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Research Article

Acute toxicity and anti-ulcerogenic activity of an aqueous extract from the stem bark of *Terminalia superba* Engl. and Diels (Combretaceae)

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ABSTRACT

Terminalia superba is a plant used in traditional medicine to treat many illnesses particularly gastro-intestinal disorders. This study was aimed to evaluate the acute toxicity and gastric anti-ulcer activity of an aqueous extract of *Terminalia superba* (AETs). The LD_{50} was determined by the graphic method of Miller and Tainter (1944) and the calculation method of Dragsted and Lang (1957) in mice. The preventive anti-ulcerogenic action of the extract was assessed using four models of gastric ulcer induction namely HCl/Ethanol solution, indomethacin solution, pylorus ligation and cold restraint stress in rats. The LD_{50} obtained by the oral administration of AETs was 12.2 ± 0.21 g/kg b.w. and 12.33 ± 0.87 g/kg b.w. by the graphic method and the calculation method respectively. The administration of AETs intraperitoneally gave 1.97 ± 0.29 g/kg b.w. (graphic method) and 1.93 ± 0.21 g/kg b.w. (calculation method) as LD_{50s}. The preventive gastric anti-ulcer study revealed that for doses ranging from 125 to 500 mg/kg body weight, EATs significantly ($P<0.05$) inhibited ulcers induced in the four models. The inhibition values were 96.25, 96.01, 98.10 and 96.75 % on ulcerations induced respectively by HCl/Ethanol, indomethacin, pylorus ligation and cold restraint stress at the dose of 500 mg/kg b. w. At the same dose, AETs significantly ($P < 0.05$) increased mucus production and reduced gastric acid secretion. Phytochemical screening of the aqueous extract of the stem bark of *Terminalia superba* showed the presence of polyphenols, tannins, flavonoids, quinones, coumarines, saponins, reduced sugar, sterols and polyterpenes. These results suggested that the preventive anti-ulcer activity of AETs may be due to a cytoprotective effect. The LD_{50s} found indicated that the extract was not toxic and that the phytochemical coumpounds present in EATs could be responsible for its effects. In conclusion, the preventive gastric anti-ulcer and the non toxic effects of the aqueous extract of *Terminalia superba* could justify the use of this plant in traditional medicine to treat abdominal disorder and pains.

Key words: *Terminalia superba*, Acute toxicity, LD_{50}, Gastric anti-ulcer, Phytochemical screening.

INTRODUCTION

Herbal remedies mainly used in traditional medicine are employed in the treatment of diseases of diverse origins. A study reported that 25 % of medical prescriptions contain substances derived from plants so that the herbal remedies received a great attention as alternative to synthetic pharmaceutical products, leading to the increase in their demand [1,2]. It was also admitted that traditional medicine was used by about 80 % of the world population in developing countries [3]. Traditional healers most often utilize and recommend plant extracts over a short or long

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period without a proper dosage monitoring and consideration of toxic effects that might result from such practices. So, traditional healers must be informed of the reported incidence of renal and hepatic toxicity resulting from the ingestion of medicinal herbs [4].

The use of herbal preparations in the treatment of gastric ulcer is popular in many parts of Africa and in Côte d'Ivoire. In the scientific literature, a large number of medicinal plants with gastric anti-ulcer potential were highlighted [5,6,7]. *Terminalia superba* Engl. et Diels (Combretaceae), in many countries referred to as «fraké» or «limbo», was extensively recognized as being effective in folk medicine in the treatment of various illnesses like gastric ulcer [8-14]. Gastric ulcer is an illness that affects a considerable number of people worldwide. The etiological factors of this disorder include stress, smoking, nutritional deficiencies, infections, frequent and indiscriminate use of non-steroidal anti-inflammatory drugs [15]. The pathogenesis of gastro-duodenal ulcers is influenced by various aggressive and defensive factors, such as mucus secretion, mucosal barrier, acid pepsin secretion, blood flow, cellular regeneration and endogenous protective agents [16]. Despite its extensive and intensive employment in folk medicine, no or few studies were initiated to explain the toxicological profile and anti-ulcer activity of the stem bark of *Terminalia superba*.

The aim of this study was to evaluate the safety and the anti-ulcer activity of the aqueous extract of the stem bark of *Terminalia superba* and to determine the phytochemical constituents present in the extract.

MATERIALS AND METHODS

Plant material: The stem barks of *Terminalia superba* were collected locally from the forest of Ebillassokro village in the East of Côte d'Ivoire in December 2009. Taxonomical identification of those stem barks was established by Professor Aké-Assi Laurent from the National floristic Center of University of Felix Houphouet Boigny, Cocody-Abidjan, Côte d'Ivoire, voucher n°2456, *Terminalia superba* Engl. et Diels in June 4, 1954; n°4207 in March 26, 1957; n°10477, February 26, 1969 and n°416 in April 03, 1974 of Côte d'Ivoire national herbarium.

Preparation of aqueous extract from the stem bark of *Terminalia superba*: The stem bark of *Terminalia superba* were dried under shade and powdered with a machine (mark RETSCH, type SM 100, Germany). Powder of stem bark was extracted using aqueous infusion. One hundred grams (100 g) of the stem barks powder of *Terminalia superba* were infused in1 l hot distilled water for 15min and then filtered (Whatman n°1). The aqueous extract of the stem bark of *Terminalia superba* (AETs) was then concentrated under reduce pressure with a rotary evaporator (Büchi R110, type MKE 6540/2) at a temperature of 45°C and was stored in desiccators at 45°C. The concentrations to be tested were prepared by dilution in saline solution (NaCl 9 ‰). The pH value of the extract before being tested after dilutions was determined to be 8.43 at 60 mg/ml.

Animals*:* Albino mice (*Mus musculis*) of both sexes weighting between 25 and 30 g and aged from 12 to 16 weeks each were used to assess acute toxicity. Albino wistar rats of either sex weighing between 200 and 215 g and approximately the same age (14 weeks) were selected for gastric anti-ulcer experiments. They were bred in Animal house of Animal Physiology, Pharmacology and Phytotherapy laboratory of the University of Nangui Abrogoua (Former University of Abobo-Adjamé, Abidjan, Côte d'Ivoire) according to the principles for the care and use of laboratory animals of the Ethical Committee of the University (Nangui Abrogoua, Abidjan, Côte d'Ivoire). They were exposed to 12 hours dark/light cycle.

Acute toxicity study by oral and intraperitoneal routes: Mice were distributed into one control group and seven treated groups containing eight animals per group. They were fasted for 18 hours prior to experiments. The control group received normal saline solution orally while each treated group received orally a single administration of AETs at the following doses: 6, 8, 10, 12, 14, 16 and 18 g/kg body weight (b.w.). Behavioural changes of the 7 treated groups were observed every 30 min for a period of 2 hours after administration of the extract and mortality rate were recorded for 24 hours post treatment [17]. Two methods were used to determine the LD_{50} [18,19]. The same protocol was used except that each mouse in the control group was treated with 0.5 ml isotonic solution of NaCl 9 ‰ intraperitoneally and the 7 other groups were treated with a single intrperitoneal administration of AETs at 0.5, 1, 1.5, 2, 2.5, 3 and 3.5 g/kg b.w.

Anti-ulcer studies: The negative Control 1 is the same for all the models. Group 1 composed of 6 rats received orally distilled water.

Gastric lesions induced by a necrotizing agent (HCl/ethanol): The method described by some authors was adopted for this study with slight modifications [20]. The animals were divided into 6 groups of 6 animals each. Groups 2 received 1

ml/150 g b.w. of the necrotizing solution (150 mM HCl in 60 % ethanol) (control 2). Groups 3 and 4 (positive controls) were pretreated with Cimetidine (12 mg/kg b.w.) and Maalox (50 mg/kg b.w.) respectively. Groups 5 to 7 were pretreated with the aqueous extract at doses of 125, 250 and 500 mg/kg b.w. All treatments were administered orally. One hour after drug administration, 1 ml/150 g b.w. of the necrotizing solution was given orally to each rat except rats of negative controls. The animals were sacrificed one hour later using an over dose of ether and the stomachs were incised along the greater curvature. The mucosal erosion was determined by measuring the area of the lesions and then it was scored. The sum of the areas was expressed as ulcer index (mm^2). The scoring of stomach lesions was established according to a described method [21]. The percentage of inhibition (%I) was calculated using the following formula:

$$\% I = \frac{(US_C - US_T)}{US_C} X\ 100$$

Where US_C = ulcer surface area in control animals and US_T = ulcer surface area in treated animals. The mucus covering the gastric wall of each rat was collected and weighed.

Gastric lesions induced by Indomethacin: The method described by [22] was adopted for this study. 6 groups of 6 animals each were used. Group 2 received orally Indomethacin (30 mg/kg) at 1 ml/100 g b.w. (Control 2). Groups 3 and 4 considered as positive controls were pretreated with Misoprostol (0.012 mg/kg b.w.) and Maalox (50 mg/kg b.w.) respectively. Groups 5 to 7 were pretreated with the aqueous extract at doses of 125, 250 and 500 mg/kg b.w.

All treatments were administered orally. One hour after drug administration, each animal received orally 30 mg/kg b.w. of Indomethacin except rats of negative controls. The animals were sacrificed 5 hours after treatment by over dose of ether. The stomachs were excised, rinsed with normal saline and examined for ulceration. The ulcers produced were scored as described by [23] and modified by [24]. The ulcer index and the percentage of inhibition were estimated as describe above. The mucus covering the gastric wall of each rat was collected and weighed.

Pylorus-ligated rats: Six (6) groups of 6 animals each were used. Group 2 was pylorus-ligated and did not receive any solution (Control 2). Groups 3 and 4 (positive controls) were pretreated with Cimetidine (12mg/kg b.w.) and Maalox (50 mg/kg b.w.) respectively. Groups 5 to 7 received the aqueous extract at doses of 125, 250 and 500 mg/kg b.w. All treatments were administered orally. Pylorus ligation was made under ether anesthesia 1 hour after treatment except rats of Control 1. The rats were sacrificed 6 hours after pylorus ligation. The stomachs were removed, the contents collected, the volumes measured, centrifuged and the supernatant measured. The ulcers produced were scored as described by [22]. The ulcer index, the percentage ulcerated surface and the percentage of inhibition were estimated as described above. One milliliter of the total centrifuged gastric contents from each pylorus-ligated rat was analyzed for titratable acidity against 0.01 mol/l NaOH at pH 7 using a pH meter (HANNA instruments HI 9025).

Hypothermic restraint stress-induced ulcers: The method described by [25,26] was used with slight modifications for this study. 6 groups of 6 animals each were constituted. Group 2 was hypothermic restraint stress-induced ulcers without receiving a solution (Control 2). Groups 3 and 4 (positive controls) were pretreated orally with Misoprostol (0.012 mg/kg b.w.) and Ranitidine (50 mg/kg b.w.) respectively. Groups 5 to 7 were pretreated with the aqueous extract at doses of 125, 250 and 500 mg/kg b.w. One hour after the oral administration of AETs (125, 250 and 500 mg/kg b.w.), the rats were immobilized by strapping the hind limbs on a wooden plank and kept for 1 h 30 min at temperature of 3-5°C [26] except rats of group 1. One hour later**,** the animals were then sacrificed and the stomachs were excised. They were examined for ulceration and the severity of intraluminal bleeding according to the scale described by [27].

Drugs: The following reference drugs were used: Aluminium hydroxide (Maalox^R Sanofi Aventis, France), Misoprostol (Cytotec^R, Pfizer, Germany), Ranitidine (Zantac^R, Bristol Myers Squibb, USA) and Ether (VWR International-Geldenaakfebaan 464-B-3001 Leuven-Belgium). Cimetidine, Indomethacin, HCl, and Ethanol were purchased from Sigma chemical Company (Saint Louis, MO, USA).

Phytochemical screening: Aqueous extract from the stem bark of *Terminalia superba* (AETs) was screened for the presence of polyphenols, tannins, flavonoids, saponins, alkaloids, sterols and ployterpenes, reduced sugar, proteins, coumarines and quinones. Detection of these constituents was carried out as described by [28].

Data analysis: All values were expressed as mean ± standard error of the mean (m±s.e.m). Statistical analysis was carried out using the software GraphPad Prism 5.01 (San Diego California, USA).

The significance of the differences observed between the concentrations was implemented by analysis of variances (ANOVA) of the multiple test of comparison of Turkey-Kramer. The differences between the concentrations were considered statistically significant when $p < 0.05$.

RESULTS

Acute toxicity of AETs by oral tract: After oral administration of AETs at doses of 6 and 8 g/kg b.w., mice had difficulty in breathing and were weak. However, they continued to feed. After two hours, all the animals which received the dose of 6 g/kg b.w. found again the behavior of mice of the control group which were very mobile and fed correctly. No death was recorded in this group after 24 h. The death rate was function to the dose administered. Indeed, the death rate increased when the dose increased from 8 g/kg b.w. to 18 g/kg b.w. after 24 h.

Animals that received 10 and 12 g/kg b.w. were motionless and refused to feed the first hours after extract administration. Deaths occurred 30 min after oral administration of the extract. The survival mice found again the control group behavior 14 hours after drug administration.

At 14 g/kg b.w., all the mice were immediately immobile, with rapid breathing and the first deaths were noticed 30 min after administration of the drug. Animals died lying down on the back or the side.

At 18 g/kg b.w., all the mice of this group died few minutes post treatment. No diarrhoeic feces were observed during the experiments.

The death rate of one experiment is shown in table 1. This experiment was repeated 3 times and the LD_{50} determined graphically by the method of Miller and Tainter was 12.2 ± 0.21 g/kg b.w. and that calculated by the method of Dragsted and Lang was 12.33 ± 0.87 g/kg b.w. There is no significant difference between the two values of LD_{50} ($p > 0.05$).

Acute toxicity of AETs by intraperitoneal way: The intraperitoneal administration of AETs at the dose of 0.5 g/kg b.w. showed no toxic symptoms and no mortality in the treated mice after 24 hours. However, 3 min after extract administration at the dose of 1 g/kg b.w., signs of toxicity in all the mice included initial excitement, difficulty in breathing, loss of appetite, general weakness, convulsions and depression were observed. From 1 g/kg b.w., the mortality increased dose dependently and salivation or diarrhoea was observed. Death occurred after breathing difficulties and weakness of the mice. At 3.5 g/kg b.w., 100 % death was recorded in the treated group after extract administration. No diarrhoeic feces were observed at this dose because of the sudden death. Table 2 indicates for one experiment the death rate of mice. This experiment was repeated 3 times. The graphic method of Miller and Tainter permitted to determine a LD_{50} value of 1.97 ± 0.29 g/kg b.w. while the calculation method of Dragsted and Lang gave a LD_{50} value of 1.93 ± 0.21 g/kg b.w. There is no significant difference between the two values of LD_{50} ($p>0.05$).

Effect of AETs on necrotizing agent-induced gastric lesions: The treatment of rats with HCl/ethanol produced extensive gastric lesions in the glandular mucosa of the stomach in all the control rats (Figure 1A). The lesions (mm^2) decreased significantly ($p < 0.05$) from 198.13 ± 13.15 (Control 2) to 7.43 ± 0.24 at 500 mg/kg b.w. (AET_S) and it was also observed that protection of gastric mucosa was more prominent in rats pre-treated with the same dose of AETs (Figure 1A and Table 3). The mean ulcer index decreased significantly ($p < 0.05$) from 5.82 ± 0.41 (Control 2) to 0.29 ± 0.72 at 500mg/kg b.w. of AET_S one hour after administration of the necrotizing agent. Pretreatment of rats with AETs at doses ranging from 125 to 500 mg/kg b.w. induced a dose dependent inhibition of gastric ulceration ranging from 35.84 to 96.25 %. Cimetidine and Maalox showed cytoprotective effect on HCl/ethanol induced lesions with an ulcer surface area of 63.10 ± 1.36 and 119.57 ± 11.4 mm^2 at the dose of 12 and 50 mg/kg b.w. corresponding to 68.15 and 39.65 % inhibition respectively (Table 3). The mucus produced by rats of the Control 2 group (102.13 ± 2.47 mg) significantly ($p < 0.05$) decreased as compared to rats of control 1 (156.87 ± 2.35) (Table 3). Cimetidine, Maalox and rats pretreated with AETs from 125 to 500 mg/kg b.w. significantly ($p<0.05$) increased dose-dependently mucus weight as compared to control2 (Table 3). For AETs applied at the dose of 500 mg/kg b.w., this value attained 366.40 ± 11.21 mg (Table 3).

Effect of AETs on gastric lesions induced by Indomethacin: The oral administration of indomethacin induced acute damage in the rat glandular stomach (Figure 1B). AETs at 125, 250 and 500 mg/kg b.w. significantly ($p < 0.05$) and dose dependently prevented the development of gastric lesions in the rats stomachs corresponding to inhibition percentage of 51.66 (125 mg/kg b.w.), 81.44 (250 mg/kg b.w.) and 96.01 (500 mg/kg b.w.) as shown in Table 4. Misoprostol (0.012 mg/kg b.w.) and Maalox (50 mg/kg b.w.) exhibited respective inhibition percentage of 68.71 and 55.14. In addition, AETs elicited significant ($p <$

0.05) dose dependent increases of mucus (173.61 ± 3.18 to 479.83 ± 7.84 mg) in the treated rats as compared to Control 2 group (87.37 ± 4.17 mg) (Table 4).

Effect of AETs on pylorus ligation-induced gastric lesions: When the rats were subjected to pylorus ligation for 6 h, a considerable amount of basal gastric acid secretion was noted (9.81 ± 0.72 ml) in the Control 2 group (Table 5). In the same control group, the titratable acidity, the pH, the surface area, and the mucus were found to be 180.83 ± 3.14 mEq/l, 1.57 ± 0.01, 135.14 ± 0.76 mm^2, 49.72 ± 1.78 mg respectively and the ulcer index recorded was 5.31 ± 0.12 (Table 5). AETs (125, 250 and 500 mg/kg b.w.) produced a significant ($p<0.05$) dose dependent decrease in gastric acid secretion (116.33 ± 1.44 to 89.67 ± 2.81 mEq/l), ulcer index (4.56 ± 0.02 to 0.98 ± 0.08) and ulcer formation (76.47 ± 0.82 to 2.56 ± 1.31 mm^2) compared to Control 2 with maximal percentage of inhibition of 98.10 at the dose of 500 mg/kg b.w. (Table 5 and Figure 1C). AETs (125, 250 and 500 mg/kg b.w.), Cimetidine and Maalox increased the pH and the mucus weight.

Effect of AETs on hypothermic restraint stress-induced gastric mucosal lesions: As shown in Table 6, oral administration of AETs (125, 250 and 500 mg/kg b.w.), significantly ($p < 0.05$) inhibited intraluminal bleeding and ulcer formation induced by hypothermic restraint stress. That protection of gastric mucosa was more efficient in rats pre-treated with 500 mg/kg b.w. with AETs (Figure 1D and Table 6). The ulcer index was significantly ($p < 0.05$) reduced for Ranitidine (3.87 ± 0.52) and AETs at 500 mg/kg b.w. (0.23 ± 0.07). As for the lesions (mm^2), significant ($p < 0.05$) reduction was recorded as well. Indeed, values varied from 82.31 ± 1.72 to 4.16 ± 0.04 mm^2 for doses of AETs ranging from 125 mg/kg b.w. to 500 mg/kg b.w. as compared to Control 2 group. The mucus weight was significantly ($p < 0.05$) and dose dependently augmented by both substances (Ranitidine and AETs) as compared to Control 2 (Table 6).

Phytochemical studies: Phytochemical screening of an aqueous extract from the stem bark of *Terminalia superba* (AETs) showed positive results for polyphenols, tannins, flavonoids, quinones, coumarines, saponins, reduced sugar. Sterols and polyterpenes were present in traces. No alkaloids were found out in the extract (Table 7).

DISCUSSION

Acute toxicity studies of an aqueous extract form the stem barks of *Terminalia superba* (Combretaceae) were undertaken in mice. The results showed that the graphically determined and calculated LD_{50} were respectively 12.2 ± 0.21 g/kg b.w. and 12.33 ± 0.87 g/kg b.w. when AETs was administered orally. The intraperitoneal injection of AETs permitted the determination of LD_{50} values of 1.97 ± 0.29 g/kg b.w. and 1.93 ± 0.21 g/kg b.w. respectively by the graphic method and by the calculation method. The LD_{50} values obtained graphically and by calculation methods are quite similar by oral administration. This observation is the same with intraperitoneal injection. According to the classification of [29], AETs is not toxic by oral way and weakly toxic by intraperitoneal route. This result is similar to those of plant extracts such as the methanol extract from the stem bark of *Erythrina Senegalensis* (Fabaceae) which LD_{50} is above 12.5 g/kg b.w. when administered orally to mice [30], the methanolic extract of *Elytraria acaulis* which LD_{50} is higher than 2 g/kg b.w. [31] and the methanolic extract of *Fagara zanthoxyloides* with a LD_{50} higher than 5 g/kg b.w. in rats [32]. Those different extracts were found to be non-toxic. AETs can be considered safe when injected intraperitoneally as compared to a chromatographic fraction from *Bidens pilosa* leaves with a LD_{50} of 452.50 ± 23.10 mg/kg b.w. [33] and the aqueous leaf extract of *Sesamum radiatum* with a LD_{50} of 184.2 ± 21 mg/kg b.w. [34] which are more toxic than EATs when injected intraperitoneally in mice. The toxicity of a substance can change according to the route of administration and some authors highlighted the relation between the dose and the way of administration [35]. This fact is verified with AETs. Since the use of AETs is advised in oral route by traditional healers, it can be concluded that there is no danger in this practice. However, sub-acute and chronic toxicity must be implemented to ascertain the complete safety of AETs.

Anti-ulcerogenic effects of AETs were investigated on HCl/ethanol, indomethacin, pylorus ligation and cold restraint stress-induced gastric lesions in rats. The results of this study showed that the aqueous extract from the stem bark of *Terminalia superba* possessed significant anti-secretory, anti-ulcer and cytoprotective properties in rats. Similar effects were observed by different authors with ethanolic extract of *Gynura procumbens* leaf, rocket "*Eruca sativa* " (Brassicacae) a salad herb, ethyl acetate extract of the fruit of *Morinda citrifolia* Linn (Rubiaceae), aqueous and methanol extracts of *Solanum torvum* (Solanaceae), aqueous and organic extracts of the stem bark of *Anthocleista vogelii* (Loaganiaceae) and *Ocimum sanctum* (Labiaceae) [36-41]. According to the authors cited above, those medicinal plants exhibited anti-ulcer effects against gastric mucosal lesions in rats. Some authors suggested that *Ocimum sanctum* possessed

potent anti-ulcerogenic as well as ulcer-healing properties and could act as therapeutic agent against peptic ulcer disease [41]. Ethanol induced ulcers are due to direct necrotizing effect of ethanol on gastric mucosa [42]. Ethanol causes necrosis of superficial epithelial cells on gastric mucosa and erosion [43]. Therefore a cytoprotective agent, which increases mucus secretion, will be effective in this model. In the present studies, AETs significantly reduced the ulcer index as compared to control group in animal model of HCl/ethanol-induced ulcers. So, AETs had a cytoprotective effect which was similar to the effects of Cimetidine and Maalox. The cytoprotective ability of AETs may be due to its capacity to stimulate mucus production. According to a study, the cytoprotective property against necrotizing agent-induced gastric lesions can be mediated through a number of mechanisms that include enhancement of the gastric mucosal defense through increase in mucus and/or bicarbonate production, reducing the volume of gastric acid secretion or by simply neutralizing the gastric acidity [44]. Accordingly, AETs could either reduce the gastric acid secretion or enhance the barrier defense of the mucosal wall. The cytoprotective effect of AETs was further confirmed by evaluating its efficacy on indomethacin-induced ulcer. It is known that indomethacin is a cyclooxygenase inhibitor which suppresses gastroduodenal bicarbonate secretion, reduces endogenous prostanglandin biosynthesis and disrupts the mucosal barrier and mucosal blood flow in animals [45]. An investigation reported that prostaglandins synthesized in large quantities by gastro-intestinal mucosa can prevent experimentally induced ulcers by ulcerogens [46]. Thus, when the ulcers lesions are induced by indomethacin, the cytoprotective effect of the anti-ulcer agent can be mediated through endogenous prostanglandins. AETs elicited inhibition on indomethacin induced-gastric mucosal damage. A significant increase in gastric mucus weight in AETs treated animals is likely responsible for its gastroprotective effect against indomethacin-induced gastropathology. The mucus gel adhering to the gastric mucosal surface protects the underlying epithelium against acid, pepsin and necrotizing agents such as ethanol and indomethacin [47-50]. Moreover, gastric wall mucus plays a more important role in the defense of the gastric mucosa against chemical or mechanical aggression than the soluble mucus in the lumen of the stomach [51]. The gastric mucus coat is thought to be important in facilitating the repair of the damaged gastric epithelium [52]. It is probable that the cytoprotective activity of AETs could result, at least in part, from interaction with the adhering gastric mucus layer. It can therefore be suggested that AETs stimulates the secretion of prostaglandins or possesses prostaglandins-like substances. However, this suggestion needs to be confirmed by further investigations.

In order to probe the effectiveness of AETs in preventing gastric ulcer and also assess its anti-secretory activity, AETs was tested on pylorus ligation and hypothermic restraint stress-induced gastric mucosal lesions. In pylorus ligation, ulcers are developed due to accumulation of gastric acid and pepsin which leads to auto digestion of gastric mucosa [53,54]. Furthermore, role of free radicals is also proved in induction of ulcers [55]. Pretreatment with AETs caused a dose-dependent decrease in the volume of basal gastric secretion, titratable acidity and lesions in pylorus-ligated rats and significantly increase the mucus weight and gastric juice pH. Gastric acid is an important factor in the genesis of ulceration in pylorus-ligated rats [22]. The activation of the vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hypersecretion model of pylorus ligature is believed to increase gastric acid secretion [56]. The anti-secretory activity of AETs could be explained by the same pathway described above. Yet, the cytoprotective effect of this extract could also involve free radical scavenging activity which confirmation requires further additional experiments.

In hypothermic restraint stress-induced gastric mucosal lesions, incidence of ulcers is mainly due to increased acid secretion and generation of free radicals. A work reported that peripheral sympathetic system activation plays an important role in induction of ulcers by restraint [57]. Hypothermic restraint-stress causes disturbances of gastric mucosal circulation, alteration of gastric secretion and abnormal gastric motility which are considered to be the pathogenic mechanisms responsible for stress-induced gastric mucosal lesions and gastric mucus depletion [58]. Stress inactivates mucosal prostaglandin syntheses by accumulating hydrogen peroxide, a prostaglandin biosynthesis inhibitor, which also induces reactive oxygen species (ROS) generation [59]**.** Moreover, a positive correlation was reported between the level of gastric mucosal lipid peroxidation products, a marker of oxidative stress, and stomach damage in cold restraint-stressed rats [60]**.** AETs significantly decreased the ulcer index in this model. The protective efficacy against cold restraint-stress may be probably due to the anti-oxidant activities of AETs. This suggestion requires additional experiments to be confirmed. The possible antioxidant effect of AETs with its anti-secretagogue potential thereby strengthens the animal's physiological capacities to decrease stress ulcers.

Phytochemical tests were carried out to identify the metabolites supposed to be responsible for these pharmacological effects. The results revealed that AETs contained polyphenols, tannins, flavonoids, quinones, coumarines, saponins, reduced sugar, sterols and polyterpenes. Studies showed that tannins, saponins, flavonoids, sterols, ploy terpenes and reduced sugar possess anti-inflammatory activity [61-63]. A work showed that the anti-ulcerogenic activity of many medicinal plants is due to presence of saponins and terpenoids [64]. The anti-ulcer activity of AETs could be linked to the flavonoids since according to a study, flavonoids protect the mucosa by preventing the formation of lesions by various necrotic agents [65]. It is known that many flavonoids display anti-secretory and cytoprotective properties in different experimental models of gastric ulcer [66]. Flavonoids possess anti-oxidant properties in addition to strengthening the mucosal defense system through stimulation of gastric mucus secretion and flavonoids can scavenge for the reactive oxygen species (super-oxide anions) and free radicals produced by ethanol [67]. In addition to flavonoids, other constituents in AETs such as sterol and/or polyterpenes are known for their anti-oxidant activities, which may contribute to some of the anti-ulcer mechanisms [68].

CONCLUSION

This study showed that the high LD_{50} values obtained were a clear indication that AETs was safe for use and could protect the gastric mucosa against HCl/ethanol, indomethacin, pylorus ligation and cold restraint stress-induced gastric injury. This cytoprotective action may result to strengthening the mucosal barrier through the increase of mucus production. The exact mechanism(s) underlying this anti-ulcerogenic effect remain unknown. However, the extract contains substances which could increase endogenous prostaglandins and mucus synthesis through its potent anti-oxidant activity. It is recommended that a long-term study be conducted (sub-acute and chronic toxicity tests) in order to determine the long-term effects of the extract. The various chemical groups contained in this extract could justify the use of the plant by traditional healers. Additional experiments to isolate, purify and characterize the active constituent(s) and elucidate the exact mechanism of action of AETs are necessary.

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Table 1: Acute toxicity of AETs by oral tract in mice

			AETs	
Groups	Number of mice	Dose (g/kg b.w.)	Number of dead mice	Dead mice (%)
1	8	NS	0	00.00
2	8	6	0	00.00
3	8	8	1	12.50
4	8	10	3	37.50
5	8	12	4	50.00
6	8	14	6	75.00
7	8	16	7	87.50
8	8	18	8	100.00

Group 1 (Control group) was administered normal saline (NS) orally. Groups 2 to 8 received AETs orally from doses ranging from 6 to 18 g/kg b.w. and the mortality rate was evaluated after treatment.

Table 2: Acute toxicity of AETs by intraperitoneal tract in mice

			AETs	
Groups	Number of mice	Dose (g/kg b.w.)	Number of dead mice	Dead mice (%)
1	8	NS	0	00.00
2	8	0.5	0	00.00
3	8	1	1	12.50
4	8	1.5	3	37.50
5	8	2	4	50.00
6	8	2.5	6	75.00
7	8	3	7	87.50
8	8	3.5	8	100.00

Group 1 (Control group) was injected normal saline (NS) intraperitoneally. The 7 other groups (0.5-3.5 g/kg b.w.) was administered AETs intraperitoneally and the mortality rate was evaluated post treatment.

Table 3. Effect of AETs on necrotizing agent-induced gastric lesions

Treatment	Dose (mg/kgb.w)	US (mm^2)	UI	% I	Mucus (mg)
Control 1	-	-	-	-	156.87 ± 2.35^{z}
Control 2	-	198.13 ± 13.15^{b}	5.82 ± 0.41^{g}	-	102.13 ± 2.47^{p}
Cimetidine	12	63.10 ± 1.36^{e}	2.47 ± 0.13^{d}	68.15	155.93 ± 2.13^{z}
Maalox	50	119.57 ± 11.41^{i}	3.73 ± 0.92^{mp}	39.65	169.41 ± 2.81^{z}
EATs	125	127.12 ± 11.72^{i}	3.61 ± 0.21^{mp}	35.84	121.43 ± 0.31^{n}
	250	57.02 ± 1.01^{f}	1.82 ± 0.61^{w}	71.22	182.03 ± 10.3^{k}
	500	7.43 ± 0.24^{k}	0.29 ± 0.72^{x}	96.25	366.40 ± 11.21^{e}

AETs significantly inhibited the gastric lesions caused by the necrotizing agent (HCl/ethanol). n = 6 rats per group; US= ulcerated surface; UI=ulcer index; %I= inhibition percentage. Values with the same letter in the same column are not statistically different at $p<0.05$.

Table 4. Effect of AETs on gastric lesions induced by indomethacin

Treatment	Dose (mg/kgb.w)	US (mm^2)	UI	% I	Mucus (mg)
Control 1	-	-	-	-	156.87 ± 2.35^{z}
Control 2	30	154.78 ± 3.19^{a}	5.58 ± 0.31^{y}	-	87.37 ± 4.17^{k}
Misoprostol	0.012	48.43 ± 2.72^{b}	3.08 ± 0.38^{z}	68.71	118.11 ± 3.24^{gt}
Maalox	50	69.43 ± 5.89^{c}	3.67 ± 0.28^{x}	55.14	109.45 ± 2.12^{gt}
EATs	125	74.81 ± 4.32^{c}	4.62 ± 0.32^{ip}	51.66	173.61 ± 3.18^{n}
	250	28.73 ± 3.14^{h}	1.92 ± 0.16^{d}	81.44	301.38 ± 3.27^{zx}
	500	6.17 ± 3.43^{t}	1.38 ± 0.13^{r}	96.01	479.83 ± 7.84^{r}

Indomethacin-induced gastric lesions were significantly attenuated by the pre-treatment with AETs. n = 6 rats per group; US= ulcerated surface; UI=ulcer index; %I= inhibition percentage. Values with the same letter in the same column are not statistically different at $p < 0.05$.

Table 5. Effect of AETs on pylorus ligation-induced gastric lesions

Treatment	Dose (mg/kg b.w)	Volume (ml) (gastric juice)	pH (gastric juice)	Gastric acidity (mEq/l)	US (mm^2)	UI	%I	Mucus (mg)
Control 1	-	-	-	-	-	-	-	156.87 ± 2.35^{z}
Control 2	-	9.81 ± 0.72^{e}	1.57 ± 0.01^{es}	180.83 ± 3.14^{mk}	135.14 ± 0.76^{c}	5.31 ± 0.12^{x}	-	49.72 ± 1.78^{k}
Cimetidine	12	4.31 ± 0.21^{ij}	1.77 ± 0.06^{e}	132.16 ± 2.32^{m}	64.35 ± 0.31^{ab}	4.18 ± 0.14^{q}	52.38	63.41 ± 2.12^{at}
Maalox	50	3.28 ± 0.17^{a}	1.86 ± 0.07^{e}	129.62 ± 2.13^{m}	56.41 ± 0.53^{b}	4.09 ± 0.43^{q}	58.25	68.31 ± 1.4^{at}
EATs	125	6.34 ± 0.31^{kf}	1.97 ± 0.07^{ks}	116.33 ± 1.44^{f}	76.47 ± 0.82^{d}	4.56 ± 0.02^{t}	43.41	57.21 ± 0.15^{l}
	250	3.71 ± 0.14^{b}	1.93 ± 0.03^{ks}	112.46 ± 2.13^{f}	18.61 ± 0.47^{h}	2.12 ± 0.03^{v}	86.23	65.07 ± 0.21^{at}
	500	3.12 ± 0.13^{aj}	2.19 ± 0.05^{sg}	89.67 ± 2.81^{p}	2.56 ± 1.31^{x}	0.98 ± 0.08^{w}	98.10	74.31 ± 0.81^{o}

Pylorus ligation-induced gastric ulcers were significantly impeded by different doses of AETs. n = 6 rats per group; US= ulcerated surface; UI=ulcer index; %I= inhibition percentage. Values with the same letter in the same column are not statistically different at $p < 0.05$.

Table 6. Effect of AETs on hypothermic restraint stress-induced gastric mucosal lesions

Treatment	Dose (mg/kgb.w)	US (mm^2)	UI	% I	Mucus (mg)
Control 1	-	-	-	-	156.87 ± 2.35^{z}
Control 2	-	128.13 ± 3.15^{b}	5.97 ± 0.71^{g}	-	58.13 ± 0.17^{p}
Misoprostol	0.012	39.81 ± 0.63^{e}	2.49 ± 0.45^{d}	68.93	76.93 ± 0.14^{nq}
Ranitidine	50	76.38 ± 1.41^{i}	3.87 ± 0.52^{e}	40.39	72.41 ± 0.31^{nq}
EATs	125	82.31 ± 1.72^{i}	3.38 ± 0.81^{mp}	35.76	71.43 ± 0.42^{nq}
	250	35.86 ± 0.01^{f}	1.72 ± 0.02^{w}	72.01	82.03 ± 0.13^{k}
	500	4.16 ± 0.04^{k}	0.23 ± 0.07^{x}	96.75	136.40 ± 1.21^{e}

Gastric lesions elicited by hypothermic restraint stress were significantly reduced by preventive employment of AETs. n = 6 rats per group; US= ulcerated surface; UI=ulcer index; %I= inhibition percentage. Values with the same letter in the same column are not statistically different at $p < 0.05$.

Table 7. Phytochemical screening of AETs extract of the stem bark of *Terminalia superba*

Constituents	Reagents	AETs
Polyphenols	$FeCl_3$ test	+
Tannins	Stiasny test	+
	$FeCl_3$ test	-
Flavonoids	Cyanidine test	+
Quinones	Borntraëger test	+
Alkaloids	Bouchardat test	-
	Dragendorff test	-
	picric Acid test	-
Saponins	Frothing test	+
Sterols and polyterpenes	Liebermann test	…
Reduced sugar	Tollens test	+
Proteins	Biuret test	+
Coumarines	reaction on the lactonic cycle	+

. …: traces +: positive -: negative

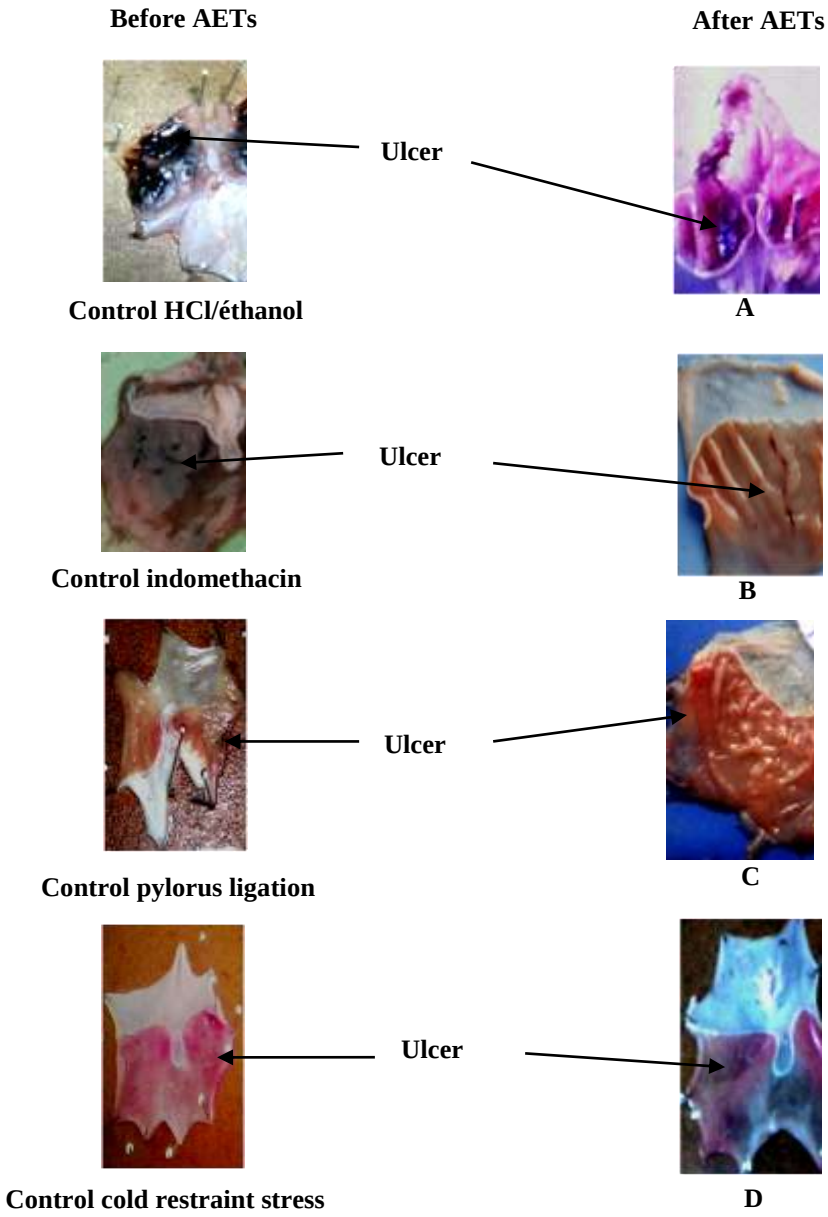

Figure 1: Gross appearance of gastric ulcers before AETs administration to the rats. The ulcerated area was larger in the control groups than in the treated.
A, B, C and D indicated the treatment of AETs at 500mg/kg b.w. on HCl/ethanol, indomethacin, pylorus ligation and cold restraint stress-induced gastric lesions in rats respectively.