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Effect of Glyphosate (GLP) on Organ Weights in *Wistar* Rats and its Amelioration with Ascorbic Acid

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Abstract: ***The aim of this study was to analyze the effect of glyphosate of organ weights and the protective role of Ascorbic Acid (AA) in male albino Wistar rats. Forty eight (48) rats were randomized into four groups consisting of twelve (12) animals in each: Group 1 served as control, group 2 received glyphosate at a dose of 500 mg/kg b.wt, group 3 received Vitamin C at a dose of 250 mg/kg b.wt and group 4 received glyphosate (500 mg/kg b.wt) and Vitamin C (250 mg/kg b.wt). The treatment regimens were administered by oral gavage once daily for three weeks. Results showed a significant reduction in the absolute weights of testes, liver and kidneys in glyphosate treated groups. However, administration of Vitamin C caused a mild ameliorative effect on the parameters investigated.***

Keywords: glyphosate, organ weights, Vitamin C and albino *Wistar*

1. Introduction

Glyphosate is the active ingredient of Roundup®, marketed as a non-selective, broad spectrum, post emergence herbicide. It is used to control weeds in emerged grasses, pastures, rice, corn and soy plantations[1].Based upon animal studies, some investigators suggested that the GLP may enhance Adenosine Triphosphatase (ATPase) activity and uncouple mitochondrial oxidative phosphorylation [2, 3, 4]. Vit. C being an antioxidant, is involved in the prevention of cellular damage by safely interacting with free radicals and terminating the chain reactions before vital molecules are damaged. It also removes free radical intermediates and inhibits other oxidative reactions[5].

2. Materials and Methods

2.1 Experimental animals

Forty eight (48) adult male albino *Wistar* rats weighing 200-240 g, bred at Jeeva Life Sciences (ISO 9001:2015 certified company), Hyderabad were used for this research. The rats were housed in solid bottom polypropylene cages at RUSKA Labs, Department of Veterinary Pathology and were maintained in controlled environment (20-22^{0}C) throughout the course of experiment. Sterile rice husk was used as standard bedding material. All the rats were provided with standard pellet diet (low fat and nutritionally balanced food) and deionized drinking water *ad libitum* throughout the experimental period.The experiment was carried out according to the guidelines and prior approval of Institutional Animal Ethics Committee(IAEC-No.01-2019).

2.2 Chemical source

Glyphosate was obtained from Seed Research and Technology Centre (SRTC), Professor Jayashankar Telangana State Agriculture University (PJTSAU), Hyderabad-30 under the trade name Roundup® (41%) and Vitamin C was obtained from S.D. Fine-Chem Ltd., Mumbai, India.

2.3 Experimental design

A total of 48 male albino *Wistar* rats were randomly divided into four (4) groups consisting of twelve (12) animals in each.
Group 1 - Control
Group 2 - GLP (@500 mg/kg b.wt)
Group 3 - AA (@250 mg/kg b.wt)
Group 4 - GLP+AA (@500 mg/kg b.wt + 250mg/kg b.wt)

The dose regimens were administered *per os*once daily for a period of three weeks. The rats were monitored for clinical signs and death.

2.4 Organ morphometry

Experimental rats were sacrificed on 7th and 21st day of experiment by gaseous anesthesia and a detailed necropsy examination was carried out as per standard procedure[6]. The testes, liver and kidneys from all the animals were weighed using electronic balance.

2.5 Statistical analysis

Data obtained were subjected to statistical analysis by applying one way ANOVA using Statistical Package for

Social Sciences (SPSS) version 16.0. Differences between the means were tested by using Duncan's multiple comparison tests and significance level was set at P<0.05 [7].

3. Results and Discussion

3.1 Absolute weights of testes

The mean values of absolute organ weights of testes in group 1 (1.38 ± 0.04, 1.57 ± 0.04), group 2 (1.28 ± 0.03, 1.29 ± 0.03), group 3 (1.53 ± 0.02, 1.57 ± 0.03) and group 4 (1.31 ± 0.06, 1.33 ± 0.03) were recorded on 7th and 21st day of experiment. No difference was observed in groups 1, 2 and 4 but, significant increase was noticed in group 3 on 7th day of experiment. Significantly (P<0.05) reduced weights were noted in groups 2 and 4 as compared to groups 1 and 3 on 21stday of experiment. (Table 1)

Table 1: Absolute weights of testes (g) in different groups

Group	Day 7	Day 21
Group 1	1.38 ± 0.04^{a}	1.57 ± 0.04^{b}
Group 2	1.28 ± 0.03^{a}	1.29 ± 0.03^{a}
Group 3	1.53 ± 0.02^{b}	1.57 ± 0.03^{b}
Group 4	1.31 ± 0.06^{a}	1.33 ± 0.03^{a}
P value	*	*

Values are Mean ± SE (n=6); One way ANOVA
Means with different superscripts in a column differ significantly at P<0.05 (*)

Contrary to this, no significant difference in the testicular weights were reported by previous authors [5, 8]. Hypothetically, in the present study direct cytotoxic action of GLP on testes by evading the anti-apoptotic pathways leading to an over production of Reactive Oxygen Species (ROS), these phenomenal changes might have triggered the initiation of atrophy and necrosis of seminiferous tubules.

3.2 Absolute weights of liver

The mean values of absolute organ weights of liver in group 1 (9.79 ± 0.06, 9.89 ± 0.06), group 2 (8.58 ± 0.19, 8.37 ± 0.12), group 3 (10.01 ± 0.15, 10.34 ± 0.17) and group 4 (8.89 ± 0.23, 8.82 ± 0.13) on 7th and 21st day of experiment. Significantly (P<0.05) reduced weights were recorded in groups 2 and 4 as compared to groups 1 and 3 on 7th and 21stday of experiment. (Table 2)

Table 2: Absolute weights of liver (g) in different groups

Group	Day 7	Day 21
Group 1	9.79 ± 0.06^{b}	9.89 ± 0.06^{b}
Group 2	8.58 ± 0.19^{a}	8.37 ± 0.12^{a}
Group 3	10.01 ± 0.15^{b}	10.34 ± 0.17^{b}
Group 4	8.89 ± 0.23^{a}	8.82 ± 0.13^{a}
P value	*	*

Values are Mean ± SE (n=6); One way ANOVA
Means with different superscripts in a column differ significantly at P<0.05 (*)

The results are in agreement with the findings of previous authors [9, 10, 11]. In the present experiment, the decrease in the liver weights might be due to the major pathological changes in the hepatocytes including degeneration, necrosis and leakage of enzymes.

3.3 Absolute weights of kidneys

The mean values of absolute organ weights of kidneys in group 1 (1.29 ± 0.07, 1.33 ± 0.08), group 2 (0.91 ± 0.03, 0.96 ± 0.03), group 3 (1.26 ± 0.06, 1.42 ± 0.07) and group 4 (1 ± 0.01, 1.09 ± 0.05) on 7th and 21st day of experiment were recorded. Significantly (P<0.05) reduced organ weights were recorded in groups 2 and 4 as compared to groups 1 and 3 on 7th and 21st day of experiment. (Table 3)

Table 3: Absolute weights of kidneys (g) in different groups

Group	*Day 7*	*Day 21*
Group 1	1.29 ± 0.07^{b}	1.33 ± 0.08^{b}
Group 2	0.91 ± 0.03^{a}	0.96 ± 0.03^{a}
Group 3	1.26 ± 0.06^{b}	1.42 ± 0.07^{b}
Group 4	1 ± 0.01^{a}	1.09 ± 0.05^{a}
P value	*	*

Values are Mean ± SE (n=6); One way ANOVA

Means with different superscripts in a column differ significantly at P<0.05 (*)

Results are similar to the findings of previous authors [11]. The decline in the weights of kidneys in the present study might be due to atrophy and necrosis of renal parenchyma. This also infers that the capability of Vitamin C alone was not sufficient to counteract the toxic effects of GLP.

4. Conclusion

The GLP (500 mg/kg b.wt)induces toxic changes in testes, liverand kidneys. It shows marked alterations in organ morphometry. Administration of Vitamin C alone hasnot completely ameliorated the toxic changes induced by GLP despite of its high antioxidant properties.

5. Acknowledgement

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