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## Effects of Aqueous Leaf Extract of *Acalypha wilkesiana* on some Serum Biochemical Profiles of Mice in Sub-Acute Toxicity Studies

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### ABSTRACT

*Acalypha wilkesiana* has been used for various medicinal purposes such as in the treatment of hypertension, fungal and bacterial infections. In this study, the effects of prolonged oral administration of the aqueous leaf extract on some biochemical profiles of mice were evaluated. A total of 80 mice were allocated to 4 groups (A, B, C and D) comprising of 20 mice each. Mice in group A (control) were given (1 mL) of distilled water orally while 1 mL of the extract was given to the mice in groups B, C and D daily at the concentrations of 800, 1000 and 1200 mg/kg, respectively. This was continued for three weeks and at the end of each week, 4 mice from each group were humanely sacrificed and sera from blood samples collected were used for determination of activities of biochemical parameters. The results showed that administration of the extract resulted in significantly ( $p < 0.05$ ) lower concentrations of total protein and cholesterol in the treatment groups at the 3rd week of treatment when compared to control values. Glucose level was significantly ( $p > 0.05$ ) higher in the extract-treated groups by the 3rd week of treatment, while activities of AST, ALT and ALP were not significantly ( $p > 0.05$ ) altered by the extract. There were no significant ( $p > 0.05$ ) differences between the levels of calcium, total and conjugated bilirubin in treatment and control groups, while sodium concentration was significantly ( $p < 0.05$ ) higher at day 14 after withdrawal of treatment, compared to the control. This study suggests that the extract of *Acalypha wilkesiana* has a hyperglycaemic effect.

**Keywords:** *Acalypha wilkesiana*, Aqueous Leaf Extract; Serum Biochemistry; Liver

### INTRODUCTION

*Acalypha wilkesiana* Muell-Arg (copper leaf, fire dragon plant, beef steak plant, Jacob's coat, match-me-if-you-can and locally called Eela or jiwene jinwinini), is a plant from the family *Euphorbiaceae*. The genus *Acalypha* comprises of about 570 species (Riley, 1963), a large proportion of which are weeds while the others are ornamental plants. The plants are found all over the world especially in tropical Africa, America and Asia (Ogundaini, 2005).

Some of the species of *Acalypha* are well known in traditional medicine and a few have actually appeared in Africa and in the Mascarene Islands (Seebaluck et al., 2015). *Acalypha wilkesiana* was reported to be used in the treatment of hypertension (Ikewuchi et al., 2008). The plant was found to have antibacterial and antifungal properties (Akinde, 1986; Alade and Irobi 1993; Adesina et al., 2000; Ogundaini, 2005), and was earlier reported to be used in treatment of gastrointestinal disorders and fungal infections such as *Pityriasis versicolor*, *Impetigo contagiosa*, *Taenia versicolor* and other similar skin infections which affect the back, chest and the axillae of many infants in Nigeria (Oliver, 1959). Due to the wide usage of *Acalypha wilkesiana*, this study was conducted to

evaluate the effect of prolonged oral administration of its aqueous leaf extract on serum biochemical profiles in mice experimental model.

### MATERIALS AND METHODS

#### Collection, Identification and Processing of Plant

Fresh leaves and stem of *Acalypha wilkesiana* were collected in the month of June from Maiduguri, Borno State, Nigeria. The plant was authenticated to be *Acalypha wilkesiana* from the Department of Forestry, at the University of Agriculture, Makurdi, Benue State. The leaves were dried under shade, crushed and made into powder using a mortar and pestle in the laboratory and then stored in a plastic container.

#### Aqueous Extract Preparation

Two hundred gram (200 g) of the dried powdered leaf material was placed into a round bottom flask and 1 liter of distilled water was added after which the mixture was placed on a hot plate and allowed to boil for 1 hr. It was then allowed to cool and then filtered using Whatman filter paper No. 1. The filtrate was then evaporated to dryness using hot air oven at 50 °C and, thereafter, stored at 4 °C until used. The extract

was made into a concentration of 100 mg/ml and the yield was 22.85 %<sup>w/w</sup>.

### Experimental Animals

A total of 100 adult mice, weighing 15-25 g, procured from National Veterinary Research Institute Vom were used for the experiments. They were kept in plastic cages in the Veterinary Physiology, Pharmacology and Biochemistry Laboratory of the University of Maiduguri; they were given two weeks to acclimatize, before the commencement of the experiments. They were fed with growers' mash (Vital feeds® Nig. Ltd. Jos) and clean water was given *ad libitum*.

### Ethical Statements

Ethical procedure according to Council for International Organisations of Medical Sciences and the International Council for Laboratory Animal Science (2012), were used.

### Sub-acute Toxicity Studies

A total of 80 mice were used for the study. The experimental animals were caged into 4 groups (A, B, C and D), of 20 mice each. Group A served as the control and were administered 1 mL of distilled water only throughout the period of the study. Group B, C and D mice were orally administered, daily, 1 mL of the leaf extract at doses of 800, 1000 and 1200 mg/kg, respectively, for three weeks. At the end of each week, 4 mice were humanely sacrificed. Blood samples were collected from the jugular vein of the sacrificed mice and serum was obtained by centrifugation of the clotted blood and used for the biochemical assays.

### Determination of some Biochemical Parameters

The sera obtained was used for analysis of activities of alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, and concentrations of total protein, total cholesterol, total bilirubin, conjugated bilirubin, glucose, calcium and sodium.

### Determination of Serum Enzyme Activities

Alanine aminotransferase (ALT), aspartate amino transferase (AST) and alkaline phosphatase activities were assayed using commercial test kits (Randox Lab Ltd, Ardmore, U.K.) as described (Babson *et al.*, 1966; Gueroui and Kechrid, 2016).

### Determination of Serum Bilirubin

Serum total and conjugated bilirubin concentrations were determined using colorimetric method (Jendrassik and Grof; 1938; Gueroui and Kechrid, 2016) with commercial test kit (Randox, Lab Ltd. Armore, U.K.).

### Determination of Serum Total Protein

The serum total protein was determined spectrometrically (Peter *et al.*, 1982).

### Determination of Cholesterol

The serum cholesterol was determined as described by Allain *et al.* (1974) using the commercial test kit (Randox Lab Ltd, Ardmore, U.K.).

### Determination of Blood Glucose

Immediately on collection, a drop of the fresh whole blood was placed on the sample-spot of glucose test strips. The stripe was placed in one-touch 11 glucose meter (Astoor and Kings, 1954) and results obtained were recorded.

### Statistical Analyses

All values were expressed as Mean  $\pm$  Standard (S.D). Analysis of variance (ANOVA) was used to analyse the extent of variation among groups and p value equal to or less than 0.05 ( $p \leq 0.05$ ) were considered significant, the computer statistical software GraphPad InStat® (2003) was used to analyse the data.

## RESULTS

### Effects of the Leaf Extract Administration on Biochemical Profiles

The effect of sub-acute oral administration of the aqueous extract of *A. wilkesiana* leaves on blood glucose and serum concentrations of total bilirubin, conjugated bilirubin, protein and cholesterol on treated mice is shown in Table 1. The glucose values of the treated mice indicated a significant ( $p < 0.05$ ) increase only at the third week of treatment in group (B and C) and first week of withdrawal with no significant changes in the first two weeks of treatment and second week of withdrawal when compared to the control.

The aqueous leaf extract administration induced no significant ( $p > 0.05$ ) changes in total bilirubin and conjugated bilirubin values (group B, C and D) when compared to control. The total protein values of the leaf extract-treated groups C and D significantly ( $p < 0.05$ ) decreased at first and second week of treatment for the respective doses of 1000 mg/kg and 1200 mg/kg, while a significant increase in this parameter occurred in group B leaf extract-treated mice with the dose of 800 mg/kg. There were no significant ( $p > 0.05$ ) changes in the total protein values of leaf extract-treated groups at third week of treatment and at the withdrawal periods, when compared to the control group.

The total cholesterol values of the leaf extract-treated mice significantly ( $p < 0.05$ ) decreased at the third week of treatment, with no significant ( $p > 0.05$ ) changes at week 1 and 2 of treatment, and two weeks of withdrawal compared with the control.

### Liver Enzymes (AST, ALT, and ALP)

Results in Table 2 indicated that the liver enzymes (AST, ALT and ALP) based on the treatment using the extract, showed no significant ( $p > 0.05$ ) decrease. Similarly, days after extract withdrawal showed no significant ( $p > 0.05$ ) changes, but at day 7 of extract withdrawal of AST indicated a significant ( $p < 0.05$ ) increase when compared to the control.

## DISCUSSION

The finding that administration of the aqueous leaf extract did not significantly ( $p > 0.05$ ) affect the serum enzyme levels (ALT and ALP) in the leaf extract-treated mice suggests that it had low hepatic toxicity. The observed increase in serum

AST activity in group B mice treated with 1000 mg/kg leaf extract at day 7 of extract withdrawal when compared to the control group in absence of such changes in ALT and ALP activities may indicate the source of this change was extra-hepatic, perhaps skeletal and cardiac muscles, which were reported to contain the enzyme (Coles, 1974; Mayne, 1998; Gueroui and Kechrid, 2016). The absence of changes in serum ALT and ALP activities may also be an indication that mouse liver does not contain significant levels of the enzymes. For example, it was reported that liver of ruminants does not contain significant concentration of ALT (Coles, 1974). Liver enzymes are known to be liberated into the blood whenever liver cells are damaged and enzymes activity in the plasma is, thus, increased (Edwards *et al.*, 1995).

An elevation in the serum activity of AST is known to occur in association with liver diseases in a variety of animal species and it is of diagnostic importance in the assessment of the level of liver cell damage only when no disease exists in other tissues, such as the skeletal and cardiac muscles, in which it is found in high concentration (Coles, 1974). The elevation of AST activity is suggestive of damage to the cytoplasmic and or mitochondrial membranes of liver or the skeletal and cardiac muscles resulting to altered membrane permeability and subsequent escape of the enzyme into interstices and eventually the plasma (Coles, 1974; Mayne, 1998; Gueroui and Kechrid, 2016).

**Table 1:** Effects of Sub-acute Oral Administration of Aqueous Extract of *Acalypha wilkesiana* Leaves on some Biochemical Parameters

	Extract Treatment Dose (mg/kg)	Treatment Days			Days Post Treatment (withdrawal)	
		7	14	21	7	14
Glucose (mg/dl)	Control (Distilled water)	130.00±21.51 <sup>a</sup>	117.40±29.27 <sup>a</sup>	110.60±17.56 <sup>a</sup>	119.40±31.21 <sup>a</sup>	155.40±37.37 <sup>a</sup>
	800 mg/kg	110.20±24.79 <sup>a</sup>	103.60±13.43 <sup>a</sup>	124.20±19.97 <sup>b</sup>	147.80±31.26 <sup>a</sup>	139.60±26.76 <sup>a</sup>
	1000mg/kg	123.20±11.08 <sup>a</sup>	108.20±12.28 <sup>a</sup>	149.40±25.19 <sup>b</sup>	124.20±17.59 <sup>a</sup>	135.00±40.84 <sup>a</sup>
	1200mg/kg	103.60±19.53 <sup>a</sup>	114.20±19.69 <sup>a</sup>	118.00±18.89 <sup>b</sup>	132.20±17.89 <sup>a</sup>	129.60±45.37 <sup>a</sup>
Total Bilirubin (µmol/L)	Control (Distilled water)	3.60±1.14 <sup>a</sup>	3.20±0.84 <sup>a</sup>	7.60±1.34 <sup>a</sup>	8.40±0.89 <sup>a</sup>	7.40±1.67 <sup>a</sup>
	800 mg/kg	3.40±0.55 <sup>a</sup>	3.20±1.30 <sup>a</sup>	6.60±1.67 <sup>a</sup>	6.80±2.28 <sup>a</sup>	6.80±2.28 <sup>a</sup>
	1000mg/kg	4.20±2.95 <sup>a</sup>	3.40±1.14 <sup>a</sup>	7.80±1.30 <sup>a</sup>	8.20±1.30 <sup>a</sup>	8.00±1.00 <sup>a</sup>
	1200 mg/kg	2.40±0.55 <sup>a</sup>	3.00±0.71 <sup>a</sup>	6.20±1.92 <sup>a</sup>	7.00±0.71 <sup>a</sup>	7.60±1.34 <sup>a</sup>
Conjugated bilirubin (µmol/L)	Control (Distilled water)	6.60±1.34 <sup>a</sup>	6.60±1.52 <sup>a</sup>	4.00±1.00 <sup>a</sup>	3.80±0.84 <sup>a</sup>	3.80±0.84 <sup>a</sup>
	800 mg/kg	7.40±1.14 <sup>a</sup>	6.20±1.30 <sup>a</sup>	2.80±0.84 <sup>a</sup>	3.20±1.30 <sup>a</sup>	3.00±1.00 <sup>a</sup>
	1000mg/kg	6.60±2.30 <sup>a</sup>	6.20±1.92 <sup>a</sup>	3.60±1.14 <sup>a</sup>	3.60±0.89 <sup>a</sup>	3.80±0.84 <sup>a</sup>
	1200 mg/kg	3.60±1.14 <sup>a</sup>	6.20±1.30 <sup>a</sup>	2.60±0.89 <sup>a</sup>	3.80±0.45 <sup>a</sup>	3.60±0.55 <sup>a</sup>
Total Protein (g/L)	Control (Distilled water)	70.40±9.66 <sup>a</sup>	69.20±9.01 <sup>a</sup>	73.60±7.64 <sup>a</sup>	70.40±1.14 <sup>a</sup>	79.40±1.67 <sup>a</sup>
	800 mg/kg	82.40±5.89 <sup>a</sup>	81.80±5.12 <sup>a</sup>	72.60±7.19 <sup>a</sup>	70.80±6.09 <sup>a</sup>	78.60±7.40 <sup>a</sup>
	1000mg/kg	63.80±12.11 <sup>b</sup>	65.20±10.62 <sup>b</sup>	79.00±2.45 <sup>a</sup>	75.60±3.65 <sup>a</sup>	68.00±4.85 <sup>a</sup>
	1200 mg/kg	66.00±6.32 <sup>b</sup>	67.40±5.13 <sup>b</sup>	72.60±7.44 <sup>a</sup>	68.80±4.38 <sup>a</sup>	72.60±5.59 <sup>a</sup>
Total cholesterol (mg/L)	Control (Distilled water)	4.34±0.94 <sup>a</sup>	5.16±1.71 <sup>a</sup>	5.96±2.27 <sup>a</sup>	6.18±4.55 <sup>a</sup>	5.14±2.25 <sup>a</sup>
	800 mg/kg	5.70±4.15 <sup>a</sup>	5.04±0.84 <sup>a</sup>	3.80±1.29 <sup>b</sup>	2.90±0.43 <sup>a</sup>	4.90±2.26 <sup>a</sup>
	1000mg/kg	5.00±1.84 <sup>a</sup>	5.36±1.61 <sup>a</sup>	3.10±1.23 <sup>b</sup>	4.80±2.38 <sup>a</sup>	2.50±0.95 <sup>a</sup>
	1200 mg/kg	4.70±1.01 <sup>a</sup>	4.22±1.08 <sup>a</sup>	3.00±0.99 <sup>b</sup>	3.10±0.88 <sup>a</sup>	3.30±0.57 <sup>a</sup>

<sup>a, b</sup>, means with different superscript across the rows are significantly ( $P < 0.05$ ) different ( $n=5$ )

**Table 2:** Effects of Sub-acute Oral Administration of Aqueous Extract of *Acalypha wilkesiana* Leaves on Liver Enzymes in Mice

Parameters	Extract Treatment Dose(mg/kg)	Treatment Days			Days Post Treatment (withdrawal)	
		7	14	21	7	14
		Control (Distilled water)	231.20±89.04 <sup>a</sup>	149.80±37.98 <sup>a</sup>	175.20±53.92 <sup>a</sup>	97.80±8.41 <sup>a</sup>
Aspaartate	800	186.20±122.59 <sup>a</sup>	154.00±33.71 <sup>a</sup>	140.40±54.97 <sup>a</sup>	98.20±7.92 <sup>b</sup>	154.40±56.59 <sup>a</sup>
Aminotransferase (AST)	1000	153.00±46.32 <sup>a</sup>	145.60±43.98 <sup>a</sup>	115.60±39.23 <sup>a</sup>	183.80±80.75 <sup>c</sup>	115.40±48.12 <sup>a</sup>
	1200	205.60±15.42 <sup>a</sup>	114.60±34.14 <sup>a</sup>	109.80±12.11 <sup>a</sup>	102.40±7.50 <sup>a</sup>	101.00±9.79 <sup>a</sup>
Alanine	Control (Distilled water)	81.00±35.62 <sup>a</sup>	51.80±10.83 <sup>a</sup>	36.80±16.92 <sup>a</sup>	29.20±4.87 <sup>a</sup>	42.20±20.13 <sup>a</sup>
	800	54.80±18.09 <sup>a</sup>	42.40±10.36 <sup>a</sup>	32.20±11.26 <sup>a</sup>	31.20±3.37 <sup>a</sup>	35.20±14.29 <sup>a</sup>
Aminotransferase (ALT)	1000	70.00±17.19 <sup>a</sup>	45.60±14.36 <sup>a</sup>	22.20±6.91 <sup>a</sup>	38.40±25.66 <sup>a</sup>	20.60±4.04 <sup>a</sup>
	1200	67.60±28.13 <sup>a</sup>	34.80±14.94 <sup>a</sup>	19.60±5.03 <sup>a</sup>	23.80±8.14 <sup>a</sup>	23.60±6.35 <sup>a</sup>
Alkaline Phosphatase (ALP)	Control (Distilled water)	147.80±75.72 <sup>a</sup>	91.60±13.96 <sup>a</sup>	84.60±31.18 <sup>a</sup>	74.20±33.97 <sup>a</sup>	54.20±10.52 <sup>a</sup>
	800	119.40±46.66 <sup>a</sup>	100.40±7.37 <sup>a</sup>	84.20±39.46 <sup>a</sup>	102.20±16.15 <sup>a</sup>	72.60±27.36 <sup>a</sup>
	1000	126.00±28.22 <sup>a</sup>	84.40±22.73 <sup>a</sup>	85.60±31.42 <sup>a</sup>	97.60±30.44 <sup>a</sup>	58.80±35.46 <sup>a</sup>
	1200	126.20±35.56 <sup>a</sup>	80.40±31.29 <sup>a</sup>	73.40±24.35 <sup>a</sup>	66.40±25.13 <sup>a</sup>	45.60±13.76 <sup>a</sup>

<sup>a, b, c</sup> means with different superscript across the rows are significantly ( $p < 0.05$ ) different ( $n=5$ ).

However, in acute injury to the liver, as in viral hepatitis, the level of the ALT and AST may be used as a general measure of the degree of liver inflammation or damage. In chronic liver disease, this is not the case, for these enzymes may be entirely within the normal range, even in the presence of cirrhosis (scarring of the liver) (Anon, 2010). ALT is the more liver specific enzyme and therefore generally more sensitive to changes in activity levels than AST (Sodipo *et al.*, 2009).

There was no significant ( $p > 0.05$ ) decrease in total bilirubin and conjugated bilirubin. Treatment with the extract of *A. wilkesiana* leaves indicated a significant ( $p < 0.05$ ) decrease in total protein and cholesterol level in treated mice when compared to the control. When the levels of serum cholesterol are lowered by diet or drugs, the rate of progression of atherosclerotic disease is slowed, some atherosclerotic plaques regress and the risk of cardiovascular events is reduced (Gotto, 1997). Glucose level in this study was observed to significantly ( $p < 0.05$ ) increase from 110.60 ± 17.56 to 124.20±19.97, 149.40 ± 25.19 and 118.00 ± 18.89. This is probably an indication that the extract has the ability to cause hyperglycaemia.

### Conclusion

The liver enzymes were not affected except on day 7 post-withdrawal which indicated a decrease in AST with 800 mg/kg which may be an indication of degenerative changes in the liver one week later and at 1000 mg/kg there was increase in AST which may also be a delay in the body response to infection. Protein and cholesterol show decrease indicating that the extract had a hyperglycaemic effect when taken in lower dosage and as a fat reducing agent. Calcium and Sodium levels were not affected during the treatment. Body weight was also not affected which may be why there was no increase in the cholesterol level.

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### Conflict of Interest

The authors declare that they do not have any conflict of interest.

### Author's Contribution

Project design; MHA, SS. and SUK. Original draft of manuscript; MHA. and SUK. Review and editing of manuscript MHA and CM. Interpretation of result; MHA, CM, SOA. and SUK. All authors read and approved the final manuscript.

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