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STUDIES ON THE POSSIBLE TOXICITY OF *ARTOCARPUS HETEROPHYLLUS*

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SUMMARY. Investigations with *Artocarpus heterophyllus* showed that the extracts of this plant exerted no adverse effects on liver function, haematological parameters (haemoglobin concentration, red blood cell count, white blood cell count and packed cell volume), on the reproductive ability of experimental animals, and on the the histology of heart, lung, kidney, intestines and pancreas.

INTRODUCTION

Artocarpus heterophyllus Lam. (family Moraceae) is a plant whose various parts are used by traditional and ayurvedic medical paractitioners in Sri Lanka to treat many conditions such as skin disease, diarrhoea, fever, bilious colic and diabetes (1). In previous investigations Fernando (2) has confirmed the presence of oral hypoglycaemic activity in the mature leaves of this plant.

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. There are no reports on toxic or unacceptable effects of *A. heterophyllus*. Investigations were therefore conducted to look for any possible toxic effects mediated by the long-term administration of an aqueous extract of *A. heterophyllus*. The possible effects of the plant extract on (*a*) some haematological parameters, (*b*) liver function, as assessed by the effects on alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase levels in serum, (*c*) fertility and (*d*) the histological appearance of various organs, were investigated using Sprague Dawley rats as experimental animals.

MATERIALS AND METHODS

Experimental animals

In all experiments, Sprague Dawley rats, litter mates of weight 150 $\pm$ 10g were used. They were provided *ad libitum* with a standard laboratory diet (natural diet obtained from Moosajee's Ltd., Sri Lanka) and water.

Preparation of plant extract

The plant extract was prepared according to the method by which it is normally made by traditional medical practitioners for administration to diabetic patients. The method quoted in books on medicinal plants was confirmed by discussions with several reputed traditional medical practitioners in Galle and at the Bandaranaike Memorial Ayurvedic Research Institute, Nawinna.

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Fresh mature leaves (200g) were collected in the morning, boiled with distilled water (1,000ml) for 3h and the final volume reduced to 200 ml.

Dosage and administration of drug

The plant extract was administered orally via a stomach tube. The dosage administered was 1ml/100g body weight.

Study 1

Male rats (n=20) were randomly divided into two groups of ten each. The mean weights of animals in each group were similar. Group 1 served as the controls and were given distilled water (1ml/100g body weight) once daily for 30 days. Group 2, which served as the test animals, were given the *A. heterophyllus* extract (1ml/100g body weight) once daily for 30 days. At the end of the experimental period, all animals were sacrificed by decapitation and blood collected for liver function tests and haematology.

Effect of *A. heterophyllus* extract on liver function

For liver function tests, blood was collected into clean, dry, centrifuge tubes. After separation of the serum, the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase were estimated. Serum ALT and AST were estimated by using methods of Mohun and Cook (3) and Reitman and Frankel (4) as described by BDH Chemicals Ltd., Pool, England in their assay kits for the respective enzymes. The method of King et al. (5) was employed in the estimation of serum alkaline phosphatase.

Effect of *A. heterophyllus* extract on haematological parameters

For use in these experiments, 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 and MacMillan (6). The WBC count was estimated according to the method of Berg (7). The PCV and Hb concentration were estimated according to the methods described by Willard and John (8).

Histological study of the effects of treatment with *A. heterophyllus*

From the animals killed for obtaining the blood for liver function tests and blood cell counts, the livers, kidneys, pancreas, intestines, hearts and lungs were excised and fixed in formalin buffered with sodium phosphate buffer for histological assessment of tissue damage, after haematoxylin-eosin staining.

Study 2

Effect of *A. heterophyllus* 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 the controls and were given 1ml/100g. body wt, of distilled water once daily for 10 days.

Group 2 was given *A. heterophyllus* leaf extract for 10 days. Vaginal smears were examined daily to check whether the animals showed persistent dioestrus or regular cyclicity. Different stages of the oestrus cycle were determined according to Soejarto et al.(9)

Study 3

Effect of *A. heterophyllus* 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 copulation plugs or sperms in the vaginal smear in the following morning was regarded as day 1 of pregnancy. The anti-implantation effect was studied following the method by Soejarto et al(9).

Pregnant female rats (n=20) were randomly divided into two groups of ten each. Group 1 was given distilled water (1ml/100g body wt.) from days 1 to 7 of pregnancy. Group 2 was given *A. heterophyllus* extract (1ml/100g body wt.) from days 1 to 7 of pregnancy. The animals were autopsied on the 10th day. The number of pregnant animals, the number of implantation sites, the number of live and dead fetuses and the number of corpora lutea of pregnancy were recorded. The results of each index were analysed statistically by comparing the experimental group with the control group.

Study 4

Abortifacient effect of *A. heterophyllus* extract

A. heterophyllus extract was investigated for possible abortifacient activity by the method described by Soejarto et al(9). Adult regularly cyclic female virgin rats (8—10 weeks of age) were mated. Pregnant female rats (n=12) were randomly divided into two groups of 6 each. Group 1 served as the control and were given distilled water (1ml/100g body wt.) from day 5 to 7 of pregnancy. Group 2 were given *A. heterophyllus* extract (1ml/100g body wt.) from day 5 to 7 of pregnancy. The females were then observed for vaginal bleeding from the 6th to 10th day. The animals were autopsied on the 16th day and observations recorded as in the study on implantation activity.

Study 5

Effect of *A. heterophyllus* extract on sperm mobility

The method described by Soejarto et al(9) was used to determine the effect of the plant extract on sperm mobility. Masturbated human sperm was obtained from proven human males and allowed to liquefy at 37°C for 30 min. Two slides were taken and one drop of sperm placed on each slide. Two drops of the *A. heterophyllus* 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, coverslips were placed on them and the slides examined under the light microscope for sperm immobiltity.

RESULTS

The effects of *A. heterophyllus* extract on the serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase are shown in Table 1. Statistical analysis by the Student's t-test revealed that there were no siginificant differences between the mean enzyme levels in the A. *heterophyllus* treated group and in the water treated control group ($p > 0.5$ for all enzymes).

TABLE 1. Effect of the aqueous extract of *A. HETEROPHYLLUS* on serum levels of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase

Treatment and dosage administered	Alanine amino transferase (IU/L)	Aspartate amino transferase (IU/L)	Alkaline phosphatase (K.A. units/100ml)
Distilled water, 10 ml/kg	24.31 ± 2.1	51.67 ± 2.2	52.17 ± 2.0
A. heterophyllus extract 10 ml/kg.	24.15 ± 1.1*	53.51 ± 2.1*	53.65 ± 2.4*

Each value is the mean of 10 determinations ± S.E.M. *p>0.5

TABLE 2. Effect of aqueous extract of *A. HETEROPHYLLUS* on red blood cell (RBC) count, white blood cell (WBC) count, packed cell volume (PCV) and haemoglobin concentration (Hb).

Treatment and dosage administered	Hb concentration g/dl	RBC count (10^6/mm^3)	WBC count (10^3/mm^3)	PCV %
Distilled water 10 ml/kg. (Control group)	11.09 ± 0.3	7.90 ± 0.21	5.425 ± 0.193	52.0 ± 1.0
A. heterophyllus extract 10 ml/kg.	11.20 ± 0.2*	7.70 ± 0.20*	5.525 ± 0.197*	53.0 ± 1.5*

Each value is the mean of 10 determinations ± S.E.M. *p>0.5

In Table 2 are summarized the results of the effects of *A. heterophyllus* extract on the RBC count, WBC count, PCV and Hb concentration in the rats. In all parameters studied, there were no statistically significant differences ($p>0.5$) between the mean values in the test groups and in the control groups.

On comparison of histological sections of the hearts, lungs, intestines 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.

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

DISCUSSION

In spite of the establishment of many hospitals and dispensaries where western medical aid is available, approximately 70% of Sri Lankans resort to the use of medicinal plant preparations prescribed by ayurvedic and other traditional medical practitioners. *A. heterophyllus* extracts have long been used by patients for many conditions, including diabetes mellitus (1, 10).

As evident from the results obtained in the present study, even after administration of *A. heterophyllus* 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 (Hb%, PCV, blood cell counts) or on the reproductive ability of the experimental animals. The general conditions of the animals also did not change and all the animals remained in good health throughout the experimental period. *A. heterophyllus* therefore appears to be free from any major toxic or unacceptable effects. However, for a more definite 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.

TABLE 3. Effect of aqueous extract of *A. HETEROPHYLLUS* given orally from day 1 to day 7 of pregnancy on implantation in rats

Treatment (10ml/kg)	Rate of pregnancy	Average foetuses/pregnant rat			Foetus mortality %	Implantation loss/rat %	Fertility rate
		Alive	Dead	Total			
Distilled water (Controls)	10/10	8.0 ± 0.37	0	8.0 ± 0.37	0	2.08 ± 2	1
A. hetero phyllus extract	10/10	7.0 ± 0.47*	0	7.0 ± 0.47*	0	2.8 ± 2.2*	1

Each value is the mean of 10 determinations ± S.E.M. *$p>0.5$

TABLE 4. Effect of aqueous extract of *A. HETEROPHYLLUS* given from day 5 to day 7 of pregnancy on early abortifacient activity

Treatment (10ml/kg.)	Rate of pregnancy	Average foetuses/pregnant rat			Foetus mortality	Implantation loss/rat	Fertility rate
		Alive	Dead	Total			
Distilled water (Controls)	6/6	7.6 ± 0.34	0	7.6 ± 0.34	0	2.2 ± 1.8	1
A. heterophyllus extract	6/6	7.0 ± 0.42*	0	7.0 ± 0.42*	0	1.8 ± 1.1*	1

Each value is the mean of 6 determinations ± S.E.M. *p>0.5

TABLE 5. Effect of the aqueous extract of *A. HETEROPHYLLUS* on ovulatory activity

Treatment (10ml/kg.)	Mean duration (days)	
	Oestrus cycle	Dioestrus stage
Distilled water (Controls)	4.7 ± 0.27	1.3 ± 0.17
A. heterophyllus extract	4.3 ± 0.33*	1.17 ± 0.16*

Each value is the mean of 6 determinations ± S.E.M. *p>0.5

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