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Hematological alterations induced by glyphosate and ameliorative effect of ascorbic acid in *wistar* rats

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ABSTRACT

This experimental research was executed to record the adverse toxic effects of glyphosate (GLP) on blood of male albino *Wistar* rats and its mitigation with administration of Ascorbic Acid (AA). We used a total of 40 adult male rats of similar body mass and age. All the rats were blindly divided and placed in 4 different groups and each experimental group contained12 rats. The rats in group 2 received glyphosate @ 500 mg/kg body weight, rats kept in group 3 received only ascorbic acid @ 250 mg/kg body weight and the rats of group 4 were exposed to glyphosate (500 mg/kg b.wt) and ascorbic acid (250mg/kg b.wt) in combination orally for period of 21 days. All the treatments were given to rats daily by oral route. A total of 6 rats were arbitrarily picked from each group on day 7 and 21 for blood collection. The blood samples were then used for determination of total red blood cells, total white blood cells, hemoglobin (Hb) quantity, hematocrit values and different other blood parameters. The results on blood profile of treated rats exhibited a significant ($P<0.05$) reduction in the mean values of erythrocyte count, white blood cell count, Hb, haematocrit, MCHC, MCV and MCH in comparison to unexposed rats. Results exhibited that the administration of ascorbic acid (Vitamin C) lowered the adverse toxic ailments of glyphosate on blood of treated rats. In conclusion, the results of this experimental trial suggested that vitamin C partially mitigate the toxic effects induced by glyphosate in rats.

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Introduction

Glyphosate is a well-known broad-spectrum weedicide and is extensively and persistently employed in agriculture and different food crops to eliminate/kill the undesirable weeds (Temple and Smith 1992). Pesticides, insecticides, herbicides and fungicides after application can easily enter to nearby surface water by runoff and cause detrimental effects on freshwater species (Ronco et al. 2016; Vandenberg et al. 2017). The production of crops and animals since 1961 has been increased fivefold due to application of pesticides and fertilizers (Godfray et al. 2010; Fumetti and Blaurock 2018). Glyphosate herbicide is the world's largest selling broad-spectrum agrochemical (Baylis 2000). In agriculture sector, there are various different glyphosate-resistant crops which facilitate frequent application of glyphosate (Persch et al. 2018). Extensive application of glyphosate in different areas has increased its load in environment, surface water and ground waters (Benbrook 2016). Glyphosate under a trade name (Roundup®) was brought in the market for agricultural purposes in 1974 by Monsanto. Earlier reports have shown that different environmental contaminants like pesticides cause alterations in water ponds nearby cultivation areas via surface runoff, spray drift, chemical-biological degradation and rainwater (Rebelo and Caldas 2014). Different studies have detected the residues of glyphosate in various food stuffs including contaminated drinking water through rain and surface runoffs entering into groundwater (Bohn et al. 2014; EFSA 2014). The extensive use of glyphosate has increased its residues in various foods and food products and in water (Bøhn et al. 2014). Moreover, different studies in human have indicated

Table 1: Hematological parameters of rats exposed to glyphosate and ascorbic acid at different intervals of trial

GROUP	GROUP-1		GROUP-2		GROUP-3		GROUP-4	
TIME INTERVAL	Day 7	Day 21	Day 7	Day 21	Day 7	Day 21	Day 7	Day 21
Erythrocyte count	10.21±0.08[c]	10.73±0.24[c]	8.86±0.1[a]	7.61±0.33[a]	10.2±0.07[c]	10.4±0.18[c]	9.55±0.13[b]	9.31±0.07[b]
Leukocyte count	18.28±0.12[c]	18.53±0.11[c]	13.95±0.19[a]	11.76±0.38[a]	17.98±0.19[c]	18.23±0.23[c]	15.4±0.15[b]	14.33±0.19[b]
Hemoglobin quantity	17.55±0.18[b]	17.76±0.32[c]	14.98±0.21[a]	13.25±0.5[a]	17.48±0.2[b]	17.23±0.24[c]	15.71±0.39[a]	15.55±0.27[b]
Pack cell volume	53.68±0.87[c]	55.35±1.1[c]	46.75±0.57[a]	42.35±2.51[a]	53.46±0.87[c]	53.95±0.97[c]	49.95±1.17[b]	48.78±0.82[b]
MCV (fL)	52.31±0.41	51.43±0.39[a]	52.38±0.65	55.11±1.09[b]	52.18±0.43	51.76±0.78[a]	51.96±0.76	52.01±0.68[a]
MCH (pg)	17.11±0.13	16.53±0.17[a]	16.8±0.26	17.3±0.11[b]	17.08±0.14	16.56±0.26[a]	16.35±0.27	16.6±0.25[a]
MCHC (g/dL)	32.71±0.39[b]	32.1±0.36	31.46±0.1[a]	31.46±0.69	32.71±0.38[b]	31.96±0.7	32.06±0.23[ab]	31.88±0.14

Values (Mean ± SE) containing different superscripts in each column significantly differ days 7 and 21 of trial at $P<0.05$

metabolites of glyphosate in urine, blood, umbilical cord blood and in maternal blood (Conrad et al. 2017; Kongtip et al. 2017). Some studies have indicated that glyphosate inhibits an important and major enzyme (5-enolpyruvylshikimate-3- phosphate synthase) in plants (Rueppel et al. 1977). Therefore, due to absence of this enzyme in animals, it does not cause adverse toxic impacts in non-target organisms (Williams et al. 2000). The exposure to glyphosate causes clinical ailments like hepatotoxicity, gastrointestinal problems, multi-organ toxicity and nephrotoxicity along with reproductive and cardiovascular disorders (Lee et al. 2009; Roberts et al. 2010; Sribanditmongkol et al. 2012; Dos Santos et al. 2017; Namratha et al. 2020). Research in animal studies suggested that glyphosate can increase the quantity of Adenosine Triphosphatase (ATPase) resulting in induction of increased process of oxidative phosphorylation in mitochondrial (Bababunmi et al. 1979; Olorunsogo et al. 1979; Olorunsogo et al. 1982). It has been also recorded that glyphosate induces remarkable changes in blood and serum profiles of exposed organisms (Jasper et al. 2012). Glyphosate is classified and known as organophosphorus chemicals which have been reported to cause neurotoxic disorders/impacts (Torretta et al. 2018). However, different international agencies have classified glyphosate as non-neurotoxic chemical even at high concentrations (EFSA 2014), which is controversial to many reports (Roy et al. 2016; Martinez et al. 2018).

Different studies have reported that Vitamin C (ascorbic acid) acts as an electron donor during several metabolic reactions and has potential scavenging activity. Moreover, Vitamin C also causes protective effects and prevents abnormal apoptotic cell death in different tissues via interferences with reactive oxygen species (ROS) and reducing oxidative stress (Ikpeme et al. 2012).

Materials and Methods

Experimental animals

The experimental research was planned and executed according to the procedures, guidelines and welfare regarding the use of laboratory animals. The study was approved by the Institutional Animal Ethics Committee (IAEC-No.01-2019). This study included a total of 48 mature (adult) male albino *Wistar* rats having approximately body mass (200-240 g). All the experimental rats were purchased from Jeeva Life Sciences (ISO 9001:2015 certified company), Hyderabad, India.

All the experimental animals were placed under normal laboratory conditions (20-22°C) in solid bottom polypropylene cages (RUSKA Labs) at Department of Veterinary Pathology. The experimental trial was executed at RUSKA Labs, Department of Veterinary Pathology, College of Veterinary Science, Rajendranagar, Telangana, India during 2019. The rats were provided controlled laboratory conditions throughout the trial. During the research and acclimatization period, all the animals were offered standard pellet diet and sterile rice husk as bedding material. The pellet diet and clean fresh water was provided to rats *ad libitum* twice a day early morning and evening throughout the trial.

Chemical source

Glyphosate (Roundup® 41%) was procured from Seed Research and Technology Centre (SRTC, Hyderabad-30), while ascorbic acid (Vitamin C) was procured from S.D. Fine-Chem Ltd., Mumbai, India.

Experimental design

Prior to start of trial, all the experimental rats (48) were blindly placed in cages for a duration of two weeks for acclimatization purpose. After that all the rats were indiscriminately picked and divided into 4 equal groups as follow; Group 1 (untreated control group), Group 2 exposed to glyphosate (500 mg/kg b.wt), Group 3 exposed to vitamin c (250 mg/kg b.wt) and Group 4 exposed to glyphosate (500 mg/kg b.wt) and ascorbic acid (250mg/kg b.wt) in combination for a period of 21 days.

All the experimental treatments were administered daily via oral route to each rats. All the rats were carefully observed for any clinical and behavioral disorders till the completion of trial.

Hematological evaluation

For hematological changes, a total of 6 rats were blindly picked at days 7 and 21 of trial from each experimental group. About1-1.5 mL of blood obtained from retro-orbital plexus of each animal in EDTA coated glass test tubes. All the rats were kept without diet for 12 h prior to collection of blood. All the blood samples collected at days 7 and 21 of trial were used for the estimation of different hematological profile including total and differential white blood cell count red blood cell count, hemoglobin quantity, hematocrit value, Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Hemoglobin (MCH) and Mean Corpuscular Volume (MCV) using an automated hematological analyzer (Huma count, med source ozone biomedical Faridabad, Haryana).

Statistical analysis

Data of our trial was normally distributed and were investigated by using suitable statistical analysis. for any significant difference using statistical software version 16.0. All the mean+SD were compared by using Duncan's multiple test with $P<0.05$ significance level (Snedecor and Cochran 1994).

Results and Discussion

A significant ($P<0.05$) decrease in the mean values of TEC, TLC, Hb, PCV and MCHC on 7^{th} and 21^{st} day except MCHC which was insignificant on 21^{st} day only and increased mean values of MCV and MCH on 21^{st} day were detected in GLP treated rats when compared to control group rats which might be due to hypoplasia of bone marrow. The production of Reactive Oxygen Species (ROS) induced by Roundup® could be the root cause for all hematological alterations. In the present study, significantly lower mean values were recorded in TEC, PCV and Hb concentration in group 2 rats, which could be responsible for mild anemia. The GLP might have interfered in erythropoietin production and also indirectly affecting erythropoiesis and leucopoiesis of bone marrow. The anemia could be due to the ability of GLP to cause extravascular hemolysis or it might be due to its ability to cause oxidative stress to erythrocytes (Modesto and Martinez 2010). Similar causes for anemia ware also opined by previous authors (Jasper et al. 2012; Tizhe et al. 2013). The decrease in TLC in the present study may be attributed to the toxic effects of GLP on leucopoiesis. In the present observation, reduction in TLC could be due to GLP induced oxidative stress on liver, kidneys and bone marrow. The reduction in hematological parameters like red blood cell, hemoglobin concentrations, lymphocyte, and monocyte cell counts could be due to toxic effects of glyphosate on hematopoietic tissues. The lower values of red blood cells could also be due to erythrocyte destruction in blood-forming tissues, abnormal heme synthesis, increase the production of free radicals, poor delivery of oxygen by gills and dysfunction osmoregulatory tissues (El-Murr et al. 2015). Similar hematological results in different other species of fish exposed to pure and different formulations of glyphosate have also been recorded (de Moura et al. 2017; Kondera et al. 2018). In contrast to hematological results of the present study, reduced white blood cells in fish exposed to glyphosate have been reported (Kreutz et al. 2011; Gholami-Seyedkolaei et al. 2013). The increased increments in the number of white blood cells and neutrophil populations in the present study are suggestive of tissue damages due to increasing the stress-induced inflammatory response of fish. Moreover, increased the number of eosinophilic, monocytes, and basophilic lineage cells due to glyphosate has also been reported in fish (Kondera et al. 2018).

In group 4 (GLP + AA) rats, a significant improvement in all the above parameters were observed in comparison to group 2 (GLP) rats. These changes could be due to the protective action and antioxidant property of Vitamin C against free radical induced oxidative stress in different tissues including blood cells.

Conclusion

A significant difference in the hematological parameters in the present experiment indicate eventual changes in hemopoietic system due to direct and indirect toxic effects of GLP as a result of free radical injury. The results exhibited that ascorbic acid partially reduces the toxic effects induced by glyphosate on hematopoietic tissues of rats.

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