

Effect of ethanolic extract of *Anacardium occidentale* leaves on haematological and biochemical parameters of albino rats

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Medicinal plants are used in the treatment and management of most diseases worldwide. One of such diseases is diabetes. It is a metabolic disorder characterized by lack of insulin or inadequate supply of insulin. It affects one quarter of the world's population as reported by World Health Organization. *Anacardium occidentale* parts are used in the management of diabetes hence the need to study its effect on the biochemical and haematological parameters. The ethanolic extract of *A. occidentale* leaves at 2000, 4000 and 6000mg/kg B.W were administered on albino rats for a period of 42 days (i.e. 6 weeks) and haematological and biochemical parameters investigated. The packed cell volume (PCV) and red blood cell values fluctuated during the study period. However, there was no significant difference compared to the control. There was initial increase in white blood cell values for all the groups but fluctuated for the remaining period of study. The liver enzymes (ALT and AST) and protein levels significantly decreased. However, the creatinine and urea levels increased during the study period. The results indicate that *A. occidentale* leaves at doses examined had significant ($P<0.05$) effect on the parameters investigated hence further investigation on its use as a medicinal plant may be necessary.

Keywords: Haematological, Alanine transaminase, Aspartate transaminase.

INTRODUCTION

Despite several efforts made in the treatment and management of diseases by synthetic drugs, the search for safe and improved natural agents is on-going. The plant kingdom offers a wide area for the oral hypoglycemic drugs. About 400 plant species have been reported to display the effects, but only few of them were investigated and reported (Miura, *et al.*, 2002). World Health Organization has recommended the need for research into this area (WHO, 2002). As a result of toxic effects that may be envisaged on using plants, liver disease remains one of the serious health problems that may arise.

A number of plants were known to possess hepatoprotective properties (Barar, 2000). *Anacardium occidentale* has been in use as a folk remedy for some diseases e.g. diabetes mellitus (Longuefosse, *et al.*, 1996; Kamtchouing *et al.*, 1998). Thus, at lower doses, *A.occidentale* may possess hepatoprotective properties.

This work focused on the effect of ethanolic extract of *A. occidentale* on haematological and biochemical parameters of albino rats.

MATERIALS AND METHODS

Materials

The leaves of *A. occidentale* were obtained from Minna and suburbs and identified in the Department of Biological Sciences, Federal University of Technology, Minna. While albino rats weighing 80g to 200g were purchased and used for the study. Blood samples were collected through the tail tips of the rats for analysis.

Chemicals and Reagents

Chemicals used were of analytical grade and obtained from reputable companies such as: May and Baker Ltd, Sigma, BDH chemicals Ltd. The chemicals were: 97%

Ethanol, Assay kits for in vitro determination of ALT (SGPT), AST (SGOT) in serum, and kits for total protein.

Special Equipment

The instruments used in this work are viz

Hematocrit centrifuge (SH120-F), Spectrophotometer (20D+), Rotary evaporator, Hematocytometer (YSG-750-T), Microscope.

Methods

Extraction

1.4g of the leaves were dried and ground into powdered form. The dried material was refluxed in 97% ethanol for another 2 hrs and filtered. The filtrate was concentrated using rotary evaporator at 78°C and the resulting concentrate was air dried.

Sub-Chronic Toxicity Studies

4 groups of 3 rats each were used for the sub-chronic studies. The first group serve as control and to the three other groups, doses of 2000, 4000 and 6000mg/kg B.W. were administered orally respectively. The administration was carried out for 42 consecutive days. These animals were allowed free access to food and water. The body weight and haematological parameters were monitored weekly for any possible toxic effect. At the end of six weeks (42 days), the animals were sacrificed and the blood samples were collected. The serum was subjected to biochemical analysis. Total protein, aspartate amino transferase (Clin. Chem. Acta 105; 1980), alanine amino transferases (IFCC, 1976) were determined using specific enzyme kits. Blood samples were collected into EDTA-tubes for biochemical analysis.

Haematological Parameters

Packed Cell Volume (PCV)

Blood samples were collected by tail clip method into heparinised capillary tubes until it is two third filled with blood. Two hematocrit tubes were placed in the radial grooves of the centrifuge head exactly opposite each other with the sealed end away from the centre of the centrifuge. The centrifuge was covered and spurned for

10mins. The PCV was determined using microhematocrit reader.

Red Blood Cell Count (RBC)

4ml of RBCs diluting fluid was disposed into a tube and mixed with 20µl of blood sample using a micropipette. The counting of cells was carried out using Hematocytometer.

White Blood Cell Count (WBC)

0.4ml of WBCs diluting fluid was dispensed into small tube. 20µl of blood sample was measured and mixed with the diluent. Mixture was allowed to stand for 30 mins. Some diluted blood was drawn using capillary tube and used in charging the chamber. The chamber is allowed to stay for 20 mins and all the white cells are counted.

Biochemical Parameters

ALT – Alanine Transaminase

1.0ml of the reagent was pipetted into the tube marked standard and 1.0ml of distilled water was pipette into blank tube. 1ml of the working reagent was pipette and mixed with 1.0ml of the sample in the sample tube. They were mixed and incubated at 37°C for 1min. Absorbances were taken at 340nm.

AST – Aspartate Transaminase

1.0ml of the mixed reagents was pipette into marked standard tube and 1.0ml of reagent pipette into blank. 1.0ml or working reagent was pipette and mixed with 1.0ml of the sample. The tubes were incubated at 37°C and absorbances read at 340nm.

Creatinine Determination

0.5ml of serum was pipetted into the labeled test-tube and 1.5ml of distil-deionised water was added to all the tubes followed by 0.5ml of sodium tungstate and sulphuric acid and mixed. A milky precipitate was formed which was then centrifuged for 2mins. After centrifugation, 1.5ml of the supernatant was pipette into the tube. 15ml of standard was pipetted into the tube labeled standard and blank. 0.5ml of NaOH and 0.5ml of picnic acid was pipetted into each of the tubes. They

Table 1. Biochemical parameters of rats administered Ethanolic Extract of *A. occidentale* leaves.

Parameters	Control		Group I		Group II		Group III	
	0	42	0	42	0	42	0	42
ALT (U/L)	49.94±0.2	49.73±0.3	50.12±0.5	48.27±0.2*	49.93±0.8	46.39±0.2**	50.49±0.4	41.88±0.9+
AST (U/L)	52.8±0.4	53.4±0.1	53.0±1.2	51.7±0.5*	52.5±2.0	48.2±0.6**	52.6±1.6	44.5±0.6+
Total protein (g/dl)	6.76±0.01	6.74±0.04	6.84±0.04	6.29±0.04	6.79±0.207	6.36±0.05	6.82±0.05	6.39±0.09
Creatinine (mmol/l)	10.3±0.1	14.5±0.2	15.3±0.21	17.2±0.12*	15.1±0.31	16.8±0.118**	18.5±0.21	19.1±0.22+
Urea (mmol/l)	32.1±0.21	35.3±0.11	30.4±0.2	34.3±0.41*	36.2±0.21	37.2±0.2**	39.1±0.22	40.1±0.11+

The Values are expressed as mean ± SEM

Group I (rats fed 2000mg) Vs Control *P<0.05

Group II (rats fed 4000mg) Vs Control **P<0.05

Group III (rats fed 6000mg) Vs Control +P<0.05

were mixed and allowed to stand at room temp for 20mins. Absorbances were read at 520nm.

Urea Determination

2.0ml of distilled deionised water was pipette into three labeled tubes, 0.02ml of serum and standard were pipetted into the tubes respectively. 1.0ml of working reagent and mixed acid reagent were added to each of the tubes and mixed. The tubes were placed in water bath for 20mins at 100°C. A purple colour was produced indicating the presence of urea. Absorbances were read at 340nm.

Total Protein Determination

1.0ml of the reagent was pipetted into a tube and 0.02ml of the sample was added. A standard reagent was prepared by adding 1.0ml of reagent to 0.02ml of the standard. 1.0ml of the reagent was added to 0.02ml of the serum. All were mixed and incubated for 5mins at 37°C. Absorbance was read at 520nm against blank.

Statistical Analysis

The results are reported as mean ± SEM. Statistical analysis was carried out using analysis of variance (ANOVA).

RESULTS

Figures 1, 2 and 3 shows the effect of ethanol extract of *A. occidentale* leaves on PCV, RBC and WBC parameters while Table 1 shows the effect of ethanolic extract of *A. occidentale* leaves on biochemical parameters.

DISCUSSION

Toxicity studies are usually undertaken to define the toxicity and effect of extract, access the susceptible species, identify target organs, provide data for risk assessment in case of acute exposure to the chemical or drug, provide information for the design and selection of dose levels for prolonged studies (Wallace, 2001). The toxicity of plants are mostly dependent on the plant organs which may be as a result of some factors such as the storage form of the organ and seasonal variation considering the phytochemicals (Jaouad *et al.*, 2004). The parameters often used for the toxicity studies are the haematological and biochemical parameters hence the use of such parameters in this study. The Packed Cell Volume (PCV) of the experimental rats (Figure 1) fluctuate which may be an indication that the fluid intake by the rats as a result of extract administration was irregular and there was no complete dehydration that will lead to constant increase in the PCV values. The same trend was observed for the red blood cells count (figure 2) and white blood cells count (figure 3). These findings are in agreement with those reported by Tedong, *et al.*, (2007). It was also reported by Shermer (1967) that animals, showed appreciable fluctuations in their hematological parameters as a result of changes in nutrition and/or the environment. Although, leaves of *A. occidentale* are known to be rich in tannins (about 23%), they are a group of polyphenols. They sometimes cause hemolysis of the red blood cells and interfere with the iron absorption thereby influencing red blood cell production but are seen to be insignificant on treating the rats with the extract.

The liver enzymes (ALT and AST) significantly decreased (Table 1). The decreased levels of the enzymes suggest impaired protein synthesis process in the liver which is in agreement with the findings of other researchers (Chaudan *et al.*, 1991; Kamis *et al.*, 2003; Tedong *et al.*, 2007; www.essrtment.com). Since there

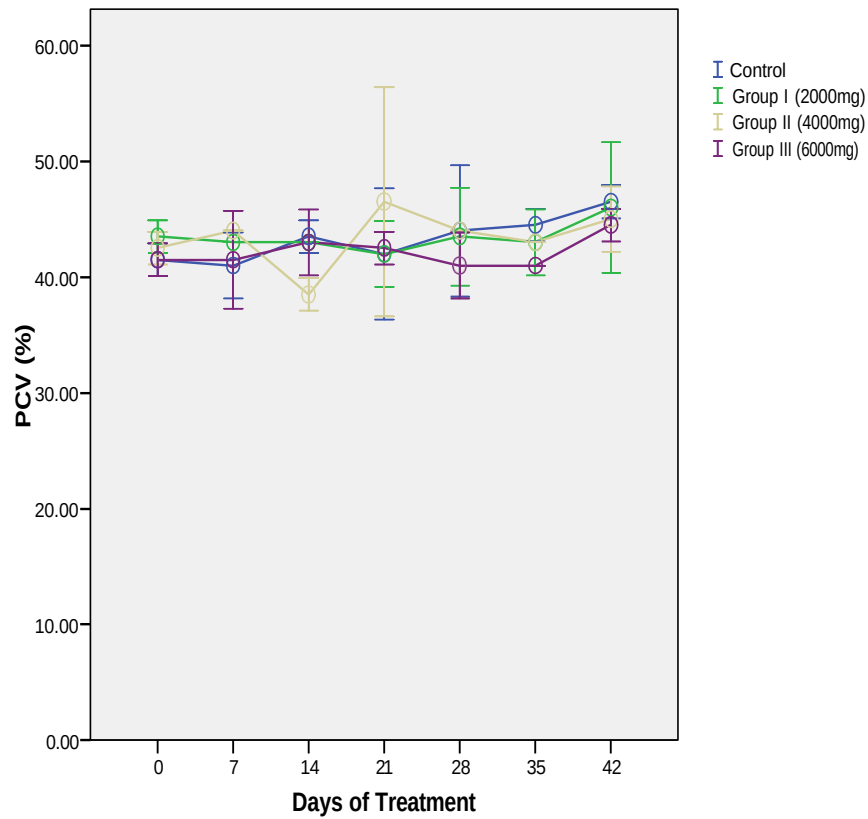


Figure 1. Mean percentage PCV values of rats fed with ethanolic extract of *A. occidentale* leaf

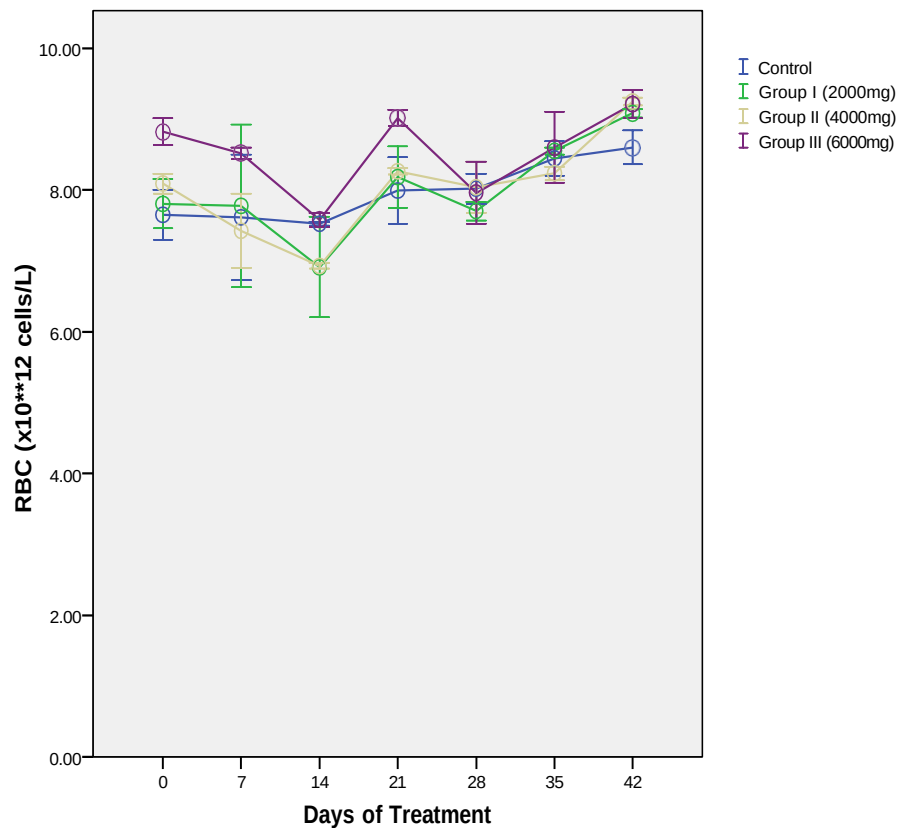


Figure 2. Effect of ethanolic extract of *A. occidentale* leaf on RBC of rats.

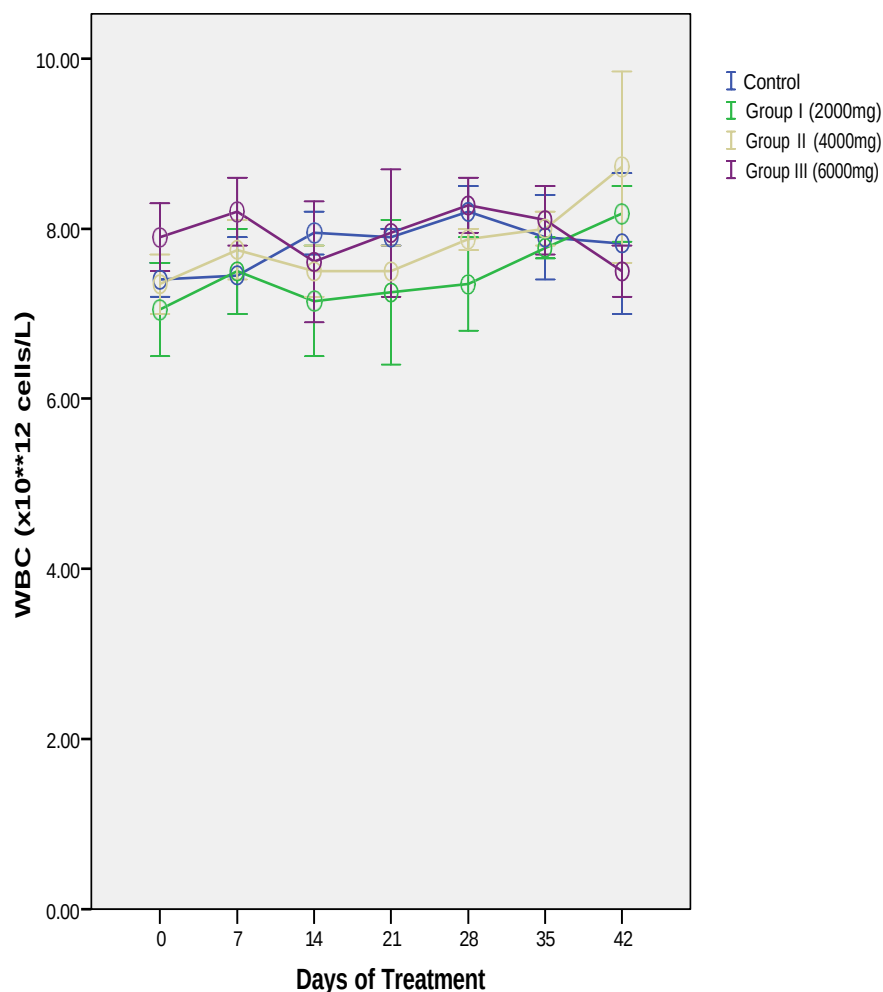


Figure 3. Effect of ethanolic extrac of *A. occidentale* leaf on WBCs of rats

was decrease in the levels of liver enzymes at varying doses, it's reasonable to deduce that ethanolic extract of *Anacardium occidentale* may induce liver damage (Kamtchoung *et al.*, 1998). Elevations in ALT levels are rarely observed except in chronic liver disease (Katchmar *et al.*, 1973; Gad, 2001; Haschek and Rauseax, 1991), while elevations in AST levels are usually associated with skeletal and heart muscles. The prognosis most times cannot be conclusive unless after parameters and histological evidences are considered (Loeb and Qainby, 1989).

The significant increase observed for creatinine and urea may induce kidney damage in rats (Zaki and Dafallah, 2004). However, the significant decrease observed in the protein levels is in agreement with the decrease in enzyme levels because all enzymes are protein. This is in agreement with the findings of Tedong, *et al.*, (2006) in which the rats were fed hexane extract of *A. occidentale* leaf and a decrease in protein levels was observed.

In conclusion, the ethanolic extract of *A. occidentale* leaves at doses below 2000mg/kg B.W could be used as a medicinal plant in the treatment of diseases.

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