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ANTI GASTRIC ULCER ACTIVITY OF LEAVES OF *Pyracantha angustifolia* (Franch.) C. K. Schneid IN RATS

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Abstract

In vivo study to evaluate the anti-gastric ulcer activity of the hydro alcoholic extract of the leaves of *Pyracantha angustifolia* on rat has been conducted. Gastric ulcer was induced by the administration of indomethacin and absolute ethanol *per os* and pylorus ligation. The results obtained with the use of absolute ethanol showed that the extract protects gastric mucus. The extract, at a dose of 600mg/kg, reduced the ulcer index from 56.58% in the control group to 5.16% in the treated animals ($p<0.05$). The indomethacin provoked lesions of 11.36±0.08 mm^2 in the control group and 1.21±0.12mm^2 in the experimental group which received the 600mg/kg of the extract ($p<0.05$). After pylorus ligation, the pH of the gastric content of the control group animals was 2.13±0.21versus 3.89±0.49 for the animals which received 600mg/kg of the extract ($p<0.05$). These findings show that the extract of the leaves of *Pyracantha angustifolia* protects the gastric wall against the ethanol aggression, accelerates the healing of indomethacin-provoked gastric lesion and decreases gastric acidity after pylorus ligation.

Keywords: *Pyracantha angustifolia*, anti-gastric ulcer

INTRODUCTION

Gastric ulcer affects 4 million persons in the world each year and thus is a major public health problem (Thorsen K. *et al.*, 2013). Ethnopharmacological investigations carried by us in different parts of Madagascar, revealed gastric ulcer as a disease that affects most people in rural areas. Gastric ulcer affects 5% of the population in Madagascar and ranks 12th of the diseases which make people go for consultation (Cassel-Beraud A.M. *et al*. 1996)

Pyracantha angustifolia is one of the many plants that were cited as used for gastric ulcer treatment during our field missions. It was selected for investigation, to verify its anti-ulcer activity and to valorize this plant generally considered ornamental in many places and finally in the context of biodiversity conservation. Its use will mean diversifying plants used for gastric ulcer and thereby reduce or take off threats of over using some rare plants.

The anti-gastric acid secretion, wound healing activity and the mucoprotective property of the ethanolic extract of *Pyracantha angustifolia* leaves were carried out in this study.

MATERIALS AND METHODS

1. Extraction

Branch leaves of *Pyracantha angustifolia* were collected in the south of Antananarivo in November 2014. The leaves were ground and macerated in an ethanol/water mixture (60:40) at room temperature for 15 days. The macerate was afterward filtered and evaporated to dryness under vacuum with a Rotavapor (BUCHI®) 240 at 80°C. This extract was used for the phytochemical screening and the biological tests.

2. Phytochemical screening

Phytochemical screening of the hydro-alcoholic extract of the leaves of *Pyracantha angustifolia* was carried out following the method described by Fong H. S. (1977) using specific reactive for each chemical family that formed a precipitate and/or changed coloration in the presence of a corresponding chemical family.

3. Animal experiment

Albino rats, Wistar strain, of both sexes, aged 7 to 9 weeks weighing 180 to 200 g were used during the experiments. The animals were bred in the animal house of the *Laboratoire de Pharmacologie Générale, de Pharmacocinétique et de Cosmétologie* of the Faculty of Science, University of Antananarivo at a temperature of 22±2°C and kept under 12h/12h light/dark cycle.

4. Mucoprotective activity study

The animals were fasted 18 hours prior to tests and had free access to water. They were divided into 5 groups of 5 rats per group. The first group was used as reference and received distilled water by oral administration. The second, the third and the fourth groups respectively received the extract at a dose of 150, 300 and 600mg/kg. The fifth group was given Misoprostol® at a dose of 1.43µg/kg and served as the positive reference (Sayanti B. and Susri R.C., 2007). All the products were administered by oral route in a fixed volume of 10ml/kg (Dielh-Heinz K., 2010).

One hour after the administration of the products, 1mL/200g of absolute ethanol (Hollander D. *et al*., 1985) was administered to each rat to induce ulcer (Oates P. J. and Hakkinen J. P., 1988; Hui W.M. *et al*., 1990). One hour later, the animals were anesthetized by intra peritoneal injection of barbiturate at the dose of 100mg/Kg and exsanguinated by cutting the carotids. The stomachs were isolated then cut open along the greater curvature and rinsed with physiological solution to remove their contents. The mucus surface hyperemia was measured by direct planimetry method using transparent millimeter paper (Manjusha K. *et al*., 2013). The ulcer index (UI), the relation between the hyperemia surface and the total stomach surface was calculated as follows:

$$\text{UI (\%)} = \frac{\text{hyperemia surface}}{\text{stomach surface}}.\text{x } 100$$

5. Wound healing activity investigation

Five groups of 5 WISTAR rats per group of both sexes were used. They weighed between 180 and 200g, and were 7 to 9 weeks old. The animals were fasted 18 hours prior to tests and had free access to water. Each animal received indomethacin, orally, at the dose of 30mg/kg daily for 3 days to provoke gastric mucus lesions. After the 3 days, the first group of animals (control group) were given distilled water; the second group, served as positive reference, received Misoprostol® at the dose of 1.43µg/kg (Sayanti B. and Susri R.C., 2007) while the remaining 3 groups respectively received 150, 300, 600mg/kg of the extract. The products were administered by oral route in a fixed volume of 10ml/kg (Dielh Heinz K., 2010).

At the end of the treatment, the animals were anesthetized with inhalation of diethyl ether and then exsanguinated. The animals'stomachs were isolated then cut open along the greater curvature. The mucosa was rinsed with water and the surface macroscopic lesions measured by direct planimetry method using transparent millimeter paper (Ganguly A. K., 1969, Manjusha K. *et al*., 2013).

6. Anti-acid activity study

The anti-acid activity of the extract was conducted *in vivo* with WISTAR rats of both sexes, aged of 7 to 9 weeks, weighing between 180 and 200 g. The animals were divided into 5 groups of 5 rats and fasted for 17 hours before the experimental manipulation and had free access to water. The first group (control group) received distilled water; the second group received Cimetidine at a dose of 100mg/kg (Iyyam P.S. et al., 2010) and the remaining three groups received the extract at a dose of 150, 300, 600mg/kg respectively. All these products were administered by oral route in a fixed volume of 10ml/kg (Dielh Heinz K., 2010).

One hour after the administration of the products, the animals were anesthetized by the inhalation of diethyl ether. An incision at the upper part of the abdomen was made to spot the pylorus which was then ligatured. The incision was then closed by points of suture and 16 hours after ligation the animal was euthanized using diethyl ether. Stomachs were isolated and the contents collected in test tubes. These gastric contents were centrifuged at 3000 rpm for 10 minutes and the pH of the supernatant was measured using a pH meter (PIERRON®) (Kinger K. H. & Gupta M. K., 2012).

7. Expression and Analysis of results

The results were expressed as mean ± sem. The means were compared using the Student ‘’t’’ test. A value of $p<0.05$ was considered significant.

RESULTS

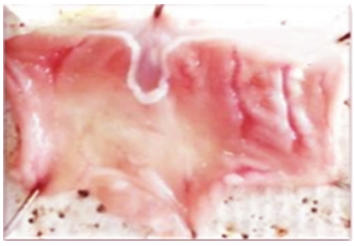
1. C**hemical families present in the extract**

Phytochemical screening done on the extract has shown the presence of high content of flavonoids, average content of tannins, polyphenols and polysaccharides and a low content of alkaloids .

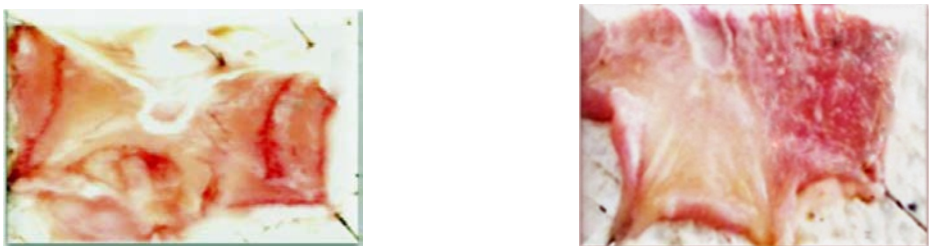
2. **Mucoprotective activity**

One hour after administering the absolute ethanol, the gastric mucosa of all of the rats presented hyperemia (fig 1).

a



b



c d

Figure 1: Stomach of control group animal (a), treated animals with the extract at the dose of 150 mg/kg (b), 300 mg/kg (c) and 600mg/kg (d).

The surface of hyperemia in the animals treated with the extract was inferior compared to the control animals. The ulcer index was 56.58% for the control group, 26.57±0.12 %, 17.28±0.02% and 5.16±0.03% for the animals treated respectively with 150, 300, 600mg/kg, and 9.07±0.02% for the animals which received Misoprostol®. The results show that the extract protects the gastric mucosa of the rats against the ulcerogenic effect of the absolute ethanol ($p<0.05$) (figure 2).

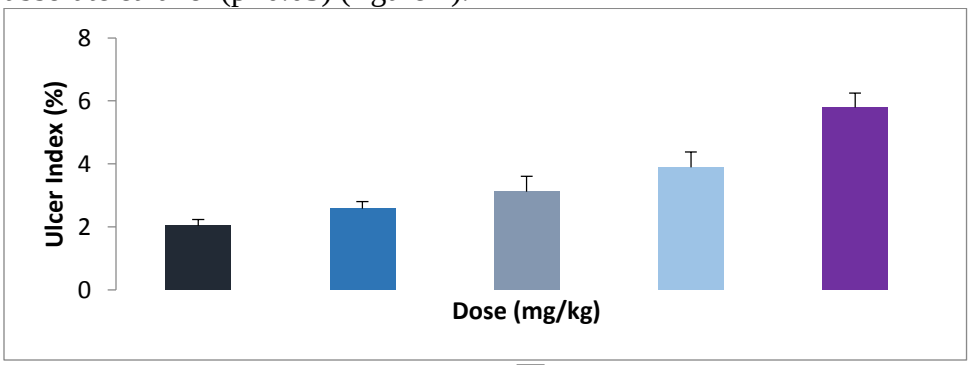


Figure 2: Ulcer Index in the control animals and the treated animals treated with the extract at 150, 300 and 600mg/kg and Misoprostol via oral administration, expressed in % of hyperemia area in relation to the total stomach surface ($\bar{X}$±SD; n=5; $p<0.05$).

3. Wound healing activity

Administration of indomethacin during 3 days induced lesions on the gastric mucosa of the animals. However, the lesion surface on the gastric mucosa of the treated animals was less than that observed in the control animals (Figure 3).

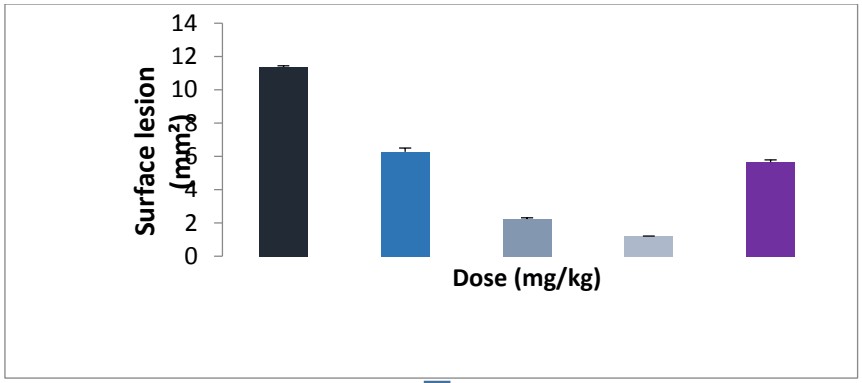


Figure 3: Surface lesion observed in control animals and that of treated animals with the dose of 150mg/kg 300mg/kg 600mg/kg and cimetidine ($\bar{X}$±SD; n=5; p<0.05).

The control animals had gastric mucosa lesions surface of 11.36 ± 0.08mm^2, whereas the animals treated with the extract at the dose of 150, 300, 600 mg/kg presented gastric mucosa surface lesions of 6.27 ± 0.24 mm^2, 2.23 ±0.08 mm^2, and 1.21± 0.012 mm^2 respectively. The animals that were given cimetidine presented surface lesion of 5.66 ±0.14 mm^2 (p<0.05). According to the results, the extract promotes the healing of the lesions induced by indomethacin on gastric mucosa.

4. **Anti-acid activity**

Pylorus ligation induced gastric ulcer in the stomach was used to investigate the effect of the extract on gastric acid secretion. Sixteen hours after pylorus ligation, the pH of the gastric content of the control animals was 2.03± 0.21. For the animals which received the extract at the dose of 150, 300, 600 mg/kg, the pH values were 2.58±0.23, 3.12 ±0.49, 3.89 ±0.49 respectively. The pH value for the animals that received cimetidine was 5.79 ±0.46 (P<0.05) (figure 4).

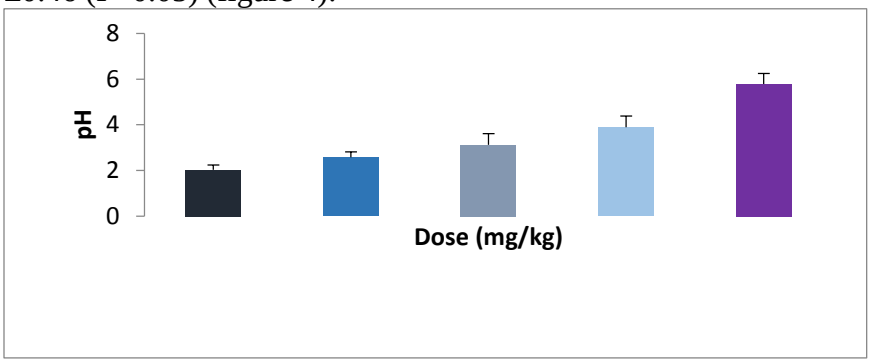


Figure 4: pH values for control animals and that of treated animals with the dose of 150mg/kg 300mg/kg 600mg/kg and cimetidine ($\bar{X}$±SD; n=5; p<0.05).

These results show an increase in pH value of the gastric content pH increased, which means the acidity has diminished (fig.5). Thus the extract reduced gastric acidity provoked by pylorus ligation.

DISCUSSION

Gastric ulcer is due to the unbalance between the protective and aggressive factors in the stomach, to the advantage of the aggressive ones (Rao C.V. *et al*., 2000). The etiology of gastric ulcer is influenced by divers factors, aggressors as well as protectors. Pepsin and hydrochloric acid secreted by the parietal cells as well as ingested food constitute aggressive factors whereas the protection is assured by the mucus, bicarbonate, blood flux, cellular regeneration and the endogenous agents of protection (prostaglandin and the epidermal growth factors). Hydrochloric acid and pepsin secretion are controlled by histamine, gastrin and acetylcholine whereas the secretion of mucus and bicarbonate are under the control of prostaglandin (Valle, D. L. 2005).

Our results show that extract protects the gastric mucosa against the ulcerogenic effect of absolute ethanol. It is also able to reduce the acidity of gastric content produced as a result of pylorus ligature and accelerate the healing of lesions provoked by indomethacin. That is why it is effective against gastric ulcer, which justifies its use in traditional medicine.

Ethanol was used to produce gastric ulcer in rats during this study because the damages that it provokes resemble to acute peptic ulcer in humans (Brzozowski T. *et al*., 1998). It is capable of dissolving mucus and thus expose the gastric mucosa to the proteolytic action of pepsin and hydrochloric acid which causes the destruction of underlying structure (Oates P.J. and Hakkinen P.J., 1988; Sener G. *et al*., 2004). This model is equally appropriate for studying products which possess cytoprotective activity and/or anti-oxydant (Hollander D. *et al*., 1985). The reduction of the ethanol-induced hyperemia in the animals treated with extract show that it is able to protect the gastric mucosa against the ethanol aggression. This could be due to the cytoprotective action thus avoiding the destruction of the mucosa, or by increasing the mucus secretion to protect the mucosa against the aggressive effect of ethanol, and another possibility is that polysaccharides present in extract might protect the mucosa.

The healing of the wounds provoked by repetitive oral administration of indomethacin is also accelerated in the rats treated with the extract, which involves PGE2. Indomethacin is an anti-inflammatory which inhibits the enzyme COX_1 required for the synthesis of prostaglandin (PGE1) and that's why lesions were produced in the gastric mucosa after its administration (Rainsford K. D. 1987; Jyoti G. *et al*., 2012). Acceleration of the wound healing process may be also due to an important amount of mucus which

protects the wounded mucosa to allow its recovering, and/or the reduction of the intra gastric acidity as result of increase of bicarbonate secretion or reduction of acid secretion (Wallace J.L., 1997). This increase of mucus secretion could also have protected the gastric mucosa from the aggression of ethanol (Mizuno H. *et al*., 1997).

On the other hand, the increase in the value of pH signifying a reduction in gastric content acidity which could be due to either an increase in bicarbonate secretion (Kierszenbaum. A.L., 2006) or a reduction in the secretion of H^+ from the parietal cellular (Brodie A. and Knapp P.G., 1966). During our preliminary investigation, we found out that the pH of the direct mixture of gastric content after pylorus ligation and the extract is lower than what we got when extract was administrated after pylorus ligation, and comparing to the value of pH provoked by cimetidine suggest more of an increase of bicarbonate secretion than an inhibition of acid secretion .

These results suggest an effect of the extract via prostaglandin synthesis which increase bicarbonate and mucous secretion and wound healing acceleration.

Our results also shows that the extract of *Pyracantha angustifolia* can prevent and heal gastric ulcer. The protection could be the result of the polysaccharides in the extract or mucus secretion increasing. It could be due to the anti oxydant activity of the flavonoids, polyphenols and tannins in the extract (Lenoir L., 2011).

CONCLUSION

Pyracantha angoustifolia leaves' extract has shown an anti-gastric ulcer activity. It protects the gastric mucosa against the aggressive agents by increasing the amount of mucus and bicarbonate. It also accelerates the wound healing. These activities involve the prostaglandin synthesis and the presence of flavonoids, tannins, polyphenols and polysaccharides in the extract.

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