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Evaluation of the gastroprotective activity of 3-carbomethoxypyridine from *Pyrenacantha staudtii* Engl. (Icacinaceae) in rats

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Abstract
3-carbomethoxypyridine (3-CMP, also known as methyl nicotinate) is a compound isolated and characterized from the leaves of a local plant, *Pyrenacantha staudtii* Engl. (Icacinaceae), and known to possess anti-ulcer effects. The present study was undertaken to evaluate the possible gastroprotective effects of 3-CMP. Various models of ulcer such as pylorus ligation ethanol-, ethanol/HCl- and indomethacin-induced ulcer in rats were employed. Anti-ulcer effect was assessed on the basis of the number of reduction in gastric mucosal lesions, increase in pH, and decrease in volume, free and total acidity of gastric juice. The compound produced a non-dose dependent protection of the gastric mucosa in ethanol-, ethanol/HCl- and indomethacin-induced ulcerations with the highest effect at 25mg/kg. 3-CMP significantly decreased the secretion of gastric aggressive factors; free acidity and total acidity as well as superoxide dismutase and catalase. Results obtained suggest that 3-CMP has anti-ulcer and antioxidant properties which may be attributed to its ability to reduce total acidity and free acidity, or mediated through the production of prostaglandins and free radical scavengers which protect the gastric mucosa.

Keywords: 3-Carbomethoxypyridine; Pylorus ligation; Indomethacin; Ethanol; Ranitidine; Catalase

INTRODUCTION

Peptic ulcer disease (PUD) is characterized by disruption of mucosal integrity leading to local injury due to active inflammation. Gastric ulcer is caused by an impairment of the balance between offensive factors such as acid, pepsin and *Helicobacter pylori*, and defensive factors (mucus bicarbonate, blood flow, prostaglandins), as a result of increase in either factor or interaction between the two factors (Sun, 1974; Piper and Stiel, 1986). In order to regain this balance, different therapeutic agents including medicinal plants are used to inhibit gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production.
It had long been suggested that herbal drugs should be looked for as better alternatives for the treatment of peptic ulcer disease (Akhtar *et al.*, 1992). This is because of their perceived lower incidence of relapse, side effects and drug interactions compared to orthodox drugs (McQuaid, 2007).

Pyrenacantha staudtii Hutch and Dalz (Icacinaceae) is an annual herb found in light tropical forest and farmland bushes, with

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intensely bitter leaves. The aqueous extract is used for treatment of dysmenorrhea, intestinal colic and threatened abortion (Agbakwuru *et al.*, 1988; Akubue *et al.*, 1983). Other effects include antimalarial (Mesia *et al.*, 2005), anti-ulcer (Aguwa and Mittal, 1981; Aguwa and Okunji, 1986) and tocolytic (Falodun *et al.*, 2005). An alkaloidal bitter chemical constituent, 3-carbomethoxypyridine (3-CMP) had been isolated and characterized from the plant, using bioactivity guided techniques (Falodun *et al.*, 2006a) and found to possess smooth muscle relaxant activity on the isolated rat uterus (Falodun *et al.*, 2006b). This compound has been reported to have a vasodilating effect on the skin of humans (Lepold and Lippold, 1995) and is a useful pharmacological tool in inducing skin inflammation (Duteil *et al.*, 1990). It is also found in terrestrial plants, marine invertebrates, algal sources (Sebastiano *et al.*, 1988) and *Platymonas subcordifo* (Chia-Ying *et al.*, 2005).

EXPERIMENTAL

Animals. Sprague-Dawley rats (207±12g) of both sexes were obtained from the Laboratory Animal Centers of Department of Pharmacology & Toxicology, University of Benin, Benin City and Ambrose Alli University, Ekpoma, Edo State, Nigeria. The animals maintained under standard diet (Bendel Feeds Ltd. Ewu. Edo State, Nigeria) and water *ad libitum*, were acclimatized for two weeks. Approval for the use of animals in the experiments was obtained from the Ethical Committee on the Use of Laboratory Animals, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. Animals were handled according to the protocol outlined in "*Principles of Laboratory Animal Care*" (National Institute of Health Guide for Care and Use of Laboratory Animals, Pub No. 85 – 23, revised 1985).

Drugs and reagents. Absolute ethanol, Diethyl ether and Indomethacin (Sigma-Aldrich Laborchenikalien, GmbH Germany); Formaldehyde (Merck, Germany); Ranitidine (Ranbaxy Pharmaceutical Company, India); Dipotassium hydrogen phosphate, Epinephrine, Hydrochloric acid, Sodium carbonate, Sodium hydroxide and Sulphuric acid (BDH Chemicals, England).

Pharmacological tests

Ethanol-induced ulceration. Rats were randomly selected into 4 groups of 5 animals each and starved for 24 hours but had free access to water. Water was however withdrawn 2 hours before experiments.

Group 1, which served as the control received normal saline orally. Group 2 received ranitidine (100mg/kg) orally, while groups 3, 4 and 5 received by oral intubation 10, 25 and 50 mg/kg of the extract, respectively (Robert, 1979). One hour later, 1ml of absolute ethanol was administered by intragastric intubation to all the groups. One hour following ethanol administration, the animals were sacrificed by overdose of ether and the stomachs examined macroscopically. Ulcer lesions were counted using a magnifying glass while the diameter of the ulcers was measured using a vernier caliper and scored (Martin, 1988).

Ethanol/ HCl-induced ulceration. Rats were randomly divided into 5 groups of at least 4 animals per group and starved for 24 hours with free access to water which was withdrawn 2 hours before experiments.

Group 1 served as the control and received distilled water (3ml/kg) orally. Group 2 received ranitidine (100mg/kg) orally, while groups 3, 4 and 5 received 10mg/kg, 25mg/kg and 50mg/kg of 3-carbomethoxypyridine (3-CMP), respectively, by oral intubation.

One hour later, ulcer was induced by intragastric instillation of 0.15M Hydrochloric acid in 70% v/v ethanol (4ml/kg) to all the groups (Hara and Okabe, 1985). One hour following HCl-ethanol administration, the animals were sacrificed by ether anaesthesia. The stomach was isolated, placed in formol saline (2%v/v) for 10 minutes, opened along

the greater curvature and rinsed with tap water. Macroscopic examination of the stomachs of the animals in all the groups was done. The presence of ulcers was counted using a magnifying glass. The diameter of the ulcers was measured using a vernier caliper and scored on a scale of 0-10 (Martin, 1988).

Indomethacin-induced ulceration. Rats were randomly allotted to 5 groups of at least 6 animals each. Test samples were administered to animals one hour before oral administration of indomethacin (20mg/kg). Six hours later, each rat was sacrificed by ether anaesthesia and the stomach removed. Formal saline (2%) was injected into the totally ligated stomach for overnight storage. The stomach was then opened along the greater curvature, rinsed with water and scored for ulcer (Martin, 1988).

Pylorus ligation-induced ulcer. Rats were divided into 5 groups of 5 animals each and fasted for 30 hours with access to drinking water *ad libitum*. Group 1(control) received distilled water (3ml/kg), group 2 received ranitidine (100mg/kg), while groups 3, 4 and 5 were administered 10, 25 and 50mg/kg of 3-CMP, respectively, one hour prior to pyloric ligation. The abdomen was opened by a midline incision, under light ether anaesthesia, and pyloric ligation was done without causing any damage to its blood supply (Shay *et al.*, 1954). The stomach was replaced carefully and the abdomen was closed with interrupted sutures. Eighteen hours later, the animal was sacrificed with overdose of anaesthetic ether. The stomach was isolated and gastric juice collected and centrifuged at 5000 rpm for 20 min. The volume and pH of the resulting supernatant were recorded after which they were subjected to analysis for free and total acidity using 0.1N NaOH for titration, with phenolphthalein and Toffer's reagent as indicators. Superoxide dismutase (SOD) in gastric juice collected from the pylorus-ligated rats was determined by method of Mishra and Fridovich (1972) while catalase (CAT) activity was estimated by the method of Beers and Sizer (1952). The emptied stomach was opened along the greater curvature and washed. Ulcer lesions were counted using a magnifying glass while the diameter of the ulcers was measured using a vernier caliper and scored on a scale of 0-10 (Martin, 1988).

Statistical analysis. Results are presented as mean $\pm$ standard error of mean (SEM) and n represents the number of animals per group. Data comparisons were made using the Student's t-test for unpaired data or one way ANOVA with Tukey-Kramer post hoc test (GraphPad InStat 3). Values were considered statistically significant at $p<0.05$.

RESULTS

Ethanol-induced ulceration. Pretreatment with 3-CMP significantly ($p<0.001$) reduced in a non-dose dependent manner the severity of the ulcers induced by ethanol. The type of ulceration seen in this model was the long and deep type with wide diameters visible from the outside the stomach as thick black or red lines especially in the control groups. All the doses of 3-CMP (10, 25 and 50 mg/kg) showed significant reductions in the number and severity of ulcers when compared to control (Table 1). Ranitidine showed a slightly greater reduction in the ulcer index than the 3-CMP (25mg/kg).

Ethanol/HCl-induced ulceration. Pretreatment with 3-CMP significantly ($p<0.001$) reduced in a non dose-dependent manner the severity of the ulcers induced by ethanol-hydrochloric acid mixture. 3-Carbomethoxypyridine, at a dose of 25mg/kg, showed significant reduction in the number and severity of ulcers when compared to control (Table 2). Ranitidine (100mg/kg), like the lowest dose of 3-CMP, showed a slight reduction in the ulcer index but this was not significant ($p>0.05$).

Indomethacin-induced ulceration. The effect of 3-CMP on indomethacin-induced ulceration is shown on Table 3. Small round, red and dispersed lesions of the mucosa were evident in control rats that received indomethacin (40mg/kg). Pre-treatment with 3-CMP (10mg/kg) caused a significant reduction in both the number and severity of these lesions. However, the standard anti-ulcer drug, ranitidine showed a significantly higher reduction ($p<0.001$) in ulcer index than this dose of the compound. Higher doses of 3-CMP did not exert any significant inhibitory effects on the ulcerations produced by indomethacin.

Pylorus ligation induced ulceration. Pylorus ligation caused the accumulation of gastric secretion and hence, intense lesions in the ruminal/ antral portion of the stomach in control rats.

Table 1: Effect of 3-CMP on ethanol-induced ulceration in rats

Groups	Dose (mg/kg)	Ulcer index (UI)	% inhibition
Control	-	22.17 ± 1.97	-
3-CMP	10	14.00 ± 3.96*	36.85
	25	12.00 ± 2.00**	45.72
	50	14.60±1.35*	34.15
Ranitidine	100	9.34 ± 1.32**	57.87

Values are Mean ± SEM. *P < 0.01, **P<.001, significantly different from control; (ANOVA; Tuckey-Kramer post hoc test). n= 5 animals.

Table 2: Effect of 3-CMP on ethanol/HCl-induced ulceration in rats

Groups	Dose (mg/kg)	Ulcer index (UI)	% inhibition
Control	-	20.75 ± 1.44	-
3-CMP	10	15.80 ± 2.60	23.86
	25	7.80 ± 2.73*[a]	62.41
	50	20.60±3.22	0.72
Ranitidine	100	16.00 ± 3.28	22.89

Values are Mean ± SEM. *P<0.001, significantly different from control; [a]P<0.05, compared with 50mg/kg, 3-CMP (ANOVA; Tuckey-Kramer post hoc test). n = 4-5 animals.

Table 3: Effect of 3-CMP on indomethacin-induced ulceration in rats

Groups	Dose (mg/kg)	Ulcer index (UI)	% inhibition
Control	-	22.89 ± 1.45	-
3-CMP	10	12.83 ± 1.31*[a]	43.94
	25	19.17 ± 1.54	16.25
	50	19.50±1.12	14.81
Ranitidine	100	1.97 ± 0.57*[b]	91.39

Values are Mean ± SEM. *p<0.001, significantly different from control; [a]P<0.05, compared with 25 and 50mg/kg (3-CMP); [b]p<0.001, compared with 10mg/kg - 3CMP (ANOVA; Tuckey-Kramer post hoc test). n = 6-9 animals.

Table 4: Effect of 3-carbomethoxypyridine on pylorus ligation in rats

Group	Dose (mg/kg)	Vol. of acid (ml/100g rat)	pH	Free acidity (mEq/L)	Total acidity (mEq/L)	SOD (units/ml)	Catalase (units/ml)
Control	-	3.14±0.70	3.20±0.26	5.82±1.83	14.23±3.33	2.86±0.48	0.13 ±0.04
3-CMP	10	1.68.±0.46	4.74±0.29	7.42±3.60	17.63± 8.68	1.84±0.62	0.32±0.09
	25	1.83±0.26	5.04±0.28*	3.45±0.44	7.9±1.03*	3.77±0.39	0.34±0.02
	50	3.36±0.72	4.45±0.29	2.12±0.54*	11.84±3.05	2.43±0.68	0.47±0.06
Ranitidine	100	3.06±0.30	4.39±0.31	2.28±0.55*	7.90±1.05*	2.39±0.18	0.58±0.07

Values are mean±SEM of experiments from 5 animals , *p<0.05 vs. control (ANOVA).

Pylorus ligation decreased the SOD and catalase levels in the gastric juice of the control rats but increased in those treated with 3-CMP. Ranitidine did not significantly alter the volume and pH of the gastric juice but caused significant reductions in free acidity and total acidity (Table 4).

DISCUSSION

3-Carbomethoxypyridine was evaluated against gastric lesions induced by ethanol, hydrochloric acid-ethanol, indomethacin and pyloric ligation. The results obtained show a non dose-dependent gastro-protective effect in the models of ulcer studied.

Ethanol induced gastric ulcer was employed to study the cytoprotective effect of 3-CMP. Absolute ethanol is known to induce gastric lesion, due to its corrosive effect. It rapidly penetrates the gastric mucosa to disrupt the mucus-bicarbonate barrier, causing cell and plasma membrane damage, leading to increased membrane permeability to sodium and water (Mincis *et al.*, 1995). There is also massive intracellular accumulation of calcium, which represents a major step in the pathogenesis of gastric mucosal injury and this leads to cell death and exfoliation in the surface epithelium (Soll, 1990). In addition, there is development of oxygen-derived free radicals (Oates and Hakkinem, 1988), hyperoxidation of lipids (Jainu and Devi, 2006) and stimulation of histamine and serotonin release from mast cells (Alarcon de la lastra *et al.*, 1997).

All the doses of 3-CMP and ranitidine were effective in preventing development of ethanol induced gastric ulcers, indicating that the compound possesses gastric cytoprotective effect.

The compound, at a dose of 25 mg/kg, evoked a greater than 60% inhibition of the ulcerations caused by ethanol-hydrochloric acid mixture, an effect far greater than those produced by the other doses of the compound and ranitidine.

Ethanol alone rapidly penetrates the gastric mucosa causing cell and plasma damage. Combination of hydrochloric acid with ethanol increases the level of acidity in the stomach; hence they act additively causing massive necrotizing lesions in the gastric mucosa which are more severe than those caused by ethanol alone, as a result of reduction in the protective layer of mucus and an increase in peptic acid secretion (Mizui and Douteuchi, 1983).

In indomethacin-induced ulceration, the lowest dose of 10mg/kg exerted a significant ($p<0.001$) protection from gastric damage. However, this effect was significantly ($p<0.001$) lower than that of ranitidine. The higher doses (25 and 50 mg/kg) exerted protective effects which were, however, not significantly different from control.

Nonsteroidal anti-inflammatory drugs (NSAIDs), like indomethacin, damage the gastric mucosa by inhibiting COX-1 thereby reducing the production of prostaglandins E_2 and I_2 (Wallace, 2001) which are involved in the synthesis of mucus and bicarbonate. There is also an enhancement of the lipooxygenase pathway thereby liberating leukotrienes and these are reported to have a role in ulcerogenesis (Malairajan *et al*., 2008). Therefore, the gastroprotective effect of 3-CMP may be due to its ability to inhibit the synthesis of leukotrienes. Since prostaglandins are known to protect gastric mucosal cells against injury caused by indomethacin (Robert, 1975; Whittle, 1977), it is possible that the compound stimulates the production of endogenous prostaglandins, which then provide the protection.

3-CMP decreased the secretion of gastric aggressive factors including free acidity, total acidity. Agents that decrease gastric acid secretion and/or increase mucus secretion are effective in preventing ulcers

induced by pylorus ligation. The ligation of the pyloric end of the stomach causes accumulation of gastric acid in the stomach, leading to the development of ulcers in the stomach (Khare *et al..*, 2008).

The superoxide radicals have been implicated in pylorus ligation-induced gastric mucosal injury (Rastogi *et al.*, 1998) and are known to be responsible for elevated oxidative stress in several pathological disorders. High vulnerability of the gastric mucosa to oxidative damage is mainly due to a decline in the level of free radical scavengers (Koc *et al.*, 2008). Superoxide dismutase (SOD) and catalase (CAT) accelerate the degradation of the harmful H_2O_2 and convert it into water and oxygen (Halliwell, 1990). The SOD and CAT activities were found to be lower in untreated pylorus ligated rats. The inhibition of SOD and CAT in these rats may be due to increased generation of reactive free radicals which can create oxidative stress in cells. The administration of 3-CMP increased, though not significantly, the SOD and CAT levels activities in the stomach and protected from free radical-induced oxidative stress. This suggests a possible antioxidant effect of the compound.

The finding commonly noted in the different models used was a tendency to a decreased inhibition of gastric injury formation (or none at all) at the highest dose (50mg/kg). This suggests a ceiling effect in the activity of the compound.

Conclusion. 3-carbomethoxylpyridine (3-CMP) was shown to possess a clear gastroprotective effect in the four models of gastric ulcer used. Although the mechanism through which it exerts this effect is not clear, it could be through reduction of total acid output or via the production of gastrointestinal prostaglandins and free radical scavengers, which protect the gastric mucosa.

3-carbomethoxylpyridine has the potential of a good anti-ulcer agent. However, more detailed studies are needed to elucidate the exact mechanism(s) through which it produces its effects.

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