compared with the mean value obtained from the control rats (Table 2) ($P<0.05$). There was however, a significant reduction in low dose (LD) animals. Omeprazole and Misoprostol at various doses used caused significant increase in gastric mucus cell counts compared to control ($P<0.05$).

Table 1: Mean gastric mucus secretion in control and animals treated with Methanolic extract of the root of Tetracera potatoria (MeTp)

Animal Treatment	*No of Animals*	*Gastric mucus secretion (mg/g) Mean ±S.E.M*
Control (non-treated)	5	4.16 ± 0.08
Low-dose (100mg/kg)	5	4.55±0.09*
Medium-dose (400mg/kg)	5	6.44±0.13*
High Dose (800mg/kg)	5	5.67±0.08*
Omeprazole (0.67mg/kg)	4	2.61± 0.02*
Misoprostol (0.875µg/kg)	4	5.54± 0.02 *

P-value at P< 0.05 *Significantly different from control.

Table 2: The mean gastric mucus cell count (mm^2) in control and animals treated with the Methanolic Extract of the Root of Tetracera potatoria (MeTp)

Group Treatment	*No of Animals*	*Gastric mucus cell count(mm^2) Mean±SEM*
Control (non-treated)	5	1.98 ± 0.00
Low-dose (100mg/kg)	5	1.83±0.01*
Medium-dose(400mgkg)	5	2.34±0.02*
High dose (800mg/kg)	5	2.93±0.00*
Omeprazole (0.67mg/kg	5	2.09±0.05*
Misoprostol(0.875µg/kg)	5	2.08±0.01*

P-value at P< 0.05. *Significantly different from control.

Effect of Tetracera potatoria on Superoxide dismutase (SOD) activity

Methanolic extract of *Tetracera potatoria* increased SOD activity in a dose dependent fashion in all the groups (Table 3). The increase demonstrated in each group was significant conpared to the control (P<0.05).

Effect of Tetracera potatoria on malonialdehye concentration

The extract reduced the concentration of assayed maloniaaldehye from 1.888±0.011 in the control to 1.714±0.009 (LD), 1.561±0.005 (MD) and 1.304±0.005 (HD). The reduction in the MD and HD pretreated animals were significant (P<.0.05). This is illustrated in Table 4.

Table 3: The Mean superoxide dismutase activity in (µg/ml) control and animals treated with Methanolic Extract of the root of Tetracera potatoria (MeTp).

Group Treatment	*No of Animals*	*Superoxide dismutase activity(µg/ml) Mean±SEM*
Control (non-treated)	5	19.90 ± 1.25
Low-dose (100mg/kg)	5	21.86±0.64*
Medium-dose(400mg/kg)	5	32.08±1.50*
High Dose (800mg/kg)	5	49.40±4.40*

P-value at P< 0.05. *Significantly different from control.

Table 4: Mean malonialdehyde concentration in control and animals treated with the Extract of the root of Tetracera potatoria (MeTp)

Group Treatment	*No of Animals*	*Malonialdehye concentration ($\mu mol/l$) $x10^{-6}$ Mean±SEM*
Control (non-treated)	5	1.888 ± 0.011
Low-dose (100mg/kg)	5	1.714±0.009
Medium-dose (400mg/kg)	5	1.561±0.005*
High dose (800mg/kg)	5	1.304±0.005*

P-value at P< 0.05. *Significantly different from control.

Discussion

The role of MeTp as an antiulcer agent had earlier been reported by Adesanwo *et al* (2003), having discovered a reduction in gastric acidity in animals treated with MeTp. Acute pretreatment of rats with MeTp and Misoprostol (15days) caused significant increase in gastric mucus secretion in all doses administered (100,400, and 800mg/kg) in comparison to 4.16±0.08mg/g in the control group (P<0.05). However, omeprazole, a proton pump inhibitor significantly reduced gastric mucus secretion (P< 0.05). Gastric mucus cells counts also increased significantly at doses of 400 and 800 mg/kg compared with the control (Table 2) (P<0.05). This

finding is indicative of the fact that MeTp enhances the growth of mucus secreting cells and thus agree with the report of Mojzis *et al.* (1995) that gastric mucus is an important factor in gastric mucosal defense. Other reports of the gastro-protective property of mucus opined that a decrease in gastric mucus secretion renders the mucosa more susceptible to injury induced by various factors with the converse being very correct (Nosalova *et al,* 1991; Farre *et al,* 1995).

These stimulatory effects of MeTp on gastric mucus cells and gastric mucus secretion may be similar to that of known drugs such as sucralfate and misoprostol (Slomiany *et al*, 1991, Takahashi and Okabe, 1996). Percentage increase in gastric mucus has been reported to be associated with graded doses of misoprostol in man (Wilson *et al,* 1986). Misoprostol, by virtue of its ability to stimulate mucus secretion, is an anti-ulcer agent in man (Poonam *et al*, 2003). Cellular antioxidant enzymes such as superoxide dismutase, glutathione peroxide and catalase normally challenge oxidative stress. In this study, MeTp significantly increased the concentration of superoxide dismutase (an antioxidant enzyme) from 19.90±1.25 in the control to 49.40± 4.40 in high dose treated animals ($P<0.05$). Increasing doses of MeTp (LD, MD and HD) significantly decreased the level of malondialdehyde (MDA), a marker of lipid peroxidation ($P<0.05$). The findings support other studies that demonstrated a reduction in lipid peroxidation of the gastric mucosa shown to be associated with increased activities of antioxidant enzymes (Melchiorri *et al*, 1997; Dela *et al*, 1999).

The mechanism of action of MeTp in ameliorating ulcer might be due to increased gastric mucus secretion as a result of increased number of gastric mucus cells through cell-proliferation, a mucogenic effect. The extract raises the concentration of one of the primary endogenous enzymes; superoxide dismutase which improves free radical scavenging property in the stomach.

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