Possible toxicity of a medicinal plant, "*Asteracantha longifolia*"

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Abstract

Asteracantha longifolia is a medicinal plant that is extensively used for the treatment of diabetes mellitus in Sri Lanka. Experiments were carried out to evaluate any possible toxicological effects mediated by the long term administration of an aqueous extract of this plant. Investigations with *Asteracantha longifolia* showed that the aqueous extracts of this plant had no adverse effects on the histology of liver, heart, lung, kidney, intestine and pancreas, on liver function, haematological parameters (haemoglobin concentration, red blood cell count, white blood cell count and packed cell volume) or on the reproductive ability of rats.

Keywords: *Asteracantha longifolia*; toxicity; medicinal plants, rats.

Introduction

Asteracantha longifolia L. of the family Acanthaceae (**S:** Katuikiriya, Neeramulliya, **T:** Neremulli, Nirmalli) is recommended by ayurvedic and folk medical practitioners in Sri Lanka to treat various diseases such as diabetes mellitus, oedema, dropsy, jaundice, anasarca and gonorrhoea (1,2), In our previous investigations we have shown that aqueous extracts of *Asteracantha longifolia* can significantly lower the fasting blood glocose level and markedly improve the glucose tolerance of rats (3), healthy human subjects and maturity onset diabetic patients (4).

The value of any hypoglycaemic agent depends not only on its hypoglycaemic potency but also on its lack of toxicity. Any hypoglycaemic agent with therapeutic value would have to be administered over a relatively long period. It must therefore be free of acute as well as chronic toxic effects. Although there are no previous reports of toxic or unacceptable effects of *Asteracantha longifolia* this may be due to the lack of controlled scientific investigations with this plant. Investigations were therefore conducted to evaluate any possible toxicological effects mediated by the long term administration of the aqueous extract of *Asteracantha longifolia.*

Objectives

The effects of this plant extract on (a) the histopathology of various organs in the body, (b) some haematological parameters, (c) liver function, as assessed by the effects on alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase levels in serum and (d) fertility, were investigated using Sprague Dawley rats as the experimental model.

Material and Methods

In all experiments, Sprague-Dawley rats, litter mates of weight 150 ± 10g maintained on a standard laboratory diet (obtained from Moosajee's Ltd, Sri Lanka) and water *ad libitum* were used.

The plant extract was prepared according to the method used by ayuvedic and other traditional medical practitioners for administration to diabetic patients. The method quoted in books on medicinal plants (Attygalle, 1952; Jayaweera, 1982) was confirmed by discussions with several well-regarded traditional medical practitioners in southern Sri Lanka and at the Bandaranaike Memorial Ayurvedic Research Institute, Nawinna,

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Sri Lanka. Fresh whole plants (100g) including roots were cut into small pieces and boiled with water (800mL) for 3h, filtered and final volume reduced to 200mL by boiling. The botanical identity of the plant was determined by using the descriptions in books (Attygalle, 1952: Jayaweera, 1982) and confirmed by comparison with authentic samples from the herbarium of the University of Peradeniya, Sri Lanka. A voucher specimen (R.F.M. 020) has been deposited in the herbarium maintained by the Faculty of Medicine, University of Ruhuna, Sri Lanka. The plant extract was administered orally via a stomach tube. The dosage administered was 1 mL/100g body weight which was comparable to that given to humans on a weight for weight basis.

Male rats (n=20) were randomly assigned into two groups of ten each. The mean weights of animals in each group were similar. Group 1 served as the control and was given distilled water (1mL/100g body weight) once daily for 30 days. Group 2 which served as the test animals was given the *Asteracantha longifolia* extract (1mL/100g body weight) once daily for 30 days. On the 31st day of the experiment all animals were sacrificed by decapitation at 9.00 a.m. and blood was collected for liver function tests and haematology. Blood from rats subjected to decapitation were collected into clean, dry centrifuge tubes. The serum was allowed to separate and used in enzyme assays. The serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase were estimated using previously reported method (5) as described by BDH chemicals Ltd., Poole, England in their assay kits for the respective enzymes. The method of King et al (6) was employed in the estimation of serum alkaline phosphatase. For haematology tests blood from the decapitated rats was collected into clean, dry, sample bottles containing dry anticoagulant (Sodium EDTA). The RBC count, WBC count, PCV and Hb concentration in these samples were then estimated. The RBC count was estimated according to the method described by Biggs amd MacMillan (7). The WBC count was estimated by using the method of Berg (8). The PCV and Hb concentrations were estimated according to the method described by Willard and John (9). These assays were carried out at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna.

Histological study of the effects of treatment

From the animals killed for obtaining blood for liver function tests and blood cell counts, the organs (liver, kidney, pancreas, intestine, heart and lungs) were excised and fixed in formalin, buffered with sodium phosphate buffer for histological assessment of tissue damage, after haematoxylin-eosin staining. The histological examination of sections were carried out in the Department of Pathology, University of Ruhuna.

Effects on ovulatory activity

Twelve female rats (8-10 weeks of age) were divided into two groups, each consisting of 6 animals. Group 1 served as control and was given 1 mL/100g body weight of distilled water once daily for 10 days. Group 2 was given *Asteracantha longifolia* extract for 10 days. Vaginal smears were examined daily to check whether animals showed persistent dioestrus or regular cyclicity. Different stages of the oestrus cycle were determined according to Soejarto et al (10). For collection of vaginal smears, a few drops of saline (0.9% NaCl solution) were taken into a dropper. Each rat was held with one hand while inserting the tip of the dropper into the vagina with the other hand, taking care not to touch the cervix. The saline was expelled into the vagina and withdrawn. The contents were expelled into a microscopic slide and examined under a light microscope while wet or after staining with haematoxylin and eosin. Under the microscope, three types of cells could be observed. They were leucocytes, round epithelial cells with easily distinguishable nuclei and cornified cells in which nuclei were difficult to observe or were absent. Examination of the vaginal smears was carried out by the first author at the Department of Biochemistry, Faculty of Medicine, University of Ruhuna.

Effects on implantation

Adult, regularly cyclic female virgin rats (8-10 weeks of age) and male rats (12-16 weeks of age) of proven fertility were mated. Presence of copu-

lation plugs or sperms in the vaginal smear in the following morning was regarded as day 1 of pregnancy. The anti-implantation effect was studied by using the method described by Soejarto et al. (10).

Pregnant female rats (n=20) were randomly divided into two groups of ten each. Group 1 was given distilled water (1mL/100g body weight) from days 1 to 7 of pregnancy. Group 2 was given *Asteracantha longifolia* extract (1mL/100g body weight) from days 1 to 7 of pregnancy. Autopsies were performed on the 10th day. The number of live and dead fetuses and the number of corpora lutea of pregnancy were recorded in two groups. The student's test for unpaired data was used to test for significant differences between the groups.

Abortifacient effect

Asteracantha longifolia extract was investigated for possible abortifacient activity by the method of Soejarto et al. (10). Adult regularly cyclic female virgin rats (8-10 weeks of age) were mated. Pregnant female rats (n=12) was randomly divided into two groups of 6 each. Group 1 served as the control and was given distilled water (1mL/100g body weight) from day 5 to 7 of pregnancy. Group 2 was given *Asteracantha longifolia* extract (1mL/body weight) from day 5 to 7 of pregnancy. The females were then observed for vaginal bleeding from 6th to 10th day. The animals were autopsied on the 16th day and observations recorded as in the study on implantation activity.

Effects on sperm motility

The method described by Soejarto et al (10) was used to determine the effect of the plant extract on sperm motility. Masturbated human sperm was obtained from fertile males and allowed to liquefy at 37°C for 30 min. Two slides were taken and one drop of semen placed on each slide. Two drops of *Asteracantha longifolia* extract were added to one slide, while two drops of distilled water were added to the other. After mixing the contents on each slide for 5 seconds, cover slips were placed on them and the slides examined under the light microscope for sperm motility

Results

The effects of *Asteracantha longifolia* extract on the serum levels of ALT, AST and alkaline phosphatase are shown in Table 1.

Table 1

Effects of aqueous extracts of *Asteracantha longifolia* on liver enzymes

Treatment and dosage administered	Alanine amino transferase (IU/L)	Aspartate amino transferase (IU/L)	Alkaline phosphase (K. A. units/100mL)
Distilled water, 100 mL/kg	24.31±2.1	51.67±2.2	52.17±2.0
A. longifolia extract 10 mL/kg	25.28±1.2^{NS}	55.13±1.3^{NS}	53.41±1.6^{NS}

Mean ± S.E.M. are shown, (n=10); NS: Not Significantly different from the controls

In Table 2 the results of the effect of *Asteracantha longifolia* extract on haematological parameters are summarized.

Table 2

Effects of the aqueous extract of *Asteracantha longifolia* on haematological parameters

Treatment and dosage administered	HB concentration g/dL	RBC count (10^6/mm^3)	WBC count (10^3/mm^3)	PVC %
Distilled water 10mL/(control group)	11.09±0.3	7.90±0.21	5.425±0.193	52.0±1.0
A. longifolia extract 10mL/kg	10.99±0.2^{NS}	7.80±0.31^{NS}	5.517±0.127^{NS}	50.0±0.9^{NS}

Mean ± S.E.M. are shown, (n=10); NS: Not significantly different from the controls

Results given in these two tables show that administering the extract for one month had no effect on liver function or on the haemoglobin concentration and blood cell counts.

Effects on organ histology

On comparison of histological sections of the organs (liver, heart, lung, intestines, kidney and pancreas) of control animals with those of animals treated with the plant extract for 30 days, no differences could be observed between the two groups (data not shown).

Effects on the reproductive ability of rats

As shown in Tables 3, 4 and the 5 plant extract exhibited no anti-implantation or abortifacient effects and did not significantly increase the duration of the dioestrus phase or the total length of the oestrus cycle. The plant extract also had no apparent effect on sperm motility as evident from microscopic examination of the sperm samples treated with or without the plant extract.

Table 3

Effects of the aqueous extract of *Asteracantha longifolia* on implantation in rat

Treatment (10mL/kg)	Rate of pregnancy	Average foetuses/pregnant rat			Foetal mortality %	Implantation loss/rat %	Fertility rate
		Alive	Dead	Total			
Distilled water (controls)	10/10	8.0±0.4	0	8.0±0.4	0	2.1±2.0	1
A. longifolia extract	10/10	7.0±0.6^{NS}	0	7.0±0.5^{NS}	0	2.5±3.0^{NS}	1

Mean ± S.E.M. are shown, (n=10); NS: Not significantly different from the controls

Table 4

Effects of the aqueous extract of *Asteracantha longifolia* on early abortifacient activity

Treatment (10mL/kg)	Rate of pregnancy	Average foetuses/pregnant rat			Foetal mortality %	Implantation loss/rat %	Fertility rate
		Alive	Dead	Total			
Distilled water (controls)	6/6	7.6±0.3	0	7.6±0.3	0	2.2±1.8	1
A. longifolia extract	6/6	7.4±0.3	0	7.4±0.3	0	2.1±1.3^{NS}	1

Mean ± S.E.M. are shown, (n=10); NS: Not significantly different from the controls

Table 5

Effects of the aqueous extract of *Asteracantha longifolia* on ovulatory activity

Treatment (10mL/kg)	Mean duration (days)	
	Oesturs cycle	Dioestrus stage
Distilled water (controls)	4.7±0.3	1.3±0.17
A. longifolia extract	4.5±0.2^{NS}	1.4±0.14^{NS}

Mean ± S.E.M. are shown, (n=10); NS: Not significantly different from the controls

Discussion

The therapeutic value of any plant extract depends not only on its pharmacological potency but also on its lack of toxicity. This is specially important in the case of hypoglycaemic plant extracts which have to be administered over a relatively long period of time. Some plants with hypoglycaemic properties have been shown to have toxic effects (11).

As evident from the results obtained in the present study, administration of *Asteracantha longfolia* extracts to rats for one month, no toxic effects of importance were detectable. The extracts used had no significant effects on the histology of various body organs, haematological parameters or on the reproductive ability of the experimental animals. The general condition of the animals also did not

change and all of them remained in good health throughout the experimental period.

Asteracantha longifolia therefore appers to be free from any major toxic or unacceptable effects when administered for a period of one month. However, for a more definitive conclusion with regard to the non-toxicity of the plant extract, a greater variety of animal species should be studied and the plant extract administered for a longer period of time.

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