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Toxicological implications of the therapeutic use of *Acalypha wilkesiana* leaves in traditional medicine

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Abstract

Background: In traditional medicine, *Acalypha wilkesiana* is frequently used solely or as a composite part of many herbal preparations for therapeutic purposes. This study was therefore conducted to evaluate the effects of oral administration of extracts of *Acalypha wilkesiana* leaves, on some serum diagnostic enzymes in normal experimental rabbits.

Methods: Eighteen adult male experimental rabbits were randomized into three groups (A, B and C), comprising of six animals each. Group A animals were given aqueous extracts of *Acalypha wilkesiana* leaves, while group B animals were given ethanol extracts of *Acalypha wilkesiana* leaves. The extracts were administered orally at a dose of 300 mg/kg body weight for a period of twenty-one (21) days. Group C animals were given water, thus they served as control. Data are represented as Mean ± S.E.M ($n = 6$). Significance of Difference was tested by ANOVA at $P < 0.05$.

Results: Administration of the aqueous or ethanol extracts, to the experimental animals resulted in a significantly ($P < 0.05$) higher alkaline phosphatase (ALP) and lactate dehydrogenase (LDH), non-significantly ($P > 0.05$) lower serum total bilirubin, direct bilirubin, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), gamma glutamyl transferase (GGT) and creatinine kinase (CK), as compared with the control animals.

Conclusion: In view of the effects of the plant extracts on ALP and LDH levels, the use of *Acalypha wilkesiana* leaf in traditional medicine should be with caution.

Keywords: *Acalypha wilkesiana*, Ethanol extract, Aqueous extract, Diagnostic enzymes, Bilirubin

Background

The increase in demand for therapeutic drugs from natural products is traceable to the realization that plant products contain active constituents that are capable of curing majority of man's diseases. The use of plant, plant extract or plant-derived chemicals to treat diseases (whether topical, subcutaneous or systemic) has stood the test of time [1]. Medicinal herbs are plants which contain substances that can be used for therapeutic purposes, some of which are precursors for the synthesis of drugs [2]. Herbal medicines are the mainstay of about 75–80% of the world population (mainly in developing countries) for primary health care because of better cultural acceptability, regarding compatibility with the human body and less adverse effects [3–5]. In West Africa, new drugs are often beyond the reach of the poor and not less than 80% of the population use medicinal plants as remedy against infections and diseases [6, 7].

Acalypha wilkesiana is native to the south pacific islands and belongs to the Euphorbiaceae family. It is widely cultivated in the tropical and subtropical countries. The plant reportedly contains terpenoids; sesquiterpenes, monoterpenes, triterpenoids and polyphenols [8, 9]. The leaves are known to contain steroids, alkaloids, phytates, anthraquinones, oxalates, saponins, tannins, and glycosides [1, 9]. In view of the benefits of these compounds detected in *Acalypha wilkesiana* leaves, the plant can be seen as a potential source of useful drugs [10]. In traditional medicine, *Acalypha wilkesiana* is frequently used solely or as a composite part of many herbal preparations for therapeutic purposes. Although the leaves of this plant are eaten as vegetables in Southern Nigeria for therapeutic purposes,

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the scientific basis for its safety has not been fully established. Thus, the aim of this research is to evaluate the safety or otherwise of the use of *Acalypha wilkesiana* leaves in traditional medicine, by evaluating the effects of oral administration of extracts of *Acalypha wilkesiana* leaves on some serum diagnostic enzymes, in normal experimental rabbits.

Methods

Plant materials

Fresh *Acalypha wilkesiana* leaves, obtained from a local garden within Benin City were authenticated at the Department of Plant Biology and Biotechnology, of the University of Benin, Benin City. The leaves were properly washed, air-dried and ground into fine powder.

Preparation of ethanol extract

One hundred gram of the powdered leaves was soaked in 400 ml of absolute ethanol (95%) for 72 h. The mixture was occasionally stirred using a magnetic stirrer, to ensure proper mixture of the vessel content. After 72 h, the mixture was filtered using a sintered funnel and the extract (filtrate) concentrated using a rotary evaporator. The concentrate of the extract was weighed and used for the study [10].

Preparation of aqueous extract

One hundred gram of the powdered leaves was soaked in 400 ml of distilled water for 72 h, and treated as described above for ethanol extract [10].

Experimental animals

Eighteen adult male experimental rabbits of the New Zealand strain, weighing between 0.9–1.5 kg were used for the study. The rabbits were housed in clean disinfected cages in the animal house of the Department of Biochemistry, University of Benin and maintained on a 12-h light and dark cycle. They were allowed to acclimatize to the new environment for a period of three weeks, with free access to feed (standard pelletized growers feed from UAC-Vital Feed, Jos, Plateau State) and water. The experimental procedures performed on the animals were approved by the Animal Ethics Committee of the Faculty of Life Sciences, University of Benin, Nigeria. The use of rabbits for the study was also according to the Ethical Guidelines Involving Whole Animal Testing of the Animal Ethics Committee, Faculty of Life Sciences, University of Benin. The experimental animals were then randomized into three groups (groups A, B and C) of six rabbits each [11].

Experimental design

Groups A, B and C animals were treated as follows;

Group A Rabbits: Given Aqueous Extract of *Acalypha wilkesiana* leaves
Group B Rabbits: Given Ethanol Extract of *Acalypha wilkesiana* leaves
Group C Rabbits: Given distilled water (Control) [11]

Administration of extracts

Five grams of the concentrated extracts were suspended in distilled water and measured amounts (dose) administered to the experimental animals with respect to their body weight. The extracts (aqueous or ethanol) were administered orally at a dose of 300 mg/kg body weight to the experimental rabbits for a period of twenty-one (21) days [11].

The dose "300 mg/kg body weight" was selected after a preliminary study (though unpublished) was done with graded doses (i.e. 200, 250, 300, 350 and 400 mg/kg body weight) of the plant extracts administered to salt loaded rabbits. The 300 mg/kg body weight dose was found to be more effective.

Collection of blood

Prior to administration (Basal/day 0) with the extract, blood samples were collected from the veins located on the dorsal side of the ear lobes of the experimental rabbits, using sterilized hypodermic needles. At days 7, 14 and 21 after administration of the extracts, blood samples were also collected. Blood samples were collected in plane (universal) bottles immersed in ice. Immediately after collection of blood, the tubes were centrifuged at 3500 rpm for 10 min and clear serum obtained which were used for further analysis [11].

Assay methods

Alanine aminotransferase (ALT) assay was done by the method of Reitman and Frankel (1957) [12]. ALT was measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4 – dinitrophenyl hydrazine at 546 nm. Aspartate aminotransferase (AST) assay was also done by the method of Reitman and Frankel (1957) [12]. AST was measured by monitoring the concentration of oxaloacetate formed with 2, 4- dimtrophenyl hydrazine at 546 nm. Gamma Glutamyl Transferase (GGT) was determined by the method of Szasz (1969) [13]. The substrate L-γ-glutamyl-3-carboxy-4-nitroanilide, in the presence of glycylglycine was converted by γ-GT in the sample to 5-amino-2-nitrobenzoate which was measured at 405 nm, to determine the increase in absorbance. Alkaline Phosphatase (ALP) was determined by the method of Rec. GSCC (1972) [14], which measured the intensity of the yellow colour PNP formed from PNPP by ALP action at 405 nm. Lactate Dehydrogenase (LDH) was done by the method of Weissharr et al., (1975) [15]. The reduction of pyruvate to lactate involved the concomitant oxidation of NADH to

NAD^+ which was monitored at 340 nm. Creatinine Kinase was by the method of Bauer (1976) [16]. The creatine that was liberated reacted with diacetyl and α-naphtol to form a colored product which was read at 520 nm. Bilirubin was determined by the method of Jendrassik and Grof, (1938) [17]. The principle involves the reaction of direct (conjugated) bilirubin with diazotized sulphanilic acid in alkaline medium to form a blue colored complex. Total bilirubin was determined in the presence of caffeine, which released albumin bound bilirubin, by the reaction with diazotized sulphanilic acid and absorbance was read at 578 nm.

Statistical analysis

Data are represented as Mean ± S.E.M (n = 6). Significance of Difference was tested by Student T-Test, ANOVA and Turkey-Kramer test, using the GraphPad Instat Version 3 (GraphPad Software Inc. San Diego, California U.S.A.). Statistical Significance was set at $P < 0.05$.

Results

The effects of oral administration of extracts (aqueous or ethanol) of *Acalypha wilkesiana* leaves on some serum parameters in normal experimental rabbits, are as described below.

In Table 1, the treated groups (A & B) showed fluctuations in their mean serum alanine aminotransferase (ALT) levels, after extract administrations, with the changes significant ($p < 0.05$) at day 14 of extract administration. When compared with the control (group C), group A (given aqueous extract) showed ALT levels significantly ($p < 0.05$) lower at day 7.

At day 7 of treatment, the aqueous extract (group A) resulted in significantly ($P < 0.05$) lower AST levels as compared with the control, while the ethanol extract (group B) resulted in non-significantly ($P > 0.05$) lower AST levels as compared with the control. At day 14 of treatment, group A showed a non-significantly ($P > 0.05$) higher AST levels while group B showed a non-significantly ($P > 0.05$) lower AST levels, as compared with the control. At day 21 of treatment, group A showed a significantly ($P < 0.05$) lower AST levels, while group B showed a non-significantly ($P > 0.05$) lower AST levels as compared with the control.

Alkaline phosphatase (ALP) levels were also affected by treatment with *Acalypha wilkesiana* leaf extracts (aqueous or ethanol) as indicated in Table 2. At day 7 of extract administration, group A (given aqueous extract) showed a significantly ($P < 0.05$) higher ALP levels, while group B (given ethanol extract) showed a non-significantly ($P > 0.05$) higher ALP levels, as compared with group C (control). At day 14 of extract administration, there was no significant ($P > 0.05$) difference in the values of ALP (A & B compared with C). But at day 21 of extract administration, both test groups (A & B) showed significantly ($P < 0.05$) higher ALP levels, as compared with the control group (C).

At day 7 of extract administration, group A showed a non-significantly ($P > 0.05$) higher LDH levels, while group B showed a significantly ($P < 0.05$) higher LDH levels, as compared with group C. At day 14 of treatment, both test groups (A & B) showed significantly ($P < 0.05$) higher LDH levels, as compared with the control group (C). But, at day 21 of treatment, group A showed a non-significantly ($P > 0.05$) higher LDH levels, while group B showed a significantly ($P < 0.05$) higher LDH levels, as compared with group C. Thus, administration of the extracts (aqueous and ethanol) resulted in

Table 1 Serum ALT and AST of normal rabbits treated with extracts of *Acalypha wilkesiana* leaves

	Treatment or group		
	A (Aq. Ext)	B (Et. Ext)	C (Control)
Serum ALT (U/L)			
DAY 0	30.83 ± 5.05^{xa}	32.50 ± 3.50^{xa}	37.83 ± 4.00^{xa}
DAY 7	25.00 ± 0.87^{xa}	34.00 ± 1.53^{ya}	38.00 ± 3.88^{ya}
DAY 14	48.83 ± 2.35^{xb}	51.83 ± 3.09^{xb}	45.33 ± 5.86^{xa}
DAY 21	33.83 ± 0.67^{xa}	43.50 ± 2.18^{xa}	45.67 ± 4.76^{xa}
Serum AST (U/L)			
DAY 0	45.67 ± 2.03^{xa}	36.00 ± 8.54^{xa}	60.67 ± 5.36^{xa}
DAY 7	24.00 ± 3.62^{xa}	28.00 ± 1.80^{ya}	52.83 ± 18.70^{ya}
DAY 14	89.67 ± 0.33^{xb}	41.83 ± 4.97^{ya}	60.17 ± 15.48^{ya}
DAY 21	32.33 ± 1.96^{xa}	54.00 ± 7.51^{yb}	62.83 ± 8.73^{ya}

Data represent Means ± S.E.M (n = 6). Means with different letter $^{a, b}$ superscripts, along column, are significantly different ($p < 0.05$). Means with different letter $^{x, y}$ superscripts, along row, are significantly different ($p < 0.05$)

Table 2 Serum ALP and LDH of normal rabbits treated with extracts of *Acalypha wilkesiana* leaves

	Treatment or group		
	A (Aq. Ext)	B (Et. Ext)	C (Control)
Serum ALP (IU/L)			
DAY 0	10.55 ± 0.36^{xa}	11.52 ± 1.44^{xa}	11.37 ± 1.68^{xa}
DAY 7	17.69 ± 0.77^{xb}	14.50 ± 1.67^{ya}	13.93 ± 0.53^{ya}
DAY 14	11.95 ± 0.66^{xa}	11.22 ± 0.12^{xa}	11.53 ± 1.52^{xa}
DAY 21	24.31 ± 1.19^{xb}	20.61 ± 0.34^{xb}	15.93 ± 1.03^{ya}
Serum LDH (U/L)			
DAY 0	12.91 ± 3.23^{xa}	16.14 ± 6.46^{xa}	29.05 ± 14.79^{xa}
DAY 7	48.42 ± 11.18^{xb}	87.15 ± 29.58^{yb}	41.96 ± 17.08^{xa}
DAY 14	96.83 ± 5.59^{xc}	119.42 ± 46.89^{xc}	58.10 ± 14.79^{ya}
DAY 21	167.84 ± 26.42^{xd}	203.34 ± 39.13^{yd}	151.70 ± 44.84^{xa}

Data represent Means ± S.E.M (n = 6). Means with different letter $^{a, b, c, d}$ superscripts, along column, are significantly different ($p < 0.05$). Means with different letter $^{x, y}$ superscripts, along row, are significantly different ($p < 0.05$)

a steady and significant ($p < 0.05$) increases in serum LDH levels all through the period of administration.

In Table 3, the test groups, given the aqueous or ethanol extract showed non-significantly ($P > 0.05$) higher gamma glutamyl transferase (GGT) levels, as compared to the control group, at day 7 of treatment. But at day 14 of treatment, the test groups showed non-significantly ($P > 0.05$) lower GGT values, as compared with the control. However, at day 21 of treatment, there was no significant difference in the values of GGT in all the groups (A & B compared with C).

After 7 days of extract administration, group A (given aqueous extract) showed a significantly ($P < 0.05$) lower CK levels, while group B (given ethanol extract) showed a non-significantly ($P > 0.05$) lower CK levels, as compared with group C (control). After 14 days of treatment, both test groups (A or B) showed non-significantly ($P > 0.05$) lower CK levels, as compared with the control group (C). However, at day 21 of treatment, group A showed a significantly ($P < 0.05$), lower CK levels while group B showed a non-significant ($P > 0.05$) lower CK levels, as compared with group C. Thus, treatment with the extracts resulted in decreases in serum CK levels.

Serum total bilirubin (mg/dl) levels (Table 4) were shown to be non-significantly ($P > 0.05$) lower in groups A & B as compared to group C, at day 7 of extracts administration. But at day 14, group A (given aqueous extract) showed a significantly ($P < 0.05$) lower total bilirubin levels as compared with group C (control), while group B (given ethanol extract) showed a non-significantly ($P > 0.05$) lower values as compared with group C. However, at day 21 of extract administration, there was no significant difference ($P > 0.05$) in serum total bilirubin levels of all the groups (tests compared with control).

Table 3 Serum GGT and CK of normal rabbits treated with extracts of *Acalypha wilkesiana* leaves

	Treatment or group		
	A (Aq. Ext)	B (Et. Ext)	C (Control)
Serum GGT (U/L)			
DAY 0	3.86 ± 0.39^{xa}	3.86 ± 0.39^{xa}	4.63 ± 0.67^{xa}
DAY 7	5.02 ± 0.39^{xa}	4.63 ± 0.67^{xa}	4.24 ± 0.39^{xa}
DAY 14	3.09 ± 0.38^{xa}	3.48 ± 1.16^{xa}	4.24 ± 0.77^{xa}
DAY 21	4.24 ± 0.39^{xa}	4.63 ± 0.67^{xa}	4.63 ± 0.67^{xa}
Serum CK (U/L)			
DAY 0	53.65 ± 2.38^{xa}	53.65 ± 4.13^{xa}	48.15 ± 3.64^{xa}
DAY 7	31.64 ± 3.64^{xb}	39.89 ± 1.38^{yb}	44.02 ± 4.96^{ya}
DAY 14	42.65 ± 1.38^{xb}	33.01 ± 4.13^{xb}	45.40 ± 4.77^{xa}
DAY 21	15.13 ± 1.38^{xc}	23.39 ± 3.64^{yc}	31.64 ± 1.38^{ya}

Data represent Means ± S.E.M ($n = 6$). Means with different letter $^{a, b, c,}$ superscripts, along column, are significantly different ($p < 0.05$). Means with different letter $^{x, y}$ superscripts, along row, are significantly different ($p < 0.05$)

Table 4 Serum total bilirubin and direct bilirubin of normal rabbits treated with extracts of *Acalypha wilkesiana* leaves

	Treatment or group		
	A (Aq. Ext)	B (Et. Ext)	C (Control)
Serum total bilirubin (mg/dl)			
DAY 0	2.34 ± 0.83^{xa}	2.53 ± 0.22^{xa}	2.20 ± 0.45^{xa}
DAY 7	1.52 ± 0.20^{xa}	1.24 ± 0.10^{xa}	1.83 ± 0.33^{xa}
DAY 14	1.04 ± 0.78^{xa}	2.65 ± 0.45^{ya}	3.67 ± 0.45^{yb}
DAY 21	0.87 ± 0.30^{xa}	0.66 ± 0.11^{xb}	0.82 ± 0.08^{xa}
Serum direct bilirubin (mg/dl)			
DAY 0	4.31 ± 0.38^{xa}	4.66 ± 1.57^{xa}	4.69 ± 0.56^{xa}
DAY 7	5.20 ± 1.69^{xa}	3.36 ± 0.29^{xa}	4.56 ± 1.86^{xa}
DAY 14	5.03 ± 0.25^{xa}	3.59 ± 0.29^{xa}	5.28 ± 1.22^{xa}
DAY 21	2.21 ± 0.29^{xb}	2.76 ± 0.41^{xa}	3.26 ± 0.57^{xa}

Data represent Means ± S.E.M ($n = 6$). Means with different letter $^{a, b}$ superscripts, along column, are significantly different ($p < 0.05$). Means with different letter $^{x, y}$ superscripts, along row, are significantly different ($p < 0.05$)

Serum direct bilirubin levels (Table 4) were shown to be non-significantly ($P > 0.05$) higher in group A and non-significantly ($P > 0.05$) lower in group B as compared with group C, at day 7. But at day 14, only group B showed a non-significantly ($P > 0.05$) lower values as compared with group C. While at day 21 of treatment, both groups (A & B) showed non-significantly ($P > 0.05$) lower serum direct bilirubin levels as compared with the control group (C).

Discussion

The rationale for assaying enzyme activities is based on the premise that changes in activities reflect changes that have occurred in a specific tissue or organ. ALT is found mainly in the liver, but also in smaller amounts in the kidneys, heart, muscles, and pancreas. Low levels of ALT are normally found in the blood. But when the liver is damaged or diseased, it releases ALT into the bloodstream, which makes ALT levels increase. Most increases in ALT levels are caused by liver damage. The treated groups showed fluctuations in their mean serum alanine aminotransferase (ALT) levels, with the changes significant at day 14 of extract administration. Fluctuation of ALT levels is normal over the course of the day, and ALT levels can also increase in response to strenuous physical exercise. Significantly elevated levels of ALT (SGPT) often suggest the existence of other medical problems such as viral hepatitis, diabetes, congestive heart failure, liver damage, bile duct problems, infectious mononucleosis, or myopathy. For this reason, ALT is commonly used as a way of screening for liver problems.

Mean serum aspartate aminotransferase (AST) levels, showed a different trend from that of ALT. As observed, treatment with the plant extracts resulted in lower levels of serum AST of the test groups as compared with the

control. Here, the effect of the aqueous extract is shown to be more significant. This possibly suggests a protective role or a non toxic effect of the plant on the various organs or tissues where this enzyme is found. AST is found in many tissues throughout the body, including the liver, heart, muscles, kidney, and brain. If any of these organs or tissues is affected by disease or injury, AST is released into the bloodstream. This means that AST is not as specific an indicator of liver damage as ALT. Damage to these tissues normally causes the enzymes to leak out into the blood, thus increasing the levels in the blood above normal. The decreases in the activities of AST as occasioned by the administration of the extract, tends to portend a protective role of the plant on this tissues. Aspartate aminotransferase (AST) exists in human tissues as two distinct isoenzymes, one located in the cytoplasm (c-AST), and the other in mitochondria (m-AST). Striated muscle, myocardium, and liver tissues are the main sources of AST. A growing body of information suggests that determination of AST isoenzymes in human serum is useful in evaluating damage to some of these organs [18].

Alkaline phosphatase (ALP) levels were also affected by treatment with *Acalypha wilkesiana* leaf extracts (aqueous or ethanol). Administration of the extracts resulted in significantly higher serum ALP levels of the test groups, with the aqueous extract more implicated. This may portend possible toxicity of the plant. The majority of sustained elevated ALP levels are associated with disorders of the liver or bone, or both. Therefore, these organ systems are of prime consideration in the differential diagnosis. Diseases of bone associated with increased serum ALP are restricted to the presence of osteoblastic activity. High ALP levels can show that the bile ducts are blocked. Levels are significantly higher in children and pregnant women. Also, elevated ALP indicates that there could be active bone formation occurring as ALP is a byproduct of osteoblast activity (such as the case in Paget's disease of bone). Levels are also elevated in people with untreated Celiac Disease [19]. Because of the cellular distribution of ALP, increased serum activity may be caused by a wide variety of disorders involving multiple organs. Attempts to define organ source by isoenzyme study may be met with limited success because of technical limitations; accurate measurement of different isoenzymes contributing to total serum ALP activity is not currently possible. However, the presence of the intestinal or placental isoenzyme may be revealed by selected methods.

Administration of the extracts (aqueous or ethanol) resulted in a steady and significant increases in serum LDH levels all through the period of administration. Increases in serum LDH levels of the test groups (as compared with the control), all through the period of treatment with the extract portends a negative implication. LDH is present in almost all body tissues, so the LDH test is used to detect tissue alterations and as an aid in the diagnosis of heart attack, anemia, and liver disease [20]. When disease or injury affects tissues containing LDH, the cells release LDH into the blood stream, where it is identified in higher than normal levels. The LDH is also elevated in disease of the liver, in certain types of anemia, and in cases of excessive destruction of cells, as in fractures, trauma, muscle damage and shock [20]. Even though an LDH test is useful in diagnosing tissue damage, other tests are usually necessary to pinpoint the location of the damage. One such test is the LDH isoenzymes test. LDH isoenzymes are five kinds of the LDH enzyme that are found in specific concentrations in different organs and tissues. By measuring the blood levels of these isoenzymes, the type, location, and severity of the cellular damage, can be understood. Thus, to ascertain the origin of the increased serum LDH as occasioned by treatment with extract, the differential diagnosis may need to be carried out. However, the plant may be selectively toxic with respect to the tissues. The ethanol extract was shown to be more potent in this respect.

Gamma-glutamyl transferase (GGT) is a metabolic enzyme expressed primarily in the liver, kidneys and other organs. Organ damage, especially damage to the liver, causes the release of this enzyme into the blood. Elevation of GGT levels is often an indication of liver damage and has been associated with liver injury as well as pancreatic and myocardial disorders. The results indicate that the plant (extracts) had no adverse effects on the serum GGT levels of the experimental animals. The slight increase and subsequent reduction in the serum GGT levels may be more of protective function than damage to the tissues of the test animals. GGT is also a very useful tool for preclinical investigation of experimental drug formulations and GGT levels are commonly used to monitor and attenuate the toxic effects of experimental drug formulations in rodents [21]. Although it is considered to be an index of hepatobiliary dysfunction and alcohol abuse [22], recent epidemiology and pathology studies have suggested its independent role in the pathogenesis and clinical evolution of cardiovascular diseases brought on by atherosclerosis [22, 23].

Serum creatinine kinase (CK) levels were affected after administration of extracts. Treatment with the extracts resulted in decreases in serum CK levels. The aqueous extract, as observed caused more significant decreases in the serum creatinine kinase levels than the ethanol extract, all through the treatment period. CK is an important enzyme involved in energy maintenance and energy transfer in muscle and brain cells. Four different isoforms of CK are expressed in a tissue-specific and

developmentally regulated manner. Elevation of CK is an indication of damage to muscle. It is therefore indicative of injury, rhabdomyolysis, myocardial infarction, myocitis and myocarditis. For years, the gold standard for diagnosis of myocardial necrosis was the cardiac-specific isoenzyme of creatinine kinase (CK-MB). Previously, the myocardial fraction of lactate dehydrogenase and even aspartate aminotransferase were used to diagnose myocardial necrosis [24]. Isoenzyme determination has been used extensively as an indication for myocardial damage in heart attacks. The plant may therefore be protective against damages to muscles and possibly brain cells.

As observed, the ethanol extract tends to be more effective at reducing the levels of both serum total and direct bilirubin. Total bilirubin concentration reflects the levels of both the conjugated and unconjugated fractions of bilirubin. Total bilirubin levels are elevated in various forms of liver disease such as cirrhosis, hepatitis, and obstructions of the hepatobiliary system such as gallstones or tumors. Elevated total bilirubin levels are also observed in cases of intravascular hemolysis [25]. A high level of conjugated bilirubin in the blood can also be detected in the urine. In hepatitis, fibrosis, and cirrhosis, high amounts of unconjugated bilirubin means the liver cells are not conjugating bilirubin normally, causing it to build up in the blood. Bilirubin is not normally found in the urine but if it is, that is an indication of either liver cell damage or blockage of the flow of bile from the liver or gallbladder. These adverse effects of increased or elevated bilirubin in the system may possibly be countered by the plant, as it tends to help in the excretion of bilirubin, thus reducing its concentration in the blood.

Conclusion

Treatment with both extracts resulted in fluctuations (no significant effect) in the levels of serum ALT and decreases in the levels of serum AST, total bilirubin and direct bilirubin of the test animals. Administration of the extracts resulted in significant increases in serum ALP and LDH levels of the test animals (which portends a negative implication). However, the serum GGT levels were not significantly affected, while the serum CK levels were reduced. In view of the effects of the plant extracts on ALP and LDH levels, the use of *Acalypha wilkesiana* leaf in traditional medicine should be with caution.

Abbreviations

ALP: Alkaline Phosphatase; ALT: Alanine Aminotransferase; Aq.: Aqueous; AST: Aspartate Aminotransferase; CK: Creatinine Kinase; Et.: Ethanol; Ext.: Extract; GGT: Gamma Glutamyl Transferase; LDH: Lactate Dehydrogenase; S.E.M: Standard Error of Mean

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Declaration

We the authors declare that this manuscript is original, has not been published before and is not currently being considered for publication elsewhere. We confirm that the manuscript has been read and approved by all authors

Authors' contributions

OK: Concepts, Design, Experimental Studies, Data acquisition and Analysis, Manuscript preparation, Statistical Analysis. AAM: Concepts, Design, Experimental Studies, Data acquisition and Analysis, Manuscript preparation, Statistical Analysis. ONEJ: Concepts, Design, Experimental Studies, Data acquisition and Analysis. IOS: Design, Experimental Studies, Data acquisition and Analysis. All authors read and approved the final manuscript.

Competing interest

The authors wish to state that there are no competing interests associated with this publication and there has been no significant financial support for this work that could have influenced its outcome.

Publisher's Note

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