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Glyphosate Caused Detrimental Changes in Enzymatic Antioxidants in Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Background: Glyphosate is the most enormously used broad spectrum herbicide in the world. Current assessment of carcinogenic capability of glyphosate-based herbicides by various regional, national, and international agencies have endangered the controversy. Antioxidant enzymes are often used as biomarkers of oxidative stress. Among the biomarkers superoxide dismutase, catalase and glutathione peroxidase were essential in conservation of homeostasis of cell to function as normal being.
Aim: To investigate glyphosate induced detrimental changes in the enzymatic antioxidants in experimental rats.
Materials and Methods: Adult male wistar albino rats were divided into 4 groups, each consisting of 6 animals. Group 1 consists of Normal control rats, Group 2 consists of Glyphosate treated at a dose of 50mg/kg body weight/day. Group 3 consists of Glyphosate treated at a dose of 100 mg/kg body weight/day. Group 4 consists of Glyphosate treated at a dose of 250 mg/kg body weight/day. The experimental period was 16 weeks. All chemicals and reagents used in this study were purchased from sigma chemical company, USA. Adult male albino rats weighing 180-200g were used for the study. Parameters analyzed were assay of Superoxide Dismutase Catalase, Glutathione Peroxidase. The data were analyzed statistically by one-way analysis of variance

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followed by Duncan's multiple range test, and it was used to see the statistical significance among the groups. The results with the $p<0.05$ level was considered to be statistically significant.
Results: The results indicated that there was a significant decrease in the activities of enzymatic antioxidants in all the Glyphosate induced rats, and it decreases with increase in dose of Glyphosate.
Conclusion: Glyphosate has induced oxidative stress in experimental animals by decreasing the expression of Enzymatic Antioxidants.

Keywords: Detrimental changes; enzymatic antioxidants; experimental rat.

1. INTRODUCTION

Glyphosate is the most enormously used broad spectrum herbicide in the world. Current assessment of carcinogenic capability of glyphosate-based herbicides by various regional, national and international agencies have endangered the controversy [1]. In glyphosate-based herbicides the revealed principle G is mixed with many adjuvants which help to pierce the cell membrane of plants [2]. Glyphosate, an N-(Phosphonomethyl) glycine is the main constituent in Monsanto ROUNDUP Herbicide. Antioxidant enzymes are often used as bio markers of oxidative stress. Among the biomarkers Superoxide dismutase (SOD), Glutathione peroxidase (GPx), Catalase (CAT) were essential in conservation of homeostasis for cell to function as normal. The mRNA expression of Catalase, Glutathione Peroxidase, Cu/Zn Superoxide Dismutase after the 2 weeks the introduction of diabetes was not different from that in the control rats [3]. Antioxidant system (TAS, TOS, OSI, PON, Arytelase, Catalase and MDA) and the level of GST plays an important role in mechanism of detoxification in various organs such as liver, lung, kidney and mitochondrial fractions in rats with hyperglycemia where non chronically developed with STZ (Streptozotocin) which is a diabetogenic chemical agent [3,4]. Recent findings indicate glyphosate and its metabolites can also diffuse by wind and soil erosion [5]. Glyphosate which is used as weed control for both domestic horticulture and large crops [6].

In reality, excessive development of ROS (Reactive Oxygen Species) brings about issues like Lipid Peroxidation and Injury of DNA. Extreme evaluation of ROS gives on to construction of cytotoxic metabolites which can induce irreversible disorders like damage of DNA, Peroxidation of lipid. These changes were in agreement with the worsening of kidney function, signifying that renal I/R(Ischaemia\Reperfusion)induced kidney ROS formation, reduced the capacity of cells to get rid of ROS, augmented endogenous antioxidant depletion, and deteriorated renal injury [7]. Glyphosate (N-phosphonomethyl [glycine], is an organophosphorus compound with herbicide properties discovered in 1970. It's A competitive inhibitor of the 5-enolpyruvylshikimate-3-phosphate synthase, an enzyme involved in aromatic amino acid biosynthesis in plants and microorganisms [8]. In 1974, Monsanto started its commercialization as a broad-spectrum herbicide. This first glyphosate-based herbicide (GBH), RoundUpVR, and the others that followed such as GlyphoganVR , TouchdownVR , or Golflogix VR , are mixtures of glyphosate and various adjuvants used to boost its penetration in plants and enhance its activity [9].

Antioxidants are the constituents manufactured by the body to neutralize the cause of free radicals, but the outcome will be restricted to particular antioxidants. In the human body oxidants and antioxidant ratio will be continued, any changes in these oxidants and antioxidants will bring about collection of ROS within the body, this process is called as oxidative stress [10]. Oxidative stress has a fundamental part in harm of tissue and watches out for neurotic conditions like malignancy, [11,12]. Glyphosate-based herbicides mainly represented by roundup are the most widely used commercial formulations of pesticides worldwide [12,13]. Glyphosate has low harmfulness in warm blooded creatures like mammals. Our team has extensive knowledge and research experience that has translate into high quality publications in various fields like Cancer Biology, Molecular Biology, Nanotechnology, Diabetes, Phytochemistry etc [14-33]. Hence the present study has been designed to fulfill the deficiency about Glyphosate induced detrimental changes in enzymatic antioxidants in experimental rats.

2. MATERIALS AND METHODS

2.1 Chemicals

All chemicals and reagents used in this study were purchased from Sigma Chemical Company

St. Louis, MO, USA; Invitrogen, USA; Eurofins Genomics India Pvt Ltd, Bangalore, India; New England Biolabs (NEB), USA; Promega, USA. glyphosate was procured from Sigma Chemical Company St. Louis, MO, USA; Total RNA isolation reagent (TRIR) was purchased from Invitrogen, USA. The reverse-transcriptase enzyme (MMuLv) was purchased from Genet Bio, South Korea purchased from Promega, USA. Dopamine Receptor, Serotonin receptor (The serotonin 1A receptor) and β-actin primers were purchased from Eurofins Genomics India Pvt Ltd, Bangalore, India.

2.2 Experimental Animals

Adult male Wistar albino rats, weighing 180–200g, were obtained and maintained in clean propylene cages at the Biomedical Research Unit and Laboratory Animal Centre (BRULAC), Saveetha Dental College and Hospitals, Saveetha University, India) in an air-conditioned animal house, fed with standard rat pelleted diet (Lipton India Ltd., Mumbai, India), and clean drinking water was made available ad libitum.

2.3 Experimental Groups

Rats were divided into 3 groups, each consisting of 6 animals. Experimental Design is explained in Table 1.

At the end of the treatment, animals were anesthetized with sodium thiopentone (40 mg/kg b.wt), blood was collected through cardiac puncture, sera were separated and stored at −80°C, and 20 ml of isotonic sodium chloride solution was perfused through the left ventricle to clear blood from the organs. Gastronemous muscle from control and experimental animals was immediately dissected out and used for assessing the various parameters.

2.3.1 Assessment of Fasting Blood Glucose (FBG)

After the overnight fasting, the blood glucose was estimated using On-Call Plus blood glucose test strips (ACON Laboratories Inc., USA). From the rat tail tip the blood was collected and results were expressed as mg/dl

2.3.2 Oral Glucose Tolerance Test (OGTT)

For the oral glucose tolerance test, animals were fasted overnight. After giving the oral glucose load (10 ml/kg; 50% w/v). blood glucose level was estimated at various time periods (60, 120, and 180 min) by using On-Call Plus blood glucose test strips. Before giving glucose load, the value of blood glucose is considered as 0 min value. Results were marked as mg/dl.

2.4 Procedure

2.4.1 Assay of Superoxide Dismutase (SOD)

Superoxide dismutase was assayed by the method of Marklund and Marklund [34]. Procedure To 1 ml of tissue homogenates 0.25 ml of ethanol and 1.25 ml of chloroform were added, kept in a mechanical shaker for 15 min and centrifuged at 20000xg for 15min. To 0.5 ml of the supernatant, 2.0 ml of 0.1 M Tris-HCl buffer pH 8.2; 1.5 ml of distilled water and 0.5 ml of pyrogallol were added. Change in optical density at 0, 1 and 3 min was read at 420 nm in a spectrophotometer. Control tubes containing 0.5 ml of distilled water were also treated in a similar manner against a buffer blank. The enzyme activity is expressed as Units/mg protein. One enzyme unit corresponds to the amount of enzyme required to bring about 50% inhibition of pyrogallol auto-oxidation.

Table 1. Experimental Design

Groups	
Group I	Normal control rats fed with normal diet and drinking water
Group II	Glyphosate treated (dissolved in water at a dose of 50 mg/kg body weight/day at 8 to AM) orally for 16 weeks
Group III	Glyphosate treated (dissolved in water at a dose of 100 mg/kg body weight/day at 8 to AM) orally for 16 weeks
Group IV	Glyphosate treated (dissolved in water at a dose of 250 mg/kg body weight/day at 8 to AM) orally for 16 weeks

2.4.2 Assay of Catalase (CAT)

Catalase activity was assayed by the method of Sinha [35]. To 0.1 ml of tissue homogenates 1.0 ml of buffer and 0.5 ml of hydrogen peroxide were added and the time was noted, and then 2.0ml of Dichromate acetic acid was added. The green colour developed was read at 570 nm using a spectrophotometer. Catalase activity is expressed as μ moles of H_2O_2 consumed/min/mg protein.

2.4.3 Assay of Glutathione Peroxidase (GPX)

Glutathione peroxidase was assayed by the method of Rotruck et al. [35,36]. 0.2 ml each of EDTA, sodium azide, GSH, H_2O_2, buffer and tissue homogenates were mixed and incubated at 37°C for 10 min. The reaction was arrested by the addition of 0.5 ml of TCA and the tubes were centrifuged. To 0.5 ml of supernatant, 3.0 ml of phosphate solution and 1.0 ml of DTNB were added and the colour developed was read at 420 nm immediately against blank containing only phosphate solution and DTNB reagent. Graded amounts of standards were also treated similarly. GPX activity is expressed as μg of glutathione utilized/min/mg protein.

2.5 Statistical Analysis

The triplicate analysis results of the experiments performed on control and treated rats were expressed as mean ± standard deviation.Results were analyzed statistically by a one-way analysis of variance (ANOVA) and significant differences between the mean values were measured using Duncan's multiple range test using Graph Pad Prism Version 5. The results with the $p<0.05$ level were considered to be statistically significant.

3. RESULTS AND DISCUSSION

Fig. 1 represents the impact of glyphosate on antioxidant- SOD (Superoxide Dismutase) activity in adult male albino Wistar rats. The X-axis represents the amount of glyphosate which was exposed to rats. The Y-axis represents the fold change over in Superoxide Dismutase enzyme (SOD). Green colour represents the controlled rats. Orange colour represents Group 1 rats exposed to about 50 mg of glyphosate. Blue represents Group 2 rats exposed to about 100 mg of glyphosate. Purple colour represents Group 3 rats exposed to about 250 mg of glyphosate. Each bar represents mean∓SEM (n=6). Significance at P <0.05glyphosate.

Fig. 2 represents represents the impact of glyphosate on antioxidant- CAT(Catalase) activity in adult male albino Wistar rats. The X-axis represents the amount of glyphosate which was exposed to rats. The Y-axis represents the fold change over in Catalase enzyme (CAT).Green colour represents the controlled rats. Orange colour represents Group 1 rats exposed to about 50 mg of glyphosate. Blue Group 2 represents rats exposed to about 100 mg of glyphosate. Purple colour represents Group 3 rats exposed to about 250 mg of glyphosate. Each bar represents mean∓SEM (n=6). Significance at P<0.05.

Fig. 3 represents the impact of glyphosate on antioxidant- GPx (Glutathione peroxidase) activity in adult male albino Wistar rats.The X-axis represents the amount of glyphosate which was exposed to rats. The Y-axis represents the fold change over in Glutathione peroxidase enzyme (GPx).Green colour represents the controlled rats. Orange colour represents Group 1 rats who were exposed to about 50 mg of glyphosate. Blue colour represents Group 2 rats who were exposed to about 100 mg of glyphosate. Purple colour represents Group 3 rats who were exposed to about 250 mg of glyphosate. Each bar represents mean∓SEM (n=6). Significance at P<0.05

From the Fig. 1 we know that, Group 1 rats were exposed to about 50 mg of glyphosate there is a decrease in the activity of SOD from change over value, Group 2 rats were exposed to about 100 mg of glyphosate there is more decrease in the activity of SOD from group 1, Group 3 rats were exposed to about 250 mg of glyphosate there is also a decrease in the activity of SOD. From the figure 2 it was clear that, there was a decrease in the activity of catalase in all the glyphosate treated rats in a dose dependent manner, with more decreased activity in group treated with glyphosate at a dose of 250 mg. Figure 3 also showed a dose dependent decrease in the activity of GPx in all the glyphosate treated rats with more decrease in rats treated with glyphosate at a dose of 250 mg.

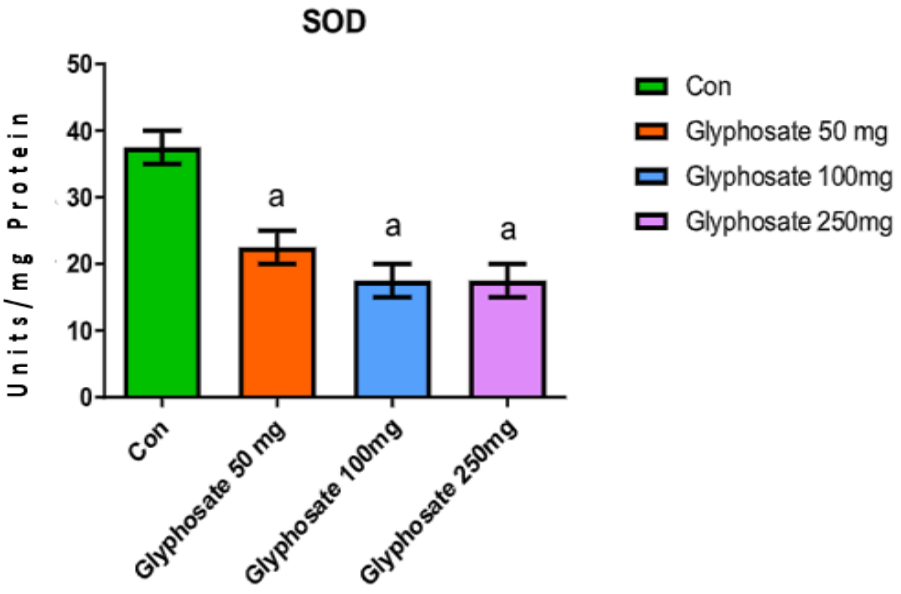

Fig. 1. Activity of SOD

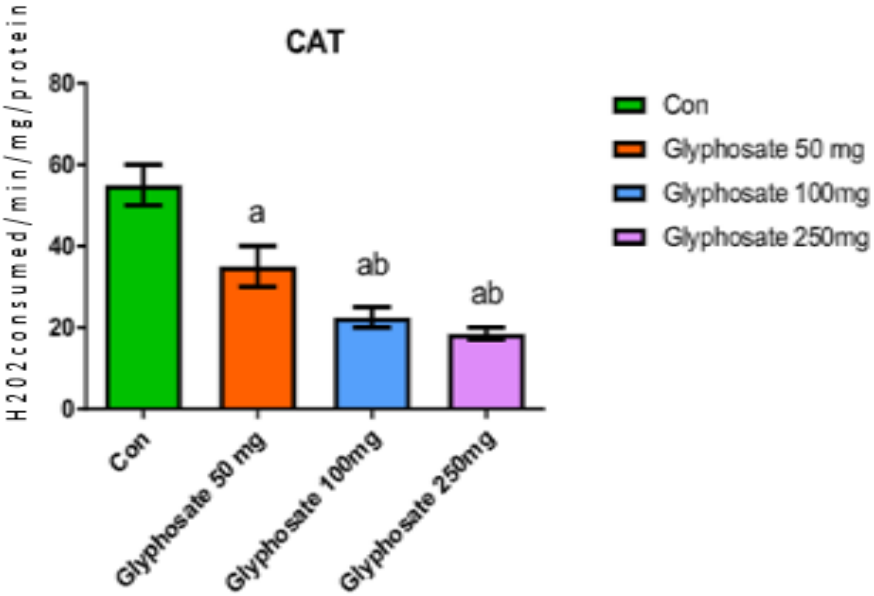

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Fig. 2. Activity of Catalase

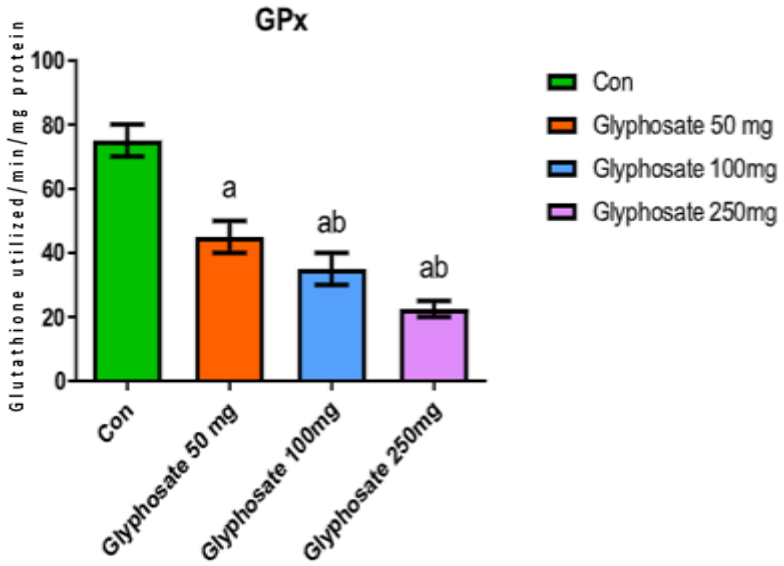

Fig. 3. Activity of glutathione peroxidase

Antioxidants play a significant role in protecting the cells by inhibiting the oxidative stress while glyphosate is exposed to rats. These enzymatic antioxidants level gets decreased and there is a dose dependent decrease in the activity of these enzymes on glyphosate exposure. SOD, CAT, GPx are three important antioxidants which play a major role in protecting the cell. In this study we observed the effect of glyphosate on SOD, CAT, GPx (Figs. 1-3). When glyphosate was given to rats, these enzymatic antioxidant levels got decreased because of glyphosate induced oxidative stress. Antioxidants are a natural defense mechanism which fights against free radicals which result in oxidative stress. In most of the cases, free radical accumulation may lead to various non pathological disorders like Diabetes mellitus, Cancer, Ageing related disorders etc. The imbalance between the antioxidant and free radical levels is the root cause for the generation of free radicals and its associated disorders. In the current study, the administration of non-specific herbicide-Glyphosate has resulted in decreased expression of the natural defense mechanism. The study has focused on the alarming decrease in the levels of the natural antioxidants and other related consequences. A previous study proved that the administration of Rutin (polyphenolic flavonoid) in streptozotocin-induced diabetes Wistar rats has resulted in the increase of non-enzymatic antioxidants, thus showed that Rutin exhibits anti-hyperglycemic and antioxidant activity in streptozotocin-induced diabetic rats [37]. The study by Magdalena Gorny et al has proved the alternations in the antioxidant enzyme activities in the neurodevelopmental rats model. Glutathione deficiency during early postnatal life has resulted in the decrease in SOD activity. Comparison of CAT activity in kidney examined groups did not show significant differences between them [4,38]. Previous study by Cindy Peillex etal has proved the role of antioxidants in diabetes-induced oxidative stress in glomeruli of daibetic rats. Decreased antioxidant levels has resulted in excessive oxidative stress [9]. From the study it was evident that prolonged use of Glyphosate can lead to detrimental decrease in the enzymatic antioxidants, which needs to be taken seriously. The examination was finished with only three fluctuated portions of glyphosate. Studying more shifted dosages will help in acquiring more precise consequences for articulation with additional time and test. In future, other associated parameters also need to be checked in order to prove the ill effects of prolonged exposure to this non specific herbicide.

4. CONCLUSION

Antioxidants play a significant chemical role in protecting the cells by inhibiting oxidative stress. Prolonged exposure of glyphosate has resulted in the decreased activities of enzymatic antioxidants, which might be due to the oxidative stress caused by this herbicide. This may lead to imbalances in the natural antioxidant

mechanisms and can result in oxidative stress induced non pathological disorders. In future, detailed studies will help to understand the mechanism of action of Glyphosate in inducing the oxidative stress related toxicity.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The present experimental study was approved by the institutional animal ethics committee (IAEC no.: BRULAC/SDCH/SIMATS/IAEC/02-2019/ 015).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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38. Website [Internet]. [cited 2021 Mar 8]. Available from:)Alterations in the Antioxidant Enzyme Activities in the Neurodevelopmental Rat Model of Schizophrenia Induced by Glutathione Deficiency during Early Postnatal Life by Magdalena Górny 1,Anna Bilska-Wilkosz 1,Małgorzata Iciek 1,Marta Hereta 2,Kinga Kamińska 2,Adrianna Kamińska 3,Grażyna Chwatko 3,Zofia Rogóż 2 andElżbieta Lorenc-Koci 2,*Antioxidants. 2020;9(6): 538. Available:https://doi.org/10.3390/antiox9060538

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