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Research paper

Expected adverse health influences due to exposure to glyphosate-based herbicide (GBH) and its environmental outcomes using Wistar rats as experimental animals

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Abstract

A sum of 32 Wistar rats were divided into four equal groups, each containing 8 rats. Study animals were freely subject to normal rodent diet and tap water. One group was put as control. The applied agents were exposed to glyphosate-based herbicide (GBH-400 mg kg–1) GSH (nmol g–1 tissue), (GBH-600 mg kg–1) GSH (nmol g–1 tissue) and (GBH-850 mg kg–1) GSH (nmol g–1 tissue) once per day as oral gavage for 6 weeks. Study results clearly revealed that (GBH) significantly ($p \leq 0.05$) decreased the levels of GSH in liver, kidney, and brain tissues, due to many reasons including poor diet, chronic disease, infection and constant stress, besides aging. Biological negative influences of glyphosate herbicide through the scientific community, including government and non-government organizations have increased their interest in detecting and controlling the environmental agents responsible for damages to the human health and sustainability of the ecosystems.

Keywords: *Glyphosate Exposure; Industrial Revolution; Mutagenic Products; Scientific Community; Urbanization.*

1. Introduction

During the last decades, the scientific community, including government and non-government organizations have increased their interest in detecting and controlling the environmental agents responsible for damages to the human health and sustainability of the ecosystems. This interest has been intensified by the frightening increase on the reports of the anthropogenic action on the environment responsible for damages to the ozone layer, accidental release of wastes and radioactive gases, as well as contamination by pesticides used in agriculture. However, the growth of the human population and of the activities associated with agriculture, industrialization and urbanization have contributed to the depredation of the biodiversity and genetic variability, resulting in the compromise of several species, including man [17]. After the industrial revolution, a great number of chemical substances have been released into the terrestrial and aquatic environments and in the atmosphere. These substances can be transported and transformed by different processes, whose transformation by-products can cause adverse effects on man, as well as damages to the terrestrial and aquatic ecosystems. Several studies have shown the presence of residues of several chemical substances in the air, water, soil, food and organisms in general [5]. Glyphosate is the active ingredient of several widely used herbicide formulations. Glyphosate targets the shikimate metabolic pathway, which is found in plants but not in animals. Despite the relative safety of glyphosate, various adverse developmental and reproductive problems have been alleged as a result of exposure in humans and animals. To assess the developmental and reproductive safety of glyphosate, an analysis of the available literature was conducted. Epidemiological and animal reports, as well as studies on mechanisms of action related to possible developmental and reproductive effects of glyphosate, were reviewed. An evaluation of this database found no consistent effects of glyphosate exposure on reproductive health or the developing offspring. Furthermore, no plausible mechanisms of action for such effects were elucidated. Although toxicity was observed in studies that used glyphosate-based formulations, the data strongly suggest that such effects were due to surfactants present in the formulations and not the direct result of glyphosate exposure. To estimate potential human exposure concentrations to glyphosate as a result of working directly with the herbicide, available biomonitoring data were examined. These data demonstrated extremely low human exposures as a result of normal application practices. These available literature shows no solid evidence linking glyphosate exposure to adverse developmental or reproductive effects at environmentally realistic exposure concentrations [1]. The broad-spectrum herbicide glyphosate (common trade name "Roundup") was first sold to farmers in 1974. Since the late 1970s, the volume of glyphosate-based herbicides (GBHs) applied has increased approximately 100-fold. Further increases in the volume applied are likely due to more

and higher rates of application in response to the widespread emergence of glyphosate-resistant weeds and new, pre-harvest, desiccant use patterns. GBHs were developed to replace or reduce reliance on herbicides causing well-documented problems associated with drift and crop damage, slipping efficacy, and human health risks. Initial industry toxicity testing suggested that GBHs posed relatively low risks to non-target species, including mammals, leading regulatory authorities worldwide to set high acceptable exposure limits. To accommodate changes in GBH use patterns associated with genetically engineered, herbicide-tolerant crops, regulators have dramatically increased tolerance levels in maize, oilseed (soybeans and canola), and alfalfa crops and related livestock feeds. Animal and epidemiology studies published in the last decade, however, point to the need for a fresh look at glyphosate toxicity. Furthermore, the World Health Organization's International Agency for Research on Cancer recently concluded that glyphosate is "probably carcinogenic to humans." In response to changing GBH use patterns and advances in scientific understanding of their potential hazards, we have produced a Statement of Concern drawing on emerging science relevant to the safety of GBHs. Our Statement of Concern considers current published literature describing GBH uses, mechanisms of action, toxicity in laboratory animals, and epidemiological studies. It also examines the derivation of current human safety standards [8]. It was conducted that the initial assessments of glyphosate toxicity (in the 1970s) and approved a wide array of agricultural and non-agricultural uses, only limited and fragmentary data on GBH toxicity and risks were available. This previous study indicated minimal mammalian toxicity. Many articles published in 2000, written by consultants associated with the registrant and drawing on unpublished industry reports, confirmed that GBH toxicity and risks were poorly researched [8]. Glutathione peroxidase 3 (GPX3) catalyzes the reduction of H2O2 and is highly abundant in the thyroid gland. Therefore, excess of H2O2 and other reactive oxygen species (ROS) generated by DUOX system may be catalyzed by GPX3, protecting the cells against the oxidative damage. This is a finely regulated process between the balance among ROS generation and removal. Besides GPX3, thyrocytes express other members of the enzymatic antioxidant system, such as catalase, superoxide dismutase 1, 2 and 3 (with higher levels of SOD1), glutathione-disulfide reductase (GSR), several members of glutathione-S-transferase family (with higher levels of GSTP1 and GSTK1, respectively), several members of peroxiredoxin family (with higher levels of PRDX1, PRDX5 and PRDX3, respectively), thioredoxin (TXN) and thioredoxin reductases 1, 2 and 3 (with higher levels of TXNRD1) [13]. Glyphosate is an herbicide which inhibits the synthesis of aromatic amino acids in plants such as tryptophan, tyrosine and phenylalanine. 5-Enolpyruvylshikimate 3-phosphate synthase (EPSPS) is inhibited by the glyphosate in plants. EPSPS is primarily present in plastids, and its inhibition causes shikimate-3-phosphate accumulation. Thus, the production of aromatic amino acids is hindered, and this means prevention of protein synthesis [5]. Since only plants and some micro-organisms have the shikimate aromatic synthesis pathway, the toxic effects it is thought to have on mammals and other animals are caused by different reasons. Although the toxicity mechanism of GBH in animals has not been clarified yet, it has been reported in many studies that formation of reactive oxygen species (ROS) or oxidative stress, known as deterioration of the antioxidant defense mechanism, may have a role [18]. Non-target organisms such as humans and animals are exposed to GBH residues more, and this may cause different health problems in these living beings [5]. GBHs, classified as class E in 1991, are believed to be safe and non-toxic compounds for both humans and animals [11]. However, a previous study revealed that GBHs, which are commonly used around the world, may have toxic effects even below the acceptable limit values [17]. A previous study on frogs, found that rats and human cell cultures exposed to commercial formulations of glyphosate were only more toxic than glyphosate active substances. [15] concluded that isopropyl amine alone was more toxic than glyphosate on aquatic phytoplankton such as cyanobacterial strains.

2. Materials and methods

Knockdown 48 SL (Safa Agriculture Corp., Turkey), which contained 480 g L^{-1} a glyphosate isopropyl amine salt, was used to induce GBH toxicity in this study. Res, administered to the treatment groups, was purchased from Treatment (Santa Clara, CA, USA). The chemicals which would be used to determine the parameters to be analyzed were procured from the relevant firms. Wistar rats with different age and gender were used in this study, which were encaged in Meck – Nimir Research Unit, Khartoum, Sudan during June – July 2022. All experimental procedures were performed in compliance with the ARRIVE guidelines for the ethical treatment of experimental animals. Study animals were freely subject to normal rodent diet and tap water. The rats were divided into 4 groups, 8 rats/ each group. Distilled water was provided to the control group. Different doses of GBH (GBH-400 mg kg^{-1}), (GBH-600 mg kg^{-1}) and (GBH-850 mg kg^{-1}) were administered once per day as oral gavage for 6 weeks.

2.1. Preparation of homogenate

Cervical dislocation was utilized to euthanize the rats, the liver, renal and brain tissues were washed immediately with ice-cold 0.9% NaCl. Each tissue was trimmed free of extraneous tissue rinsed in a 0.15 M chilled Tris–HCl buffer (pH 7.4). The aforementioned tissues were blotted dry and homogenized in a 0.15 M Tris–HCl buffer (pH 7.4) to yield a homogenate of 10% (w/v). Later, they were centrifuged at 2100g for 10 min at 4 °C. The nuclear fraction was symbolized by the pellets, and the supernatants were subjected to centrifugation at 18 600g for 20 min at 4 °C. The mitochondrial fraction and the cytosolic (including microsomal fraction) fraction were symbolized by the emerging pellets and the supernatants, respectively. Formation of reactive oxygen species was seen in all the fractions, as well as the entire homogenate.

2.2. Measuring GSH in tissue homogenates

In accordance to method described by [13] tissue homogenates were prepared. The method described by [7] was used to measure the GSH concentration in the whole blood and tissue homogenates. The results were explained as nmol g^{-1} protein.

1) Statistical analysis

Study results were statistically analyzed in accordance to SPSS version 2021, Anova, One sample T – test.

2) Results

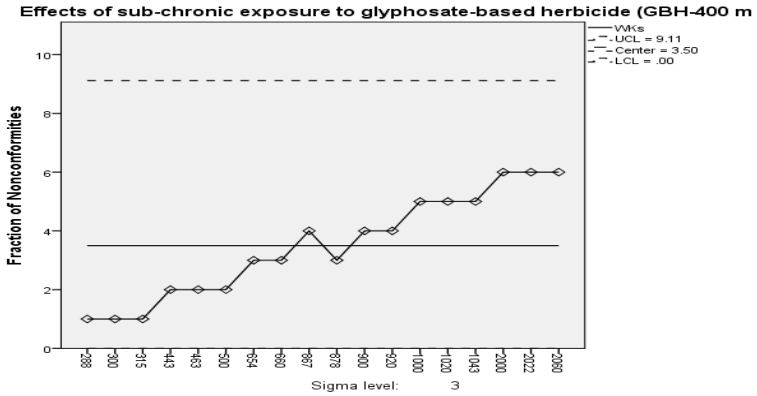

Fig. 1: Effects of Sub-Chronic Exposure to Glyphosate-Based Herbicide (GBH-400 Mg/ Kg) on Liver GSH (Nmol/ G Tissue).

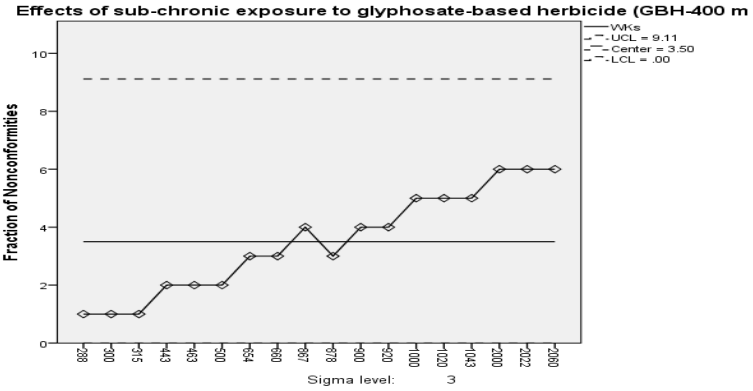

Fig. 2: Effects of Sub-Chronic Exposure to Glyphosate-Based Herbicide (GBH-600 Mg/ Kg) on Liver GSH (Nmol/ G Tissue).

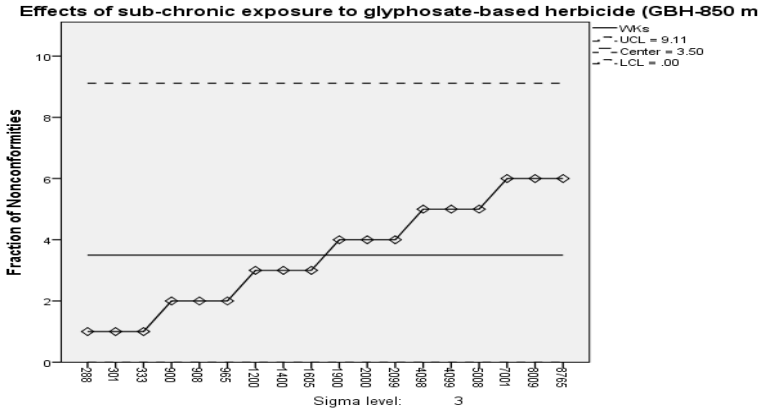

Fig. 3: Effects of Sub-Chronic Exposure to Glyphosate-Based Herbicide (GBH-850 Mg/ Kg) on Liver GSH (Nmol/ G Tissue).

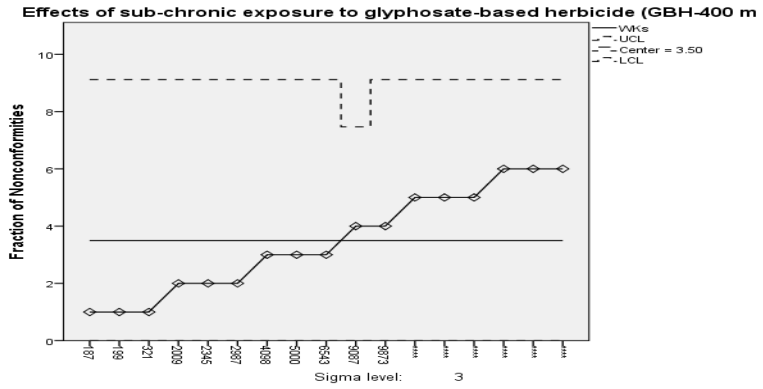

Fig. 4: Effects of Sub-Chronic Exposure to Glyphosate-Based Herbicide (GBH-400 Mg/ Kg) on Kidney GSH (Nmol/ G Tissue).

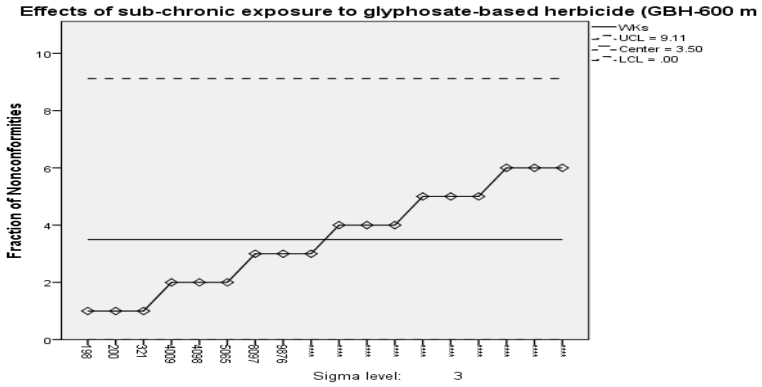

Fig. 5: Effects of Sub-Chronic Exposure to Glyphosate-Based Herbicide (GBH-600 Mg/ Kg) on Kidney GSH (Nmol/ G Tissue).

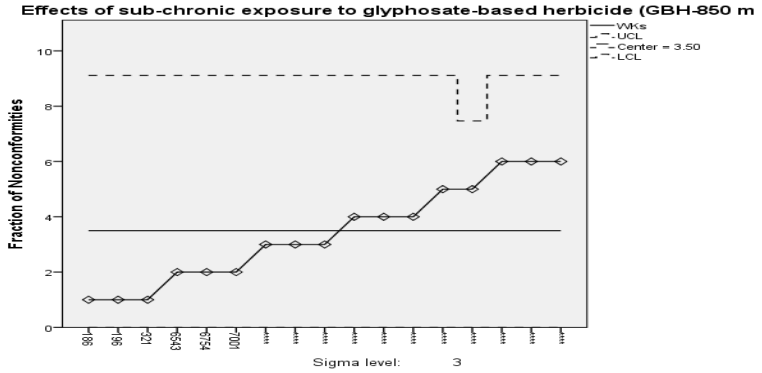

Fig. 6: Effects of Sub-Chronic Exposure to Glyphosate-Based Herbicide (GBH-850 Mg/ Kg) on Kidney GSH (Nmol/ G Tissue).

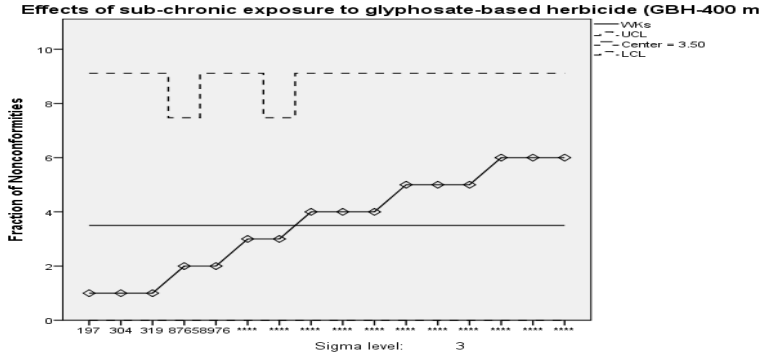

Fig. 7: Effects of Sub-Chronic Exposure to Glyphosate-Based Herbicide (GBH-400 Mg/ Kg) on Brain GSH (Nmol/ G Tissue).

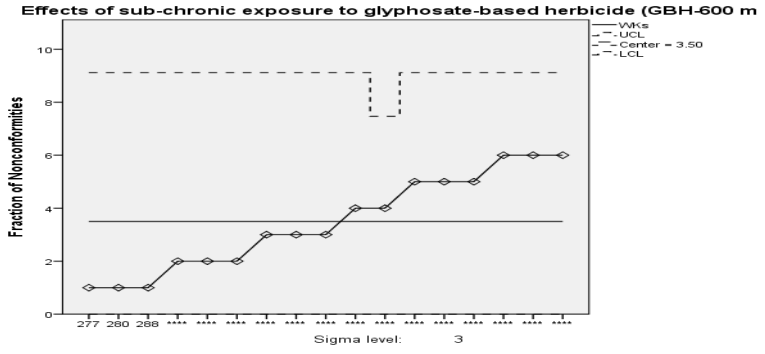

Fig. 8: Effects of Sub-Chronic Exposure to Glyphosate-Based Herbicide (GBH-600 Mg/ Kg) on Brain GSH (Nmol/ G Tissue).

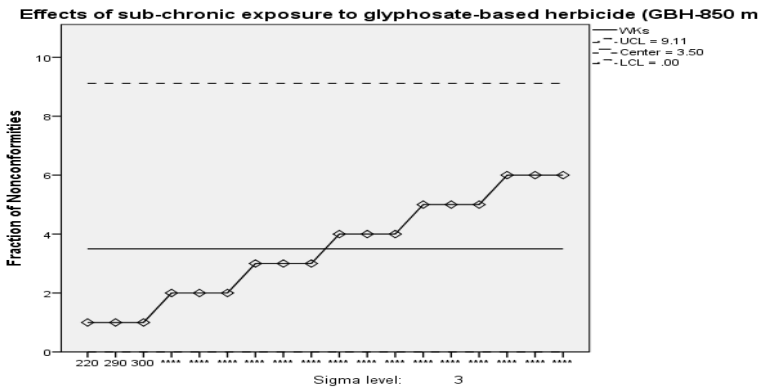

Fig. 9: Effects of Sub-Chronic Exposure to Glyphosate-Based Herbicide (GBH-850 Mg/ Kg) on Brain GSH (Nmol/ G Tissue).

Table 1: Effects of Sub-Chronic Exposure to Glyphosate-Based Herbicide (GBH- 400, 600, and 850 Mg/ Kg) on Liver, Kidney, and Brain GSH (Nmol/ G Tissue)

ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
WKs	Between Groups	52.500	5	10.500	.	.
	Within Groups	.000	12	.000		
	Total	52.500	17			
Effects of sub-chronic exposure to glyphosate-based herbicide (GBH-400 mg kg–1) on liver GSH (nmol g–1 tissue).	Between Groups	5576207.611	5	1115241.522	344.754	.000
	Within Groups	38818.667	12	3234.889		
	Total	5615026.278	17			
Effects of sub-chronic exposure to glyphosate-based herbicide (GBH-600 mg kg–1) on liver GSH (nmol g–1 tissue).	Between Groups	93707865.611	5	18741573.122	102.819	.000
	Within Groups	2187328.667	12	182277.389		
	Total	95895194.278	17			
Effects of sub-chronic exposure to glyphosate-based herbicide (GBH-850 mg kg–1) on liver GSH (nmol g–1 tissue).	Between Groups	123462282.944	5	24692456.589	133.275	.000
	Within Groups	2223295.333	12	185274.611		
	Total	125685578.278	17			
Effects of sub-chronic exposure to glyphosate-based herbicide (GBH-400 mg kg–1) on kidney GSH (nmol g–1 tissue).	Between Groups	590772646.500	5	118154529.300	211.569	.000
	Within Groups	6701614.000	12	558467.833		
	Total	597474260.500	17			
Effects of sub-chronic exposure to glyphosate-based herbicide (GBH-600 mg kg–1) on kidney GSH (nmol g–1 tissue).	Between Groups	854446795.111	5	170889359.022	214.337	.000
	Within Groups	9567524.667	12	797293.722		
	Total	864014319.778	17			
Effects of sub-chronic exposure to glyphosate-based herbicide (GBH-850 mg kg–1) on kidney GSH (nmol g–1 tissue).	Between Groups	2194802002.278	5	438960400.456	89.550	.000
	Within Groups	58822034.000	12	4901836.167		
	Total	2253624036.278	17			
Effects of sub-chronic exposure to glyphosate-based herbicide (GBH-400	Between Groups	18607835394.000	5	3721567078.800	15159.225	.000
	Within Groups	2945982.000	12	245498.500		

3. Discussions

Glyphosate significantly ($p \leq 0.05$) depletes liver, kidney and brain tissues glutathione (GSH), as illustrated in Figures; 1, 2, 3, 4, 5, 6,7,8 and 9, besides Table 1, [15] and glutathione S transferase (GST) is a critical enzyme for liver detoxification of arsenic [16]. As a consequence, excess arsenic in the kidney causes acute kidney failure, without evidence of other symptoms such as diabetes usually preceding kidney failure. Glutathione participates in detoxification of potentially harmful molecules, such as pesticides or heavy metals. Conjugation process can be accomplished spontaneously or in the presence of GST [7]. Glyphosate causes decrease of glutathione (GSH) and glutathione peroxidase (GSH-Px) activity in the kidney tissue. It is suggested that glyphosate caused obvious damage to rats' liver and caused various mineral elements content imbalances in various organs of rats. Ion imbalance could weaken antioxidant capacity and involve in the mechanism of liver oxidative damage caused by glucagon-like peptide (GLP) [7]. A postmortem study of brains of autistic individuals showed a striking decrease in aconitase activity in the cerebellum associated with a similar decrease in glutathione redox antioxidant capacity (GSH/GSSG), with the plot producing a near 100% separation between cases and controls [18]Moreover, increase of hydrogen peroxide (H_2O_2) level and catalase (CAT) activity in the serum and liver and decrease of glutathione (GSH) and glutathione peroxidase (GSH-Px) activity in the kidney tissue further confirmed the occurrence of oxidative stress [18]. A postmortem study of brains of autistic individuals showed a striking decrease in aconitase activity in the cerebellum associated with a similar decrease in glutathione redox antioxidant capacity (GSH/GSSG), with the plot producing a near 100% separation between cases and controls [18] Inadequate clearance of superoxide due to Mn-SOD inactivation can easily account for this observation. Aconitase is a crucial participant in the citric acid cycle in the mitochondria, so this effect has catastrophic consequences on the renewal of adenosine triphosphate (ATP) as an energy source for the neurons.

4. Conclusions

It is concluded that Glyphosate significantly depletes liver, kidney and brain tissues glutathione (GSH), resulting in many cellular abnormalities.

5. Recommendations

It is highly recommended to deal with glyphosate herbicide with care, in order to decreases risks associated with inhalatory, dermal or oral exposure. Further studies considering potential negative health influences are also recommended.

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