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Acute Toxicity of Chloroform Extract of *Artemisia macivera* Linn in Swiss Albino Mice

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Authors' contributions

This work was carried out in collaboration between all authors. Authors ACE and SEA respectively designed the study. Author ACE did all the laboratory work, performed the statistical analysis, wrote the protocol, managed the literature searches and wrote the first draft of the manuscript. Author SEA managed the analyses of the study. Author MYF carried out the histopathological analysis of the animals' organs. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

The acute toxicity of chloroform extract of *Artemisia maciverae* Linn was studied in Swiss albino mice. The mice were randomly distributed into four groups of three animals each. The groups were respectively administered both intraperitoneally and orally chloroform extract of *Artemisia maciverae* at 0, 10, 100 and 1000mg/kg in a single dose and monitored frequently for 24h and daily for 13 days in the first phase of the experiment. In the second phase of the experiment, the animals were administered single doses of the extract at 0, 200, 400 and 800mg/kg both intraperitoneally and orally and monitored frequently for 24h and 13 days respectively. The number of deaths in a group was recorded. The results of the second phase experiment were used to calculate the LD_{50} of the plant extract. All surviving animals were sacrificed after 14 days. Selected organs of the animals i.e. heart, lungs, liver, kidney, spleen, stomach and intestine of both the dead and sacrificed animals were removed and stored in 10% formal saline ready for histopathological analysis. Tissue specimens of the organs were examined histopathologically after processing and staining with haematoxylin and eosin. Lesions

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were observed in the liver, kidney and intestine of mice administered 800 and 1000mg/kg of chloroform extract of *Artemisia maciverae.* From this result, the LD_{50} of the chloroform extract of *Artemisia maciverae* was calculated to be 566 mg/kg. The results indicate that the extract may be toxic at a high dose and short term exposure.

Keywords: Artemisia maciverae; chloroform extract; albino mice; acute toxicity; organs Histopathology.

1. INTRODUCTION

Plants have always been among the common sources of medicines, either processed as traditional preparations or used to extract pure active principles. Because of the large chemical diversity among natural products, many research groups screen plant extracts for new promising therapeutic candidates for infectious diseases [1]. Traditional healers and local people in Africa rely heavily on medicinal plants for curing illnesses. *Artemisia maciverae* Linn is one of such plants widely used for these purposes, however, there is limited scientific evidence regarding safety and efficacy to back up the continued therapeutic application of these remedies. The rationale for their utilization has rested largely on long-term clinical experience [2]. The acute or sub-chronic toxicity data may be required to predict the safety or otherwise of long term low dose exposure to a particular medicinal product [3].

Artemisia maciverae Linn is a small herbaceous plant belonging to the family *Asteraceae*, and is found in the northern part of Nigeria where it is locally known as “tazargade' "in Hausa. The Hausa people of northern Nigeria use this plant in treating malaria when it is boiled in water with lemon and red potash or soaked in local gin to treat malaria [4]. We have demonstrated that indeed, this plant possesses a very high anti-malarial activity [5].

In as much as many health problems have been solved using medicinal plants, a number of them are toxic when not properly prepared or dispensed [6]. As such a scientific approach needs to be applied towards the use of plant extracts in managing ailments, especially in the developing countries where the level of literacy is low and the status of health management is poor, and about 80% of the population patronize herbal drugs. However, the safety of *Artemisia maciverae* is important in relation to its therapeutic actions. From literature, nothing is known of *Artemisia maciverae* toxicity, therefore, this study was aimed at determining the possible acute toxicity of chloroform extract of *Artemisia maciverae* in Swiss albino mice.

2. MATERIALS AND METHODS

2.1 Plant Material and Extract Preparation

The plant *Artemisia maciverae* L. was collected in Zaria, Kaduna State, Nigeria, and identified by a Taxonomist at the Herbarium Section of the Department of Biological Sciences, Ahmadu Bello University, Zaria. The Voucher number of the plant is 70737.

The whole plants of *Artemisia maciverae* used for this study was collected in April. The plants were dried under the shade for two weeks and ground into powdered form using laboratory mortar. Extraction of this powdered form of the plant was carried out by first defating it with petroleum ether for 8h before extracting with chloroform for (4hx2) using

Soxhlet apparatus. The extract was stored at -4ºC until required. Prior to use, the extract was dissolved in 0.3% Tween 80 solution.

2.2 Animals

The male Swiss albino mice used for this study were purchased from the Department of Pharmacology, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria, Nigeria. The animals were about seven weeks old and weighed between 22-3g initially.

2.3 Acute Toxicity Evaluation/Determination of Median Lethal Dose LD_{50}

The method of Lorke [7] was used to study the acute toxicity of *Artemisa maciverae*. For the first phase of treatment, the doses of 10mg/kg, 100mg/kg and 1000mg/kg of chloroform extracts of *A. maciverae* were administered intraperitoneally to the test animals in each treatment group respectively (ie 10, 100 and 1000mg/kg groups). Three mice were used for each of the test/treatment groups and control. The control groups received isovolumetric amounts of 0.3 % Tween 80 solution which was used in dissolving the extract. The animals were observed continuously for the first 1h and then every 30 min during the first 24h for the onset of any immediate toxic signs and daily during the 13 days observation period to record any delayed acute effects. The number of deaths in a group was also recorded.

For the second phase of treatment, the doses of 200mg/kg, 400mg/kg and 800mg/kg of chloroform extract of *A. maciverae* were equally administered intraperitoneally to the test animals in each treatment group respectively (ie 200, 400 and 800mg/kg groups) This second phase doses were chosen based on the results of the first phase of treatment. The animals were observed as previously described and the number of deaths in a group was equally recorded. The results of the second phase experiment were used to calculate the LD_{50} of the plant extract.

The LD_{50} was calculated according to the method outlined by Lorke [7] as:

Geometric mean of the doses for which there was no mortality x the next doses for which mortality occurred = x mg/kg.

All surviving animals were sacrificed after 14 days. Selected organs of the dead or sacrificed animals (i.e. heart, lungs, liver, kidney, spleen, stomach and intestine) of both the dead and sacrificed animals were removed and stored in 10% saline solution ready for histopathological analysis. Detailed histopathological study of the animals' organs administered with the chloroform extract of *A. maciverae* was carried out.

2.4 Histopathological Analysis of Organs

Tissue specimens of the organs were examined histopathologically after processing and staining with haematoxylin and eosin. The method of Drury et al. [8] was used in the processing and embeeding of organs, while the method of Van der Merwe [9] was used in staining the processed organs.

4. RESULTS

4.1 Acute Toxicity Evaluation of Chloroform Extract of *Artemisia maciverae*

In the first phase of treatment, mice administered the chloroform extract of *Artemisia maciverae* developed clinical signs of toxicity (loss of appetite, loss of agility, convulsion and loss of vision) after 2h of the post treatment period with 1000mg/kg of the extract. All the mice in this 1000mg/kg group died after 6h of treatment. There were no clinical signs of toxicity observed with the mice in the 100mg/kg, 10mg/kg and the control groups either immediately or during the post treatment period. No mortality occurred in these groups either immediately or during the 14- day observation period (Table 1).

In the second phase of the experiment, mice administered the extract developed clinical signs of toxicity (loss of appetite, loss of agility, convulsion and loss of vision) after 10h of the post treatment period with 800mg/kg of the extract. Two-third of the mice in this 800mg/kg group died after 20h of treatment. The only clinical sign of toxicity observed in the 400mg/kg group was loss of agility and appetite after 10h of treatment. No clinical signs of toxicity were observed with the mice in the 200mg/kg and control groups. No mortality occurred in the 400mg/kg, 200 mg/kg and the control groups during the 14-day observation period (Table 1).

From this result, the LD_{50} of the chloroform extract of *Artemisia maciverae* was calculated to be 566mg/kg (Table 1). Body weight loss was observed in the 1000mg/kg and 800mg/kg treated groups compared to the normal control and the other treatment groups.

Table 1. Acute Toxicity Studies (LD_{50}) of chloroform extract of *Artemisia maciverae*

Plant	Experiment	Dose (mg/kg)	Proportion of death	
			After 24 h	After 2 weeks
Artemisia maciverae (Chloroform extract)	Phase 1	10	0/3	0/3
		100	0/3	0/3
		1000	3/3	-
	Phase 2	200	0/3	0/3
		400	0/3	0/3
		800	2/3	2/3

LD_{50} of Artemisia maciverae = 566mg/kg

The extract produced significant decrease ($p<0.05$) in the body weights of the animals at the doses of 800 and 1000mg/kg compared to the normal control and the smaller doses (Fig. 1). There were also significant changes ($p<0.05$) in the relative weights of the liver and kidney of mice treated with 800mg/kg and 1000mg/kg of chloroform extract of *A. maciverae* compared to the other treated groups and untreated controls (Tables 2 and 3).

Macro and microscopic observations indicated congestion/ focal necrosis in the liver of the animals treated with 1000mg/kg and 800mg/kg of chloroform extract of *Artemisia maciverae* (Fig. 2). There was congestion/swollen mesangium observed in the kidney of the treated animals with 1000mg/kg and 800mg/kg of the extract (Fig. 3). There was also epithelial necrosis observed in the intestine of animals treated with 1000 mg/kg of the extract (Fig. 4). There were no lesions observed in the organs of the normal control (Fig. 2-4).

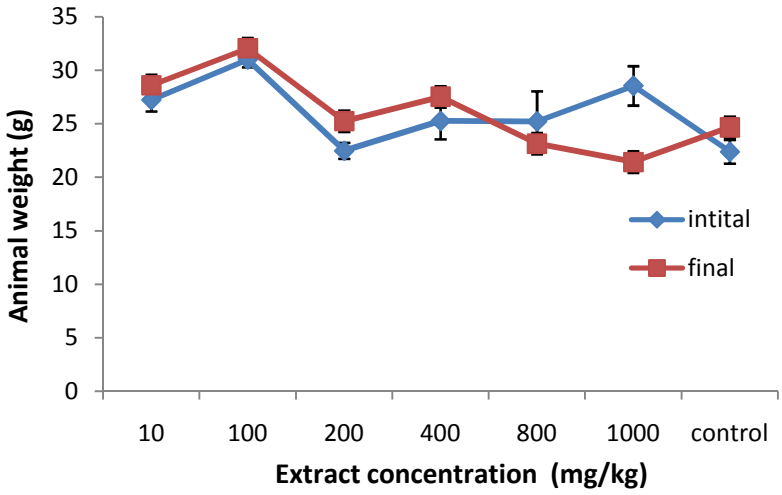
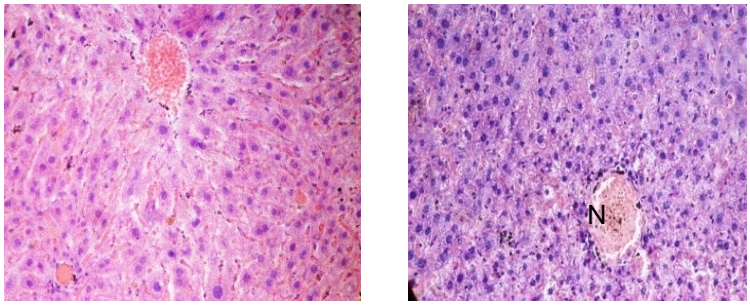


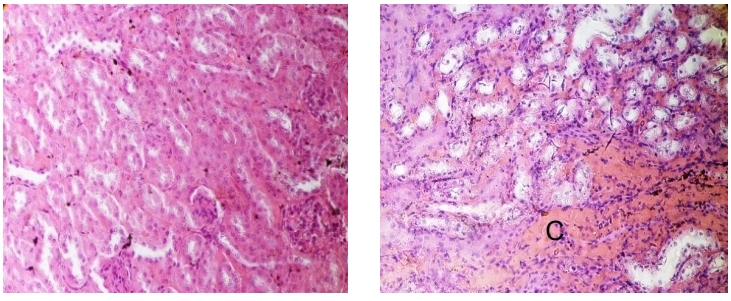
Fig. 1. Effect of acute administration of different doses of chloroform extract of *Artemisia maciverae* on the body weights of Swiss albino mice



Normal control: No lesion

Congestion in sinusoids and focal necrosis (N)

Fig. 2. Histopathology of Liver of Mice Following Intraperitonial Acute Administration of *A. maciverae* (x400)



Normal control: No lesion

Congestion/swollen Mesangium (C)

Fig. 3. Histopathology of Kidney of mice following intraperitoneal acute administration of *A. maciverae* (x400)

Table 2. Body weight and relative organ weights of mice for acute toxicity studies of *A. maciverae*

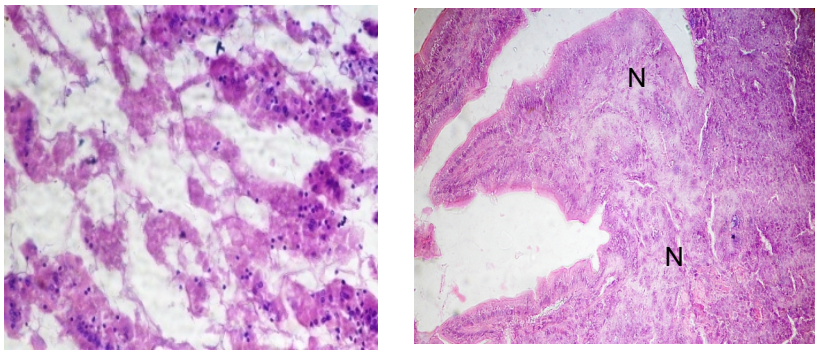
S/N	Dose (mg/kg)	N	Weight of mice (g)		Organ/body weight ratio				
			Initial	Final	Heart	Lungs	Kidney	Liver	Spleen
1	1000	3	31.43±0.59	30.76±0.21	0.005±0.001	0.008±0.004	0.015±0.009[e]	0.064±0.003[h]	0.006±0.001
2	100	3	27.71±1.89	29.63±1.56	0.004±0.001	0.007±0.001	0.010±0.002	0.047±0.020	0.005±0.003
3	10	3	27.75±1.43	30.22±1.57	0.005±0.002	0.007±0.002	0.009±0.001	0.043±0.050	0.005±0.002
4	800	3	29.79	28.97	0.006±0.003	0.008±0.003	0.014±0.005[e]	0.062±0.020[h]	0.007±0.011
5	400	3	27.14±1.82	28.48±1.43	0.005±0.001	0.006±0.002	0.009±0.012	0.041±0.010	0.006±0.001
6	200	3	22.21±2.75	24.31±3.07	0.004±0.002	0.006±0.003	0.010±0.004	0.044±0.030	0.005±0.014
7	TW	3	23.68±0.84	26.05±1.32	0.004±0.001	0.007±0.002	0.009±0.003[f]	0.041±0.020[g]	0.005±0.012

Values with different superscripts vertically differ statistically (P<0.05) from the control. TW= 0.3% Tween 80 (0.2 ml/kg)
N = number of animals in a group. Foot note: Superscripts e,f,h and g represents significant differences between groups

Table 3. Relative organ weights of mice for acute toxicity studies of *A. maciverae*

S/N	Dose (mg/kg)	N	Organ weight/body weight x 100				
			Heart	Lungs	Kidney	Liver	Spleen
1	1000	3	0.5±0.001	0.8±0.004	1.5±0.009[e]	6.4±0.003[h]	0.6±0.001
2	100	3	0.4±0.001	0.7±0.001	1.0±0.002	4.7±0.020	0.5±0.003
3	10	3	0.5±0.002	0.7±0.002	0.9±0.001	4.3±0.050	0.5±0.002
4	800	3	0.6±0.003	0.8±0.003	1.4±0.005[e]	6.2±0.020[h]	0.7±0.011
5	400	3	0.5±0.001	0.6±0.002	0.9±0.012	4.1±0.010	0.6±0.001
6	200	3	0.4±0.002	0.6±0.003	1.0±0.004	4.4±0.030	0.5±0.014
7	TW	3	0.4±0.001	0.7±0.002	0.9±0.003[f]	4.1±0.020[g]	0.5±0.012

Values with different superscripts vertically differ statistically (P<0.05) from the control. TW= 0.3% Tween 80 (0.2 ml/kg)



Normal control: No lesion Epithelial necrosis (N)

Fig. 4. Histopathology of Intestine of Mice Following Intraperitoneal Acute Administration of *A. maciverae* (x400)

5. DISCUSSION

The toxicological evaluation of a plant extract seeks to determine its possible collateral effects, to ensure the safety of use. Some factors are capable of interfering with the toxicity of medicinal plant extract. This toxicity can be intrinsic of the vegetable drug or happens during the process of extract preparation [10]. The toxicity of the drug is related to its compound (s) and could be attributed to the active principle or not.

The acute toxicity of the chloroform extract of *Artemisia maciverae* was established. Acute toxicity study indicated that the chloroform extract of *Artemisia macvierae* is toxic when administered by intraperitoneal route to experimental animals at a dose greater than 566 mg/kg. In a similar study, Atawodi et al. [11] reported the toxic effects of sub-chronic administration of chloroform extract of *Artemisia maciverae* Linn in Swiss albino rats. They reported that the extract at higher doses caused significant elevation in serum urea and creatinine with concurrent tubular epithelial necrosis observed in the kidney. Similar results were also reported by Ibrahim et al. [12] in a study with ethanolic extract of *Vernonia kotschyana* (Asteraceae). In their own study, they reported that the leaf ethanolic extract of *Vernonia kotschyana* have an LD_{50} value of 471.2mg/kg i.p in mice. They showed that the plant extract was modestly toxic to the experimental animal. In the first phase of treatment, they used the doses of 10, 100 and 1000mg/kg, and the result of the phase one treatment determined the doses used in phase 2 treatment. This is in agreement with the methodology and results of this present study.

6. CONCLUSION

A single oral and intraperitoneal dose of 800mg/kg and 1000mg/kg of chloroform extract of *Artemisia maciverae* (whole plants) was able to induce mortality or toxic effects in mice, while lower doses did not induce mortality. This shows that this extract is toxic at higher doses but safe at lower doses.

CONSENT

Not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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