

CYTOPROTECTIVE AND ANTIOXIDANT EFFECTS OF THE METHANOL EXTRACT OF *EREMOMASTAX SPECIOSA* IN RATS.

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* E-mail: pvernyuy@yahoo.com**Abstract**

Background: Ethno-botanical information shows that *Eremomastax speciosa* is used in the traditional management of various stomach complaints including gastro-duodenal ulcers.

Materials and Methods: In this study, we tested the cytoprotective potential of the whole plant methanol extract (100-200 mg/kg, *p.o.*), against HCl/ethanol, absolute ethanol, cold/restraint stress rats, and pylorus ligated rats pre-treated with indomethacin. The effects of the extract on gastric lesion inhibition, the volume of gastric juice, gastric pH, gastric acid output, mucus production and gastric peptic activity were recorded. Oxidative stress parameters were measured in blood and gastric tissue samples obtained from the animals in all the models tested.

Results: The extract significantly ($p < 0.05$), reduced the formation of cold/restraint ulcers by (31-60%, inhibition), completely inhibited (100%) the formation of lesions induced by HCl/ethanol at the highest dose, but was less effective against absolute ethanol (22-46% inhibition). The extract (200 mg/kg), significantly reduced lesion formation ($P < 0.01$), gastric acidity ($P < 0.01$), and volume of gastric secretions ($P < 0.05$), in the indomethacin/pylorus ligation model, and did not affect the activity of pepsin in gastric juice. Blood concentrations of antioxidant enzymes (catalase, SOD and GSH), increased significantly and MDA concentrations decreased in all models tested.

Conclusion: Cytoprotection by *E. speciosa* methanol extract was attributed to its ability to reduce acid secretion, and to enhance mucosal defence and *in vivo* antioxidant status.

Key words: *Eremomastax speciosa*, cytoprotection, gastric ulcers, antioxidant status

Introduction

Eremomastax speciosa (Hochst.) Cufod. (Acanthaceae) is widely distributed in tropical Africa and is the only species of the genus *Eremomastax* (syn.: *Paulowilhelmia* (Lindau); and *Ruellia* (S. Moore)) (Heine, 1966). The plant is a plant used in Cameroonian ethno-medicine for the traditional treatment of various complaints including dysentery, irregular menstruation, spurious labor pains, constipation, urinary tract infections and hemorrhoids. The plant is commonly referred to, 'in Cameroon', as 'blood plant', due to its reputed use in the traditional treatment of cases of anemia. This robust, polymorphous shrub grows to 2m in height and has a quadrangular stem. The characteristic violate underside of the leaves has earned for itself, the local name *Pang nyemshe* and *Pang ndjenit* (meaning 'red on one side') among the Bamiléké (Western Region) of Cameroon, and the pidgin English common name, "purple leaf". It is known as "Ntamir" in the Nso tribe of Cameroon (Adjanohoun et al., 1996). The plant is also used in Cameroonian ethno-medicine for the treatment of various stomach complaints and information from trado-practitioners suggested that it possesses antiulcer effects. The only literature report till date on the anti-ulcer potential of *E. speciosa* is the preliminary study (Tan et al., 1996) which showed that the water extract significantly inhibited the formation of HCl/ethanol-inflicted gastric lesions in rats. In the present experiment, various animal models of gastric ulcer were used to test the cytoprotective potential of *E. speciosa* methanol extract, to elucidate its possible mode of antiulcer action.

Materials and Methods**Animals**

Male albino Wistar rats (190 – 240g), raised in the Animal house of the Animal Physiology laboratory, Faculty of Science, University of Yaounde I, were used. They were fed a standard laboratory diet (supplied by SPC Ltd, Bafoussam, Cameroon), and given tap water *ad libitum*. The animals were deprived of food 12 h prior to experimentation but access to water was maintained. Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethics Committee (Reg. No. FWA-IRB00001954). The use, handling and care of animals were done in adherence to the European Convention (Strasbourg, 18.III.1986) for the protection of vertebrate animals used for experimental and other purposes (ETS-123), with particular attention to Part III, articles 7, 8, and 9.

Preparation of the plant extract

E. speciosa were collected in (May/June) in Yaounde (Centre Region), and identified botanically (Cameroon National Herbarium voucher specimen No. HNC/136984). The leaves and stems were chopped and rapidly sun dried to avoid the leafy parts getting moldy. 1 kg of the fine ground powder was macerated in 3 L of methanol for 48 h. After filtration, the extract solution was evaporated in a rota vapor to obtain a paste which was further evaporated to dryness at 50°C, using a Jencons-PLS, UK, convection air oven (yield, 6.3% w/w). The resulting product dissolved in vehicle (distilled water plus a few drops of Tween 20) which was given to the control animals.

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Phytochemical analysis

Standard protocols (Bruneton, 1993) were used to screen the extract for the qualitative presence of flavonoids, alkaloids, sterols, triterpenes, and carotenoids.

HCl/ethanol-induced gastric lesions in rats

The rats were deprived of food for 12 h prior to experimentation, but all the animals had free access to tap water. The HCl/ethanol solution was used to induce ulcers in the gastric mucosa according to the method of Hara and Okabe (1985). The animals received the plant extract by oral route, 1 h before they were given the necrotizing solution. An hour later, blood samples were collected in heparinized tubes from each rat by cardiac puncture under light ether anaesthesia and preserved for subsequent analyses for antioxidant status. They rats were sacrificed using ether and the abdomen of each opened and the stomachs removed. The ulcers produced in the glandular region of each stomach were measured and scored as earlier described (Tan et al., 1996) and the ulcer index (UI), percent inhibition (% I) and percentage of ulcerated surface (%US) were calculated.

Absolute ethanol-induced ulcers

The method described above for the HCl/ethanol method was used, the only difference being that 1 ml of absolute ethanol was used as the necrotizing solution. Blood samples were also collected at the end of the experiment for analyses for antioxidant status

Pylorus ligated gastric secretion in rats pre-treated with indomethacin

The pylorus ligation method of Shay et al. (1945) was used but with a modification, namely, the association of pylorus ligation with a pre-ligation administration of indomethacin by oral route. The test rats received the extract 100, and 200mg /kg, or sucralfate (50 mg/kg), while the controls were given the vehicle (1 ml). One hour later, all the animals were given indomethacin by oral route (50 mg/kg). An hour later, laparotomy was performed under light ether anaesthesia, and the pylorus of each rat was ligatured and the abdominal incisions stitched up. Six hours later, blood samples were collected in heparinised tubes and stored for subsequent analyses. The gastric juice produced under pyloric ligation was collected from each rat, the volume measured and 1 ml aliquots assayed for gastric acid content. Lesions observed in the glandular region of the stomachs were measured and expressed according to the score described by Tan et al. (1996) and ulcer index (UI) and percent inhibition (%I) were determined. Stomach tissue samples were also kept for subsequent antioxidant status study. Two extra groups of experimental animals were subjected to either pylorus ligation or oral indomethacin treatment alone for comparison.

Cold stress-induced gastric lesions

Stress-induced gastric ulcers were provoked in rats using a modification of the method earlier described by Takagi and Okabe (1968). Following 12 hours of food (but not water) deprivation, test rats were given the extract (100 and 200 mg/kg) by oral route while control rats received the vehicle. One hour later, the rats were placed in small individual wire cages and immersed in cold water (3-5°C), up to the level of the xiphoid. 120, minutes later blood samples were taken and the animals were sacrificed using ether and the stomachs removed for the assessment of lesion formation and mucus production (lesion indices expressed as the sum of the lengths of the lesions for each rat). Stomach samples were also preserved frozen awaiting measurement of antioxidant parameters.

Mucus production assessment

The mucus covering of each stomach was gently scraped using a glass slide and the mucus weighed carefully using a sensitive digital electronic balance. The same experimenter performed this exercise each time.

Measurement of pepsin activity

The Biuret method (Henry et al., 1974) was used to measure pepsin hydrolytic activity in gastric juice after incubating a solution of albumin (50 mg/ml), with the gastric juice (1ml), at 37°C, for 10 min.

Measurement of in vivo antioxidant capacity

Parameters of oxidative stress were measured in blood and gastric tissue samples obtained from the experimental animals. Lipid peroxidation was assessed by measuring the levels of malondialdehyde (MDA) (Slater, 1984). Cellular glutathione was measured using a modification of the standard "Ellman's Test" as described by Bulag et al. (1998). The method is based on the reaction between 2,2-dithio-5,5'-dibenzoic acid and the thiol (SH) groups of glutathione to yield a complex whose absorbance was read at 412nm. Superoxide dismutase (SOD) activity was measured using a standard method (Misra and Fridovich, 1972) and expressed in U/mg of protein, while catalase (CAT) was determined (Sinha, 1972) and expressed as $\mu\text{mol of H}_2\text{O}_2/\text{min/mg}$ of protein.

Statistical analysis

Values in tables are given as arithmetic means, $[\pm]$, standard error of the mean (S.E.M). The significance of differences between means was calculated using the student's t-test.

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Results

Thin layer chromatography of *E. speciosa* extract revealed the presence of five major classes of compounds of which triterpenes, sterols and carotenoids were in predominance. Table 1 shows the preventive effects of *E. speciosa* extract against HCl/ethanol-induced gastric lesions and on mucus production. The gastric mucosal preventive effect was dose-dependent, lesion index scores decreasing from 1.23, to 0.00, for the 100 and 200mg/kg, doses, respectively, compared with 3.92, for the controls. A similar dose-dependent trend was observed for mucus production, the quantity of mucus increasing by 4.2, and 25.8, per cent for the 100 and 200 mg/kg, doses, respectively, compared with the controls.

Table 1: Effect of *E. speciosa* extract on HCl/ethanol-induced gastric lesions in rats.

Treatment	Dose (mg/kg)	N	% ulcerated surface	Ulcer index (mean \pm SEM)	Inhibition (%)	Mucus production (mg)
Control	-	6	11.60	3.92 \pm 0.08	-	263.71 \pm 13.74
<i>E. speciosa</i>	100	6	0.59	1.23 \pm 0.23*	68.62	274.81 \pm 7.48
<i>E. speciosa</i>	200	6	0.00	0.00 \pm 0.00**	100	331.87 \pm 20.10*
Cimetidine	200	6	0.00	0.00 \pm 0.00**	100	309.99 \pm 11.87

Statistically different, relative to control; *p<0.05; **p<0.01; N, number of rats.

Dose-dependent effects were also obtained when the extract was used to prevent absolute ethanol-induced lesions. However, absolute ethanol considerably reduced mucus production values both in the controls and in the rats given the extract compared with the HCl/ethanol solution. The highest dose of extract provided only 45.8, per cent inhibition (Table 2) compared to 100, per cent with the HCl/ethanol method, although the increase in mucus production (129%), was highly significant (P<0.001) at the 200 mg/kg dose.

Table 2: Effect of *E. speciosa* extracts on absolute ethanol-induced gastric lesions in rats.

Treatment	Dose (mg/kg)	N	% ulcerated surface	Ulcer index (mean \pm SEM)	Inhibition (%)	Mucus production (mg)
Control	-	6	14.4	4.85 \pm 0.62	-	49.60 \pm 5.60
<i>E. speciosa</i>	100	6	6.7	3.78 \pm 0.36	22.0	73.66 \pm 6.79*
<i>E. speciosa</i>	200	6	3.9	2.63 \pm 0.55*	45.8	113.83 \pm 10.35**
Sucralfate	200	6	4.5	3.48 \pm 0.28	26.6	47.26 \pm 7.54

Statistically different relative to control; *p<0.05; **p<0.001; N, number of rats.

In the control rats subjected to pyloric ligation alone or oral indomethacin alone, gastric lesion indices were, respectively, 3.25 \pm 0.28, and 2.39 \pm 0.33, compared to control rats that were challenged with a combination of indomethacin pre-treatment followed by pylorus ligation (3.80 \pm 0.33). Increasing doses of *E. speciosa* extract inhibited lesion formation by 45 and 60 per cent, respectively, for the 100 and 200 mg/kg doses compared with 100 per cent for sucralfate (Table 3). Increasing doses of extract reduced gastric acid secretion from 51.47 mEq/l in the indomethacin/pylorus ligated controls to 41.45, and 28.18 mEq/l, respectively, for the 100 and 200mg/kg, doses but had no significant effects on gastric pH and pepsin activity. The gastric protective effects of the extract against the combined effect of indomethacin and acid gastric juice was associated with a significant increase in mucus production up to 25 per cent (P<0.01) for the 200 mg/kg dose compared with the controls (Table 4).

The effects of subjecting the rats to a combination of restraint and cold stress are shown in Table 5. *E. speciosa* (100 – 200 mg/kg), dose-dependently prevented the formation of gastric lesions, mean ulcer indices reducing from 1.68, in the controls to 1.16, and 0.66 for the 100 and 200mg/kg doses, respectively. Cimetidine (100mg/kg), prevented lesion formation by 41, per cent. Mucus production increased significantly (P<0.001), from 74mg, in the controls to 146, mg (98%), at the highest dose of the extract

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Tables 6 to 9, show the effects of *E. speciosa* extract on oxidative stress parameters measured in blood and stomach tissue samples obtained from rats subjected to various experimental ulcer models. For all methods of ulcer induction, dose-dependent exhibition of extract antioxidant capacity were observed with varying degrees of significance. Thus, blood concentrations of antioxidant enzymes (Catalase, SOD and GSH) increased significantly, while dose-dependent decreases in MDA, the major product of lipid per-oxidation, were observed compared with the negative controls.

Table 3: Effect of *E. speciosa* extract on pylorus-ligated gastric ulceration in rats pre-treated with indomethacin.

Treatment	Dose (mg/kg)	N	% ulcerated surface	Ulcer index (mean ± SEM)	Inhibition (%)	Mucus production (mg)
Control	-	6	4.01	3.80 ± 0.33	-	161.50 ± 7.20
<i>E. E. speciosa</i>	100	6	2.15	2.06 ± 0.08**	45.26	189.77 ± 4.09*
<i>E. speciosa</i>	200	6	1.09	1.52 ± 0.15**	60.00	201.69 ± 2.78**
Sucralfate	200	6	0.00	00.00 ± 0.00**	100	98.52 ± 3.26**

Statistically significant relative to control, *p<0.05; **p<0.001; N, number of rats.

Table 4: Effect of *E. speciosa* extract on gastric secretion in pylorus-ligated rats pre-treated with indomethacin.

Treatment	Dose (mg/kg)	N	Gastric contents (ml) (mean ± SEM)	pH of gastric contents (± SEM)	Gastric acidity (mEq/L)	Pepsin activity: (% hydrolysed protein) (± SEM)
Control	-	6	4.67 ± 0.33	3.49 ± 0.32	51.47 ± 1.55	83.97 ± 0.86
<i>E. speciosa</i>	100	6	3.75 ± 0.35	4.01 ± 0.12	41.45 ± 3.24*	84.45 ± 0.98
<i>E. speciosa</i>	200	6	3.33 ± 0.26*	4.14 ± 0.13	28.18 ± 3.04**	82.59 ± 0.58
Sucralfate	200	6	3.38 ± 0.33*	4.05 ± 0.28	34.33 ± 2.48**	84.62 ± 0.97

Statistically different relative to control, *p<0.05; **p<0.01; N, number of rats..

Table 5: Effect of *E. speciosa* extract on cold/restraint-induced gastric lesions in rats.

Treatment	Dose (mg/kg)	N	% ulcerated surface	Ulcer index (mean ± SEM)	Inhibition (%)	Mucus production (mg)
Control	-	6	1.43	1.68 ± 0.20	-	73.91 ± 5.12
<i>E. speciosa</i>	100	6	0.30	1.16 ± 0.28	30.9	111.02 ± 14.71*
<i>E. speciosa</i>	200	6	0.02	0.66 ± 0.21*	60.1	146.38 ± 9.50**
Sucralfate	200	6	0.05	0.99 ± 0.16	41.1	98.09 ± 1.40

Statistically different relative to control; *p<0.05; **p<0.01; N, number of rats.

Table 6: Effect of *E. speciosa* extract on oxidative stress parameters in rats subjected to HCl/ethanol treatment.

Treatment (mg/kg):	Catalase (umol H ₂ O ₂ /min/mg)	SOD (U/mg)	GSH (mmol/g x 10 ⁻³)	MDA (mmol/g x 10 ⁻⁶)
- Normal rats	10.44 ± 1.35	12.46 ± 0.37	9.13 ± 0.50	10.45 ± 1.19
- Negative control	7.34 ± 1.40	9.45 ± 0.44	6.17 ± 0.39	22.26 ± 0.89
- <i>E. speciosa</i> 100	12.17 ± 1.38*	10.40 ± 0.37	9.09 ± 1.01*	14.64 ± 0.74**
- <i>E. speciosa</i> 200	16.66 ± 1.27**	13.53 ± 0.41**	9.69 ± 0.52**	10.91 ± 1.28**
- Cimetidine 200	10.88 ± 0.66	11.37 ± 0.29**	7.14 ± 0.37	18.52 ± 0.77*

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Statistically significant relative to the control, * (P<0.05); ** (P<0.01); *** (P<0.001). SOD, superoxide dismutase; GSH, reduced glutathione; MDA, malondialdehyde. Each value is a mean from 6 rats \pm SEM.

Table 7: Effect of *E. speciosa* extract on oxidative stress parameters in rats subjected to *Absolute ethanol treatment*.

Treatment (mg/kg):	Catalase ($\mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg}$)	SOD (U/mg) ($\text{mmol}/\text{g} \times 10^{-3}$)	GSH ($\text{mmol}/\text{g} \times 10^{-6}$)	MDA
- Normal rats	29.84 \pm 2.57	10.11 \pm 0.82	6.26 \pm 0.90	2.79 \pm 0.20
- Negative control	15.20 \pm 0.75	5.07 \pm 0.47	4.05 \pm 0.30	6.75 \pm 0.65
- <i>E. speciosa</i> 100	18.33 \pm 1.06	14.10 \pm 1.70**	6.48 \pm 0.69	4.12 \pm 0.86**
- <i>E. speciosa</i> 200	23.46 \pm 1.10*	21.78 \pm 2.88***	6.70 \pm 0.50	3.14 \pm 0.63***
Succralfate 200	19.56 \pm 0.67	15.18 \pm 1.89**	5.48 \pm 0.58	5.98 \pm 0.67

Statistically significant relative to the control, * (P<0.05); ** (P<0.01); *** (P<0.001). SOD, superoxide dismutase; GSH, reduced glutathione; MDA, malondialdehyde. Each value is a mean from 6 rats \pm SEM.

Discussion

The present experiments were designed to validate the folk use of *E. speciosa* in gastric upset and to suggest a possible mode of its cytoprotective action. The results presented here showed that the methanol extract protected the gastric mucosa against damage induced by HCl/ethanol, absolute ethanol, pylorus ligation and cold/restraint stress, models commonly used to evaluate gastric ulceration in rodents. In contrast to the HCl/ethanol ulcers which were completely inhibited at the highest dose, the ethanol-induced ulcers were less susceptible to the cytoprotective action of the extract (22 – 46%, inhibition), and sucralfate (26%, inhibition). The highly corrosive nature of absolute ethanol to the gastric mucosa is well known. Absolute ethanol causes gastric mucosal lesions through the release of tissue-derived mediators such as histamine and leucotriene C₄ as well as by superficial aggressive cellular necrosis. The action of these mediators on gastric microvasculature results in both mucosal and sub mucosal gastric mucous tissue destruction (Oates and Hakkinen, 1985). *E. speciosa* extract offered the lowest cytoprotection against absolute ethanol, suggesting little effect on the generation or action of these mediators on the gastric microvasculature.

Unlike with the simple pylorus ligation technique, we challenged the animal gastric mucosa with a combination of pylorus ligation and oral indomethacin. In pyloric ligation, the pain-induced stress created by laparotomy and pylorus ligation combines with the accumulated gastric acid, and pepsin to create characteristically pointed ulcers or raised gastric mucosal inflammations. These are macro-morphologically different from the stripped lesions caused by absolute ethanol or HCl/ethanol. Indomethacin, on its part, is a non-steroidal anti-inflammatory drug which reduces prostaglandin and bicarbonate secretion, as well as gastric mucosal blood flow in animals (Whittle, 1977; Flemstrom et al., 1982; Miller, 1982; Selling et al., 1987), and the role of prostaglandins in gastric mucosal protection is well known (Konturek et al., 1981, 1982; Robert, 1981). When cytoprotection is significantly reduced by pre-treatment with indomethacin, the cytoprotection is interpreted to be mediated by endogenous prostaglandins. The involvement of endogenous PGs in *E. speciosa* cytoprotection is therefore possible since, in a separate experiment, pylorus ligation alone yielded an ulcer index (3.27 \pm 0.15), that was significantly higher compared with the indomethacin/pylorus ligation lesion index (2.06 \pm 0.08), at the dose of 100mg/kg, of the extract.

The results show that with the combination of the indomethacin and pylorus ligation techniques, *E. speciosa* extract produced significant dose-dependent reductions in ulcer indices (45 – 60% inhibition), accompanied by significant dose-dependent reductions in gastric acidity (41.45 \pm 3.24 - 28.18 \pm 3.04 mEq/L), compared with the controls (51.47 \pm 1.55 mEq/L). Sucralfate (ULCAR) treatment also significantly decreased gastric acidity to 34 mEq/L. Such significant drops in gastric acidity, which usually accompany the use of anti-secretory agents (histamine H₂ receptor blockers) like Cimetidine and Ranitidine and many antacid drugs, usually cause gastric indigestion and constipation during ulcer treatment. In the present experiment, pepsin activity of the gastric juice remained high (82 – 84%), compared with the control. In addition, the pH of the gastric juice for the controls (3.49 \pm 0.32), and the extract-treated animals (4.01 \pm 0.12 - 4.14 \pm 0.13) was not far from the upper limit of the optimal pH range (1.6 – 3.2) for pepsin activity. Indigestion and constipation are therefore not likely to occur as side effects in the use of *E. speciosa* extract.

E. speciosa methanol extract also prevented cold/restraint stress ulcers in a dose-dependent manner. The mechanism of acute stress ulcer involves an increase in intra-luminal gastric acid which causes mucosal damage by reducing the gastric adherent mucus (Meserau and Hinchey, 1973; Lambert and Kinsley, 1993), and substances that reduce gastric acid secretion and improve gastric mucus secretion are useful in the prevention of stress ulcers. In all models of gastric ulcer tested, *E. speciosa* methanol extract showed dose-dependent increases in gastric mucus production compared with the controls. The recorded increases for the highest dose of extract were 25-26% for the HCl/ethanol and indomethacin/pylorus ligation techniques and 98–129% for the stress and absolute ethanol methods. The remarkable increase in mucus production when the extract was faced with the highly necrotic action of absolute ethanol lends further credence to the possible involvement of endogenous PGs in the cytoprotective action of *E. speciosa* methanol extract.

Blood concentrations of antioxidant enzymes (Catalase, SOD and GSH) increased significantly, while dose-dependent decreases in MDA, the major product of lipid peroxidation, were observed compared with the negative controls. The stress ulcer model stimulates the production of oxygen-derived free radicals (in endothelial cells and polymorphonuclear neutrophils) which cause gastric mucosal tissue damage by inducing ischemia at the level of gastric microvasculature, and the direct role of the oxygen free radicals in the etiopathology of acute stress ulcers has been established (Coskun et al. 1995, Ohara et al. 1990; Manicelli et al. 1990). It is well known that the exposure of gastric mucosa to damaging factors such as water immersion/restraint stress and NSAIDs produce acute ulcers that are mainly mediated by ROS (Carnevale et al. 2011), and gastric protection against absolute ethanol injury in rats has been linked to antioxidant activity (de-Faria et al. 2012). The formation of water/restraint stress ulcers also involves the corrosive action of gastric acid on gastric mucosal cells as well as the cell wall lipid peroxidation which results in the formation of MDA (Tandon et al. 2004). Since SOD converts superoxide free radicals into H₂O₂ which is subsequently degraded by CAT, the dose-dependent increases in the enzyme concentrations following *E. speciosa* extract treatment are evidence of the extract-induced enhancement of the antioxidant status of the animals. Carotenoides and triterpenes, which were found in significant quantities in the

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methanol extract, are natural plant substances with well-known preventive antioxidant and antiulcer activities (Favier, 2003; Vera-Arzave et al 2012). Beta-carotene and other carotenoids are capable of scavenging reactive free radicals and quenching singlet oxygen (Paiva and Russel, 1999; Takashima et al., 2012).

Table 8. Effect of *E. speciosa* extract on oxidative stress parameters in rats subjected to *Indomethacin/pylorus ligation treatment*.

Treatment (mg/kg):	Catalase ($\mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg}$)	SOD (U/mg)	GSH ($\text{mmol}/\text{g} \times 10^{-3}$)	MDA ($\text{mmol}/\text{g} \times 10^{-6}$)
- Normal rats	6.07 + 0.56	8.36 + 0.72	5.76 + 0.39	6.11 + 1.11
- Negative control	2.01 + 0.39	2.90 + 0.58	3.74 + 0.50	43.08 + 2.82
- <i>E. speciosa</i> 100	2.94 + 0.74	4.45 + 0.53	4.37 + 0.20	15.27 + 1.08***
- <i>E. speciosa</i> 200	5.25 + 0.65***	6.49 + 0.38***	4.47 + 0.30	7.05 + 0.49***
- Sucralftae 200	3.38 + 0.32	4.52 + 0.47	6.19 + 0.30**	8.12 + 0.78

Statistically significant relative to the control, * (P<0.05); ** (P<0.01); *** (P<0.001). SOD, superoxide dismutase; GSH, reduced glutathione; MDA, malondialdehyde. Each value is a mean from 6 rats \pm SEM.

Table 9. Effect of *E. speciosa* extract on oxidative stress parameters in rats subjected to *Cold/restraint stress treatment*

Treatment (mg/kg):	Catalase ($\mu\text{mol H}_2\text{O}_2/\text{min}/\text{mg}$)	SOD (U/mg)	GSH ($\text{mmol}/\text{g} \times 10^{-3}$)	MDA ($\text{mmol}/\text{g} \times 10^{-6}$)
- Normal rats	5.13 + 0.90	8.26 + 1.02	6.99 + 0.12	3.08 + 0.59
- Negative control	3.07 + 0.70	5.53 + 0.20	6.26 + 0.20	4.98 + 0.82
- <i>E. speciosa</i> 100	7.34 + 1.20*	6.78 + 0.17	10.90 + 1.14*	1.20 + 0.49*
- <i>E. speciosa</i> 200	8.30 + 1.40*	9.99 + 1.30*	13.60 + 0.07*	2.10 + 0.63*
- Cimetidine 100	7.01 + 1.25*	6.31 + 0.31	7.57 + 1.12	1.90 + 0.52*

Statistically significant relative to the control, * (P<0.05); ** (P<0.01); *** (P<0.001). SOD, superoxide dismutase; GSH, reduced glutathione; MDA, malondialdehyde. Each value is a mean from 6 rats \pm SEM.

In conclusion, cytoprotection by *E. speciosa* methanol extract can be attributed to its ability to enhance gastric mucosal defense and *in vivo* antioxidant status. The results also confirm our previous findings which suggested that the extract of *E. speciosa* possesses cytoprotective effects that may be associated to an antisecretory potential. Possible mechanism for anti-secretion need to be investigated.

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