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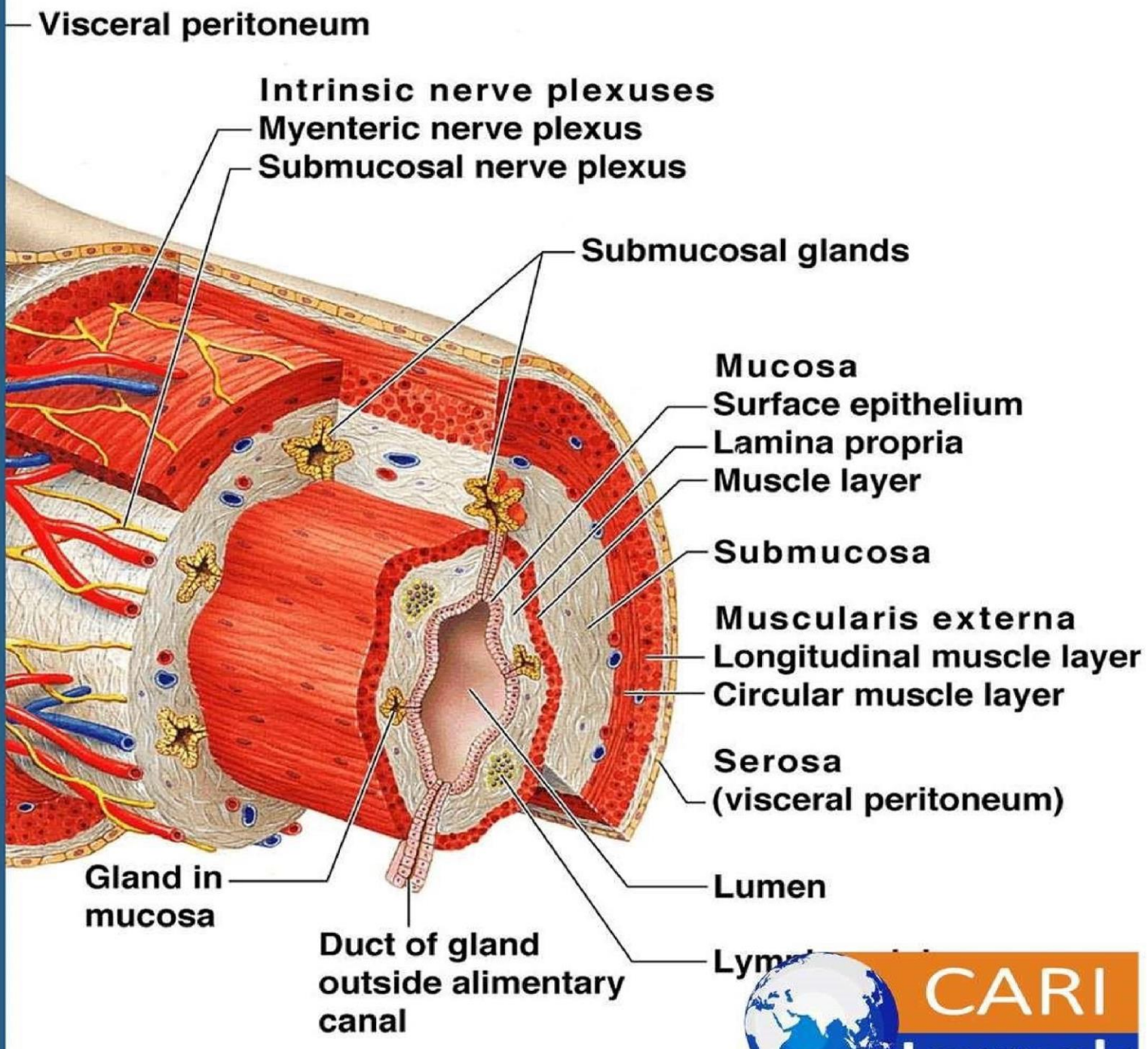

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Gastroprotective and Curative Effects of the Aqueous Extract of Stem Bark of *Pittosporum Mannii* Hook F. (Pittosporaceae) on Some Models of Gastric Ulcer in Rats

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Abstract

Purpose: This work assesses the antiulcerogenic and antiulcer properties of the aqueous extract of *Pittosporum mannii* and shows at what dosage this plant would not be of any danger to the patient.

Methodology: The aqueous extract of its bark, orally administered at the doses of 35, 75, 150 and 300mg/kg, has been experimented on acute (HCl/EtOH (150mM HCl in 60% ethanol); ligature of the pylorus) and chronic ulcers (acetic acid; 0.05 ml of 30% acetic acid) induced on rats.

Findings: The results from these experiments show that: the aqueous extract of the barks of *Pittosporum mannii* possesses antiulcerogenic properties. At 300mg/kg dose, the extract completely inhibited (100%) the ulcer induced by the mixture HCl/EtOH. For the three patterns of gastric ulcers induction used, the mucus weight did not vary significantly ($p>0.05$) on the treated animals as compared to the negative control groups. In the group that underwent pylorus ligature, the extract did not induce any significant variation in both the gastric acidity and the gastric volume. However these results do not bring out the exact mechanism of action of the plant extract. Furthermore, the extract neither acts in reinforcing the protection barrier of the gastric mucus membrane nor its antisecretory properties. The nitric oxide proportion of the gastric mucous membrane presents a significant variation ($p<0.05$) at the dose of 300mg/kg. With regards to these results, we realized that this extract increases the nitric oxide rate of the gastric mucosa which intervenes in the healing process. The aqueous extract of the stem bark of *Pittosporum mannii* provokes the death of all animals of the groups from a dose of 3g/kg (LD_{100}) whereas half of the population of the experimental rats succumbed at the dose of 2.66g/kg (LD50). After two weeks of treatment, the rats that received the extract at the doses of 75, 150 and 300mg/kg presented no significant variation of the hepatic and plasmatic protein levels. The levels of plasma

transaminases (AST; 300mg/kg), hepatic ALT (300mg/kg) and plasmatic ALT (150mg/kg) present a significant drop. The stem bark of *Pittosporum mannii* possess gastroprotective and ulcer-healing effect although at this time it is difficult to explain the exact mechanism involved in these two processes.

Unique contribution to theory, policy and practice: According to the results obtained, it would be important to realise a histological test of the detoxification organs such as the liver, the heart and the kidneys. Moreover, the cytotoxicity test has to be done to determine the effect of the extract at the cellular level before any therapeutic use.

Keywords: *Pittosporum mannii*, gastric ulcer, toxicity.

1. INTRODUCTION

Gastric ulcer disease is known as a major cause of morbidity and mortality (Tsoi et al., 2022). The pathophysiology of gastric ulcer has been centralized on an imbalance between aggressive (gastric-acid-pepsin secretion) and protective (gastric mucosal integrity) (Gugliandolo et al., 2021). Most of the drugs available are thought to act on the offensive factor, which neutralizes acid secretion, H2 receptor blockers, anticholinergics and proton pump blockers, which interfere with acid secretion. These products, some time, are very expensive and present low effectiveness. To solve this problem, traditional medicine also uses herbal preparation to threat this pathology. The natural substances provided by the plants present many advantages: effectiveness, availability of the products used and accessibility of the ingredients (Kamanyi, 2006). Some information obtained from the African phytomedicine indicated the use of the stem bark of *Pittosporum mannii* (P. m.), a tree which grows in the mountains and littoral rainforest of Africa, for curing many diseases such as syphilis, fever, malaria, sexual failure, high blood pressure, intestinal and infectious diseases, pain, inflammation and gastric ulcers (Adjanohoun et al., 1996; Momeni et al., 2010; Njiaza et al., 2015). The current study was undertaken to determine the antiulcer potential of the aqueous extract of the stem bark of P. m. (AEPM), using three experimental gastric ulcer methods, namely HCl/ethanol, pylorus ligation and acetic acid induction. This study was also undertaken to determine at what dosage level this plant will not be of any danger to the patient.

2. MATERIALS AND METHODS

2.1. Plant material identification, collection and extraction

The stem bark of P. mannii were harvested in Foumban in November and were identified at the Cameroon National Herbarium, Yaounde´ by comparison to existing voucher specimen number 22420HNC. These stem bark s were dried in shade, ground and extracted as a decoction to yield 6.35% of aqueous extract (AEPM).

2.2. Animals

Wistar rats (150-175g) and swiss mice (20-25g) of either sex obtained from the animal house of the Department of Animal Biology of University of Dschang were used for the study. They were raised in the animal house of the Faculty of Science of University of Dschang and fed with normal laboratory rat diet and water was given ad libitum under standard conditions of 12 hours of darkness and 12 hours of light. Fasting was used prior to all assays and aqueous extract was always administered orally.

2.3. Drugs and chemicals

Formol, HCl, ethanol, NaOH, acetic acid, cimetidine (tagamet), Maalox were obtained from local pharmacy.

2.4. Anti-ulcers study

2.4.1. HCl/ethanol induced ulcers

Gastric mucosal lesions were induced in male rats using the HCl/ethanol method as described by Hara and Okabe (1985). The animals were divided into 6 groups of 5 rats each. Group 1, the negative control group which received 1ml/100g body weight distilled water. Group 2, the positive control group, received 1ml/100g body weight of Maalox (50mg/kg). Group 3 to 6 also received 1ml/100g body weight aqueous extract (35, 75, 150 and 300mg/kg).

One hour after the drug treatment, 1ml per 150g body weight of the necrotizing solution (150mM HCl in 60% ethanol) was given to each rat and was sacrificed one hour later using the solution of thiopental (50mg/kg). The stomach was removed and observed for ulcer in the glandular region. The ulcer area was measured and scored as described by Tan et al. (1996). The ulcer index for each rat was taken. The percentage of ulcerated surface was calculated. The percentage of inhibition was calculated using the following formula:

% I = ulcer surface area of negative control group – ulcer surface control of treated group x 100 / ulcer surface area of negative control group

2.4.2. Pylorus-ligature induced ulcers

According to the method described by Shay et al. (1945) quoted by Cao et al., 2005, male Wistar rats fasted for 48h were used in this experiment. Rats were divided into 6 groups of 5 rats each. Group 1, the negative control group, received 1ml/100g body weight of distilled water. Group 2, the positive control group, received 1ml/100g body weight of cimetidine (12mg/kg). Group 3 to 6 received also 1ml/100g body weight of aqueous extract (35, 75, 150 and 300mg/kg). All substances were administered orally at the rate of 1 ml per 100 g of body weight. One hour later, the animals were anesthetized with ether. The abdomen was opened and the stomach ligated at the level of pylorus. The incised abdomen was then sutured. Six hours after the operation, the animal was sacrificed by over inhalation of ether solution, the abdomen reopened, the stomach was ligated at the level of the cardia and then isolated from the rest of the animal. The stomach contents were collected in a tube and then subjected to centrifugation at a speed of 2000 rpm for 10 minutes. The

supernatant was collected, then its volume and its pH were measured. The total acid content of gastric secretion was also determined by titration to pH 7.0 with 0.01N NaOH using a digital burette. The stomach was opened along the greater curvature and ulcers produced were graded as following: 0: absence of ulceration, 1: dilation of vessels and small points, 2.5: small ulcers < 4 mm long, 3.5: 4 mm long < ulcer ≥ 5 mm long, 5: large ulcer > 5 mm long (Tan et al., 1997b). The percentage of inhibition were estimated as described above.

Measurement of gastric acidity

1 ml of gastric juice was diluted 10 times and titrated with 0.01N sodium hydroxide solution using pH meter. The experiment was done three times for each animal.

2.4.3. Acetic acid-induced ulcer.

The experiments were done according to the method described by Takagi et al (1969) with some modifications. Thirty male Wistar rats were starved for 24 hours prior to the experiments and were divided into 6 groups. Under thiopental (50mg/kg) anaesthesia, a laparatomy was done in all animals through a midline epigastric incision after exposing the stomach, 0.05 ml of 30% acetic acid were injected into subserosal layer in the glandular part of the anterior wall (Hara et Okabe 1985). The stomach was bathed in saline solution 0.9 %) to avoid adherence to the external surface of the ulcerated region. The abdomen was then closed and all animals where fed normally. The distilled water, the aqueous extract (75, 150 and 300mg/kg) and Maalox (50mg/kg) was administered orally once a day for 14 consecutive days beginning one day after surgery. Body weight was recorded daily throughout the experiments to evaluate the possible chronic toxicity induced by the treatments on the day after the last drug administration. The rats were killed, the stomach removed to assess the ulcer area.

2.4.4. Mucus weight

Gastric mucus production was measured in the rats subjected to Hcl/ethanol, pylorus ligation, acetic acid induced lesions according to the method described by Tan and Nyasse (2000). The gastric mucus from each rat was gently scraped off using a glass slide and the resulting mucus was carefully weighed using a sensitive digital electronic balance.

2.5. Acute toxicity

The experiment was done according to the method described by Sousa Brito (1995). In the first experiment, a single dose of the aqueous extract was administered orally to 5 groups of 6 mice (20-24g). The dosage level were 1500, 3000, 6000 and 12000mg/kg body weight. The number of deaths that occurred within three hours post gavage was recorded.

In the second experiment, the negative control group received distilled water and a single dose of the aqueous extract was administrated orally to 4 others groups (500, 1000, 1500, 2000 mg/kg. The number of deaths that occurred within three hours post gavage and animals' behaviour (reaction to noise, mobility, pinch, aggressiveness, faecal statement) was recorded.

2.6. Statistical analysis

Statistical analysis was performed using ANOVA and t-test, and the significance of difference between treatments was accepted at $p \leq 0.05$. Results are expressed as Mean ± SEM (standard error of the mean)

3. RESULT

3.1. HCl/ethanol induced ulcers

The injury caused by HCl/EtOH confers a direct topical mucosa with an undesirable effect. It is a good model to investigate product with a possible cytoprotective activity. The oral administration of a 150mM/ 60% EtOH solution produced characteristic lesions in the glandular portion of the negative control rats' stomach with a total ulcer area of 195.08± 9.92 mm^2. As table 1 shows, the pre-treatment with AEPM at doses 35, 75, 150, and 300mg/kg) significantly ($p \leq 0.05$) and dose dependently decreased the severity of lesion with 82.54, 88.12, 98.51 and 100% respectively (Table 1).

The animals treated with Maalox at dose 50mg/kg produced a significant ($p \leq 0.05$) decrease in ulcer area from 195.08 ± 9.92 mm^2 to 56.33 ± 9.12 mm^2. The mean ulcer index scored decreased significantly from 3.81 ± 0.28 in negative control group to 0.66 ± 0.42 mm^2 and 0.00 ± 0.00 mm^2 in aqueous extract respectively in dose 150 and 300mg/kg.

3.2. Pylorus-ligature ulcer technique

The results obtained by pylorus ligation are shown in Table 2. The control group produced pointed lesions or raised inflammation. The total ulcerated area obtained in the negative control group was 15.81 ± 1.51 mm^2. The AEPM at doses 35, 75, 150 and 300mg/kg produced a significant decrease in ulcer area, from 15.81 ± 1.51mm^2 in the control group to 53.74±5.65 and 5.6±2.19 mm^2 in the group that received aqueous extract at dose of 300mg/kg corresponding to the inhibition percentage of 64.16%.

The ulcer index significantly decreases at dose of 150 mg/kg (1.37 ±0.45) compared to the negative control group (2.55 ± 0.10). But no significant difference was observed at 300mg/kg (1.74 ± 0.39). The mucus weights present no difference in all groups. The volume of gastric juice, gastric acidity (35, 75, and 150mg/kg) and pH of animals treated with aqueous extract of the plant (35, 150 and 300mg/kg) present no difference compared to the negative control group.

3.3. Acetic acid-induced ulcer

The subserosal administration of 0.05 ml of acetic acid solution 30% presented 2 weeks later in the negative control animal a deep crater . In this group the ulcerated area and the mucus weight were respectively of 53.11 ± 5.69 mm^2 and 118.33 ± 10.46 mg (Table 3). No significant difference of the mucus weight was observed in the groups of animals treated with various doses of *P. mannii* aqueous extract compared with the negative control group.

The administration of the Maalox at dose 50mg/kg produced a significant reduction ($p < 0.05$) of the ulcer surface area (9.81 ± 4.22 mm^2) compared to the negative control group (53.11 ± 11.69mm^2). On the other hand, no significant difference was observed as regards the mucus weight between the groups of animals having received the Maalox (101.67 ± 12.22 mg) and the negative control group (118.33±10.46 mg) (Table 3).

3.4. Assessment of the relative body weight of rats

The aqueous extract of the stem barks of Pittosporum mannii does not show any significant difference in weight between the different groups of animals treated at different doses. However, a progressive weight gain is observed from the 1st to the 15th day (Figure 1).

3.5. Biochemical analysis

3.5.1. Effects of the AEPM on proteins (plasmatic and hepatic) and nitric oxide.

For hepatic proteins, there was no significant difference between the various groups of animals treated with the aqueous extract of the plant compared with the negative reference group. As for plasmatic proteins we noted a significant decrease of concentration in the group of animals treated with the Maalox (39.55 ± 3.04 mg/ml) compared to the negative control group. In addition a significant increase in the rate of nitric oxide was observed only with the dose 300mg/kg (0.20 ± 0.024 nmol/g) compared to the negative reference group (0.03± 0.01nmol/g) (Table 4).

3.5.2. Effects of the AEPM on plasma and hepatic transaminases

Table 5 presents the various values obtained after the proportioning of the concentration of alanine aminotransferase (ALT) and the aspartate aminotransferase in plasma and the hepatic surpernatant. We observed no significant difference ($p>0.05$) for the hepatic AST. The hepatic ALT and the plasmatic AST respectively presented a significant decrease at the dose 300mg/kg (3.24 ± 0.00 and 38.83 ± 2.29UI/l) compared to the negative control group (16.84 ± 2.97 and 65.44 ± 3.45UI/l). For the plasmatic ALT, a significant decrease was observed only with the dose 75mg/kg (6.48 ± 1.02UI/l) compared to the negative reference group (18.79 ± 1.21UI/l).

3.6. ACUTE TOXICITY

3.6.1. General behaviour of the mice after administration of the extract

The administration of the AEPM modified the mobility of the animals (Table 6). With the doses of 0.5 and 1.5g/kg, they moved slowly. From dose 3g/kg this movement became very difficult. The communication between the animals decreased at the dose 1.5g/kg and became deep starting from dose 6g/kg. The sensitivity to the pain due to the pinching of the tail and the sensitivity to the sound were manifested starting from dose 1g/kg and became deep starting from dose 2g/kg. The aggressiveness of the animals dropped slightly at dose 1 and 1.5 g/kg, decrease starting from dose 2g/kg. At dose 1.5g/kg the state of the saddles started to be soft and became completely liquid with the amount 6g/kg. The animals which did not die after the experiment were comparable 24 hours later with the negative control group of animals.

3.6.2. Mortality of the mice during the acute treatment by the AEPM

Table 7 shows the rate of mortality in the mice during the acute treatment at the aqueous extract of the stem barks of *Pittosporum mannii*. Between the groups of the animals having received the extract at the dose 0.5g/kg and those having received 1.5g/kg, one-third of the animals died. In addition, the death rate was 100% in the groups treated at doses 3, 6 and 12g/kg. The death rates obtained during the test of mortality were 55.55%.

3.6.3. Determination of the average lethal dose or lethal dose 50 (Method by calculation)

The lethal dose 50 (LD50) was determined by Spearman and Kärber quoted by Chippaux et al (1997) formula as following:

$DL50 = DL100 - \sum [(z \times d) / n]$.

DL100 = lethal dose which killed all the animals.

z = half of the sum of dead animals in two groups corresponds to a successive dose.

d = difference between the successive doses administered to 2 successive batches.

n = number of rats per group.

4. DISCUSSION

The results of this study show that the aqueous extract of the stem barks of *Pittosporum mannii* at doses 35, 75, 150 and 300mg/kg has significant antiulcerogenic properties on the ulcer induced by HCl/ethanol and the pylorus ligation as well as antiulcerous effects on the ulcer induced by the acetic acid. HCl/ethanol has been reported to cause disturbances in gastric secretion, damage to the mucosa, alterations in the permeability, gastric mucus depletion and free radical production. Our results confirmed that administration of acidified ethanol caused severe gastric damage accompanied by significant increases in hemorrhagic lesion. The AEPM showed significant cytoprotector effects. The cytoprotection of the gastric mucosa against the irritating agents uses several mechanisms such as the reduction of the secretion of acid or quite simply the neutralization of acidity and the intensification of the mucosal barrier through the increase in the production of mucus and bicarbonate (Nguelefack et al, 2005a, Yoo et al., 2018). The mucus weight did not present a significant difference ($p>0.05$) in the animals of the groups treated with the solution of Maalox at dose 50 mg/kg and with the aqueous extract of the stem barks of *Pittosporum mannii* at doses 35, 75 and 300 mg/kg. However we noted a significant decrease of the mucus weight in the animals of the group treated with the aqueous extract of the stem bark of *Pittosporum mannii* at dose 150mg/kg. This significant decrease of the mucus weight at the dose 150mg/kg excluded the assumption according to which the substance tested would act by intensification of the mucosal barrier through the increase in the production of mucus. This model of the gastric ulcers induction by necrotic mixture HCl/EtOH, although fast and being indicated (Tan et al., 2000), does not allow us to appreciate exactly the mechanism of action of the aqueous extract.

In order to check the antisecretory and antacid activities of the aqueous extract of the stem barks of *Pittosporum mannii*, the ulcer induced by pylorus ligation technic was carried out. It is known that, the pyloric ligation of the stomach causes accumulation of gastric acid which leads to development of ulceration in stomach. The agents that decrease gastric acid secretion are effective in preventing the ulcers induced by this method (Deshpande et al., 2003; Saroj Kumar Sahoo et al., 2016). This antiulcerogenic test shows that the aqueous extract of the stem bark of *Pittosporum mannii*, dose-independently and significantly reduced the ulcer surface area produced by pylorus ligation, meaning that, aqueous extract of the stem bark might prevent the ulcer by antisecretory action. We observed a significant increase ($p<0.05$) of the pH at the dose 75mg/kg and the volume of the gastric juice at the dose 300mg/kg. This increase could translate the antacid activity of the extract. But the gastric acidity rate and the mucus weight did not differ significantly whatever the dose of aqueous extract of the stem bark of *Pittosporum mannii* administrated to these animals. The absence of significant variation of the mucus weight goes in line with the results obtained during the test with HCl/EtOH and justifies once more the assumption that the extract of this plant would not act by intensification of the mucosal barrier. In the same way, the absence of significant variation of the gastric acidity rate takes us along to exclude the assumption that the aqueous extract of this plant would have an antisecretory activity.

The model of induction of the ulcer by the acetic acid was selected because this method produces in the rats the lesions similar to the chronic ulcers in Man in terms of location, severity, and chronicity as well as in the physiological processes related to healing (Okabe et Amagase, 2005; Batista et al., 2015). In this model, the acetic acid produces a restricted wound in the form of a crater on the glandular part of the stomach. The results of this study show that the average of ulcer area of the stomach of the animals which have received extract (300mg/kg) and mucus weight at all groups of animals treated with the aqueous extract of the stem barks of *Pittosporum mannii* were not significantly changed. These results let us to believe that the aqueous extract of the stem bark of *Pittosporum mannii* would have curative properties only with amounts 75mg/kg and 150mg/kg. In view of this result, AEPM may accelerate the healing of gastric ulcer by acting on other factors such as growth factors which have been identified as the first to have a healing effect on chronic gastric ulcer (Skarstein et al., 1979; Ateufack et al., 2015).

The study of acute toxicity of the aqueous extract of the stem bark of *Pittosporum mannii* revealed that the maximum dose tolerated by the mice is at least equal to 0.5g/kg. On the top of this amount the number of deaths increases gradually and reaches the maximum at 3g/kg (LD100). This progressive increase in the death rate could result from the accumulation of the toxic compounds in the mice; of which the proportions would increase with the amount. The mean lethal dose (LD50), was 2,66g/kg. According to Delongeas et al. (1983) and Schorderet (1992), all products which have an LD50 >5g/kg are regarded as nontoxic. The aqueous extract of the stem bark *Pittosporum mannii* would thus be a toxic extract, because the values of DL100 and DL50 obtained are lower than 5g/kg. After administration of a single dose of the aqueous extract of *Pittosporum mannii*, the animals presented a depressive state dose-dependent with reduction on mobility,

sensitivity and aggressiveness. This extract possesses similar effects to those of *Bidens Pilosa* which would act like tranquillizing or myorelaxant (Bouquet and Debray, 1974). This reduction of the sensitivity to the noise and the pinching could result from an attack of the nervous system, either at the level of the receptor cells, or at the level of the conducting elements (Olivier and Bever, 1986; Stuart, 1999). The reduction in the social interaction observed could translate the sedative or tranquillizing effect of the extract (Nguelefack et al., 2002).

The saddles of the mice which passed from the granulous state to liquid state, starting from the dose 1.5g/kg, could translate an acceleration of the intestinal transit involving the fast elimination of saddles before their consolidation (Emerson et al., 1993). The results of the proportioning of plasma and hepatic proteins during the test of subchronic toxicity reveal no significant difference ($p>0.05$) in the groups of animals treated with the AEPM compared to the negative reference group. The plasma proteins are synthesized mainly by hepatocytes which constitute approximately 60% of the hepatic cells (Gelbhardt, 1992). Then, the aqueous extract of this plant would have the chemical compounds having very little effect on the hepatocytes but which could act on the other liver cells.

For a long time, the medicinal plants wrongly enjoyed the reputation of non-total toxicity. But today the toxicity of certain plants is not doubted any more. Therefore the study of the degree of toxicity of all the medicinal plants is an important precaution to take before advising a product to the treatment of an infection. Studies undertaken in this view have shown that the majority of the compounds resulting from the toxic plants accumulate in the liver where they are detoxicated (Clarke and Clarke, 1977). Also, the liver is one of the major targets of xenobiotic biotransformation (Hoff-Brand et Pettit, 2000; Unuofin et al., 2018). The hepatic and plasma transaminases (ALT and AST) rates were determined to evaluate the hepatotoxicity. This test shows a significant decrease ($p<0.05$) of the rates of hepatic ALT and the plasma AST with the dose 300mg/kg and that of the plasma ALT to the dose of 150mg/kg. The significant decrease of these enzymes could mean that the hepatic cells are not affected, since hepatic necrosis cells rather involve an increase in the plasma rate of ALT and AST. This shows that the aqueous extract of *Pittosporum mannii* would be saved toxic effects at the hepatic level after 14 days of treatment. That takes us along to confirm that the great therapeutic dose (300 mg/kg) that we tested is not toxic for the organism. This dose and this treatment time (14 days) may be indicated for the treatment of the gastric ulcer.

To investigate the possibility of a chronic toxicity, the animals' weight was also measured during the experiment (14 days). The result shows that the average weights of the various groups of animals did not present any significant difference. This means that the therapeutic doses of the extract do not affect the animals' growth.

The results of this study reveal that the aqueous extract of the stem bark of *Pittosporum mannii* possess gastroprotective effect and ulcer-healing effect although at this time it is difficult to explain the exact mechanism involved in these two processes.

Before any therapeutic use, it would be important to realise a histological test of the detoxification organs such as the liver, the heart and the kidneys. Moreover, the cytotoxicity test has to be done to determine the effect of the extract at the cellular level.

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Table and figure

Table 1: Effects of the stem bark aqueous extract of *Pittosporum mannii* on HCl/ethanol induced gastric lesions in rats

Treatment	Dose (mg/kg)	n	SU (mm^2)	% SU	IU	% I	Mucus weight (g)
distilled water	/	6	195.08 ± 9.93 a	15.01 ± 87 a	3.81 ± 0.28 a	/	198.63 ± 39 a
Maalox	50	6	56.33 ± 9.12 bc	5.15 ± 0.90 bc	3.78 ± 0.38 ac	71.124	155 ± 13.10 ac
Aqueous extract	35	6	34.05 ± 5.95 bc	2.75±0.47 bc	2.95 ± 0.28 ac	82.542	141.17 ± 32.52 ac
	75	6	23.16 ± 3.00 bd	2.12 ± 0.31 bd	2.87 ± 0.34 ac	88.123	101.33 ± 20.89 ac
	150	6	2.89 ± 1.83 bd	0.24 ± 0.15 bd	0.66 ± 0.42 bd	98.517	89.83 ± 6.935 bc
	300	6	0.00 ± 0.00 bd	0.00 ± 0.00 bd	0.00 ± 0.00 bd	100	104.33 ± 0.66 ac

n = number of rats per group; I = inhibition; IU= ulcer Index; SU = ulcer surface area. In the same column, the affected values of the same letter do not differ significantly ($P < 0.05$). $^{b}P < 0.05$: statistically significant compared to the negative control group; $^{d}P < 0.05$: statistically significant compared to the positive control group; a: not statistically significant compared to the negative control group; c: not statistically significant compared to the positive control group. Data was analyzed using one way ANOVA followed by Dunnet's t-test.

Table 2: Effects of the stem bark aqueous extract of *Pittosporum mannii* on Pylorus-ligation induced gastric lesions in rats.

Treatment	Dose (mg/kg)	n	SU (mm2)	% SU	IU	% I	Mucus weight g	gastric	Gastric
Distilled water (Neg C)		6	15.81 ± 1.51 a	4.26 ± 0.93 a	2.55 ± 0.10 a	/	57.66 ± 3.90 a	493.33 ± 89.74	3.42 ± 0.26
Cimetidine	12	6	14.62 ± 1.61 ac	2.24 ± 1.33 ac	2.11 ± 0.44 ac	7.51	82.00 ± 15.48 a	464.00	3.33
Aqueous extract	35	6	7.91 ± 0.49 bc	1.23 ± 0.12 bc	1.31 ± 0.16 bc	49.94	64,33 ± 8.73 a	221.17 ± 38.76 a	3.88 ± 0.54 a
	75	6	7.73 ± 0.65 bc	0.81 ± 0.23 bc	1.11 ± 0.05 bc	51.1	82.16 ± 14.65 a	482.50 ± 131.95 a	4.73 ± 0.36 b
	150	6	5.00 ± 4.89 bd	0,73 ± 0,31 bc	1.37 ± 0.45 bc	61.32	52.66 ± 6.34 a	489.83 ± 186.92 a	3.64 ± 0.31 a
	300	6	5.66 ± 2.19 bd	0.82 ± 0.35 bc	1.74 ± 0.39 ac	64.16	82.28 ± 12.00 a	478.29 ±79.07 a	4.19 ± 0.44 a

n = Number of rats per group; I = inhibition; IU= ulcer Index; SU = ulcer surface area. In the same column, the affected values of the same letter do not differ significantly ($P < 0.05$);). $^{b}P < 0.05$: statistically significant compared to the negative control group; $^{d}P < 0.05$: statistically significant compared to the positive control group; a: not statistically significant compared to the negative control group; c: not statistically significant compared to the positive control group. Data was analyzed using one way ANOVA followed by Dunnet's t-test.

Table 3: Variation of the ulcer surface area and the mucus weight in the rats whose ulcer was induced by the acetic acid.

Treatment	N	Dose (mg/kg)	Ulcer area (mm^2)	Mucus weight (mg)
Distilled water	6	/	0.00 ± 0.00 b	105.00 ± 31.59 a
Distilled water	6	/	53.11 ± 5.69 a	118.33 ± 10.46 a
Maalox	6	50	9.81 ± 4.22 bc	101.67 ± 12.22 a
Aqueous extract	6	75	21.94 ± 3.53 bc	100.00 ± 11.54 a
	6	150	15.80 ± 4.35 bc	141.67 ± 29.72 a
	6	300	40.86 ± 6.42 ad	138.33 ± 18.87 a

n = number of rats per group. In the same column, the affected values of the same letter do not differ significantly ($P < 0.05$). $^{b}P < 0.05$: statistically significant compared to the negative control group; $^{d}P < 0.05$: statistically significant compared to the positive control group; a: not statistically significant compared to the negative control group; c: not statistically significant compared to the positive control group. Data was analyzed using one way ANOVA followed by Dunnet's t-test.

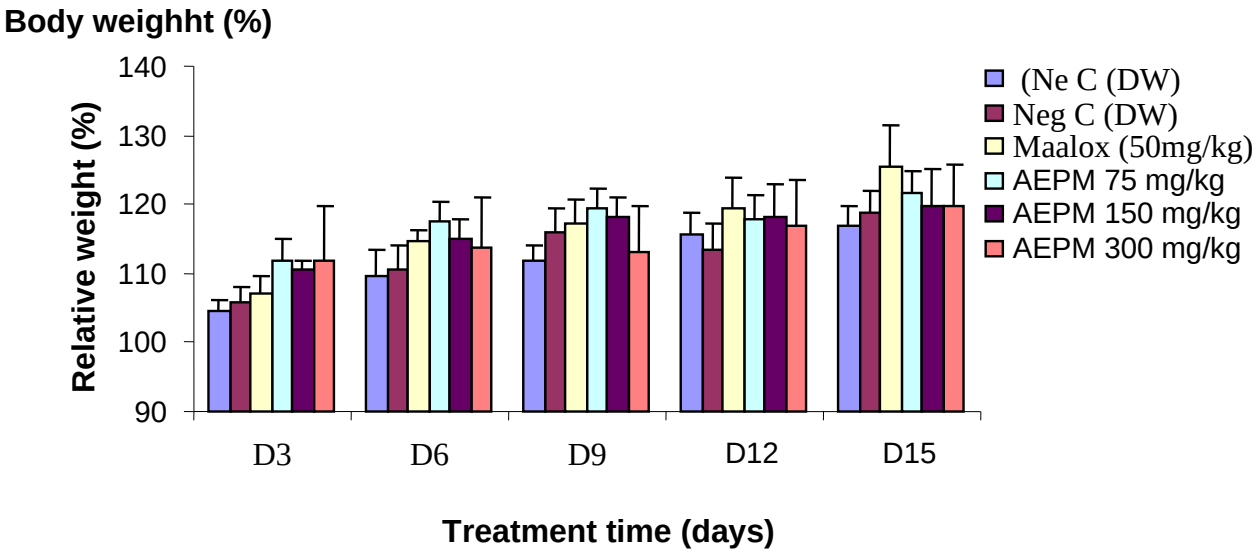

Figure 1: body weight gain in rat treated orally with aqueous extract (AE) of *Pittosporum mannii* (75,150 and 300mg/kg) during 14 days after ulcer formation by 30 % acetic acid solution injected into the subserous layer of the stomach. Data was analyzed using one way ANOVA followed by Dunnet's t-test; DW: Distilled water; D=day; NeC=Neutral control; NegC= Negative control.

Table 4: **effects of the aqueous extract of *Pittosporum mannii* on proteins (plasma and hepatic) and nitric oxide.**

Treatment	n	Dose (mg/kg)	Plasmatic protein (mg/ml)	Hepatic protein (mg/g liver)	Nitric oxide nmol/g organ
Neutral control	6	/	51.47 ± 1.72 a	249.52 ± 24.20 a	0.26 ± 0.11 a
Negative control	6	/	51.22 ± 1.11 a	279.87 ± 30.37 a	0.03 ± 0.01 a
Maalox	6	50	39.55 ± 3.04 b	263.34 ± 41.10 a	0.02 ± 0.00 a
aqueous extract	6	75	55.86 ± 3.62 a	270.16 ± 13.37 a	0.06 ± 0.01 a
	6	150	57.14 ± 3.15 a	241.54 ± 10.40 a	0.13 ± 0.04 a
	6	300	53.44 ± 0.71 a	260.16 ± 21.49 a	0.20 ± 0.02 b

n = number of rats per group. In the same column, the affected values of the same letter do not differ significantly. $^{b}P < 0.05$: statistically significant compared to the negative control group; a: not statistically significant compared to the negative control group;. Data was analyzed using one way ANOVA followed by Dunnet's t-test.

Table 5: Effects of the aqueous extract of *Pittosporum mannii* on plasma and hepatic transaminases in the rats.

Treatment	n	Dose (mg/kg)	Hepatic AST (UI/l)	Hepatic ALT (UI/l)	Plasmatic AST (UI/l)	Plasmatic ALT (UI/l)
Distilled water (Ne C)	6	/	8.10 ± 0.93 a	31.59 ± 16.84 a	99.63 ± 11.09 b	12.31 ± 0.64 a
Distilled water (Neg C)	6	/	10.53 ± 2.03 a	16.84 ± 2.97 a	65.44 ± 3.45 a	18.79 ± 1.21 a
Maalox	6	50	13.60 ± 1.88 a	23.49 ± 5.35 a	61.56 ± 7.36 a	16.20 ± 4.22 a
Aqueous extract	6	75	7.29 ± 4.05 a	12.15 ± 2.76 a	69.33 ± 4.17 a	6.48± 1.02 b
	6	150	8.91 ± 1.55 a	13.60± 1.21 a	59.13 ± 5.01 a	11.66± 1.29 a
	6	300	12.96 ± 1.87 a	3.24 ± 0.00 b	38.83 ± 2.29 b	10.36 ± 2.38 a

n = number of rats per group. In the same column, the affected values of the same letter do not differ significantly. $^{b}P < 0.05$: statistically significant compared to the negative control group; a: not statistically significant compared to the negative control group; Data was analyzed using one way ANOVA followed by Dunnet's t-test.

Table 6: Effects of the aqueous extract obtained from the stem barks of *Pittosporum mannii* on the behaviour of the mice during the test of the letal dose 50 (LD50) and letal dose (LD100)

Treatment	N	Dose (mg/kg)	Parameter					
			Mobility	Communi-cation bet-ween mice	Sensitivity to the pain	Sensitivity to the sound	Agres-siveness	State of saddles or stools
Distilled water	6	/	N	N	N	N	N	G
Aqueous extract	6	0.5	N	N	N	N	N	G
	6	01.5	D	D	D	D	D	L (12.5
	6	3	D-	D-	D=	D=	D=	L (50%)
	6	6	D=	D=	D=	D=	D=	L (100%)
	6	12	D=	D=	D=	D=	D=	L (100%)

N: normal; D: decrease very slightly; D-: decrease slightly; D=: decrease deeply; G: granular; L: liquid.

Table 7: Variation of the mortality of the mice during the acute treatment with the extract of *Pittosporum mannii* during the test of the DL100.

Treatment	**n**	**Dose (g/kg)**	**Number of Animal dead per day**	**Total mortality rate (%)**	**DL50**
Distilled water (Neg C)	6	/	0	0	
Aqueous extract	6	0.5	0	0	2.83 g/kg
	6	1.5	2	33.33	
	6	3	6	100	
	6	6	6	100	
	6	12	6	100	
Total	36	/	20	55.55	