



Antioxidant Potentials of Aqueous Leaf Extract of *Acalypha godseffiana* on Hydrogen Peroxide-Induced Oxidative Stress in Wistar Rats

Samson Eruke Okoro^{1*}, Stephen I. Omeodu¹ and Favour Uzoma Iyke-David¹

¹Department of Biochemistry, University of Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration among all authors. Authors SEO and SIO designed the study, performed the statistical analysis and wrote the protocol. Author SEO wrote the first draft of the manuscript. Authors SEO and FUID managed the analyses of the study. Author FUID carried out the laboratory work. Authors SEO and FUID managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aim: This study evaluated the efficacy of aqueous extract of *Acalypha godseffiana* leaves against H₂O₂-induced oxidative stress and organ damage in wistar rats by measuring hepatotoxic biomarkers, nephrotoxicity and status of the antioxidant defense system.

Study Design: Twenty-five rats, randomly divided into five groups, were used in this study. Fresh leaves of *Acalypha godseffiana* leaves were air-dried, ground into fine powder and used in the preparation of an aqueous extract.

Place and Duration of Study: Department of Biochemistry, and Department of Physiology, University of Port Harcourt, Nigeria between November 2020 and April 2021.

Methodology: Oxidative stress and toxicity was induced using 5ml of 6% H₂O₂. Treated rats received *A. godseffiana* aqueous leaf extract at various doses: 100, 200 and 400 mg/kg b.w. The rats were fasted for 24 h prior to sampling and then mildly anesthetized with chloroform. Biochemical assays and histological studies were conducted at days 14 and 28 respectively.

*Corresponding author: E-mail: samson.okoro@uniport.edu.ng;

Results: There was significant ($P<0.05$) increase in plasma levels of AST in the H_2O_2 -treated group as compared with the negative control which recorded 105.00 ± 3.00 and 107.50 ± 7.50 (μ/L) at day 14 and 28 respectively. The H_2O_2 group showed significant ($P<0.05$) increases in plasma levels of K^+ , Na^+ , Urea, Cl^- and HCO_3^- as compared with the negative control. *A. godseffiana*-treated rats showed significant ($P<0.05$) increase in serum levels of GSH, Catalase and SOD. Photomicrographs obtained showed histologically distorted liver and kidney tissues in the H_2O_2 group at days 14 and 28. Overall, the architecture of the liver was preserved by the administered aqueous leaf extract of *A. godseffiana*.

Conclusion: Data obtained from this study suggest that *A. godseffiana* leaves exhibit promising antioxidant and hepatoprotective potency, and consequently has ameliorative effects against H_2O_2 -induced oxidative stress and organ damage in wistar rats.

Keywords: Oxidative stress; hydrogen peroxide; plant extract; *Acalypha godseffiana*; antioxidant; ameliorative effect.

1. INTRODUCTION

Oxidative stress refers to a cellular state characterized by the immoderate release of reactive oxygen species (ROS) or the weakening of the antioxidant defense system [1,2]. Oxidative stress results from an imbalance between radical generating and radical-scavenging systems with increased production of ROS or reduced activity of antioxidant defenses or both [3].

Increased ROS production lowers cellular antioxidant levels and enhances the oxidative stress in many tissues, especially the liver [4]. There have been found many mechanisms contributing to the cell death – impairment of mitochondrial respiration, induction of oxidative stress and lipo-peroxidation [5]. The imbalance between the systemic manifestation of reactive oxygen species (ROS) and a biological systems ability to readily detoxify the reactive intermediates or repair the resulting damage can cause oxidative damage to DNA, proteins, and lipids [6,7]. Free radicals and peroxides are responsible for cell damage, cell death also known as (apoptosis), mutations leading to cancer, development of cardiovascular diseases, infections and many other diseases. Oxidative stress crucially affects the onset and progression of most human diseases such as diabetes, arthritis, aging-related diseases and reproductive system disorders [8-10].

Organisms combat ROS accumulation through antioxidant defense systems, including non-enzymatic antioxidants, such as glutathione, and antioxidant enzymes, such as glutathione peroxidase, superoxide dismutase, and catalase. Superoxide dismutase catalyses the dismutation of toxic superoxide (O_2^-) radical to molecular oxygen (O_2). Catalase and glutathione peroxidase are enzymes which catalyse the

decomposition of hydrogen peroxide to water and oxygen [11,12]. Diet-derived antioxidants may play an important role to prevent the chronic diseases [13].

Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free radicals that damage all components of cell including proteins, lipids and DNA. Oxidative stress from oxidative metabolism causes base damage as well as strand-breaks in DNA. Base damage is mostly indirect and caused by reactive oxygen species generated, e.g. superoxide radical (O_2^-), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2) [14]. The oxidative effect of these reactive oxygen species can be reduced or obliterated by enzymatic antioxidants, such as superoxide dismutase (SOD) and glutathione peroxidase, and non-enzymatic antioxidants [9].

Hydrogen peroxide itself is not very reactive, but it can sometimes be toxic to cell because of it may give rise to hydroxyl radical in the cells [15] and it together with superoxide radical anion can damage many cellular components [16]. Recently, the hydrogen peroxide was viewed mainly as a toxic cellular metabolite.

Phytochemicals which are chemical compounds found in plant are known for their potentials in reducing the effects of these free radicals. Phytochemicals such as alkaloids, flavonoids, tannins are known for their antioxidant effects. *Acalypha godseffiana* is a plant that is attracting research interest due to its widely reported application in traditional health care systems. In an earlier study, phytochemical analysis of the leaves of *A. wikesiana* showed the presence of alkaloids, flavonoids cardiac glycosides, phenols, terpenoids and steroids [17]. *Acalypha godseffiana* is reportedly used in the treatment of

a number of diseases including diarrhea, gastrointestinal disorders, fungal skin infections, hypertension and diabetes mellitus among others [18]. Previous studies have also reported that the aqueous extract of *A. godseffiana* had a lowering effect on blood cholesterol level as well as blood sugar, thereby explaining its use in the treatment of cardiovascular related diseases. Further studies on fractions of the plant extract report its inhibitory effects on the production of methicillin-resistant staphylococcus aureus [19] as well as bactericidal activities [20] and antioxidant activities [21].

The present study was designed to evaluate the efficacy of aqueous extract of *Acalypha godseffiana* leaves against H₂O₂-induced oxidative and organ damage in wistar rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

Wistar rats weighing 125-250g were obtained from the Animal House of the Department of Biochemistry, University of Port Harcourt, Nigeria. The rats were acclimatized in a temperature-controlled animal house (25 ± 2 °C) under 12 h of light and 12 h of darkness for 10 d before the experiment. The rats were then housed in standard iron cages in five groups of five rats each and given *ad libitum* access to standard food and water.

2.2 Chemicals

The reagents used for the experiments were of analytical grade: (6% dilute Hydrogen Peroxide, 5,5' -dithiobis-2-nitrobenzoic acid (DTNB), Sodium hydroxide (NaOH), ALP substrate, Randof reagents, Haematoxylin and Eosin (H&E) stain, Xylene, Paraffin wax, 10% formalin, Caffeine reagent).

2.2.1 Preparation of aqueous extract of *Acalypha godseffiana* leaves

Fresh leaves of *Acalypha godseffiana* plant were obtained from the garden of Ignatius Ajuru University of Education (St. John's Campus), Port Harcourt, Nigeria. The plant was identified by a Plant Technologist in the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria. The freshly collected leaves of *Acalypha godseffiana* were thoroughly washed with tap water followed by distilled water. The leaves were air-dried in the shade at 33°C ± 2°C and ground into a fine powder. *A. godseffiana* aqueous extract was prepared according to a previously reported method [22].

Dry powder (10 g) was placed in a Soxhlet extractor with 300 ml distilled water and continuously heated at 40°C for more than 10 h until the powder color faded. The water extract was then concentrated under reduced pressure at 40°C in a rotary evaporator and the solid material residue stored in sealed dark glass bottles under free moisture conditions in a deep freezer until use.

2.3 Experimental Protocol

Twenty-five rats were randomly divided evenly into five groups as follows:

Group 1: Normal rats; received standard food and water (Negative Control)

Group 2: Received 5ml of 6% H₂O₂ (Positive Control)

Group 3: Received 5ml of 6% H₂O₂ + 100 mg/kg b.w. of aqueous leaf extract of *A. godseffiana*

Group 4: Received 5ml of 6% H₂O₂ + 200 mg/kg b.w. of aqueous leaf extract of *A. godseffiana*

Group 5: Received 5ml of 6% H₂O₂ + 400 mg/kg b.w. of aqueous leaf extract of *A. godseffiana*

The experiment lasted for 28 days. At days 14 and 28, the rats were fasted for 24 h prior to sampling and then mildly anesthetized with chloroform. Blood samples were collected for biochemical analysis. The serum was separated by centrifugation at 3,000 g for 5 min and kept at -20°C until use for biochemical assay. The kidney and liver were excised and placed in a formalin bottle to preserve the tissue for histology analysis.

2.4 Biochemical Analysis

2.4.1 Hepatotoxic biomarkers

Plasma L - alanine aminotransferase (ALT) and L - aspartate aminotransferase (AST) activities were determined using the method described by Reitman and Frankel [23] while Alkaline phosphatase (ALP) activity was measured using the colorimetric method using phenolphthalein monophosphate as substrate [24]. Bilirubin was determined using the method described by Jendrasik & Grof [25]. Serum Total Protein level was measured using the Biuret Method [26]. Determination of serum Albumin concentration was by the Biromoeresol Green Method described by Rodkey [27].

2.4.2 Nephrotoxic biomarkers

Plasma sodium determination followed the precipitation method described by Henry [28].

Serum Potassium ion concentration was measured using the Tetraphenylborate method described by Tietz [29]. A Cecil 7400 series UV visible spectrophotometer was used for the determination of chloride concentration in serum samples at wavelength of 480nm. The back titration method was used to measure bicarbonate levels as described by Kenkel [30]. Urea Estimation Blood urea concentrations were determined using Berthelot's reaction as described by Kaplan and Teng [31]. Creatinine Estimation Assay for creatinine was carried out using the Reflotron Dry Chemistry Analyzer as described by Estridge et al. [32].

2.4.3 Antioxidant assays

Determination of malondialdehyde concentration was by the lipid peroxide assay method described by Varshney and Kale [33]. Superoxide dismutase (SOD) activity was assayed using the method described by Fridorich [34] and as contained in the Randox commercial kit. Catalase activity was assayed using the method of Aebi [35]. The concentration of glutathione was determined according to the method of Habig et al. [36].

2.4.4 Histological studies

The tissues were fixed in 10% formalin, routinely processed for dehydration, and embedded in paraffin wax. Section cuts were stained with hematoxylin and eosin for light microscopic examination [37]. The histological sections were examined as the slides were mounted using Canada balsam and examined using $\times 400$ objective lens.

2.5 Statistical analysis

Statistical analysis of data All values were expressed as mean \pm SD and then subjected to analysis of variance (ANOVA) using the Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc., Chicago Illinois). Statistical significance was considered at $P=0.05$.

3. RESULTS AND DISCUSSION

3.1 Effects of *Acalypha godseffiana* Aqueous Leaf Extract on Hepatotoxic Biomarkers

Table 1 shows the effect of *Acalypha godseffiana* aqueous leaf extract on liver biomarkers: Plasma

L - aspartate aminotransferase (AST), L - alanine aminotransferase (ALT), Alkaline phosphatase (ALP), Total Protein, Serum Albumin, Total Bilirubin and Conjugated Bilirubin.

There was significant increase ($P<0.05$) in plasma levels of AST in group 2, recording 120.00 ± 3.00 and 120.50 ± 8.50 (μL) at day 14 and 28 respectively, as compared with the negative control which recorded 105.00 ± 3.00 and 107.50 ± 7.50 (μL) at day 14 and 28 respectively. Also, significant increase ($P<0.05$) in plasma total protein was recorded in group 2. However, plasma AST levels in groups 3 and 4 were 106 ± 1.00 and 111.50 ± 1.50 (μL) at day 14, and 111.00 ± 1.00 and 108.00 ± 14.00 (μL) at day 28 indicating significant improvement when compared with values for the positive control group. At day 28, Total Bilirubin levels in groups 3, 4 and 5 (4.30 ± 0.20 and 4.80 ± 1.70 ($\mu\text{mol/L}$)) were lower than those of the positive control group (5.15 ± 0.95 ($\mu\text{mol/L}$)). There was no significant variation in plasma ALT and plasma total proteins in groups 2, 3, 4 and 5 at day 14. There were significant differences in plasma conjugated bilirubin levels between group 2, and groups 3 and 4 at day 14. However, group 5 recorded significant ($P<0.05$) increase in plasma ALT levels at day 14.

3.2 Effect of *Acalypha godseffiana* Aqueous Leaf Extract on Nephrotoxic Biomarkers

Table 2 illustrates the effect of *Acalypha godseffiana* aqueous leaf extract on kidney function parameters. Rats administered H_2O_2 (negative control) showed significant ($P < 0.05$) increases in plasma levels of K^+ , Na^+ , Urea, Cl^- and HCO_3^- as compared with the negative control at days 14 and 28 respectively. With the administration of *Acalypha godseffiana* aqueous leaf extract, groups 3, 4 and 5 recorded 5.20 ± 0.20 , 7.25 ± 0.25 and 5.95 ± 0.05 mmol/L for K^+ , 114.00 ± 4.00 , 137.50 ± 2.50 and 126.50 ± 1.50 mmol/L for Na^+ , 50.50 ± 0.50 , 45.00 ± 0.00 and 45.50 ± 0.50 mmol/L for Cl^- , all at day 14, an indication of improved plasma electrolytes levels. At day 28, there were significant ($P < 0.05$) decreases in K^+ , Na^+ and Cl^- in group 5.

3.3 Effects of *Acalypha godseffiana* Aqueous Leaf Extract on Antioxidant Enzymes

The effects of *Acalypha godseffiana* aqueous leaf extract on antioxidant enzyme levels is

shown in Table 3. The positive control group showed a significant increase ($P < 0.05$) in serum GSH (4.24 ± 0.04 , $3.77 \pm 0.24 \mu\text{g/ml}$) and SOD (0.90 ± 0.005 , $0.64 \pm 0.145 \mu\text{g/ml}$) when compared with the negative control group at days 14 and 28 respectively. Values obtained for the serum in the H_2O_2 group showed significant decrease ($P < 0.05$) in catalase content ($7.40 \pm 0.05 \mu\text{g/ml}$) at day 14, MDA (0.16 ± 0.04 and $0.17 \pm 0.025 \mu\text{g/ml}$) at day 14 and 28 respectively. Rats treated with 400 mg/kg b.w. of *A. godseffiana* showed a significant difference ($P < 0.05$) in serum levels of GSH ($3.84 \pm 0.54 \mu\text{g/ml}$ at day 28), Catalase ($14.40 \pm 0.40 \mu\text{g/ml}$ at day 14), SOD (0.84 ± 0.06 and $0.65 \pm 0.25 \mu\text{g/ml}$ at day 14 and 28 respectively).

3.4 Results for Histological Studies

3.4.1 Liver Photomicrographs

Plates A – J are defined as follows: Light microscope photographs of liver paraffin sections, stained with hematoxylin and eosin, obtained from negative control at day 14 & 28 (A, F), H_2O_2 group at day 14 & 28 (B, G), 100 mg/kg *A. godseffiana* group at day 14 & 28 (C, H), 200 mg/kg *A. godseffiana* group at day 14 & 28 (D, I), 400 mg/kg *A. godseffiana* group at day 14 & 28 (E, J).

3.4.2 Kidney Photomicrographs

Plates K – T are defined as follows: Light microscope photographs of liver paraffin sections, stained with hematoxylin and eosin, obtained from negative control at day 14 & 28 (K, P), H_2O_2 group at day 14 & 28 (L, Q), 100 mg/kg *A. godseffiana* group at day 14 & 28 (M, R), 200 mg/kg *A. godseffiana* group at day 14 & 28 (N, S), 400 mg/kg *A. godseffiana* group at day 14 & 28 (O, T).

Data from the present study showed that animals induced with toxicity using hydrogen peroxide showed elevated plasma AST activity. This finding corroborates previous studies that demonstrated rise in plasma AST and ALT activities, indicating hepatic damage [38, 39]. Also, another study on the chemoprotective activity of aqueous leaf extract of *Acalypha wilkesiana* against cyclophosphamide-induced toxicity in rats [40]. reported elevated plasma AST and ALT activities in the untreated group. The elevated levels of these hepatic enzymes in the H_2O_2 group agrees with the alteration in hepatocellular integrity. However, experimental

groups treated with aqueous extract of *A. godseffiana* leaf recorded reduced plasma AST and ALT activities when compared with H_2O_2 alone treated group. This is suggestive that *A. godseffiana* leaf extract possesses hepatoprotective and antioxidant properties which may be due to the presence of bioactive compounds such as flavonoids, proanthocyanidins, alkaloids, terpenoids, steroids and phenols [18, 21, 41, 42]. This inference is supported by Asekunowo et al. [43] who reported a positive relationship between phenolic content and antioxidant activity of extracts of *A. godseffiana*. Flavonoids are the most diverse and widespread group of natural polyphenolic compounds known for their antioxidant, anti-inflammatory and anti-carcinogenic properties [44]. Polyphenolic compounds are very important plant metabolites which defend the body from diverse types of oxidative damage [45]. The non-significant change in the activities of ALT and ALP in the wistar rats that received both H_2O_2 and *A. godseffiana* extract, is similar to documented reports on hepatoprotective activity of similar plant extracts [46]. This could be adduced to the anti-peroxidative effect of the extract, thereby maintaining cell membrane integrity [47].

The liver and kidneys work in synergy to maintain homeostasis in the body. This ensures the proper excretion of waste materials and reabsorption of some useful materials by the kidneys [48]. Finding in this study of a significant ($P < 0.05$) decrease in urea in the H_2O_2 group, compared to the negative control group, suggests that the urea cycle may have produced systemic toxicity, invariably leading to a reduction in the production of urea, reduced ability to excrete waste and failure to maintain balance in body fluid and electrolytes [49-51]. Creatinine clearance in the glomerulus of the kidney is a useful tool to assessing the functionality of the kidney [52, 53]. Creatinine is produced endogenously in the muscle by a non-enzymic action on creatine phosphate. When creatinine and urea are retained in the blood, it shows a possible impairment of the kidneys [54]. The result of creatinine obtained in this study showed a significant ($P < 0.05$) increase on administration of *A. godseffiana* aqueous leaf extract compared to the control. The significant increase in creatinine may have resulted from glomerular inflammation and interstitial nephritis. This result corroborates the result of a previous research using extract of *Acalypha wilkesiana* on wistar rats which suggested that the presence of tissue

damaging alkaloid and saponins may have contributed to the increase in creatinine concentration [55]. This showed the constituents of leaf extract of *A. godseffiana* may possess renal toxicity and result in impaired kidney function.

Body fluid compartments (both extracellular and intracellular fluids) comprises of inorganic electrolytes which in its dissociated forms help to facilitate the movement of water and electrolytes between the body fluid compartments. Healthy functioning of the kidneys, heart and liver can be assessed using the electrolytes balance in the blood. Rats administered H₂O₂ only (negative

control) showed significant ($P < 0.05$) increases in plasma levels of K⁺, Na⁺, Cl⁻ and HCO₃⁻ as compared with the negative control. When the level of serum or plasma electrolytes are abnormal, it is believed that the kidney function has been impaired [56]. The results of this study (Table 2) showed that the concentrations of serum electrolytes (chlorides, potassium and sodium) favorably improved in all groups administered with *A. godseffiana* aqueous leaf extract, when compared with the negative control. This shows that the plant extract could aid electrolyte balance, to ensure proper maintenance of homeostasis [51].

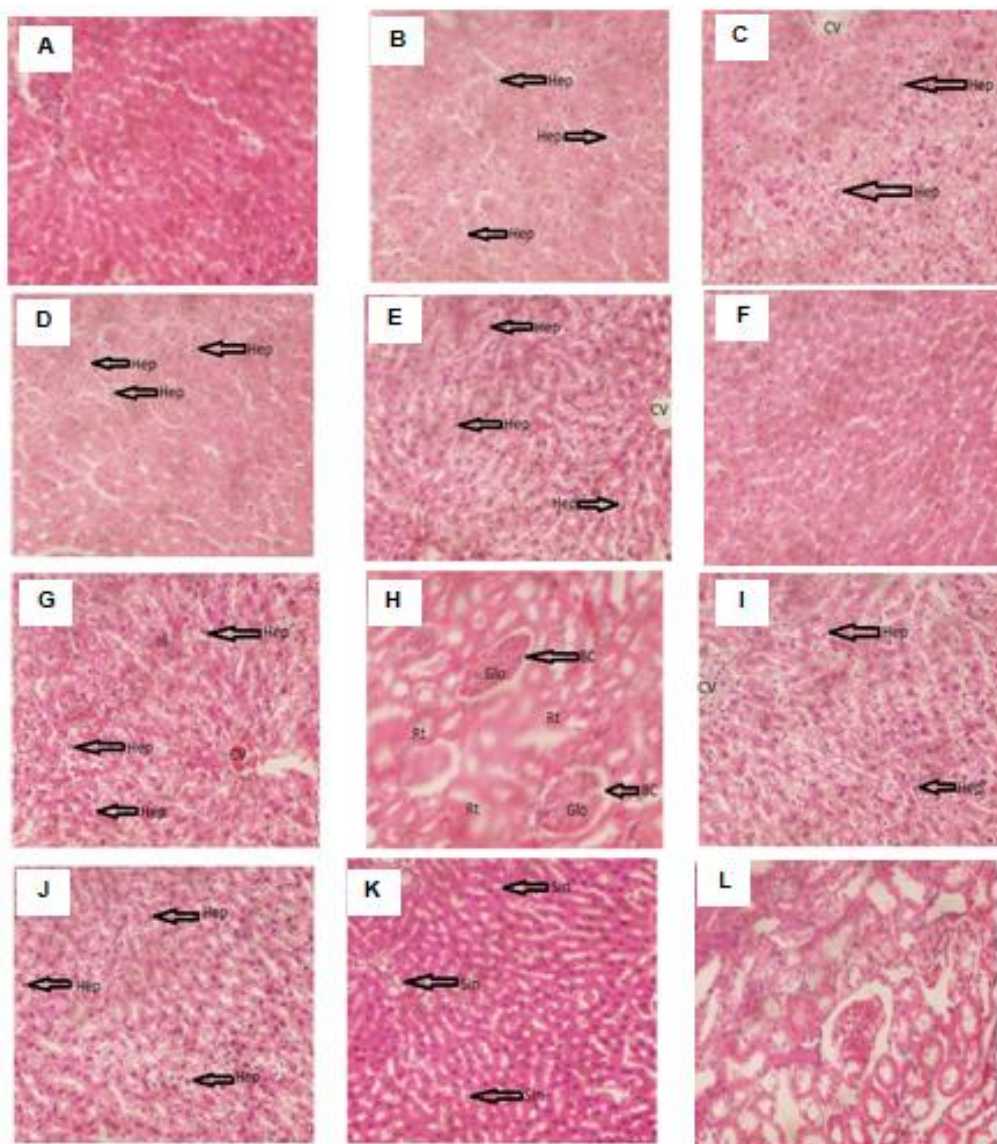


Fig. 1a. Plates A-L. Light microscope photographs of liver and kidney paraffin sections (H & E stained), (Mag *400)

Table 1. Effects of *Acalypha godseffiana* aqueous leaf extract on Hepatotoxic biomarkers

	Group 1 (Negative Control)	Group 2 (H ₂ O ₂) Positive Control	Group 3 (H ₂ O ₂ + 100mg/kg <i>A. godseffiana</i>)	Group 4 (H ₂ O ₂ + 200mg/kg <i>A. godseffiana</i>)	Group 5 (H ₂ O ₂ + 400mg/kg <i>A. godseffiana</i>)
AST (μ/L)					
Day 14	105.00 ± 3.00 ^a	120.00 ± 3.00 ^a	106 ± 1.00 ^a	111.50 ± 1.50 ^a	57.50 ± 7.50 ^b
Day 28	107.50 ± 7.50 ^a	120.50 ± 8.50 ^a	111.00 ± 1.00 ^a	108.00 ± 14.00 ^a	54.00 ± 4.00 ^b
ALT (μ/L)					
Day 14	22.50 ± 1.50 ^{ab}	23.50 ± 6.50 ^{ab}	16.50 ± 1.50 ^{ab}	26.50 ± 1.50 ^{ab}	29.50 ± 0.50 ^a
Day 28	25.00 ± 5.00 ^{ab}	21.50 ± 3.50 ^{ab}	22.00 ± 2.00 ^{ab}	24.50 ± 2.50 ^{ab}	22.50 ± 2.50 ^{ab}
ALP (μ/L)					
Day 14	59.00 ± 9.00 ^a	54.50 ± 0.50 ^{ab}	29.50 ± 0.50 ^d	40.00 ± 2.00 ^{bcd}	29.50 ± 1.50 ^d
Day 28	59.50 ± 0.50 ^a	53.50 ± 3.50 ^{ab}	36.50 ± 8.50 ^{cd}	45.50 ± 2.50 ^{abc}	29.00 ± 1.00 ^d
Total Protein (g/L)					
Day 14	64.50 ± 4.50 ^a	72.50 ± 2.50 ^a	68.00 ± 1.00 ^a	69.50 ± 0.50 ^a	72.50 ± 2.50 ^a
Day 28	70.00 ± 2.00 ^a	70.00 ± 7.00 ^a	66.00 ± 1.00 ^a	69.00 ± 1.00 ^a	66.00 ± 2.00 ^a
Serum Albumin (g/L)					
Day 14	45.50 ± 2.50 ^a	42.50 ± 0.50 ^{abc}	39.50 ± 1.50 ^{bc}	39.00 ± 1.00 ^{bc}	44.00 ± 1.00 ^{ab}
Day 28	42.50 ± 2.50 ^{abc}	39.50 ± 1.50 ^{bc}	42.50 ± 0.50 ^{abc}	40.50 ± 0.50 ^{bc}	39.50 ± 1.50 ^{bc}
Total Bilirubin (μ mol/L)					
Day 14	7.15 ± 0.15 ^{abc}	7.95 ± 0.25 ^{abc}	7.10 ± 0.10 ^{abc}	8.30 ± 0.50 ^{ab}	5.85 ± 0.15 ^{cd}
Day 28	6.00 ± 1.50 ^{cd}	8.40 ± 0.60 ^a	8.35 ± 0.15 ^{ab}	8.10 ± 1.60 ^{abc}	4.65 ± 0.15 ^d
Conjugated Bilirubin (CB) (μ mol/L)					
Day 14	3.60 ± 0.10 ^{bc}	3.90 ± 0.60 ^{bc}	3.35 ± 0.15 ^{bc}	6.05 ± 0.15 ^a	3.50 ± 0.50 ^{bc}
Day 28	3.50 ± 0.50 ^{bc}	5.15 ± 0.95 ^{bc}	4.30 ± 0.20 ^{abc}	4.80 ± 1.70 ^{ab}	2.40 ± 0.40 ^c

Values are Mean ± Standard Deviation. Data with the same alphabets (a,b,c,d,e,f) as superscript shows non-significant differences ($p \geq 0.05$), while that with different alphabets as superscript shows significant differences ($p \leq 0.05$)

Table 2. Effects of *Acalypha godseffiana* aqueous leaf extract on renal biomarkers

	Group 1 (Negative Control)	Group 2 (H ₂ O ₂) Positive Control	Group 3 (H ₂ O ₂ + 100mg/kg <i>A. godseffiana</i>)	Group 4 (H ₂ O ₂ + 200mg/kg <i>A. godseffiana</i>)	Group 5 (H ₂ O ₂ + 400mg/kg <i>A. godseffiana</i>)
K ⁺ (mmol/L)					
Day 14	4.30 ± 0.40 ^c	6.95 ± 1.75 ^a	5.20 ± 0.20 ^{bc}	7.25 ± 0.25 ^a	5.95 ± 0.05 ^{abc}
Day 28	5.90 ± 0.10 ^{abc}	6.10 ± 0.50 ^{abc}	6.85 ± 0.65 ^{ab}	6.35 ± 0.05 ^a	5.55 ± 0.05 ^{abc}
Na ⁺ (mmol/L)					
Day 14	112.50 ± 2.50 ^c	136.50 ± 8.50 ^a	114.00 ± 4.00 ^c	137.50 ± 2.50 ^a	126.50 ± 1.50 ^{ab}
Day 28	128.00 ± 1.00 ^{ab}	133.50 ± 3.50 ^{ab}	134.00 ± 4.00 ^{ab}	131.50 ± 1.50 ^{ab}	124.00 ± 4.00 ^{bc}
Urea (mmol/L)					
Day 14	6.95 ± 0.15 ^{bc}	6.75 ± 0.15 ^{bcd}	9.85 ± 0.15 ^a	6.7 ± 0.2 ^{bcd}	7.35 ± 0.15 ^{bc}
Day 28	4.55 ± 0.25 ^e	3.80 ± 0.10 ^f	5.70 ± 0.10 ^{de}	7.80 ± 1.10 ^b	6.30 ± 0.30 ^{cd}
Creatinine (mmol/L)					
Day 14	136.00 ± 6.00 ^b	135.00 ± 3.00 ^b	165.50 ± 2.50 ^a	139.00 ± 1.00 ^b	147.00 ± 3.00 ^b
Day 28	90.50 ± 1.50 ^d	76.00 ± 2.00 ^d	114.00 ± 2.00 ^c	145.00 ± 12.00 ^b	136.00 ± 4.00 ^b
Cl ⁻ (mmol/L)					
Day 14	51.00 ± 1.00 ^{abcd}	58.50 ± 1.50 ^a	50.50 ± 0.50 ^{bcd}	45.00 ± 0.00 ^d	45.50 ± 0.50 ^d
Day 28	47.50 ± 2.50 ^{cd}	55.50 ± 0.50 ^{ab}	55.00 ± 5.00 ^{abc}	48.50 ± 4.50 ^{bcd}	48.50 ± 1.50 ^{bcd}
HCO ₃ ⁻ (mmol/L)					
Day 14	27.00 ± 1.00 ^{ab}	25.00 ± 1.00 ^{ab}	27.50 ± 1.50 ^{ab}	26.50 ± 1.50 ^{ab}	29.50 ± 0.50 ^a
Day 28	24.00 ± 2.00 ^b	29.00 ± 1.00 ^{ab}	26.00 ± 4.00 ^{ab}	29.00 ± 1.00 ^{ab}	29.00 ± 1.00 ^{ab}

Values are Mean ± Standard Deviation. Data with the same alphabets (a, b, c, d, e, f) as superscript shows non-significant differences ($p \geq 0.05$), while that with different alphabets as superscript shows significant differences ($p \leq 0.05$).

Table 3. Effects of *Acalypha godseffiana* aqueous leaf extract on Antioxidant Enzymes

	Group 1 (Negative Control)	Group 2 (H ₂ O ₂) Positive Control	Group 3 (H ₂ O ₂ + 100mg/kg <i>A. godseffiana</i>)	Group 4 (H ₂ O ₂ + 200mg/kg <i>A. godseffiana</i>)	Group 5 (H ₂ O ₂ + 400mg/kg <i>A. godseffiana</i>)
GSH (µg/ml)					
Day 14	3.87 ± 0.50 ^{ab}	4.24 ± 0.04 ^a	2.76 ± 0.05 ^d	3.18 ± 0.02 ^{bcd}	3.59 ± 0.05 ^{abcd}
Day 28	3.28 ± 0.18 ^{bcd}	3.77 ± 0.24 ^{abc}	2.94 ± 0.32 ^{cd}	3.01 ± 0.13 ^{bcd}	3.84 ± 0.54 ^{ab}
Catalase (µ/g)					
Day 14	13.50 ± 0.70 ^{ab}	7.40 ± 0.05 ^{cd}	8.40 ± 0.10 ^{cd}	9.95 ± 0.05 ^{bcd}	14.40 ± 0.40 ^a
Day 28	9.80 ± 2.80 ^{bcd}	10.45 ± 2.35 ^{abc}	9.20 ± 0.40 ^{cd}	9.65 ± 0.25 ^{cd}	6.30 ± 0.54 ^d
SOD (µ/mol)					
Day 14	0.75 ± 0.10 ^a	0.90 ± 0.005 ^a	0.87 ± 0.025 ^a	0.68 ± 0.005 ^{ab}	0.84 ± 0.06 ^a
Day 28	0.40 ± 0.065 ^b	0.64 ± 0.145 ^{ab}	0.83 ± 0.12 ^a	0.72 ± 0.035 ^{ab}	0.65 ± 0.25 ^d
MDA (µmol/ml)					
Day 14	0.27 ± 0.07 ^b	0.16 ± 0.04 ^a	0.17 ± 0.035 ^b	0.17 ± 0.025 ^b	0.22 ± 0.025 ^b
Day 28	0.61 ± 0.065 ^a	0.17 ± 0.025 ^b	0.15 ± 0.055 ^b	0.19 ± 0.01 ^b	0.15 ± 0.045 ^b

Values are Mean ± Standard Deviation. Data with the same alphabets (a, b, c, d, e, f) as superscript shows non-significant differences ($p \geq 0.05$), while that with different alphabets as superscript shows significant differences ($p \leq 0.05$)

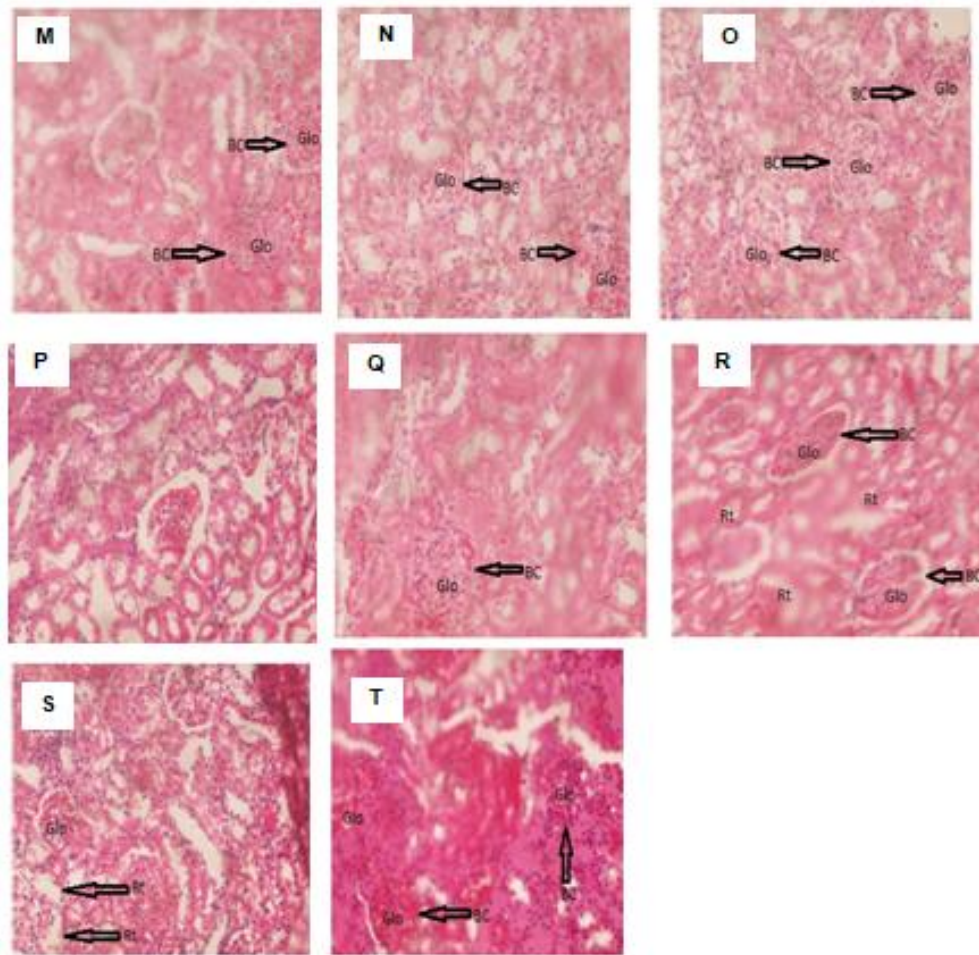


Fig. 1b. Plates M-T. Light microscope photographs of liver and kidney paraffin sections (H & E stained), (Mag *400)

Endogenous antioxidant enzymes (e.g., CAT and SOD) and non-enzymatic enzymes (e.g., GSH) protect cell constituents from oxidative damage. SOD and CAT form part of the enzymatic oxidative system of RBCs while MDA is an end product of lipid peroxidation in the liver and other tissues. SOD is a metalloproteinase enzyme involved in antioxidant defense, which acts by lowering the steady state level of O_2^- while catalase protects cells against radical toxicity by catalyzing the decomposition of hydrogen peroxide to water and molecular oxygen. Decreased levels of CAT in H_2O_2 group rats may be due to the overuse of this enzymatic antioxidant. The significant increase in the activities of GSH, SOD and CAT suggests a greater level of endogenous antioxidant associated with the *A. godseffiana* treatment resulting in an enhanced free radical scavenging activity. Plants are the sources for a wide variety of compounds like flavonoids and polyphenols.

These compounds may be responsible for increasing antioxidant status.

Photomicrographs of the normal liver showed normal hepatic architecture in rats in the negative control group. The hepatocyte, portal tract and sinusoids containing capillaries and kupffer cells were normal at day 14 and 28 respectively (Fig. 1, plates A, F). A histologically distorted liver with hepatocytes showing microvesicular steatosis was observed in the H_2O_2 group at day 14 and 28 (Fig. 1, plates B, G). Conversely, the same tissues were characterized by little necrosis and good recovery, fewer hepatocytes with microvesicular steatosis and fusion of nuclei in the groups treated with *A. godseffiana* as shown by photomicrographs from liver section of rat administered *A. godseffiana* leaf extract. The H_2O_2 + 400 mg/kg *A. godseffiana* group recorded mildly distorted liver with sinusoids a filled with

only few inflammatory cells at days 14 and 28 (plate J).

Photomicrographs of the kidney for rats in the negative control group (Fig. 1, plates K & P) showed histologically normal kidney at days 14 and 28; the glomeruli, bowman's capsular space (BC) and renal tubules (RT) were observed to be normal. H₂O₂ toxicity resulted in the distortion of the kidney tissues (plates L & Q) at days 14 and 28. The administered aqueous leaf extract of *A. godseffiana* seem not to have done much to ameliorate the tissue distortion previously observed. The H₂O₂ + 400 mg/kg *A. godseffiana* group recorded histologically distorted kidneys at days 14 and 28 (plates O & T) just as observed *ab initio* in the H₂O₂ group.

Overall, the architecture of the liver was preserved by the aqueous leaf extract of *A. godseffiana* administered, with the liver photomicrographs of the experimental animals showing mildly distorted liver with sinusoids a filled with only few inflammatory cells.

4. CONCLUSION

From the foregoing, *A. godseffiana* exhibit promising antioxidant and hepatoprotective potency, supported by previously reported pharmacologically active compounds, with antioxidant potentials, in the leaves. These potentials should be explored further as it may serve as a future therapy in the management of oxidative stress.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that "Principles of Laboratory Animal Care" (NIH Publication no. 85-23, revised 1985) were followed. All experiments were examined and approved by the appropriate ethics committee.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Yesilbursa D, Serdar Z, Serdar A, Sarac M, Jale C. Lipid peroxides in obese patients

and effects of weight loss with orbital on lipid peroxides levels. *Int. J. Obesity*. 2005; 29:142–145.

2. Seung H, Kyeong M, Yong LK, Sang L, Jinwoong HK, Young C. Chemical constituents isolated from *Paeonia lactiflora* roots and their neuroprotective activity against oxidative stress in vitro. *J. Enz. Inhib. Med. Chem*. 2009;25:1138–1140.

3. Maritim CA, Sanders RA, Watkins III JB. Diabetes, oxidative stress, and antioxidants: a review, *Journal of Biochemical and Molecular Toxicology*. 2003; 17(1): 24–38.

4. Hye-Lin H, Hye-Jun S, Mark, AF, Dae-Yeul Y. Oxidative stress and antioxidants in pathogenesis. *World J. gastroenterol*. 2010; 16(48): 6035 - 6043.

5. Johnson F, Giulivi C. Superoxide dismutases and their impact upon human health. *Molecular Aspects of Medicine*. 2005; 26(4-5): 340–52.

6. Roušar T, Kučera O, Křiváková P, Lotková H, Kandár R, Mužáková V, Červinková Z. Evaluation of Oxidative Status in Acetaminophen-Treated Rat Hepatocytes in Culture. *Physiol. Res*. 2009; 58: 239-246.

7. Tsao AS, Kim ES, Hong WK. Chemoprevention of Cancer. *CA Cancer J Clin*. 2004;54:150–180.

8. Nwobodo EI, Nwosu DC, Ogbodo SO, Ugwuene FO, Ihim AC, Onuabuchi Ani N, Nnodim JK, Ani O. Effects of *Azadirachta indica* leaf aqueous extract on the antioxidant enzymes in paracetamol-induced hepatotoxicity in Wistar rats *Int. J. Biol. Chem. Sci*. 2018;12(1): 1-10.

9. Valacchi G, Davis P. *Oxidants in Biology: A Question of Balance*. 1st edition, Springer, New York; 2008.

10. Makker K, Ashok A, Rakesh S. Oxidative stress and male infertility. *Indian J. Med. Res*. 2009; 129:357–367.

11. Ozen OA., Kus MA, Kus I, Alkoc OA, Songur A. Protective effect of melatonin against formaldehyde-induced oxidative damage and apoptosis in rat testes: An immunohistochemical and biochemical study. *Syst Biol Reprod Med*. 2008; 54:169–176.

12. Krizanovic D, Susic V, Bozic P, Stokovic I, Kabalin AK. Changes of bovine blood lipid peroxides and some antioxidants in the

- course of growth. *Veterinarski Arhiv*. 2008; 78:269–278.
13. Gulcin I, Mshvildadze V, Gepdiremen A, Elias R. Screening of antiradical and antioxidant activity of monodesmosides and crude extract from *Leontice smirnowii* tuber. *Phytomedicine*. 2006;13:343–351.
 14. Chandra K, Syed, SA, Abid M, Sweetey R, Najam AK. Protection Against FCA Induced Oxidative Stress Induced DNA Damage as a Model of Arthritis and in vitro Anti-Arthritic Potential of *Costus speciosus* Rhizome Extract. *Int. J. Pharmacog. Phytochem. Res.* 2015; 7(2): 383 -389.
 15. Halliwell B. Reactive oxygen species in living systems-source, biochemistry, and role in human-disease. *Am J Med.* 1991; 91:14–22.
 16. Kaur H, Perkins J. The free radical chemistry of food additives. In: Aruoma OI Halliwell B (Ed): *Free radicals and food additives*. 17–35, Taylor and Francis Ltd, London; 1991.
 17. Davey MW, Stals E, Panis B, Keulemans J, Swennen RL. High-throughput determination of malondialdehyde in plant tissues. *Analytical Biochemistry*. 2005; 347(2):201–207.
 18. Ikewuchi JC, Onyeike EN, Uwakwe AA, Ikewuchi CC. Effects of aqueous extract of the leaves of *Acalypha wilkesiana* ‘Godseffiana’ Muell Arg (Euphorbiaceae) on the hematology, plasma biochemistry and ocular indices of oxidative stress in alloxan induced diabetic rats. *J. Ethnopharmacol.* 2011; 137:1415-1424.
 19. Santiago C, Lim K, Loh H, Ting KN. Prevention of cell-surface attachment and reduction of penicillin-binding protein 2a (PBP2a) level in methicillin-resistant *Staphylococcus aureus* biofilms by *Acalypha wilkesiana*. *BMC Complement Altern. Med.* 2015;15:79.
 20. Din WM, Chu J, Clarke G, Jin KT, Bradshaw TD et al. Antioxidant and cytoprotective effects of an ethanol extract of *Acalypha wilkesiana* var. *macafeana* from Malaysia. *Nat. Prod. Commun.* 2013; 8: 375-380.
 21. Anokwuru CP, Sinisi A, Samie A, Taghialatela-Scafati O. Antibacterial and antioxidant constituents of *Acalypha wilkesiana*. *Nat. Prod. Res.* 2015; 29:1180-1183.
 22. Lemhadri A, Zeggwagh NA, Maghrani A, Jouad H, Eddouks M. Anti-hyperglycaemic activity of the aqueous extract of *Driganum vulgare* growing wild in Tafilalet region. *J. Ethnopharmacol.* 2004; 90:251–256.
 23. Reitman S, Frankel S. Glutamic – pyruvate transaminase assay by colorimetric method. *Am. J. Clin. Path.* 1957; 28:56.
 24. Plummer DT. *An introduction to Practical Biochemistry*, 3rd edition. McGraw – Hill Book Company, Maidenhead, Berkshire; 1978.
 25. Jendrassik L, Grof P. Colorimetric Method of Determination of bilirubin. *Biochem Z.* 1938; 297:81-82
 26. Lubran MM. The Measurement of Total Serum Proteins by the Biuret Method. *Annals of Clinical and Laboratory Science.* 1978; 8(2).
 27. Rodkey FL. Direct spectrophotometric determination of albumin in human serum. *Clin Chem.* 1965; 11: 478-87.
 28. Henry RJ. *Clinical Chemistry*. 2nd edition. Harper & Row, New York; 1974.
 29. Tietz NW. *Fundamentals of Clinical Chemistry*, 2nd edition, Saunders, Philadelphia; 1976.
 30. Kenkel J. Back titrations. In: Kenkel J, editor. *Analytical chemistry for technicians*. Boca Raton, Florida: Lewis; 2003.
 31. Kaplan A, Teng LL. In *Selected Methods of Clinical Chemistry*, Vol. 9, Faulkner WR and Meites S Eds., AACC, Washington; 1982.
 32. Estridge BH, Reynolds AP, Walters NJ. *Basic medical laboratory techniques*, 4th edn, Thomson Learning, USA; 2000.
 33. Varshney R, Kale, RK. Effects of calmodulin antagonists on radiation induced lipid peroxidation in microsomes. *Int. J. Rad. Biol.* 1990; 58:733743.
 34. Fridorich I. Superoxide dismutases: An adaptation to a paramagnetic gas. *J. Biol. Chem.* 1989; 264:7761-7764.
 35. Aebi HE. Catalase. In *Methods of Enzymatic Analysis*, 3rd ed; Bergmeyer H, Verlag C, Weinheim A (Eds.); Verlag Chemie: Weinheim, Germany; 1983.
 36. Habig WH, Pabst MJ, Jakoby WB. Glutathione s-transferases: The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* 1974; 249:7130-7139.

37. Bankroft JD, Stevens A. Theory and practice histological technique. Churchill Livingstone, Edinburgh, London; 1982.
38. Adikwu E, Bokolo B. Effect of cimetidine on cyclophosphamide induced liver toxicity in albino rats. Asian. J. Med Sci. 2018; 9(5): 50-56.
39. Adeshina AJ, Fakunle PB, Oloyede AO. Some Protective Effects of Aqueous Leaf Extract of *Azadirachta indica* on Paracetamol-induced Hepatotoxicity in Adult Wistar Rats. American Journal of Tropical Medicine & Public Health. 2011; 1(3): 97-106.
40. Anokwuru CP, Anyasor GN, Shokunbi OS, Sopekan B, Osinuga OK, Afolabi OE, Arojjoye OB, Ezekiel CN, Ramaite ID. Chemoprotective activity of aqueous leaf extract of *Acalypha wilkesiana* against cyclophosphamide-induced toxicity in rats. Asian Pacific Journal of Tropical Medicine. 2019; 12(9): 409-415.
41. Olubodun S. Phytochemical studies and antimicrobial activity of a Nigerian medicinal plant *Acalypha godseffiana* (Euphobiaceae) leaf extract used in skin infection. International Journal of Chemistry. 2007; 17(2):113-117.
42. Aladejimokun AO, Daramola KM, Osabiya OJ, Arije OC. Comparative Study of Phytochemical Constituents and Antimicrobial Activities of *Acalypha wilkesiana* and *Acalypha godseffiana* Extracts. Journal of Advances in Microbiology. 2017; 4(1): 1-7.
43. Asekunowo AK, Ashafa AOT, Okoh O, Asekun OT, Familoni OB. Polyphenolic constituents, antioxidant and hypoglycaemic potential of leaf extracts of *Acalypha godseffiana* from Eastern Nigeria: *In vitro* study. Journal of Medicinal Plants for economic development. 2019; 3(1).
44. Samatha T, Shyamsundarachary R, Srinivas P, Swamy RS. Quantification of total phenolic and total flavonoid contents in extracts of *Oroxylum indicum* L. Kurz. Asian Journal of Pharmaceutical and Clinical Research. 2012; 5(4): 177-179.
45. Shukla S, Mehta A, John J, Singh S, Mehta P, Vyas SP. Antioxidant activity and total phenolic content of ethanolic extract of *Caesalpinia bonducella* seeds. Food and Chemical Toxicology. 2009; 47(8): 1848-1851.
46. Chattopadhyay RR, Bandyopadhyay M. Effect of *Azadirachta indica* leaf extract on serum lipid profile changes in normal and streptozotocin induced diabetic rats. African J. Biomed. Res. 2005; 8(2):101 - 104.
47. Nwobodo EI. Evaluation of Antilipid Peroxidation and Hypolipidemic Potentials of *Azadirachta indica* Leaf Aqueous Extract in Paracetamol Induced Hepatotoxicity in Wistar Rats. Int. J. Inform. Res. Rev. 2017; 4(2): 3615-3619.
48. Aderogba MA, Akinkunmi EO, Mabusela WJ. Antioxidants and Antimicrobial activities of flavonoid glycosides from *Dennettia tripetala* G. Baker Leaf extract. Niger J Nat Prod Med. 2011; 15: 49-52.
49. Imo C, Arowora KA, Ezeonu CS, Ikwebe J, Yakubu OE, Imo NG, Danlami GC. Biochemical and histological effects of ethanolic extracts of fruits of *Xylopiya aethiopia* and seeds and leaves of *Piper guineense* on liver and kidney function in male albino rats. Future Journal of Pharmaceutical Sciences. 2021;7:35.
50. Toafeeq O, Ibrahim BG, Abdul-waheed A., Gassal R, Godwin A. Hepatotoxicity and nephrotoxicity evaluation in Wistar albino rats exposed to *Moringa lucida* leaf extract. North American J. Med. Sci. 2010;2:230-233.
51. Yakubu MT, Bilbis LB, Lawal M, Akanji MA. Evaluation of selected parameters of rat Liver and kidney function following repeated administration of yohimbine. Biochemistry. 2003; 15: 50-56.
52. Ifeanacho MO. Biochemistry of the Kidney in Biochemistry for Medical and Basic Sciences. 2nd edn. Uwakwe, A. A., Agomuo, E. N. (eds). Supreme Publishers, Owerri; 2017.
53. Devlin TM. Textbook of Biochemistry with Clinical Correlation. 6th edn. Wiley-liss New Jersey; 2011.
54. Okpala JC, Sani I, Abdullahi R, Ifedilichukwu HN, Igwe JC. Effects of nbutanol fraction of *Gongronema latifolium* leave extract on some biochemical parameters in CCl₄- induced oxidative damage in Wistar albino rats. Afr. J. Biochem. Res. 2014; 8(2):52-64.

55. Oladunmoye MK. Comparative evaluation of antimicrobial activities and phytochemical screening of two varieties of *Acalypha wilkesiana*. Trends Appl. Sci. Res. 2006;1:537-541.
56. Chatterjea MN, Sinde R. Textbook of Medical Biochemistry. 8th edn. Jaypee Brothers Medical Publishers Ltd, New Delhi; 2012.

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