

The Hepato-Renal Effect of Graded Doses of a Glyphosate-Based Herbicide (Roundup) in Rat

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Abstract

Roundup, one of the most widely used glyphosate-based herbicides is classified as hazardous to the environment. Its low persistence leads to repeated applications and consequently, large portions contaminate soil and water bodies, causing harm to non-target species. The hepato-renal toxicity of graded doses (300, 400 and 600 mgKg-1body wt) of roundup on selected serum metabolites, enzymes and hematological parameters was investigated in rats (Wistar strain; 207g± 6 g). Thirty-two male rats were divided into four groups; control (n = 8) and test groups (n = 24), divided into 3 groups of eight rats each that received graded doses of the herbicide, respectively for 8 days. Hepatotoxicity was investigated by quantitative analysis of serum alanine transaminase (ALT), alkaline phosphatase (ALP), total protein, albumin, globulin and cholesterol levels. Renal-toxicity was determined by quantitative analysis of serum creatinine, urea, K⁺ and Na⁺ levels. Relative to the control, there was significant (p<0.05) reduction in the activities of ALT and ALP while serum total protein, albumin and globulin levels were significantly (p<0.05) elevated in the groups that received high doses of the herbicide. Creatinine, urea, Na⁺ and hematocrit were significantly (p<0.05) elevated while K⁺, white blood cell and lymphocyte were significantly (p<0.05) reduced in the groups that received the high doses of the herbicide, relative to the control. Histopathological studies revealed periportal steatosis and mild necrosis in the liver and thickened alveolar septae filled with inflammatory cells in the lung tissues. This study shows that high doses of roundup may be toxic to the hepatorenal tissues of rat.

Keywords: Roundup, hepato-renal, toxicity, histopathology, rat

Introduction

Herbicides are a group of economically important chemicals used in agriculture to increase crop yield (1). Organophosphate herbicides constitute a special class of these pesticides. The extensive international use of organophosphate herbicides amongst other pesticides results in diverse acute intoxications each year (2).

Glyphosate (N-Phosphonomethyl-glycine) is one of the organophosphate herbicides. It is the active ingredient of Roundup®, a non-selective broad-spectrum and post-emergent herbicide, used in the control of grasses, broad-leaf weeds, pastures, and rice, maize and soy plantations (3, 4).

Roundup is reported to be harmful to the environment (5). It is a mixture of glyphosate and surfactants such as polyoxyethylene amine (POEA) (6). Roundup, in comparison with other formulations, has a major characteristic of being rapidly absorbed, aided by the presence of the surfactants (Jasper et al., 2012). There are reports (7, 6, 8) that Roundup is more toxic than glyphosate alone.

The effect of acute pesticide poisoning is well known for most pesticides currently used (9). Chronic pesticide poisoning is associated with carcinogenesis, neurotoxicity, reproductive, developmental and immunological defects (2).

Studies have shown that application of glyphosate based herbicides result in residues of glyphosate and its primary metabolite, Amino Methyl Phosphoric Acid (AMPA) in crops at harvest as well as in processed foods. It was initially thought that glyphosate has limited hazard in vertebrates since it targets enzymes in plants that are not present in vertebrates. However, this assumption has been refuted by reports that the herbicide and its metabolites can act through pathways that are present in vertebrates (10).

Thus, the aim of this study was to investigate the hepato-renal effect of graded doses of the glyphosate - based herbicide Roundup in rat.

Materials and Methods

Thirty two adult male rats (Wistar strain), obtained from the laboratory animal unit of the Department of Anatomy, University of Benin Benin city, Edo state, Nigeria, were housed in wire gauze cages and used for this study. These rats were left to acclimatize for two weeks before the commencement of the experiment and were allowed free access to water and chow (BFFM Ltd, Ewu, Nigeria).

The rats were organized into four groups of 8 rats each, the first group being the control group received distilled water, while the second, third and fourth groups received 300, 400 and 600 mg Roundup Kg-1 body wt respectively. The glyphosate based herbicide was administered orally by gavage for 8 days. Rats were observed before and during exposure for signs of toxicity. At the end of the treatment period, the animals were sacrificed

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by chloroform anesthesia. Blood was removed by cardiac puncture into EDTA and heparinized tubes respectively while the liver, kidney, heart and lungs were excised, trimmed free of fatty tissue, weighed and used for histopathological studies. Body weight of animals was taken at the start and end of the experiment. Relative organ weight (organ / body weight) of each animal was calculated.

Preparation of Serum

The blood which was collected in plain tubes was centrifuged at 2,500 rpm, for 10 min and the neat supernatant separated and used for biochemical studies.

Biochemical and Hematological evaluation

Aspartate transaminase (AST) and Alanine transaminase activity were determined by the method of Reitman and Frankel (11) while Alkaline phosphatase (ALP) was estimated by the method described in GSCC (1972) and modified by Amino and Giese (12), in each case, using the commercially available kit by Randox Laboratories, U.K.

Total protein was determined using the Biuret method as described by Henry (13) while albumin was estimated by the method of Dumas (14). Globulin was obtained by difference between total protein and albumin. Creatinine was estimated by the Jaffe reaction method as described by Bonsnes and Taussky (15) and cholesterol was determined by the method of Richmond (16). Renal function was evaluated using plasma concentrations of urea (17), sodium and potassium ions were estimated by the method described by Tietz, (18). All the tests in this study were performed using kits obtained from Randox Diagnostics Laboratories (United Kingdom).

Some of the blood samples were collected in EDTA tubes and used for hematological studies using MS 4 CBC analyzer.

Histopathological analysis

Lung, liver and kidney samples were taken from all the groups and fixed in 10% formalin solution. The tissues were embedded in paraffin and sectioned (5µm). The tissue sections were stained with hematoxylin-eosin (H&E) and examined under light microscopy

Statistical analysis.

Values were expressed as mean ± SD. Statistical analysis of data was conducted by one-way analysis of variance (ANOVA) using SPSS package program, version 16.0 followed by the multiple Duncan’s test. Level of significance was set at p<0.05.

Results

In this study, reduced appetite and activity, weakness, nasal bleeding, erosion of the epithelial lining of the mouth, diarrhea and death of 8 of the herbicide–treated rats was observed.

The effect of Roundup on gravimetric parameters in rat is presented in Table1. The eight days treatment with the glyphosate based herbicide caused a significant (p<0.05) reduction in the body weight of rats that were treated with 400 and 600 mg Roundup Kg-1 body wt when compared with the control. However, there were no differences in the organ/body wt ratio of the liver, kidney, heart and lungs relative to the control.

Table 2 shows the effect of the graded doses of Roundup on serum ALT and ALP.

TABLE 1: Effect of Graded Doses of Glyphosate-based Herbicide, Roundup on Gravimetric Parameters in Rat

Parameters	Change in Body Weight	Liver /Body Weight	Kidney /Body Weight	Heart /Body Weight	Lung /Body Weight
Control	19.25±8.51	40.63±4.30	3.45±0.39	3.36±0.39	7.10±1.51
300mg/kg	2.75±18.20 ^a	34.16±2.04	3.25±0.39	3.59±0.23	6.78±1.04
400mg/kg	18.33±20.72 ^a	37.14±7.87	3.45±0.27	3.57±0.34	8.68±2.43
600mg/kg	14.50±8.35 ^a	40.87±2.80	3.30±0.30	3.72±0.55	7.66±2.20

Values are mean ± SD.

^aSignificantly different from control (p<0.05).

TABLE 2: Effect of Graded Doses of Glyphosate-based Herbicide, Roundup on Serum AST and ALP in Rat.

PARAMETERS	ALT	ALP	ALT/ALP
Control	39.42±2.62	72.62±12.69	0.38±0.03
300mg/kg	34.40±2.30 ^a	61.18±5.78	0.51±0.07 ^a
400mg/kg	36.42±5.76 ^a	58.80±8.65 ^a	0.55±0.07 ^a
600mg/kg	34.00±1.50 ^a	55.56±7.76 ^a	0.65±0.07 ^a

Values are mean ± SD.

^aSignificantly different from control (p<0.05).

Results show a significant ($P<0.05$) reduction in ALT activity in all the test groups relative to the control. Although the ALP activity in all the test groups were reduced, it was significantly ($p<0.05$) so in the groups that received 400 and 600 mg roundup/Kg-1 body wt respectively. The study also shows a sequential elevation in ALT/ALP ratio of all the test groups relative to control. This increase in the ALT/ALP ratio was significant ($p<0.05$).

Relative to the control, results also show that total protein, albumin and globulin were elevated in all the herbicide-exposed rats (Table 3). However, this increase was only significant ($p<0.05$) in the total protein and globulin levels. Total cholesterol was significantly ($p<0.05$) reduced while albumin was significantly ($p<0.05$) increased in the group that was treated with the highest dose of the herbicide (Table 3).

Results also show that creatinine was elevated in all the treatment groups compared with the control. This increase was significant ($p<0.05$) in the groups that received the highest and lowest dose of the herbicide relative to the control. Though generally elevated, urea level was only significantly ($p<0.05$) increased in the group that received 600 mg Roundup Kg-1 body wt., relative to the control. Sodium ion was elevated while K^+ concentration was reduced in all the Roundup-exposed rats relative to the control (Table 3).

TABLE 3: The Effect of Graded Doses of the Glyphosate-based Herbicide, Roundup on Selected Serum Metabolites in Rat

GROUPS	PARAMETERS							
	Total Protein	Albumin	Globulin	Cholesterol	Creatinine	BUN	Na ⁺	K ⁺
Control	5.34±0.15	2.99±0.09	2.42± 0.42	107.46±3.34	0.81±0.11	39.71±1.80	116.48±3.49	6.21±0.42
300 mgK ⁻¹	7.16±0.17 ^a	3.35±0.24	3.7 1± 0.14 ^a	107.80±3.46	1.19±0.23 ^a	43.28±2.15	134.72±3.82 ^a	5.35±0.96
400 mgK ⁻¹	6.13±0.58 ^a	3.30±0.18	3.40± 0.68 ^a	105.96±2.46	0.94±0.05	39.78±1.65	122.84±3.55	4.59±0.23 ^a
600 mgK ⁻¹	7.42±0.27 ^a	3.18±0.30 ^a	4.24 ±0.57 ^a	94.84±5.98 ^a	1.34±0.30 ^a	45.77±1.7 ^a	143.77±5.50 ^a	4.45±1.1 ^a

Values are mean ± SD

^aSignificantly different from control ($p<0.05$).^b

TABLE 4: The Effect of Graded Doses of the Glyphosate-based Herbicide, Roundup on Some Hematological Parameters in Rat

PARAMETERS	HCT (%)	MCHC (g/dl)	WBC (/μl)(10 ³)	LYM (%)	ABSOLUTE LYM
Control	38.16±1.95	35.91±0.83	11.28±2.17	92.87±2.05	1047.57± 4.45
300 mg/dl	41.45±3.21 ^a	35.92±0.61	8.47±1.85	89.29±9.27	756.29 ± 11.15 ^a
400 mg/dl	41.47±2.55 ^a	34.72±1.28 ^a	10.58±1.79	89.30±4.12	944.79 ± 7.37 ^a
600 mg/dl	41.43±2.61	33.90±0.74 ^a	4.65±3.77 ^a	70.53±26.31 ^a	327.96 ± 15.22 ^a

Values are mean ± SD.

^aSignificantly different from control ($p<0.05$).

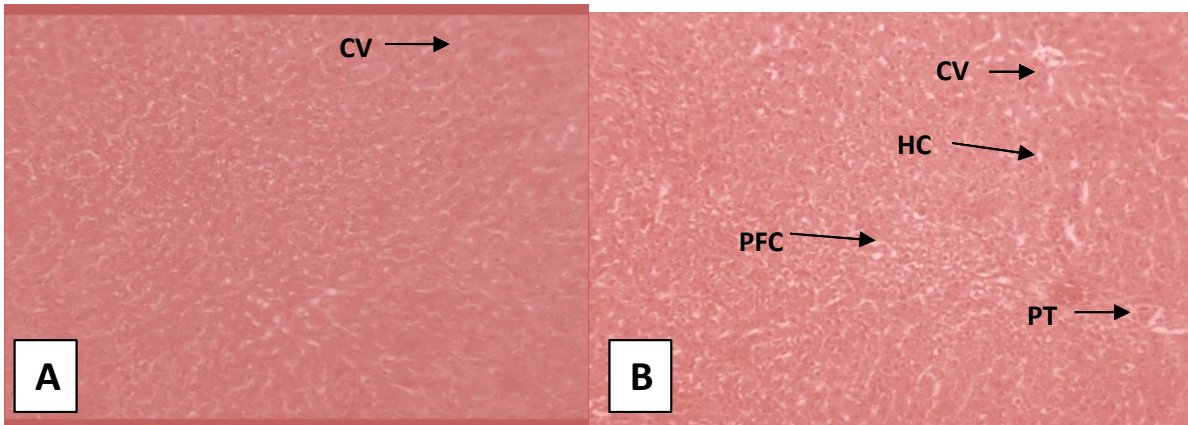


Plate 1: In the Control (A), histologic sections show normal hepatocyte cords, portal tracts and central veins while in (B), which received 300mg/kg bw glyphosate, there is presence of periportal steatosis and mild necrosis (H & E staining $\times 100$)
CV: central vein, HC: hepatocyte, PFC: periportal fatty change, PT: portal change.

The effect of the Roundup on hematological parameters is shown in Table 4. Percentage hematocrit (HCT) was significantly ($p < 0.05$) increased while the mean cell hemoglobin concentration (MCHC) was significantly ($p < 0.05$) reduced in the group that received 400 mg Kg^{-1} body wt. Relative to the control, the MCHC, white blood cell (WBC) and % lymphocyte levels were significantly ($p < 0.05$) reduced in the group that was treated with the highest dose of the herbicide (600 mg Kg^{-1}).

The histologic sections through the liver show the presence of periportal steatosis and mild necrosis in the rat exposed to 300 mg Kg^{-1} when compared with the control (Plate 1) while studies of the histological sections of the kidney of all the herbicide-treated groups did not show any changes when compared with the control (Plate 2).

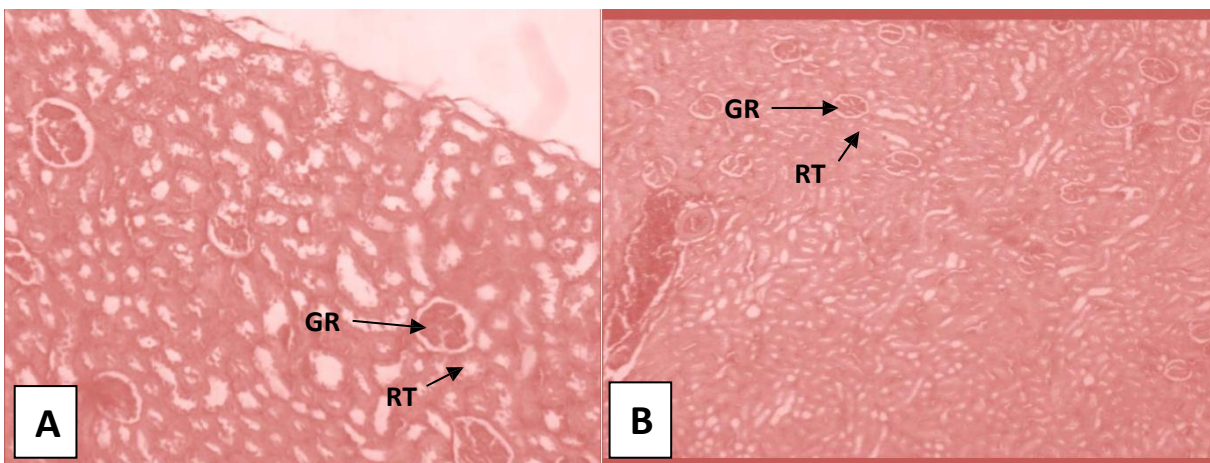


Plate 2: In the Control (A) and the group that received 600mg roundup kg^{-1} bw (B), histologic sections of the kidney show normal glomeruli and tubules (H & E staining $\times 100$)

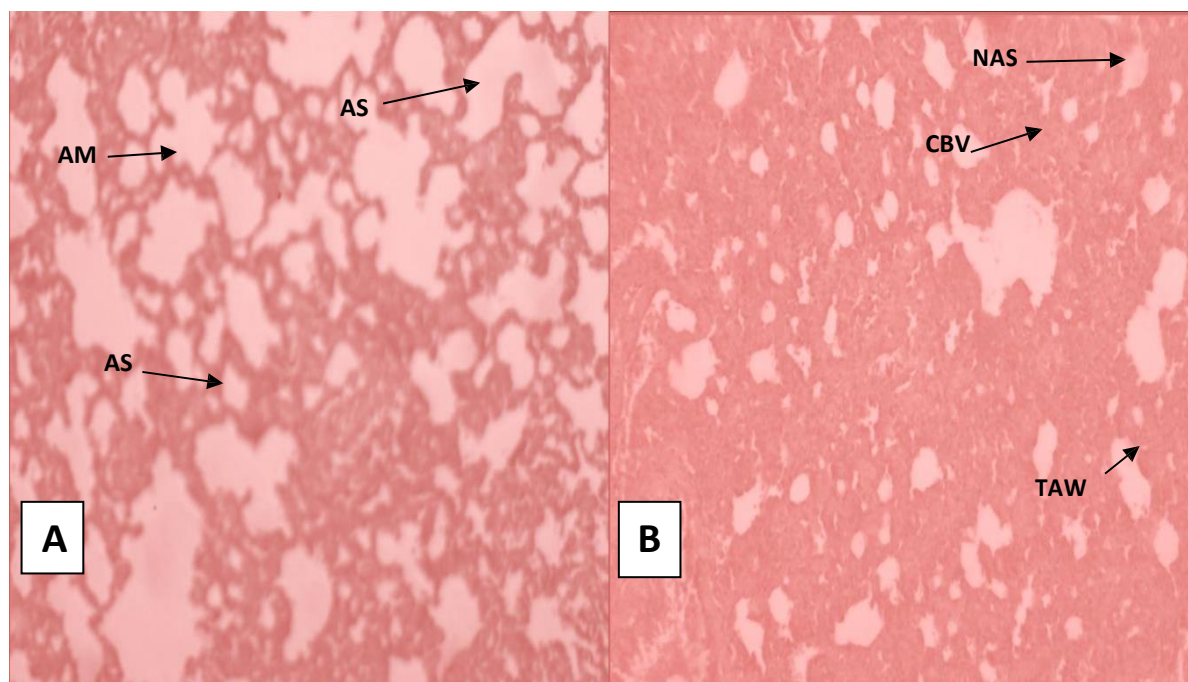


Plate 3: The control (A), histologic sections of the Lung show normal alveoli spaces and alveoli septae while in (B) which received 400mg roundup kg^{-1} bw, there were thickened alveolar septae filled with inflammatory cells including neutrophils, lymphocytes and plasma cells. Presence of oedema and haemosiderin laden macrophages (H & E staining x100).

AS: alveolar space, AM: alveolar membrane, NAS: narrow alveolar space, CBV: congested blood vessels, TAW: thickened alveolar wall.

However, the histological sections of the lungs of rats that were treated with 400 mg Kg^{-1} body wt showed thickened alveolar septae filled with inflammatory cells including neutrophils, lymphocytes and plasma cells. Oedema and haemosiderin laden macrophages were also observed in the lungs of this group (Plate 3).

Discussion

In this study, the effect of graded doses of the glyphosate-based herbicide roundup, on selected serum metabolites and tissues in rats was investigated.

Due to its involvement in the biotransformation of environmental xenobiotics, the liver is always at risk of damage when animals are exposed. In this study, low Alanine aminotransferase (ALT) and Alkaline phosphatase (ALP) activities were recorded in the animals that were treated with high doses of roundup relative to the control group. This result is at variance with those reported by Benedetti *et al.*, (19) and Jasper *et al.*, (5). The difference is probably due to the length of exposure to the herbicide. Although most low serum ALT level results indicate normal healthy liver, this may not always be the case since low enzyme activities may also indicate a low functioning or non-functioning liver, lacking normal levels of ALT activity. Such hepatocytes lacking normal levels of ALT will not release a lot of enzyme into the blood when damaged. Low ALT levels may also be an indication of malnutrition (20, 21). Although the results of the present study show weight loss in all the herbicide-treated groups, total protein and albumin were also increased in these groups. It is therefore unlikely, that malnutrition is the cause of the low ALT activity. However, it is possible that though ALT and ALP levels in the blood might be high, the activities of these enzymes might have been inhibited by Roundup. In this study, enzyme activity and not concentration was estimated.

Total protein levels in the blood are important indexes for assessing the state of health of the organism. The increase in this parameter is often associated with chronic inflammatory processes or infection, tissue dysfunction, hepatic cell membrane damage and/or elevation in the enzymes involved with detoxification of a toxicant (22). Since the serum activities of ALT and ALP were not elevated in this study, it is unlikely that the increase in total protein observed is due to damage of hepatic cell membranes, but rather a consequence of increased inflammation process and increased secretion of enzymes that are involved in detoxification of roundup in these rats. This hypothesis is supported by the elevation in total globulin levels in the rats exposed to 400 and 600 mg roundup Kg^{-1} body wt. Infection in these groups is ruled out by the low levels of white blood cell (WBC) and lymphocytes observed in this study. Also, the elevation in total protein is accompanied by increased serum albumin in all the rats exposed to high doses of the herbicide. Studies (23) have shown that albumin in blood, play many important roles including binding of toxins to avoid toxic effects, transport of

various substances and neutralization of free radicals. The elevated albumin level observed in all the groups that were treated with high doses of the herbicide is probably a defense mechanism to neutralize the toxic effect of Roundup.

Renal toxicity in this study was determined by creatinine, urea and electrolyte levels in the serum. The level of serum creatinine is maintained by the balance between its generation and its excretion by the kidneys. Under normal conditions, plasma creatinine has a reciprocal relationship with glomerular filtration rate (GFR). Elevated serum creatinine is therefore, commonly associated with reduced Glomerular filtration (24). Blood Urea Nitrogen (BUN) is the most frequently determined clinical index for renal function. Urea, a major nitrogenous end product of protein and amino acid metabolism is produced by the liver and distributed through intracellular and extracellular fluid. The glomerulus of the kidney filters urea from the blood and as it passes down the tubules, it is partially reabsorbed along with water. Serum creatinine and urea are readily excreted by the normal kidney thus keeping their levels in the blood fairly constant. The elevated level of creatinine and urea, observed in this study, is therefore an indication of impaired renal function. The primary function of the kidney is to maintain fluid and electrolyte homeostasis (25). The electrolytes measured in this study are sodium and potassium ions. Sodium ion is the major cation of extracellular fluid and is crucial in osmoregulation as well as signaling in the body. Potassium ion, on the other hand, is the major cation of the intracellular fluid and functions in the regulation of the heartbeat and muscular functions. Studies have shown that hypernatremia (26) and hypokalemia (27) can result from kidney dysfunction. The elevated Na⁺ and reduced K⁺ levels observed in this study therefore indicate kidney dysfunction.

These results characterize high doses of the Roundup formulation of glyphosate as probable hepato-renal toxins.

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