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Effects of chloroform extract of *Artemisia maciverae* Linn on blood cholesterol levels and pathological lesions in the heart of rats

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ABSTRACT: The effect of chloroform extract of *Artemisia maciverae* Linn was studied in male Swiss albino rats. The rats were randomly distributed into four groups of 24 animals each. Group 1, the normal control was administered 0.3% Tween 80 solution. Groups 2 to 4 rats were administered chloroform extract of *Artemisia maciverae* at 50, 100 and 200 mg/kg body weight (b.wt) respectively for 60 days (for those that survived) and monitored until day 90 when they were sacrifice. The blood cholesterol levels of the rats were determined. The hearts were subjected to gross and histopathological examination. At the onset of treatment (week one), the mean (+ SD) serum cholesterol levels in the control, 50, 100 and 200 mg/kg treatment groups were 53.2+2.20, 78.5+6.40, 83.3+2.30 and 92.5+13.80 mg/kg respectively. A statistically significant difference ($p<0.05$) was observed in the cholesterol levels in the treated animals compared to the untreated controls at the onset of treatment. By day 90 (week 12), the cholesterol levels in the groups with the exception of 200mg/kg group were 42.2+3.20, 54.8+1.40 and 61.3+0.00 mg/kg in control, 50 and 100 mg/kg groups respectively. However, no gross and histopathological lesions were observed in the heart of rats in all groups. It is therefore concluded that the plant extract is associated with hypercholesterolemia in high doses.

Key words: *Artemisia maciverae*, Chloroform extract, Cardio-toxic effect, Swiss albino rats

INTRODUCTION

Medicinal plants play a very significant role in health care needs of rural populations in African and other third world countries especially in treatment of diseases[1]. The use of herbal prescriptions and natural remedies for the treatment of various diseases in developing countries compensate for some perceived deficiencies in orthodox pharmacotherapy[2,3]. 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[3]. But now, with the upsurge in the use of herbal medicines, a thorough scientific investigation of these plants will go a long way in validating their folkloric usage[2].

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[4]. It is rich in phytochemicals such as flavonoids, triterpenes, phlobatannins, tannins, anthraquinones, steroids, saponins and alkaloid.

There is a high degree of concern regarding the safety use of plant extracts. The medicinal uses of some plants are well documented in the literature. However, there are few records in the literature of the toxicity profiles of some of these plants[5]. 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[6].

The heart is the primary organ that pumps blood throughout the body. Damage to the heart could arise due to the administration of plant extracts, but there is paucity of scientific information because the incidence of toxicity in local settings are hardly reported nor documented. A study of the toxicity of *Artemisia maciverae* is imperative because of its positive therapeutic effect. Moreover, a study of the effect of the drug extract on the heart is essential because of the cardinal role the organ plays in maintaining life. The determination of the histopathological effect on the heart and some biochemical parameters are relevant in the establishment of safety of this plant extract. Therefore, the present study aims at determining the levels of cholesterol and pathological effects of chloroform extract of *Artemisia maciverae* in Swiss albino rats.

MATERIALS AND METHODS

Plant material and extract preparation

The plant *Artemisia maciverae* was collected in Zaria 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 grounded into powder using laboratory mortar. Extraction of this powdered form was carried out by defating with petroleum ether followed by extraction with chloroform. The extract was stored at -4 ^{o}C until required.

Animals

Adult Swiss albino mice weighing between 20-35g were used for the assessment of the acute toxicity. Animals of both sexes were randomly assigned to 4 groups of 3 rats each. For the cardio-toxicity study, adult male Swiss albino rats weighing between 150g-280g were employed. Animals were randomly assigned to control and treated groups (24 animals per group).

Cardiotoxicity evaluation

The method described by Chan *et al* [7]and adopted by Adeyemi *et al* [8] 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 one week before the commencement of study. Group 1 which served as the normal control was administered 0.3% Tween 80 solution, while rats in groups 2, 3, and 4 were administered the chloroform extract of *Artemisia maciverae* at daily dose of 50 mg/kg, 100 mg/kg and 200 mg/kg b.wt respectively for sixty days. Administration of extract was stopped after 60 days of dosing the animals and the surviving animals were monitored till day 90 before sacrifice.

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. Animals were sacrificed at the end of weeks 1, 2, 4, 8, 10 and 12. At the end of the study, all surviving animals were sacrificed. Blood samples were collected for biochemical analysis. Heart of both the dead and sacrificed animals were removed and fixed in 10 % formal saline ready for histopatholgical analysis.

Histopathological Studies

The heart were embebbed in paraffin wax, sectioned at 5µ and stained with haematoxylin and eosin [9]. Detailed microscopic examination was carried out in the heart of both control and test/treatment groups.

Determination of serum cholesterol

Whole blood samples were collected and allowed to clot. The sera collected were analyzed for cholesterol [10], employing standard kit (Biosystems S. A; Costa Brava 30, Barcelona, Spain), Iso 13485-TUV Rheinland-Reg: SX 60010383 0001).

Statistical analysis

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

RESULTS

Administration of chloroform extract of whole plant of *Artemisia maciverae* produced clinical signs of toxicity like 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 50 mg/kg treatment group in week one of treatment, while 25% mortality was recorded in the 100 mg/kg treatment group in week one and four of treatment.

There was 100% mortality in the 200 mg/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. The LD50 of the extract was found to be 570 mg/kg. A drop in water and food consumption was observed in the animals as the dose of the extract administered to them increases.

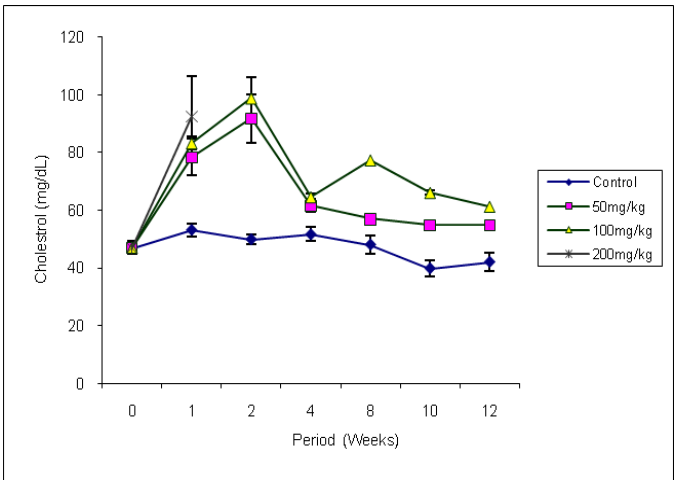

Figure 1. Cholesterol levels in rats treated with chloroform extract of *Artemisia maciverae* for 90 days

Serum cholesterol profile showed an elevation in the levels of cholesterol in the treated groups at the onset of treatment compared to the normal control (Figure 1). This elevation in serum cholesterol is equally presented in table 3. By one week of treatment, the mean serum cholesterol levels in the control, 50, 100 and 200mg/kg treatment groups were 53.2±2.20, 78.5±6.40, 83.3±2.30 and 92.5±13.80mg/kg respectively (Table 1). There was a statistically significant difference ($P< 0.05$) observed when the levels of cholesterol in the treated groups were compared with the normal control. This increase in the cholesterol levels was more prominent in weeks one and two of treatment. The elevation in this biochemical parameter was highest in the animals treated with 200mg/kg body weight of the extract compared with the other treatment groups and the normal control group. The cholesterol levels remained elevated in all the treated groups when compared with the control throughout the duration of this study. At week 12, the cholesterol levels in the above treatment groups were found to be 42.2±3.20, 54.8±1.40 and 61.3±0.00mg/kg respectively (Figure 1).

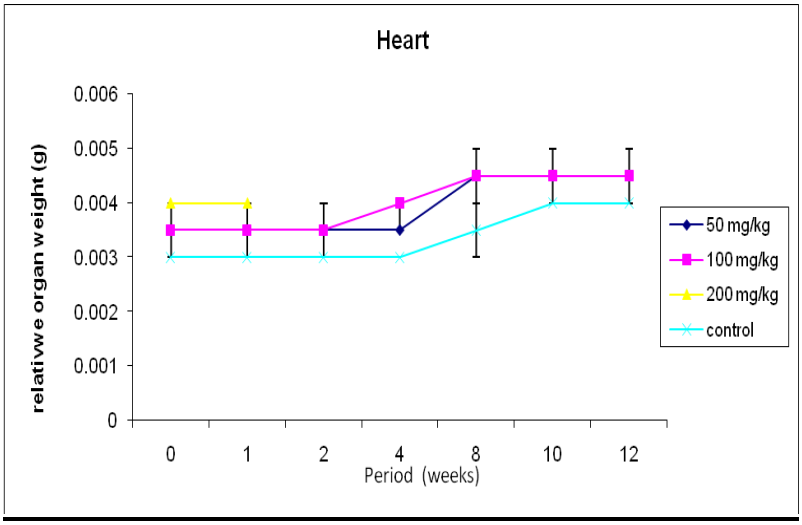

Figure 2. Relative weight of heart of rats receiving chloroform extract of *Artemisia maciverae* at different period of time

For cardio-toxicity studies, macro and microscopic observations indicated no lesions in the hearts of the animals treated with 50mg/kg, 100mg/kg and 200mg/kg of the extract. There were also no lesions observed in the heart of the normal control. Normal heart of rat is shown in plate 1. The relative weight of the heart of the treated animals were not significantly different ($p>0.05$) from that of the controls at the beginning and end of experiment (Figure 2). There was a significant difference ($p<0.05$) 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.

Table 1. Cholesterol (mg/dL) levels of rats treated with chloroform extracts of *Artemisia maciverae* for 90 days

Groups	Period						
	Week 0	Week 1	Week 2	Week 4	Week 8	Week 10	Week 12
Control	47.1±2.20^{c}	53.2±2.20^{c}	50.0±1.80^{c}	51.7±2.40^{c}	48.2±3.20^{c}	49.9±2.80^{c}	42.2±3.20^{c}
50mg/kg	47.1±2.20^{c}	78.5±6.40^{d}	91.8±8.40^{f}	61.6±2.30^{g}	57.0±0.70^{g}	54.8±0.00^{g}	54.8±1.40^{g}
100mg/kg	47.1±2.20^{c}	83.3±2.30^{d}	98.9±7.20^{f}	64.6±1.20^{g}	77.4±0.00^{d}	66.1±0.70^{h}	61.3±0.00^{h}
200mg/kg	47.1±2.20^{c}	92.5±13.80^{d}	-	-	-	-	-

All values were compared with each other at P = 0.05
Number of animals in a group (n) = 6
- = 100% mortality
Values with different superscript vertically and horizontally differ statistically ($P<0.05$)

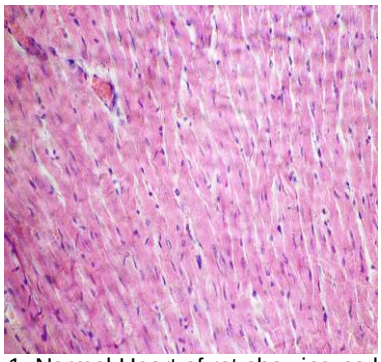

Plate 1. Normal Heart of rat showing no lesion

DISCUSSION

The therapeutic importance of the chloroform extract of the whole plant of *Artemisia maciverae* in folk medicine have been documented[4]. Nevertheless, there is paucity of information regarding the adverse or toxic effect of the plant extract in spite of its use in folk medicine practice. Current study showed that the chloroform extract of this plant could be potentially cardio-toxic when the dose is high and the duration of use extended.

Sub-chronic/cardio-toxicity studies are almost always invaluable in evaluating the safety profile of phytomedicines. This is because acute toxicity data are of limited clinical application since cumulative toxic effects do occur even at very low dose.

Cardio-toxicity/sub-chronic study revealed that the chloroform extract of *Artemisia maciverae* was toxic to the experimental animals during the onset of the experiment particularly weeks one and two. This toxic effect was observed with all the doses of the extract administered to the animals with the highest toxicity observed in the 200 mg/kg treatment group. This can be explained by the fact that all the animals in this group died in week one of treatment. The extract was associated with consistent dose and duration dependent increase in serum cholesterol with significant increase ($p<0.05$) observed in the 50, 100 and 200 mg/kg treatment groups compared to the untreated controls. The toxic effect of this plants extract was reversed towards the end of the experiment when the administration of the extract was stopped.

The elevation observed at the onset of treatment in the level of cholesterol, may be attributed to the toxic effect of the plant extract to the heart. Surprisingly no lesions were observed in the heart throughout the experiment. Cholesterol level is one of the markers indicating the health status of the heart. Therefore, marked increase in serum cholesterol is an indication of hypercholesterolemia[11]. The significant increase ($p<0.05$) in

cholesterol levels at high dose of the extract and longer duration showed that the cardiovascular system could be adversely affected by the extract, but the adverse effect is prominent when high dose of the extract was given for a long period. Increased total cholesterol values are associated with a progressively escalating risk of atherosclerosis and coronary heart disease[10,12]. The serum concentrations of cholesterol are also elevated due to skeletal or cardiac muscle disease among various other diseases arising from administration of various drugs[13].

Similar results were reported by Al-Sultan and Hussein[14]. They reported that in a toxicity study carried out with the ethanol extract of *Euphorbia heliscopia* in Swiss albino rats, there was a significant increase in the levels of serum cholesterol. They stated that there were no lesions observed in the heart of the treated animals during the toxicity study.

CONCLUSIONS

Sub-chronic toxicity/cardio-toxicity test in rats dosed 50 mg/kg, 100 mg/kg and 200 mg/kg b.wt demonstrated that the extract is toxic at the onset of treatment. However, towards the end of this study which lasted for 90 days, the toxic effect of this plants extract was slightly reduced.

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