Cytoprotective and Antioxidant Properties of the Stem Bark Aqueous extract of *khaya grandifoliola* (Meliaceae) in Rats

ABSTRACT

Aims: To evaluate the qualitative chemical composition of the aqueous extract of the stem bark of *Khaya grandifoliola* and test the antiulcer actions on gastric lesions induced by HCl/Ethanol, HCl/Ethanol/Indomethacin, indomethacin, absolute ethanol, cold/restraint stress and pylorus ligation in experimental Wistar rats.

Study design: Random allocation of male rats to groups of five rats each.

Place and Duration of Study: Department of Animal Biology and Physiology, Animal Physiology Laboratory (Gastroenterology Unit), University of Yaoundé 1.

Methodology: Gastric ulcers were produced in the glandular regions of rat stomachs using standard models of gastric ulcer induction. Ulcers produced were scored and mucus production and the severity of ulceration were compared between control groups and those given the plant extract or reference drugs. Oxidative stress parameters (superoxide dismutase (SOD), malondialdehyde (MDA), reduced glutathione (GSH), catalase (CAT)) were measured in tissue samples of rats subjected to the cold/restraint stress method.

Results: Phenols, saponinins, flavonoids, proteins, acids, anthocyanins, tannins, alkaloids, ketones, sugars, coumarins, quinones, and amino acids were among the phytochemicals detected. The extract (250–500 mg/kg) inhibited the formation of gastric ulcers and significantly reduced the ulcer index in all models used (81.8 % ($p < 0.001$) with HCl/ethanol; 88.2 % ($p < 0.001$) with absolute ethanol; 100 % ($p < 0.05$) with HCl/ethanol/indomethacin; 72.6 % with cold/restraint stress ulcers, and 69.6 % ($P<0.01$) with pylorus ligation at the highest dose of 500 mg/kg. Gastric acidity significantly ($p<0.01$) dropped from 88 mEq/L in the controls to 34 mEq/L at the dose of 500 mg/kg. In cold/restraint-induced stress, *K. grandifoliola* (500 mg/kg) lowered the increased levels of malondialdehyde (MDA) from 2.90 (control group) to 0.46 nmol/g tissue. The reduced levels of catalase were also significantly improved in rats treated with extract.

Conclusion: *K. grandifoliola* aqueous extract possesses gastric antisecretory potential. Its cytoprotective activity can be attributed to its ability to increase the antioxidant status and to enhance gastric mucosal defense possibly through the mediation of endogenous prostaglandins.

Keywords: Khaya grandifoliola, gastric ulcer, cytoprotection, antioxidant activity.

1. INTRODUCTION

Gastric ulcers are caused by the creation of an imbalance between gastric mucosal integrity and aggressive factors. For the maintenance of mucosal integrity, different therapeutic agents, including plant extracts, are used to inhibit gastric acid secretion or to stimulate the mucosal defense mechanism by increasing the mucosal production of mucus, bicarbonate, endogenous prostaglandins and surface epithelial cells [1]. Various factors can contribute to the formation of gastric ulcer including infection of the stomach by *Helicobacter pylori* [2] and the frequent use of nonsteroidal anti-inflammatory drugs (NSAIDs) [3]. In the West, peptic ulcer disease frequently touches 8 to 10 persons out of 100 residents [4]. The introduction of endoscopy in Africa at the beginning of the 1980s helped to reveal the high degree of prevalence of the disease in the pathology of the black Africans [5], and the prevalence of gastric ulcers in Cameroon has been estimated at about 31.65 % [6]. The success of commercially available antiulcer drugs in the treatment of gastric ulcer is usually overshadowed by various side effects. For example, H_2-receptor antagonists like cimetidine may cause gynecomasia in men and galactorrhea in women [7] while proton-pump inhibitors (e.g. omeprazole and lanzoprazol) can cause nausea, abdominal pain, constipation and diarrhea [8, 9]. Due to these side effects, there is a need to find new antiulcer compounds with potentially less or no side effects and medicinal plants have always been the main source of new drug candidates for the treatment of gastric ulcer [10, 11].

Khaya grandifoliola (WELW) C.DC. (Meliaceae) is also called African Mahogany, Benin Mahogany, Large-leaved Mahogany, Senegal Mahogany. The species occurs in all of inter-tropical Africa (Benin, The Democratic Republic of the Congo, Ivory Coast, Ghana, Guinea, Nigeria, Sudan, Cameroun, Togo and Uganda) at the transition zone between dense forest and savanna [12]. This important timber species, commonly confused with *Khaya anthotheca,* occurs more frequently in dry semi-deciduous forest and forest outliers than *K. anthotheca. K. grandifoliola* is classified under the Red List Category & Criteria as "Vulnerable A1 cd". It has been threatened by comprehensive exploitation of mature stands from subpopulations as well as by its poor regeneration capacity. For these reasons, various countries have created protected subpopulations and continue to enforce log export bans [13].

K. grandifoliola is used in Cameroonian folk medicine for the treatment of pneumonia, intestinal helminthiasis [1], hepatitis and other liver related-diseases [14, 15]. The stem bark extract is used in Nigeria as an anticonvulsivant [16]. Bark extracts of various species of the genus *Khaya* are used in West African ethnomedicine to treat fever, cough, lumbago, rheumatism, stomach ache gastric pains, and diarrhea in horses and camels [17]. Limonoids obtained from *K. grandifoliola* [18] were shown to be

responsible for the antimalarial activity of the stem bark extract [19], whose schizozonticidal activity in early *Plasmodium berghei berghei* infection in mice had earlier been demonstrated [20]. The bark extract of *K. grandifoliola* enhanced the antiplasmodial effects of two commercialized antimalarial drugs, halofantrine and chloroquine, in a mouse model of *Plasmodium yoelii nigerense* [21]. The n -hexane extract, the crude and purified fractions from *K. grandifoliola* bark gave significant (91%) chemosuppression of a multi-drug resistant clone of *Plasmodium berghei berghei in vivo* and significant *in vitro* antiplasmodial activities against Nigerian *P. falciparum* isolates [19]. The bark extract of *K. grandifoliola* has been shown to possess antiinflammatory [22], antioxidant [23], anti-insecticidal [24], hepatoprotective [25] and antimicrobial activity against bacterial isolates of *Bacillus subtilis, Klebsiella pneumonae and Proteus mirabilis* [26]. The effects of the bark extract on red blood cells and bone mineral content in rats [27] and on some biochemical parameters in rats [28] have also been demostrated. Analysis of the proximate, phytochemical and mineral element composition of *K. grandifoliola* revealed that the bark extract is rich in proteins, carbohydrates, minerals such as magnesium, calcium, sodium, potassium, magnesium, iron and manganese, as well as in secondary metabolites including saponins, tanins, alkaloids, anthraquinones, flavonoids, reducing sugars and phlobatanins [29]. Previous work has shown the antisecretory potential, and the cytoprotective activity of the bark methanol extract of a sister species (*K. senegalensis*) against absolute ethanol-induced gastric lesions [30]. Although *K. grandifoliola* was not cited for its antiulcer potential by the OAU/STRC-sponsored ethnobotanical survey in Cameroon [12], the plant is well known in the Bamoun area (local name, *Fah, Faturtu, Fatiti*) for its usefulness in the treatment of peptic ulcers. In the present study, we evaluated the cytoprotective and antioxidant actions of the decoction of *K. grandifoliola* against various ulcerogens. The possible modes of action of the extract are discussed in relation to the pathogenic mechanisms of action of the various necrotizing agents use.

2. MATERIAL AND METHODS

2.1 Preparation of plant extract

The fresh stem-bark of *K. grandifoliola*, was collected in Mbokam village (Jakiri) in the North West Region of Cameroon. Botanical identification was done at the National Herbarium in Yaoundé by comparison with existing herbarium specimen No. PM 098 /95. The fresh bark was cut up, dried and ground to a powder. 1 kg of the dried material was boiled in 5 liters of water for 30 minutes. The extract solution was filtered through four layers of cheesecloth, then through Whatman filter paper No. 3. The resulting extract solution was evaporated at 40°C using a convection air oven (Jencons-PLS, UK) to obtain 66.35 g of a red powder. The extract re-dissolved readily in distilled water which was used as the vehicle.

2.2 Animals

Male Wistar rats (147–180 g) raised on a standard laboratory diet and tap water in the animal house of the Faculty of Science, University of Yaounde 1, were used. 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).

2.3 Phytochemical tests

Phytochemical tests for the major metabolites of the extract were performed. The aqueous extract of *K. grandifoliola* was screened for the presence of biologically active compounds such as tannins, alkaloids, saponins, flavonoids, anthocyanins, phenols, quinones, coumarins, sterols, triterpenoids, glycosids and proteins. Based on the intensity of coloration, the lather or the precipitate formed during the test, secondary metabolite proportions were characterized as present (++) or weakly present (+) when the test result was positive, and absent (–) when the test result was negative.

2.4 Induction of gastric ulcers

2.4.1 HCl/ethanol-induced gastric lesions in rats

The rats were deprived of food for 36 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 [31]. The animals received the extract by oral route, 1 h before they were given the necrotizing solution. Positive control rats received Sucralfate in place of the extract. They were killed another hour later 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, and the ulcer index (UI), percentage of inhibition (% I) and percentage of ulcerated surface (%US) were calculated [32].

2.4.2 Absolute ethanol-induced gastric lesions

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.

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

Indomethacin was given to the rats (20 mg/kg) by intra peritoneal route at the end of the 24 h fast. This was followed 1 h later by the HCl/ethanol ulcer procedure as described above.

2.4.4 Indomethacin-induced gastric lesions

The animals were deprived of food for 36 hours. The vehicle and the extract (250 and 500 mg/kg) were given to them 3 times at 12-hour intervals. Indomethacin (50 mg/kg) was given to the rats by oral route 1 hour after the animals received the last administration of the plant extract and vehicle. They were sacrificed another hour later and the ulcers produced in the glandular region of the stomachs were measured and expressed according to the score described by [32]. Petechial lesions were counted and every five lesions were taken as 1 mm of ulcer [33].

2.4.5 Pylorus ligated gastric secretion and ulceration in rats

The method of Shay et al. [34] 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, while the controls received distilled water (1ml) or Cimetidine. One hour later, laparotomy was performed under 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 kept for gastric acid measurement. The ulcers produced in the glandular region of the stomachs were measured and ulcer index, % of inhibition, and % of ulcerated surface were determined.

2.4.6 Cold stress-induced gastric lesions

Stress-induced gastric ulcers were provoked in rats using a slight modification of the method earlier described by [35]. The animals were deprived of food for 36 hours (but not water deprivation). Test rats were given the extract (250 and 500 mg/kg) by oral route while control rats received the vehicle or Cimetidine three times at 12-hour intervals. One hour later, after the last administration of vehicle or extract, the rats were placed in small individual wire cages and immersed in cold water (20 ± 1°C), up to the level of the xiphoid. Three hours later blood samples were taken and the animals were sacrificed using ether and the stomachs removed. The same protocol used with the indomethacin model for the assessment of lesion formation was performed. Blood and gastric tissue samples were taken, prepared and preserved frozen for the measurement of different oxidative stress parameters.

2.5. Measurement of mucus production

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

2.6 Measurement of *in vivo* antioxidant capacity

Blood and gastric tissue samples were taken and prepared for the measurement of different oxidative stress parameters: Cellular glutathione (GSH) was measured 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 412 nm [36]. The glutathione concentration was calculated using the molar extinction coefficient ε= 1.36 104 M-1 cm-1. Superoxide dismutase (SOD) activity was measured using a standard method [37], and expressed in U/mg of protein, while catalase was determined [38] and expressed as mM of H_2O_2/min/mg of protein, and tissue protein was measured using the Biuret method of protein assay. Lipid peroxidation was assessed by measuring the levels of malondialdehyde [39]. Quantification of MDA was done using an extinction coefficient of ε = 1.56 105 M-1 cm-1.

2.7 Statistical analysis

The data were analyzed using the one way analysis of variance (ANOVA) followed by the student-Newman- Keuls test. *P* values <.05 were considered significant. Values in tables are given as arithmetic means ± standard error of the mean (S.E.M.)

3. RESULTS

3.1 Phytochemical screening

Phytochemical screening of the bark extract of *K. grandifoliola* revealed the presence of many phytoconstituents. These included phenols, saponinins, flavonoids, proteins, acids, (+++), anthocyanins (++), tannins, alkaloids, ketones, sugars, coumarins; quinines, and amino acids (+). Oils, sterols, triterpenoids, glycosides and resins (-) were absent.

3.2 Anti-ulcer activity

The effects of HCl/ethanol-induced gastric lesions in rats are shown in Table 1. Control rats developed hemorrhagic lesions in the glandular portions of their stomachs 1 hour after induction of the lesions. *K. grandifoliola* (250–500 mg.kg) prevented the formation of gastric lesions, inhibition attaining 81.8 % at the dose of 500 mg/kg. Sucralfate (100 mg/kg) prevented lesion formation by 30.5%. Mucus production increased from 85.4 mg in the controls to 105.9 mg for Sucralfate and 129.4 mg for the highest dose of extract.

Table 1. Effect of *K. grandifoliola* extract on HCl/ethanol-induced gastric lesions in rats.

Treatment	***Dose (mg.kg)***	***N***	***Ulcer index***	***% ulcerated surface***	***Inhibition %***	**Mucus production (mg)**
Control	-	5	4.03 ± 0,13	5.29	-	85.41 ± 5.55
K. grandifoliola	250	5	2.25 ± 0.33*	2.87	44.16	119.8 ± 20.90*
K. grandifoliola	500	5	0.73 ± 0.045**	0.43	81.77	129.4 ± 9.23*
Sucralfate	100	5	2.80 ± 0.97*	1.13	30.45	105.9 ± 12.17*

*Statistically different relative to control; **P<0.01; N, number of rats. The values are expressed as mean± SEM.*

Table 2 shows that pre-treatment with indomethacin followed by HCl/ethanol significantly increased the ulcerated surface area (7.3%) compared with the HCl/ethanol treatment alone (5.3%). Ulcer index reduced significantly from 4.04 ±0.13 for the vehicle control to 2.99 ± 0.09 for the maximal dose of extract. Although per cent inhibition of ulcer in all the extract-treated groups dropped considerably compared with those obtained with the HCl/ethanol model, the cytoprotection was accompanied by significant increase in mucus production, from 70.03 mg in the vehicle control to 103 mg for the highest dose of extract.

Table 2. Effect of *K. grandifoliola* extract on HCl/ethanol-induced gastric lesions in rats pre-treated with indomethacin.

Treatment	***Dose (mg.kg)***	***N***	***Ulcer index***	***% ulcerated surface***	***Inhibition %***	**Mucus production (mg)**
Control	-	5	4.04 ±0.13	7.32	-	70.03 ± 9.87
K. grandifoliola	250	5	3.13 ± 0.09*	6.42	23.32	79.96 ± 18.37
K. grandifoliola	500	5	2.99 ± 0.09**	5.36	25.94	103.30 ± 11.11**
Sucralfate	100	5	2.55 ± 0.33***	4.18	36.98	59.40 ± 6.81

*Statistically different relative to control; *P<0.05; **P<0.01; ***P<0.001; N, number of rats. The values are expressed as mean ± SEM.*

Table 3 shows that the extract significantly prevented gastric lesions induced by absolute ethanol, with 88.2% protection at the maximal dose, (ulcer index 0.48 ± 0.30, compared with 4.08 ± 0.29 for the negative control).

Table 3. Effect of *K. grandifoliola* extract on absolute ethanol-induced gastric lesions in rats.

Treatment	***Dose (mg.kg)***	***N***	***Ulcer index***	***% ulcerated surface***	***Inhibition %***	**Mucus production (mg)**
Control	-	5	4.08 ± 0.29	6.53	-	95.96 ± 4.34
K. grandifoliola	250	5	1.20 ± 0.51***	0.43	70.57	82.00 ± 3.74
K. grandifoliola	500	5	0.48 ± 0.30***	0.07	88.23	86.00 ± 9.27
Sucralfate	100	5	2.43 ± 0.47*	1.44	40.31	77.44 ± 10.32

*Statistically different relative to control; *P<0.05; ***P<.001; N, number of rats. The values are expressed as mean ± SEM*

Treatment with indomethacin produced lesions in the stomach glandular region (ulcer index, 2.87 ± 0.60) of control rats (Table 4). Extract administration significantly protected the glandular stomach against indomethacin-induced lesions (inhibition, 79 and 100% for the 250 and 500 mg/kg doses, respectively). Mucus production increased significantly with Cimetidine and was poor with extract doses compared to the values obtained with HCl/ethanol and HCl/ethanol-Indomethacin pre-treatment.

Table 4. Effect of *K. grandifoliola* extracts on Indomethacin-induced gastric lesions in rats.

Treatment	***Dose (mg.kg)***	***N***	***Ulcer index***	***% ulcerated surface***	***Inhibition %***	**Mucus production (mg)**
Control	-	5	2.87 ± 0.60	0.86	-	26.01 ± 5.10
K. grandifoliola	250	5	0.47 ± 0.29***	0.13	79.07	16.0 ± 1.40
K. grandifoliola	500	5	0.00 ± 0.00***	0.00	100	42.0 ± 3.74
Sucralfate	100	5	0.20 ± 0.20***	0.003	93.02	54.0 ± 7.90**

*Statistically different relative to control; ***p<0.001; N, number of rats. The values are expressed as mean ± SEM.*

Tables 5 and 6 show the results obtained using the pylorus ligation ulcer induction method. *K. grandifoliola* aqueous extract protected the stomachs against lesions with a protection percentage of 65.31 and 72.9 at the 250 and 500 mg/kg dose, respectively. The cytoprotection was accompanied by a significant decrease of ulcer indices at all the doses of *K. grandifoliola* extract, and increase in mucus protection from 30.76 ± 0.01mg (control) to 57.86 ± 0.23 and 60.25 ± 0.22 mg, respectively, for the 250 and 500 mg/kg doses (Table 5). In comparison with the negative control, the volume of gastric juice (3.21 ± 1.37 mL) did not change significantly with extract and Cimetidine administration, but gastric acidity significantly ($p<0.01$) dropped from 88 mEq/L in the controls to 34 mEq/L for Cimetidine and the 500 mg/kg dose of extract (Table 6).

Table 5. Effect of *Khaya grandifoliola* extract on pylorus-ligated gastric ulceration in rats.

Treatment	***Dose (mg/kg)***	*N*	***Ulcer index***	***% ulcerated surface***	***Inhibition %***	***Mucus production (mg)***
Control	-	5	3.95 ± 0.28	1.29	-	30.76 ± 0.01
K grandifoliola	100	5	1.68 ± 0.00**	0.59	57.47	43.92 ± 0.49**
K grandifoliola	250	5	1.54 ± 0.04**	0.59	61.01	57.86 ± 0.23**
K grandifoliola	500	5	1.20 ± 0.4**	0.19	69.62	60.25 ± 0.22**
Cimetidine	50	5	1.50 ± 0.61**	0.31	62.02	88.80 ± 0.13**

*Statistically different relative to control; **p<0.01; N, number of rats. The values are expressed as mean± SEM.*

Table 6. Effect of *Khaya grandifoliola* extract on gastric secretion in pylorus-ligated rats.

Treatment	*Dose (mg/kg)*	*N*	*gastric pH*	*Gastric contents (ml)*	*Gastric acidity (mEq/L)*
Control	-	5	2.59 ± 0.14	5.38 ±0.33	88.80 ± 0.13
K grandifoliola	100	5	2.85 ± 0.01	4.42 ± 0.39	78.5 ± 0.50
K grandifoliola	250	5	2.92 ± 0.01	4.36 ± 0.46	71.00 ± 2.45
K grandifoliola	500	5	4.44 ± 0.02**	3.61 ± 0.22**	34.00 ± 1.78
Cimetidine	50	5	4.30 ± 0.34**	4.2± 0.21	35.75 ± 0.58

*Statistically different relative to control; **p<0.01; N, number of rats. The values are expressed as mean± SEM.*

The effects of subjecting the rats to a combination of restraint and cold stress are shown in Table 7. Control rats developed many lesions in the glandular portions of their stomachs 6 hours after cold water immersion. *K. grandifoliola* extract (250–500 mg/kg) prevented the formation of gastric lesions, inhibition attaining 72.6% at the dose of 500 mg/kg. Cimetidine (50 mg.kg) prevented lesions formation by 53.8%.

Table 7. Effect of *K. grandifoliola* extract on cold/restraint stress-induced gastric lesions in rats.

Treatment	*Dose (mg.kg)*	*N*	*Ulcer index*	*% ulcerated surface*	*Inhibition %*	**Mucus production (mg)**
Control	-	5	1.44 ± 0.21	0.63	-	55.91 ± 1.69
K. grandifoliola	250	5	1.00 ± 0.27	0.10	31.60	57.86 ± 8.03
K. grandifoliola	500	5	0.40 ± 0.24*	0.01	72.64	63.60 ± 3.72
Cimétidine	50	5	0.68 ± 0.28	0.31	53.76	71.02 ± 8.10

*Statistically different relative to control; *P<0.05; N, number of rats. The values are expressed as mean ± SEM.*

Table 8 shows that subjection of the rats to cold restraint stress significantly decreased antioxidant enzyme concentrations (GSH and SOD) compared with controls. Treatment with extract and cimetidine did not prevent the drop in the concentration of these enzymes. The cold stress method reduced catalase enzyme levels from 5.13 ± 0.90 µmol H_2O_2/min/mg of protein in normal rats to 4.39 ± 0.59 µmol H_2O_2/min/mg of protein. The highest dose of extract raised catalase concentrations to above normal values. The high MDA concentrations (2.90 ± 0.44 mmol/g protein $.10^{-6}$) created by the stress method were significantly lowered in all extract-treated groups.

Table 8. Effect of *K. grandifoliola* extract on oxidative stress parameters in stomach tissues of rats subjected to cold/restraint stress-induced gastric lesions.

Treatment	*Dose (mg/kg)*	*N*	*SOD (U/mg protéine)*	*Catalase (µmol H_2O_2/min/mg of protein)*	*GSH (mol/g protein . 10^{-4})*	MDA (mmol/g protein .10^{-6})
Normal rats	-	5	8.26 ± 1.020	5.13 ± 0.90	6.99 ± 0.12	2.26 ± 0.19
Control	-	5	4.55 ± 0.003	4.39 ± 0.59	2.52 ± 0.56	3.30 ± 0.02
K. grandifoliola	250	5	4.56 ± 0.002	6.63 ± 0.17	2.61 ± 0.18	0.65 ± 0.18***
K. grandifoliola	500	5	4.56 ± 0.002	11.94 ± 1.66**	2.74 ± 0.31	0.46 ± 0.01***
Cimétidine	50	5	4.55 ± 0.003	7.12 ± 2.20	2.27 ± 0.30	1.05 ± 0.33**

*Statistically different relative to control; **p<0.01; ***p<0.001; N, number of rats. The values are expressed as mean± SEM.*

4. DISCUSSION

The present experiments were designed to validate the folk use of *K. grandifoliola* in the management of gastric ulcer, and to suggest possible modes of its cytoprotective action. Peptic ulcer and gastritis have been associated with multipathogenic factors that disturb the natural equilibrium between endogenous mucosal defense mechanisms and the mucosal aggressive factors (acid and pepsin). Experimental ulcerogenic models involving alcohol, HCl hypersecretion, NSAIDs and stress are therefore designed to tip the equilibrium in favour of gastric ulcer generation [40, 41], and the ability of candidate antiulcer agents to attenuate and possibly block the gastric acid secretion or to enhance the mucosal defense mechanisms are then tested. The results presented here show that the aqueous extract of *K. grandifoliola* protected the gastric mucosa against damage induced by pylorus ligation, HCl/ethanol, absolute ethanol, indomethacin and cold/restraint stress, models commonly used to evaluate gastric ulceration in rodents.

HCl/ethanol- and absolute ethanol-induced ulcers were significantly inhibited (81.8 % and 88.2 %, respectively) at the highest dose of extract whereas Sucralfate showed 30.5 and 40.3 % inhibition against the two models. The HCl/ethanol solution directly irritates the stomach mucosa, reduces mucosal resistance and erodes the mucosal barrier. 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 C_4 as well as by superficial aggressive cellular necrosis. The action of these mediators on gastric microvasculature result in both mucosal and sub mucosal gastric tissue destruction [42]. *K. grandifoliola* extract offered significant cytoprotection against absolute ethanol (70 – 88% inhibition). This effect was not accompanied by a significant increase in mucus production, suggesting important inhibitory effects on the generation of the destructive tissue-

derived mediators, or inhibition of their action on the gastric microvasculature [43, 44]. Pre-treatment with indomethacin led to a significant drop in cytoprotection (23.2 and 25.9% inhibition for the negative 250 and 500 m/kg doses of extract, respectively). When cytoprotection against HCl/ethanol is significantly reduced by pre-treatment with indomethacin, the cytoprotective action is usually interpreted to be mediated through endogenous prostaglandins. Although indomethacin administered alone by oral route significantly decreased mucus production in the controls (26.01 ± 5.10 mg), *K. grandifoliola* extract raised mucus levels to 42.0 ± 3.74 at the dose of 500 mg/kg, and offered the highest degree of cytoprotection (79 – 100% inhibition) compared with the other models. Indomethacin and other NSAIDs are well known for their ability to reduce prostaglandin secretion as well as gastric mucosal blood flow, factors that are highly critical to the early events in the pathogenesis of gastric ulceration. The reduced microcirculation can negatively impact on the secretion of bicarbonate and mucus by the gastric and duodenal epithelium and on the proliferation of epithelial cells [45, 46]. The results further lend support to the suggestion that endogenous prostaglandin and gastric mucus production are involved in the cytoprotective action of the extract.

Gastric acid plays a major role in the pathogenesis of gastric and duodenal ulcers [47]. Gastric acid secretion is mediated by the enzyme H^+/K^+-ATPase or by the proton pump localized on the luminal membrane of parietal cells [48]. In the pyloric ligation-induced ulcer model, ulceration is caused by the accumulation of acidic gastric juice in the stomach [46]. The accumulated acid, in addition to its corrosive action on gastric glandular epithelium, provides the optimum pH (1.6– 3.2) for the conversion of pepsinogen to pepsin. Both HCl and pepsin are important ingredients for the formation of pylorus ligated ulcers [49,50]. *K. grandifoliola* extract (100, 250 and 500 mg/kg) significantly reduced the pylorus ligated ulcer index, gastric acidity and the volume of gastric contents in a dose-dependent manner compared with the negative controls. Gastric acid concentrations at 500 mg/kg of extract (34.0 mEq/L) were comparable to those obtained with 50 mg/kg of cimetidine (35.6 mEq/L), and with 400 mg/kg of *Khaya senegalensis* bark aqueous extract (40 mEq/L) by [30].

It was reported that doses of *K. grandifoliola* aqueous extract as low as 12.4 mg/kg completely inhibited the formation of cold stress-induced lesions in rats [51]. Our results (31and 72% inhibition for 200 and 400 mg/kg extract, and 54% inhibition for 50 mg/kg of cimetidine) do not confirm these unprecedented reports even though our bark samples were harvested from the same ecological zone. We did not observe noticeable cytoprotective effects at extract doses below 200 mg/kg.

Water immersion/restraint stress-induced gastric injury is a useful tool in the examination of the pathomechanism of acute gastritis. In acute stress ulcer, intraluminal acid must be present for mucosal damage to occur [52] and gastric adherent mucus plays an important role in protecting the mucosa against ulceration. The stress ulcer model increases gastric acid secretion [42] and reduces gastric adherent mucus.

In addition, the model also stimulates the production of oxygen-derived free radicals by endothelial cells and polymorphonuclear neutrophils. The free radicals, among other mechanisms, provoke tissue damage by inducing ischemia and vascular endothelial cell damage through membrane lipid peroxidation, but endogenous antioxidants (superoxide dismutase, glutathione and catalase) are effective in reducing the adverse effects of free radicals on the gastric mucosa. The neutrophils, also produce pro-inflammatory mediators that inhibit gastric ulcer healing [53-55]. In experimental rats submitted to cold/immersion stress, blood concentrations of SOD, catalase and GSH decreased compared with normal rats. *K. grandifoliola* extract (500 mg/kg) and cimetidine reverted the blood concentrations of catalase (but not SOD and GSH) back to levels greater than normal. SOD converts superoxide free radicals into H_2O_2 which is subsequently degraded by catalase. In control rats, the stress model also increased blood levels of MDA, the major product of cell membrane lipid peroxidation, but both doses of *K. grandifoliola* extract significantly blocked the production of MDA. These findings are evidence of the extract–induced enhancement of the antioxidant status of the animals. Antioxydant activity of *K. grandifoliola* has been reported by [25]. In addition, phenols and flavonoids which were found in significant quantities in the extract, are natural plant substances with well-known preventive antioxidant and antiulcer activities [11, 41, 56, 57]. These compounds most likely inhibit gastric mucosal injury by scavenging the indomethacin- or stress-generated oxygen metabolites [40]. The gastroprotective effect may be due to the action of these compounds.

5. CONCLUSION

In conclusion, *K. grandifoliola* aqueous extract possesses gastric antisecretory potential. Its cytoprotective activity can be attributed to its ability to increase the antioxidant status and to enhance gastric mucosal defense possibly through the mediation of endogenous prostaglandins. The possible mechanism for anti-secretion need to be investigated.

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