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Ameliorative effect of quercetin on body weights and haematology of glyphosate induced toxicity in albino Wistar rats

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Abstract
The ameliorative potential of Quercetin was studied against Glyphosate induced toxicity. Total 24 male *Wistar* rats were separated into four groups (n = 4). Group 1 (control) and groups 2, 3, 4 were received glyphosate, quercetin, glyphosate and quercetin at the rate of 600, 80, 600 and 80 mg/kg b.wt respectively orally for 14 days. All the animals were sacrificed on 15th day and evaluated for haematological alterations.
In the present study, Weekly body weight gains were significantly ($P< 0.05$) decreased in group 2 rats when compared to group 1. In group 4, there was a significant increase in body weights on 14th day of experiment when compared to group 2 rats. In group 2 Total erythrocytic count, Haemoglobin were significantly ($P< 0.05$) reduced and Packed cell volume, Total leucocytic count were increased which were ameliorated in group 4 rats. In conclusion, quercetin was found to possess moderate protective action against glyphosate induced toxicity.

Keywords: Quercetin, glyphosate, Wistar rats, body weights and hematology

1. Introduction
GLP is one agrochemical that has been the focus of discussions worldwide, for a variety of reasons. Unlike other herbicides, this particular chemical has transcended boundaries of pesticide usage and application. About 77 per cent of farmers and 41 per cent of workers reported use of GLP in weed control for several crops, all of them are non-approved uses for this herbicide in India [1, 2]. Stated that GLP caused an increase in reactive oxygen species (ROS), cytosolic calcium ions levels and changed mitochondrial membrane permeability (MMP), activated caspases - 8, 9, 3 and caused chromatin condensation, which showed that they were capable of inducing apoptosis both *via* extrinsic and particularly intrinsic pathway.
Flavonoids are a class of natural substances present in plants, fruits, vegetables, wine, bulbs, bark, stems, roots and tea. Quercetin (QE) is the major polyphenolic flavonoid found in food products, including berries, apples, cauliflower, tea, cabbage, nuts, and onions that have traditionally been treated as anticancer and antiviral, and used for the treatment of allergic, metabolic, and inflammatory disorders, eye and cardiovascular diseases a nd arthritis [3]. QE is a bioactive compound that is widely used in botanical medicine and traditional Chinese medicine due to its potent antioxidant activity. In recent years, antioxidant activities of QE have been studied extensively, including its effects on glutathione, enzymatic activity, signal transduction pathways, and ROS caused by environmental and toxicological factor [4]. Therefore, we aimed to investigate whether quercetin could offer any protection against glyphosate- induced toxicity using rat as the animal model in the present study.

2. Material and Methods
2.1 Chemicals
GLP was obtained from Seed Research and Technology Centre (SRTC), PJTSAU Hyderabad-30 under the trade name Roundup® (41%) and QE was obtained from Healthvit, West Coast Pharmaceutical Works Ltd., Ahmedabad, India.

2.2 Experimental animals
A total of 24 male albino *Wistar* rats weighing 180 to 200g, bred at Vyas labs (CPCSEA registered No.2085/PO/ RcBiBt/S/19/CPCSEA), Medchal, Malkajgiri were used for this research.

2.3 Experimental design
A total of 24 male albino *Wistar* rats were randomly divided into four (4) groups consisting of six (06) animals in each. The rats were housed in solid bottom polypropylene cages and were maintained in controlled environ- ment with a temperature of 20 to 220C at Animal house facility, College of Veterinary Science, P.V Narsimha Rao Telan- gana Veterinary University, Rajendranagar, Hyderabad throughout the course of experiment. Sterile rice husk was used as standard bedding material.

All the rats were provided with standard balanced pellet diet and deionized drinking water ad libitum any during the entire period of experiment. All the experimental rats were closely observed thrice daily for clinical signs and mortality, if any during the entire period of experiment. The experiment was carried out according to the guidelines and prior approval of Institutional Animal Ethics Committee (IAEC-No. 5/23/C.V. Sc., Hyd)

Table 1: Experimental design with group wise treatment protocol

Groups	No. of rats	Treatments
Group 1	6	Control
Group 2	6	GLP @ 600 mg/kg b.wt, orally
Group 3	6	Quercetin @ 80 mg/kg b.wt, orally
Group 4	6	GLP @ 600 mg/kg b.wt + Quercetin @ 80 mg/kg b.wt, orally

2.4 Blood collection
Six rats from each group were sacrificed on 15th day of experiment. Prior to blood collection, the experimental rats were put to fast for 12 h. Just before sacrifice, 1 mL of blood was collected from retro-orbital plexus with the help of capillary tube (3 mm) in an anticoagulant (K3- EDTA) coated vacationers {13 mm x 75 mm, 4 mL (Rapid Diagnostics Pvt, Ltd., Delhi)} to carry out all haematological parameters.

2.5 Analysis
Individual body weights of all the rats were recorded using electronic balance on 1st day and subsequently on 7th and 14th day of experiment to study the body weight gains. All the blood samples were used for the estimation of TEC, TLC, Hb concentration, PCV, MCH, MCV and MCHC by using automated whole blood analyzer (Huma count, med source Ozone Biomedical Faridabad, Haryana)

2.6 Statistical analysis
Data were subjected to statistical analysis by applying one-way analysis of variance (ANOVA) using the statistical package for social sciences (SPSS). Differences between means were tested using Duncan's multiple comparison tests and significance was set at $P< 0.05$.

3. Results
3.1 Effect of GLP on weekly body weight gain (g)
Significantly ($P< 0.05$) higher mean values were recorded in group 1 and group 3 rats. Significantly ($P< 0.05$) lower mean values of weekly body weights were recorded in group 2 rats on 7th and 14th day of experiment. In group 4 rats, there was a significant ($P< 0.05$) increase in the mean values on day 7th and 14th day of experiment when compared to group 2 rats (Fig.1).

3.2 Effect of GLP on haematological parameters
Haematological studies revealed that in group 2 there were significantly reduced mean values of total erythrocytic count (TEC), haemoglobin (Hb) concentration, packed cell volume (PCV), significantly elevated mean values of total leucocytic count (TLC), insignificant mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) when compared to group 1 rats on 15th day of experiment. Significantly ($P< 0.05$) increased mean values of TEC, Hb, PCV, decreased TLC values, insignificant MCV, MCH and MCHC were recorded in group 4 when compared to group 2 rats on day 15th of the experiment. Statistically, no significant difference were observed between the groups 1 and 3.

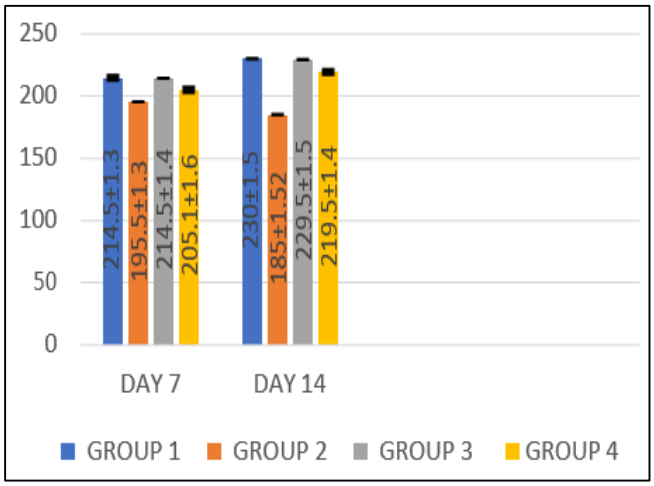

Fig 1: Weekly body weight gain (g) in different group

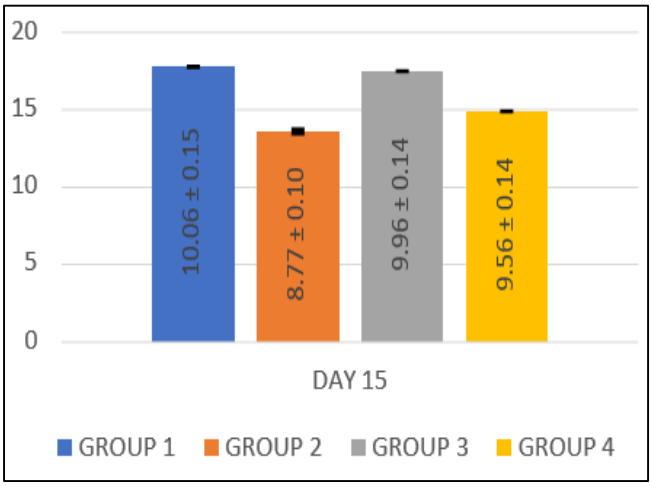

Fig 2: Total Erythrocytes Count: (TEC-millions/µL) in different groups

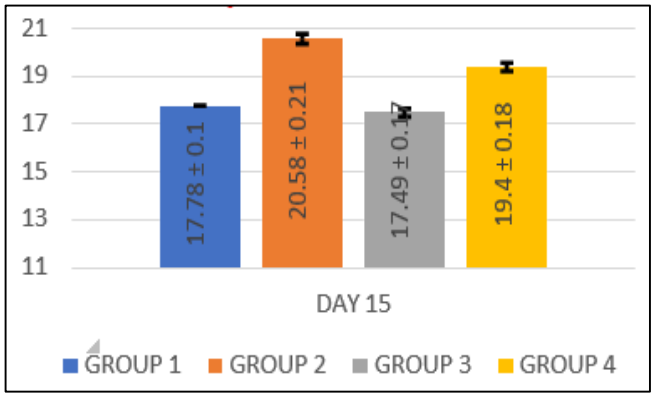

Fig 3: Total Leucocyte Count: (TLC-thousands/µL) in different groups

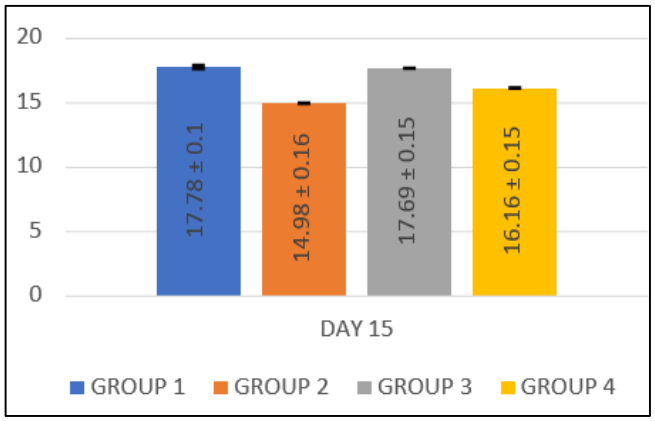

Fig 4: Hemoglobin concentration: (Hb-g %) in different groups

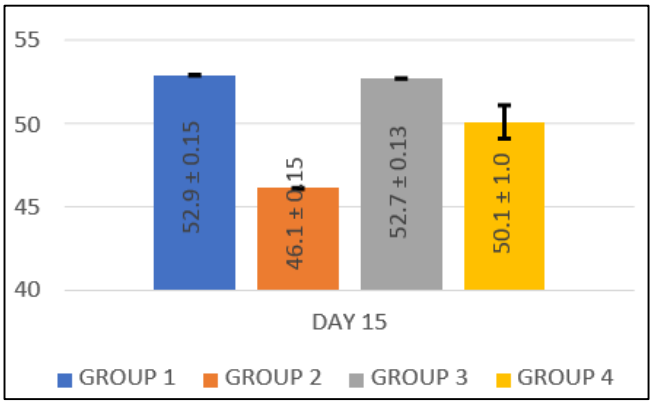

Fig 5: Packed Cell Volume (Hematocrit): (PCV-%)

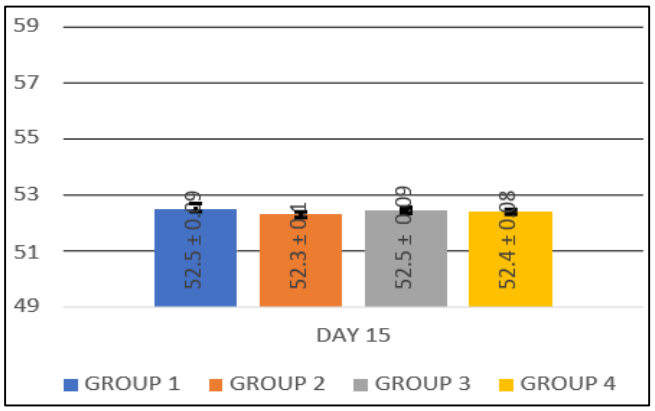

Fig.6: Mean Corpuscular Volume: (MCV-fL) in different groups

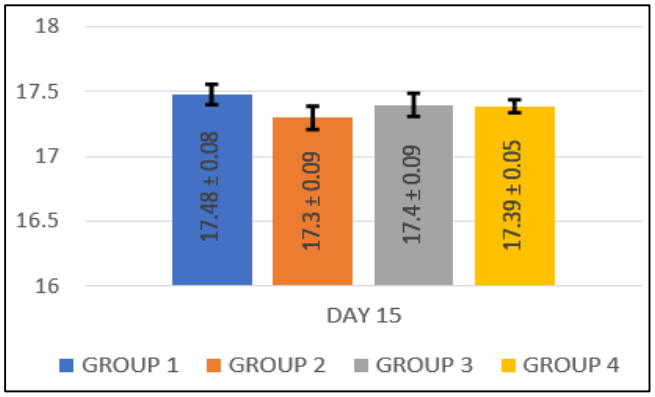

Fig 7: Mean Corpuscular Hemoglobin: (MCH-pg) in different groups

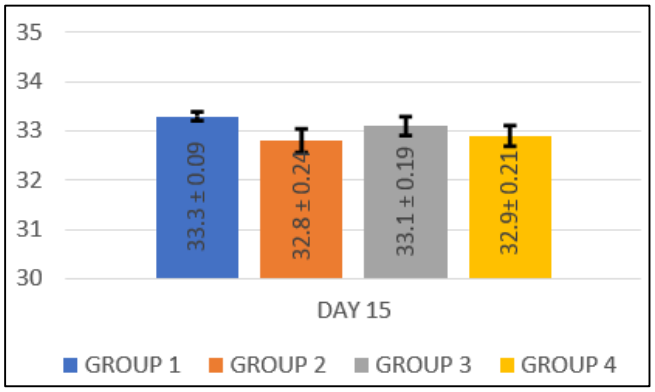

Fig 8: Mean Corpuscular Hemoglobin Concentration: (MCHC-g/dL) in different groups

Table 2: Weekly body weight gain (g) in different groups

Group	Day 7	Day 14
1	214.8 ± 1.3a	230 ± 1.5a
2	195.5 ± 1.4b	185 ± 1.52c
3	214.5 ± 1.4a	229.5 ± 1.5a
4	205.1 ± 1.6c	219.5 ± 1.4b
P value	*	*

Table 3: Effect of GLP and QE on haematological parameters

Group	(TEC- millions/µL)	(TLC- thousands/µL)	(Hb-g %)	(PCV-%)	(MCV-fL)	(MCH-pg)	(MCHC- g/dL)
1	10.06 ± 0.15a	17.78 ± 0.1c	17.78 ± 0.1a	52.9 ± 0.15a	52.5 ± 0.09	17.48 ± 0.08	33.3 ± 0.09
2	8.77 ± 0.10c	20.58 ± 0.21a	14.98 ± 0.16c	46.1 ± 0.15c	52.3 ± 0.1	17.3 ± 0.09	32.8 ± 0.24
3	9.96 ± 0.14a	17.49 ± 0.17c	17.69 ± 0.15a	52.7 ± 0.13a	52.45 ± 0.09	17.4 ± 0.09	33.1 ± 0.19
4	9.56 ± 0.14b	19.4 ± 0.18b	16.16 ± 0.15b	50.1 ± 1.0b	52.4 ± 0.08	17.39 ± 0.05	32.9 ± 0.21
P value	*	*	*	*	NS	NS	NS

Values are Mean + SE (n =6); One way ANOVA with Duncan's post hoc test (SPSS).
Means with different alphabets as superscripts differ significantly (P< 0.05) among the groups (Vertically). NS=non-significant.

4. Discussion

A significant ($P< 0.05$) reduction in the mean values of body weights were recorded in group 2 rats on 7th and 14th day of experiment. The weight loss could be due to reduced feed and water intake [5, 6], on the account of toxic action of GLP on GIT which alters the gut microbiota and also might be due to free radical induced oxidative damage at sub cellular level in different vital organs. This observation is in accordance with the earlier studies of [7, 8, 9, 10, 11, 12]. Group 4 rats showed a significant increase in the mean values of body weights when compared to that of group 2 which could be due to the protective effect of QE by scavenging of free radicals generated due to GLP induced toxicity.

Haematology is the study of the numbers and morphology of the cellular elements of the blood - the red cells (er- ythrocytes), white cells (leucocytes), and the platelets (thrombocytes) and the use of these results in the diagnosis and monitoring of disease [13]. The blood reflects the pathological status of the exposed laboratory animals to toxicants and other conditions. Blood parameters change in relation to the physiological status of an animal. Haematological exami- nation provides the opportunity to clinically investigate the presence of metabolites, other constituents in the body of ani- mals and it plays a vital role in the physiological, nutritional and pathological status of an animal.

Significantly ($P< 0.05$) decreased mean values of various haematological parameters *viz*., TEC, Hb concentration and PCV were observed in the present study among group 2 rats when compared with group 1 rats on 15th day of experiment. These changes might be due to imbalance in antioxidant and prooxidant defence, which might have been due to the ability of the herbicide to cause extravascular haemolysis or it might be due to its ability to cause oxidative stress [14]. The erythrocyte membrane was reported to be highly vulnerable to oxidative stress condition because it contains high amounts of lipid, iron and is bathed in serum that has low antioxidant properties [15]. The sub cellular changes observed in kidneys might be due to the interference of the GLP in erythropoietin production and also indirectly affecting erythropoiesis and leucopoiesis of bone marrow. The increase in TLC in the present study may be the result of the activation of the immune system in the presence of toxic contaminants might have resulted in an adaptive response of the rat in a more effective immune defence [16, 17, 18].

A significant increase in TEC, Hb concentration, PCV values and a decrease in TLC in group 4 rats were observed when compared to group 2 rats. This change might be due to the QE antioxidant defence action against free radical induced oxidative stress in different tissues including blood cells. Membrane lipids are vital for the maintenance of cellular integrity and survival. Peroxidation of membrane lipids can result in the inactivation of enzymes and cross-linking of membrane lipids and proteins and in cell death.

5. Conclusion

In conclusion, glyphosate caused significant reduction in body weights, TEC, TLC, and PCV by the formation of free radicals and increased TLC due to immune response. However, quercetin supplementation to GLP treated rats exhibited comparatively reduced adverse effects indicating its protective antioxidant, anti-inflammatory property. Thus, present investigation confirmed the moderate protective role of quercetin against glyphosate toxicity.

6. Acknowledgment

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