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Research Paper

Changes in the Hematological Parameters of Rats Administered Sub-chronic Doses of Chloroform Extract of *Artemisia maciverae* Linn

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Abstract: *The effect of sub-chronic doses of chloroform extract of Artemisia maciverae on the hematological parameters of male Swiss albino rats was studied. The rats were randomly distributed into four groups of 24 animals each. The control group was administered the solvent while the other groups were intraperitoneally administered chloroform extract of Artemisia maciverae at 50, 100 and 200mg/kg b.wt, respectively, for 60 days, and then monitored for another 30 days before sacrifice. Results showed no significant changes (p>0.05) in the hematological parameters at doses of 50mg/kg and 100mg/kg treated animal groups, but statistically significant (p<0.05) elevations were observed in the packed cell volume (PCV), white blood cell count (WBC), hemoglobin concentration (Hb) and lymphocyte count of the animals treated with 200mg/kg body weight of the extract after one week of treatment. Neutrophil, monocyte, eosinophil and basophil counts remained statistically unchanged (p>0.05) for all the treatment groups during the experimental period. The elevation observed in the PCV, Hemoglobin, WBC and lymphocyte counts of the rats treated with 200mg/kg of the extract at the onset of treatment suggests some possible breach of the integrity of blood synthesis and regulatory system.*

Keywords: *Artemisia maciverae*, Chloroform extract, Sub-chronic toxicity, Hematological parameters.

Introduction

Artemisia maciverae Linn is a small herbaceous plant belonging to the family Asteraceae. This medicinal plant is found in the northern part of Nigeria and is locally known in Hausa as "Tazargade". Pharmacological studies on this plant have revealed that the chloroform extract possess anti-malarial and anti-trypanosomal activities [6]. The Hausa people of Northern Nigeria use this plant traditionally in treating malaria after boiling in water with lemon, red potash and mixing with local gin.

In spite of the fact that medicinal plants continue to provide solutions to many health problems, a number of them are toxic if not properly prepared or properly dispensed [5]. As such, scientific approach needs to be applied in the use of plant extracts in managing ailments, especially in developing countries where about 80% of the population patronize herbal drugs due to low literacy level and poor health services. Therefore, the safety of *Artemisia maciverae* is important in relation to its therapeutic actions. Since no study on the systemic toxicity of *Artemisia maciverae*, especially with respect to the blood system has been previously reported, we evaluated the safety of chloroform extract of whole plant of *Artemisia maciverae* in Swiss albino rats by determining some hematological parameters following sub-chronic administration.

Material and Methods

Plant Collection and Extract Preparation: The plant *Artemisia maciverae* was collected from Zaria town in northern 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 powder using laboratory mortar. Extraction of this powdered form of the plant was carried out by first defating it with petroleum ether before extracting it exhaustively with chloroform

using Soxhlet apparatus. The extract was stored at - 4 ^{o}C until required.

Animals Groupings and Experimentation: 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 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 the dose levels of 50mg/kg, 100mg/kg and 200mg/kg body weight (b.wt) respectively for sixty days. The extract was administered intraperitoneally. Administration of extract was stopped after 60 days of dosing the animals and the surviving animals were monitored till day 90 before sacrifice. Blood samples were collected for the analysis of hematological parameters. The protocols for the use of animals were approved by the Institutions Animal Ethics Committee (IAEC) of the Nigerian Institute of Medical Research, Yaba Lagos, Nigeria.

Hematological analysis: Two milliliters of blood samples were collected into vacutainer containing 0.1ml of 10% EDTA/saline pH 7.2. These blood samples were used for hematological analysis such as determination of packed cell volume, hemoglobin concentration, total and differential white blood cell counts using standard techniques [8,9,10].

Determination of Packed Cell Volume (PCV): The method as described by Moura, (1982) [9] was used. Recommended anticoagulant used is ethylene-diamino-tetra-acetic acid (EDTA). Capillary haematocrit tubes approximate 7 cm in length, with a bore of about 1 cm was used.

Procedure

(i) The tubes were filled with blood by capillary action (to about 7.5% of its length.
(ii) The outside was dried carefully with a piece of gauze.
(iii) The opposite end of the tube was sealed with special plasticine or with a flame from the bunsen burner.
(iv) The sealed tubes were placed in a special high-speed microhaematocrit centrifuge, with the sealed ends near the outside rim of the centrifuge.
(v) The microhaematocrit centrifuge cover was tightened into place.
(vi) The centrifuge was turned on for 5 min at the speed recommended by the manufacturers.
(vii) At the end, the spun microhaematocrit tube was placed on the microhaematocrit tube reader to determine the packed cell volume in percentage.

Determination of Hemoglobin Concentration (Hb): The method as described by Smith, (1995) [10] was used.

Hemoglobin determination reflects the ability of the erythrocyte to carry oxygen since hemoglobin is the respiratory pigment in the blood sample.

Procedure

(i) Exactly 5 mL of cyanmethaemoglobin reagent was placed into a chemically clean cuvette.
(ii) Exactly 0.02 mL of blood was added into the diluent.
(iii) The tube was covered firmly and inverted two or three times.
(iv) The blood/reagent mixture was allowed to stand for about 10 min for maximum conversion of hemoglobin to cyanmethaemoglobin.
(v) The cuvette was wiped with tissue paper and placed in a spectrophotometer. Reading was taken and the absorbance or the percentage transmission recorded at 540 nm.
(vi) Values obtained were compared with the readings obtained using standard cyanmethaemoglobin solution.

Unit: gm of hemoglobin per deciliter of blood (gm/dL)

Determination of Total White Blood Cell Count (WBC)

The haemocytometer method method as described by Kjeldsberg, (1998) [8] was used.

Procedure:

(i) The blood samples were gently agitated by inversion, after bringing them to room temperature (i.e. if the samples were stored at 4°C).
(ii) A chemically clean white cell micropipette was taken. An aspiration rubber tube with its mouth piece was attached to the end closer to the bulb.
(iii) Blood sample was carefully drawn by aspiration to the 0.5 mark of the pipette.
(iv) Excess blood from the stem of the pipette was wiped off with gauze. The pipette was then placed horizontally at the eye level.
(v) The pipette was plunged into a cuvette containing the diluting fluid which was: (a) 2 ml glacial acetic acid in 100 ml of distilled water (2%) to which 1 ml of a 1% aqueous solution of Gentian violet has been added. (b) N/10 hydrochloric acid made by adding 1 ml concentrated HCl to 100 ml of distilled water was used.
(vi) The diluting fluid was carefully drawn by aspiration into the pipette past the bulb and to the 11 mark.
(vii) The aspiration rubber was fixed between the thumb and the index finger.
(viii) The above was mixed gently and properly by inversion.
(ix) The diluted blood was discharged into a chemically clean haemocytometer counting chamber, allowing one or two minutes for the white blood cells to settle. This was examined under the low objective for cell distribution in the corner ruled areas or squares.
(x) The total number of cells in the sixteen (16) squares was counted within the larger ruled areas in the corner.
(xi) This total of the four corner squares was multiplied by 50. This was the total leukocytes per microlitre (b x 10^{3}/UL).

Determination of Eosinophils, Neutrophils, Lymphocytes, Monocytes, Basophils and Band Neutrophil

The methods of Smith, (1995) [10] and Kjeldsberg, (1998) [8] were used.

Differential Leucocyte count was carried out by the examination of stained blood smear.

Procedure:

(i) Microscopic glass slides with already stained thin blood smear was retrieved and placed on the microscope.

(ii) The examination was started from the thin end of the smear (where particularly erythrocytes are well separated and particularly the leucocytes are thinly spread).

(iii) The oil immersion objective was used for proper identification and detailed study of abnormal cells.

(iv) From the thin end, a systematic meander system of slow, careful and detailed examination of the leucocytes was made. The edges and other areas were examined.

(v) As the individual cell types were identified, they were recorded using a multiple unit tally counter.

(vi) For a differential leucocytes count, a minimum of 100 cells were counted.

(vii) The individual cell types were recorded as per cent (relative count).

(viii) Results were then converted to absolute values, the question in mind being: what were the identifying features of Neutrophil, Lymphocyte, Eosnophil, Monocyte, Basophil and Band cells?

Statistical analysis

All the results generated were analyzed using students 't' test and Analysis of Variance (ANOVA) at 95% confidence level ($p<0.05$).

Results

Clinical Observations: Signs of toxicity such as loss of appetite, loss of agility and dizziness occurred in the treated groups after administration of chloroform extract of whole plant of *Artemisia maciverae*. The severity of signs of toxicity was found to increase as the dose of the extract was increased. Mortality was recorded in the 50, 100 and 200mg/kg -treatment groups in week one of treatment. All the animals in the 200mg/kg body weight (b. wt) treatment group died within week one of treatment with convulsion as a terminal sign of toxicity. Two animals died in the 50mg/kg b. wt treatment group, while six animals died in the 100mg/kg b. wt -treatment groups in the course of this study. No casualty was recorded in the control group. A drop in water and food consumption was observed in the animals as the dose of the extract administered to them was increased.

Hematological Parameters: The hematological parameters of the rats that were examined following treatment with the chloroform extract of *Artemisia maciverae* were as presented in Figures 1 – 3 and tables 1-6. No significant changes were observed in the neutrophil, monocyte, eosinophil, band neutrophil and basophil counts of the 50mg/kg and 100mg/kg treated animal groups when compared with those of the control animals. It can be seen clearly that no significant changes were observed in the aforementioned hematological parameters in all the treatment groups throughout the experimental period. Changes were only observed in the Packed cell volume (PCV), hemoglobin concentration (Hb), white blood cell count (WBC) and lymphocyte count of the animals treated with 200mg/kg body weight of the extract in week one of treatment when compared with the other treatment groups and the control animals (Figures 1 – 3 and Table 2). The changes were very significant ($p<0.05$) with the 200mg/kg treatment groups compared to the control and other treatment groups in week one of treatment (Figures 1 – 3), but afterwards, these hematological parameters normalized.

Table 1
Neutrophil Count (cells/mm^3) of rats receiving chloroform extract of *Artemisia maciverae* for 3 months

Groups	Period						
	Week 0	**Week 1**	**Week 2**	**Week 4**	**Week 8**	**Week 10**	**Week 12**
Control	46.00 ± 1.20^b	48.8 ± 3.50^b	47.7 ± 4.90^b	46.0 ± 0.60^b	46.0 ± 3.20^b	64.7 ± 2.60^c	56.70 ± 3.30^d
50mg/kg	46.00 ± 1.20^b	57.8 ± 3.80^d	44.2 ± 3.10^b	58.7 ± 3.90^d	40.0 ± 1.30^b	66.7 ± 1.70^c	71.7 ± 0.80^e
100mg/kg	46.00 ± 1.20^b	61.5 ± 2.80^c	50.8 ± 3.20^d	60.3 ± 4.20^d	42.0 ± 0.00^b	64.0 ± 0.00^c	56.0 ± 0.00^d
200mg/kg	46.00 ± 1.20^b	49.5 ± 3.90^b	-	-	-	-	-

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 ($P<0.05$)

Table 2
Lymphocyte Count (cells/mm^{3}) of rats receiving chloroform extract of *Artemisia maciverae* for 3 months

Groups	Period						
	Week 0	Week1	Week 2	Week 4	Week 8	Week 10	Week 12
Control	52.50±1.20^{e}	49.8±3.60^{e}	51.0±5.20^{e}	53.0±0.60^{e}	51.0±3.20^{e}	31.3±2.60^{f}	42.70±3.30^{g}
50mg/kg	52.50±1.20^{e}	39.5±3.90^{h}	55.0±3.20^{e}	40.7±3.70^{f}	57.3±0.40^{e}	32.7±1.50^{f}	28.0±0.60^{j}
100mg/kg	52.50±1.20^{e}	35.7±2.00^{h}	47.3±3.30^{e}	38.0±4.10^{g}	57.0±0.00^{e}	32.0±0.00^{f}	41.0±0.00^{g}
200mg/kg	52.50±1.20^{e}	59.7±4.00^{e}	-	-	-	-	-

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)

Table 3
Monocyte Count (cells/mm^{3}) of rats receiving chloroform extract of *Artemisia maciverae* for 3 months

Groups	Period						
	Week 0	Week 1	Week 2	Week 4	Week 8	Week 10	Week 12
Control	1.0±0.00^{a}	1.3±0.20^{a}	1.0±0.00^{a}	1.0±0.00^{a}	1.7±0.20^{a}	2.3±0.20^{c}	1. 0±0.00^{a}
50mg/kg	1.0±0.00^{a}	1.7±0.20^{a}	1.0±0.00^{a}	0.0±0.00^{b}	3.0±0.00^{c}	1.0±0.00^{a}	1.0±0.00^{a}
100mg/kg	1.0±0.00^{a}	1.6±0.40^{a}	1.0±0.00^{a}	1.0±0.00^{a}	1.0±0.00^{a}	0.0±0.00^{b}	1.0±0.00^{a}
200mg/kg	1.0±0.00^{a}	1.0±0.00^{a}	-	-	-	-	-

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)

Table 4
Eosinophil Count (cells/mm^{3}) of rats receiving chloroform extract of *Artemisia maciverae* for 3 months

Groups	Period						
	Week 0	Week 1	Week 2	Week 4	Week 8	Week 10	Week 12
Control	0.7±0.20^{b}	0.5±0.30^{b}	0.5±0.30^{b}	0.3±0.20^{b}	1.3±0.20^{c}	1.0±0.00^{c}	0.3±0.20^{b}
50mg/kg	0.7±0.20^{b}	1.0±0.40^{b}	0.2±0.20^{b}	0.7±0.20^{b}	0.7±0.20^{b}	0.0±0.00^{d}	0.0±0.00^{d}
100mg/kg	0.7±0.20^{b}	1.2±0.50^{c}	1.3±0.30^{c}	1.0±0.40^{c}	0.0±0.00^{d}	2.0±0.00^{c}	2.0±0.00^{c}
200mg/kg	0.7±0.20^{b}	0.3±0.20^{b}	-	-	-	-	-

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)

Table 5
Band Neutrophil Count (cells/mm^{3}) of rats receiving chloroform extract of *Artemisia maciverae* for 3 months

Groups	Period						
	Week 0	Week 1	Week 2	Week 4	Week 8	Week 10	Week 12
Control	0.0±0.00^{e}	0.0±0.00^{e}	0.2±0.10^{f}	0.0±0.00^{e}	0.0±0.00^{e}	0.3±0.20^{f}	0.0±0.00^{e}
50mg/kg	0.0±0.00^{e}	0.0±0.00^{e}	0.3±0.20^{f}	0.0±0.00^{e}	0.0±0.00^{e}	0.0±0.00^{e}	0.0±0.00^{e}
100mg/kg	0.0±0.00^{e}	0.0±0.00^{e}	0.3±0.20^{f}	0.5±0.20^{f}	0.0±0.00^{e}	0.0±0.00^{e}	0.0±0.00^{e}
200mg/kg	0.0±0.00^{e}	0.3±0.30^{f}	-	-	-	-	-

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)

Table 6
Basophil Count (cells/mm^3) of rats receiving chloroform extract of *Artemisia maciverae* for 3 months

Groups	Period						
	Week 0	**Week 1**	**Week 2**	**Week 4**	**Week 8**	**Week 10**	**Week 12**
Control	0.0 ± 0.00^g	0.2 ± 0.20^h	0.0 ± 0.00^g	0.0 ± 0.00^g	0.0 ± 0.00^g	0.0 ± 0.00^g	0.0 ± 0.00^g
50mg/kg	0.0 ± 0.00^g	1.0 ± 0.40^l	0.0 ± 0.00^g	0.0 ± 0.00^g	0.0 ± 0.00^g	0.0 ± 0.00^g	0.0 ± 0.00^g
100mg/kg	0.0 ± 0.00^g	0.5 ± 0.20^h	0.0 ± 0.00^g	0.0 ± 0.00^g	0.0 ± 0.00^g	0.0 ± 0.00^g	0.0 ± 0.00^g
200mg/kg	0.0 ± 0.00^g	0.0 ± 0.00^g	-	-	-	-	-

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)

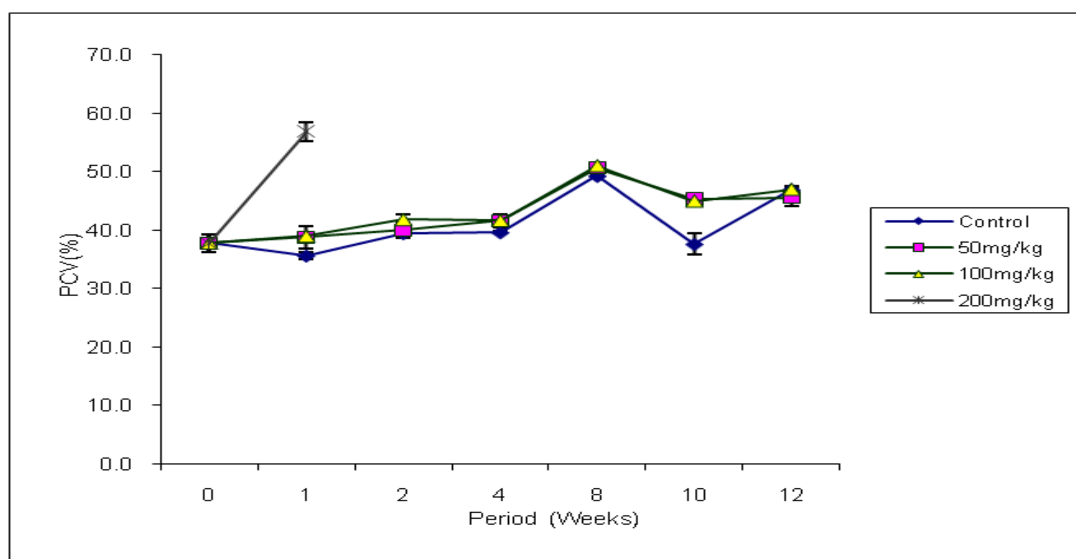

Figure 1: Packed cell volume (PCV) levels of rats receiving chloroform extracts of *Artemisia maciverae* for 3 months

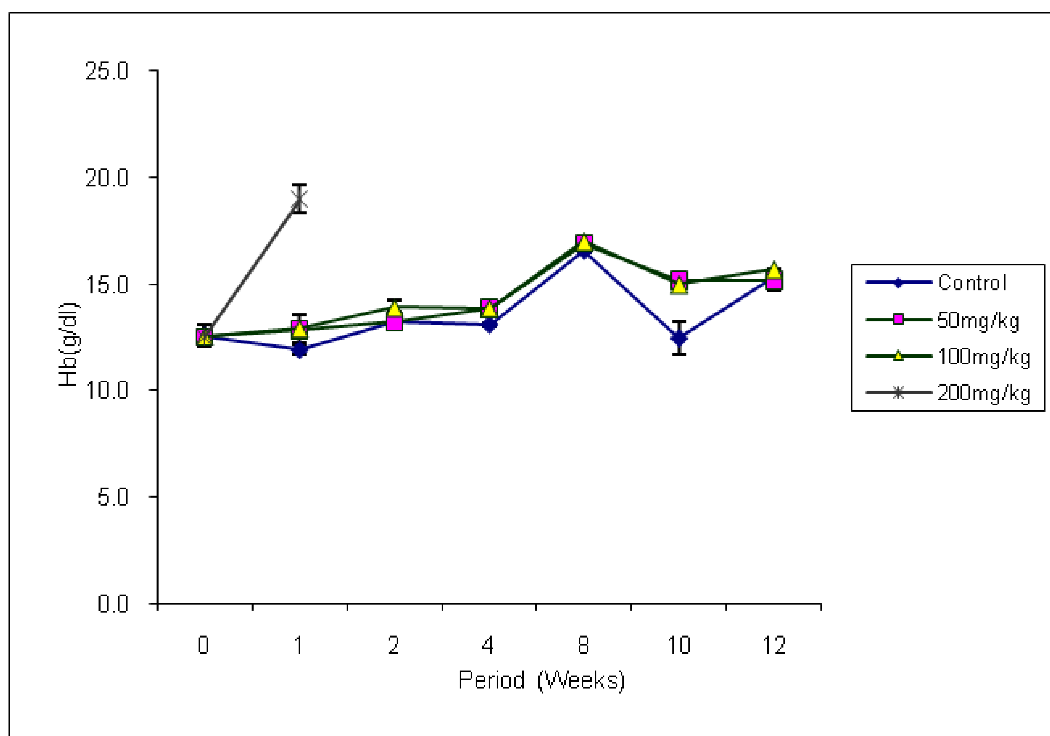

Figure 2: Hemoglobin concentration (Hb) of rats receiving chloroform extracts of *Artemisia maciverae* for 3 months

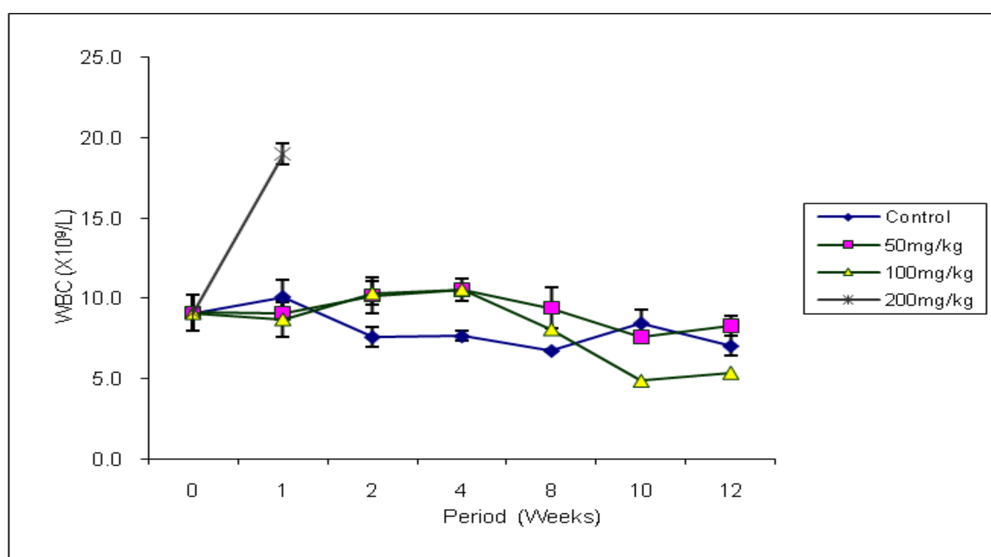

Figure 3: White blood cell count (WBC) of rats receiving chloroform extracts of *Artemisia maciverae* for 3 months

The PCV levels at the onset (ie week 1) of extract administration in the control, 50, 100 and 200mg/kg treatment groups were 35.7+0.60, 38.8+1.30, 39.0+0.60 and 56.8+1.60 (%) respectively, while that of hemoglobin (Hb) were found to be 11.9+0.20, 12.9+0.70, 12.9+0.20 and 19.0+0.60 (g/dL) respectively. Also in the above treatment groups during week one of treatment, the WBC counts were 10.1+1.10, 9.1+0.70, 8.7+1.10 and 19.0+0.60 (X 109/L) respectively and lymphocyte counts were 49.8+3.60, 39.5+3.90, 35.7+2.00 and 59.74+4.00 (cells/mm^3) respectively. These changes were statistically significant ($p<0.05$) when compared with the normal control. After the onset of treatment, no more changes were observed in the above hematological parameters (Figures 1-3). At day 90 (ie week 12), the PCV levels in the control, 50 and 100mg/kg treatment groups were found to be 47.0+0.60, 45.7+1.50 and 47.0+0.00 (%) respectively, while that of hemoglobin (Hb) were found to be 15.3+0.20, 15.2+0.50 and 15.7+0.00 (g/dL) respectively. At day 90, the WBC counts were found to follow the same trend in the above treatment groups. The values were 7.10+0.60, 8.3+0.60 and 5.4+0.00 (X 10^9/L) respectively and lymphocyte counts were 42.7+3.30, 28.0+0.60 and 41.0+0.00 (cells/mm^3) respectively.

Discussion

Sub-chronic toxicity evaluation revealed that the chloroform extract of *Artemisia maciverae* was toxic to the experimental animals during the onset of the experiment particularly week one. The clinical signs of toxicity observed were dizziness, loss of appetite, loss of agility and convulsion. This toxic effect was observed with all the doses of the extract administered to the animals. The toxic effect of this plant extract was reversed after the onset of the experiment.

Hematological parameters of treated rats were not markedly changed in the 50mg/kg and 100mg/kg treatment groups from the beginning and the end of this experiment, showing that this extract is not toxic at these doses (Figures 1-3, Tables 1-5). The changes observed in the PCV, Hb, WBC and lymphocyte counts of the animals treated with 200mg/kg of the extract, one week following treatment (Figure 1 -3) shows that this extract may be more toxic at a higher dose. In a similar study, Chunlaratthanaphorn *et al*, (2007) [3]reported that the administration of1,200mg/kg/day of water extract from the root of *Imperata cylindrical* Linn led to a significant increase in mean hemoglobin concentration of male rats when compared to that of the control group but did not produce marked changes in the rats' parameters like PCV, RBC, hemoglobin concentration etc. when administered at lower doses of 300 and 600mg/kg/day. In this study, the increases in PCV, Hb and WBC observed (Figure 1 – 3) were most probably the result of dehydration, as there was a decrease in water intake (Figure 4) and loss of appetite (Figure 5) during the course of this experiment. The reduced water intake especially could result in dehydration with subsequent haemoconcentration and hence the higher values of the PCV, Hb and WBC observed in this study [4]. In addition, the extract might have caused some damage within the gastrointestinal tract and peritoneum of the rats administered 200mg/kg of the extract. Such damage could result in loss of body fluid due to the compromised membrane integrity consequent to the toxic effect of the extract [1,2,7].

Conclusion

Sub-chronic administration of *A. maciverae* at doses of 50mg/kg, 100mg/kg and 200mg/kg b.wt caused changes on hematological parameters such as PCV, Hb and WBC at the onset of treatment particularly at a higher dose of 200mg/kg, but this effect may be corrected spontaneously or through withdrawal of treatment. The changes in the above hematological parameters were more prominent at higher doses of the extract compared to the lower dose of 50 mg/kg. This signifies that the extract is safer when administered at a lower dose.

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