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Research Article

Chemoprotective Potentials of Selected Dietary Supplements in Glyphosate-based Herbicide-induced Nephrotoxicity and Dyslipidemia in Albino Wistar Rats

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Abstract

Background and Objectives: There is an immediate and urgent need to fashion out workable strategy for the amelioration of intentional and/or unintentional exposure to commercially formulated glyphosate based herbicide. This is important because of the multiple biochemical and organ toxicity arising from the surge in its use. To achieve this, nutritional supplements were used in this chemoprotective study to ameliorate glyphosate-based herbicide-induced nephrotoxicity and dyslipidemia in albino Wistar rats. **Materials and Methods:** Forty two (160±20 g) rats were divided into 7 groups of 6 each. The groups were treated as follows: Group 1 received only feed and water (normal control), Groups 2-7 received intraperitoneally on alternate days 50 mg kg^{-1} b.wt., (bw) uproot herbicide. Also, Groups 3 -7 received oral daily dose of 20 mg kg^{-1} bw garlic, glutathione, vitamin C single and in combination for 4 weeks. **Results:** This exposure induced significant increase in lipid peroxidation product, creatinine and urea concentrations. Also kidney GSH concentration and total antioxidant capacity reduced and fluctuations in lipid profile were discussed. The kidney histopathological studies recorded mild ultrastructural changes. Furthermore, some of the rat groups administered dietary supplements showed levels of the determined parameters within that of the normal group. **Conclusion:** These findings indicate sub-lethal dose of glyphosate-based herbicide can induce renal dysfunction and adverse changes in lipid profiles in mammals. However, the administration of garlic, glutathione and vitamin C singly and/or in combination confers chemoprotective effects to oxidative changes and damages.

Key words: Glyphosate, herbicide, nephrotoxicity, dyslipidemia, antioxidants, chemoprotection, dietary-supplement

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The challenge of feeding the world's growing population has necessitated the enormous use of agrochemicals such as herbicides for increased food production to maintain the human race[1]. Uproot is a glyphosate-based herbicide[2,3] used for agricultural, silvicultural and non-agricultural weed control world-wide[4,5]. On exposure, plants absorb glyphosate and it leads to the inhibition of 5-enolpyruvylshikimate-3-phosphate synthase (*EPSPS*), vital for the shikimic acid pathway and important in the biosynthesis of aromatic amino acids and most plant phenolics[4]. This inhibition terminates affected organism's growth. Since the shikimic acid pathway operates only in plants and microorganisms, the mechanism is not considered to be a risk for humans.

However, genotoxic, hormonal and enzymatic effects of glyphosate in mammals have been reported[6] and numerous studies have shown glyphosate-based pesticides as potential hazard to animal health and environment[7,8]. Some studies recorded that mammalian toxicity of glyphosate is by generation of radicals that triggers oxidative stress[9] and this affects concentration of glutathione[10], metabolism of lipids and important antioxidant enzyme system[11,12]. Oxidative stress when not compensated by antioxidant defense systems, the pro-oxidants and radicals can directly damage cells, tissues or organs[13] via lipid peroxidation[12].

Glutathione is an endogenous non-enzymatic antioxidant involved in the regulation of cellular oxidative processes[14,15]. Glutathione detoxifies by conjugating and priming xenobiotics for elimination[16] via the kidneys. More than half of dietary glutathione needs are supplied by fruits and vegetables, with less than quarter from meat sources[17]. There are biochemical link among GSH, vitamin C and E (α-Tocopherol). Vitamin C is sourced majorly from fruits and vegetables and is synthesized in the liver of some mammals[18,19]. Vitamin C is an antioxidant that donates electrons to various enzymatic and non-enzymatic reactions[20]. α-Tocopherol reduces lipid peroxy radical generated from lipid peroxidation process to stable and relatively non-reactive tocopheryl radicals. The α-Tocopherol is regenerated by ascorbate (vitamin C), which donates its electrons and is restored to a reduced state by glutathione and NADPH-dependent enzymatic mechanisms[21]. Garlic (*Allium sativum*) is another important dietary material with antioxidant properties[22]. It contains active sulfur compounds (S-allyl-l-cysteine sulfoxide), peptides, steroids, terpenoids, flavonoids and phenols[23]. Dietary consumption of whole or extracts of garlic protect animals from oxidative actions of xenobiotics, as it inhibits biochemical derangement and confers physiological advantages[24,25].

There are abundant evidences of glyphosate-based herbicide residues in food, water and the environment and the rampant use without cognizance to the ecotoxicological impacts[7], especially to humans and other mammals. It is based on this toxicity evidences and dearth of research on the amelioration strategy on exposure that the current study was conducted with the objectives of determining the changes in kidney function parameters, lipid metabolism and tissue structures of albino Wistar rats on exposure to sub-lethal dose of glyphosate-based herbicide and the chemoprotective effect of selected dietary supplements. This dietary strategy may provide protection to consumers of crops and other foods, bearing residual load of glyphosate-based herbicide and those occupationally exposed.

MATERIALS AND METHODS

Experimental animal: This study was carried out at the Department of Biochemistry, Federal University of Technology Owerri, Nigeria, from March 2017 to October 2017. The study used 42 male Wistar albino rats (160±20 g) acquired from the Dave Animal House Federal University of Technology Owerri. The animals were kept in metal cages in animal house properly ventilated and at an ambient room temperature (24-28°C) and natural dark and light conditions. The rats were maintained *ad libitum* on water and growers mesh and after 14 days of acclimatization were divided into 7 groups of 6 rats each, labeled as follows: Group 1 (Normal control) received only feed and water. Group 2 (Uproot control) received 50 mg kg^{-1} b.wt., of uproot herbicide (UPH) only. Others in addition to 50 mg kg^{-1} b.wt., UPH received the following: Group 3 received 20 mg kg^{-1} b.wt., Garlic (GA); Group 4 received 20 mg kg^{-1} b.wt., Glutathione (GSH); Group 5 received 20 mg kg^{-1} b.wt., Vitamin C (VC); Group 6 received 20 mg kg^{-1} b.wt., GA+20 mg kg^{-1} b.wt., vitamin C; Group 7 received 20 mg kg^{-1} b.wt., GSH+20 mg kg^{-1} b.wt., vitamin C.

All groups except group 1 were intraperitoneally intoxicated with the uproot herbicide prepared in distilled water every other day for 4 weeks while dietary supplements (glutathione, vitamin C and garlic) were orally administered everyday for 4 weeks. This animal experiment was approved by the Ethics Committee of the Department of Biochemistry, Federal University of Technology Owerri, Nigeria (FUTO/BCH/EC/2017/24) and was conducted in accordance with the guidelines on the care and wellbeing of research animals[26].

Sample collection: After 4 weeks of treatment, blood samples were collected by ocular puncture into plain

tubes, allowed to stand for 30 min to clot and centrifuged at 3000×g for 15 min to obtain serum. Afterwards, the animals were sacrificed and kidney samples were excised and thoroughly rinsed in cold saline. Some portions of kidney sample were stored in 0.3% formaldehyde for histopathology. The other portions of the kidney samples were cut into small pieces and homogenized in phosphate buffer saline (PBS) to give a 10% (w/v) kidney homogenate. The homogenates were centrifuged at 12,000 rpm for 50 min and the supernatant obtained for biochemical analysis.

Biochemical evaluations: Renal function was determined using serum concentrations of urea and creatinine. Serum urea and creatinine were determined using commercial kits provided by BioSystems S.A. Costa Brava, 30.08030 Barcelona (Spain). Lipid profile (cholesterol, triglycerides, HDL and LDL) were also determined using the commercial kits provided by BioSystems S.A. Serum concentration of uric acid and nitric oxide were determined using commercial kits provided by Randox United Kingdom.

Oxidative stress indices: Reduced glutathione was determined by the method described by Raja *et al.*[27]. Lipid peroxidation product determined by measuring the concentration of malondialdehyde (MDA) using the method described by Wallin *et al.*[28]. Total antioxidant capacity (TAC) of the kidney was determined by the ferric reducing ability method described by Benzie and Strain[29].

Histological studies: The method of Okoro[30] and Conn *et al.*[31] were adopted for the histological studies of the kidney sections.

Statistical analysis: The data obtained from the laboratory result of the tests were subjected to one way analyses of variance ANOVA. The results were expressed as mean±standard deviation. *Post hoc* multiple test comparison was used to compare group means. Significant differences were observed at $p \leq 0.05$.

RESULTS

Kidney function parameters: The concentrations of serum urea, creatinine, uric acid and nitric presented in Table 1, shows that animals which received uproot pesticide without dietary treatment presented significant increase in urea, creatinine and uric acid compared to control and groups administered dietary supplements. However, uric acid concentration increased significantly in groups administered Vitamin C+Garlic and Vitamin C+Glutathione.

Oxidative stress parameters: The results of oxidative stress parameters presented in Table 2 showed significant decrease and increase in kidney glutathione and malondialdehyde concentration respectively in uproot control animals compared to the normal control and groups administered dietary supplements. Total antioxidant capacity recorded no significant difference amongst the groups.

Table 1: Liver function parameters of uproot herbicide intoxicated albino wistar rats and the effects of dietary supplements

Groups	N	Urea (mmol L^{-1})	Creatinine (umol L^{-1})	Uric acid (mmol L^{-1})	Nitric oxide (mg dL^{-1})
Normal control	4	9.02±0.54^{a}	8.30±1.10^{a}	5.98±0.02^{d}	0.49±0.06^{a}
Uproot control	4	10.82±0.42^{b}	14.37±1.65^{c}	6.33±0.41^{de}	0.44±0.02^{a}
Garlic	4	9.31±0.13^{a}	9.50±0.47^{a}	4.31±0.21^{b}	0.54±0.13^{ab}
GSH	4	9.76±0.53^{ab}	11.73±0.68^{b}	4.90±0.22^{c}	0.54±0.05^{ab}
Vitamin C	4	8.62±0.85^{a}	7.75±1.02^{a}	3.82±0.22^{a}	0.64±0.05^{b}
Garlic+VC	4	9.30±0.66^{a}	11.73±1.77^{b}	6.44±0.16^{e}	0.44±0.04^{a}
GSH+VC	4	9.12±1.51^{a}	7.60±1.84^{a}	7.18±0.16^{f}	0.47±0.07^{a}

Columns represent mean±standard deviation of quadruplet determinations. Columns with different superscript letters are statistically different ($p<0.05$)

Table 2: Oxidative stress parameters of uproot herbicide intoxicated albino wistar rats and the effects of dietary supplements

Groups	N	Glutathione (mg dL^{-1})	Malondialdehyde (mg dL^{-1})	Total antioxidant capacity (ng mL^{-1})
Normal control	4	2.82±0.53^{b}	0.07±0.03^{a}	0.92±0.06^{a}
Uproot control	4	1.59±0.07^{a}	0.80±0.11^{e}	0.87±0.03^{a}
Garlic	4	2.83±0.56^{b}	0.47±0.04^{cd}	0.92±0.07^{a}
Glutathione	4	2.61±0.73^{b}	0.30±0.04^{b}	0.89±0.06^{a}
Vitamin C	4	2.34±0.38^{ab}	0.53±0.03^{d}	0.92±0.05^{a}
Garlic+VC	4	2.08±0.37^{ab}	0.37±0.13^{bc}	0.95±0.07^{a}
GSH+VC	4	1.98±0.16^{ab}	0.27±0.57^{b}	0.93±0.05^{a}

Columns represent mean±standard deviation of quadruplet determinations. Columns with different superscript letters are statistically different ($p<0.05$)

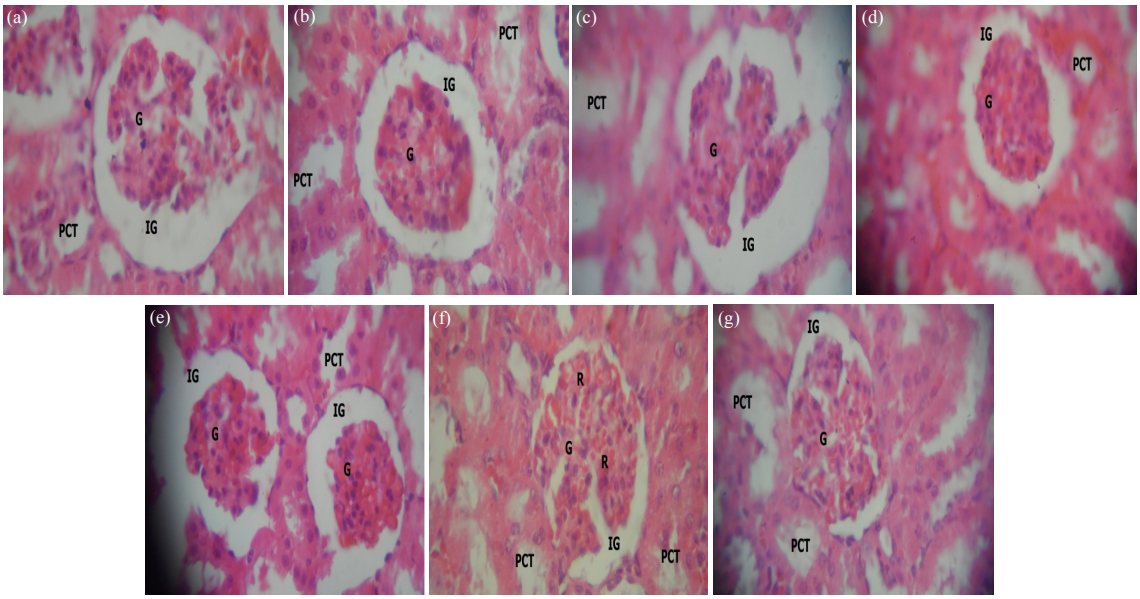

Fig. 1(a-g): Histological sections (400x) of kidney of albino Wistar rats exposed to uproot herbicide and treated with dietary supplements (a) Normal control (NC), (b) Uproot control (UPC), (c) Garlic group (GA), (d) Glutathione group (GSH), (e) Vitamin C group (VC), (f) Garlic+Vitamin group (GAVC) and (g) Glutathione+Vitamin C group (GSHVC). The markings on the slides indicate, Glomerulus (G) intraglomerular space (IG), proximal convoluted tubule (PCT) and red blood cells (R)

Table 3: Lipid profile of uproot herbicide intoxicated albino wistar rats and the effects of dietary supplements

Groups	N	Cholesterol	Triacylglyceride	LDL-cholesterol	HDL-cholesterol
Normal control	4	3.28±0.07^{a}	1.61±0.08^{a}	1.16±0.08^{a}	1.80±.00^{b}
Uproot control	4	4.41±0.27^{b}	1.78±0.10^{a}	2.58±0.12^{d}	1.25±0.13^{a}
Garlic	4	3.96±0.80^{ab}	1.64±0.15^{a}	1.59±0.36^{ab}	1.46±0.26^{ab}
Glutathione	4	3.91±0.56^{ab}	1.75±0.18^{a}	1.73±0.09^{b}	1.46±0.13^{ab}
Vitamin C	4	3.89±0.36^{ab}	1.66±0.05^{a}	1.88±0.25^{bc}	1.46±0.10^{ab}
Garlic+VC	4	4.05±0.37^{ab}	1.50±0.09^{a}	1.93±0.46^{bc}	1.61±0.22^{ab}
GSH+VC	4	4.09±0.28^{ab}	1.77±0.25^{a}	2.32±0.03^{cd}	1.57±0.17^{ab}

Columns represent mean±standard deviation of quadruplet determinations. Columns with different superscript letters are statistically different ($p<0.05$)

Lipid profile: Serum concentration of cholesterol, triacylglyceride, LDL-cholesterol and HDL-cholesterol is presented in Table 3. Except HDL-cholesterol concentration, all other determined lipids increased significantly in uproot control compared to normal. However, groups administered dietary supplements showed non-significant variations in serum lipids when compared to the normal control.

Kidney histology: The histological sections of kidney samples of rat groups are presented in Fig. 1. The normal control section shows a renal corpuscle with normal glomerulus (G) and slightly dilated intraglomerular space (IG) and proximal convoluted tubule (PCT) which appeared normal. The uproot control section shows renal corpuscle with shrunken glomerulus (G) and slightly dilated intraglomerular space (IG), with slightly widened proximal convoluted tubules (PCT). However, histological sections of groups administered the dietary supplements presented features similar to that recorded in the normal control.

DISCUSSION

The toxicological effects of glyphosate and glyphosate-based pesticides on mammals have generated a lot of debate since the introduction. Despite the discrepancies and conflicting classification of this herbicide by some regulatory bodies[32-35], it is important the risk[36] for human health is always considered. Equally imperative is consideration of biochemical remedies in cases of intoxication using readily available compounds.

In the present study, the altered concentrations of urea and creatinine in uproot control rats was indicative of renal dysfunction and an impetus for kidney damage[37] and diminished renal function[38]. This result is in line with the results of Caglar and Kolankaya[39] and Tizhe *et al.*[40]. However, groups administered dietary supplement, recorded significantly lower serum creatinine and urea concentration indicating effective ameliorative potentials of the supplements. The supplements may have provided glutathione and Vitamin C needed for the synthesis of depleted antioxidants. This assertion is consistent with the report of Cavusoglu *et al.*[41] and Jasper *et al.*[12] who related the observed alterations to lower levels of glutathione and increased lipoperoxidation. The ability of the supplements to inhibit possible renal complication[42] was evident by the reduced serum uric acid observed in groups administered single dietary supplement. The sub-lethal dose used may have caused the non-significant variation recorded for nitric oxide and because of nitric oxide host's adaptive response to noxious stimuli and virulent pathogens it may be beneficial to the animal[43].

The increase in LDL-cholesterol recorded in the present study agrees with reports linking hyperlipidemia with increase in glyphosate use[44], due to disruption of lipid metabolism[45,46]. Therefore, suggesting that glyphosate is causal in hyperlipidemia[44]. This disruption could enhance hepatic synthesis of cholesterol[47] which may increase serum cholesterol as recorded in this study. However, contrary to the non-significant variation of triacylglycerides concentration observed in this study, Hernandez *et al.*[48] reported increased levels of triacylglycerides.

Furthermore, the significant decrease in HDL-cholesterol in uproot control indicates loss of HDL-scavenging of cholesterol. In contrast to other epidemiological studies[49,50], data obtained in this study did not indicate significant variation in the serum triacylglyceride concentration. The significantly reduced values recorded for total cholesterol and LDL-cholesterol in groups administered dietary supplements indicate chemoprotection. The administered vitamin C in the presence of other antioxidants decreased lipid peroxidation of cellular membranes by donating electrons to uproot-induced free radicals, quenching their reactivity[51,52].

The recorded decrease in glutathione concentration of uproot control rats can be attributed to depletion of antioxidants due to increased activities of glutathione peroxidase and glutathione reductase. This is consistent with the report of El-Shenawy[53] and Mesnage *et al.*[54]. However, El-Shenawy[53] recorded decreased GSH with 3-5 times glyphosate doses and in a shorter duration compared to the present study. Furthermore, the elevated concentration of kidney malondialdehyde (MDA) of uproot control in the present study agrees with the study of Manas *et al.*[55]. However, this finding was not consistent with the work of Milic *et al.*[13], who reported no significant increase in lipid peroxidation. The recorded significant decrease in MDA concentration in groups administered the nutritional supplement indicated that the supplements contain bioactive compounds for protection against free radical damage induced by uproot herbicide. The reduced concentration of lipid peroxidation product in the presence of garlic-constituents may have increased the activities of antioxidant enzymes, thereby reducing oxidative lipid peroxidation. The non-significant difference in total antioxidant capacity amongst the groups may be attributed to gradual cellular recovery from oxidative stress. This agrees with the report of Milic *et al.*[13] who stated that cellular antioxidants tend to be elevated after long-term exposure to oxidants.

Kidneys are important in xenobiotic metabolism and excretion[56], therefore, renal lesions are prominent indicators of xenobiotics exposure. The present study recorded mild to moderate ultrastructural changes on glomerulus, intraglomerular and proximal convoluted tubule on kidney tissues of rats exposed to sub-lethal dose of uproot herbicide having antioxidant capacity. This was further confirmed by the structural presentations of the groups administered dietary supplements which were similar to that of normal control. Furthermore, studies using higher concentrations of glyphosate-based herbicide on the underlying mechanism for renal toxicity such as that reported by Dedeke *et al.*[45] showed severe histopathological lesions in the kidneys of rats exposed to Roundup. Similarly, Samanta *et al.*[57] demonstrated histological changes in the kidneys of *Heteropneustes fossilis* after exposure to glyphosate.

CONCLUSION

The values recorded in this study showed that Uproot, a glyphosate-based herbicide induced nephrotoxicity and variations in lipid metabolism of albino Wistar rats through generation of oxidants. However, administration of selected dietary supplements showed significant chemoprotection against adverse oxidative changes and amelioration of toxic damage.

SIGNIFICANCE STATEMENT

This study discovered that sub-lethal exposure to Uproot, a glyphosate-based herbicide induced renal dysfunction and adverse changes in lipid profiles of albino Wistar rats. The study also established that the administration of dietary supplements conferred chemoprotection. These supplements can be beneficial for treating cases of the herbicide exposure via consumption of contaminated foods, occupational and/or accidental poisoning. This study will help in the development of treatment and ameliorative strategies for this herbicide and others that on exposure are associated with organ and oxidative damage.

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