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Acute toxicity and laxative activity of *Aloe ferox* resin

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Abstract: *Aloe ferox* Mill., Xanthorrhoeaceae, resin is the solid residue obtained by evaporating the latex that drains from the leaves transversally cut. *Aloe ferox* has been used in folk medicine as anti-inflammatory, immunostimulant, anti-bacterial, anti-fungal, antitumor, laxative and to heal wounds and burns. The effects of the oral administration of *A. ferox* resin (10, 25, 50, 100 and 200 mg/kg) were evaluated on intestinal transit in mice and its acute toxicity (5.0 g/kg) in Wistar rats. The hydroxyanthracene derivatives present in the resin were expressed as aloin, identified by thin layer chromatography and quantified by spectrophotometry. The aloin (R_f 0.35) was identified and the percentage of hydroxyanthracene derivates expressed as aloin was 33.5%. *A. ferox* resin extract (50, 100 and 200 mg/kg) increased the gastrointestinal motility at a 30 min interval at 93.5, 91.8 and 93.8%, respectively, when compared to control group (46.5%). A single oral dose of the *A. ferox* resin extract did not induce signs of toxicity or death. Thus, the results demonstrate that *A. ferox* has laxative activity and that it is nontoxic, since LD50 could not be estimated and it is possibly higher than 5.0 g/kg.

Article

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Introduction

Aloe ferox Mill., Xanthorrhoeaceae, commercially known as Cape aloe, is a plant native of South Africa and Lesotho (Sibuyi et al., 2007). Cape aloe was a local traditional medicine adopted by colonists at the Cape of Good Hope and it was the first exported product to Europe in the eighteenth century (Hodge, 1953; Grace et al., 2008). *A. ferox* resin is a solid residue obtained by evaporating latex which drains from the leaves transversally cut (Westendorf, 1993). It is soluble in alkalis, concentrated acetic acid, absolute alcohol, glycerin and hot ethanol. It is partially soluble in boiling water and it is practically insoluble in ethyl ether (Brazilian Pharmacopoeia, 2010).

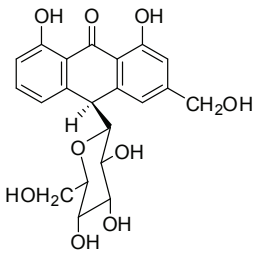
In the Brazilian folk medicine, the plant is known as "babosa" and the largest producers are in the states of São Paulo and Santa Catarina and in the Northeast region (Magalhães, 2005). Plants of *Aloe* genus have been used as laxative, anti-inflammatory, immunostimulant, anti-bacterial, anti-fungal, antitumor, hypoglycaemic, to treat gastric disorders and to wound and burn healing. These treatments are based on ethnopharmacological evidence or research findings with *Aloe vera* (Loots et al., 2007) or *A. ferox* (Chen et al., 2012).

Miscellaneous bioactive constituents have been identified from the leaves and roots of *A. ferox*. These components belong to different classes such as polysaccharides, alkaloids, anthraquinones, saccharides, enzymes, amino acids, inorganic mineral. However, the main components of *Aloe* are aloin, aloe-emodin and aloeresin (Vogler & Ernst, 1999; Eshun & He, 2004).

One of the main biologically active constituents of *Aloe* extracts is aloin or barbaloin (10-glucopyranosyl-1,8-dihydroxy-3-(hydroxymethyl)-9(10*H*) - anthracenone) which is found in nature as a mixture of two diastereomers, aloin A (**1**) ((10*S*)-10-glucopyranosyl-1,8-dihydroxy-3-(hydroxymethyl)-9 (10*H*)-anthracenone) and aloin B (**2**) ((10*R*)-10-glucopyranosyl-1,8-dihydroxy-3-(hydroxymethyl)-9 (10*H*)-anthracenone) (Fanali et al., 2010).

Aloin, a *C*-glycoside derivative of anthraquinone, may release the quinine when ingested, and the quinone (aloe-emodin), in turn, may accelerate the rate of ethanol metabolism *in vivo* (Chung et al., 1996). Aloeresin, a chromone, seems to be a potent antioxidant with the oxygen radical absorbance capacity of 33 and 299 higher than the green tea and the grape seed extracts, respectively. It is also well documented that aloe resin promotes an anti-aging effect by restoring the immune function in UV-damaged cells (Jones et al., 2002).

Taking into account the commercialization and use of *A. ferox* resin in the treatment of intestinal constipation and that few studies has been published in the scientific literature about its toxicological profile, the aim of the present study was to evaluate the effects of *A. ferox* on intestinal transit in mice, as well as the safety of this use through a study of acute toxicity.



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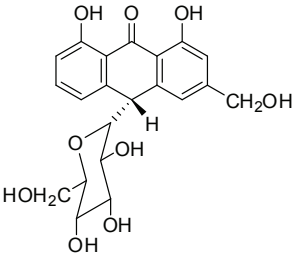

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Material and Methods

Preparation of the extract

Aloe ferox Mill., Xanthorrhoeaceae, resin was prepared by cutting transversely the leaf near the base and taking it inclined so that the latex contained in the specialized pericyclic cells and sometimes in the adjacent parenchyma flowed out in about 6 h. A slow evaporation, carried out in an appropriate temperature of 45º C in a incubator for 6 h, produced an opaque mass, with a wax-like fracture. The resin used in the current study was a commercial preparation produced and kindly donated by Odaly Soares Pharmaceutical Laboratory, Ceará, Brazil, used in a medicine registered in Anvisa number 105190028. In previous analysis in our laboratory concerning the resin solubility, the glycerin was the solvent that showed better solubility and compatibility with *in vivo* experiments. The resin was crushed in automatic processor and it was solubilized in 40% glycerin (Vetec®) (v/v).

Drugs

Physostigmine (eserine salicylate salt) was purchased from Sigma-Aldrich (USA).

Quantification of hydroxyanthracene derivates by UV-Vis spectrophotometry

Powdered sample (400 mg) was put in a 250 mL Erlenmeyer flask, together with 2 mL of methanol and 5 mL of water previously heated (60 ºC). The components were mixed and 75 mL of the same water was added and stirred for 30 min. After cooling, the solution was filtered and transferred to a volumetric flask and its volume was completed to 1000 mL. Then, 10 mL of this solution was added in a round bottom flask containing 1 mL of a solution 60% ferric chloride (w/v) and 6 mL of hydrochloric acid. The solution was heated in a water bath under reflux for 4 h and, after cooling, it was transferred to a dropping funnel. After successive washing with polar and apolar solvents, the organic phase was collected for UV-analysis. The UV absorption spectrum was obtained in the wavelength range from 200 to 600 nm (50 UV-Vis Spectrophotometer), using the methanol as the compensation liquid. The specific absorbance of aloe-emodin, obtained by oxidation of aloin, is at 512 nm. The assay was performed in triplicate. The percentage of hydroxyanthracene derivates expressed as aloin was calculated from the expression (Brazilian Pharmacopoeia, 2010).

$$HAD = A \times 19.6/m$$

where: HAD; hydroxyanthracene derivates (%); A: absorbance at 512 nm; m: mass of the substance examined in grams.

Identification of aloin by thin layer chromatography

In 250 mg of the powdered sample, it was added 20 mL of methanol and the solution was heated up to boiling.10 µL of this solution were applied silica gel plates (Merck® art. 105553, UV 250-366 nm). After drying, the plate was eluated with water-methanol-ethyl acetate (13:17:100 v/v). The plate was allowed to dry in air, sprayed with 100 g/L solution of potassium hydroxide in methanol. The retention factor (R_f) was obtained and the plate was examined in ultraviolet light at 365 nm. It was used an aloin solution as reference standard (Gutterman & Chauser-Volfson, 2000; Brazilian Pharmacopoeia, 2010).

Animals

Adult female and male Wistar rats (*Rattus norvegicus* var. *albinus*), aged three months and weighing 180-200 and 240-260 g, respectively, and male Swiss albino mice (*Mus musculus*), 6-8 weeks of age, weighing between 25-35 g were obtained respectively from Department of Physiology and Pharmacology and from Aggeu Magalhães Laboratory at the Federal University of Pernambuco. The animals were maintained in standard environmental conditions (22±2 ºC; 12:12 h dark/light cycle). Water and chow (Labina®, Purina, Brazil) were available *ad libitum*. The experimental protocol was approved by the Animal Experimentation Ethics Committee of UFPE (Process no. 007340), in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Experimental protocol

Acute toxicity

"Up and down" acute toxicity studies were performed in rats of both sexes as described by OECD (2001), with slight modifications. The animals were randomly divided into four groups (n=4/group/sex) and deprived of feed for 12 h with access to water *ad libitum.* The treated groups received *A. ferox* resin in a single dose of 5 g/kg, by oral route, and the control groups received a same volume of vehicle (40% glycerin solution - v/v). The observations were performed at 30, 60, 120, 180 and 240 min after the oral treatment and daily for fourteen days. Behavioral changes (piloerection, tremors, sedation, loss of corneal reflex, motor activity), weight, food and water intake, clinical signs of toxicity and mortality were recorded daily (Malone, 1977).

Gastrointestinal motility test

The intestinal transit of *A. ferox* resin was evaluated in mice using the method of Stickney & Northup (1959). Mice fasted for 16 h were randomly divided into eight groups (n=8/group). G1-control group (received water), G2-received vehicle (40% glycerin solution - v/v), G3-received physostigmine 0.001 mg/kg, G4-G8-resin extract of *A. ferox* (Af) at doses of 10, 25, 50, 100 and 200 mg/kg, respectively. The treatments were administered orally by gavage in a single dose. After 60 min of the treatments, a 10% activated charcoal solution was given orally (0.1 mL/10 g b.w.) to all groups and, after 30 min of this administration, the mice were killed. The small intestines were removed and their length was measured from the pyloric sphincter to the ileocaecal junction. The distance traversed by activated charcoal (it was considered the last portion of the small intestine that showed at least one centimeter continuous of the marker) was recorded as a percentage of the total length of the small intestine (gastrointestinal transit percent).

Statistical analysis

Statistical analyses were performed by using the software GraphPad Prism 5®. The results were expressed as mean value±SEM. The differences between control and treated groups were compared using one-way ANOVA followed, when necessary, by Dunnett's test. A probability level of less than 5% ($p<0.05$) was considered significant.

To the intestinal transit protocol, a concentration-effect curve was constructed by subtracting the values of the control group from the treated groups and the ED50 was calculated using the nonlinear regression by the software GraphPad Prism 5®.

Results

Aloin identification and quantification

The chromatogram obtained with the solution test showed in the central part a zone of yellow fluorescence (aloin-R_f0.35) similar in position to the zone corresponding to aloin in the chromatogram obtained with the reference solution. The percentage of hydroxyanthracene derivates expressed as aloin was 33.5%.

Acute toxicity

It was observed that *Aloe ferox* resin (5.0 g/kg, *p.o.*) induced moderate diarrhea and reduced motor activity at the first hour of observation. However, the animals treated with the same volume of vehicle (40% glycerin solution-v/v) did not show these symptoms in any time of the experiment. The *A. ferox* resin produced no sign of acute toxicity or death in the treated animals. No significant changes in food and water intake or body weight were observed during the fourteen days of observation (data not shown). Therefore, the LD50 could not be estimated and it is possibly higher than 5.0 g/kg.

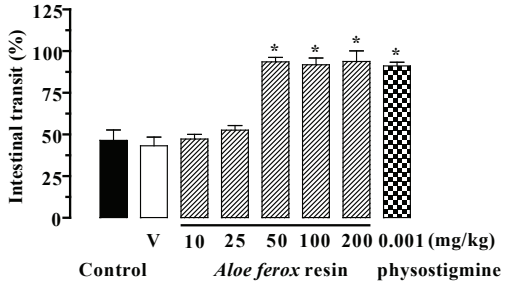

Figure 1. Effect of the oral administration of *Aloe ferox* resin on gastrointestinal motility in mice (n=8/group). Groups: control (water), vehicle (V, 40% glycerin solution), Aloe ferox resin (10 to 200 mg/kg) and physostigmine (0.001 mg/kg). The results were expressed as mean±SEM. The ordinates indicate the percentage of distance traveled by the marker (activated charcoal) in relation to the total length of the small intestine (intestinal transit) 30 min after ingestion of the marker. *Statistically different from control group (ANOVA followed by Dunett's test, $p<0.05$).

Gastrointestinal motility

The results of the gastrointestinal motility test are shown in the Figure 1. *A. ferox* resin extract at doses of 50, 100 and 200 mg/kg induced significant increase of the gastrointestinal motility at 30 min of 93.5, 91.8 and 93.8%, respectively, in relation to control group (46.5%). As expected, the group treated with physostigmine (0.001 mg/kg) significantly potentiated (91.2%) the intestinal transit, with a estimated ED50 of 19,1 mg/kg. As it was observed, the groups treated with *A. ferox* resin and physostigmine increased about two times the distance traveled by the marker when compared to vehicle and control groups.

Discussion

The use of herbal remedies in the treatment of constipation is a common practice in many countries of the world (Kim, 2005). The constipation is the most common gastrointestinal complaint being a risk factor of colorectal cancer. The pathophysiological mechanisms that could explain the constipation are reduction of the colonic transit speed (colonic inertia) and difficulty of removing the faeces due to functional changes of the pelvic floor, rectum or anus (Lennard-Jones, 2002).

In Brazil, *Aloe ferox* Mill., Xanthorrhoeaceae, resin is widely used in the ethnomedicine as laxative, particularly by women during pregnancy (Bradley, 1992; Akao et al., 1996). However, there is little scientific information to support this hypothesis. Therefore, the study was designed to evaluate the effects of the oral administration of *A. ferox* resin on gastrointestinal motility in mice and its safety of use in rats Wistar.

The laxative effect of *Aloe* species is due to the presence of anthranoid glycosides derivates, mainly aloin (Bradley, 1992; Akao et al., 1996). These derivates were quantified in the *A. ferox* resin (33.5%) and they could explain the increased intestinal transit in the animals treated with the highest doses of *A. ferox*. According to Brazilian Pharmacopoeia (2010), *A. ferox* resin consists of at least 18% hydroxyanthracene derivates expressed as aloin and Costa (1975) has reported that this level can reach 40%.

The intestinal transit measurement model in mice has been used to prove the effectiveness of laxatives and it showed to be suitable for this purpose (Mesia-Vela et al., 2004). The treatment with *A. ferox* resin (50, 100 e 200 mg/kg, b.w.) showed evident laxative effect 30 min after its administration, supporting its ethnomedicinal use. In fact, Wintola et al. (2010) recently showed that oral administration of the leaves aqueous extract of *A. ferox*, in the same doses, exhibited laxative activity on constipated rats induced by loperamide. In our study, activated charcoal solution was used as a marker to measure the intestinal transit. Its propulsion through the gastrointestinal tract did not differ significantly between the groups treated with *A. ferox* resin (10 and 25 mg/kg, b.w.) and the control and vehicle groups. Although the glycerin, used as vehicle, is considered an osmotic laxative by producing water retention in the intestinal lumen, stimulating the secretion of fluid and intestinal motility (Fuchs et al., 2004), it was not observed increased peristaltic activity in the group treated with vehicle alone when compared to control. As expected, since the standard drug physostigmine (an anticholinesterase agent) inhibits the enzyme acetylcholinesterase and therefore augments the concentration of acetylcholine, it accelerated the intestinal transit.

The increase in intestinal motility may be due to the presence of anthranoid glycosides derivatives, of which aloin is the main compound (Ishii et al., 1994). According to Bradley (1992) and Akao et al. (1996), after oral administration, the aloin is not absorbed in the upper intestine, it is hydrolyzed in the colon by *Eubacterium* sp. and then reduced to active metabolite aloe-emodinanthrone. This one stimulates colonic motility, augmenting propulsion and accelerating colonic transit, which reduces fluid absorption from the fecal mass. It also increases paracellular permeability across the colonic mucosa probably owing to an inhibition of $(Na^{++}K^{+})$-ATPase or an inhibition of chloride channels, which results in an increase in the water content in the large intestine (Witte, 1993). Aloe-emodin stimulates the release of Platelet-Activating Factor (PAF) in human ileal and colonic mucosa contributing to the laxative effect of Aloe (Tavares et al., 1996). Izzo et al. (1999) also studied the role of oxide nitric (NO) on aloe-induced diarrhea in the rats. The results suggested that inhibition of basal calcium-dependent NO synthesis activity by aloe could reduce its diarrheal effect.

Moreover, the acute toxicity study did not show any toxic symptoms, changes in behavior or mortality at the dose of 5.0 g/kg. According to Kennedy et al. (1986), substances that present LD50 higher than 5 g/kg, by oral route, can be considered practically nontoxic. Therefore, it can be suggested that acute toxicity of the *A. ferox* resin is low by oral route.

In conclusion, our results showed that the *A. ferox* resin extract increases the gastrointestinal motility, proving its laxative action. In addition, revealed to be nontoxic, when oral acute administration was performed. However, chronic and reproductive toxicities, mutagenicity and others carcinogenicity studies are necessary to further support the safety of use.

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Authors' contributions

VRLC and HMLM (PhD students) contributed in running the laboratory work, analysis of the data and drafted the paper. CFBV, CRB and GCRM contributed in the laboratory work. AVA and AGW designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

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