Acute toxicity effects of the methanolic extract of *Fagara zanthoxyloides* (Lam.) root-bark

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ABSTRACT

Background: *Fagara zanthoxyloides* is a well known medicinal plant in Uganda. It is used extensively in malaria and other infections. However nothing is known about its toxicity.

Objective: The objective of the study was to evaluate the acute toxicity of the methanolic extract of the root-bark of *F. zanthoxyloides*, in mice.

Methods: Methanolic extract of the root-bark of the plant was administered orally to mice at various dose levels to determine the acute toxic effects and the median lethal dose (LD_{50}) in mice.

Results: The LD50 of the methanolic extract was found to be 5.0 g/Kg body weight within 95 % confidence limits. The mice showed signs of cerebral irritation before dying. Histopathological examinations of the viscera showed congestion and focal necrosis of the liver and renal tubules.

Conclusion: It was concluded that the extract of *F. zanthoxyloides* is safe, however the cerebral mechanism that lead to the death of the mice need to be investigated further.

Keywords: *Fagara zanthoxyloides*, medicinal plant, toxicity, Uganda.

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BACKGROUND

Fagara zanthoxyloides (Lam.) a Rutaceae, is widely distributed in Uganda and other African countries. It is well known for its varied uses in traditional medicinal practices. The root-bark extract is used in treating elephantiasis, toothache, sexual impotence, gonorrhoea, malaria, dysmenorrhoea and abdominal pain[1, 2, 3]. Workers in West Africa have reported the anti-sickling and antimicrobial activity of the extracts of the plant[4]. In Nigeria *F.zanthoxyloides* is used as a chewing stick. Water extracts from the plant showed activities against bacteria significant to periodontal disease[5, 6]. The anthelmintic activity of the methanolic extract of the root-bark of *F.zanthoxyloides* was also reported[7], and it is a very popular anthelmintic amongst the various tribes in Uganda. It has also been found that the alcoholic extracts of the root-bark possesses considerable antibacterial activity[8]. An anti-sickling agent[9], and an anti-inflammatory amide were isolated from the plant[10].

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As euphoria about the usefulness of medicinal plants grow, researchers have become aware of the toxicity of their extracts[4]. It is necessary to carry out toxicity studies on medicinal plants even though they have been used for decades to determine acceptable from non-acceptable toxicity levels. While *F.zanthoxyloides* has such diverse traditional medicinal use in Uganda and other African countries, there appears to be limited toxicity reports on it. The root extracts of the plant was shown to have some cytotoxic effects on the human erythroleukaemia cell line[11]. Compounds with cytotoxic activities have the potential for adverse effects to the human body. It is based on this premise that its short-term safety was assayed using mice in order to highlight any unrecognized toxic manifestations.

OBJECTIVES

The general objective of this study was to determine the toxicity level of the methanolic extract of the root-bark of *F.zanthoxyloides* in mice.

Specific Objectives

a) To determine the acute toxicity value (LD_{50}) of the methanolic extract of the root-bark of *F.zanthoxyloides* in mice.
b) To determine histologically the acute toxicity effects of *F.zanthoxyloides* on the internal organs of mice.

MATERIALS AND METHODS

Samples of *F.zanthoxyloides*, including the root, stem and aerial parts, were collected from Apac District, Northern

Uganda, during the dry season. It was identified by a taxonomist at the Natural Chemotherapeutics Research Laboratory, Kampala, and confirmed by the Department of Botany, Makerere University. A voucher specimen has been deposited at the Department of Pharmacology and Therapeutics herbarium, Makerere University. A total of 40 male albino mice, weighing 27 - 35 g were used in the experiments.

The methanolic extract

Powdered root-bark of *F.zanthoxyloides* (100 g) was first extracted with ether at room temperature to remove oil soluble components of the sample. This yielded 22.2 g (7.4%) of the crude ethereal extract which was discarded. The marc was then soaked in methanol. The extract was dried under reduced pressure giving a yield of 31.5 g (10.5%) of the crude methanolic extract, which was used in the study. A stock solution was prepared from the alcohol extract by dissolving 2.0 g in 10 ml of distilled water.

Toxicity Tests

An initial test was done to determine the approximate lethal and non-lethal doses of the extract according to Turner [12]. Five groups of eight mice each were used in the experiments. The extract, in doses of 2.0, 3.0, 4.0, 6.0 and 10.0 g/Kg body weight respectively was administered orally, using intragastric tubes, to the animals as a single dose. The control group was given an equal volume of water. The animals were observed for 24 hours and the number of dead mice was recorded and used in the calculation of the acute toxicity value (LD_{50}). The mice were also observed for other signs of toxicity, such as, excitation, tremors, twitches, motor coordination, righting reflex and respiratory changes. A pathologist carried out postmortem examinations of the viscera (stomach, liver, heart, kidney and brain) of the animals.

RESULTS

Within six hours of administration of the extract, all the mice that received 10.0 g/Kg of the extract had died. All the animals that received 2.0 g/Kg of the extract survived beyond the 24 hours of observation. The median acute toxicity value (LD_{50}) of the methanolic extract of *F. zanthoxyloides* determined to be 5.0 g/kg body weight within 95 % confidence limits (3.45-7.25g/kg), Table 1.

Table 1: Acute toxicity of the methanol extract of *F.zanthoxyloides* in mice.

Number of Mice	Doses of extract g/kg	Number of mice dead	Percentage of mice dead
8	0.0	0	0
8	2.0	0	0
8	3.0	2	25
8	4.0	3	37
8	6.0	5	62
8	10.0	8	100

On administration of the extract, no immediate behavioural changes were noted. The mice move and fed normally. After twenty minutes, piloerection was noticed and the animals became restless, some trying to escape through the holes in the cages. The animals did not vomit, neither was there ptosis. The animals that received higher doses went into convulsions and died in hyperextention. Post mortem examination did not reveal any gross abnormality of the brain, the organs of the chest and abdominal cavities. Histopathological examination showed congestion and focal necrosis in the liver and renal tubules [13].

DISCUSSION

According to Ghosh[14], *F.zanthoxyloides* can be classified as slightly toxic, since the LD_{50} was found to lie between 0.5 - 5.0 g/Kg. The gram equivalent of the LD_{50} of the extract in an adult man would be 300g, that is, a plate full of the extract, making it relatively safe. It has been observed that overdose of *F. zanthoxyloides* extract is usually non-fatal; the victims tend to suffer self-limiting gastrointestinal disturbances[2]. Likewise, the extract was administered orally to the test animals. This way the same route used by the traditional healers in treating their patients was used in the test animals. This would make any findings in the mice easily translatable to what would be expected in the human subjects. In any case the extract was not pure enough for parenteral route administration.

The viscera of the animals did not show any macroscopic changes that could point to the cause of death. However, since death occurred just after convulsions, it is postulated that the extract killed the mice by some action on the nervous system. Other workers found active principles from the stem bark extract of the plant that appeared to exert their action on the neuromuscular transmission either by blockage of the post-junctional end-plate or by enhancing release of neurotransmitters[15]. Similarly, the stem bark extract caused tonic-clonic seizures when injected intra-peritoneally to mice[16].

The histological changes that were demonstrated in the liver and kidney is however significant. However, because of genetic variation in response to drugs by different species, it is difficult to directly translate the results of this study to other animal species or to man. This has been recognized as a limitation in this study. Never the less, in view of the above findings, patients receiving larger doses, or under-going prolonged medication with the extracts of the plant, should have renal as well hepatic functions evaluated regularly. These effects are indeed a warning regarding the potential toxicity of the extracts of the plant.

Conclusions

It can be concluded the methanol extract of *F.zanthoxyloides* is relatively safe within the normal doses. However it is recommended that:

a) Further toxicity studies using different animal species is necessary.

b) Sub-acute and chronic toxicity tests is planned in order to determine the long-term effects of the extract.

c) Further studies to elucidate the exact mechanism and loci of the fatal action of the extract need to be carried out.

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