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Full Length Research Paper

Anti-*Helicobacter* and gastroduodenal cytoprotective actions of the leaf aqueous extract of *Ocimum suave* (Lamiaceae)

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The gastroduodenal cytoprotective potential of the leaf aqueous extract of *Ocimum suave* (Lamiaceae) was investigated in rats using several ulcer-inducing methods. The anti-*Helicobacter* property of the extract was further assessed *in vitro* against *Helicobacter pylori* CCUG-39500. The results obtained indicated that in all cases, orally administered extract (125 to 500 mg/kg) dose-dependently prevented lesion formation, with significant increases in gastric mucus production. The dose-500 mg/kg completely inhibited HCl/ethanol lesions but intraperitoneal indomethacin did not significantly reduce this effect. Honey-250 mg/kg, in combination with the extract-250 mg/kg, offered 50% prevention of ethanol-induced lesions, but *O. suave* alone (500 mg/kg) was more potent (62% inhibition) compared with honey alone (35% inhibition) at the same dose. In pylorus ligation, gastric acidity (91 mEq/L) was high compared with the controls (68 mEq/L), but 79% lesion inhibition was achieved. Mucus secretion (116 mg at 500 mg/kg) was most significant in highly acidic gastric environments compared with the controls (53 mg). The extract (12 mg) had anti-*H. pylori* effects (inhibition diameter, 33 mm; minimum inhibitory concentration (MIC), 1.17 mg/ml; minimum bactericidal concentration (MBC), 2.34 mg/mg). The cytoprotective actions of the extract are associated with its gastric mucosal re-enforcing effects. The extract possesses both anti-*Helicobacter* and gastro-duodenal cytoprotective effects, and may be useful in an antiulcer regimen without antimicrobial, antacids and antisecretory agents.

Key words: *Helicobacter pylori, Ocimum suave*, cytoprotection, gastroduodenal ulcer.

INTRODUCTION

World Health Organisation (WHO) guidelines for the use of herbs as medicines stipulate that preparation methods for scientific evaluation of medicinal plants should be as close as possible to the described ethno medical procedure. Although the nation-wide OAU/STRC-sponsored ethno botanical survey in Cameroon and Ghana (Adjanohoun et al., 1996; Mshana et al., 2000) did not attribute antiulcer effects to any of the main *Ocimum* species (*Ocimum viride* Linn, *Ocimum gratissimum* Linn, *Ocimum basillicum* Linn and *Ocimum canum* Sims), we have found new evidence that the leaf water extract of *Ocimum suave* wild (Lamiaceae) is a potent anti-ulcer preparation used in the North west region of Cameroon. A handful of the fresh leaves are boiled in water and honey is added to the filtered solution which is taken orally for about two weeks (Dr Kinni Khon, Personal communication). The mosquito repellent, antimalarial, analgesic and antibiotic activities of *O. suave* have been cited in the literature (Seyoum et al., 2002; Makonnen

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et al., 2003a, b; Nguta et al., 2010). We have also observed the antimicrobial potency of the water soluble fraction of the methanol extract of *O. suave* against *Helicobacter pylori* and C*ampylobacter sp.,* as well as on some nosocomial bacteria (Boda et al., 2006, Tan et al., 2006). The Maandi, Koongo and Sundi peoples of Congo Brazzaville use the leaves of *O. suave* in combination with other plants to treat fever in children, menstrual problems, stomachache and brocho-pneumonic diseases (Bouquet, 1969). The oil extracted from the leaves of *O. suave* contains phenols (Watt et al., 1962) and our preliminary studies have shown the high presence of triterpenes.

O. suave wild (Lamiaceae, syn.: Labiatae (Thonner, 1915), syn.: Menthaceae (Burger, 1967) is a seasonal, ruderal and anthropophilic plant which is sometimes cultivated. This small aromatic ramified shrub grows to an average height of 1 m (Hutchinson et al., 1963; Raynal et al., 1979). Its geographical distribution stretches from tropical Asia to tropical West and East Africa where it is limited to mountainous areas. The Massai people of East Africa refer to it as Olamora (Watt et al., 1915). In Equatorial Africa, *O. suave* is limited to Congo and Cameroon. In Cameroon, the plant is found only in the Bamenda highlands of the North West Province, especially on grazing areas but it does not form part of the diet of grazing animals. The seasonal appearance of the plant as well as the wide annual propagation by wind and grazing animals make it a good candidate for large scale medicinal use since it is not consumed by man or animals as food.

In the present study, we have examined the effects of the leaf water extract of *O. suave* against duodenal ulcers generated using cysteamine, against gastric lesions induced using HCl/ethanol, absolute ethanol, indomethacin and pylorus ligation, and its effect on the growth of *Helicobacter pylori in vitro*. The effect of the extract with or without natural honey in ethanol-induced lesions was also tested. Mucus production, gastric acidity and peptic activity of gastric juice were used as parameters to explain the possible mode of gastric cytoprotective action.

EXPERIMENTAL

Animals

Male Wistar rats (160 to 200 g) and mice (20 to 25 g) were used for the experiments. The animals were raised on a standard laboratory diet and tap water in the Animal house of the Faculty of Science, University of Yaounde I. Prior authorization for the use of laboratory animals in this study was obtained from the Cameroon National Ethics Committee (Reg. No. FWA-IRB00001954).

Test organism and culture media

The *H. pylori* isolate (strain CCUG 39500) was obtained in lyophilized form from the Culture Collection, University of Göteborg (CCUG), Sweden. The revival of the strain, the preparation of the culture media as well as the standard inoculum was done as previously described (Tan et al., 2010). The antimicrobial agar diffusion and dilution tests were performed on 5 to 6 mm- thick Columbia agar supplemented with 5% (v/v) lacked horse blood and 1% (v/v) Vitox (CA-Vitox).

Preparation of the plant extract

The plant material was collected in September from the Wainama hills of Jakiri in the North West Region of Cameroon. Botanical identification was done at the National Herbarium in Yaoundé by comparison with existing herbarium voucher specimen (Herbariun No. HNC: 6077/6914 (R. Letouzey)). The dried ground leaves were extracted in water by boiling 1 kg in 1 L of water for 15 min. The resulting extract was dried in a convection air oven (Jencons-PLS, UK) at 50°C to obtain 20 g of a brown solid (2% yield w/w). The extract re-dissolved readily in distilled water which was used as the vehicle. The natural honey used was a polyfloral specimen purchased from the local market in Jakiri.

Phytochemical tests

The extract was subjected to the Libermann Buchard, Schinoda, Meyer and Molish tests (for triterpenes, flavonoids, alkaloids and sugars, respectively), as well as characterisation tests for the presence of phenols, sterols, and multiple bonds (Bruneton, 1993).

In vitro anti-*Helicobacter* tests and determination of antimicrobial parameters

These tests were performed as previously described (Tan et al., 2010). For the agar well diffusion test to determine diameters of inhibition zones, serially decreasing quantities of extract (0.36 to 12 mg) prepared using sterile distilled water were dropped into wells (6 mm diameter) drilled on CA-Vitox agar plate already inoculated with 100 µl bacterial suspension. Metronidazole (5 µg) and Amoxicillin (10 µg) were used as positive controls. The plates were incubated under microaerophilic conditions at 37°C for 72 h, after which the dilution (or concentration) showing a diameter of inhibition ≥ 7 mm was considered as active. The tests were run in triplicate for each concentration and the mean of the diameters of inhibition (DI) zone for each concentration was considered. The minimum active quantity (MAQ) was determined as the minimum quantity of test compound producing the smallest inhibition zone.

For the agar dilution test used for the determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC), two-fold decreasing concentrations of extract prepared and incorporated in CA-Vitox at concentrations ranging from 150 to 0.57 mg/ml were inoculated and incubated as described above for 72 h, after which the lowest concentration of extract that prevented visible growth was considered as the MIC. The surface of each plate that showed no visible growth was washed with 100 µl of sterile distilled water and the resulting suspension was plated on an extract-free plate of CA-Vitox. The plates were incubated for 72 h and the lowest concentration corresponding to the plate that yielded no growth was considered as the MBC.

Induction of gastric ulcers

HCl/ethanol-induced gastric lesions in rats

The rats were deprived of food for 48 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. They were killed using ether, 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.

HCl/ethanol-induced lesions in rats pre-treated with indomethacin

Indomethacin (Mark Sharp and Dohme, U.K) was given to the rats (20 mg/kg) by oral route at the end of the 24 h fast. This was followed 1 h later by the HCl/ethanol ulcer procedure as described earlier.

Indomethacin-induced gastric lesions

Experimental rats were manipulated as described earlier for the HCl/ethanol lesion induction method. Indomethacin (50 mg/kg) was given to the rats by oral route, 1 h after the animals had received 250 and 500 mg/kg of the plant extract. They were sacrificed another hour later and the degree of gastric lesion formation observed and scored.

Absolute ethanol-induced ulcers

The method described previously for the HCl/ethanol method was used, the only difference being that 1 ml of absolute ethanol was used as the necrotizing solution.

Effect of extract/honey on absolute ethanol-induced ulcers

Gastric lesions were induced using absolute ethanol. One hour prior to lesion induction, experimental animals were given distilled water (controls), 250 to 2500 mg/kg of pure honey, or a 1:1 mixture (500 mg/kg) of honey and *O. suave* extract. The animals were sacrificed another hour later and the degree of lesion formation observed and scored.

Pylorus ligated gastric secretion and ulceration in rats

The method of Shay et al. (1945) was used to study the ability of the extract to reduce gastric acid secretion as well as prevent gastric ulceration resulting from auto digestion by stomach secretions. The test rats received the extract or Cimetidine (Smith Kline and French) while the controls received distilled water (1 ml). One hour later, laparotomy was performed under light ether anesthesia, the pylorus of each rat was ligatured, and the abdominal incisions stitched up. The gastric juice produced during six subsequent hours was collected from each rat, the volume measured and 1 ml aliquots assayed for gastric acid content. On examination, the ulcers produced in the glandular region of the stomachs were measured and expressed according to the score described by Tan et al. (1996), and UI, %I, and %US were determined.

Induction of duodenal ulcers

Cysteamine-induced duodenal ulcers

Duodenal ulcers were induced in rats using a modification of the method described by Pallai and Santhakumari (1984). Female albinos rats (140 to 200 g) were kept on a normal laboratory diet. They were deprived of food 48 h prior to the experiment. They were given the aqueous extract of *O. suave* by oral route at the dose of 250 and 500 mg/kg. One hour later, the rats were each given four doses of cysteamine (200 mg/kg orally) at 2 h intervals. Forty-eight hours after the first dose of cysteamine, all the animals were sacrificed under light ether anesthesia and the abdomens were cut open. The duodenum of each was opened and the severity of the ulcers created was assessed by measuring the area covered by each ulcer.

Measurement of mucus production

The mucus covering of each stomach was gently scraped using a glass slide and the mucus weighed carefully using a sensitive digital electronic balance.

Measurement of gastric acidity

One ml of centrifuged gastric contents from each rat was assayed for hydrogen ion concentration by pH metric titration against 0.1 N NaOH using a digital pH meter. Gastric acidity was expressed as mEq/L.

Measurement of pepsin activity in gastric juice

Peptic activity of gastric juice obtained from pylorus ligated animals was determined by subjecting a solution of albumin (50 g/l) to the digestive action of the gastric juice. 2.5 ml of the albumin solution were incubated with 1 ml of gastric juice at 37°C for 2 h. The quantity of hydrolyzed protein was estimated using the Biuret method.

Statistical analysis

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

RESULTS AND DISCUSSION

The extract of *O. suave* had a dose-dependent *in vitro* activity against *H. pylori* (Table 1). Diameters of the inhibition zones increased from 8 to 33 mm with increasing quantities of extract (1.5 to 12 mg) in the well. The MAQ value was 1.5 mg while the MIC and MBC values were, respectively, 1.17 and 2.34 mg/ml. Given the major role that *H. pylori* plays in the aetiology of peptic ulcers, its eradication is strongly recommended as key for the effective management of these pathologies (Soll, 1996; European *H. pylori* Study Group, 1997). This justifies the current use of the triple therapy regimen which includes antibiotics, antacids and antisecretory

Table 1. Diameter (mm) of inhibition zones, minimum active quantities (mg), minimum inhibitory concentrations (mg/ml) and the minimum bactericidal concentrations (mg/ml) of *O. suave* water extract against *H. pylori* CCUG 39500.

Parameter	Diameter of inhibition							MAQ	MIC	MBC
	Quantity of the test product in the well									
	5 µg	10 µg	0.75 mg	1.5 mg	3 mg	6 mg	12 mg			
O. suave water extract	nd	nd	0	8	12	20	33	1.5 mg	1.170	2.340
Amoxicillin	nd	24	nd	nd	nd	nd	nd	nd	0.006	0.013
Metronidazole	28	nd	nd	nd	nd	nd	nd	nd	0.002	0.003

MIC: minimum inhibitory concentration, MBC: minimum bactericidal concentration, MAQ: minimum active quantity; nd: not done.

Table 2. Effect of the leaf aqueous extract of *O. suave* on mucus production and gastric lesions induced by HCl/ethanol solution in rats.

Treatment	Dose (mg/kg)	*N*	% ulcerated surface	Ulcer index (Mean ± SEM)	Inhibition (%)	Mucus production (mg)
Control	-	6	2.68	2.27 ± 0.67	-	65.55 ± 6.49
Extract	125	6	0.46	1.60 ± 0.67	29.5	82.60 ± 9.06**
Extract	250	6	0.14	1.20 ± 0.58*	47.1	99.88 ± 4.35**
Extract	500	6	0	0.00 ± 0.00*	100	101.99 ±6.39**
Sucralfate	100	6	0.44	1.55 ± 0.43	32.0	55.32 ±1.95

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

agents for the proper management of peptic ulcers. The development of a single drug endowed with both anti *Helicobacter* and cytoprotective properties will be a welcome contribution to peptic ulcer management since high cost and low patient compliance to multiple drugs are major setbacks of the triple therapy regimen. The *in vitro* anti-*Helicobacter* activity of the water extract of *O. suave* obtained in the present study (MAQ = 1.5 mg; MIC = 1.17 mg/ml; MBC = 2.34 mg/ml) was superior compared with that previously reported (Tan et al., 2006) for the methanol extract (MAQ = 2.5 mg; MIC = 1.56 mg/ml; MBC = 6.25 mg/ml), suggesting that the active anti-*Helicobacter* components may be more concentrated in the aqueous extract. These results explain, at least in part, the degree of efficacy that is reported when the aqueous extract is used in traditional medical practice for peptic ulcer disease management.

Table 2 shows the effect of the water extract on HCl/ethanol-induced gastric lesions. The extract dose-dependently inhibited lesion formation, with complete inhibition at the highest dose of 500 mg/kg. This was accompanied by an equally progressive increase in mucus production up to 35% for the highest extract dose compared with the controls. This protective effect dropped non significantly to 88% when the animals were pre-treated with indomethacin prior to HCl/ethanol treatment. The accompanying increase in mucus production also dropped non significantly from 101.99±6.39 to 96.74±3.32 mg with indomethacin pre-treatment (Table 3). In both cases, inhibition by sucralfate (32 and 24%) was poor although it significantly raised mucus production following pre-treatment with indomethacin. The lower dose of 250 mg/kg of the aqueous extract yielded barely 50% of the complete inhibition (100%) earlier observed with the methanol extract at the same dose, although mucus production (99.88±4.35) doubled compared with the methanol extract (50.44±0.74) (Tan et al., 2002). In addition, the presence of indomethacin significantly reduced the effectiveness of the methanol extract in the previous study. These findings reveal that even with crude plant extracts, the type of vehicle significantly influences the efficacy of the product and the mode or mechanism of action of the extract. Thus, the water extract does not confer cytoprotection through the mediation of endogenous prostaglandins in contrast to the methanol extract. Usually, subcutaneous injection of indomethacin reduces bicarbonate and PG secretion, predisposing the gastro duodenal mucosa to damage by irritant substances (Miller, 1982; Konturek et al., 1982; Robert, 1983). Table 4 shows that when indomethacin was administered alone by oral route, the poorest degree of gastric protection (0.4 to 20%) was obtained both for the extract and sucralfate, ulcer index scores (2.00±0.50 to 2.49±0.21) remaining close to control values (2.50±0.01). Instead of the characteristic striated HCl-and and pointed inflammations with raised borders. The lesions were widely scattered and varied in size. Mucus production was also poor compared to values obtained

Table 3. Effect of the leaf aqueous extract of *O. suave* on gastric lesions induced by HCl/ethanol solution in rats pre-treated with indomethacin.

Treatment	Dose (mg/kg)	*N*	% ulcerated surface	Ulcer index (Mean ± SEM)	Inhibition (%)	Mucus production (mg)
Control	-	6	3.58	3.30 ± 0.31	-	57.09±4.13
Extract	250	6	0.97	1.60 ± 0.66*	51.5	75.73± 4.30*
Extract	500	6	0.6	0.40 ± 0.01*	87.9	96.74± 3.32**
Sucralfate	100	6	1.13	2.52 ± 0.21	23.6	70.73± 3.30*

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

Table 4. Effect of the leaf aqueous extract of *O. suave* on gastric lesions induced by oral indomethacin in rats.

Treatment	Dose (mg/kg)	*N*	% ulcerated surface	Ulcer index (Mean ± SEM)	Inhibition (%)	Mucus production (mg)
Control	-	6	1.85	2.50 ± 0.01	-	47.98 ± 7.23
Extract	250	6	0.62	2.08 ± 0.52	16.8	56.23 ± 7.95
Extract	500	6	0.29	2.00 ± 0.50	20.0	58.82 ± 1.38
Sucralfate	100	6	1.23	2.49 ± 0.21	0.40	48.97 ± 4.92

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

Table 5. Effect of the leaf aqueous extract of *O. suave* on mucus production and gastric lesions induced by absolute ethanol solution in rats.

Treatment	Dose (mg/kg)	*N*	% ulcerated surface	Ulcer index (Mean ± SEM)	Inhibition (%)	Mucus production (mg)
Control	-	6	16.57	5.75 ± 0.41	-	58.50 ± 2.01
Extract	250	6	6.35	4.38 ± 0.33	23.8	81.33 ± 7.08**
Extract	500	6	3.55	2.21 ± 0.98**	61.5	95.22 ± 11.21**
Sucralfate	100	6	6.48	4.4 0± 0.28*	23.5	28.55 ± 0.98

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

with the other irritants even though extract-treated animals had higher but non-significant values compared with the controls. The direct necrotic effect of indomethacin on the gastric mucosa is more severe compared to the parenteral effects. In addition to reduced gastric mucosal blood flow, reduced bicarbonate and PG secretion, indomethacin also inactivates gastric peroxidase to induce reactive oxygen-mediated gastric mucosal injury (Chattopadhyay et al., 2006). Indomethacin-induced oxidative damage by reactive oxygen species can be demonstrated by increased lipid peroxidation and thiol depletion. Indomethacin causes nearly a fivefold increase in hydroxyl radical (•OH) and significant inactivation of gastric mucosal peroxidase to elevate endogenous H_2O_2 and H_2O_2-derived •OH (Chattopadhyay et al., 2006). Indomethacin also reduces superoxide dismutase and glutathione S-transferase (Halici et al., 2005). The results indicate that the extract of *O. suave* does not efficiently counteract the mechanism of oxidative damage at the higher dose of 500 mg/kg. The water extraction procedure might have been incapable of yielding sufficient concentrations of some vital ingredients.

Table 5 shows that both the extract and sucralfate were poor inhibitors of lesion formation when the gastric environment was challenged with absolute ethanol solution, the 500 mg/kg dose of extract yielding only 61% inhibition. Although mucus production in absolute ethanol medium was high with extract treatment (81 to 95 mg), mucus yield with sucralfate (28 mg) was significantly low. In all the three preceding experiments, the characteristic striped lesions that resulted from HCl and ethanol treatment were observed. Considering the highly aggre-the activity of antioxidant enzymes such as catalase,

Table 6. Effect of extract and honey mixture on absolute ethanol-induced gastric lesions.

Treatment	Dose (mg/kg)	*N*	% ulcerated surface	Ulcer index (Mean ± SEM)	Inhibition (%)	Mucus production (mg)
Control	-	5	16.6	5.75 ± 0,41	-	58.50 ± 2.01
Honey	250	4	8.51	4.29 ± 0.15	25.4	112.75 ± 2.49
Honey	500	4	5.1	3.76 ± 0.36	34.0	130.01 ± 19.57
Honey	2500	4	0	0.00 ± 0.00**	100	250.00 ± 17.79
Honey + *O. suave*	500	4	2.9	2.90 ± 0.16*	50.3	142.50 ± 21.74

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

Table 7. Effect of the leaf aqueous extract of *O. suave* on pylorus-ligated gastric ulceration in rats.

Treatment	Dose (mg/kg)	*N*	% ulcerated surface	Ulcer index (Mean ± SEM)	Inhibition (%)	Mucus production (mg)
Control	-	6	1.90	3.32 ± 0.23	-	52.99 ± 4.66
Extract	250	6	0.45	2.12 ± 0.54	36.1	76.13 ± 4.86*
Extract	500	6	0.19	0.70 ± 0.48**	78.9	115.67 ± 2.85*
Cimetidine	200	6	0.25	1.01 ± 0.20**	69.6	122.00 ± 8.80*

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

ssive nature of absolute ethanol and the complicated physiological events involved in the pathogenesis of ethanol-induced gastric mucosal damage (Oates and Hakkinen, 1988), it is evident that the cytprotection offered by the extract goes beyond simple acid neutralization when the HCl/ethanol mixture is used. Gastric mucosal damage by absolute ethanol can attain mucosal as well as sub mucosal tissues. In all the experimental models of gastric irritation employed, sucralfate yielded lower lesion inhibition levels and mucus production compared with the extract of *O. suave*.

In Cameroon, the aqueous extract of *O. suave* is taken in combination with honey for complaints symptomatic of peptic ulcer disease. Honey is used in many traditional herbal preparations to veil the bitter taste that often reduces patient compliance. However, the gastric cytoprotective effects of natural mono- and polyfloral honey against lesions induced by absolute ethanol, indomethacin and acidified acetyl salicylic acid have been demonstrated (Gharzouli et al., 1999, 2001, 2002). In one experiment (Gharzouli et al., 2002), protection of the stomach elicited by both types of honey was almost total against ethanol-induced lesions. When honey was administered alone at the dose of 500 mg/kg in the present experiment, it inhibited ethanol-induced lesions by 35% compared to the extract of *O. suave* (62%) at the same dose. However, when the 500 mg/kg dose composed of equal amounts of honey and *O. suave* extract were given to the animals, the level of inhibition increased to 50% (Table 6) compared to 24% for the 250 mg/kg of *O. suave* alone (Table 5) and 25% for the 250 mg/kg of honey alone (Table 6). Complete inhibition was achieved when honey was given alone at 2500 mg/kg, a dose which represents 175 g of honey per day for a 70 kg adult human. Although the results of the present experiment indicate that the water extract of *O suave* can act in synergy with honey to produce good antiulcer effects at low doses, it is evident that *O. suave* extract alone is more potent. Research findings suggest that the mechanism of action of natural honey against ethanol-induced gastric lesions may involve sulfydryl-sensitive processes. While preventing gastric mucosal damage, natural honey can reverse the changes in gastric pH induced by ethanol (Ali, 1991). The use of honey in many traditions for open wound treatment suggests its possible usefulness in the treatment of deep-seated gastroduodenal ulcer craters.

In Shay-ligated rats, a dose-dependent inhibition of gastric ulceration was observed (36 to 79%), accompanied by a highly significant increase in mucus production compared with the controls. Mucus production values obtained at the extract dose of 500 mg/kg (116 mg) and with cimetidine (122 mg) were more than double the control values (53 mg) (Table 7). Although the volume of gastric juice did not differ much between extract-treated and control rats, the acidity of the gastric juice rose significantly (91 mEq/l) at the extract dose of 500 mg/kg compared with the controls (68 mEq/l) (Table 7). However, the degree of pepsin activity in the gastric juice (59 to 66%), indexed by the percentage of hydrolyzed incubated protein, did not differ between the control and extract-treated rats (Table 8), but lesion index dropped

Table 8. Effect of the leaf aqueous extract of *O. suave* on gastric secretion and mucus production in rats.

Treatment	Dose (mg/kg)	*N*	Gastric contents (ml) (Mean ± SEM)	Gastric acidity (mEq/l)	Pepsin activity (% hydrolysed protein)
Control	-	6	6.31 ± 0.47	67.8 ± 1.9	66.74 ± 2.38
Extract	250	6	7.36 ± 0.36	62.2 ± 4.0	66.42 ± 4.12
Extract	500	6	5.30 ± 0.53	91.1 ± 3.3*	59.27 ± 4.26
Cimetidine	200	6	8.55 ± 1.52	80.2 ± 2.1*	60.45 ± 3.34

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

Table 9. Effect of the leaf aqueous extract of *O. suave* on cysteamine-induced duodenal ulcers in rats.

Treatment	Dose (mg/kg)	*N*	Number of ulcer craters	Ulcer index (mm^2 ± SEM)	Inhibition (%)
Control	-	6	5	16.60 ± 5.49	-
Extract	250	6	3	7.20 ± 3.38*	56.6
Extract	500	6	3	7.60 ± 3.70*	54.2

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

from 3.32±0.23 in the controls to 0.70±0.48 at the extract dose of 500 mg/kg (Table 7). Cimetidine also maintained high gastric acid levels (Table 8). The results indicate that the extract of *O. suave* has no antisecretory effects similar to H_2 receptor blockers of histamine. The accumulated gastric acidity due to pylorus ligation can cause auto digestion of the superficial mucous layer and expose the underlying tissues to the digestive action of gastric pepsin. Mucus production at 500 mg/kg of extract which is more than doubled compared with the control values, must have been responsible for the physical cytoprotective effect of the extract. We did not observe any increase in the total protein content of mucus samples to suggest a chemical effect. Although many experiments have demonstrated that gastric acid levels between 40 and 65 mEq/l following Shay ligation usually lead to severe gastric mucosal ulceration (Martin et al., 1993; Tan et al., 1996), plant extracts have also been shown to prevent gastric mucosal ulceration in highly acidic gastric environments (Akhtar et al., 1995; Tan et al., 2000). The low pH levels that are maintained by the high acidity can be useful during protein digestion by pepsin, thus avoiding the constipation that is a common side effect brought about by acid-neutralizing antacids and H_2 receptor blockers of histamine (Feldman and Burton, 1990). The unexpected inability of cimetidine to show its well-known antisecretory effect has been a common observation in our laboratory.

Repeated administration of cysteamine to the experimental rats produced ulcer craters in the duodenum with perforations into the peritoneal cavity in some cases (Table 9). The ulcer craters were similar in appearance to acetic acid-induced chronic gastric ulcers, and cases of perforation were limited to the controls. The number of ulcer craters reduced from 5 in the controls to 3 in the extract-treated animals, and the surface areas reduced significantly from 16.6 mm^2 in the controls to 7.6 mm^2 at the highest extract dose. Duodenal ulcers are four times more common than gastric ulcers even though the ulcerating acid and pepsin are secreted in the stomach (Grossman, 1981). Redox-dependent regulatory mechanisms are involved in the early stages of duodenal ulceration, and cysteamine is a reducing agent which markedly increases the redox status in duodenal mucosa. Cysteamine has been shown to reduce duodenal mucosal oxygenation by 19% compared with baseline values (Khomenko et al., 2004). The mode of action of *O. suave* extract may therefore involve increased mucosal blood flow and oxygenation or by mechanisms which lower the redox status of duodenal mucosa. Cysteamine also inhibits the release of somatostatin and induces duodenal ulcers by a mechanism which may be associated with increased plasma ghrelin levels (Fukuhara, 2005). The mode of action of *O. suave* extract may also involve a beneficial effect on somatostatin secretion.

Although the cytoprotective mechanisms of the extract differ from one experimental procedure to the other, it was noted that the EC_{50} values (250 mg/kg for the HCl/ethanol, HCl/ethanol-indocid, cysteamine methods; 316 mg/kg for pylorus ligation; 400 mg/kg for absolute ethanol method) were reasonably similar. We have carried out acute and sub-acute toxicity tests, as well as teratogenic studies on the water extract of *O. suave.* The extract was not toxic in acute study up to 5000 mg/kg. Because the non-toxic nature of the extract in teratogenic

and sub chronic study (250 to 1000 mg/kg) was well supported by haematological analysis and blood biochemical data, we have proposed that the 500 mg/kg dose may be used in phytomedical formulations with a low risk of adverse effects. Details of toxicity studies are published elsewhere (Tan et al., 2008).

The qualitative phytochemical tests revealed the predominant presence of flavonoids, polyphenols and triterpenes in *O. suave* extract, as well as the presence of small amounts of sugars and compounds with multiple bonds (unpublished data). The extract has also shown significant histological healing of chronic ulcers, and in the cold/restraint-induced oxidative stress model, it raised tissue levels of reduced glutathione, superoxide dismutase and catalase. *In vitro*, its high phenolic content is associated with significant DPPH radical scavenging activity (89.29%) and FRAP (antioxidant capacity) (212.64 mg/g catechin equivalent) (unpublished data).The preventive antioxidant and anti-inflammatory roles of flavonoids and polyphenols are well known (Herto et al., 1993; Favier, 2003), and this may explain, at least in part, the absence of toxic effects (Tan, 2008) as well as the cytoprotective effects observed in the present experiment. This study confirms the positive healing effects derived from *O. suave* in traditional use for the management of peptic ulcer disease. The water extract possesses both anti-*Helicobacter* and gastro-duodenal cytoprotective effects, and may be useful in an antiulcer regimen without antimicrobial, antacids and antisecretory agents.

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