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Anti-ulcer Activity of Hydro Alcoholic Extract of *Acridocarpus excelsus* a. Juss Leavesin Rat

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ABSTRACT

The anti ulcer activity of the hydro alcoholic extract of the leaves of *Acridocarpus excelsus* was studied *in vivo* using WISTAR rats. This study investigated the extract's effect on gastric ulcer induced by indomethacin orally administered at the dose of 30mg/kg, acid secretion by pylorus ligation and mucus secretion. The results obtained showed that ulceration provoked by the indomethacin on the gastric mucosa surface was reduced from 13.12 ± 0.96 mm^2 found inthe control group to 8.15± 0.12 mm^2, 2.87 ± 0.41 mm^2 and 1.25 ± 0.05 mm^2 respectively in the rats treated with the extract at doses 150 mg/kg, 300 mg/kg and 600mg/kg ($p<0.05$). After pylorus ligation, the gastric content pH was 2. 01 ± 0. 07 for the control group; 3.67 ± 0.14, 5.82 ± 0.04 and 6.82 ± 0.27 respectively for the rats that received the extract at doses 150 mg/kg, 300 mg/kg and 600mg/kg ($p<0.05$). The mucus secretion of the control group was 18.06±1.02 mg but was 25.60±0.35 mg, 30.00±0.28 mg and 40.80±0.55 mg respectively in the groups that received the extract at doses 150mg/kg, 300mg/kg and 600mg/kg. These results show the anti-ulcer activity of *Acridocarpus excelsus* leaves. The extract of the leaves protected the gastric mucosa against damages induced by indomethacin, decreased gastric acidity by increasing the pH of the gastric content and increased mucus production.

Keywords: anti-ulcer, *Acridocarpus excelsus*, rats.

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INTRODUCTION

Ethnopharmacological surveys that we conducted in the rural areas of the northern part of Madagascar revealed gastric ulcer as one of the diseases that people reported to affect them the most in those areas. The disease, peptic ulcer, manifests as a break of the lining of the gastro-duodenal mucosa bathed by acid and pepsin. Generally, it is due to the imbalance between the gastric aggressive factors and the mucosal defensive factors[1, 2]. Dietary habits, stress and *Helicobacter pylori* are among the agents responsible for the development of peptic ulcer disease[3]. Several orthodox medicines are employed to treat this disease. In Madagascar, medicinal plants are mostly used due to their efficiency, ease of accessibility and affordability. We have collected data on medicinal plants used to treat gastric ulcer, and *Acridocarpus excelsus* is one of the plants. Our choice of this plant for this study was based on the analysis of the results of our interviews with people that indicated that the decoction of the plant is the most effective of the various plants they use for the treatment of their gastric ulcer. This led us to investigate the possible anti-acid and the mucus-protection properties of *Acridocarpus excelsus*.

MATERIALS AND METHODS

Extraction and Phytochemical Screening

The leaves of *Acridocarpus excelsus* were collected from the northern part of Madagascar in May 2015. They were dried under shade and ground with an electric grinder. The powder was macerated in a mixture of ethanol and water (60:40) for 48 hours. The macerate was filtered and evaporated to dryness under vacuum with a rotavapor (Evapotec®). Phytochemical screening was conducted on the dry extract following the method of Fong H. H.S[4]. The dry extract was also used for the biological tests.

Animals used in the Experiments

Male and female albino rats WISTAR strain of 7 weeks, weighing from 180 to 200 grams were used for the biological tests. The animals were reared on a standard laboratory diet and given tap water *ad libitum*. They were kept in a room where temperature (24 ± 2°C), humidity (65–70%), and day/night cycle (12 h/12 h) were controlled. The experiments were conducted following the guidelines of the ethic committee of Sciences Faculty, University of Antananarivo, Madagascar ((Ref: CE/Fac Sciences/Pharmacol./001, 06/21/2015). Rats were deprived of food but had free access to tap water 24 h before ulcer induction.

Effect of the extract on indomethacin induced lesions

Twenty-four rats of both sexes were fasted 24 hours prior to this experiment. They were separated into 4 groups of 6 rats per group: the first group served as the control group and received distilled water by oral route; the other 3 groups were given the extract orally at the dose of 150mg/kg 300mg/kg and 600mg/kg respectively. Indomethacin was administered by oral route at the dose of 30mg/kg 30 minutes after administration of the extract for 5 days[5, 6]. All the products were administered in a volume of 10 ml/kg[7]. On the fifth day, the animals were anesthetized using 100 mg/kg of Phenobarbital *i.m.* and exsanguinated by cutting the carotids. A laparotomy was practiced and the animals' stomach was isolated, opened along the big curvature and rinsed with saline solution (NaCl 9‰). The lesions on the gastric mucosa were measured by direct planimetry with a transparent millimetric paper.

Effect of the Extract on Gastric acid Secretion

Four groups of 6 rats per group of both sexes were used during this experiment. The animals were fasted 24 hours prior the tests. The first group received distilled water, the remaining 3 groups of animals received the extract at dose 150 mg/kg, 300 mg/kg and 600 mg/kg respectively. The different products were administered in a volume of 10ml/kg by oral route[7]. After 1 hour, the animals were anesthetized using 40mg/kg of Phenobarbital *i.m.* The upper part of the abdomen was incised for pylorus ligation, and afterwards sutured[8,9]. During the six hours following ligation, the animals were deprived of food, after which they were anesthetized with 100mg/kg of Phenobarbital and exsanguinated. A laparotomy was practiced and the esophagus was clamped and the stomach isolated[6]. The gastric content was poured into test tubes and centrifuged at 3000 rpm for 10 minutes. The pH of the supernatant was measured with a pH meter (PIERRON).

Anti acid Effect of the Extract

Twenty four rats were fasted 24 hours prior to the test. Their pylori were ligatured, and 1 hour after, the stomach was isolated and its content collected into test tubes and centrifuged for 10 minutes at 3000rpm. The supernatant of the gastric content of each animal was transferred in a test tube. Those 24 test tubes were divided into 4 sets of 6 tubes per set. The first set of test tubes had nothing added to the gastric content; the second set had 1ml of distilled water added in the tubes; in the third set, 1 ml of the extract used during the test was added in the tube; and the last set of test tubes had only the extract. The pH of the content of each test tube was measured using a pH meter PIERRON)

Effect of the Extract on Mucus Secretion

Four groups of 6 rats per group were fasted 24 hours prior the test. Afterwards, the first group was given distilled water, while the rest received the extract at the dose of 150 mg/kg, 300 mg/kg and

600 mg/kg respectively for 10 days. All the products were administered by oral route in a volume of 10ml/kg[7]. After 10 days of treatment, the animals were anesthetized with Phenobarbital at the dose of 100mg/kg *i.m.* and exsanguinated by cutting the carotids. Their stomachs were isolated and rinsed, and the mucus was grated and weighed.

Expression and Analysis of Results

The results were expressed as mean ± s. e.m, and Student's *t*-test was used to detect any significant difference ($p < 0.05$) between the groups in this study.

RESULTS ANDDISCUSSION

Results of the Phyto-Screening

The screening done on the hydro alcoholic extract of *Acridocarpus excelsus* using the method of FONG H.H.S (1977) revealed the presence of a large quantity of saponins, flavonoids, tannins and polyphenols, while steroids, alkaloids and polysaccharides are present in small quantity**.**

Effect of the Extract on the Indomethacin Induced Lesion

The oral administration of 30 mg/kg b.w. of indomethacin caused a significant ulceration in the rats. A significant improvement in the level of inhibition against ulceration was however observed in the animals treated with the extract. The extract at 600 mg/kg b.w. offered best protection against induced ulceration compared to the 150 and 300mg/kg b.w. regimens and the control group. The ulcerated surface observed on the stomach wall of the control group was superior to the one observed on the stomach of the extract-treated rats. It was 13.12 ± 0.96 mm^2 for the control group versus 8.15± 0.12 mm^2, 2.87 ± 0.41 mm^2 and 1.25 ± 0.05 mm^2 respectively for rats treated with the extract at dose 150 mg/kg, 300 mg/kg and 600mg/kg ($p<0.05$) (figure 1).

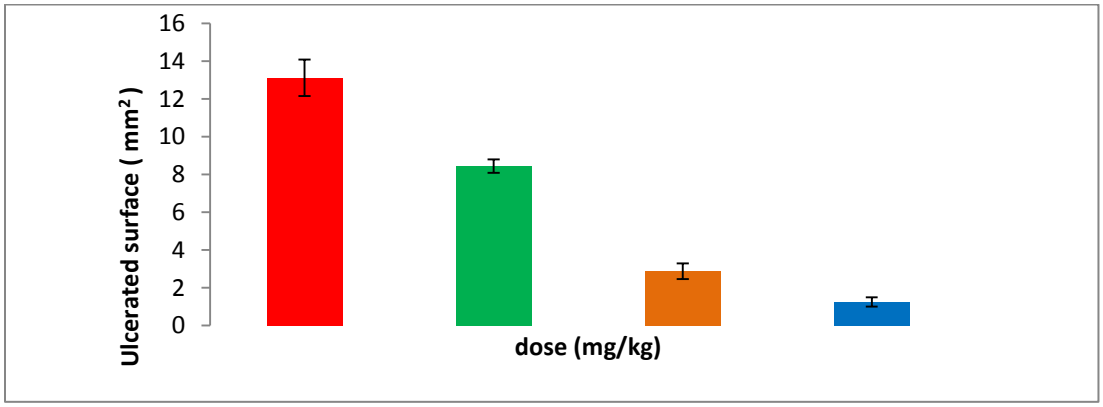

Figure 1: Ulcerated surface induced by indomethacin (30mg/kg, administered by oral route) in the control group and rats treated during 5 days with the hydro alcoholic extract of *Acridocarpus excelsus* at 150mg/kg 300mg/kg and 600 mg/kg administered by oral route ($\bar{x}$ ± s.e.m, n= 6, p<0.05).

These results show that the leaf extract of *Acridocarpus excelsus* protected the gastric mucosa against the indomethacin effects. Indomethacin is an anti-inflammatory which inhibits the synthesis of prostaglandin in the cyclooxygenase pathway which provokes mucosal lesions[10]. Furthermore, indomethacin disrupts the layer of surface of active phospholipids on the mucosal surface, independent of effects on the prostaglandin synthesis[11]. Such an action would render the mucosa less able to resist damage induced by luminal acid[12]. The reduction of the lesion' surface induced by the indomethacin observed in the rats treated with the extract would be the consequence of the state of the gastric mucus due to its effect against the indomethacin activity or its muco-protective effect[13].

Effect of the Extract on Gastric acid Secretion

The intragastric liquid pH of the pylorus ligated rats treated with the extract increased compared to the control group. The pH value was 2. 01 ± 0. 07 for the control group but 3.67 ± 0.14, 5.82 ± 0.04 and 6.82 ± 0.27 for the rats respectively treated with 150mg/kg, 300mg/kg and 600mg/kg of the extract administrated by oral route ($p<0.05$) (figure 2).

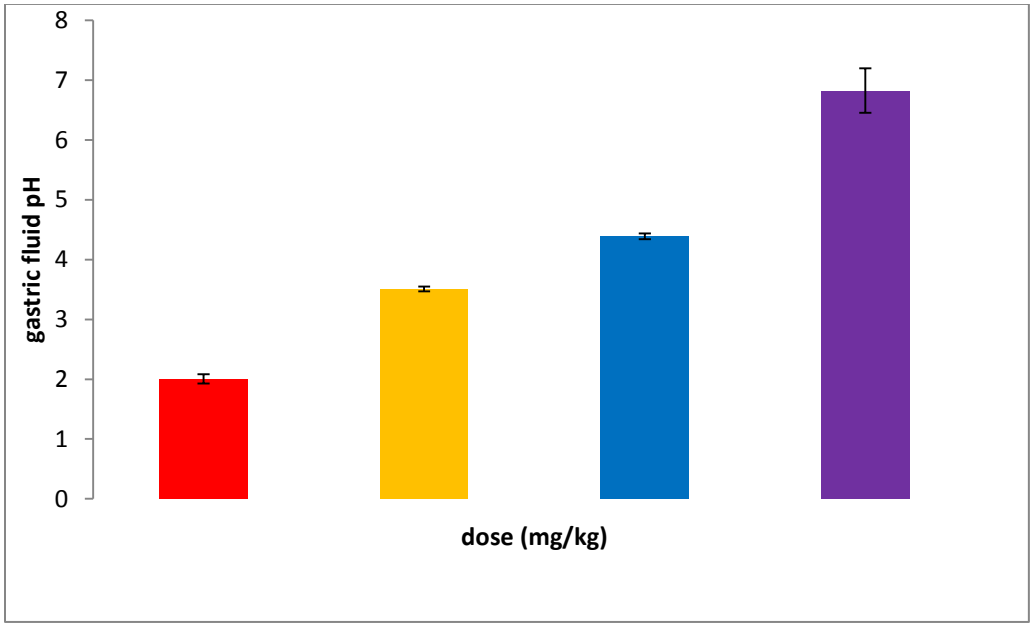

Figure 2: pH of the intragastric liquid after pylorus ligation for the control group and the rats treated with the hydro alcoholic extract of *Acridocarpus excelsus* administrated by oral route at the dose 150 mg/kg 300 mg/kg and 600 mg/kg ($\bar{x}$ ± s.e.m, n= 6, $p<0.05$).

On the other hand, the intragastric liquid pH after the pylorus ligation was equal to 2.01 ± 0.07 for the control group, the extract pH was 4.93 ± 0.07, and the pH of the mixture of gastric liquid content and extract was 4.18 ± 0.04 ($p<0.05$). Normally, pylorus ligation increases gastric fluid acidity[8], meanwhile the pH of the gastric content of the rats treated with the extract increased. The

pH of the mixture: gastric content after pylorus ligation and extract was lower than the pH of the gastric content of the rat treated with the extract after pylorus ligation. These results show that the extract didn't only neutralize directly the acidity of the gastric content but either the extract had inhibited the gastric acid secretion, or it had increased the secretion of bicarbonate.

Effect of the Extract on Mucus Secretion

The weight of the mucus increased in the rats that received the extract administrated orally for 10 days compared to the control group. The mucus weight for the control group was 18.06 ± 1.02 mg, versus 25.60 ± 0.35 mg, 30.00 ± 0.28 mg and 40.80 ± 0.55 mg ($p<0.05$) respectively for the animals treated with the extract at 150mg/kg, 300mg/kg and 600mg/kg (Figure 3).

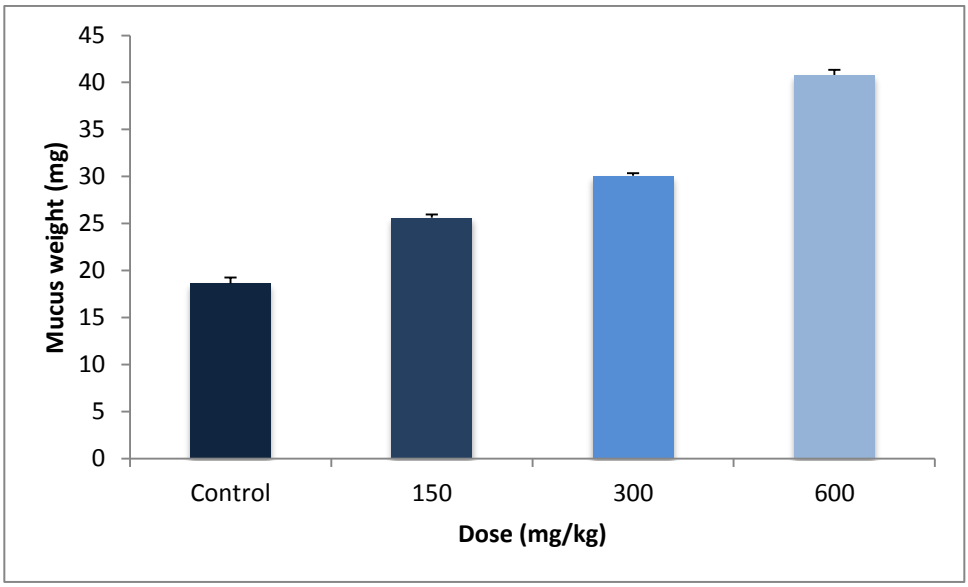

Figure 3: The mucus weight for the control group ■ and the rats treated with the hydro alcoholic extract of *Acridocarpus excelsus* administered by oral route at the dose of 150mg/kg ■ 300 mg/kg ■ and 600 mg/kg ■ ($\bar{x}$ ± s.e.m., n= 6, $p<0.05$).

In fact the increase of bicarbonate and mucus secretion is related to the increase of prostaglandin activity[14]. These results mean that the prostaglandin activity had increased, thus, we could advance the hypothesis that the extract worked through stimulating prostaglandins. The inhibition of acid secretion observed after the pylorus ligation, might have been due to the inhibition of proton pump by the flavonoids present in the extract, as Vinothapoosha G. and Sundar K.[15] (2010) have found when they have investigated the anti gastric ulcer activity of *Mimosa pudica.* Borelli F. and Izzi A.[16] have also reported that flavonoids increase the prostaglandin rate and mucus secretion. As the extract contains these products, one can advance a hypothesis that the flavonoids in the extract have increased the rate of prostaglandin leading to the increase of bicarbonate and mucus secretion to protect the gastric mucosal against the aggressive activity of indomethacin. The reduced lesion

surface observed after indomethacin administration in the rats treated with the extract might also have been due to the mucoprotective action of the tannins in the extract[17].

CONCLUSION

The aim of this study was to investigate the anti ulcer property of the leaf extract of *Acridocarpus excelsus* in rats. The results show that the extract reduced the surface of lesion induced by indomethacin, increased the pH of the gastric fluid after pylorus ligation and the weight of the mucus. These activities might be due to the stimulation if the prostaglandins' activities leading to the increase of bicarbonate and mucus secretion.

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