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# The Hepatoprotective Effect of *Acalypha wilkesiana* Muell Arg. Leaves on some Biochemical Parameters in Wistar Albino Rats


**O. J. Sule[1*], A. R. Abdu[2] and I. Elekwa[3]**

*[1]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa-State, Nigeria.*
*[2]Department of Medical Microbiology and Parasitology, Faculty of Basic Medical Sciences, College of Health Sciences, Niger Delta University, Wilberforce Island, Amassoma, Bayelsa-State, Nigeria.*
*[3]Department of Biochemistry, Faculty of Biological and Physical Sciences, Abia State University, Uturu, Abia State, Nigeria.*


***Authors' contributions***

*This work was carried out in collaboration between all authors. Author OJS designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author ARA managed the literature searches, analyses of the study and author IE wrote the final draft of the manuscript. All authors read and approved the final manuscript.*


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## ABSTRACT


The effects of *Acalypha wilkesiana* leaves on biochemical indices in wistar albino rats were studied. Activity of total Protein and bilirubin (direct and indirect bilirubin) in the serum were determined colorimetrically, while lipid peroxidation products, thiobarbituric acid-reactive substances (TBARS), were measured in liver homogenate. Histopathological studies of the liver of test and control animals were also, carried out. Rats in group1 were fed with 100% food and administered $CCL_4$, while those in groups (2, 3 and 4) were pretreated with 10%, 30%, and 50% of dried leaves of *A. Wilkesiana* respectively. Rats in group5 were pretreated with 30% *A. wilkesiana* without administration of $CCL_4$ (positive control), while rats in group6 were fed with 100% food without



**Corresponding author: Email: j_sule@yahoo.com;*

$CCL_4$ administration (general control). The $CCL_4$ administered (0.5 ml/kg body weight in 0.5 ml olive oil intraperitoneally) on the 28$^{th}$ day of study caused significant increases ($p< 0.05$) in the levels of biochemical indices (malondialdehyde, total bilirubin, direct bilirubin and indirect bilirubin), only the total protein levels significantly decreased ($p< 0.05$), when compared with the controls. Pre-treatment of rats with 10% and 30% *A. wilkesiana* resulted in significant decreases ($p< 0.05$) in the levels of these biochemical indices, only the levels of total proteins significantly increased in a dose dependent manner. These biochemical indices also significantly increased in rats group 4 pretreated with 50% *A. wilkesiana* when compared with the controls. Histopathology of the liver showed reduced level of injury in pretreated rats while; those not pretreated were presented with varying degrees of injuries. The study suggests that at low doses, *A. wilkesiana* possess hepatoprotective ability, while it could be hepatotoxic at high doses.

*Keywords: Histopathological; carbon tetrachloride; hepatoprotective; Acalypha wilkensiana.*

## 1. INTRODUCTION

The liver plays a significant role in biochemical and physiological processes. Physicians have known this from ancient times, as is evident from descriptions in the earliest medical treatises (Ibn-e-Sina) [1]. All substances absorbed by the gastrointestinal tract pass through the hepatic system before entering the circulation. This makes the liver a focal point of metabolic activities and hence its exposure to injury. In various studies, Dean et al. [2] and Aruoma [3,4], reported that lipid peroxidation and protein oxidation are involved in the aetiology of several human diseases which includes atherosclerosis, ischemia-reperfusion injury, ageing, and liver-related diseases. According to Lin et al. [5], Shenoy et al. [6], and James et al. [7], the most widely used animal models for the study of the hepatocurative or preventive effect of many medicinal plantsare paracetamol- and $CCl_4$-induced hepatitis. Investigations by various researchers have shown that lipid peroxidation and protein oxidation play a major role in the development of diseases Recknagel, [8] Fleurentin and Joyeux, [9], Vuletich and Osawa, [10], Michael et al. [11]. Thus, the inhibition of these oxidation phenomena may be important in the alleviation of the resulting diseases. In the Nigerian folk medicine, several numbers of plants have been reportedly used for the treatment of hepatitis and other liver related-diseases Mongbet, [12], Moundipa et al. [13]. In their study, Kumars and Mishra [14] documented the hepatoprotective activity of fumaric acid from *Sida cardifolia*. In addition, ursolic acid, which occurs in many plants, with hepatoprotective properties was documented by Shukla et al. [15] and Saraswat et al. [16,17]. Since toxic hepatitis is often associated with the oxidative destruction of lipids and proteins, the plants used by the herbal medicine practioners in Nigeria to alleviate liver-related diseases may contain compounds that protect lipids and proteins from oxidation since such compounds have been suggested as prophylactic agents, Aruoma [18]. *Acalypha wilkesiana* Muell Arg belongs to the family Euphorbiaceae (spurge family). Its other names include *A. amentacea* and *A. tricolor*, while its common names are copperleaf, Joseph's coat, fire dragon, match-me-if-you-can. A popular outdoor plant that provides color throughout the year, although it is also grown indoors in a container plant. They are found all over the world most especially in the tropics of Africa, including Nigeria, America and Asia. *A. wilkesiana* has been reported to have antimicrobial properties, Ogundaini [19], Akinyemi et al. Oladunmoye [20], antihypertensive properties, Ikewuchi et al. [21], antidiabetic activity Atef, [22], amongst others. However, there have not been adequate scientific data to support the hepatoprotective potentials of *A. wilkesiana* and provide information on its mechanism of action. In this study, we have investigated the ability of *A. wilkesiana* leaves to protect the liver against $CCL_4$-induced hepatocellular damage and oxidative stress in wistar rats.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

Plant under investigation (*A. wilkensiana* fresh leaves) was collected from the herbal garden of Department of Pharmacognosy, Faculty of Pharmacy, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The plant was promptly identified and authenticated by a Taxonomist, Prof S.K. Adesina of Department of Pharmacognosy. The leaves were removed from the stems, washed thoroughly with distilled water to remove adulterants. It was dried under natural conditions for two weeks, ground into powder using an electric blender and stored in airtight containers.

## 2.2 Animals

Male wistar albino rats (30) weighing between (180–190) g were purchased from the animal house unit of the Department of Pharmacology, College of Health Sciences, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria. The rats were divided into stainless metabolic cages comparising of five rats per cage. They were observed under a 12- hour/12-hour light/dark cycle in a well-ventilated room at 26–27°C. They were fed with standard rat chow purchased from Bendel Feed and Flour Mill Limited, Ewu, Benin City, Nigeria and water *ad libitum*. This experimental protocol was in compliance with internationally accepted guidelines for animal use and care (EEC Directive of 1986; 86/09/EEC; National Institutes of Health publication 85-23, revised 1985).

## 2.3 Induction of Hepatocellular Damage/ Experimental Design

The induction of hepatocellular damage/experimental design was carried out as earlier outlined by Sule, et al. (2012a and b) [23,24]. In brief, thirty (30) wistar albino rats already obtained were acclimatised for a period of seven days. They were weighed and then divided into six groups with five animals in each group. Rats in groups (2-5) were fed with formulated food substances for a period of twenty eight (28) days, while rats in groups (1 and 6) fed on 100% food for the same number of days. Rats in groups (1-4) were then injected with 0.5 ml/kg $CCL_4$, dissolved in 0.5 ml olive oil on the $29^{th}$ day and allowed free access to water. They were then fasted for 24hrs. Detail of the experimental grouping is shown below.

Group1 : Rats were fed with 100% food and served (negative control)
Group 2 : Rats were pretreated with 10% *Acalypha wilkesiana* + 90% food (w/w)
Group 3 : Rats were pretreated with 30% *Acalypha wilkesiana* + 70% food (w/w)
Group 4 : Rats were pretreated with 50% *Acalypha wilkesiana* + 50% food (w/w)
Group 5 : Rats were pretreated with 30% *Acalypha wilkesiana* + 70% food (w/w) (positive control)
Group 6 : Rats were fed with 100% food (w/w) and served (general control)

## 2.4 Sample Collection

Twenty four hours after injection of $CCL_4$, the rats were anaesthetized in chloroform saturated chamber, scarified and blood samples were obtained through cardiac puncture, in non-heparinized tubes, centrifuged at 3000 rpm for 10 minutes. Blood sera were then collected and stored at 4ºC priorto determination of biochemical parameters (total protein, total bilirubin). The liver from both control and test animals were removed and weighed to the nearest 0.01g. The livers were washed with ice-cold saline and a 10% homogenate was prepared in phosphate buffer (pH 7.0). The homogenate was centrifuged at 3000 rpm for 10 min at 4ºC and the supernatant was used for the estimation of TBARS (MDA). The pieces of liver were preserved in 10% formaldehyde solution for histological study using Haematoxylin and Eosin (H&E) stains

## 2.5 Biochemical Assay

Totalprotein was determined by colorimetric method (Biuret method), as modified by Gornallet et al. [25], 1994 method. Bilirubin was estimated by colorimetric method of Jendrassik and Grof, [26]. Lipid peroxidation products [thiobarbituric acid-reactive substances (TBARS)] was according to Hunter et al. [27] and Buege and Aust [28] as modified by Gutteridge and Wilkins, [29].

## 2.6 Histopathological Examination

The pieces of liver were preserved in 10% formal saline for histopathological examination according to the method of Baker and Silverton, [30]. In brief, tissues were processed using automatic tissue processor, embedded in paraffin wax and further stained with Haematoxylin and Eosin (H&E) for microscopy.

## 2.7 Statistical Analysis

The results were statistically analysed using one-way analysis of variance (ANOVA) followed by Student-Newman-Keuls test. P values<0.05 were considered significant.

## 3. RESULTS

The experiment lasted for a period of four weeks (30 days). One death was recorded in animals in group4 (pre-treated with 50% *A. Wilkesiana*, weighing 196.0g), on the seventeenth ($17^{th}$) day of the experiment. There was no death recorded in other pre-treated groups and controls. There was significant decrease in the water and food consumption between the rats in group 4 (pre-treated with 50% *A. wilkesiana*) and rats in groups 1,2,3,5 and 6. The rats in group 4 appeared emaciated and inactive compared with rats in other pre-treated groups and controls.

### 3.1 Effect of *A. wilkesiana*on some Biochemical Parameters

The results of some biochemical indices shown in Table 1, indicated significant increases ($p \leq 0.05$) in the levels of total proteins (TP) in rats in groups 2 and 3 that were pre-treated with 10% and 30% *Acalypha wilkesiana* respectively, when compared with rats that were treated with $CCl_4$ only (group 1). Whereas, significant decreases ($p \leq 0.05$) were obtained in rats in group 4, that were pre-treated with 50% *Acalypha wilkensiana* when compared with rats in groups 2 and 3 (fed with 10% and 30% *Acalypha wilkensiana* respectively). However, the total protein levels were still significantly decreased ($p \leq 0.05$), when compared with the total proteins levels in rats not administered with $CCl_4$ (groups 5 and 6) i.e rats pre-treated with 30% *Acalypha wilkensiana* without administration of $CCl_4$ (positive control) and general control respectively.

Rats administered with $CCl_4$ only (group 1) showed significant increase ($p \leq 0.05$) in total bilirubin (TB) levels. However incorporation of 10 % and 30% *Acalypha wilkensiana* (diet groups 2 and 3 respectively) significantly decreased ($p \leq 0.05$) the total bilirubin (TB) levels, when compared with rats treated with $CCl_4$ only (group 1). The total bilirubin levels in rats pre-treated with 50% *Acalypha wilkensiana* (group 4) also increased significantly ( $p \leq 0.05$) when compared with rats in groups 2 and 3 (fed 10% and 30% *Acalypha wilkensiana* respectively). Levels of direct and indirect bilirubin were significantly decreased ($p \leq 0.05$) in rats in groups 2 and 3, when compared with rats group1 (that received $CCl_4$ only). Whereas rats in group 4 that were pre-treated with 50% *Acalypha wilkensiana* showed significant increase ($p \leq 0.05$), when compared with rats in groups 2 and 3 (pre-treated with 10% and 30% *Acalypha wilkensiana* respectively). Malondialdehyde (MDA) levels also increased significantly ($p \leq 0.05$) in rats group1 (that received $CCl_4$ only). However, there were significant reduction ($p \leq 0.05$) in the levels of MDA in rats in groups 2 and 3, when compared with rats that received $CCl_4$ only. MDA levels also increased significantly ( $p \leq 0.05$) in rats pre-treated with 50% *Acalypha wilkensiana*, when compared rats pre-treated with 10% and 30% *Acalypha wilkensiana* (groups 2 and 3 respectively). However, the least levels of MDA were recorded in rats in groups 5 and 6 i.e. (30% *Acalypha wilkensiana* without $CCl_4$ (positive control) and general control respectively).

### 3.2 Histopathological Examination

Histopathological examinations of liver of rats were carried out to ascertain the effects of various treatments on the organs. Tissue slides of liver of rats in test and control groups were prepared and the results are as shown in Plates 1 through 6.

## 4. DISCUSSION

The need to investigate the hepatoprotective nature of *Acalypha wilkensiana* is due to the need to ascertain how well locally available plants in Nigeria can protect the liver from damage. The activities of some biochemical parameters; Total protein (TP), Total bilirubin (TB), Direct bilirubin (DB), Indirect bilirubin (IB) and malondialdehyde (MDA) were determined in experimental rats at the end of the feeding study. Carbon tetrachloride ($CCl_4$) is one of the most commonly used hepatotoxins in the experimental study of liver diseases. It has been established that $CCl_4$ is accumulated in hepatic parenchyma cells and metabolically activated by cytochrome-P450 dependent monooxygenases to form a trichloromethyl radical ($CCl_3^-$) Suja et al. [31]. These activated radicals bind covalently to the macromolecules and induce peroxidative degradation of membrane lipids of endoplasmic reticulum rich in polyunsaturated fatty acids. This leads to the formation of lipid peroxides. The lipid peroxidative degradation of biomolecules is one of the principal causes of hepatotoxicity of $CCl_4$ Balasubramanian et al. [32]. Thus, antioxidant or free radical generation inhibition is important in protection against $CCl_4$ induced liver lesions.

**Table 1. The effects of *A. wilkensiana* on some biochemical parameters in $CCl_4$ induced Hepatotoxicity**

| Group | treatments | Total bilirubin (µmol/L) | Direct bilirubin (µmol/L) | Indirect bilirubin (µmol/L) | Total protein (g/L) | MDA µmol/L × $10^{-5}$ |
|---|---|---|---|---|---|---|
| 1 | 100% FOOD+$CCl_4$ | $26.13^a$±0.20 | $14.76^a$±0.20 | $11.37^a$±0.20 | $40.25^a$±0.20 | $4.50^a$±0.01 |
| 2 | 90% FOOD+10% *A. wilkensiana* +$CCl_4$ | $13.70^b$±0.01 | $8.65^b$±0.01 | $5.05^b$±0.01 | $61.08^b$±0.20 | $3.38^b$±0.01 |
| 3 | 70% FOOD+30% *A. wilkensiana* + $CCl_4$ | $12.01^c$±0.01 | $7.56^c$ ±0.20 | $4.45^c$±0.20 | $64.60^c$±0.20 | $2.62^c$±0.01 |
| 4 | 50% FOOD +50% *A. wilkensiana* + $CCl_4$ | $21.95^d$±0.10 | $16.00^d$±0.20 | $5.95^d$±0.20 | $51.00^d$±0.30 | $3.74^d$±0.01 |
| 5 | 70% FOOD + 30% *A. wilkensiana*-$CCl_4$ (positive control) | $11.15^e$±0.10 | $6.85^e$±0.20 | $4.30^c$±0.20 | $68.56^e$±0.20 | $2.60^c$±0.01 |
| 6 | 100% FOOD - $CCl_4$ (General Control) | $9.20^f$±0.10 | $5.20^f$±0.10 | $4.20^c$±0.10 | $72.76^f$±0.10 | $2.60^c$±0.01 |

*Values are mean ± S. D for 5 replicates (n= 5), values are mean ±S.D for 4 replicates (n=4) in group 4, Means with different superscripts are significantly different at the 0.05 levels*

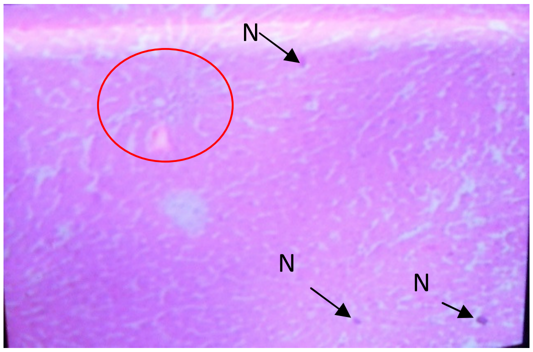


**Plate 1. Liver slide of rats administered 100% Food + $CCl_4$: mild Portal triaditis (Circle) and necrosis (N) of individual liver cells. H&E X40**

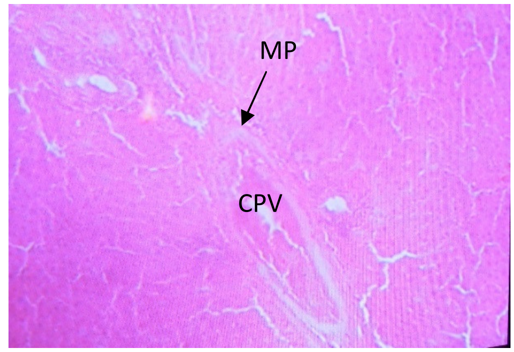


**Plate 2. Liver slide of rats administered 10% *Acalypha wilkensiana* +$CCl_4$: Normal liver architecture with Congestion of the portal vein (CPV) and mild portal triaditis (MPT). H&E X40**

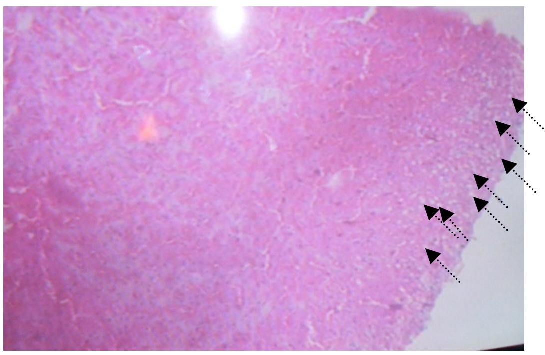

**Plate 3. Liver slide of rats administered 30% *Acalypha wilkensiana* + $CCl_4$: Normal liver architecture with mild microvesicular steatosis (Dot arrows) H&E X40**

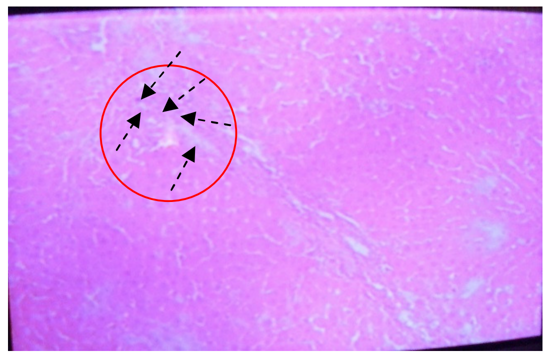

**Plate 4. Liver slide of rats administered 50% *Acalypha wilkensiana* +$CCl_4$: Spotty inflammation (Dash arrows) with mild portal triaditis (Red circle).H&E X40**

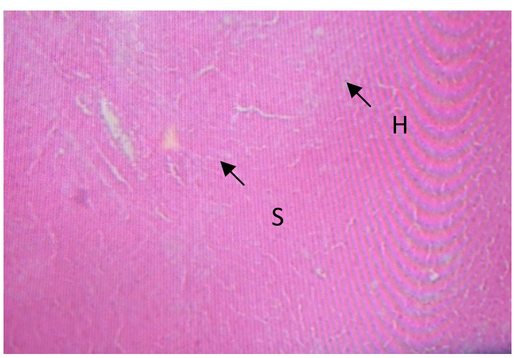


**Plate 5. Liver slide of rats administered 30% *Acalypha wilkensiana* – $CCl_4$ (positive control): Normal liver architecture. H= Hepatocyte, S= Sinusoid. H&E X40**

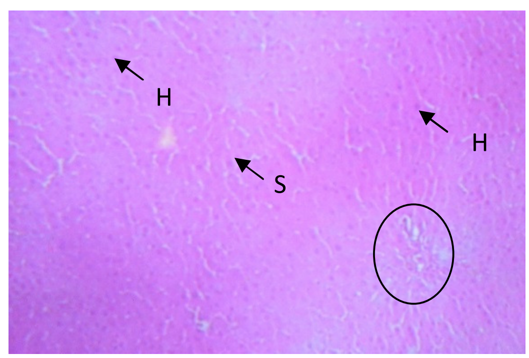


**Plate 6. Liver slide of rats administered 100% Food – $CCl_4$ (General control): Normal Liver Architecture. H= Hepatocyte, S= Sinusoid, Circle= Portal traid. H&E X40**

The efficacy of any hepatoprotective drug is essentially dependent on its ability to reduce the harmful effects or maintain the normal hepatic physiology that has been distributed by a heptotoxin Kuppuswamy et al. [33]. In this study, there were significant increases in the levels of total bilirubin, direct bilirubin, indirect bilirubin and malondialdehyde in rats not pretreated with *Acalypha wilkensiana* (group1) and levels of total proteins also, significantly decreased in rats in group 1.( administered $CCl_4$only). Such elevation is indicative of liver injury Edward et al. [34] Patrick-Iwuanyanwu et al. [35]. From this present study, pretreatments of rats with *Acalypha wilkensiana* not only decreased the $CCl_4$ induced elevated levels of total bilirubin( direct bilirubin and indirect bilirubin) and MDA, but also significantly increased levels of total proteins in groups 2 and 3 rats . This suggests the maintenance of structural integrity of hepatocytic cell membrane or regeneration of damaged liver cells by inhibiting lipid peroxidation activity of *Acalypha wilkensiana*. *Acalypha wilkensiana* has been reported to contain tannins,triterpenoids flavonoids, gallic acid, corilagin and geranin Adesina et al. [36], Gutierrez-Lugo et al. [37]. The herb may have exhibited hepatoprotective activity due to its antioxidant properties attributable to triterpeniods. The outcome of this study validates the earlier observation of Babalola et al. [38], that triterpenoids fraction of *V. amygdalina* leaf extract ameliorates carbon tetrachloride-induced hepatotoxicity in rats. The decreased levels of serum total proteins in rat group1 (administered $CCl_4$ only), is an indication of hepatotoxicity Abatan et al. [39]. Since protein degradation seems to occur by distinct mechanism, the present study corroborates suggestions made by Davies and Goldberg [40] that the herb, *Acalypha wilkensiana* has strong protein oxidation inhibitory potency). Thus, *Acalypha wilkensiana* may be a good source of medicines against diseases in which lipids and proteins oxidation are involved, such as toxic hepatitis Njayou et al. [41]. Interestingly, rat group 4 that were pretreated with 50% *Acalypha wilkensiana* showed elevated levels of total bilirubin, direct bilirubin, indirect bilirubin and malondiadehyde while corresponding decreased levels of total proteins was recorded. The elevated levels of these parameters observed in rat group4, rather suggest a physiological dysfunction arising from overdose. This finding is supported by the earlier reports of Ojiakor and Nwanjo, [42] and Ojekale et al. [43] while investigating the hepatoprotectivity of *Veronia amygdalina* and *Cissus populnea* respectively. Their findings showed that these plants are hepatoprotective at lower doses but hepatotoxic at higher doses. It can be suggested based on the reports that the dose of *Acalypha wilkensiana* (50%) used in this group 4 is above the safe dose for rats. The dose might have been too toxic to the rats to cause the death recorded in the experimental group. This agrees with the earlier report by Oyelami et al. [44], who reported an adverse reaction to ointment of *Acalypha wilkensiana* against the treatment of some superficial fungi diseases such as *Tinea corporis, Tinea pedis, Pityraiasis versicolor* and *Candida intetrigo*. Histopathologically, portal triaditis and necrosis of individual liver cells was observed in group1 rats (administered $CCl_4$ only). There are congestions of the portal veins and mild portal triaditis with normal liver architecture in rats pretreated with 10% *Acalypha wilkensiana*. Normal liver architecture with mild microvesicular steatosis was observed in rats pretreated with 30% *Acalypha wilkensiana*. However, there are spotty inflammations with mild portal triaditis in the rats group pretreated with 50% *Acalypha wilkensiana*. These changes could be because of biochemical changes that occurred in the liver cells. As observed in this study, increases in the biochemical indices are secondary to the liver dysfunction and are associated with disruption of cellular architecture. This is in agreement with the report made by Obika and Noguchi [45] and earlier findings of Krishan and Veena [46] and Kiceniuk et al. [47], who observed that xenobiotics caused elevation of biochemical indices, resulting to severe liver damage.

## 5. CONCLUSION

In conclusion, it was found that *Acalypha wilkensiana* exhibited a potent protective effect against hepatotoxicity caused by $CCl_4$ administered to the albino rats, where it produced marked amelioration of the liver histological perturbations in $CCl_4$-intoxicated animals. However, further studies including clinical trials need to be carried out to ascertain the safety of these compounds as good alternative to conventional drugs in the treatment of liver diseases.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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