Original Research Article

Effect of *Acalypha wilkesiana* on Oxidative Stress and Histopathology of Liver and Kidney in Alloxan-induced Diabetic Albino Rats

ABSTRACT

This study investigated the effect of *Acalypha wilkesiana* on oxidative stress biomarkers as well as liver and kidney histopathology. 54 Albino rats were divided into 6 groups of 9 animals each including group 1 animals received only feed and water ad libitum; group 2 received 160mg/kg alloxan by the intraperitoneal route; group 3 received 0.07mg/kg of glibenclamide follow alloxan induction, while animals in groups 4, 5 and six received the aqueous leaf extract of *Acalypha wilkesiana* at doses of 100mg/kg, 200mg/kg and 400mg/kg following the induction. Three animals from each group were anaesthetized with diethyl ether and blood samples were collected by cardiac puncture on days 5, 10 and 15 into sample bottles containing anticoagulant for evaluation of oxidative stress parameters. Liver and Kidney samples were also collected from these animals and transferred into 10% formalin solution for the histological studies. Results revealed a significant increase in oxidative stress marked by decrease in oxidative stress markers such as CAT, SOD, GSH, GPx and increase in MDA values and pathological changes of liver and kidney following alloxan administration when compared with normal control. These changes were reversed by the extract in a dose dependent manner.

Key words: Diabetes, *Acalypha wilkesiana*, Cooper Leaf, Oxidative Stress, Histopathology, Liver, Kidney

INTRODUCTION

"Plants are used for food and remedy for ailments" [1]. "Nutrition and health are interconnected and many plants are consumed as food in order to enhance health" [2,3]. "Native plant species used in daily diets such as spices, condiments, and additives are associated with a lowered incidence of degenerative diseases. These protective effects are considered to be related to the antioxidants contained in them" [4,5]. "*Acalypha wilkesiana*, commonly called Irish petticoat, Jacob's coat and Copper leaf (local name), belongs to the

family Euphorbiaceae, subfamily Acalyphoideae, tribe Acalypheae. The plant is native to the south pacific islands (Bismarck Islands, Fiji, Vanuatu)" [6]. "The plant has been reported to contain sesquiterpenes, monoterpenes, triterpenoids, and polyphenols" [7]. "The leaves reportedly contain saponins, tannins, anthraquinones and glycosides" [8]. The plant has antimicrobial and antifungal properties and in traditional medicine, the leaves are eaten as vegetables in the management of hypertension, a risk factor for cardiovascular diseases, being a diuretic plant. However, the scientific basis for its use in the management of risk factors of cardiovascular diseases has not been rationalized. Thus, the aim of this study was to evaluate the effects of oral administration of extracts of *Acalypha wilkesiana* leaves oxidative stress parameters and histology of the liver and kidney in alloxan-induced diabetic albino rats.

"Alloxan is one of the most prominent diabetogenic chemicals in diabetes research. It is a cytotoxic glucose analogue. In 1838, Wöhler and Liebig synthesized a pyrimidine derivative, which they later called alloxan" [9,10]. "In 1943, alloxan became of interest in diabetes research when Dunn and McLetchie reported that it could induce diabetes in animals as a result of the specific necrosis of the pancreatic beta cells" [11,12]. The resulting insulinopenia causes a state of experimental diabetes mellitus called "alloxan diabetes" [13]. In a study on the mechanism of alloxan-induced diabetes, Lenzen [14] reported that "alloxan has two distinct pathological effects. According to him, alloxan selectively inhibits glucose-induced insulin secretion through specific inhibition of glucokinase, the glucose sensor of the beta cell, and it causes a state of insulin-dependent diabetes through its ability to induce ROS formation, resulting in the selective necrosis of beta cells. He said these two effects were due to the specific chemical properties of alloxan, the common denominator being selective cellular uptake and accumulation of alloxan by the beta cell".

Oxidative stress is a "state where oxidative forces exceed the antioxidant systems due to loss of the balance between them" [15]. "Oxidative stress develops from an imbalance between

free radical productions and reduced antioxidant defenses" [16,17]. "Normal metabolism produces oxidant by-products (reactive oxygen species, ROS) which cause damage to DNA, proteins, and lipids which result in ageing and neurodegenerative diseases" [18]. "In normal physiological conditions, cells generate ROS which are quickly neutralized by glutathione (GSH)" [19]. "In fact, oxidative stress has been recognized to be involved in the pathophysiology of several chronic conditions including cardiovascular diseases, cancer, diabetes, arthritis, nephropathy, and skin ageing" [18,19]. "In addition, other studies have demonstrated the involvement of ROS in the etiology of liver diseases such as hepatitis, fibrosis, cirrhosis, and hepatocellular carcinoma" [20]. In other studies, Isirima and Okoroafor [21] and Ijeoma *et al*. [22] also reported oxidative stress-related liver injuries, while Isirima and Okoroafor [23] in another study reported oxidative stress-related kidney injuries. "Some of the anti-oxidant enzymes and parameters usually evaluated during oxidative stress study include Superoxide dismutases (SOD), reduced glutathione (GSH), catalase (CAT), glutathione peroxidase (GPX) and Malondialdehyde (MDA). Superoxide dismutases play a central role in the metabolism of reactive oxygen species. They greatly accelerate the dismutation of superoxide radicals" [24. The two major forms in humans are copper/zinc and manganese superoxide dismutase. All glutathione peroxidizes reduce hydrogen peroxide and alkyl hydroperoxides. The catalase activity is usually determined by its catalytic function [24]; it dismutates hydrogen peroxide in water and oxygen. Glutathione is a tripeptide (L-g-glutamyl-Lcysteinylglycine) that serves several essential functions within the cell. "The role of intracellular glutathione in the detoxification of xenobiotics and reactive oxygen species has been well established. The protective mechanism, results in an increased formation of intracellular glutathione disulphide, (oxidized form)" [25].

MATERIALS AND METHODS

Procurement of Animals

Animals used were two to three months old Albino rats. Fifty-four (54) healthy adult Albino rats with normal glucose level, weighing about 200g were used in the current experiment. All animals were left to acclimatize for two weeks before the commencement of the experiment. The animals were housed in a well-ventilated clean cages maintained under a 12-12hours light-dark cycle at a temperature of 23±3^{o}C throughout the experimental period. Drinking water and food were provided ad libitum to the animals, but the food was withdrawn 2hours before and 2hours after administration of the drugs to rule out the effect of food on the absorption of the drugs. All animals received human care according to the criteria outlined in the guide for the care and use of laboratory animals prepared by the National Research Council (US) Committee for the Update of the Guide for the Care and Use of Laboratory Animals [26]. The experimental study was conducted between 9am and 5pm.

Chemicals and Plant used

Fresh leave of *Acalypha wilkesiana* were sourced from within the Abuja part of the University of Port Harcourt environment. The plant was identified and authenticated in the department of Botany, University of Port Harcourt. The alloxan and glibenclamide were purchased from Gold Sparkle Pharmaco Nig. Ltd., 5, Aggrey Road, Port Harcourt, Rivers State of Nigeria. Distilled water was used as a vehicle for the preparation of the alloxan for intraperitoneal administration.

Plant Extraction

Plant tissue homogenization method as described by Amita and Shalini [27] was used in the extraction of the fresh plant part in distilled water. Wet, fresh plant parts were grinded in a blender to fine particles and a measured volume of distilled water was added into it and was

shaken vigorously for 5 - 10 minutes after which the extract was filtered. The filtrate was then centrifuged for clarification of the extract [28]. This was re-dissolved in the solvent to determine the concentration during administration process.

Experimental Design

In this study Albino rats were fasted overnight and were treated with 160mg/kg of alloxan by intraperitoneal injection to induce diabetes [29]. After 96 hours of alloxan administration, animals with blood glucose values of 11.1mmol/l and above were considered diabetic and were randomly divided into 5 groups of 9 animals each including group 1. Animals in group 1 received feed and water ad libitum without alloxan and served as control; those in group 2 were given no treatment following the induction and those group 3 received 5mg/70kg or 0.07mg/kg of glibenclamide. Animals in groups 4, 5 and six received the aqueous leaf extract of *Acalypha wilkesiana* at doses of 100mg/kg, 200mg/kg and 400mg/kg following the induction. Three animals from each group were anaesthetized with diethyl ether and blood samples were collected by cardiac puncture on days 5, 10 and 15 into sample bottles containing anticoagulant for evaluation of oxidative stress parameters. Liver and Kidney samples were also collected from these animals and transferred into 10% formalin solution for the histological studies.

Measurement of serum antioxidants activities and oxidative stress

Plasma levels of total antioxidant status (TAS) were determined using DPPH method (1, 1 diphenyl 2, picryl hydrazyl) [30], Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation [31], GSH was measured by the method of Beutler *et al.* [32], Plasma activities of superoxide dismutase (SOD) were determined using the method of Misra and Fridovich [33] and

measured at 480nm. Glutathione peroxidase (GPx) was assayed by the method proposed by Reddy *et al*. [34]. In the presence of the hydrogen donor pyrogallol or dianisidine, peroxidase converts H_2O_2 to H_2O and O_2. The coloured product formed was measured colorimetrically at 430nm. Catalase (CAT) was determined using direct colorimetric method of Sinha [35]. The method is based on the fact that dichromate is reduced to chromic acetate when heated in the presence of H_2O_2. The chromic acetate produced was measured colorimetrically at 570nm.

Histopathology Examination

The animals were anaesthetized with diethyl ether, dissected aseptically to remove the spleen which was then transferred into 10% chloroform and later trimmed down to a size of 2mm to 4mm thickness, to allow the fixative to readily penetrate the tissue. The tissues were exposed to different stages of processing by standard methods as described by Baker [36], including, fixation, dehydration, clearing, impregnation, embedding, sectioning and staining with hematoxylin and eosin (H&E) and finally mounting.

Method of Statistical Analysis

The results are presented as Mean ± Standard error of mean. Differences between means were assessed using Analysis of variance (ANOVA) using Dunnett method to assess any significant differences between the groups. Differences between groups at $p<0.05$ were considered to be statistically significant [37].

RESULTS

Table 1 shows the effect of *Acalypha wilkesiana* on Catalase (U/g) in diabetic Albino rats. It was observed that alloxan administration cause a significant decrease ($p<0.05$) in catalase

(2.10±0.02, 2.07±0.02 and 2.12±0.06) when compared to the normal (4.32±0.01), while administration of *Acalypha wilkesiana* to the animals significantly and dose dependently reversed this decrease. Table 2 shows the effect of *Acalypha wilkesiana* on SOD (U/mol) in diabetic Albino rats. It was observed that alloxan administration cause a significant decrease ($p<0.05$) in SOD (0.08±0.01, 0.07±0.01 and 0.05±0.01) when compared to the normal (0.25±0.01), while administration of *Acalypha wilkesiana* to the animals significantly and dose dependently reversed this decrease. Table 3 shows the effect of *Acalypha wilkesiana* on GSH (ug/ml) in diabetic Albino rats. It was observed that alloxan administration cause a significant decrease ($p<0.05$) in GSH (0.33±0.02, 0.31±0.01 and 0.29±0.01) when compared to the normal (0.87±0.01), while administration of *Acalypha wilkesiana* to the animals significantly and dose dependently reversed this decrease. Table 4 shows the effect of *Acalypha wilkesiana* on GPx (ug/ml) in diabetic Albino rats. It was observed that alloxan administration cause a significant decrease ($p<0.05$) in GPx (0.08±0.01, 0.07±0.003 and 0.06±0.003) when compared to the normal (0.16±0.02), while administration of *Acalypha wilkesiana* to the animals significantly and dose dependently reversed this decrease. Table 5 shows the effect of *Acalypha wilkesiana* on MDA (umol/ml) in diabetic Albino rats. It was observed that alloxan administration cause a significant increase ($p<0.05$) in MDA (0.97±0.01, 0.99±0.003 and 1.01±0.01) when compared to the normal (0.63±0.012), while administration of *Acalypha wilkesiana* to the animals significantly and dose dependently reversed this increase.

Table 1: Effect of *Acalypha wilkesiana* on Catalase (U/g) in diabetic Albino rats

Group	Day 5	Day 10	Day 15
C	4.32±0.01*	4.32±0.01*	4.32±0.02*
NC	2.10±0.02	2.07±0.02	2.12±0.06

G-0.07	4.01±0.04*	4.11±0.02*	4.29±0.01*
AWE-100	2.98±0.02	3.04±0.03*	3.29±0.10*
AWE-200	3.14±0.04*	3.87±0.02*	3.84±0.04*
AWE-400	3.96±0.09*	4.08±0.06*	4.16±0.03*

Significant difference is $p<0.05$
* = Significant difference when compared with NC
C = Group 1 (control); animals of the normal control group
NC = Group 2; negative control (animals exposed to alloxan without treatment)
G-0.07 = Group 3; animals treated with 0.07mg/kg Glibenclamide extract
AWE-100 = Group 4; animals treated with 100mg/kg *Acalypha wilkesiana* extract
AWE-200 = Group 5; animals treated with 200mg/kg *Acalypha wilkesiana* extract
AWE-400 = Group 6; animals treated with 400mg/kg *Acalypha wilkesiana* extract

Table 2: Effect of *Acalypha wilkesiana* on SOD (U/mol) in diabetic Albino rats

Group	**Day 5**	**Day 10**	**Day 15**
C	0.25±0.01*	0.25±0.01*	0.25±0.01*
NC	0.08±0.01	0.07±0.01	0.05±0.01
G-0.07	0.22±0.003*	0.24±0.01*	0.25±0.01*
AWE-100	0.08±0.01	0.11±0.01*	0.20±0.02*

AWE-200	0.13±0.02*	0.21±0.01*	0.24±0.003*
AWE-400	0.20±0.003*	0.230±0.01*	0.24±0.01*

Significant difference is P<0.05
* = Significant difference when compared with NC
C = Group 1 (control); animals of the normal control group
NC = Group 2; negative control (animals exposed to alloxan without treatment)
G-0.07 = Group 3; animals treated with 0.07mg/kg Glibenclamide extract
AWE-100 = Group 4; animals treated with 100mg/kg *Acalypha wilkesiana* extract
AWE-200 = Group 5; animals treated with 200mg/kg *Acalypha wilkesiana* extract
AWE-400 = Group 6; animals treated with 400mg/kg *Acalypha wilkesiana* extract

Table 3: Effect of *Acalypha wilkesiana* on GSH (µg/ml) in diabetic Albino rats

Group	Day 5	Day 10	Day 15
C	0.87±0.01*	0.87±0.01*	0.87±0.01*
NC	0.33±0.02	0.31±0.01	0.29±0.01
G-0.07	0.67±0.01*	0.75±0.02*	0.84±0.01*
AWE-100	0.40±0.01	0.59±0.01*	0.68±0.03*
AWE-200	0.47±0.01*	0.65±0.01*	0.73±0.03*
AWE-400	0.56±0.01*	0.71±0.02*	0.82±0.01*

Significant difference is P<0.05
* = Significant difference when compared with NC
C = Group 1 (control); animals of the normal control group
NC = Group 2; negative control (animals exposed to alloxan without treatment)
G-0.07 = Group 3; animals treated with 0.07mg/kg Glibenclamide extract
AWE-100 = Group 4; animals treated with 100mg/kg *Acalypha wilkesiana* extract
AWE-200 = Group 5; animals treated with 200mg/kg *Acalypha wilkesiana* extract
AWE-400 = Group 6; animals treated with 400mg/kg *Acalypha wilkesiana* extract

Table 4: Effect of *Acalypha wilkesiana* on GPx (µg/ml) in diabetic Albino rats

Group	Day 5	Day 10	Day 15
C	0.16±0.02*	0.16±0.02*	0.16±0.02*
NC	0.08±0.01	0.07±0.003	0.06±0.003
G-0.07	0.11±0.01*	0.13±0.01*	0.14±0.003*
AWE-100	0.08±0.001*	0.09±0.001*	0.10±0.01*

AWE-200	0.09±0.003*	0.10±0.01*	0.12±0.003*
AWE-400	0.10±0.003*	0.12±0.01*	0.14±0.01*

Significant difference is P<0.05
* = Significant difference when compared with NC
C = Group 1 (control); animals of the normal control group
NC = Group 2; negative control (animals exposed to alloxan without treatment)
G-0.07 = Group 3; animals treated with 0.07mg/kg Glibenclamide extract
AWE-100 = Group 4; animals treated with 100mg/kg *Acalypha wilkesiana* extract
AWE-200 = Group 5; animals treated with 200mg/kg *Acalypha wilkesiana* extract
AWE-400 = Group 6; animals treated with 400mg/kg *Acalypha wilkesiana* extract

Table 5: Effect of *Acalypha wilkesiana* on MDA (µmol/ml) in diabetic Albino rats

Group	Day 5	Day 10	Day 15
C	0.63±0.01*	0.63±0.01*	0.63±0.01*
NC	0.97±0.01	0.99±0.003	1.01±0.01
G-0.07	0.67±0.01*	0.66±0.01*	0.64±0.01*
AWE-100	0.90±0.01	0.92±0.01*	0.91±0.01*
AWE-200	0.85±0.01	0.81±0.01*	0.72±0.01*
AWE-400	0.80±0.01*	0.70±0.01*	0.65±0.01*

Significant difference is P<0.05
* = Significant difference when compared with NC
C = Group 1 (control); animals of the normal control group
NC = Group 2; negative control (animals exposed to alloxan without treatment)
G-0.07 = Group 3; animals treated with 0.07mg/kg Glibenclamide extract
AWE-100 = Group 4; animals treated with 100mg/kg *Acalypha wilkesiana* extract
AWE-200 = Group 5; animals treated with 200mg/kg *Acalypha wilkesiana* extract
AWE-400 = Group 6; animals treated with 400mg/kg *Acalypha wilkesiana* extract

Effect of *Acalypha wilkesiana* on Kidney Histopathology

Figure 1A1 presents the kidney histo-morphology from group one (normal control group), five days after the start of the experiment, showing histologically normal kidney, with intact renal tubule (RT), lined by simple epithelial cells. Glomeruli (GLO) containing; glomerular mesengial cells, glomerular matrix and capillaries, Patent Bowman's capsule (BC). Figure

1A2 presents the histomorphology from group two animals, exposed to alloxan without treatment, showsing histologically distorted kidney and collapsed but intact renal tubules (CRT). Figure 1A3, 2B3 and 3C3 present the histo-morphology of the kidney from group two (animals exposed to alloxan and treated with 0.07mg/kg of glibenclamide for 5, 10 and 15 days) respectively, showing histologically normal kidney, with Intact renal tubules (RT), Patent Bowman's capsules (BC) and glomeruli (GLO). Figure 1A4 presents the histological cross-section of the kidney from group four (animals exposed to alloxan and treated with (100mg/kg) of *Acalypha wilkesiana* for five days), showing; histologically distorted kidney, with lobulated glomeruli (GLO), patent Bowman's capsular spaces (BC), vacuolated cytoplasm in renal tubular epithelial cells, while Figure 2B4 and 3C4 presents the kidney histology from group four (animals exposed to alloxan and treated with 100mg/kg of *Acalypha wilkesiana* for 10 and 15 days), showing histologically normal kidney, with intact renal tubules (RT), and glomeruli (GLO). Figure 1A5 presents the histo-morphology of the kidney from group five (animals exposed to alloxan and treated with 200mg/kg of *Acalypha wilkesiana* for five days), showing histologically distorted kidney, but with intact renal tubules (RT), Glomeruli (GLO) with occluded Bowman's capsules, while Figure 2B5 and 3C5 present the histo-architecture of the kidney from group five animals exposed to alloxan and treated with 200mg/kg of *Acalypha wilkesiana* for 10 and 15 days, showing histologically normal kidney with intact renal tubule (RT), patent Bowman's capsule (BC) and Glomeruli (GLO). Figures 1A6, 2B6, and 3C6 present the kidney histo-mophology from group five animals exposed to alloxan and treated with 400mg/kg of *Acalypha wilkesiana* for 5, 10 and 15 days, showing histologically normal kidney with intact renal tubules (RT), Patent Bowman's capsules (BC), Glomeruli (Glo) containing; Glomerular mesengial cells, Glomerular matrix and capillaries.

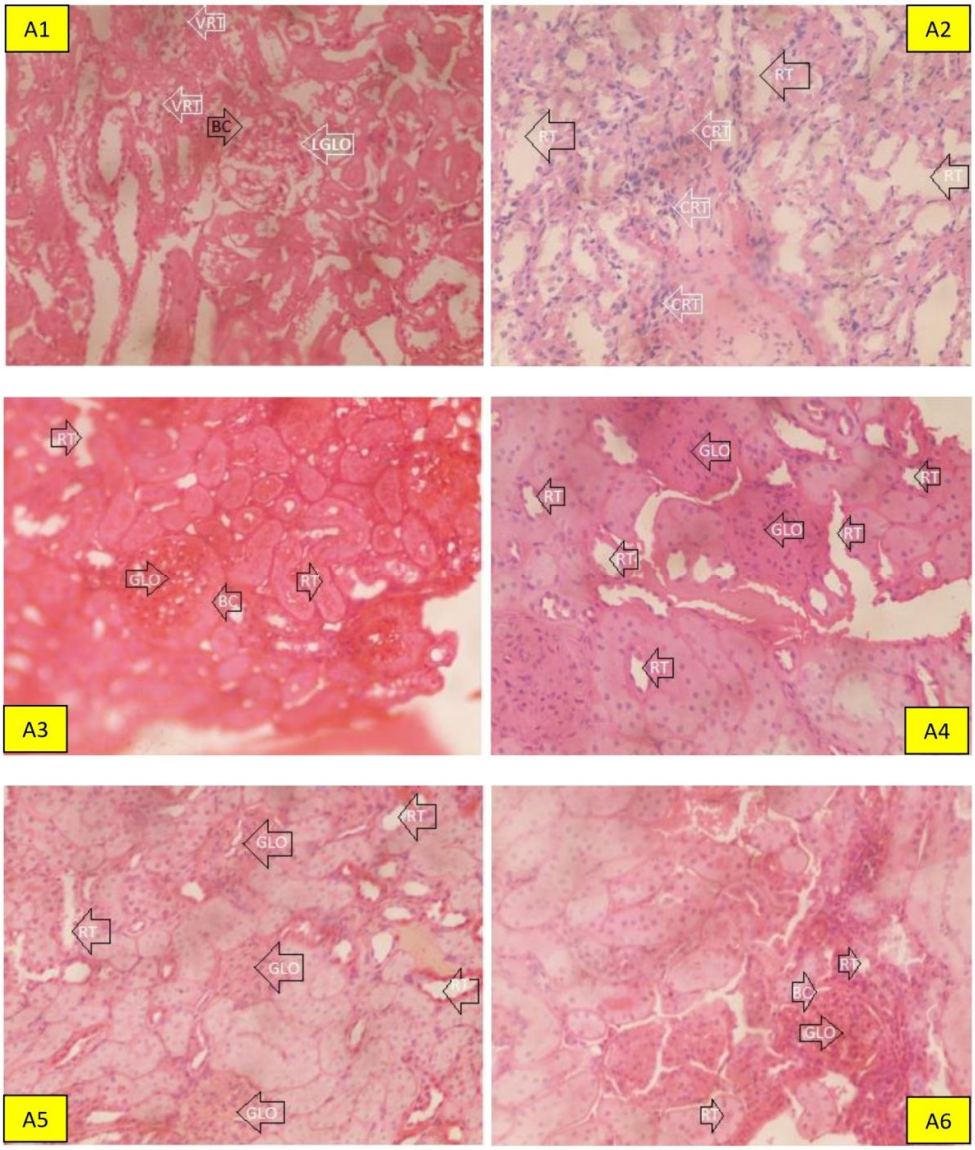

Figure 1: (A1–A6) Photomicrographs of kidney sections in each group on day 5. (A1) Normal Control, (A2) Negative Control (Alloxan), (A3) 0.07mg/kg Glibenclamide, (A4) 100mg/kg *Acalypha wilkesiana* leaf extract, (A5) 200mg/kg *Acalypha wilkesiana* leaf extract, (A6) 400mg/kg *Acalypha wilkesiana* leaf extract. Magnification: X200 and X400

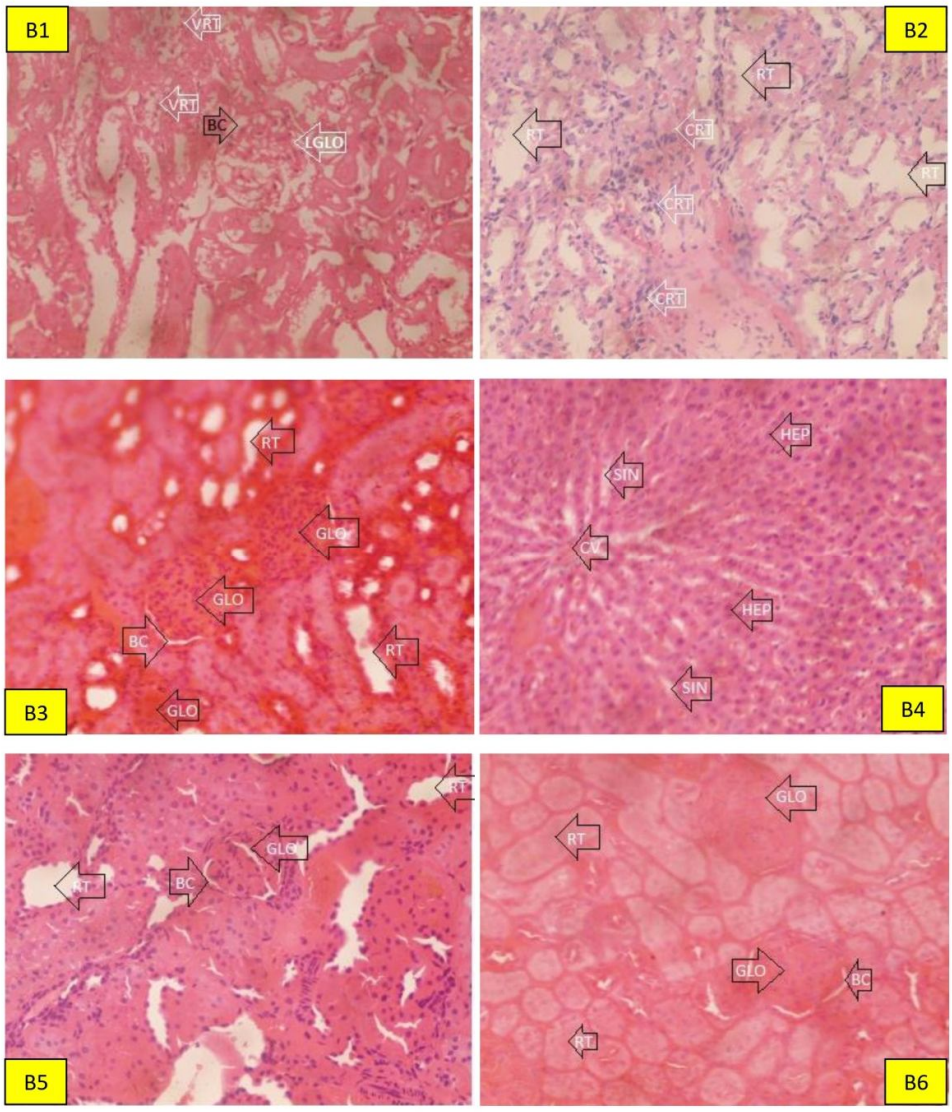

Figure 2: (B1–B6) Photomicrographs of kidney in each group on day 10. (B1) Normal Control, (B2) Negative Control (Alloxan), (B3) 0.07mg/kg Glibenclamide, (B4) 100mg/kg *Acalypha wilkesiana* leaf extract, (B5) 200mg/kg *Acalypha wilkesiana* leaf extract, (B6) 400mg/kg *Acalypha wilkesiana* leaf extract. Magnification: X200 and X400

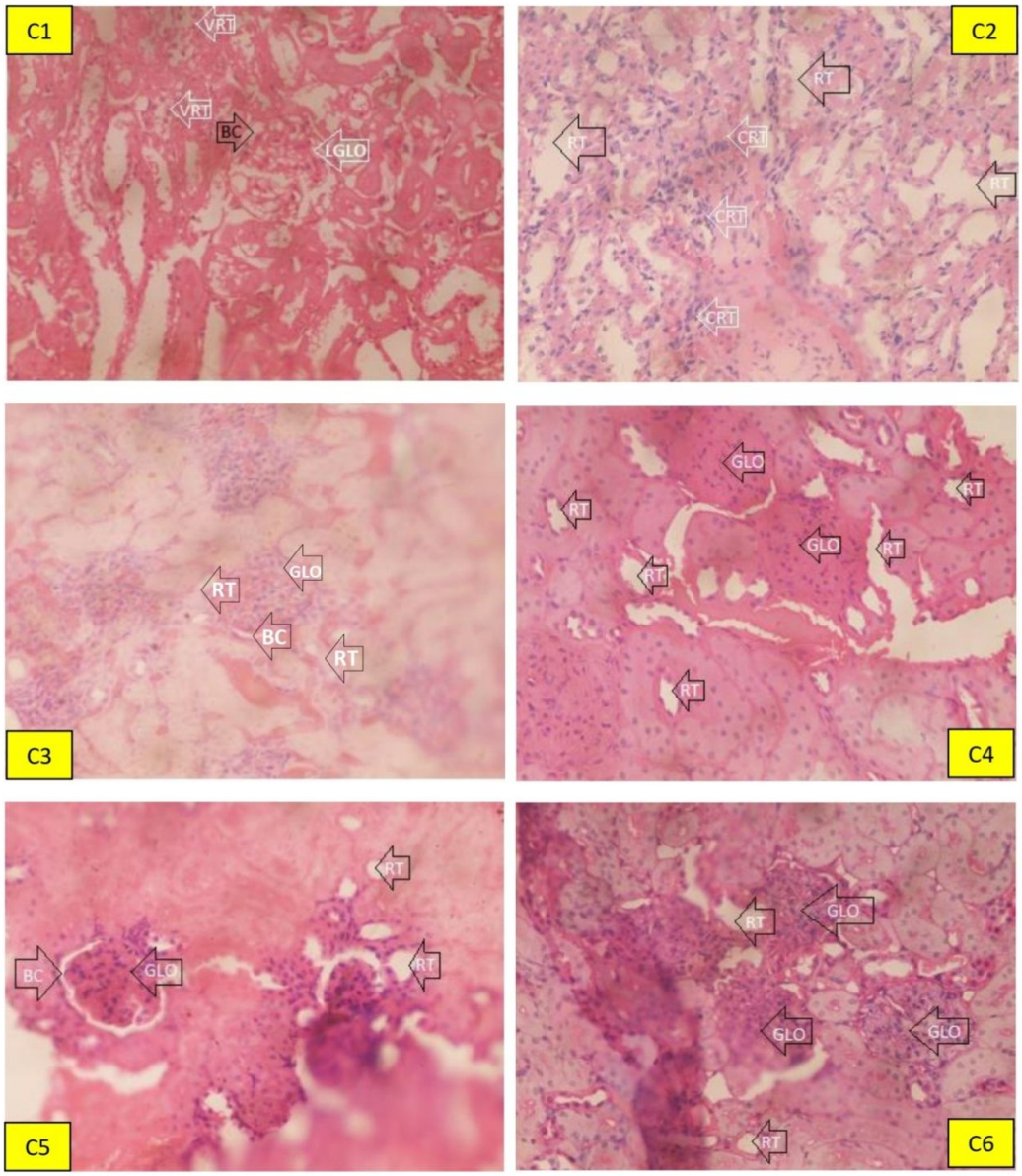

Figure 3: (C1–C6) Photomicrographs of kidney in each group on day 15. (C1) Normal Control, (C2) Negative Control (Alloxan), (C3) 0.07mg/kg Glibenclamide, (C4) 100mg/kg *Acalypha wilkesiana* leaf extract, (C5) 200mg/kg *Acalypha wilkesiana* leaf extract, (C6) 400mg/kg *Acalypha wilkesiana* leaf extract. Magnification: X200 and X400

Effect of *Acalypha wilkesiana* on Liver Histopathology

Figure 4X1 presents Liver from group one (normal control group), five days after the start of the experiment, showing histologically normal liver with normal hepatocytes (HEP) sinusoids (SIN) containing capillaries and kupffer cells, congested central vein (CV). Figure 4X2 shows liver histology of animals exposed to alloxan without treatment: showing histologically distorted liver; hepatocyte steatosis and congested central vein (CV). Figure 4X3 shows liver sections from group three (animals exposed to alloxan and treated with 0.07mg/kg of glibenclamide for 5 days), showing mildly distorted liver and hepatocyte steatosis, normal hepatocytes (HEP) and sinusoids. Figure 5Y3 and 6Z3 presents the liver histological section from group three (animals exposed to alloxan and treated with 0.07mg/kg of glibenclamide for 10 and 15 days), showing normal liver with normal hepatocytes (HEP), sinusoids (SIN); containing capillaries and kupffer cells, and congested central vein (CV). Figure 4X4, 5Y4 and 6Z4 presents liver histology from group four animals exposed to alloxan and treated with 100mg/kg of *Acalypha wilkesiana* for 5, 10 1nd 15 days respectively, showing; normal liver with normal hepatocytes (HEP), Sinusoids (sin), Patent central vein (CV). Figure 4X5, 5Y5 and 6Z5 presents histomorphology of liver from group five (animals exposed to alloxan and treated with 200mg/kg of *Acalypha wilkesiana* for five days), showing normal hepatocytes (HEP), sinusoids (SIN) containing kupffer cells and patent central vein (CV). Figure 4X6, 5Y6 and 6Z6 shows histological sections of Liver from group six (animals exposed to alloxan and treated with 400mg/kg of *Acalypha wilkesiana* for 5, 10 and 15 days), showing normal histologically normal liver with normal hepatocytes (HEP) and sinusoids (SIN).

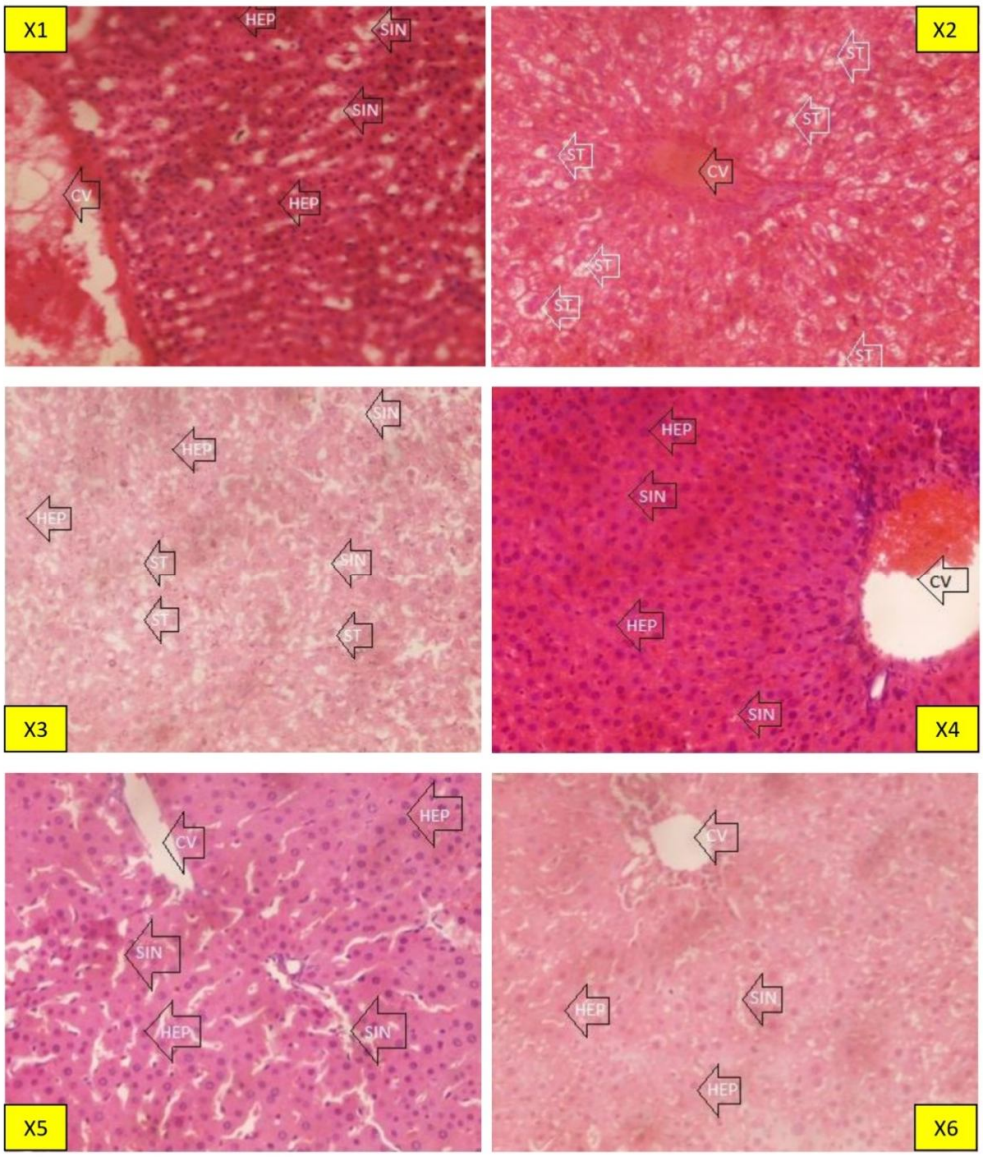

Figure 4: (X1–X6) Photomicrographs of Liver in each group on day 5. (X1) Normal Control, (X2) Negative Control (Alloxan), (X3) 0.07mg/kg Glibenclamide, (X4) 100mg/kg *Acalypha wilkesiana* leaf extract, (X5) 200mg/kg *Acalypha wilkesiana* leaf extract, (X6) 400mg/kg *Acalypha wilkesiana* leaf extract. Magnification: X200 and X400

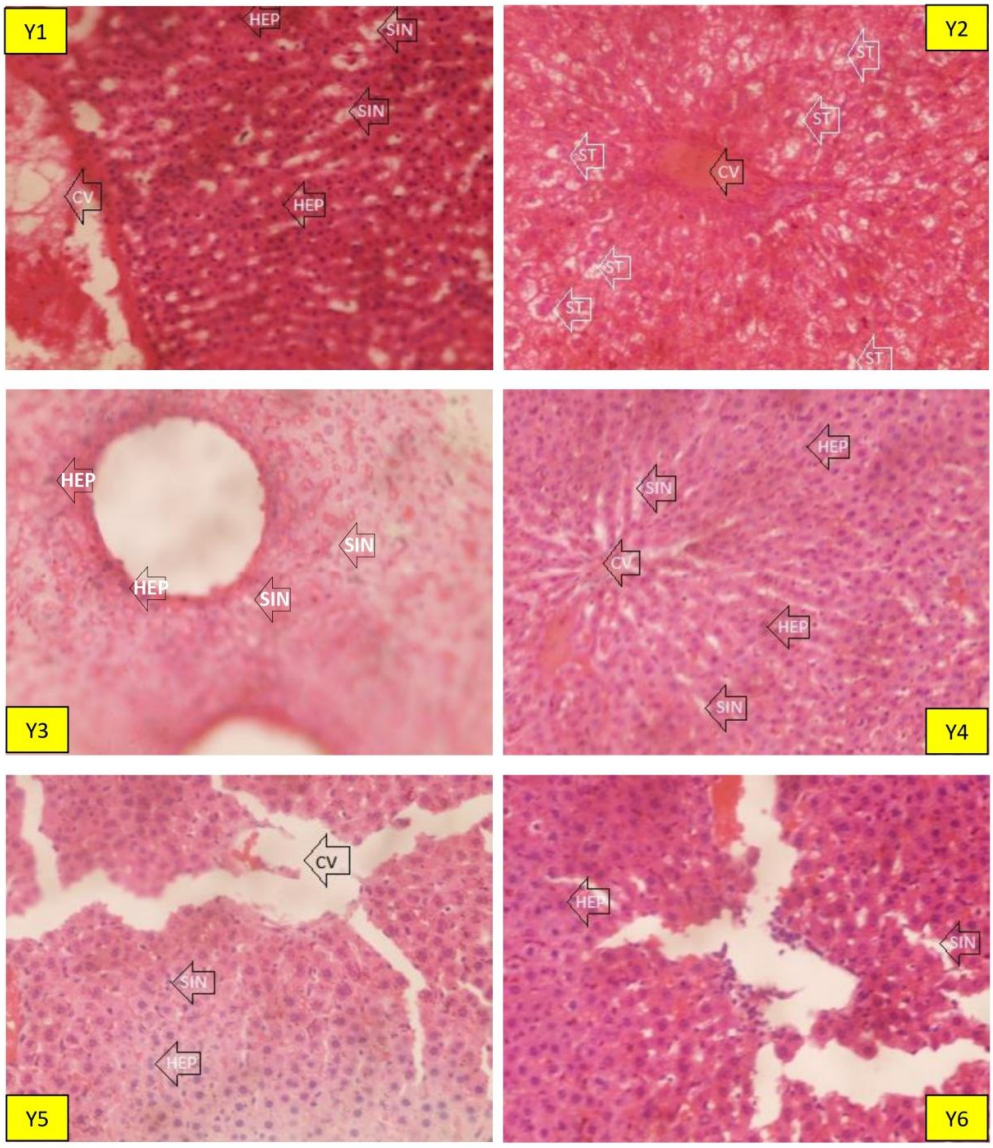

Figure 5: (Y1–Y6) Photomicrographs of Liver in each group on day 10. (Y1) Normal Control, (Y2) Negative Control (Alloxan), (Y3) 0.07mg/kg Glibenclamide, (Y4) 100mg/kg *Acalypha wilkesiana* leaf extract, (Y5) 200mg/kg *Acalypha wilkesiana* leaf extract, (Y6) 400mg/kg *Acalypha wilkesiana* leaf extract. Magnification: X200

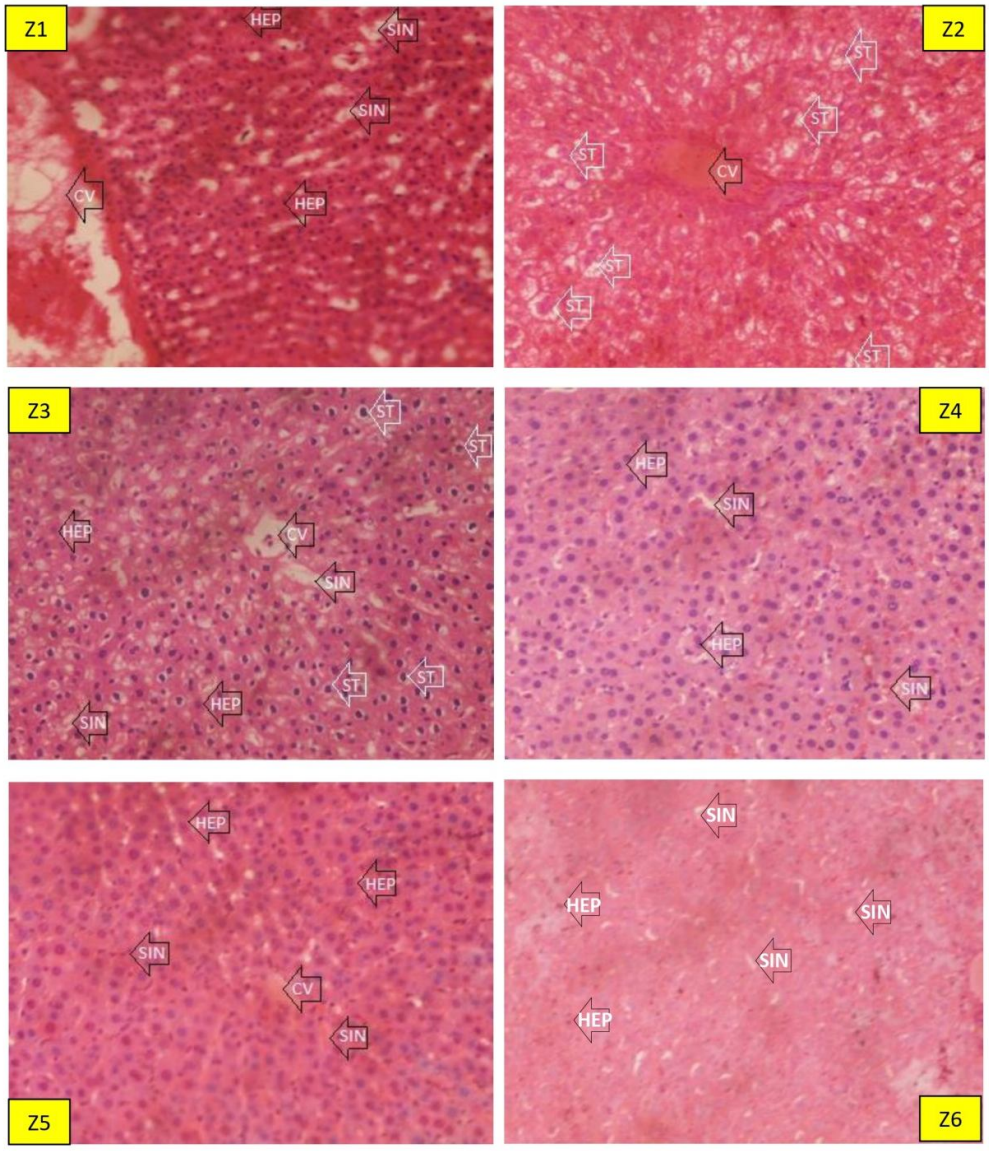

Figure 6: (Z1–Z6) Photomicrographs of Liver in each group on day 15. (Z1) Normal Control, (Z2) Negative Control (Alloxan), (Z3) 0.07mg/kg Glibenclamide, (Z4) 100mg/kg *Acalypha wilkesiana* leaf extract, (Z5) 200mg/kg *Acalypha wilkesiana* leaf extract, (Z6) 400mg/kg *Acalypha wilkesiana* leaf extract. Magnification: X200

DISCUSSION

Oxidative stress results when there is increased production of free radicals or decreased activity of counter-actors, antioxidants or both in a combination [38]. Thus by implication, alloxan may have caused an increased production of free radicals when administered to the animal, this observation agrees with the report of Lenzen [14]. Antioxidants which are either enzyme (SOD, CAT, GPx) or non-enzyme (GSH) protects against the effects of free radicals in order to maintain homeostatic balance of reactive oxygen species [39-41]. On the side of the plant, administration of *A. wilkesiana* caused a dose dependent increase in anti-oxidant activity which agrees with [22]. Other researchers including Olawale *et al*. [42] have reported the antioxidant properties of A*calypha wilkesiana*, and said that its antioxidant properties are not surprising because of the presence of the phytochemical like terpenes and tannins [43] adding that these classes of compounds have been reported in previous studies to exhibit antioxidant activities [44-46] and concluded by saying that different preparations of *A. wilkesiana* are used in ethnomedicine to treat/manage disease conditions such as wounds, inflammations and tumors amongst so many others because of it antioxidant potentials [47,48].

"SOD plays a major role as first line of the antioxidant defense system by catalyzing the dismutation of superoxide radical to form hydrogen peroxide (an oxidant) and molecular oxygen" [39,49]. Thus by implication alloxan may have caused over production of superoxide radicals, which over whelmed the intrinsic serum level of SOD thereby causing a reduction in its activity. This might have been responsible for the reduction in the serum level of SOD, following alloxan treatment and this agrees with the report of Lenzen [14]. The reversal of these deleterious effects of alloxan by *Acalypha wilkesiana* implies free radical scavenging properties of this extract and agrees with the reports of Olawale *et al*. [42], Malaya *et al*. [44], Svoboda *et al*. [45] and Oladimeji *et al.* [46].

"Catalase (CAT) catalyzes the decomposition of hydrogen peroxide to water and oxygen. It is a very important enzyme in protecting the cell from oxidative damage by reactive oxygen species (ROS)" [50]. The reduction in the serum levels of CAT following alloxan treatment, which by implication is a reduction in its activity, may not be unconnected with over production of ROS, which agrees with Lenzen [14]. Alloxan by implication may have caused over production of ROS including H_2O_2, which suppress the level of CAT in the serum, thus reducing their activities which agrees with Lenzen [14]. Administration of *Acalypha wilkesiana,* caused an increase of CAT retuning it towards normal level. This is probably due a scavenging property to eradicated the free radicals (ROS including H_2O_2), and this with Ijeoma *et al*. [22] and Olawale *et al*. [42] thus reducing the pressure exacted on the biological antioxidant (CAT), eventually returning its concentration back to normal.

"GPx is a selenium dependent enzyme with peroxidase activity whose main biological role is to protect the organism from oxidative damage. The biochemical function of glutathione peroxidase is to reduce lipid hydroperoxides to their corresponding alcohols and to reduce free hydrogen peroxide to water" [51]. Significant reduction in SOD, CAT and GPx activity might be an indication of accumulation of H_2O_2. Thus by implication alloxan may have caused over production of H_2O_2 as well as lipid peroxidation which leads to the reduction of these anti-oxidants levels in the serum and this also agrees with Lenzen [14]. With the observed effects of *Acalypha wilkesiana*, it is obvious that it possesses free radical scavenging properties, which allows their actions to reverse these toxic effects caused by alloxan administration agreeing with Ijeoma *et al.* [22] and Olawale *et al*. [42].

Reduced glutathione (GSH) reduces the oxidized form of the enzyme glutathione peroxidase, (GSSG) which in turn reduces hydrogen peroxide (H_2O_2), a dangerous reactive species within the cell [51]. Thus the significant reduction in the levels of GSH as observed in our result with alloxan administration, may be due to over production of H_2O_2 thus critically engaging

the activities of the enzyme glutathione peroxidase, to reduce cellular toxicity, which in turn caused a reduction in the levels of GSH in its attempt to reduce the oxides of the enzyme glutathione peroxidase and this agrees with Lenzen [14]. Significant reduction in plasma levels of reduced glutathione (GSH) is a result of overwhelming antioxidant effects to reduce free radicals generated. Thus the ability of *Acalypha wilkesiana* reverse the adverse effects observed of alloxan on GSH is a demonstration of anti-oxidant potentials agreeing with Ijeoma *et al*. [22] and Olawale *et al.* [42].

Malondialdehyde (MDA) is secondary products of lipid peroxidation, thus its concentration is increased during oxidative stress and decreases in the presence of anti-oxidants. The increasing concentration of MDA is an evidence of tissue damage caused by increased free radicals. Lipid peroxidation, a downstream chain reaction started by free radicals, may have been triggered by alloxan which agrees with Lenzen [14], as revealed by the increased level of lipoperoxidation product (MDA) [52]. These effects were reversed by *Acalypha wilkesiana* confirming its anti-oxidant potentials as reported by Ijeoma *et al*. [22] and Olawale *et al*. [42].

Reports have shown that increase in oxidative stress as could be cause by alloxan cause liver damage [20,21] and kidney damage [23,53]. In fact, Pavlakou *et al.* [53] added that "ROS are involved in the development of sepsis induced kidney injury on multiple levels. Inflammation induced up-regulation of inducible nitric oxide synthase leads to production of excessive nitric oxide (NO), which in turn uncouples endothelial NO synthase generating highly reactive superoxides by oxidation of oxygen". Thus the observed damage to the liver and kidney tissues in this study is not unconnected to an increase in oxidative stress. Equally, the ability of *Acalypha wilkesiana* to reverse all the adverse histological changes may not be unrelated to be its antioxidant potentials.

CONCLUSION

The results of this study revealed a significant increase in oxidative stress markers such as CAT, SOD, GSH and GPx and decrease in MDA. There were pathological changes of liver and kidney following alloxan administration when compared with normal control. These changes were reversed by the *Acalypha wilkesiana* extract in a dose dependent manner. Hence, *Acalypha wilkesiana* reversed pathological changes in the kidney and liver of alloxan-induced diabetic albino rats.

Ethical Approval:

Animal Ethic committee approval has been collected and preserved by the author(s)

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