

# Biochemical and liver histological changes in rats exposed to sub-lethal dose of Uproot-pesticide and the protective potentials of nutritional supplements

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## ARTICLE INFO

### Article history:

Received on: February 19, 2019

Accepted on: December 10, 2019

Available online: July 30, 2020

### Key words:

Hepatotoxicity, uproot, nutritional supplements, pesticides, oxidative stress, therapeutic

## ABSTRACT

This study determined the therapeutic potentials of vitamin C, glutathione, and garlic on the hepatic and histological changes induced by uproot-pesticide on albino Wistar rats. All animal groups (except normal control) received intraperitoneally 50 mg/kg body weight of uproot-pesticide (a commercially formulated glyphosate-based herbicide) on alternate days and daily oral administration (except Uproot control) of 20 mg/kg body weight of glutathione, vitamin C, and garlic as nutritional supplements singly and in combination for 28 days. Liver function and oxidative stress parameters of the liver were determined using blood and liver samples. Histological studies were done on the liver tissue. The values obtained showed significant variation of measured parameters in Uproot control compared to groups administered nutritional supplements and the control. These variations indicated oxidative liver damage and significant therapeutic potential of the nutritional supplements. Other observed significant and non-significant changes were discussed.

## 1. INTRODUCTION

Pesticides are developed for increased agricultural productivity and yield, forming part of “green revolution” [1]. The application of vast variety of pesticide and chemical fertilizer in modern agriculture practice has impacted negatively on the environment and consumer health [2–4]. Large percentage of the toxic chemicals in pesticides designed to deliberately kill pests end up in air, water, sediments, and food. Health issues, such as diabetes, cancer, Parkinson’s and Alzheimer’s disease, chronic respiratory problems, neurological disorders, spontaneous abortions, and infertility, and increased risk of development of congenital malformations, have being implicated to residues of pesticides in food [5,6].

Uproot is a glyphosate-based herbicide commonly used at home and commercially in Nigeria. Glyphosate is an important active

ingredient of many commercial herbicides, absorbed through the foliage of plants and transported to the roots through xylem and phloem, destroying the root tissue. The quality and activity of this commercial herbicide is improved by preparing it with isopropylamine salt of glyphosate and polyethoxylene amine surfactant [7,8]. The relative low persistence of glyphosate in the environment, results in repeated application [9,10] for the control of weeds in agricultural fields and household lawns, thereby, large quantities find their way into the water bodies [11].

There are records of glyphosate-induced oxidative stress [12] in mammals due to the generation of free radicals resulting in biochemical derangement and organ damage. This condition occurs due to disruption of antioxidant compounds concentration [10], and antioxidant enzyme system [13,14]. Studies have shown that the use of food containing antioxidants can be vital in amelioration of xenobiotic induced toxicity. To maintain good health, dietary supplements with antioxidant capacity play significant role in attenuating xenobiotics. Studies have shown that nutritional intake of ascorbate [15], glutathione [16], and whole or extract of garlic [17] inhibits oxidative-induced cellular damage.

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It is on the foregoing that this study was conceived, to determine the toxicity of non-lethal concentration (as may be found as residuals in food and water) of glyphosate-based pesticide on liver of Wistar albino rats and the protective potential of some nutritional supplements.

## 2. MATERIALS AND METHODS

### 2.1. Animals

Forty two matured male Wistar albino rats weighing  $160 \pm 20$  g were housed under standard room conditions of temperature  $25 \pm 3^\circ\text{C}$  and photoperiod of 12 hours light and 12 hours dark. The animals were allowed access to Vital growers mesh and clean water. Acclimation was for a week at the animal house of the Department of Biochemistry, Federal University of Technology, Owerri (FUTO), Nigeria. The Ethics Committee of the Department of Biochemistry, FUTO, Nigeria, approved this study with the reference number FUTO/BCH/EC/2017/24b. The animals used in this study were handled according to the guidelines recommended by the National Institute of Health [18]. The herbicide (Uproot) was supplied by the Ministry of Agriculture and Natural Resources, Imo State, Nigeria.

### 2.2. Hepatotoxicity Studies

At the end of acclimation, the rats were randomly allocated in seven groups with each group having six rats. Normal control group received food and water only. All the groups (less Normal control) received intraperitoneally 50 mg/kg body weight (bw) of uproot herbicide in distilled water at alternate days for the 28 days allowed for the study. Finally, all groups (except Normal and Uproot control) were administered orally, nutritional supplements daily in the following order: Garlic group received 20 mg/kg bw Garlic supplement; GSH group received 20 mg/kg bw Glutathione supplement; Vitamin C group received 20 mg/kg bw vitamin C; Garlic plus Vitamin C (GVC) group received 20 mg/kg bw garlic and 20 mg/kg bw vitamin C; Glutathione plus Vitamin C (GHVC) group received 20 mg/kg bw glutathione and 20 mg/kg bw vitamin C. Animals in each group were allowed access to Vital growers mesh and clean water for the 28 days of the study.

### 2.3. Sample Collection

At the end of four weeks of exposure and treatment, blood samples were collected from the animals via ocular puncture in plain sample bottles and ethylene diamine tetra-acetate (ETDA) bottle. The blood in plain sample bottles were allowed to clot. Serum was obtained by spinning in a centrifuge. Furthermore, the animals were sacrificed, their liver and kidney excised. A portion of the liver and kidney tissues were reserved for homogenate preparation and a portion washed in saline and preserved in 10% normal saline.

### 2.4. Liver Tissue Homogenate

Tissue homogenates (5% w/v) were prepared in cold potassium phosphate buffer of pH 7.4. The homogenate was spurned in a cold ( $4^\circ\text{C}$ ) centrifuge to remove cell debris. The supernatant obtained was used to determine oxidative stress parameters: glutathione, total antioxidant capacity, and malondialdehyde (MDA).

### 2.5. Hepatotoxicity Studies

Serum activity of liver function marker enzymes, such as ALT, ALP, and AST, were assayed using liver enzyme activity assay kits (Bio Merieux France) according to the manufacturer's instructions. Total bilirubin was determined by Jendrassik and Grof [19] method; total protein by Tietz *et al.* [20] method, and albumin by Doumas *et al.* [21] method.

### 2.6. Oxidative Studies

The concentration of glutathione was determined in tissue homogenates using the method of Raja *et al.* [22]. Briefly: To remove protein, 1.0 ml sample homogenate was mixed with 4.0 ml of 10% trichloroacetic acid (TCA). This was spurned in a centrifuge for 10 minutes at 3,000 rpm. A pipette was used to deliver 0.01 ml of the supernatant in a test tube holding 2.0 ml of phosphate buffer of pH 8.4, 0.5 ml of 5, 5-dithiobis (2-nitrobenzoic acid), and 0.4 ml doubled distilled water. Finally, the content of the tube was vigorously mixed and absorbance measurement done in spectrophotometer at 412 nm within 15 minutes.

Ferric reducing ability of Plasma [23] was adopted for the determination of total antioxidant activity. Briefly, the following compounds: acetate buffer of pH 3.6, ferric chloride and tripyridyltriazine were constituted as working reagents in 10:1:1 ratio, respectively. With the aid of a pipette, 1.8 ml of this working reagent was delivered to test tubes containing 60  $\mu\text{l}$  of Sample or Standard or Blank. This was thoroughly mixed and allowed to incubate at  $37^\circ\text{C}$  for 10 minutes. The coloured (blue) solution that developed was measured in spectrophotometer at 593 nm. The content of blank test tube received similar treatment; however, 60  $\mu\text{l}$  of distilled water was used instead of the plasma. The standard solution is made up of 1,000  $\mu\text{mol/l}$  of ferrous sulphate.

MDA concentration as a product of lipid peroxidation was determined [24]. Four test tubes were prepared and these were added into them; 0.1 ml of sample, 0.9 ml of distilled water, 0.5 ml of 25% TCA, and 0.5 ml of 17% tribarbituric acid (TBA) in 0.3% NaOH. This was allowed to incubate at  $95^\circ\text{C}$  for 40 minutes and then allowed to cool. Subsequently, with pipette, 0.1 ml of 20 % sodium dodecyl sulphate was delivered into the tubes and absorbance reading taken at 532 and 600 nm against a blank.

### 2.7. Histological Studies

Liver samples fixed in formal saline were treated to dehydration, clearing (dealcoholization), and infiltration processes and finally embedded in paraffin [25]. Furthermore, the samples were subjected to serial sectioning at defined thickness and stained using hematoxylin and eosin [26]. With the aid of light microscope set at appropriate magnification (100 $\times$  and 400 $\times$ ), the tissue sections were examined and photographed.

### 2.8. Statistical Analysis

The data obtained in this study were subjected to one-way analysis of variance, using computer software statistical package for social sciences, version 18. Significant differences were observed at  $p \leq 0.05$ . The results were expressed as mean and standard deviation.

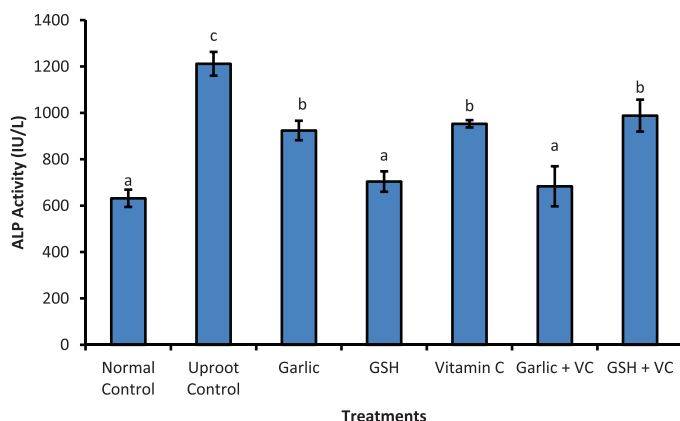
## 2.9. Biochemical Results

### 2.9.1. Liver enzymes

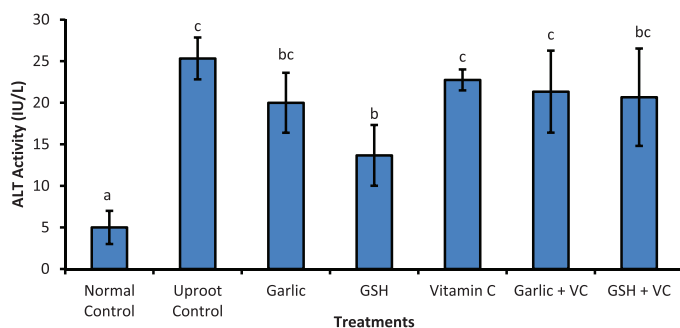
The result of ALP activity presented in Figure 1 shows a significant increase in activity of ALP in uproot control, and supplement treated groups compared to Normal control. However, activity of ALP in uproot control group increased significantly ( $p < 0.05$ ) when compared to supplement treated groups. The activities of ALT presented in Figure 2 shows significant increase in activities of ALT in uproot control and supplement-treated groups compared to normal control. However, compared to uproot control, all supplement treated groups showed non-significant reduction in ALT activities except Glutathione group which presented significant reduction. Results shown in Figure 3 reveals significant increase in activity of AST in uproot control group compared to normal control group, and supplement-treated groups. The result also showed non-significant difference in activity of AST of normal control compared to all uproot exposed and supplement-treated groups.

### 2.9.2. Liver proteins and bilirubin

Total protein concentration (Fig. 4) of uproot control showed slight (non-significant) decrease compared to normal control group and supplement-treated groups. Albumin concentration (Fig. 5) presented non-significant variation across the groups



**Figure 1:** Activities of ALP in albino Wistar rats exposed to sub-lethal dose of Uproot-herbicide and treated with nutritional supplements. Bars shows mean  $\pm$  standard deviation of quadruple determinations and those bearing different alphabets indicate significant difference at  $p < 0.05$ .



**Figure 2:** Activities of ALT in albino Wistar rats exposed to sub-lethal dose of Uproot-herbicide and treated with nutritional supplements. Bars shows mean  $\pm$  standard deviation of quadruple determinations and those bearing different alphabets indicate significant difference at  $p < 0.05$ .

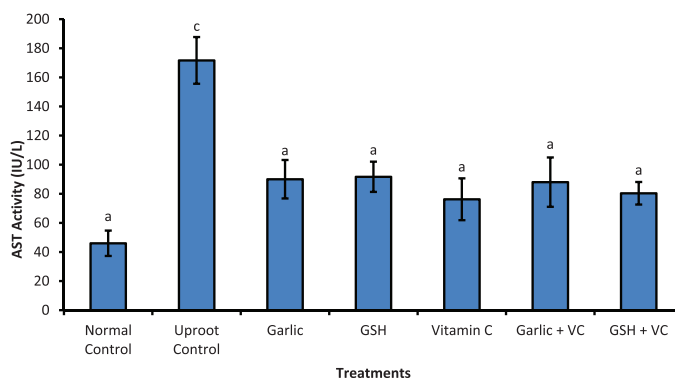
with uproot control presenting the lowest concentration. Figure 6 shows significant increases in bilirubin concentration of uproot control and treated groups compared to normal control group. Also, bilirubin reduced significantly in treated groups compared to uproot control group.

### 2.9.3. Liver oxidative stress parameters

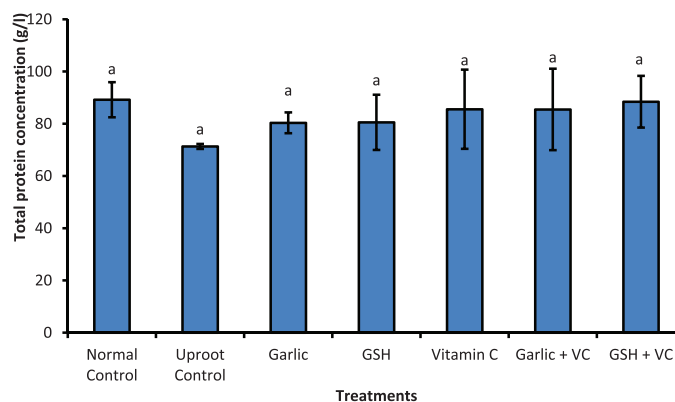
Glutathione concentrations (Fig. 7) increased significantly in supplement treated groups compared to normal control and uproot control groups. Figure 8 shows that MDA concentration increased significantly in uproot control and supplement treated groups compared to normal control group. However, groups administered selected food supplements presented significantly lower MDA concentration when compared to uproot control group. Figure 9 shows significant decrease in total antioxidant capacity of uproot control group when compared to normal control group and supplement-treated groups.

## 2.10. Histological Studies Results

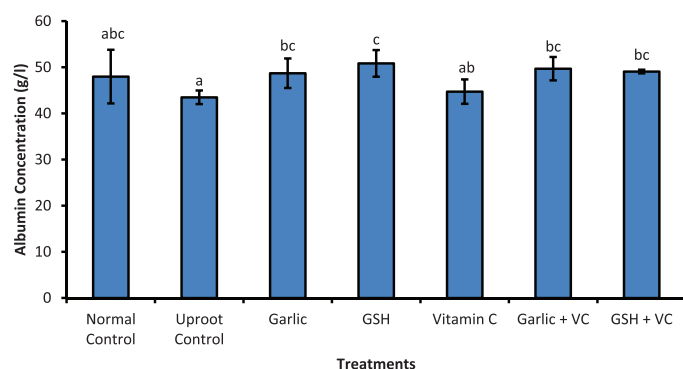
The results of the histological studies are presented in Figure 10. Normal control (NC) group shows liver tissue with relatively normal central vein (V) and hepatocytes (H); uproot control (UC)



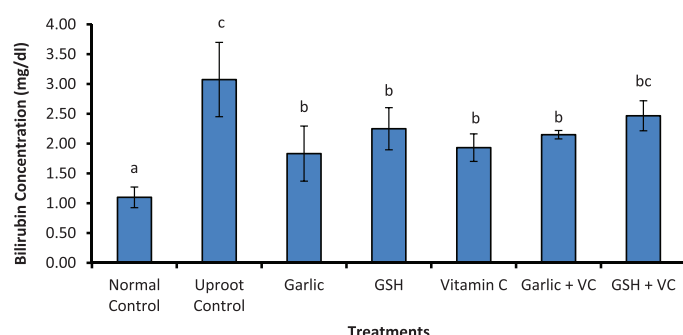
**Figure 3:** Activities of AST in albino Wistar rats exposed to sub-lethal dose of Uproot-herbicide and treated with nutritional supplements. Bars shows mean  $\pm$  standard deviation of quadruple determinations and those bearing different alphabets indicate significant difference at  $p < 0.05$ .



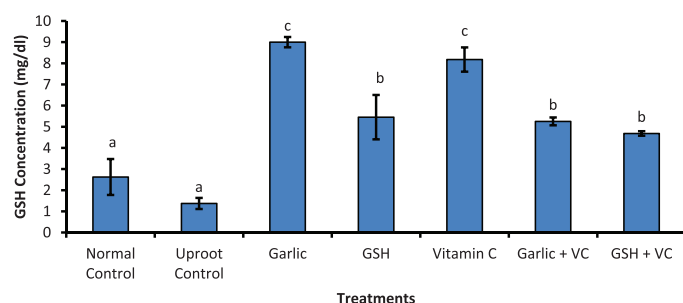
**Figure 4:** Concentration of total protein in albino Wistar rats exposed to sub-lethal dose of Uproot-herbicide and treated with nutritional supplements. Bars shows mean  $\pm$  standard deviation of quadruple determinations and those bearing different alphabets indicate significant difference at  $p < 0.05$ .



**Figure 5:** Concentrations of albumin in albino Wistar rats exposed to sub-lethal dose of Uproot-herbicide and treated with nutritional supplements. Bars shows mean  $\pm$  standard deviation of quadruple determinations and those bearing different alphabets indicate significant difference at  $p < 0.05$ .



**Figure 6:** Concentration of bilirubin in albino Wistar rats exposed to sub-lethal dose of Uproot-herbicide and treated with nutritional supplements. Bars shows mean  $\pm$  standard deviation of quadruple determinations and those bearing different alphabets indicate significant difference at  $p < 0.05$ .

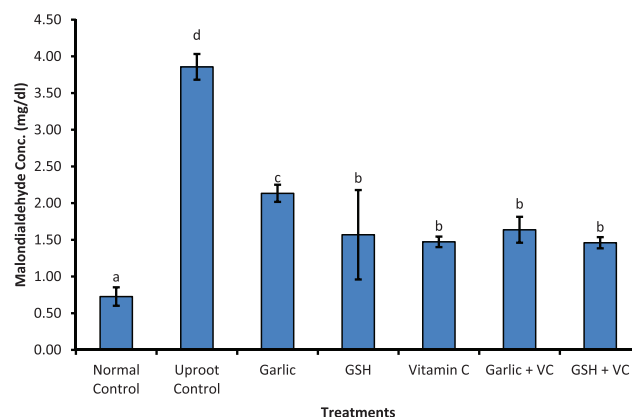


**Figure 7:** Glutathione concentration in albino Wistar rats exposed to sub-lethal dose of Uproot-herbicide and treated with nutritional supplements. Bars shows mean  $\pm$  standard deviation of quadruple determinations and those bearing different alphabets indicate significant difference at  $p < 0.05$ .

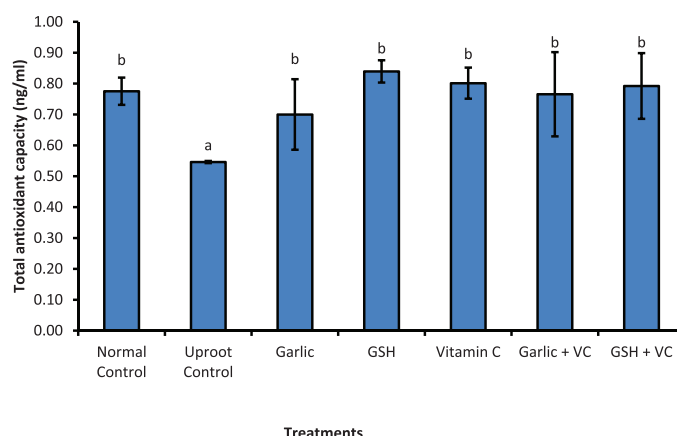
group shows liver parenchyma consisting of piecemeal loss of hepatocytes (H) and edematous sites (ED), section shows liver parenchyma with fatty deposits and inflammatory cells. However, nutritional supplements-treated groups showed mild-to-moderate recovery when compared with the uproot control.

### 3. DISCUSSION

Uproot is a commercially formulated glyphosate mix herbicide and a known terrestrial and aquatic contaminants [27–29]. This makes it very important and urgent to develop a therapy for sub-lethal and lethal exposure to Uproot-herbicide



**Figure 8:** MDA concentration in albino Wistar rats exposed to sub-lethal dose of Uproot-herbicide and treated with nutritional supplements. Bars shows mean  $\pm$  standard deviation of quadruple determinations and those bearing different alphabets indicate significant difference at  $p < 0.05$ .

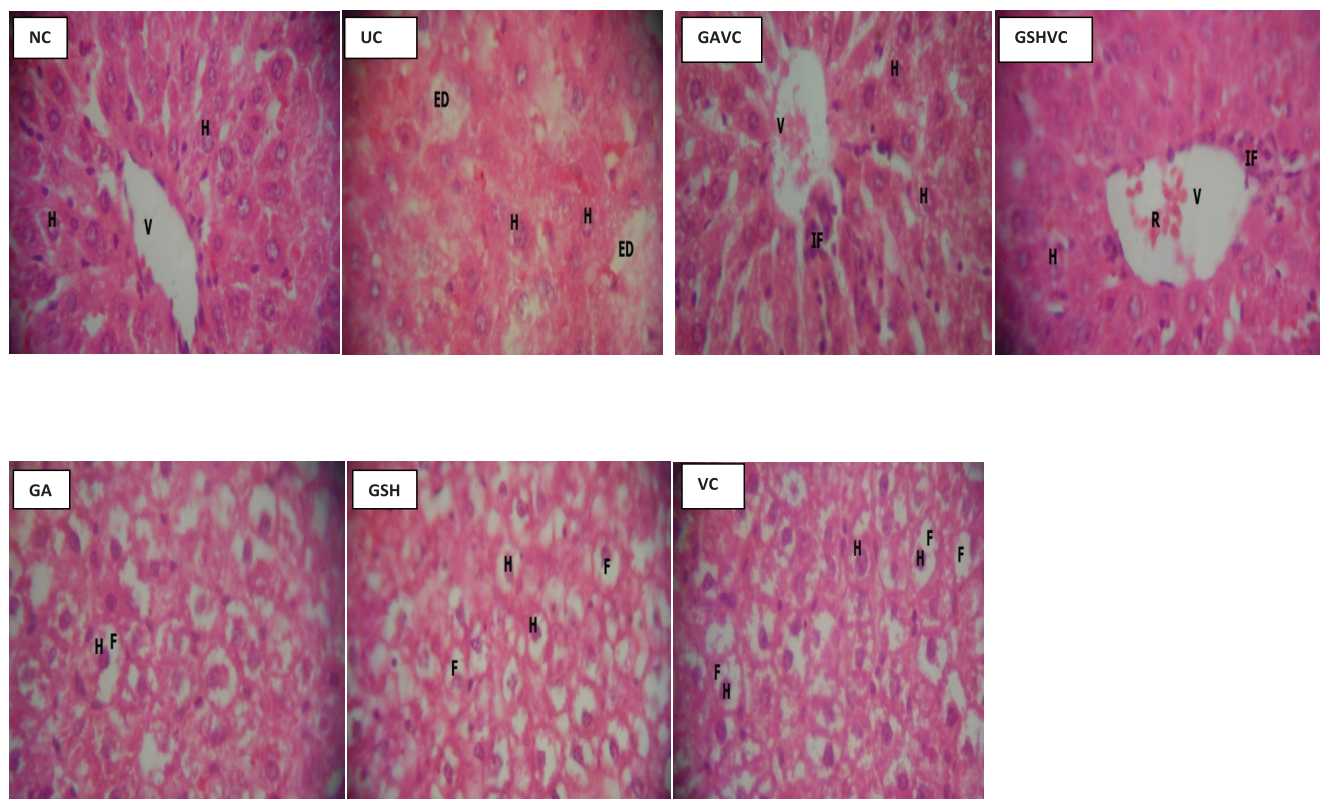


**Figure 9:** Total antioxidant capacity concentration in albino Wistar rats exposed to sub-lethal dose of Uproot-herbicide and treated with nutritional supplements. Bars shows mean  $\pm$  standard deviation of quadruple determinations and those bearing different alphabets indicate significant difference at  $p < 0.05$ .

The observed increase in activities of serum AST and ALT indicates hepatotoxic effect of uproot herbicide [30], and this agrees with the earlier observations of the effect of another glyphosate-based herbicide by Cavuşoğlu et al. [31] and dichlorvos pesticide by Celik et al. [32]. The result showed the ameliorative effect of selected food supplement as the activities of liver function enzymes of the supplement treated rats were as recorded in normal control.

The presentations of decreased total protein and albumin concentrations in uproot control rats can be a condition predicated by the hepatocytes loss of biosynthetic functions. This finding is in agreement with reports that showed decrease in total protein of albino rats exposed to glyphosate [33–35]. Fonseca et al. [36] implicated reduced serum total protein concentration to increased rate of amino acid degradation to meet the energy demand following exposure to glyphosate-based pesticide. However, groups administered the nutritional supplements (garlic, glutathione, and vitamin C) following Uproot-herbicide exposure maintained total protein and albumin concentration within the range presented by the normal control rat group. Ajith et al. [37]





**Figure 10:** Histopathological sections of liver in albino Wistar rats exposed to sub-lethal dose of Uproot-herbicide and treated with nutritional supplements. NC. Normal Control (400×); UC, Uproot control group (100×); GAVC, Garlic +Vitamin C group (100×); GSHVC, GSH+Vitamin C group (100×); GA, Garlic treated rats (100×); GSH, Glutathione group (100×); VC. Vitamin C treated rats (100×). V indicates Central vein; H indicates Hepatic parenchyma; F indicates Fatty materials, IF-inflammatory cells.

had reported earlier the ameliorative effect of Vitamin C and other supplements on herbicide-intoxicated rats.

Also, the significant increase in total bilirubin concentration of uproot control group may be due to hepatobiliary damage caused by the herbicide breakdown [38]. Bilirubin is found in bile, and also in the intestines and reticuloendothelial cells of the spleen [39]. Bilirubin is synthesized in bone marrow and in liver as breakdown product of red blood cell. The increase in bilirubin concentration may also be attributed to increased bilirubin production due to red blood cell breakdown or decreased hepatic uptake of bilirubin due to the reduced level of the transport protein-albumin. However, concomitant administration of dietary supplements significantly restored bilirubin concentration to almost normal level. Similar report showed that certain food supplements could stimulate or enhance amino acid biosynthesis and utilization leading to increased level of albumin actively involved in bilirubin transport and clearance [37].

The reduced glutathione and total antioxidant capacity of the hepatocytes of rats in uproot control group is the outcome of exhaustion of reduced glutathione, overwhelmingly used in the redox activities due to increased generation of free radicals and ROS induced by the herbicide [40]. These free radicals when unchecked can induce oxidative stress [41]. Co-administration of nutritional supplement to uproot exposed groups restored cellular glutathione concentration and total antioxidant capacity of hepatocytes as observed in normal control rats. These supplements contain antioxidants that impede and/or attenuate oxidative damage

to important cellular biomolecules [42]. Hepatic glutathione have dual actions in that in one state it is directly involved in scavenging free radicals and/or acts as an indispensable cellular substrate in the activities of glutathione peroxidase and transferase. This interplay of substrates and enzymes is an important phase in the detoxification of peroxides, electrophiles and inhibits the oxidation of thiol groups of peptides and proteins [43,44].

Also, the increased MDA concentration in uproot control indicates lipid peroxidation induced by free radicals generated by the herbicide. However, groups treated with dietary supplements showed reduced hepatic MDA, thus connoting effective antioxidant properties by the supplements. Earlier works showed that food supplements can reduce the levels of MDA [45], and phenolic fractions of garlic demonstrated radical scavenging potentials and attenuation of lipid peroxidation [46].

The uproot herbicide-induced cellular alterations, as shown by loss and subsequent death of hepatocytes and presence of edematous liver cells which may eventually rupture. These outcomes predicates increase in liver function enzymes [5] and loss of antioxidative potentials such as reduced concentration of glutathione and increased lipoperoxidation [31] as recorded in this study. corroborating the outcome of current investigation are the reports of Hazarika *et al.* [47] and Kavitha and Rao [48], which indicated that lipoperoxidation in glyphosate-based herbicide is evidenced by organophosphorus compounds acting directly with cytoplasmic membranes which results to systemic damage.

However, the systemic damage of hepatocytes membrane structure by Uproot-herbicide was significantly attenuated by the antioxidants of the supplements. Herbicide exposed and nutritional supplements treated groups, recorded mild changes in liver histoarchitecture indicating reduced toxic damage. The result of this study is corroborated by a study that reported the capacity of glyphosate-based herbicide in adverse oxidative changes and prevention of cellular death by co-administration of antioxidants [49].

#### 4. CONCLUSION

The data collected from the present study indicate glyphosate-based pesticide-induced liver dysfunction supported by histopathological lesions. These adverse changes of liver functions could be associated to induction of oxidative stress via ROS generation. However, administration of selected food supplements showed great potentials in reversing the negative consequences of systemic presence of glyphosate-based pesticide.

#### CONFLICT OF INTEREST

The authors declared that they have no conflicts of interest.

#### AUTHORS CONTRIBUTION

All the authors worked equally and approved the final manuscript.

#### FINANCIAL SUPPORT

None.

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#### How to cite this article:

Ujowundu CO, Ogamanya KI, Ujowundu FN, Adejoh VO, Iheme CI, Igwe KO. Biochemical and liver histological changes of Wistar albino rats exposed to uproot-pesticide and the protective potentials of nutritional supplements. *J Appl Biol Biotech* 2020;8(04):026–032. DOI: 10.7324/JABB.2020.80404