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## Multiple Organ dysfunction in mice exposed to Glyphosate-based Herbicides

**Hewida H. Fadel[a], Omnia A. Thabet[b], Amira A. Darwish[b], Mona Gamal[c]**

[a] *lecturer of Applied Medical Chemistry, Department of Medical Laboratory Technology, Faculty of Applied Health Sciences Technology, Pharos University in Alexandria, Egypt.*

[b]*Lecturer assistance of Molecular Biology and immunology, Department of Medical Laboratory Technology, Faculty of Applied Health Sciences Technology, Pharos University in Alexandria, Egypt.*

[d]*Lecturer assistance of Histology, Department of Medical Laboratory Technology, Faculty of Applied Health Sciences Technology, Pharos University in Alexandria, Egypt.*

**Abstract** Although, strong recent epidemiological evidence confirmed that chronic exposure to pesticide causes adverse effects on human health such as mutagenic, cancer and even death. Glyphosate, an active ingredient in widely used organophosphate pesticide “Roundup Star”, is an amino acid analog of glycine that inhibits the tryptophan metabolism in weeds. Several studies indicated that intraperitoneal administration of low dose (50 mg/kg) and high dose (300 mg/kg) of Roundup® result in significantly increased of chromosomal aberrations and micronuclei in mice. Objective: we aimed to investigate the biochemical and histopathological changes resulting from exposure to GBHs in male albino mice. The study was conducted on 80 mice divided into four groups; GPC: 20 untreated mice as control group, GP50: 20 mice were treated with 50 mg/kg/bw GBHs for 3 months; GP125: 20 mice were treated with 125 mg/kg bw GBHs for 3 months and Gp250: 20 mice were treated with 250 mg/kg bw GBHs for 3 months. Blood samples were collected for assessment of the liver, spleen, kidney, heart, lung and gastrointestinal tract (GIT) function including assessment the enzyme activity of Alanine transaminase (ALT), Aspartate transaminase (AST), Creatine kinase (CK-MB), alpha amylase, Lactate dehydrogenase (LDH) and Alkaline phosphatase (ALP), determination of the mean levels of creatinine, urea, uric acid, total protein, albumin, hemoglobin and bilirubin as well as histopathological examination of liver, kidney, spleen, brain, heart, lung and gastrointestinal tract. We found that GBHs caused deleterious effects on all examined organs accompanied with significant elevation of ALT, AST, ALP, LDH, ALP, CK-MB and alpha-amylase activities as well as elevation of the mean levels of urea, creatinine, creatinine and bilirubin levels while there was a significant decrease of total proteins, albumin, and hemoglobin in dose dependent manner compared to control group. We concluded that GBHs causes multi-organs disorders in male albino mice.

*Keywords: Glyphosate-based Herbicides (GBHs), Multiple organs dysfunction, markers, inflammation, congestion.*

### 1. Introduction

Although, strong recent epidemiological evidence confirmed that chronic exposure to pesticide led to several environmental implications and caused adverse effects on human health such as cancer, birth defects, reproductive abnormalities, toxicities, cancer and death, nearly 3 billion kg of pesticides are used every year globally with a budget of ~40 billion USD [1,2]. Over 750 products containing glyphosate for sale in USA [3]. Among sixty years, about 1 million metric tons of pesticides were used in the agricultural sector in Egypt causing diverse health and environmental problems [4]. Glyphosate, N-(phosphonomethyl) glycine, is a systemic herbicide used to kill weeds. Glyphosate, an amino acid analog of glycine, is the most widely applied organophosphate pesticide worldwide and it is an active ingredient of all glyphosate-based herbicides (GBHs), including the formulation “Roundup”. The chemical formula of Glyphosate potassium is C3H7KNO5P. Its molecular weight is 207.16 g/mol. IUPAC Name: potassium;(carboxymethylamino)methyl-hydroxyphosphinate. IUPAC Condensed: (HO)2P(O)Me-Gly-OH.K+. The main ingredient is glyphosate, a potassium salt of a glycine analogue. Glycine, the smallest and the most important amino acid which play an essential role in biosynthesis of hemoglobin, glutathione, purines, uric acid and creatine [5]. The second ingredient in Roundup is 1-4 Dioxane (C4H8O2), a stabilizer for the glyphosate in addition to a surfactant which increase the surface area

*Corresponding author e-mail: hewida.fadel@pua.edu.eg (Hewida Fadel)
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of a product to strengthen its mode of action. The carrier of Glyphosate is water. Roundup, a commercial glyphosate containing herbicide is more acutely toxic than glyphosate or surfactant (POEA) alone [6]. Roundup is also more acutely toxic than POEA. The herbicidal action of glyphosate is attributed to its ability to inhibit the shikimate pathway, an intermediate of phenylalanine, tyrosine and tryptophan metabolism in some plants and weeds. Currently, official views of respected international regulatory and health bodies remain divided on glyphosate's status as a human carcinogen, but the 2015 International Agency for Research on Cancer decision to reclassify the compound as Category 2A (probably carcinogenic to humans) [7-10]. In this aspect, it was found that Glyoxylate, an aldehyde which can react with nucleophilic amino acids on protein targets, such as cysteines, lysine, and arginine, caused disruptions in protein biochemistry, such as enzyme activity, post-translational regulation, epigenetic alterations, redox balance, metal binding, protein-protein interactions and genotoxicity [11-13]. Also, it has been shown that there is a direct link between exposure to glyphosate and increased risk of non-Hodgkin lymphoma [14]. Moreover, glyphosate acts as an endocrine disruptor (ED) in mammals altering hormonal function [15]. Roundup (CAS # 1071-83-6) is a liquid water-soluble organophosphorus herbicide, containing glyphosate as its active ingredient and surfactant (polyoxyethyleneamine) that enhances the spreading of spray droplets when they contact foliage [16].

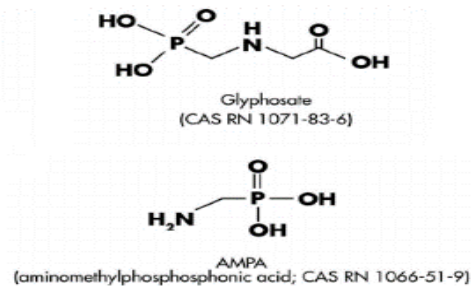


Figure 1. Structure of Glyphosate and its metabolic product [15].

## 2. Materials and methods

### Animals

Male albino mice 22–25 g of body weight were obtained from the animal house of the Faculty of Pharmacy, Pharos University in Alexandria. Standard laboratory food and water were provided ad libitum. Animal procedures were performed in accordance with the Ethics Committee of the Medical Research Institute, Alexandria, Egypt, and followed the recommendations of the Medical Research Institute Guide for the Care and Use of Laboratory Animals (the National Research Council's guide for the care and use of laboratory animals (www.nap.edu/readingroom/books/labrats/) (2004) [17]. Animals were randomly selected and housed. They were maintained in controlled atmosphere of 12 hours dark/light cycle, 25 ± 2◦C temperature, and 57 ± 7% humidity. GPC: 20 untreated mice as control group, GP50: 20 mice were treated with 50 mg/kg/bw GBHs for 3 months; GP125: 20 mice were treated with 125 mg/kg bw GBHs for 3 months and Gp250: 20 mice were treated with 250 mg/kg bw GBHs for 3 months. Random blood samples were collected from all animal's groups (control and experimental groups). Blood samples were collected by ocular vein puncture from all animal groups and prepare serum by centrifugation at 2500 r.p.m for 15 minutes, then were kept frozen at -80° C until used for biochemical investigations.

### Chemical reagents

Roundup is the brand name of a systemic, broad-spectrum glyphosate-based herbicides (GBHs) originally produced by Monsanto company. Roundup Star is a glyphosate potassium salt was applied at the doses of 50, 125, 250 mg/kg/bw (injection volume was 0.2 ml/mice).

### Biochemical investigations

Determination of liver, spleen, kidney, heart, lung and GIT functions by assessment the activities of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Lactate dehydrogenase (LDH) and alpha-amylase, creatine kinase (CK), in addition to determination the mean level of urea, creatinine, uric acid, total protein, albumin, hemoglobin and bilirubin according to manufacturer's protocol [18].

### Histopathological Examination

Three months of GBHs administration, mice were sacrified by cervical dislocation and major organs including liver, kidney, heart, lung, brain, spleen and gastrointestinal tract were dissected and fixed in freshly prepared 10 % neutral buffered formalin, processed routinely, and embedded in paraffin for histopathological examination. Sections were cut 5 µm in thickness for further investigation by a light microscope [19].

### Statistical analysis

We used ANOVA analysis to determine the statistical difference between the studied groups

## 3. Results and discussion

### 3.1. Effect of GBHs on Proteins

We found that GBHs administration caused significant decrease in the mean level of total protein in GP50 (7.668±0.837 g/dl, p=0.0015), GP125 (6.709±1.022

g/dl, p<0.0001) and in GP250 (5.515±0.9102 g/dl, p<0.0001) compared to GPC (8.779±1.011 g/dl). Also, the mean level of total protein in GP250 was significantly lower than that in GP125 (p=0.0041) as well as in GP125, its level was significantly lower than that in GP50 (p=0.0076).

GBHs administration caused significant decrease of the mean level of albumin in GP50 (3.0465±0.452 g/dl), GP125 (2.808±4.296 g/dl) and GP250 (2.373±0.207 g/dl) compared to GPC (4.473±0.9006 g/dl, p<0.0001), in addition, the mean level of albumin in GP250 was significantly lower than that in GP125 (p=0.0036) while its mean level in GP125 did not showed significant difference to GP50 (p=0.1443).

Our data showed that GBHs administration caused significant decrease of the mean level of hemoglobin in all treated groups GP50 (12.393±0.09 g/dl), GP125 (11.218±0.948 g/dl) and GP250 (10.03±1.203 g/dl) compared to GPC (15.358±1.316 g/dl, p<0.0001). Also, the mean level of hemoglobin in GP250 was significantly lower than that in GP125 (p=0.0082) as well as its mean level in GP125 was very low than that in GP50 (p=0.0052).

In contrary to the major published studies that revealed its safety as an inhibitor to biosynthesis of phenylalanine and tryptophan in weeds and some plants not mammals, our data spotlighted on the toxicity of GBHs in mammals. The results of the present study revealed that administration of GBHs in mice for three months decreased the total proteins, albumin and hemoglobin significantly. A proposed mechanism was attributed to the structure of glyphosate as a glycine analogue, the most important amino acid participating in biosynthesis of hemoglobin and generally in proteins, interferes with their biosynthesis and gene expression. Another explanation, GBHs causes epigenetic alterations including methylation and acetylation modifications in the human genome [9]. Moreover, a previous study revealed that GBHs altered the secondary structure of albumin [12].

**3.2. Effect of GBHs on liver and spleen**

We found that GBHs administration caused significant increase of the activity of ALT in GP50 (25.09±6.09 U/L, p=0.0042), GP125 (36.828±8.478 U/L, p<0.0001) and in GP250 (46.06±9.192 U/L, p<0.0001) compared to GPC (18.376±6.56 U/L). In addition, the mean activity of ALT in GP250 was significantly higher than that in GP125 (p=0.0096) as well as ALT activity inGP125 was significantly higher than that in GP50 (p<0.0001).

Also, GBHs administration caused significant increase of the mean activity of AST in GP50 (24.904±6.545 U/L, p=0.0016), GP125 (35.43±7.577 U/L, p<0.0001 and in GP250 (43.37±11.44) U/L, p<0.0001 compared to GPC (16.716±7.217 U/L). In addition, the mean activity of AST in GP250 was significantly higher than that in GP125 (p=0.042) as well as in GP125 was significantly higher than that in GP50 (p=0.0003).

Moreover, GBHs administration caused significant increase of the mean activity of ALP in GP50 (180.69±62.99 U/L, p=0.0197), GP125 (230.158±66.547 U/L, p=0.0251) and in GP250 (297.128±86.912 U/L, p<0.0001) compared to GPC (124.7±69.26 U/L). In addition, the mean activity of ALP in GP250 was significantly higher than that in GP125 (p=0.0421) as well as ALP activity inGP125 was significantly higher than that in GP50 (p=0.0321).

The histopathological examination of liver sections was in parallel to the biomarkers data as shown in Figure 1. the liver sections from GPC showed normal architecture while GP50 showed infiltration and mild congestion of central vein. GP125 showed moderate infiltration within the periportal zones, dysplastic lesions and severe congestion in the sinusoids. These findings were progressed in GP250 to severe liver injuries accompanied with high level of inflammatory cells infiltration in pericentral areas, necrotic cells and pyknotic nuclei and giant cells, in addition to cytoplasmic vacuolation, sinusoidal dilatation and congestion were observed. Collectively, it was obvious clearly that severe inflammation and congestion was the predominant harmful effect of GHBs administration on liver in mice of GP125 and GP250. A previous study explained the GBHs-induced liver damage because of hypoxic stress [20].

We also found that elevated level of serum bilirubin was highly significant in all treated groups compared to untreated control group as well as compared to each other. We found that significant elevation of the mean level of serum bilirubin in GP50 (5.324 ±1.291 mg/dl*)*, GP125 (9.617±1.857 mg/dl*)* and in GP250 (14.554±1.785 mg/dl*)* compared to GPC (2.516±0.714, $p < 0.0001$) mg/dl. In addition, the mean level of bilirubin in GP250 was significantly higher than that in GP125 ($p < 0.0001$) as well as bilirubin level inGP125 was significantly higher than that in GP50 ($p < 0.0001$). In agreement to our result, a previous study indicated that glyphosate causes the hyperbilirubinemia, hepatospleenomegaly and hypertension [21].

The spleen sections from GPC showed normal architecture as normal arrangement of white pulp (WP) containing the central arteriole (CA), surrounded by numerous lymphocytes and red pulp (RP) as well as GP50 showed no evidence of lymphoid follicular or vascular damage. The spleen sections from GP125 showed vascular damage while that from GP250 showed damage of lymphoid follicular and mild congestion.

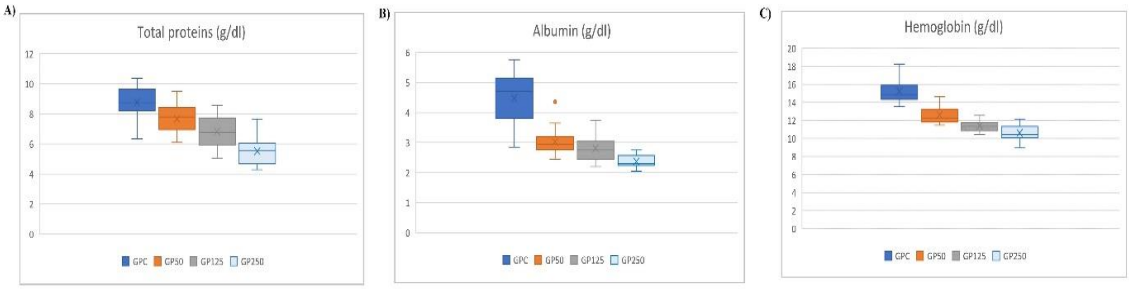


Figure 2. Boxplot presentation the comparison between the mean level of total protein (A), albumin (B), hemoglobin (C), in different groups GP50 (orange), GP125(grey), GP250 (turquoise) and GPC (blue), significant difference p≤ 0.05

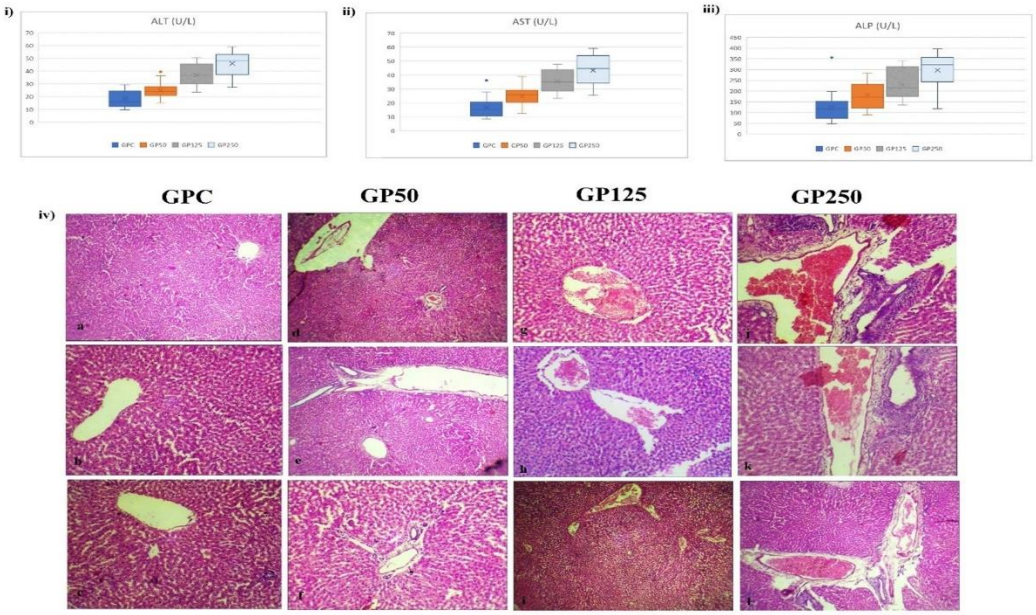


Figure 3. Upper panels showed Boxplot presenting a comparison between the mean activities of i) ALT, ii) AST, iii) ALP between different groups. iv) Microscopic slides of liver H&E stained sections from mice in GPC (a; 40x, b, c; 100x) showed normal hepatic architecture, GP50 (d,f; 40x, f; 100x) showed marked pathological changes characterized by the abundance of micro and macro vesicles, GP125 (g,h; 100x, i; 40x) showed mild congestion and aggregation of inflammatory cells, GP250 (j-l; 100x) showed multi necrotic foci filled with hemorrhage and extensive infiltration of mononuclear cells, necrotic foci surrounded by inflammatory cells.

**3.3. Effect of GBHs on the Kidney function**

We found that GBHs administration caused significant increase of the mean level of urea in GP50 (254.29±63.33 mg/dl, $p$<0.0001), GP125 (309.416±72.453 mg/dl, $p$<0.0001) and in GP250 (346.685±101.956 mg/dl, $p$<0.0001) compared to GPC (108.968±45.027 mg/dl). In addition, the mean level of urea in GP250 was significantly higher than that in GP125 (p=0.0316), however insignificant change was observed in mean level of urea between GP250 and GP125 (p=0.277).

Also, GBHs administration caused significant increase of the mean level of creatinine in GP50 (2.552±0.58 mg/dl, $p$<0.0001), GP125 (3.188±0.505 mg/dl, $p$<0.0001) and in GP250 (3.482±0.8704 mg/dl, $p$<0.0001) compared to GPC (1.392±0.472 mg/dl) . In addition, the mean level of creatinine in GP125 was significantly higher than that in GP50 (p=0.0029) while its mean level did not show significant difference between GP250 and GP125 (p=0.281). Our finding is in agreement with a previous study that indicated the effect of GBHs on kidney function causing elevation of creatinine [20]. These changes in the biomarkers of kidney function were clearly observed by the histopathological examination of kidney sections from treated and untreated groups. GPC showed normal architecture of a glomerulus as an intact Bowman's capsule with adjacent proximal and distal convoluted tubules. However, mild dilatation of glomerulus, proximal and distal tubules was observed in GP50. In GP125, sloughing of tubular epithelial cells and necrosis in large Bowman's space were noticed. In dose dependent manner, GP250 showed the most deleterious effect of glyphosate effects were noticed as marked tubular degeneration, dilatation, vacuolization in the tubules, widespread

interstitial inflammatory cells infiltration, severe glomerular degeneration and marked congestion in multiple capillaries. In support our result, a previous study reported that long term glyphosate exposure increased the risk of kidney tumors in mice. A previous study interpreted the nephrotoxicity of GBHs as a result of formation complex with metals such as Ca, Mg, Fe, Sr,---etc in drinking water and food resulting in reduced the anti-oxidants level including glutathione [22].

### 3.4. Effect of GBHs on Brain

Microscopical examination of mice brain showed normal architecture in GPC as shown in cerebellum section and a normal morphology of neurons in the cortex. GP50 group showed focal inflammatory cellular infiltration and mild neuronal injury. Marked injuries were noticed in hippocampus and cerebral cortex of GP125 including shrinkage and cellular atrophy and hyperchromatic cells. The deleterious effect of GBHs was markedly in GP250 as multipolar, neuronal swelling, vacuolated cells and vascular congestion were observed. Consistently, a previous study revealed that GBH induced anxiety and depression where cerebral venous congestion promotes disruption of blood-brain barrier resulting in dysregulation of blood flow and neuroinflammation [23,24].

### 3.5. Effect of GBHs on Heart

We found that GBHs administration caused significant increase in the mean activity of creatine kinase (CK-MB) in GP50 (57.683±19.135 U/L, p<0.0001), GP125 (64.339±18.981 U/L, p<0.0001) and in GP250 (76.901±13.412 U/L, p<0.0001) compared to GPC (31.455±8.306 U/L). However, the mean activity of CK-MB did not show significant difference between GP50, GP125 and GP250 (p=0.339,p=0.0644). The mean activity of LDH was elevated significantly in mice who received GBHs with different doses compared to the control group (P<0.0001). Also, its mean activity in GP250 was significantly higher than GP125 (P<0.0001) as well as its mean activity in GP125 was significantly higher than in GP50 (P<0.0001). In accordance, the histopathological examination of heart sections from GPC showed normal architecture while GP50 showed mild cellular infiltration of lymphocytes. GP125 showed focal degenerating myocytes, and vascular dilatation, steatosis and congestion. Gp250 showed extensive vascular congestion and severe tissue injuries. Consistently, a previous study revealed that GBHs reduced calcium intracellular uptake causing disruption of the electric potential, atrioventricular conduction blocks and arrhythmias [25]. GBHs increased Central venous pressure, disrupted the conduction and suppressed the myocardiac [26].

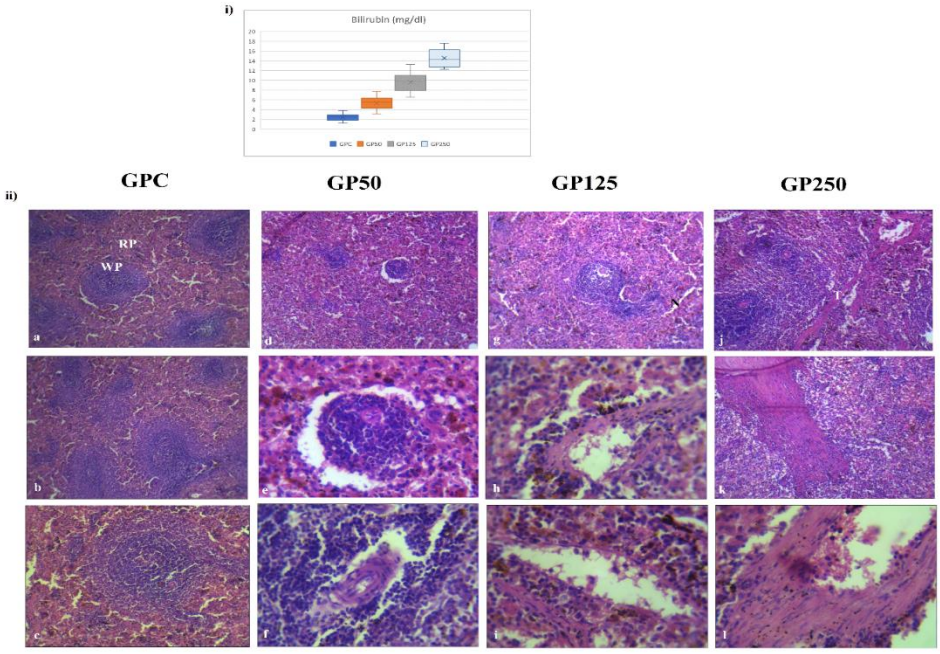


Figure 4. i): Boxplot presenting a comparison between the mean level of bilirubin between different groups. ii): Microscopic slides for H&E stained spleen sections from mice in control group GPC (a,b; 40x, c; 100x) showed normal arrangement of red pulp and white pulp, mice from GP50 (d; 40x, e,f; 100x) showed distorted lymphoid follicles, densely packed lymphocytes comprise the thick periarteriolar sheaths, mice from GP125(g; 40x, h,i; 100x) showed moderate fibrosis and necrosis, mice from GP250 (j,k; 100x, l; 400x) showed extensive fibrosis and mild congestion.

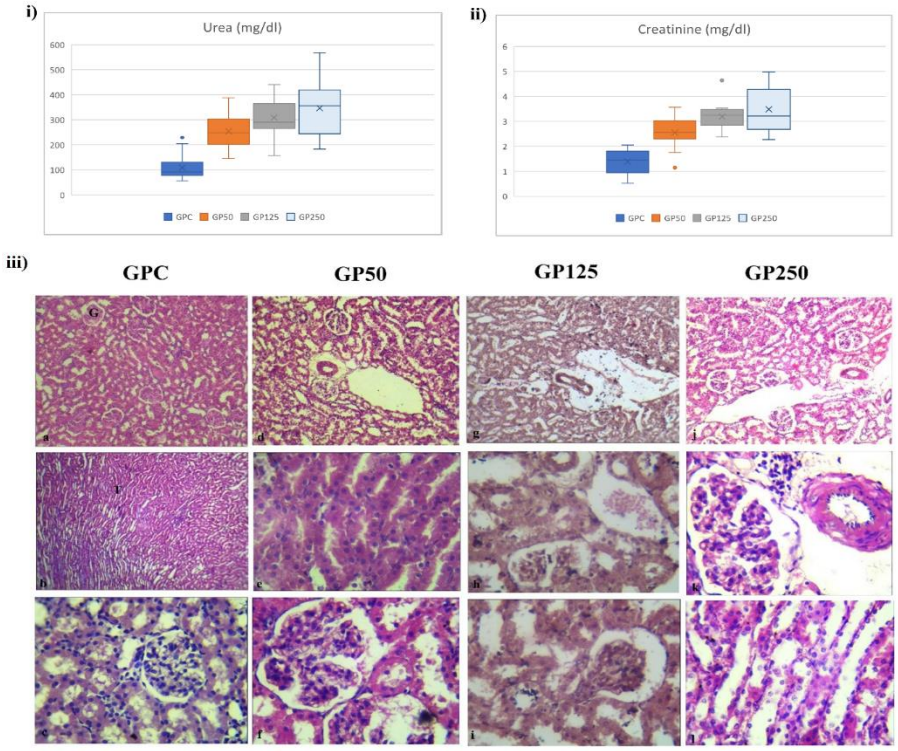


Figure 5. Upper panels showed Boxplot presenting a comparison between the mean level of i) urea, ii) creatinine between the different groups. iii): Microscopic slide of H&E stained kidney sections of mice from GPC (a,b; 40x, c; 100x) showed normal architecture glomerulus and tubules, GP50 (d;100x, e,f;400x) showed remarkable alterations of glomerulus components, tubules dilation and vacuolation, mice from GP125 (g; 100x, h,i; 400x) showed dilatation in the blood vessel, edema, tubular degeneration and congestion, GP250 showed distortion of glomerulus (j,100x), fibrotic tissues (k,400x) and extensive dilation of tubule (l,400x)

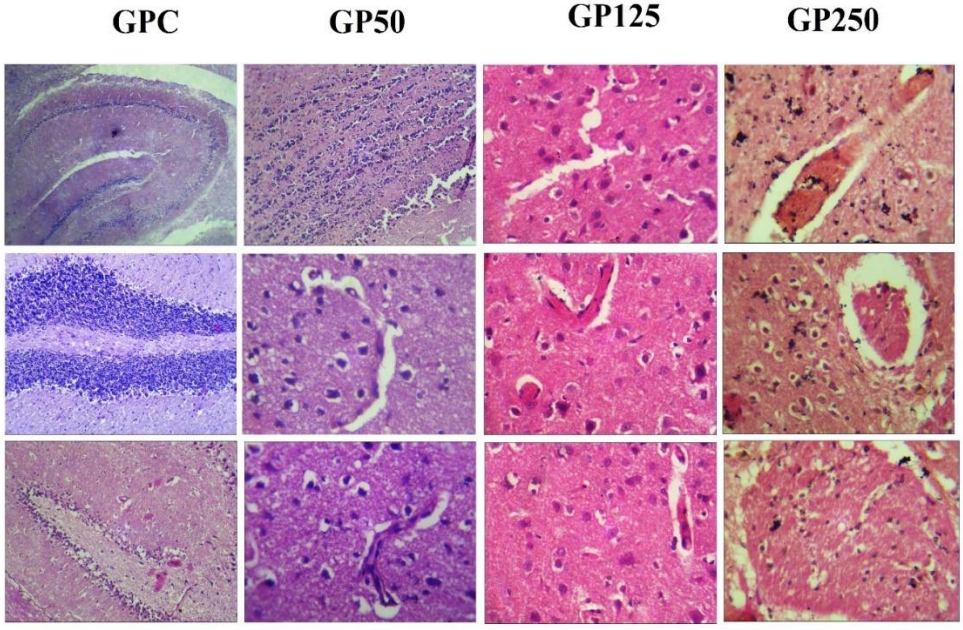


**Figure 6.** Microscopic examination of H&E stained brain sections from mice in GPC (a,b; 40x, c; 100x) showed normal hippocampus, GP50 (d; 100x, e,f; 400x) showed remarkable alterations of disorganized pyramidal cells, mice from GP125 (g-i 400x) showed degeneration and fibrosis, GP250(j-l 400x) showed extensive congestion.

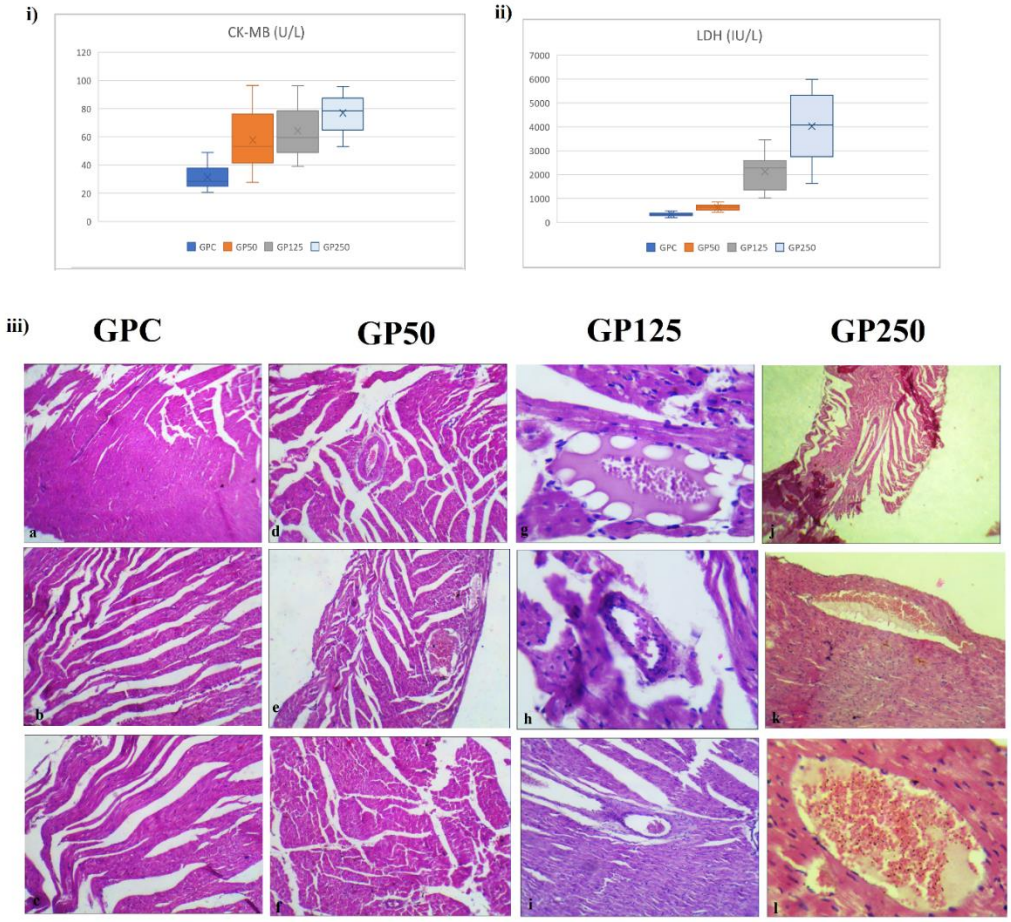


Figure 7. i): Upper panels showed Boxplot presenting a comparison between the mean enzyme activity of i) CK-MB and ii) LDH between different groups. ii) iii):Microscopic slides of H&E stained heart sections from mice in GPC (a; 40x, b,c; 100x) showed normal architecture of cardiac myocytes, GP50 (d-f; 100x) showed mild focal myocytic necrosis, GP125 (g,h; 400x, i; 100x) showed steatosis and congestion, remarked disorganization of the myofibrils and myocytes and myocytic necrosis, GP250 (j,k; 100x, l; 400x) showed extensive congestion.

### 3.6. Effect of GBHs on Lung

We found that GBHs administration caused significantly increased of the mean level of uric acid in GP50 (7.293±1.28 mg/dl, *P<0.0001*), GP125 (7.448±1.31 mg/dl, *p<0.0001* and in GP250 (8.305±1.1 mg/dl, *P<0.0001* compared to GPC (4.36±1.206 mg/dl. However, the mean level of uric acid did not show significant difference between GP50, GP125 and GP250. Microscopic examination of lung tissues from mice in GP50 exhibited mild tissue injuries where a notably marked thickening of some alveolar capillary membrane and focal hemorrhage. GP125 showed congestion and inflammatory cell infiltration while high dose of GBHs (250 mg/kg) caused severe lung injuries. Similarly, a recent study indicated the relevance of uric acid as a marker of hypertension, lung and cardiovascular disease [27,28]. Regarding to the effect of glyphosate on the lung, it has been demonstrated that glyphosate exposure was associated with asthma, chronic obstructive pulmonary disease (COPD) and pulmonary edema [29]. A mild degree of pulmonary congestion and edema was observed in both lungs. The toxic effect of Roundup on GIT and respiratory tracts was attributed to its ability to erode tissues including mucous membranes. [20]

### 3.7. Effect of GBHs on the Gastrointestinal tract

We found that GBHs administration caused significant increase in the mean level of alpha amylase in GP50 (345.848±103.024 U/L, p=0.009), GP125 (427.5±122.827 U/L, P= 0.0001) and in GP250 (686.853±182.651 U/L, p<0.0001) compared to GPC (249.738±98.54 U/L). Also, significant elevation of alpha amylase in GP250 compared to GP125 (p=0.0002) and GP125 compared to GP50 (p=0.05). Consistently, a recent study discovered the relevance of α-amylase as a marker of the proliferation and differentiation of small intestine epithelial cells [30].

We studied the effect of glyphosate on Histological examination of mice gastrointestinal tract including forestomach, glandular stomach and small intestine tissues. Microscopic examination of forestomach showed normal architecture in control group (GPC), distortion of gastric tissue was observed in GP50. In GP125 inflammation and congestion was observed.

Gastric ulceration, inflammatory cells infiltration, destruction of epithelium, atrophy of villi and crypts hyperplasia were observed in mice exposed to high dose of GBHs (GP250).

A previous study supported our finding in that Roundup administration caused hemorrhage in the fundus and dilatation in small intestine [31]. Also, a recent clinical study revealed that administration of Roundup for 2 months caused multiple gastric scar, upper GIT obstruction and pyloric obstruction [32]. Another mode of glyphosate action on the GIT digestion was via reduction of gut microflora in reptiles [33]. Numerous mechanisms were proposed for glyphosate-induced GIT toxicity, firstly, alteration of the morphometry of the intestinal wall, secondly; increased proportion of intraepithelial lymphocytes to the goblet cells, thirdly; alteration of the area occupied by collagen fibers, fourthly, causing atrophy of submucosal neurons and fifthly, infiltration of leucocytic inflammatory cell in the submucosal layer and muscularis mucosa. Moreover, congested blood vessels in the submucosal layer and hypertrophy of muscular coat were observed upon exposure to glyphosate. For the first time, we revealed that GBHs caused a gross systemic toxicity. In accordance, a previous study proposed three mechanisms; overexpression of pro-inflammatory cytokines, induced oxidative stress and imbalance of minerals levels [34].

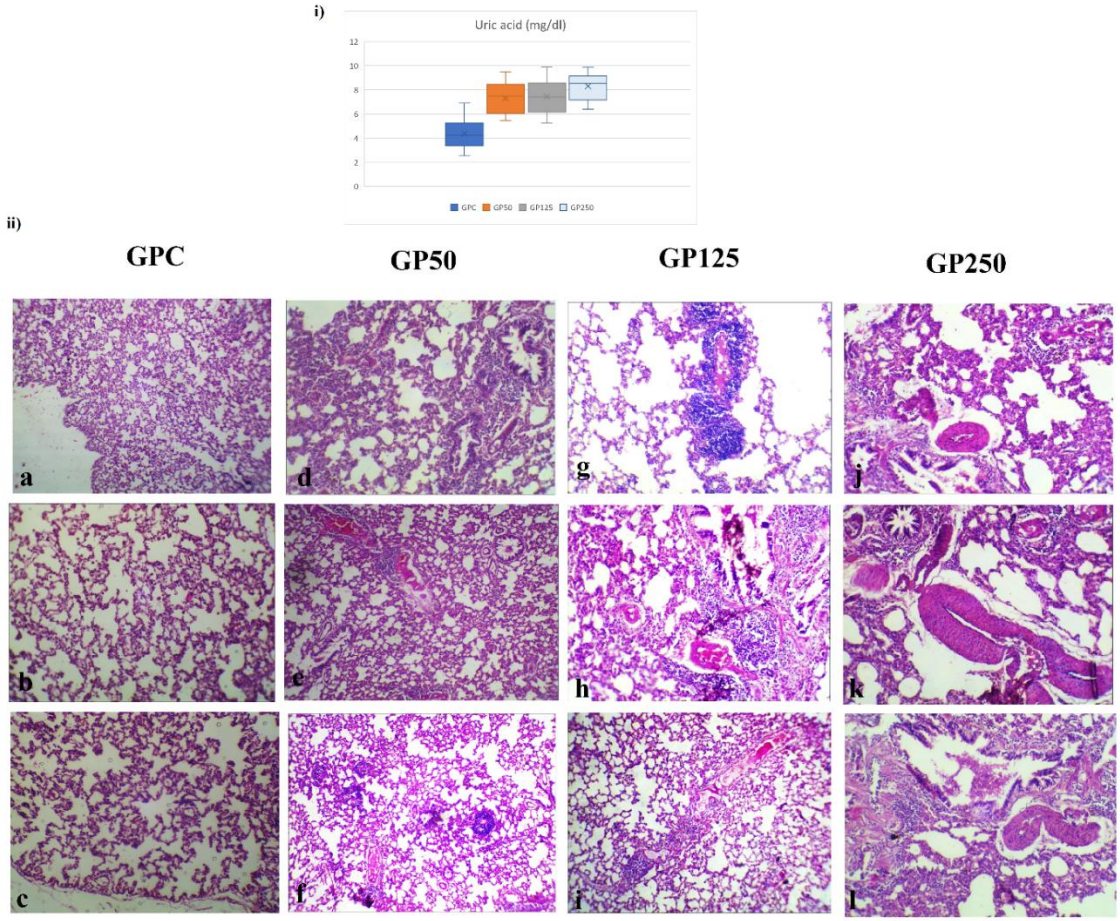


Figure 8. i) Boxplot presenting a comparison between the mean level of uric acid between different groups. ii): Microscopic slides of H&E stained lung tissue from mice in GPC (a; 40x, b.c; 100x) showed normal architecture, GP50 (d-f; 100x) showed necrotic debris in the lumen of pulmonary bronchi, mild congestion, GP125 (g,h; 100x, i; 40x) showed extensive interstitial infiltration of mononuclear inflammatory cells and moderate pulmonary congestion, GP250 (j-l 400x) showed infiltration of mononuclear inflammatory cells, fibrosis and necrotic debris.

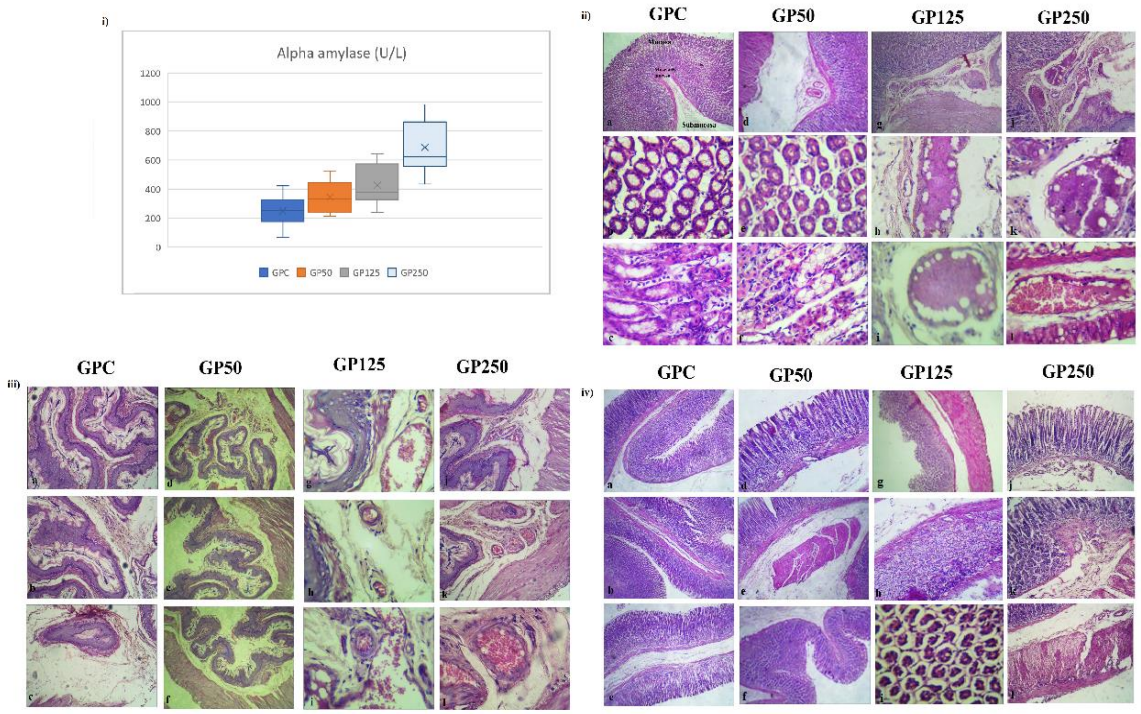


Figure 9. i) Boxplot presenting a comparison between the mean activity of alpha amylase between different groups. ii) Histopathological slides of the H&E stained glandular stomach section from mice in GPC showed normal architecture composed of normal mucosal, submucosal and musclaris layers (a,40x) and normal distribution of gastric glands (b,c; 400x), mice from GP50 showed cellular degeneration and mucosal atrophy (d;40x), gastric ulcer (e;40x), pale parietal cells (f;400x), mice from GP125 showed vascular congestion and necrosis (g;100x, h,i; 400x ), mice from GP250 showed severe congestion and lesion (j;100x, k,l; 400x). iii) Histopathological slide of the H&E stained forestomach section from mice in control group GPC showed normal architecture (a-c;40x) normal distribution of gastric glands, mice from GP50 showed mild inflammation and congestion (d-f; 100x), mice from GP125 showed moderate ulcer and congestion (g-I;400x), mice from GP250 showed ulcer and severe congestion (j,k;100x, l; 400x). iv) Histopathological slides of the H&E stained small intestine from mice in GPC showed normal architecture (a-c;40x), GP50 showed slight distortion and hyperplasia of colonic mucosa (d-f;40x), GP125 showed shortening of villi (g-i;100x), GP250 showed marked inflammation, leukocytes infiltration and fibrosis (j-l;100x).

**Conclusion:**

GBHs exerts systemic deleterious effects in mice causing multiple organ dysfunction.

**Recommendations:**

From these findings, a large extended study on field workers using GBHs is highly warranted.

**Conflicts of interest**

The authors declare no conflict of interest.

**Data availability**

All data for this work are available.

**Author contributions**

H.F: laboratory analysis, interpreted the biochemical data and histological examination and wrote the manuscript, O.T: and A.D: performed the practical experiments and M.G: histological examination.

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**ORCID**

Hewida H. Fadel, https://orcid.org/0000-0002-6696-0405

**LinkedIn Account (URL)**

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