



*International Journal of Current Research
and Academic Review*

ISSN: 2347-3215 Volume 3 Number 9 (September-2015) pp. 176-188

www.ijcrar.com



The Ameliorative Effect of Aqueous Leaves Extract of *Acalypha wilkessina* on Carbon Tetrachloride Induced Hepatotoxicity in Wistar Albino Rats

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KEYWORDS

Hepatoprotective,
Acalypha wilkessiana,
Toxicity,
Enzyme
activity

A B S T R A C T

The major functions of the liver can be detrimentally altered by liver injury emanating from chronic or acute exposure to toxicants. The hepatoprotective activity of the aqueous extract of leaves of *Acalypha wilkessiana* against carbon tetrachloride (CCl₄)–induced toxicity in albino wistar rats was assayed. Activities of liver enzyme markers (ALT, AST, ALP, Bilirubin, total protein, albumin and globulin) were determined colorimetrically using standard kits. The analyses were carried out on a total of 25 albino rats of both sexes divided into 5 groups of 5 rats in each. Group 1 served as normal control (NC), which received food and water only throughout the period. Group 2 served as the intoxicated control group (IC) or the (CCl₄) group, which received food and water *ad libitum* and carbon tetrachloride (0.2 ml/kg in liquid paraffin 1:1 on day 7. Group 3, served as the intoxicated test group (IT) that received food and water *ad libitum*, aqueous extract of *Acalypha wilkessiana* (AEAW) 100mg/kg and carbon tetrachloride(0.2ml/kg in liquid paraffin 1:1 on day 7 . Group 4, received food and water *ad libitum*, aqueous extract of *A. wilkessiana* (100 mg/kg). Group 5, received food and water *ad libitum*, Silymarin (50 mg/kg) and Carbon tetrachloride (0.2 ml/kg in liquid paraffin 1:1 on day 7 . Data obtained were analyzed using SPSS software. Carbon tetrachloride (CCl₄) induced toxicity resulted in liver damage which showed in the elevation of serum enzyme activities-Aspartate amino transferase(AST), Alanine amino transferase (ALT) and Alkaline phosphatase (ALP). There was also significant increase in total protein compared with normal control animals. Pre-treatment with aqueous leaves extract of *Acalypha wilkessiana* prevented those biochemical alterations. Hence, *A. wilkessiana* aqueous leaves extract can be used as a hepatoprotective agent.

Introduction

Hepatotoxicity remains a serious health problem and the management of liver damage is still a challenge to modern health practice. The liver occupies a pivotal position in the metabolism of various nutrients, xenobiotics and environmental toxicants that find their way into human or animal systems. The main function of the liver is to maintain homeostasis and the normal integrity of the various organs by the elimination of toxicants. Many medicines when taken in overdose and sometimes when taken within the therapeutic range may elicit some reactions within the system or may injure the liver. Many chemicals used in laboratories and industries, natural chemicals (eg. microcystins) and some herbal remedies can also induce hepatotoxicity. Chemicals and toxicants that cause changes in the liver architecture are called hepatotoxins.

More than 900 drugs have been implicated in liver injury [1]. Chemicals often cause subclinical injury to the liver which manifest as abnormal liver function, detected in routine liver tests. Although almost all tissues in the body possess some ability to metabolize chemicals, the smooth endoplasmic reticulum in the liver is the principal 'metabolite clearing house' for both endogenous and exogenous chemicals and nutrients(drugs)[2].The human body identifies almost all drugs as foreign substances and subject them to various biochemical transformations to make them suitable for elimination.

Drug metabolism is usually divided into two phases namely: phase 1 and phase 2. Phase 1 reactions involve oxidation, reduction, hydrolysis, hydration and many other rare chemical reactions. These processes tend to increase the solubility of the drugs in water

and can generate more metabolites in which some may be potentially more toxic. Liver damage is associated with cellular necrosis, increase in tissue lipid peroxidation and depletion of reduced glutathione levels. In addition, serum levels of many biochemical markers like liver transaminases and alkaline phosphatases[3].These biochemical markers such as the plasma enzymes are present at all times in systemic circulation of animals and perform physiological functions in blood [4].

Plasma enzymes are classified under functional plasma enzymes and non-functional plasma enzymes. Functional plasma enzymes are those enzymes although produced in the liver are present in equivalent or high concentrations than in tissues. The non-functional enzymes perform no known physiological function in the blood. Their substrates are normally absent in the plasma and are present in the blood of normal individuals at levels much lower than in tissues. Their presence in plasma at levels elevated above normal values suggest an increased rate of tissue destruction. Measurement of values of these non-functional enzymes can thus provide the physician with variable prognostic and diagnostic evidence [5]. Non-functional plasma enzymes include those of exocrine secretions and true intracellular enzymes. Examples of such enzymes include-pancreatic amylase, lipase in bile, alkaline phosphatase, glutamic oxaloacetate transaminase, glutamic pyruvate transaminase and prostrate acid phosphatase, these diffuse into the plasma. Low levels of non-functional enzymes found ordinarily in the plasma apparently arise from the normal destruction of erythrocytes, leucocytes and other cells with accelerated cell death. Although plasma enzyme levels are generally interpreted as evidence of cellular necrosis; vigorous exercise also releases

significant quantities of muscle enzymes; while damage to liver cells will cause the increase of such enzymes like–Aspartate aminotransferase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP). Viral hepatitis and obstructive liver disease are such liver problems that can elicit the release of such enzymes into the plasma at higher levels [6].

Aminotransferase

Aminotransferases are associated with hepatocellular damage. They catalyze the reversible transfer of an amino group between amino acid and α -keto acid. The two amino transferase enzymes are Aspartate amino transferase and Alanine transaminase.

Aspartate aminotransferase

Aspartate aminotransferase is also known as Aspartate transaminase or L-Aspartate glutamate oxaloacetate transaminase (GOT). The enzyme catalyzes the inter-conversion of L-Aspartate and L-glutamate and oxaloacetate in the presence of α -ketoglutarate. Aspartate aminotransferase (AST) is distributed widely in tissues. AST is produced in the muscle and can be elevated in other disease conditions. With viral hepatitis, and other forms of liver disease associated with hepatic necrosis, AST levels are elevated even before clinical signs and symptoms of diseases such as jaundice or moderate elevations of AST activity have been observed after intake of ethanol in *Delirium tremens* and also after administration of various drugs such as Opiates, Salicylates or the Ampicillins [7]. Myocardial infarction causes substantial increase in AST levels because the enzyme is abundantly present in heart muscles. In both acute and chronic alcoholic liver damage, AST elevations tend to be

somewhat greater than Alanine transaminase (ALT). Five to ten elevations of AST enzymes occur in individuals with primary or metastatic carcinoma of the liver [6]

Alanine transaminase

Alanine transaminase is an enzyme produced in the hepatocytes, the major cell type of the liver. It was formerly known as Glutamate pyruvate transaminase (GPT). It catalyzes the inter-conversion of L-Alanine to L-Glutamate and pyruvate in the presence of α -ketoglutarate and pyruvate. ALT is widely distributed in animal tissues but their activities are lower than that of AST. The level of ALT in blood is increased in certain conditions especially, if hepatocytes are damaged. As cells are damaged, ALT leaks into the blood stream. All types of hepatitis cause hepatocyte damage, which can lead to elevations in serum ALT activity. Extremely high ALT levels or activities are seen in severe cases of toxic hepatitis. ALT is also increased in cases of liver cell death resulting from such causes as shock or drug toxicity. The level of ALT may correlate roughly with the degree of cell death or inflammation. Although serum levels of AST and ALT become elevated whenever disease processes affect liver integrity. ALT is the main liver specific enzyme .

Alkaline phosphatase

Alkaline phosphatase is the main enzyme or family of related enzymes that hydrolyze monophosphate esters at an alkaline pH .The optimum pH of these enzymes is about ten [8]. ALP activity is most often measured to indicate bile duct obstruction. The physiologic role of ALP remains unclear, but it exists not only in biliary tract and liver epithelium, but also in osteoblasts which may lay down new bones in the

granulocytes of circulating blood, kidneys, placenta and lactating mammary glands. An elevation in the level of serum alkaline phosphatase especially, in the setting of normal or only moderately elevated ALT and AST activities, suggest diseases of the bile ducts and liver injury [9]. Serum alkaline phosphatase activity can be elevated in bile duct obstructive disease such as sclerosing cholangitis, active bone formation in pregnancy, some cases of intestinal dysfunction and in some renal infarcts. Alkaline phosphates is also produced in bone and blood activity and can also increase in bone disorder. Bilirubin is a yellow compound mostly formed from the breakdown of hemoglobin. Old or damaged red blood cells are broken down in the spleen releasing hemoglobin. Approximately, 35 mg of bilirubin is produced from every 1g of hemoglobin. About 30% of the bilirubin formed is derived from the breakdown of heme and the other 20% comes from the breakdown of immature blood cells. Two forms of bilirubin can exist in plasma. They are the conjugated and the unconjugated bilirubin [10]. The conjugated bilirubin is soluble in water and can be excreted with ease by the body. The unconjugated bilirubin is lipophilic and can be deposited in tissues leading to kernicterus [9].

Total protein is a biochemical marker for the assessment for the state of the liver. The liver is a vital organ for protein metabolism and detects the total amount of protein in the blood plasma or serum. Protein, a macromolecule in the plasma is made up of the albumin and globulin. Concentrations below the reference range usually reflect low albumin concentration. For instance, in liver disease or acute infection, concentrations above the reference range are found in leukemia. Albumin is a protein made recognition syndrome, where it is lost

through urine. The consequences of low albumin can be edematous changes since the intravascular oncotic pressure is higher than the extravascular space.

Herbal preparations

The search for therapeutic substances with novel properties to manage and cure diseases is still on-going. The role of traditional or ethnomedicine in solving health problems in the third world is valuable even globally. It is even more impressive by considering the fact that about 80% of the people living in less developed countries rely exclusively on traditional medicines for the health care needs. The WHO listed 21,000 plants used for medicinal purposes in all parts of the world.

Herbal medicines are low- cost and free of adverse effects used for the treatment of various ailments [11]. Since antiquity, different plant parts and herbs are used to treat various ailments. It is clear from history that pharmaceutical preparations are solely based on plant remedies or materials. Although, there is abundance or availability of synthetic drugs used for the treatment of various ailments, herbal remedies/preparations have been used for long to treat patients with liver diseases and malaria. During the past decade, plants have regained recognition as important remedies for medication, because they are relatively safe, non-toxic, and less cost effective [12]. Medicinal plants have very important place as they not only maintain the health and vitality of human beings and animals but are also used to cure several diseases. Nowadays, more than 50% of all modern drugs in clinical use are natural products. Recent findings show that over 60% of patients all over the world, use vitamins or phytomedicines or polyherbal preparations, which are now used for the treatment and

prevention of various liver disorders or hepatotoxicity cases [13]. Some of the herbal plants include: *Abelmoschus esculentus*, *Acococa catechu*, *Allium cepa*, *Allium sativum*, *Aloe barbadensi*, *Buppleurum falcatum*, *Cassia fistula*, *Citrus limettiodes*, *Mormodica diocea*, *Moringa oleifera*, *Ocimum grattisimum*, *Vernonia amygdalina*, *Phyllantus amarus*, *Phyllantus embelica*, *Phyllantus deblis*, mostly used for treatment and hepatoprotective effect [14, 15].

Acalypha wilkesiana belongs to the family *Euphorbiaceae* (sponge family). Its other names include: *A. amantaceae* and *A. tricolor*, while its common names are copper leaf, Joseph's coat, fire dragon, beef sleek plant and match-me-if-you-can [16]. The Hausas of Northern Nigeria, call it "Jiwene", and Jimwinini", while the Yorubas of South-West Nigeria, call it "awonso". It is a native of Fuji and nearby Islands in South-Pacific and a popular outdoor plant that provides color throughout the year. It is propagated by stem cutting at any time of the year. *A. wilkesiana* has been shown to possess antimicrobial properties [17]. According to [17], the expressed juice or boiled decoction of the leaves is used for the treatment of gastro-intestinal disorders and fungal skin infections such as *Pityriasis versicolor*, *impetigo contagiosa*, *intetrigo*, *tinea corporis* and *tinea pedes*. In Southern, Nigeria, the leaves of the plant is eaten and used as vegetable in the management of hypertension [18][19]. Another study also viewed the nutritional potential of the plant [20]. At present, there is no much information on the hepatoprotective efficacy or potential of *Acalypha wilkesiana*. The research work aims at unveiling the hepatoprotective potential of the plant leaves and the possible application of the herb in the management or treatment of liver diseases.

Materials and Methods

Collection and Identification of plant sample

Leaves of *A. wilkesiana* were obtained from Akabo community in Ikeduru LGA of Imo State, Nigeria and were authenticated by Mr. Francis Iwunze, a plant taxonomist at the Department of Forestry and Wild life of the School of Agriculture and Agricultural Technology (SAAT), Federal University of Technology, Owerri .

Preparation of plant samples

The leaves of *A. wilkesiana* were washed under running tap water, and air dried at room temperature of 27 °C to constant weight. The dried leaves were milled into powder with blender and sieved before analyses were carried out.

Phytochemical screening quantitative analyses

Phytochemical screening procedures were carried out using the methods [21][22]. The analyses involved the determination of the biologically active compounds available in the plant leaves which contribute to flavor, color, aromatic and medicinal characteristics of the leaves.

Test for Alkaloids

2.0 g of the powdered sample were pounded separately on a mortar. 0.2 g was boiled with 5 ml of 2% HCl on a steam bath for 5 min. The mixture was allowed to cool and filtered. The filtrate was shared in equal proportion into three test tubes, labeled A, B, and C. One milliliter (1 ml) portion of the filtrate was treated with 2 drops of the following reagents respectively. With Dragendroff reagent, a red precipitate

indicated the presence of alkaloids .On treatment with Meyer's reagent, a creamy white colored precipitate indicated the presence of alkaloid [22, 23, 24] .

Test for flavonoids

0.5 g of the milled sample of *A. wilkensisiana* was introduced into 10 ml of Ethylacetate and heated in boiling water for 1 min. The mixture was then filtered and the filtrate used for the following tests. Four milliliters (4 ml) was then filtered and the filtrate shaken with 1 ml of Aluminium chloride solution and kept .Formation of a yellow color in the presence of 1 ml dilute Ammonia solution, indicated the presence of flavonoids [24].

Preparation of the aqueous extracts of *A. wilkensisiana*

100 g of the powdered sample was soaked in 250 ml of distilled water for 48 hr. The mixture was filtered with muslin cloth and later with filter paper (Whatman No.1). The filtrate was emptied into a conical flask and evaporated to dryness at a temperature of 30°C.

Experimental design

Twenty-five male and female wistar strain of albino rats, weighing 150-180 g used for the study were purchased from the animal house of the Department of Zoology, University of Nigeria, Nsukka. They were divided into five groups of five animals each and kept in separate cages.

All animals were fed commercially formulated rat feed and water *ad libitum* and were allowed to acclimatize for two weeks. Their cages were cleaned daily, food and water changed daily.

Induction of Carbon tetrachloride (CCl₄) hepatocellular damage

Carbon tetrachloride (CCl₄) has been one of the most intensively studied hepatotoxicants up to date and provides a relevant model for other halogenated hydrocarbons used widely for this purpose. It consistently produce liver injury in many species of animals, including non-human primates and man [25][26] . It is well known to be converted by Cytochrome P₄₅₀- mixed function oxygenases in the smooth endoplasmic reticulum of the liver into toxic metabolites, mainly into trichloromethyl radical(CCl₃ ·). This free radical in the presence of oxygen may cause peroxidation of lipids on target cells and membranes, causing extensive liver damage. Seven days after acclimatization, the animals were distributed into five groups as specified above.

Group 1 served as normal control group (NC), which received food and water only throughout the experiment.

Group 2, served as the intoxicated control group (IC), which received food and water *ad libitum* and carbon tetrachloride (0.2 ml/kg in liquid paraffin (1:1) on day 7

Group 3 served as the intoxicated and treated group (ICT), that received food and water *ad libitum*, aqueous extract of *A. wilkensisiana* (100 mg/kg) and (CCl₄ 0.2 ml /kg in liquid paraffin(1:1) on day 7.

Group 4 received food and water *ad libitum*, aqueous extract of *A. wilkensisiana* (100 mg/kg of animals) ml /kg in liquid paraffin(1:1) on day 7.

Group 5 received food, water *ad libitum*, Sylamarin (50 mg/kg) and Carbon

tetrachloride (CCl₄)(0.2 mg/kg in liquid paraffin (1:1) on day 7

At the end of the experiment after 24 hr from the last treatment; the animals were anesthetized and sacrificed. Blood samples were collected from their heart by cardiac puncture and serum separated and used for assay of liver marker enzymes as well as other related parameters. Such parameters include-Aspartate aminotransferase(AST),Alanine transaminase (ALT), Alkaline phosphatase (ALP), total bilirubin, total protein (TP).

Determination of biochemical markers

Estimation of alanine transaminase

Alanine transferase was determined by the colorimetric method [27] . Alanine transaminase catalyzes the transfer of the amino group L-Alanine and α -ketoglutarate to form pyruvate and glutamate. The pyruvate formed is then reacted with 2,4-diphenylhydrazine to form pyruvate hydrazine which in alkaline medium (NaOH), gives a reddish brown color. The absorbance is read in a spectrophotometer at 546 nm. The reaction is shown below:

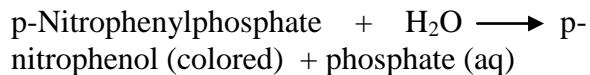


Alanine transaminase and Aspartate transaminase were determined by the colorimetric methods of

Estimation of alkaline phosphate

The determination of Alkaline phosphatase activity was carried out by employing the colorimetric method of [28]. The principle is based on the ability of the enzyme Alkaline phosphatase to catalyze the breakdown of the substrate-p nitrophenylphosphate to form

a colored complex, p-nitrophenol. The equation of the reaction is shown below:



Estimation of total bilirubin

Total bilirubin estimation was carried out by the colorimetric method described [29]. Total bilirubin is determined in the presence of caffeine, which releases albumin bound bilirubin by the reaction of diazotized sulphanilic acid.

Estimation of total protein

Total protein was estimated employing the Biuret method. Cupric ions in alkaline solution; interact with protein- peptide bonds resulting in the formation of a colored complex.

Four (4) clean and oven dried test-tubes were labeled as reagent blank, standard, and sample blank. 0.02 ml of standard reagent was also added to the tube labeled standard and 0.02 ml of serum was added to the tubes labeled sample and sample blank respectively.

1.0 ml of R1 (Biuret reagent) was added to the tubes labeled reagent blank, standard and sample and 1.0 ml of R2 (Blank reagent) was added to the tube labeled (Sample blank). The mixture was thoroughly mixed and incubated for 30 minutes at 20-25 °C. The absorbance of the sample (A sample) and of the standard (A standard) were read at 546 nm against the reagent.

$$\text{Total Protein Concentration} = \frac{\text{A (standard)}}{\text{X (std concentration)}}$$

$$\text{A (standard)}$$

Estimation of albumin

Bromocresol green (BCG) method was used for albumin estimation. Three clean and oven dried test- tubes were labeled as reagent blank, standard and sample. Into the labeled tubes, 0.01 ml of distilled water was added to the reagent; 0.01 ml of the standard reagent, to the standard and 0.01 ml of the sample. Then, 3.0 ml of BCG reagent was added to the three labeled, the mixtures were mixed thoroughly and incubated for 20 min at 20-25°C or incubated for 1 min at 37°C, and the absorbance of the sample (A sample) and the standard (A standard) were read at 578 nm against the reagent blank.

$$\text{Albumin concentration} = \frac{\text{Absorbance of sample X Conc. of standard}}{\text{Absorbance of standard}}$$

Estimation of globulin

The globulin concentration was estimated by calculation using the formula or relationship below

$$\text{Globulin concentration} = \frac{\text{T-Protein concentration} - \text{Albumin concentration}}$$

Statistical analysis

Results of groups are calculated as Mean \pm SD and subjected to ONE –WAY analysis of Variance (ANOVA). The level of significance was fixed at $p=0.05$. Analysis was done using SPSS, V.17.0 on Microsoft Excel Platform.

Results and Discussion

The results of all assays and analyses are presented in tables 1 and 2 respectively.

Table 1 shows the phytochemical composition of *A. wilkensisiana* leaves extract. The presence of the following

phytomedicines, such as, alkaloids, tannins, flavonoids and saponins were quantified. These may have contributed to the hepatoprotective effects of the plant leaves. In table 2, the effect of the aqueous extracts on various liver enzymes and proteins were determined. The effect of aqueous extracts on plasma alanine transaminase (ALT) shows that ALT activity in carbon tetrachloride intoxicated animals was elevated significantly at $p \leq 0.05$ (33.8 ± 1.92 U/L) when compared with normal control group (24.4 ± 1.44 U/L). The effect of aqueous extract of *A. wilkensisiana* on the test group (25.4 ± 0.054 U/L) and the aqueous extract of *A. wilkensisiana* on the intoxicated treated group (27.8 ± 0.83 U/L) and the Sylimarin (positive control) (24.0 ± 1.57 U/L). This is however very effective in protecting the hepatocytes against CCl_4 induced liver damage.

The effect of *A. wilkensisiana* leaves extracts on plasma Aspartate amino transferase (AST) shows that AST activity in CCl_4 -intoxicated animals were highly elevated at $p \leq 0.05$; the value being (79.6 ± 2.30 U/L) when compared with the normal control group (60.2 ± 2.68 U/L). Equally, when compared with *A. wilkensisiana* control (test group) (66.4 ± 1.14 U/L), the *A. wilkensisiana* treated (intoxicated group) (70.6 ± 2.07 U/L), and the Sylimarin (positive group) (63.6 ± 1.14 U/L). The plant extract was very effective in protecting the liver hepatocytes against carbon tetrachloride induced liver damage.

The effect of aqueous extract of *A. wilkensisiana* on plasma alkaline phosphatase (ALP) activity of CCl_4 intoxicated animals was significantly elevated at $p \leq 0.05$, the value being (8.44 ± 0.40 U/L) when compared with normal control (4.64 ± 0.23 U/L), the aqueous extract control (test group) (5.50 ± 0.022 U/L), the aqueous

extract treated (intoxicated group)(7.22±0.19U/L) and the Sylimarin positive control group(6.40±0.20 U/L). The aqueous extract was very effective in protecting hepatocytes against CCl₄ induced damage.

The effect of aqueous extract on total bilirubin concentration .The effect of CCl₄ intoxication on total bilirubin shows significant elevation at $p \leq 0.05$ in the intoxicated group. The value being (28.94±0.87µmol/L) when compared with normal control group (15.14±0.17µmol/L).The aqueous *A. wilkensisiana* control (test group) (18.54±1.40µmol/L), the effect of the extract on the treated group (intoxicated treated group) (24.12±1.91µmol/L), the effect on the aqueous *A. wilkensisiana* extract on the control (test group) when compared with the Sylimarin (positive control)(17.05±0.16 µmol/L),there was no significant difference on the values at $p \leq 0.05$ of *A. wilkensisiana* test group and the Sylimarin positive group.

The effect of aqueous extract of *A. wilkensisiana* on plasma total protein concentration. The effect of CCl₄ induced hepatotoxicity on total plasma protein concentration, result shows decreased total protein concentration at $p \leq 0.05$ in the intoxicated group; value being (52.86±1.04 g/L), when compared with the normal control group (75.58±1.18 g/L) . Also, the effect of aqueous extract of *A. wilkensisiana* on the test control group, the result is (71.78±1.30 g/L) and the *A. wilkensisiana* treated group (intoxicated group and treated), the result is (60.88±0.96 g/L). The aqueous extract was able to maintain normal homeostasis and protein balance in the intoxicated animals.

The effect of the extract on plasma albumin concentration .The effect of CCl₄ on the

intoxicated animals shows that, the albumin concentration was significantly decreased at $p \leq 0.05$, the value was (35.08±0.53 g/L), when compared with the normal control group (41.22±1.75g/L); the aqueous extract treated group (test control) (38.54±0.95g/L) and the aqueous extract treated group (intoxicated group) (37.20±0.25 g/L). The function of albumin in maintaining the osmolarity of blood and oncotic pressure, hence, the role of the aqueous extract in restoring these functions in intoxicated animals, is a measure of the hepatoprotective effectiveness of the leaves of *A. wilkensisiana*.

The effect of the aqueous extract of *A.wilkensisiana* on plasma globulin . The effect of the aqueous extract of *A. wilkensisiana* after intoxication is shown here when comparison is made between the plasma globulin concentration in the intoxicated and treated and the intoxicated (test group).Results of CCl₄ intoxication shows a significant decrease on the level of globulin at $p \leq 0.05$, value being (17.11±0.77 g/L). When compared with the normal control group (34.34±0.82 g/L), the test control group (33.24±1.53 g/L) and the treated intoxicated group (23.62±1.08 g/L). The aqueous leaves extract of *A. wilkensisiana* was able to ameliorate the intoxication and restore the hepatocytes to their normal configuration. The intoxication by CCl₄ compromised the immune system, leading to decreased synthesis of plasma immunoglobulins and lipoproteins as a consequence of lipid peroxidation [26]

Carbon tetrachloride (CCl₄) is one of the most commonly used hepatotoxins in the experimental study of liver diseases [30]. In the study, carbon tetrachloride impaired functions of liver cell was observed. This is evidenced by decreased serum total protein and albumin concentration and increased bilirubin concentration.

Table.1 Results of quantitative phytochemical analyses of leaves of *Acalypha wilkessiana*

Phytochemicals	alkaloids	Tannins	Saponins	Flavonoids
% composition	0.58±0.00	0.52±0.00	1.37±0.01	0.17±0.09

The values in the table are the Mean ± SD from triplicate determinations.

Table.2 The Effects of aqueous extract of *Acalypha wilkessiana* on liver enzymes and other parameters at a concentration of 100 mg/Kg

Grp s	Treat-ment	AST	ALP	ALT	T.Protein	Albumin	Globulin	T. Bilirubin
1	Normal Control	60.2±2.68 ^a	4.64±0.03 ^a	24.4±1.14 ^a	75.58±1.18 ^a	41.22±1.75 ^a	34.34±1.75 ^a	15.14±0.17 ^a
2	Intoxicated control	79.6±2.30 ^b	8.44±0.40 ^b	33.8±1.92 ^b	52.86±1.04 ^b	35.08±0.53 ^b	17.11±0.77 ^b	28.94±2.87 ^b
3	Intoxicate test	70.6±2.07 ^c	7.28±0.19 ^c	27.8±0.83 ^c	60.88±0.96 ^c	37.20±0.25 ^c	23.62±1.08 ^c	24.12±1.9 ^c
4	Test Control	66.4±1.14 ^d	5.50±0.22 ^d	25.4±0.54 ^a	71.78±1.30 ^d	38.54±0.95 ^{c, d}	33.24±1.53 ^a	18.54±1.40 ^a
5	Positive control	63.6±1.14 ^a	6.40±0.20 ^c	24.0±1.58 ^a	74.24±1.13 ^a	39.7±0.91 ^d	34.46±0.99 ^a	17.05±0.16 ^d

All values in the table were compared with each other at P=0.05. Values with different superscripts vertically differ statistically (P≤0.05)

This is in agreement with the report in which liver damage impaired liver functions resulting in decreased total protein and albumin concentrations in serum [31]. In this study, increases in ALT, AST and ALP activities indicated damage to hepatic cells/tissues, while elevations in total bilirubin, decreases in total protein and albumin concentrations showed impairment of hepatic functions. Most circulating proteins are synthesized in the liver and increased concentrations indicate synthetic ability of the liver. The prevention of carbon tetrachloride-induced elevation of plasma aspartate and alanine transaminases ; and alkaline phosphatase activities, and plasma bilirubin levels in animals treated with aqueous extract of the leaves of *A wilkessiana*, shows its ability to restore normal functional status of the intoxicated liver, in addition to protecting the liver and other tissues subsequent to carbon tetrachloride hepatotoxicity. The mechanism by which the extract produces its hepatoprotective activity is not understood.

However, it is possible that β-sitosterol, a constituent of the extracts of the leaves of *A. wilkessiana*, is at least partially responsible for hepatoprotectiveness of the animals. Earlier, [33] had reported that β-sitosterol was the antihepatotoxic principle in *Sambucus formosana*. Several studies on different parts of *A. wilkessiana* have demonstrated the presence of tannins, triterpenoids, flavonoids, gallic acid, corilagin and geranin[32][33][34][35]. The role of antioxidants in preventing various human diseases by preventing oxidative stress and damage in biological tissues have been demonstrated in many experiments. In this study, flavonoids have been reported to be present in *Acalypha wilkessiana*. This is consistent with the findings of [20] [31]. The efficacy of any hepatoprotective agent is essentially dependent on its ability to reduce the harmful effects or maintain normal hepatic physiology that has been disturbed by toxins [36]. Flavonoids possess antioxidant activity and can however inhibit Cytochrome P450 aromatase [37][38] and

may also inhibit lipid peroxidation by exerting membrane stabilizing action [38]. It could also be suggested that the presence of flavonoids in *A. wilkensisiana* could be responsible for the hepatoprotective activity of the leaves aqueous extract [20][31]. Many workers have reported on the hepatoprotective activities of *A. wilkensisiana* leaves extract as being able to reverse damage afflicted to liver cells by hepatotoxic agents like Carbon tetrachloride [39,40, 41].The hepatoprotective activity of the aqueous extract of *A. wikessiana* can nonetheless be attributed to the preponderance of resident phytochemicals in the leaves which act synergistically to elicit their effects[42].

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