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Toxicity of methanolic and chloroformic extract of Asistolochia brcteolata in rats

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Abstract

This study was designed to find the toxicological effect of methanolic and chlorformic extract of *Aristolochia bracteolata* in Swiss albino rats. Methanolic and chlorphormic extracts were given at doses of 250 and 500 mg/kg BW to Swiss albino rats. Oral administration of the extract caused symptoms such as depression, arching of the back and tremors. Serum analysis indicated increase in the activity of Aspartate aminotransferease (AST), Alanine amino transferase (ALT) and Alkaline phosphatase (ALP). The concentration of urea, creatinine, ammonia were also increased and the concentration of total protein decreased. The main lesions found were haemorrage in the kidney and congestion of the liver. We concluded that chloroformic extract at the rate of 500 mg/kg was more toxic than all the treatments.

Keywords: *Aristolochia bracteolata*, Toxicity, Rats

Introduction

Aristolochia bracteolata is a member of the family aristolochiaceae and is used in Kenya, Tanzania, Ethiopia and Sudan for the treatment of nematodes infections. The methanolic extract of *A. bracteolata* showed activity against some types of bacteria species (Farouk et al., 1983). There are also reports stated that *A. baracteolata* has hypotensive activity (Raq, 1980). The ethanolic extract of *Aristolochia* species roots decreased fertility in both rats and hamsters without interruption of pregnancy when administered on day six of pregnancy.

The extract of *Aristolochia bractelolata* exhibited activity against plasmodium falciparum strain. *Aristolochia bracteolata* was found to have a trypanocidal effect against *Trypanosoma evansi* (Samia et al., 2006). The quantitative analysis of aristolochiaceae determined that all the species contain aristolic acid (Hashimoto et al., 1999). It was reported that Aristolochia produce interstitial nephritis caused by aristolic acid during chronic use of treatment (Mengs and Stozen, 1993). The objective of this research was to find the effect of methanolic and chloroformic exatracts of *Aristolochia bractelolata* on serum biochemistry and histopathological alternations in albino rats.

Material and Methods

Forty Swiss albino rats (Albino Wister) weighed 150-200 gm were used in this experiment.They were housed in laboratory cages, fed with pellets and fresh vegetables and were watered *ad libitum* throughout the experimental period. The whole plant of *A. bracteolata* was collected and shade dried and then later ground to powder using a morter and pestle. The powder was extracted with chloroform and methonal successively by percolation. A ten fold quantity of solvent in relation to the plant material was mixed in 2-litre elenmeyer flask and kept over night at room temperature. It was evaporated at 40^{o}C low pressure using a rotatory evaporator completed to a themoregulatory device. The solid extract obtained was removed, weighed and was kept as the stock solution for use. The chloroformic extract was dissolved in propylene glycol while the methanolic extract was dissolved in distilled water.

The plant was extracted with chloroform and methanol, and given orally by nasogastric tube. The rats were divided into four groups. Group A was given 500 mg/kg BW Chloroformic extract, group B given 500 mg/kg BW methanolic extract, groups C given 250 mg/kg BW chloroformic extract and group D given 250 mg/kg BW methanolic extract. Group E was kept untreated control. Daily dosing was continued until the

rats died or were killed at the end of the experiments (4 weeks).

All rats were bled from oculars vein before dosing commenced and at week intervals thereafter for serum analysis and hitopathology of liver and kidneys. Blood samples were allowed to clot, serum was separated and stored at –20^{o}C until analyzed. Serum samples were examined for activities of AST and ALT by the method descrbed by Reitman (1969). The concentrations of total protein (Weichselbaum, 1946), ammonia and urea (Varley, 1967), magnesium (Gradwohp, 1956) and the activity of creatinine and alkaline phosphatase was determined by commercially available kit. For histopathological studies, tissues were fixed in 10% formal saline and 6-µm paraffin sections were stained with haemtoxylin and Eosin (HE).

Statisitcal analysis

The rsults were expressed as means and standard deviation. The significanse of differences between the means were analysed by Steel et al. (1997). Where there was significant difference, Duncan Multiple Range Test (DMRT) was used.

Results

The mortality rates for chlorofomic and methanolic extracts were shown in table 1. With 500mg/kg of the chloroformic extract, ten rats died during the observation period started from the third day of treatment till the end of experiment with percentage rate 100%. The 250 mg/kg BW of the chloroformic extract gave mortality percentage rate of 60% when 6 of the 10 rats died during the experimental period. The 500mg/kg BW methanolic extract gave percentage rate of 70% with 7 of 10 rats died, and the 250 mg/kg BW of the methanolic extract gave only 30% percentage rate of mortality. Mortality was only 10% in control rats.

The biochemical changes associated with the plant extracts was shown in table 2 and 3. The higher dose of both the chloroformic (500mg/kg) and methanolic extract (500mg/kg) gave significant increase in AST and ALT and ALP enzymes activity ($P<0.05$). There was also significant increase in the concentration of urea and creatinine ($P<0.05$). There was gradual decrease in the concentration of total protein which was found to be the significant with the chloroformic extract and non significant with the methanolic extract. The concentration of Ammonia in the group that given 500 mg/kg BW of the chloroformic extract increased from the second week of treatment and remained high till death or slaughter of the rats. With the 500mg/kg BW methanolic extract, the concentration of ammonia increased in the second week of treatment where it fluctuated after until the end of the experimental period.

The concentration of magnesium showed decrease with both plant extracts but the decrease was not found to be significant. It was found that the 250 mg/kg Bw of both the methanolic and chloroformic extract gave no significant changes in either liver enzymes or total Protein, or alkaline phosphatase, urea, creatinine, magnesium or ammonia table 4 and 5.

Table 1: Mortality rates in rats given *Aristolochia bracteolata* extracts for 4 weeks

Group No.	No. of rats used	Dose mg/kg BW	Mortality	Percentage
A	10	500 Chloroformic extract	10	100%
B	10	500 Methanolic extract	7	70%
C	10	250 Chloroformic extract	6	60%
D	10	250 Methanolic extract	3	30%
E	10	(Control)	1	1%

Table 2: Biochemical changes associated with the treatment of 500mg/kg BW of Chloroformic extract of *A. bracteola*

Parameters	Week 0	Week 1	Week 2	Week 3	Week 4
ALT (U/l)	18.76 ± 0.67^{d}	22.24 ± 0.83^{c}	29.36 ± 0.74^{b}	36.22 ± 0.64^{b}	43.21 ± 0.94^{a}
AST (U/l)	14.28 ± 2.14^{d}	18.21 ± 0.75^{c}	24.18 ± 0.82^{c}	29.12 ± 0.74^{b}	32.12 ± 0.52^{a}
Total protein (g/dl)	7.48 ± 0.19^{a}	5.65 ± 0.28^{b}	4.22 ± 0.08^{b}	3.53 ± 0.49^{c}	2.48 ± 0.21^{d}
ALP (U/l)	2056.64 ± 42.12^{d}	2216.21 ± 32.14^{d}	2500 ± 28.12^{c}	2982.47 ± 38.24^{b}	3313.38 ± 24.48^{a}
Urea (mmol/l)	4.52 ± 0.62^{b}	5.48 ± 0.54^{b}	6.88 ± 0.42^{b}	8.66 ± 0.52^{b}	11.84 ± 0.61^{a}
Creatinine (mmol/l)	0.68 ± 0.08^{c}	0.71 ± 0.07^{c}	0.82 ± 0.08^{b}	0.95 ± 0.08^{b}	1.68 ± 0.42^{a}
Magnesium (mmol/l	4.14±0.04	4.20±0.02	4.60±0.03	3.20±0.01	3.12±0.02
Ammonia	0.45±0.02	0.56±0.03	0.59±0.02	0.61±0.01	0.65±0.02

The different superscript letters a and b in columns are significantly different ($P\leq0.05$).

Table 3: Biochemical changes associated with the treatment of 500mg/kg BW of methanolic extract of *A. bracteolat*

Parameters	Week 0	Week 1	Week 2	Week 3	Week 4
ALT (U/l)	16.24±0.78^{c}	20.24±0.74^{b}	24.48±0.68^{ab}	29.00±0.62^{b}	35.21±0.64^{a}
AST (U/l)	12.32±0.82^{d}	15.82±0.25^{d}	18.25±0.83^{c}	25.25±0.83^{b}	31.50±0.68^{a}
Total protein (g/dl)	6.78±0.46	6.21±0.61	5.88±0.48	5.16±0.38	4.24±0.52
ALP (U/l)	1852.00±8.24^{c}	1984.01±11.23^{b}	2084.18±7.56^{b}	2121.32±9.24^{b}	2410.02±8.28^{a}
Urea (mmol/l)	4.23±0.52	5.56±0.45	6.84±0.48	8.21±0.26	9.86±0.36
Creatinine (mmol/l)	0.58±0.08^{d}	0.64±0.19^{d}	0.82±0.09^{c}	0.94±0.08^{b}	1.04±0.08^{a}
Magnesium (mmol/l	1.15±0.43	1.44±0.06	1.14±0.08	1.52±0.04	1.56±0.04
Ammonia	0.46±0.01	0.51±0.02	0.56±0.03	0.59±0.02	0.62±0.02

The different superscript letters a and b in columns are significantly different ($P \leq 0.05$).

Table 4: Biochemical changes associated with the treatment of 250 mg/kg BW of chloroformic extract of *A. bracteolata*

Parameter	Week 0	Week 1	Week 2	Week 3	Week 4
ALT (U/l)	15.84±0.44	16.55±0.24	18.42±0.62	19.22±0.72	21.62±0.44
AST (U/l)	14.42±0.23	15.62±0.25	16.27±0.24	18.88±0.54	19.88±0.24
Total protein (g/dl)	7.68±0.16	6.82±0.14	6.26±0.64	5.26±0.42	4.92±0.52
ALP (U/l)	1789±7.32	1886.08±6.2	1888.12±6.56	1911.92±4.42	1982.02±5.28
Urea (mmol/l)	4.85±0.12	5.68±0.52	6.94±0.42	7.86±0.25	8.84±0.18
Creatinine (mmol/l)	0.66±0.4	0.71±0.16	0.78±0.06	0.82±0.04	0.88±0.06
Magnesium (mmol/l	1.78±0.24	1.66±0.06	1.58±0.06	1.52±0.08	1.49±0.04
Ammonia	0.46±0.02	0.54±0.02	0.56±0.04	0.58±0.06	0.61±0.03

Table 5: Biochemical changes associated with the treatment of 250 mg/kg BW of methanolic extract of *A. bracteolata*

Parameter	Week 0	Week 1	Week 2	Week 3	Week 4
ALT (U/l)	15.23±0.88	16.14±0.54	17.48±0.60	18.21±0.52	20.12±0.64
AST (U/l)	13.22±0.73	14.62±0.25	15.25±0.75	16.28±0.66	18.52±0.22
Total protein (g/dl)	7.65±0.46	6.21±0.74	6.26±0.64	6.16±0.32	5.84±0.52
ALP (U/l)	1765.o±7.32	1876.01±9.22	1888.12±6.56	1898.72±7.45	1982.02±6.28
Urea (mmol/l)	4.56±0.52	5.24±0.32	6.84±0.48	7.22±0.26	7.86±0.24
Creatinine (mmol/l)	0.65±0.1	0.58±0.12	0.62±0.08	0.74±0.04	0.81±0.08
Magnesium (mmol/l	1.55±0.63	1.65±0.06	1.58±0.08	1.56±0.08	1.56±0.04
Ammonia	0.49±0.01	0.51±0.03	0.52±0.03	0.58±0.02	0.58±0.02

Rats given the high dose of chloroformic extract of the plant showed symptoms like depression, arching of the back and tremors. The histopathological changes associated with the chloroformic extract (500 mg/kg) were found to be degenerative changes in liver and kidney. The chloroformic extract resulted in vascular degenerative necrosis of the liver and focal segmental glomerulonephritis (Fig. 1 and 2).

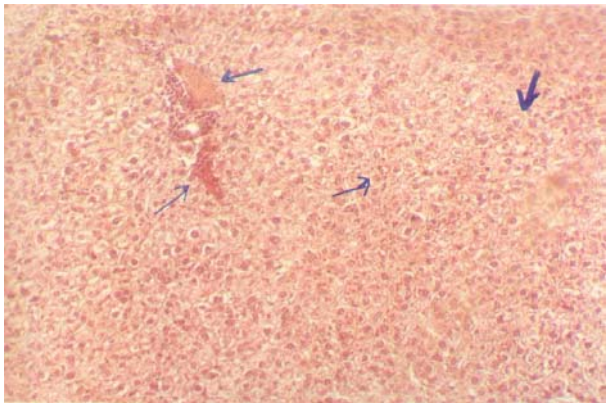

Fig 1: Haemorage in the liver and necrotic foci in response to methanolic extract (500mg/kg)

Discussion

Aristolochia bracteolata poisoning in rats caused lesions in the liver and kidney and the main signs were weakness, depression and loss of condition.

The increase in the activity of AST, ALT, ALP and the decrease in the concentration of total protein indicate liver damage. Cell damage increase the

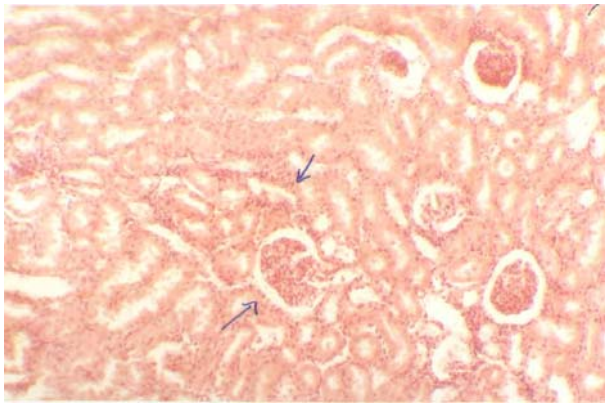

Fig 2: Degenerative changes in the kidney of rats dosed with 500mg/kg chloroformic extrac

membrane permeability, causing systolic enzymes to spill into the sinusoids and from there into peripheral blood (Carl and Edward, 2002). The increase in the concentration of urea and creatinine suggest renal disfunction. The renal tubular disorder have been known to cause hypomasgnesemia (Randel, 1969). This was probably the cause of hypomagnesemia in rats given the extract of *A. bracteolata*. Liver lesions even if relatively mild, can interfere with biluribin excretion. Similar findings have been described in goats fed with *Asristolochia bracteolata* (Barakat et al., 1983).

The mortality rates of the rats given *A. bracteolata* extracts indicate that the chloroformic extract is more toxic than the methanolic extract. This indicates that the chloroform may be capable to extract the toxic active material responsible for toxicity, this result agreed with that of Barakat et al. (1983) who found that the agous extract of *A. bracteolata* was toxic when given at high doses. The chloform extract the toxic materials found in the plant.

It was concluded that *Asristolochia bracteolate* was found to be toxic at higher doses and the chloformic extract was more toxic than the methanolic extract.

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