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STUDY ON RADICAL SCAVENGING AND GASTROPROTECTIVE EFFECTS OF ETHANOLIC STEM-BARK EXTRACT OF CARPOLOBIA LUTEA IN RODENTS

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ABSTRACT

This is a study of the antioxidant activity and gastroprotective effects of *Carpolobia lutea* ethanolic stem-bark extract (CL ESE) used as antiulcer in ethnomedicine. Experimentally induced gastric ulceration was achieved using acute models such as indomethacin, ethanol, stress and serotonin. Amino acid analysis was by cation-exchange chromatography using automated amino acid analyser. Antioxidant potential was obtained by spectrophotometric assay using 2, 2-Diphenyl-1-picrylhydrazyl DPPH. The finger print of ESE was revealed by Jasco (Tokyo, Japan) HPLC and active compounds by phytochemical screening using standard procedure. The calculated LD50 is 866.025 mg/kg (i.p) using Lock's Method. In the indomethacin model, 43.3, 86.6 and 173.2 mg/kg revealed ulcer inhibition rate of 51 %, 65 % and 76 % respectively ($p > 0.05$-0.01) when compared with the control ($p<0.05$) while the pure drugs, Cimetidine (100 mg/kg) gave ulcer inhibition rate of 74 % ($p<0.01$). In the ethanol model, 43.3, 86.6 and 173.2 mg/kg of the extract gave ulcer inhibition of 20 %, 28 % and 40 % respectively; while cimetidine produced 36 % ulcer inhibition ($p>0.05$). But combination of both 86.6 mg/kg of the extract and Cimetidine 100 mg/kg revealed the highest ulcer inhibition rate of 90% ($p<0.001$). The reaction to DPPH was less than 10%. The HPLC fingerprint of ESE revealed UV spectra of biomolecules. High concentration of Tyrosine, Glutamine, Methionine, glutamine, Phenylalanine and Arginine were observed in the CL

ESE. Phytochemical screening revealed presence of saponins, tannins, polyphenols and glycosides. These investigations indicate presence of bioactive and elemental substances which in part may play significant role in gastric ulcer management. This investigation validates the use of stem-bark of *C. lutea* in illicit gin (akpatashi) among the Effiks in Nigeria as antiulcer.

KEYWORDS: *Carpolobia lutea*, stem-bark extract, antiulcerogenic, amino acid and antioxidant profile.

INTRODUCTION

Carpolobia lutea (CL) G. Don (Polygalaceae) is a medicinal plant reported as a stomach remedy in folkloric medicine (Irvine, 1961). Recently, in our laboratoratory it has been demonstrated that the leaf extracts of CL posses gastroprotective (Nwidu and Nwafor, 2009); antinociceptive (Nwidu *et al* 2011a), antidiarrheal (Nwidu *et al*, 2011b), antimicrobial (Nwidu *et al*, 2012a), anti-inflammatory (Nwidu and Nwafor 2012b), neuropharmacological effects (Nwidu *et al*., 2012c) and antiulcer (Nwidu *et al*., 2012d). Cinnamoyl 1-deoxyglucosides and cinnamic acid derivatives have been isolated (Nwidu *et al*., 2011a). The alcoholic stem-bark extract of *Carpolobia lutea* (CL) is reported in ethnomedicine to be effective as stomach medicine, preventing sleep due to fatigue and as antidiarrheal (Personal Communication). Little is known about the pharmacological profile of the stem-bark extract in literature. Currently, effective multi-drug therapy exists for management of peptic ulcer diseases (PUD); however they are very expensive and presents with multiple side effects that limit their usage. Therefore, attention is now focused on antiulcer agents that are less expensive, less toxic and very effective (Inas *et al*., 2011). Medicinal plants are among the most attractive source of new drugs with promising results in PUD management (Borelli and Izzo, 2000). Plant remain an important resource for alleviating human ailments hence over 60% of the world's population depends on plant kindom on medication (Falbriant and Farnsworth 2001). Plants provide the raw materials for the synthesis of newer drugs (Andreo *et al*., 2006). Plants presents with arrays of amino acids and antioxidants that play significant role in wound healing and mitigation of free radical mediated injury and lipid peroxidation in numerous pathological conditions (Nwidu *et al*., 2012). No study on radical scavenging and gastroprotective effects on the ethanolic stem-bark extract of CL exist to the best of our knowledge. In this study we intend to scrutinize the radical scavenging and gastroprotective effects of ESE in rodents.

MATERIALS AND METHODS

Plant Materials

The stem and stem-bark were collected from Itak Ikpa village in Ibibo Local Government Area of Akwa Ibom State by an Herbalist named Mr. Okon Etefia attached to Pharmacognosy Department in University of Uyo. The plant was identified by a Botanist named Dr (Mrs) Margret Bassey of Botany Department in University of Uyo. A voucher specimen (UUH 998) was deposited at the University Herbarium. The leaves and stem barks were air-dried, powdered with pestle and mortar. The pulverized leaves and stem bark were stored at room temperature until used.

Extraction Procedure

The stem of *Carpolobia lutea* G. Don were harvested from the wild, air-dried, powdered and extracted by immersion in ethanol 70%. 500g of the powdered stem was soaked in one (1) Litre of ethanol. After immersing for 72hours, it was filtered with an aid of a filter paper and the residue air-dried for 24hours and subjected to the same procedure for three successive time. The filtrate of ethanol solvent was reduced in volume nearly to dryness in a rotatory evaporator (BUCCHI USA) at 40 ^{o}C. After which the extract was dried under a flow of nitrogen until constant weight was obtained. The extract was stored in an air-tight container in a refrigerator until used. Analytical HPLC-PAD chromatogram recorded at 280 nm of the compounds of CL ESE after SPE clean-up is reported [15].

Antioxidant activity with DPPH

Antioxidant potential was evaluated spectrophotometrically. A solution of 0.004 % DPPH in methanol (99.8 %) was prepared. A stock solution of CL ESE was prepared from 2.5 mg of the extract in 1ml of distilled and added to 9 mL methanol to give a stock solution of 250 µg/ml. A range of dilutions were made by adjusting 0.1, 0.2, 0.4, 0.8, 1.6 and 3.2 mL of this stock solution to 5 ml in a volumetric flask to give stock solutions with concentrations of 5, 10, 20, 40, 80 and 160 µg/ml, respectively. One ml of each concentration of extract was added 2.0 mL of DPPH solution in a test tube and allowed to react for 30 min prior to spectrophotmetric (Hach Spectrophotometer, Japan) determination at 517 nm. The reference solution was prepared by dissolving 4 mg of DPPH in 20 mL of distilled water which was then made up to 200 mL. Reduction of DPPH radical was determined at 517 nm using the method of Abe *et al*. (1998). Inhibition of the DPPH radical (RSC) by the samples was calculated as in Eq 1.

RSC = {(Ac - Ax)/Ac} x 100 …… (1)

Where Ac is absorbance of the control and Ax is absorbance of the sample after 30 min of incubation. The values obtained were plotted on a graph of % change in absorbance versus concentration of samples.

Quantification of Amino Acids

Amino acid determination was carried out before and after hydrolysis of the ESE. The extract (5 mg) was added to a mixture containing 6 mol L^{-1} of HCl (1-ml) and 5 % phenol/water (0.08 ml) and heated in a Pyrex tube with plastic Teflon-coated screw caps (13 × 1 cm) for 72 h at 110 ˚C to ensure complete hydrolysis of the peptide bonds. The hydrolyzed sample (5 mg) was dried in an oven at 70 ˚C, diluted with 1.0 ml of sodium citrate buffer pH 2.2 and filtered through a GV Millex Unity filter (Millipore). Analysis for amino acids was performed by cation-exchange chromatography using an automated amino acid analyser, Shimadzu LC-10A/C47A. Sodium was used as eluent and post-column derivatisation achieved with o-phthaldialdehyde (OPA). Identification and quantification of the amino acids were carried out by comparison of retention time and area obtained by each amino acid with 16 amino acid standards (100 $nmoL^{-1}$), respectively. The results were expressed in µM/ml extract.

Animals

Swiss albino mice (15-30g) and adult albino rats (100-150g) of both sexes were obtained from the Department of Veterinary Medicine, University of Uyo. All the animals were housed in standard cages under laboratory condition in Department of Toxicology/Pharmacology in Niger Delta University for acclimatization and animals were fed with standard pellet feeds (Vita feed®, Ibadan). The experiments were carried out between June 2011 to August 2012.

Drugs and Chemicals

Cimetidine 400mg (Targamet®), Serotonin (5-hydroxytrptamine HCL, sigma, USA) Indomethacin 25mg (Indocid®), Ethanol 99.8%, Formaldehyde 10%, and Chloroform. Stock soluton of the various drugs and CL ESE were prepared. From the sample stored in the freeze, 500 mg was weighed with an analytical balance and and 5ml of distilled water was added in aliquot in sterile container, it was corked and shaken to disolve the extract. This procedure was repeated until completely dissolved, resulting in a stock solution of 200 mg/ml (0.2g/ml) which was used for the experiment.

Toxicological Assays

The LD_{50} of the CL ESE was determined according to the procedure described by Lorke, (1983). Albino mice (20-30g) of either sexes were used. This method involved an initial dose finding procedure, in which the animals were divided into eight groups of three (3) animals per group. Doses of 10, 100, 500, 1000, 1500, 2000, 3000 and 4000 mg/kg were administered intra-peritoneally, one dose for each group. The treated animals were monitored for 24 hours for mortality and general toxicity behavioural characteristics. From the above results, four different doses of 500, 1500, 3000, and 4000 mg/kg were chosen and administered intra-peritoneally to four groups of three mouse per group. The treated animals were again monitored for 24 hours. The LD_{50} was then calculated as the square root of the mutiplication of the least dose that kill all the animals and the highest dose that do not kill any animals or the geometric mean of the lowest dose causing death and the highest dose causing no death. That is, LD_{50} = (highest dose causing no death mutiply by lowest dose causing death)[1/2]

Pharmacological Assays

The ethnopharmacological information of CL ESE employed posology is inexact. This necessitate the utilization of the 1/5th, 1/10th and 1/20th of acute toxicity dose (866.025mg/kg) as the highest, middle and highest dose used in all the experiment to determine the pharmacological profile of the antiulcer effects of CL ESE. The antiulcer assays were executed using these protocols: indomethacin-, ethanol- and stress-induced ulcer models.

Indomethacin–Induced Gastric Ulceration in Rats

This experiment was performed according to the method of Peskar *et al*. (2002) with some modification. Pilot tests aimed at determining the effective dose of indomethacin needed to produce reliable acute gastric ulceration in rats were evaluated using varying doses of indomethacin: 0.06, 0.07, 0.08, 0.09 and 0.1 g/kg on the rats. 0.09 g/kg of indomethacin per body weight of animal produced gastric ulceration in all rats in 5 h in the pilot study. Male adult albino rats weighting 100 – 140 g were used for this experiment. The rats were randomized into 6 groups of 6 rats each. Food was withdrawn 24 h and water 2 h before the commencement of the experiment. Group 1 positive control was administered with 0.09 g/kg indomethacin, orally. Group 2 – 4 were pretreated with 43.3, 86.6 and 173.2 mg/kg of CL ESE, respectively, 1 h prior to administration of 0.09 g/kg of indomethacin. While group 5

received cimetidine(0.1g/kg, p.o.) 1 hour prior to administration of 0.09 g/kg of indomethacin. Group 6 received 86.6 mg/kg of the extract 30 mins prior to administration of cimetidine (100 mg/kg). After 1 hour 0.09 g/kg of indomethacin was administered. The drugs were administered intragastrically via the aid of an orogastric cannula. 5 hours later, the animals were killed by cervical dislocation. The stomach were removed and opened along the greater curvature. The tissues were fixed with 10 % formaldehyde in saline. Macroscopic examination was carried out with a hand lens and scored for the presence of lesions using the method of Al-Said *et al*., (1986). Ulcer index (UI) of indomethacin alone, ulcer index and preventive ratio of each of the groups pretreated with CL ESE were calculated using the method of Al-Said *et al*., (1986) as follows: 0: no lesion, 1: mucosal oedema<1 mm, 2: small lesion ranged 1-2 mm, 3: lesions ranged 2-4 mm, 4: lessions >4 mm.

Ethanol–Induced Gastric Ulceration in Rats

Male adult albino rats weighing between 100 – 300 g were used for this experiment. The rats were randomized and divided into 6 groups of 6 rats each. Food was withdrawn 24 hour and water 2 hour before the commencement of the experiment. Ulcer lesion was established with 0.5 ml of 99.8 % ethanol. Group 1 was administered with 0.5ml of 99.8 % ethanol, groups 2 - 4 were pretreated with 43.3, 86.6 and 173.2 mg/kg CL ESE, respectively, 1 h prior to administration of 0.5 ml of 99.8 % ethanol while group 5 received Cimetidine (100 mg/kg, p.o.) 1 h prior to administration of 0.5 ml of 99.8 % ethanol. Group 6 received 86.6 mg/kg of the extract 30 mins prior to administration of cimetidine (100 mg/kg). After 1 hour 0.5ml of 99.8 % ethanol of was administered. The CL ESE was administered intragastrically via the aid of an orogastric cannula; 4 hours later, the animals were killed by cervical dislocation. The stomach were removed and opened along the greater curvature. The tissues were fixed with 10 % formaldehyde in saline. Macroscopic examination was carried out with a hand lens and scored for the presence of lesions using the method of Barry *et al*., (1988). The number and severity of gastric lesions were evaluated according to the following rating scale: 0: no lesion; 1: the presence of one ulcer and generalized erythema; 2: at least two ulcers of approximately 2 mm in length; 3: ulcer 1-4mm in length of 80 % of the fold; 4: lesions which follows approximately 80 % of the fold; 5: multiple ulcers along the entire length of the gastric fold; Ulcer index of ethanol alone, ulcer index and preventive ratio of each of the groups pretreated with these CL ESE were calculated using the method of Zaidi and Mukerji, (1958) with modification.

Water Immersion and Immobilization-Induced Gastric Ulceration in Rats

Male adult albino rats weighting 106 – 163 g were used for this experiment. The rats were randomized and divided into 6 groups of 6 rats each. Food was withdrawn 24 h and water 2 h before the commencement of the experiment. Group 1 positive control rats were placed individually in plastic cages measuring 30 cm. The animals were placed individually in each compartment of the cage and it was immersed vertically in water tank, water was added gradually to the level of the xiphoid. The temperature of the tank was maintained at 15 - 20 °C using ice pack to induce stress ulceration. Group 1 was immersed in water without administration of the test samples. Groups 2 - 4 were pretreated with 43.3, 86.6 and 173.2 mg/kg of CL ESE, respectively, 1 h prior to immersion and immobilization; while group 5 received cimetidine (100 mg/kg) 1 h prior to immersion and immobilization. The drugs were administered intragastrically via the aid of an orogastric cannula. One hour later, the animals were killed by cervical dislocation. The stomach were removed and opened along the greater curvature. The tissues were fixed with 10 % formaldehyde in saline. Macroscopic examination was carried out with a hand lens and scored for the presence of lesions using the methods of Takalgi and Okabe [22]. The number and severity of gastric lesions were evaluated according to the following rating scale: 0: no lesion;1: mucosal oedema and petechiae; 2: 1 - 5 small lesions (1 - 2 mm); 3: more than 5 small lesions or 1 intermediate lesion (3 - 4 mm); 4: 2 or more intermediate lesions or 1 gross lesion (greater than 4 mm); 5: perforated ulcers. Ulcer index (UI) of rats immobilized and immersed in water without drug alone, ulcer index and preventive ratio of each of the groups pretreated with CL ESE were calculated using the method of Takalgi and Okabe, (1968).

Statistical Analysis

Values for the results were expressed as a mean ± SEM. The statistical significance of each test group in relation to the control was calculated using one way analysis of variance followed by Turkey-Krammer multiple comparisons tests. A probability of less than 5% was considered significant.

RESULTS AND DISCUSSION

Phytochemistry

The HPLC fingerprint characteristics of bioactive compounds present in the CL ESE is reported [15]. A preliminary phyto-chemical screening gave positive test for saponins, polyphenols and glycosides.

Antioxidant Activity

Antioxidant activity of the ESE of CLL is shown in Fig. 1. The results show minimal radical scavenging activity when compared to ferrulic acids, caffeic acids and gallic acids standards.

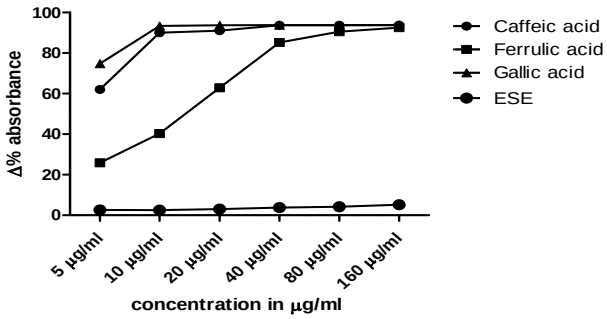

Fig 1: Antioxidant Activity of CL ESE with DPPH

Acute Toxicity (LD_{50})

The crude extracts produced mortality at the dose of 1500 mg/kg intraperitoneally. The crude extract was found to be toxic at that dose and beyond. The LD_{50} is calculated to be 866.025mg/kg. The doses used for this bioassay is chosen from 1/20 x 866.025 = 43.3mg/kg (lowest dose); 1/10 x 866.025 = 86.6mg/kg (middle dose) and 1/5 x 866.025 = 173.2mg/kg (Highest dose).

Amino acid Profile

The amino acid composition of the ESE of CLL is shown in Fig. 2. The CL ESE extract contain essential amino acids. High concentration of Tyrosine, Glutamine, Methionine, glutamine, Phenylalanine and Arginine were observed in the CL ESE.

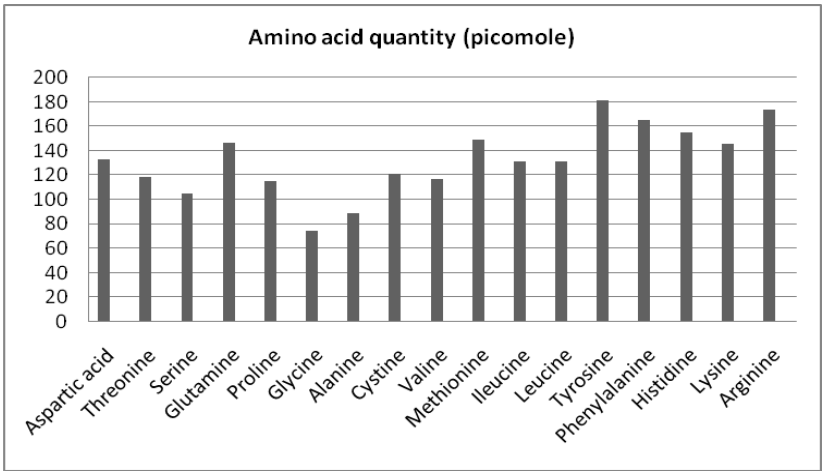

Fig 2: Amino acid profile of ethanolic stem extract of *Carpolobia lutea.*

Indomethacin–Induced Gastric Ulcer

The gastroprotective effect of *C. lutea* on indomethacin induced gastric ulcer was macroscopically determined in rats. Macroscopic lesions in various forms and sizes were observed to be distributed irregularly on all stomach surfaces tissue of the control rats that received indomethacin. From Table 1, indomethacin caused a remarkably high ulcer index (30.67± 4.07) in the control group. 43.3 mg/kg revealed the ulcer inhibition rate to 51% which was not statistically significant ($p>0.05$); 86.6 and 173.2 mg/kg of the extract showed an ulcer inhibition rate of 65% and 76 % respectively, which were statistically significant when compared with the control ($p<0.05$-0.01). Cimetidine100 mg/kg also produced an ulcer inhibition rate of 74% which was statistically significant ($p<0.01$). Pretreatment of rats with 86.6 mg/kg of extract and Cimetidine100 mg/kg produced the highest degree of mucosal protection and decreased the ulcer index (5.00±1.02) with 84% prevention ratio against gastric mucosal injury and this was significant ($p<0.01$).

Table 1: Effects of ethanolic stem extract of *C. lutea* on Indomethacin-induced ulcers in rats.

Groups	Ulcer index	ventive index
Control	30.67±4.07	-
E SE 43.3 mg/kg	15.17±2.14^{ns}	51%
ESE 86.6 mg/kg	10.83±1.48*	65%
ESE173.2 mg/kg	7.50±8.83**	76%
CME100mg/kg	8.00±1.65**	74%
ESE 86.6 mg/kg + CME 100 mg/kg	5.00±1.02**	84%

Significance relative to control: $^{*}p<0.05$, $^{**}p<0.01$, ns= not significant. Values represent mean ± SEM (n=6).

ESE; represents ethanolic stem extract of *C. lutea*, CME; Cimetidine.

Ethanol–induced gastric ulcer

Ethanol (99.8%)-induced gastric damage showed marked gross mucosal lesion including long hemorrhage bands in control given an ulcer index of 4.17±0.50 (Table 2). There was a slight decrease in the gastric ulcer index following pretreatment with 43.3, 86.6 and 173.2 mg/kg of ethanolic stem extract of *C. lutea* which gave the ulcer inhibition of 20%, 28% and 40% respectively but which was not statistically significant ($p>0.05$). Cimetidine decreased the ulcer index to 2.67±0.23 providing 36 % ulcer inhibition not statistically significant ($p>0.05$). The combination of 86.6 mg/kg of *C. lutea* ethanolic stem extract and Cimetidine 100 mg/kg

resulted in 44 % ulcer inhibition which was statistically significant ($p<0.05$). The table below summarizes the results of effects of the stem extract on Ethanol-induced ulcer.

Table 2: Effects of ethanolic stem extract of *C. lutea* on Ethanol-induced ulcers in rats.

Groups	Ulcer index	Preventive index
Control	4.17±0.50	-
ESE 43.3 mg/kg	3.33 ± 0.61^{ns}	20%
ESE 86.6 mg/kg	3.00 ± 0.40^{ns}	28%
ESE 173.2 mg/kg	2.50 ± 0.47^{ns}	40%
CME 100 mg/kg	2.67 ± 0.23^{ns}	36%
ESE 86.6 mg/kg + CME 100 mg/kg	$2.33\pm0.23^{*}$	44%

Significance relative to control: $^{*}p<0.05$, ns= not significant. Values represent mean ± SEM (n=6). ESE; represents ethanolic stem extract of *C. lutea*, CME; Cimetidine.

Water Immersion and Immobilization Induced-Gastric Ulcer

For the water immersion and immobilization stress induced ulcer model, it was observed that treatment with 43.3 mg/kg of the extract produced 46% inhibition which was not statistically significant ($p>0.05$). Treatment with 86.6, 173.2 mg/kg ethanolic stem of *C. lutea* and cimetidine 100 mg/kg reduced the ulcer indices when compared to control group significantly ($p< 0.01 - 0.05$) resulting in 52%, 64% and 76% inhibition respectively. The pretreatment of rats with both 86.6 mg/kg of the extract and Cimetidine 100 mg/kg revealed the highest ulcer inhibition rate of 90% and a decreased ulcer index of 7.83±1.91 which is statistically significance($p<0.001$). Table 3 summarizes the results of the effect of ethanolic stem extract of *C. lutea* on water immersion and immobilization-induced gastric ulcers in rats.

Table 3: Effects of Ethanolic Stem Extract of *C. Lutea* on Water Immersion and Immobilization-Induced Gastric Ulcers in Rats.

Groups	Ulcer index	Preventive index
Control	53.0±9.72	-
ESE 43.3 mg/kg	28.67 ± 6.33^{ns}	46%
ESE 86.6mg/kg	$25.50\pm6.79^{**}$	52%
ESE 173.2 mg/kg	$19.00\pm6.59^{**}$	64%
CME 100 mg/kg	$15.17\pm2.85^{**}$	76%
SE 86.6 mg/kg +CME 100 mg/mg	$7.83\pm1.91^{***}$	90%

Significance relative to control: $^{*}p<0.05$, $^{**}p<0.01$; $^{***}p<0.001$; ns= not significant. Values represent mean ±SEM (n=6).ESE; represents ethanolic stem extract of *C. lutea*, CME; Cimetidine. The effects of CL ESE on gastric ulcers induced by indomethacin, ethanol and

stress in rats revealed a gastroprotective effects. The mechanisms by which this extract produced these effects seems unclear. Non-steroidal Anti-Inflammatory Drugs (NSAIDs) is considered to be the major risk factor in causation of gastric ulcers due to its irritant property which is a major impediment to its use as anti-inflammatory drugs (Chiba *et al*., 2008). The mechanisms suggested for the gastric damage caused by NSAIDs are inhibition of prostaglandin synthesis and inhibition of epithelial cell proliferation in the ulcer margin, which is critical for the re-epithelization of the ulcer crater (Wallace and Devchand, 2005). Prostaglandins protect the gastric mucosa by maintenance of gastric microcirculation, secretion of bicarbonate and mucus (Hiruma-Lima et al., 2009).

Blockers of the prostaglandin synthesis such as indomethacin inhibit the non-parietal acid secretions; decreased prostaglandin level impairs all aspects of gastroprotection, increase acid production which, in turn, aggravate the ulcer and decrease cytoprotective mucus formation [Hiruma *et al*., 2009).

Absolute ethanol damaging effects on the gastric mucosa is attributed to generation of free radicals, increase lipid peroxidation and decreases of the level of sulphydryl proteins in the gastric mucosa (Mizui and Doteuchi, 1986). This free radical produced causes denaturation of DNA strands and protein; reduce gastric acid and increased flow of Na^+ and K^+, increased pepsin secretion, and a loss of H^+ ions and blood flow, cause haemorrhage, necrosis and solubilisation of mucus constituents in the stomach. These actions result in infiltration of histamine into the lumen (Szabo, 1987). Ethanol pretreatment causes disturbances in gastric secretion, damage to gastric mucosa, alters gastric mucosa permeability, gastric mucosa depletion and free radical production. Ethanol is one of the ulcerogenic agents that induce intense damage in gastric mucosa by promoting disturbances of mucosal microcirculation, ischemia and appearance of free radicals, endothelin release, degranulation of mast cell and inhibition of prostaglandins and decrease of gastric mucus production (Abdel-Salam *et al*., 2001).

The incidence of ethanol-induced ulcers is predominant in the glandular part of the stomach where it stimulate increase formation of leukotriene (LTC4) which cause damage of gastric mucosa (Nwafor and Bassey, 2007). Since vascular changes appears to be the most pronounced features of ethanol-induced lesions, maintenance of the mucosal vasculature and normal blood flow may be the major mechanism of cytoprotection (Matsuda *et al*., 1999). Suppression of alcohol-induced ulceration indicated that the ESE suppresses lipoxygenase

path-way, this may in part be one of its mechanisms of action. When the concentration of hydrogen ions in gastric juice decreases, it is reflective of high pH. Pretreatment with ethanolic stem extract in the positive control group produced a slight decrease in rates of ulcer inhibition but it was not statistically significant. Cimetidine has no cytoprotective effect against damage induced by ethanol (Giannarelli *et al.*, 1995). Combination of the 86.6 mg/kg of ethanolic stem extract and Cimetidine (100 mg/kg) showed statistical significant inhibition of ulcer index.

Stress plays an important role in the etiopathology of gastroduodenal ulceration (Favier *et al.*, 2005). Recently, oxygen derived free radicals have been postulated to play an important role in the pathogenesis of acute gastric mucosal injuries such as those induced by stress (Govindarajan *et al., 2006)* ethanol (Salim, 1990) and NSAIDs (Bech, 2000). Scavenging these radicals stimulates the healing process. It is well known that stress stimulates various damaging pathways, causing increased production of reactive oxygen species (ROS), such as hydrogen peroxide, hydroxyl radicals and superoxide anion radical, which lead to lipid peroxidation, protein oxidation, DNA damage and cell death, and contributes to the occurrence of pathological conditions (Heise *et al*., 2003). Locally secreted prostaglandins (PG's), sensory neuropepeptides and nitric oxide contribute to regulation of gastric blood flow and maintenance of mucosal integrity [Pawlik *et al*., 2001). In the gastric ulcer induced by water immersion and immobilization the extracts showed significant ulcer inhibition rate in a dose-dependent manner.

The phytochemical analysis of the CL ESE revealed the presence of large concentration of saponins which is the most important botanical compounds with antiulcer and gastroprotective activities (Wahida *et al*., 2007). Moreover, several plants containing high amounts of saponins have been shown to possess antiulcer activity in several experimental bioassays (Yesilada and Takaishi, 1999; Morikawa *et al*., 2006).

CONCLUSION AND SUGGESTION

The results obtained revealed that CL ESE contains pharmacologically active substance(s) with anti-ulcer properties. These properties confirm the use of *Carpolobia lutea* G. Don (Family; Polygalaceae) as an anti-ulcer drug as proposed by the traditional medicine. The plants stem extract is effective against acute ulcerogen models in rodents. Further studies are warranted to elucidate the active constituents of the CL ESE responsible for its gastroprotective effect.

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Declaration of interest

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