Pathological changes in some organs of rats administered sub-chronic doses of chloroform extract of *Artemisia maciverae* Linn

*Ene A. C*¹, Atawodi S. E², Fatihu M.Y³ and Adamu S.³

1. Department of Biochemistry, Federal University of Technology, Owerri, Nigeria.
2. Department of Biochemistry, Ahmadu Bello University Zaria, Nigeria.
3. Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Ahmadu Bello University, Zaria, Nigeria.

**Corresponding author email:** chineduene@gmail.com

**A B S T R A C T**

The effect of sub-chronic doses of chloroform extract of *Artemisia maciverae* Linn on the histology of the lungs, spleen, stomach and intestine of male Swiss albino rats was studied. The rats were randomly distributed into four groups of 24 animals each. The groups were respectively administered chloroform extract of *Artemisia maciverae* at 0, 50, 100 and 200mg/kg b.wt for 60 days (for those that survived) and then monitored till day 90 before sacrifice. Tissue specimens of the lungs, spleen, stomach and intestine were examined histopathologically after processing and staining with haematoxylin and eosin. At the onset of treatment (week one), the extract caused significant epithelial necrosis in the intestine of rats in the 50, 100 and 200mg/kg treatment groups compared with the control group. Surprisingly, no lesions were observed in the lungs, spleen and stomach of the rats in all the treatment groups and their controls. The intestinal lesions disappeared after week one of treatment. The results indicate that the extract may be toxic at a high dose, long and short term exposure.

**Key words:** *Artemisia maciverae, Chloroform extract, Swiss albino rats, Organs histopathology*

**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. 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. Unfortunately, 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. Thus there is a high degree of concern regarding the safety use of plant extracts. But, with the present upsurge in the use of herbal medicines, a thorough scientific investigation of these plants will go a long way in validating their folkloric usage. The medicinal uses of some plants are well documented in the literature. But even at that, there are few records in the literature of the toxicity profiles of some of these plants. Such acute or sub-chronic data may be required to predict the safety or otherwise of long term low dose exposure to a particular medicinal product.

*Artemisia maciverae* Linn belongs to the family Asteraceae. The whole plant of this medicinal herb is commonly used in the northern part of Nigeria in treating ailments like malaria and fever. It is commonly known in Hausa as Tazargade. This plant has been reported to have anti-malarial effect. It is rich in phytochemicals such as flavonoids, triterpenes, phlobatannins, tannins, anthraquinones, steroids, saponins and alkaloids. From literature, nothing is known of *Artemisia maciverae* toxicity. Therefore, this study is aimed at determining the pathological changes in the lungs, spleen, stomach and intestine of rats when sub-chronic doses of chloroform extract of *Artemisia maciverae* is administered to them.
Materials And Methods

Plant material and extract preparation

The plant Artemisia maciverae 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 whole plants of Artemisia maciverae were air dried at room temperature for two weeks and pounded into powdered form using laboratory mortar. Extraction was carried out by first defating it with petroleum ether before extracting with chloroform using a Soxhlet apparatus. The extract was stored at -4°C until required.

Animals

Adult Swiss albino mice weighing between 20g and 35g were used for the assessment of the acute toxicity. Animals of both sexes were randomly assigned to control and treated groups (3 animals per group). For the subchronic toxicity, adult male Swiss albino rats weighing between 150g and 280g were employed. Animals were randomly assigned to control and treated groups (24 animals per group).

Sub-chronic toxicity evaluation

The method described by Chan et al. 8 and adopted by Adeyemi et al 9 was used for this study. Ninety six male adult Swiss albino rats were randomized into four groups, each containing 24 rats. The rats were allowed to adjust to the laboratory environment for two weeks before the commencement of study. Group 1, which served as the control was administered 0.3% Tween 80 solution, while rats in groups 2, 3, and 4 were administered for 60 days the chloroform extract of Artemisia maciverae at the dose levels of 50mg/kg, 100mg/kg and 200mg/kg body weight (b.wt) respectively. After 60 days of dosing the animals, extract administration was stopped and the surviving animals were monitored till day 90 before they were sacrificed.

Food intake, water consumption and body weight of the animals were measured throughout the duration of the experiments. During the experimental period, all animals were observed daily for clinical signs and symptoms of toxicity. At the end of weeks 1, 2, 4, 8, 10 and 12, six animals were sacrificed from each of the groups. Lungs, spleen, stomach and intestine of both the dead and sacrificed animals were removed and stored in 10 % formal saline for histopatholgical analysis.

Mortality rate was calculated thus:

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\text{Mortality rate} = \frac{\text{Number of deaths}}{\text{Total number of rats in the treatment group}} \times 100 = X\%
\]

Histopathological Studies

Lungs, spleen, stomach and intestine slices were embebbed in paraffin wax, sectioned at 5µ and stained with haematoxylin and eosin 10. Detailed microscopic examination of the above organs’ sections was carried out for both control and test/treatment groups.

Statistical analysis

All the results generated were analyzed using students’ ‘t’ test and analysis of variance (ANOVA).

Results

The acute toxicity evaluation results indicated that the LD_{50} of chloroform extract of Artemisia maciverae is 570mg/kg. When the extract was administered at doses above 570mg/kg (ie at 800mg/kg and 1000mg/kg), animals’ mortality resulting from high acute toxicity of this extract occurred. There was 67% and 100% mortality in the 800 and 1000mg/kg treatment groups respectively after 24 hours of extract administration. There was no mortality when the extract was administered at doses below 570mg/kg (ie at 10, 100, 200 and 400mg/kg).

The sub-chronic toxicity results indicated clinical signs of toxicity in all the treatment groups at the onset (week one) of treatment. The clinical signs of toxicity indicated were dizziness, convulsion, loss of appetite and loss of agility in the treated groups. The signs of toxicity were found to increase as the dose increases. 8.3% mortality was recorded in the 50mg/kg treatment group in week one of treatment, while 25% mortality was recorded in the 100mg/kg treatment group in week one and four of treatment. There was 100% mortality in the 200mg/kg body weight (b. wt) treatment group within week one of treatment after convulsions as a sign of toxicity. No casualty was recorded in the control group. Epithelial necrosis was observed in the intestine of all the rats treated with 50, 100 and 200mg/kg of chloroform extract of Artemisia maciverae at the onset of treatment (Plate 1). The toxic effect of this plant extract was reversed after the onset of the experiment. This intestinal lesions disappeared after the onset of treatment. Surprisingly, no lesions were observed in the lungs, spleen and stomach of the rats in all the treatment groups at the onset or after the onset of treatment (Plates 2 to 4). No lesions were equally observed in the control groups. A drop in water and food consumption was observed in the animals as the dose of the extract administered.
to them increases. There was a significant reduction observed in the body weight of rats treated with 100 and 200 mg/kg b.wt of chloroform extract of A. maciverae when compared with the control and 50 mg/kg b.wt treatment groups (Figure 1).

Discussion

The therapeutic importance of the chloroform extract of Artemisia maciverae has been reported. Even at that, there is no much information regarding the toxicity and safety of the plant extract in spite of its positive anti-parasitic effect. Current study showed that acute administration of the chloroform extract of Artemisia maciverae is toxic when administered by oral and intraperitoneal route to experimental animals at doses greater than 570mg/kg, but not toxic when administered at doses below 570mg/kg. In a similar study, Mohajeri et al. reported that the ethanol extract of Crocus sativus L. (Saffron) stigma was toxic to experimental animals at doses of 350, 700 and 1000mg/kg following intraperitoneal acute administration. They also reported that the extract toxicity increases as the dose of the extract administered to the animals increases. Histopathological examination of organs following sub-chronic toxicity studies indicated epithelial necrosis in the intestine of the experimental animals in all the treatment groups compared to the control. This presence of lesions in the intestine of the experimental animals is a sign of toxicity of the extract at the doses used. This is because it has been reported that epithelial necrosis may occur following exposure to toxins. The epithelial cells are associated with releasing digestive enzymes which aid digestion. The epithelial necrosis observed in the intestine of the animals in the treatment groups might have hampered the release of digestive enzymes in the experimental animals. The inability of the animals to properly digest their food might have been responsible for the reduction in weight observed in the various extract treatment groups. Surprisingly, no lesions were observed in the lungs, spleen and stomach of all the animals in the treatment groups. Al-Sultan and Hussein in a similar toxicity study reported that the ethanol extract of Euphorbia heliscopa caused significant toxicity to the experimental rats used for the study. They reported that lesions were observed in the animals’ intestines and other major organs with the exception of the stomach and the heart.

Conclusions

A single oral and intraperitoneal dose of 800mg/kg and 1000mg/kg b.wt of chloroform extract of Artemisia maciverae (whole plants) was able to induce mortality or toxic effects in mice. Similarly, sub-chronic toxicity test in rats dosed 50mg/kg, 100mg/kg and 200mg/kg b.wt demonstrated that the extract causes epithelial necrosis at the onset of treatment, but that animals, at least rats, recover spontaneously from this effect. It is therefore concluded that the chloroform extract of Artemisia maciverae may be toxic especially at higher doses and on chronic application.

![Figure 1](attachment:image.png)

Figure 1. Effect of different doses of chloroform extract of Artemisia maciverae on the body weights of rats

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Figure 2. Histopathological Appearance in the Intestine of rats administered 50, 100 and 200 mg/kg b. wt. of chloroform extract of Artemisia maciverae for different period of time

Figure 3. Histopathological Appearance in the Lungs of rats administered 50, 100 and 200 mg/kg b. wt. of chloroform extract of Artemisia maciverae for different period of time

Figure 4. Histopathological Appearance in the Spleen of rats administered 50, 100 and 200 mg/kg b. wt. of chloroform extract of Artemisia maciverae for different period of time
Normal Stomach of rat showing no lesion

Figure 5. Histopathological Appearance in the Stomach of rats administered 50, 100 and 200 mg/kg b. wt. of chloroform extract of Artemisia maciverae for different period of time

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