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PRELIMINARY EVALUATION OF ANTI-POISON AND ANTI-ULCEROGENIC EFFECTS OF LEAF EXTRACTS FROM *BRILLANTAISIA CICATRICOSA* LINDAU IN GUINEA-PIG

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ABSTRACT

Brillantasia cicatricosa Lindau is used in folk medicine in the Great Lake Region to treat a number of diseases and poisonous. This study evaluated the potentiality of the plant extracts to protect against ulceration caused by anti-inflammatory drugs and against cyanide or strychnine poisoning in animal model on guinea-pigs. Dried leaves of the plant were extracted with methanol, chloroform and dichloromethane using general phytochemical procedures. Ulcer was induced with indomethacin (20 mg/kg) in propylene glycol. Potassium cyanide (6.6 mg/kg i.p.) and strychnine (4.4mg/kg sc.) were used as poisons. Solutions of extracts were administered (250-4000 mg/kg) orally thirty minutes before treating animals. The number of ulcer nodules or deaths served to measure the efficacy of the extracts. Chemical screening revealed the presence of flavonoids/phenols, terpenes/sterols, but the absence of alkaloids. Flavonoids and terpenes/sterols presented significant antiulcer activity not present in the chloroform extract. Chloroform extract presented significant antidote effect in dose dependent manner with DE_{50} of about 500 mg/kg for KCN and 1000 mg/kg for Strychnine. This preliminary study shows that the plant *Brillantaisia cicatricosa* Lindau has antiulcer activity and its use could improve the vital or functional prognosis of cyanide and strychnine poisoning. It is worth undertaking deep chemical study to isolate active terpene or flavonoid second metabolites.

KEY WORDS: ***Brillantaisia cicatricosa*, ulcer, antidote, cyanide, strychnine,**

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INTRODUCTION

The use of plants is regaining worldwide enthusiastic consideration to manage various diseases and conditions. It is obvious that in some Asian and African countries, more than 80% of the populations rely on traditional healers for their health care[1]. Usually, plant remedies are administered regardless of scientific based-evidence for their effectiveness and safety. Not all plants are safe and many contain powerful poisons[2]. The mystery of the plant kingdom, however, is that both poisons and antidotes are near. For instance, the cyanide poisoning has existed for centuries if not millennia following the ingestion of certain herbs like cassava, bitter almonds, beans Java, kernels of apricots, plums, cherries, etc.[3] The leaves and tubers of cassava constitute a staple diet in many tropical countries, but studies have found a link between long term consumption of cassava and neurological disorders or thyroid abnormalities[4]. These neurological disorders called "konzo" and the subsequent paralysis have in the recent past affected thousands of people living in the African Great Lakes region[5]. Also, strychnine is a poisonous alkaloid that is obtained from seeds of the nux vomica tree of the genus *Strychnos.* Strychnine has been introduced in the 16th century as a rodenticide, and until recently it was used as a respiratory, circulatory and digestive stimulant[6].On the other hand, some plants, such as *Brillantaisia cicatricosa Lindau,* are popularly renowned for their wide spectrum of usages including anti-poisonous action. This plant of Acanthaceae family, order and class caryophyllales, is a bushy soft-wooded aromatic shrub or even rarely a small tree, up to 5 m tall which is common in moist and marshy places found in Democratic Republic of Congo (DRC), Rwanda, Burundi, Uganda, Kenya Tanzania, Malawi, Mozambique, Zambia, Zimbabwe. It is widely used in traditional medicine to cure multiple diseases and as antidote according to ethnopharmacological reports. In Rwanda, the maceration of ikiroga leaves is used in case of blood parasitism (babesiosis in human, piroplasmosis in cattle), gastric ulcer, gastroenteritis, and various infections[7] In DRC the leaves of the plant are used in decoction to treat snake bites or venomous animal stings[8]. In western Burundi, a decoction of the leaves is rectally used to treat childhood diseases and orally for headache and fever[9]. In the international literature no study is reported dealing with chemical composition or pharmacological effects of *B.cicatricosa* Lindau.

Given the various therapeutic properties claimed of this plant, there was a need to bring about scientifically evidence-based proofs of its efficacy and safety for a further rational use in modern therapy, particularly as a mithridatum or generally all-purpose antidote. This research aimed at evaluating its toxicity and the probable usage as antiulcer and antidote in cyanide and strychnine intoxications. A quick phytochemical test was also carried out to determine the major chemical groups of secondary metabolites.

MATERIALS AND METHODS

Botanical material

The plant studied is cultivated in the botanical garden of Mamba Traditional Medicine Dispensary located at Huye in the Southern Province of Rwanda at altitude 1650 m, longitude 0470352 and latitude 9710600. The identification of the plant was made at the Institute of Research in Science and Technology (IRST). The species has been identified by referring to the herbarium of this institution (N ° specimen INRS/14/ harvest place: road Gisovu Gikongoro-2km Gisovu / Harvester: Stephen C. / Date: 07/02/1972). The leaves were collected in the morning during the rainy season.

Animals

The pharmacological tests were realized on guinea pigs weighing 400 to 500g from a homogeneous breeding. These guinea pigs were prepared and used according to the standards required for experiments on

laboratory animals (health, food, light cycle, labeling) (EEC Directive 1986)[10]. Sixteen hours before each experiment, animals were fasted in order to avoid food interference with the absorption of aqueous extracts of the plant. Healthy animals were randomly assigned whether to control or treatment groups in a way to have animals with comparable weight in each group..

Extracts Preparation

General phytochemical procedures of plant screening were used for extraction and identification [11,12,13]. The leaves of *Brillantaisia cicatricosa* were air-dried and crumbled into coarse fragments and powdered. Various aliquot quantities (60-300g) of the powder were then subjected to extraction by percolation with petroleum ether, methanol, chloroform, and dichloromethane for a period of approximately 24 hours. The extract from each source was evaporated under a vacuum at a low temperature (40-60^{o}C) until dry. Preliminary phytochemical screening and thin layer chromatography were carried out to identify presence of main secondary metabolites in the crude extract[14].

Indomethacin induced ulcer test

Ulcer was produced by orally administration of indomethacin powder suspended in propylene glycol (a dose of 20 mg/kg) on the day of experiment. Five groups of five guinea pigs in each were constituted. The first group served as negative control, the second group served as positive control; the third, fourth and fifth served as test groups for chloroform, methanol and dichloromethane extracts. Each guinea pig of negative group received only 2 ml of propylene glycol alone. Each guinea in positive control received only 2 ml of indomethacin propylene glycol suspension. The third, fourth and fifth groups received 100mg/kg BW of chloroform extract, terpenoids extract and flavonoids extracts respectively. The animals were anesthetized with chloroform and sacrificed 5 hours later, and stomach was opened to calculate the ulcer index by Kunchandy method[15]. The stomach of each was removed and observed under the magnifying glass to detect bleeding nodules on the mucosa.

Poison Antidote test

They were placed into 6 groups each of 5 animals and housed in separate cages. One cage housed one animal during the experiment. Animals of first group received only plant methanol extract 4g/kg to test the intrinsic toxicity of the extracts. The second group was given orally 1ml distilled water and the lethal dose of poison without plant extracts. Those in group 3, 4 and 5 were pre-treated orally with B*. cicatricosa* Lindau extracts (0.2 g/kg to 2 g/kg) thirty minutes before intoxication. Lethal doses were 6.6 mg/kg i.p. for KCN and 4.4 mg/kg s.c. for strychnine. Antidote activity profile was assessed by monitoring external toxicity signs related to each poisoning compound after oral administration of the plant extract. The parameters measured were severe symptoms including agitation, tremors, convulsions, palsy, coma, respiratory arrest or death.

RESULTS

Chemical constituents

The phytochemical screening detected tannins, flavonoids/phenol acids and terpene-sterols, but failed to detect the presence of alkaloids, saponosides, anthocyanosides, or mucilage. However tannins, flavonoids/phenol acids and terpene-sterols have been detected. TLC reveals the presence of an important flavonoid/phenol acid likely luteolin/quercetin/ type and chlorogenic acid. There is no flavonolignane, like sylimaryn or otherwise in trace.

Table 1
Yield of extraction of dry leaves powder from B. cicatricosa with different solvents

Solvent (Fraction)	Starting aliquot quantity	Yield (g)	Yield (%)
Chloroform extract	50 g	2.4 g	4.8
Dichloromethane (flavonoids)	100 g	17.1g	17.1
Methanol (terpene sterols)	120 g	5.5 g	4.6

Acute Toxicity

The acute toxicity test has demonstrated that the methanol extracts, up to 4g/kg, do not kill or affect any physical signs of animals when administered by oral route.

Antidote activity

Survival curves are presented in Figure-1 with approximate ED_{50} of 500mg/kg against KCN and 1000 mg/kg against strychnine. After administration of strychnine or cyanide, the intensity of early severe intoxication symptoms (agitation, tremors, convulsions, coma, respiratory arrest) were reduced even abolished, and the onset time prolonged from 10 minutes for control animals to 180 minutes for tested animals. The activity was more expressed by the chloroform extract.

Antiulcer activity

No animal in the control group who received pure propylene glycol developed stomach ulcer. This shows that propylene glycol has no influence in the development of gastric ulcer in guinea pigs treated. All guinea pigs in the reference group who received indomethacin suspended in propylene glycol at a dose of 20 mg/kg developed bleeding nodules on the stomach (ulcer index =6.20±0.34). All guinea pigs treated with chloroform extract also showed nodules of ulceration meaning this extract is not very active (ulcer index=4.40±0.23). However, those in the test groups who received 300 mg/kg BW of flavonoids and terpenes were completely protected and did not develop ulcer (index=0).

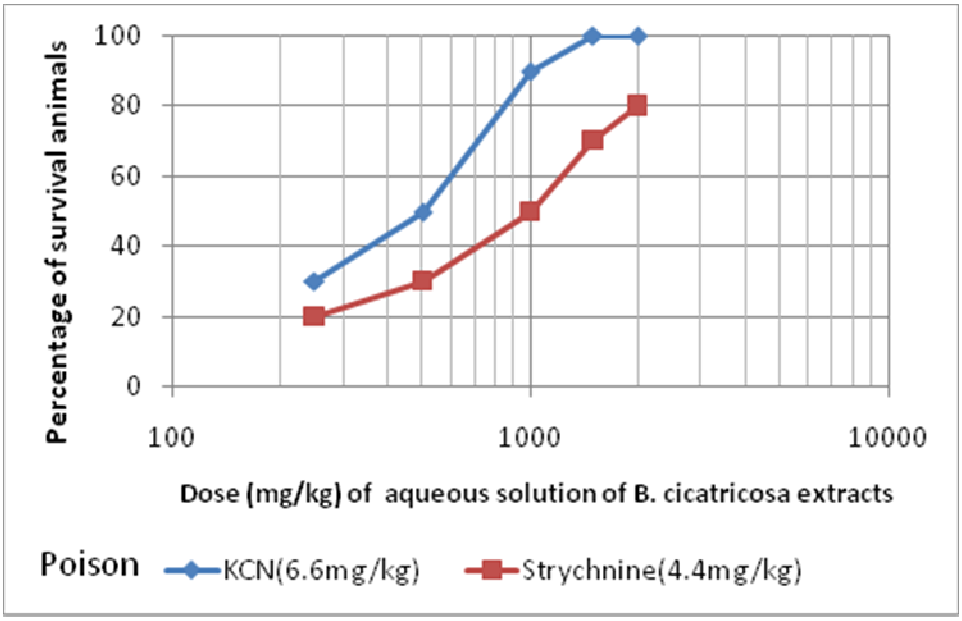

Figure 1
Antidote action of leaf extracts from B. cicatricosa in guinea pigs poisoned with cyanide or strychnine. **Each point data is the mean of 5 animals.**

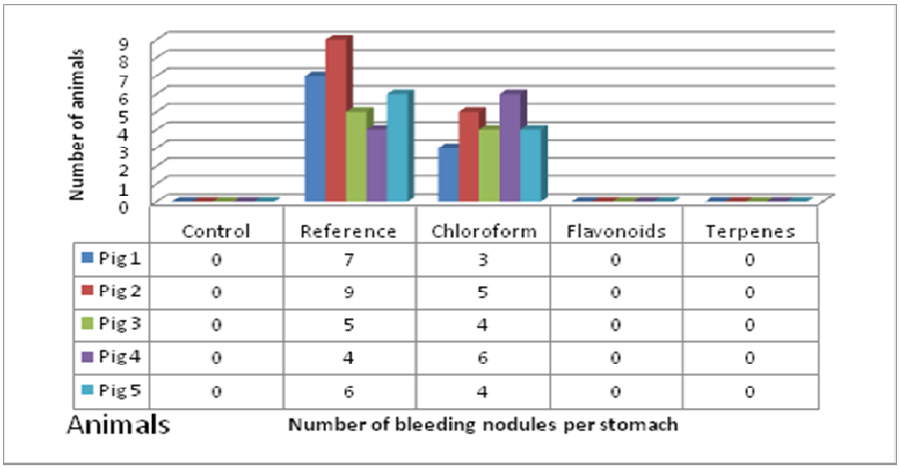

Figure 2
Protective action of leaf extract from B.cicatricosa in guinea-pigs against ulcer induced with indomethacin 20mg/kg BW. The ulcer nodules on the stomach were observed with magnifying glass after dissection.

DISCUSSION

Phytochemical reactions failed to reveal the presence of alkaloids, saponosides, anthocyanosides, or mucilage. The major components of *B.cicatricosa* Lindau may share the structure of some constituents of *Brillanataisia palisatii* Lind reported in the study of Berrondo et al.[16] on brazilian species. This study revealed the presence of a mixture containing sitosterol and stigmasterol, a new triterpene 3-*epi*-ursolic acid, and another triterpene mixture comprising α-amyrin, β-amyrin and lupeol, verbascoside, a phenylpropanoid glycoside, and lespedin, a glycosyl flavonoid. The leaves of *B.cicatricosa* Lindau are used often in mixture with other plants to treat various human and veterinary ailments in traditional medicine[7,8,9]. Traditional healers also administer it as antipoison and antiulcer[7]. Dry crude extracts from the leaves were able to prevent intoxication of guinea pigs by cyanide and strychnine in dose-dependent manner. However the protection was seen only when the extract was administered before or very early after the ingestion of the poison. Cyanide and strychnine are violent poisons acting the first by blocking respiratory cytochrome a3 and the second by competing at glycine receptors.Concerning antiulcer activity, all guinea pigs treated with chloroform extract were not protected meaning this extract is not very active. However, those treated with flavonoids and terpenes fractions were completely protected and did not develop ulcer at the dose given. In the literature, various flavonoids and terpenes sterols have shown anti-ulcerogenic activity in various in vivo experimental models[17,18]. Flavonoids are a group of polyphenolic compounds with known properties that include free-radical scavenging, inhibition of hydrolytic enzymes, anti-inflammatory and antiulcer action[19,20]. At this stage no mechanism of antiulcer or antidote activity can be formulated as the chemical second metabolites are still unknown.

CONCLUSION

This preliminary study shows that the plant *Brillantaisia cicatricosa* Lindau has antiulcer action and its use could improve the vital or functional prognosis of cyanide and strychnine poisoning. It is worth undertaking deep chemical study to isolate active terpene or flavonoid second metabolites.

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