Research Article

Effect of glyphosate on hematological and biochemical parameters of Rabbit (*Oryctolagus cuniculus*)

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Abstract

To improve agriculture, herbicides are extensively used all over the world. In this study toxicity of glyphosate was assessed on rabbits. For this reason, 36 female rabbits were divided into four groups including treated and control having 9 rabbits in each. Three concentrations of glyphosate were selected as BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) for treated groups and no any treatment for the control group C. For checking the herbicide effects hematological parameters including white blood cells (WBC), red blood cells (RBC), hemoglobin, hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelets and biochemical parameters including bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase, urea and creatinine were selected. The rabbits were exposed to glyphosate and effect of glyphosate was studied after 15,30 and 45 days of exposure. This study indicated that glyphosate showed remarkable toxicity for hematological and biochemical parameters under investigation and glyphosate toxicity was directly related to the increasing concentration and time of exposure.

Keywords: Biochemical parameters; Glyphosate; Hematological parameters; Herbicides or pesticides; Rabbit; Toxicity

Introduction

Glyphosate is a non-selective herbicide which is used to eradicate weeds efficiently without ploughing. In Kuwait its use is about 0.98 kg ai ha⁻¹/year. It inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase

which is involved in the shikimic acid (aromatic amino acid) metabolic pathway [1-4] and declared as less toxic as compared to some other herbicides [5].

Glyphosate is considered as environmentally friendly because their residues are easily

degradable and highly adsorbed into soil which cause less leaching, but it remains on crops for longer time. Although, lot of evidences about its toxic effects on non-targeted organisms were observed. Many studies have exposed its negative effect on the vertebrates and on invertebrates. But it is the widely and utmost used herbicide in the world [2, 6-8].

Now a day's all over the world 90% genetically modified glyphosate resistance crops are grown which has dramatically enhanced the use of glyphosate herbicide on crops as a result there is a vast indication of its residues in food material [9].

In milk its tolerated residue level is about 0.05 mg/kg reported from Europe. Glyphosate is considered as one of the primary main pollutant of aquatic system [10, 11]. As previously observed that even its very low amount of agriculture uses cause endocrine disruption in addition toxicity to kidney, liver cell line and human placenta [12-16].

Significant amount of glyphosate residues detected in urine of rabbits, human and cattle by Gas Chromatography-Mass Spectroscopy and ELISA. Fattening rabbits have higher number of residues. It is also observed in different organs including liver, lung, kidney, intestine, spleen and muscles of animals. It inhibits the cytochrome enzyme Cyp450, which is in charge for the formation of steroid hormones similarly it also affects some other P450 family enzymes, which is critical for xenobiotic metabolism. Several gut bacterial populations also get troubled by glyphosate [9]. It also inhibits the growth of food microorganisms and micro biodiversity of soil [11].

In June (1991) Environmental Protection Agency (EPA) declared that glyphosate was not carcinogenic [17]. But recently, in March (2015) World Health Organization (WHO) declared that glyphosate is carcinogenic and should be banned. Glyphosate have adverse

effect on biological systems proved by previous studies such as DNA damage, glycine homeostasis disturbance, inhibition of enzyme succinate dehydrogenase, manganese chelation, variation in glyoxylate and N-nitrosoglyphosate. There are lot of evidences about glyphosate use on crops and resulting into multiple types of cancers including liver cancer, kidney cancer, bladder cancer, thyroid cancer, breast cancer, pancreatic cancer and leukemia etc. [18].

Higher degree of DNA damaged was observed in individuals exposed to glyphosate and show genotoxicity [19]. At molecular level study [20] indicate that glyphosate-based herbicides cause change in transcript cluster resulting into damage in kidney tissues and regeneration of liver followed by this change cause damage in the liver tissues. Oxidative and cellular stress is also caused by transcriptional changes due to toxic effect of glyphosate [21]. The present study was done to investigate the effect of glyphosate on animals at cellular level and to check the toxicity of this important herbicide.

Materials and methods

Animals

For this study colony of rabbits was established and 36 female rabbits were randomly chosen and divided into 4 groups having 9 animals in each including three treated groups BC1, BC2, BC3 and group C as control. Throughout study animals were kept in stain less steel cages, with temperature ($21 \pm 2^\circ\text{C}$), humidity 40-70% [22] and cage sanitation was daily. The animals were fed with *Trifolium resupinatum* L. and tap water was for drinking.

Herbicide

For treatment herbicide glyphosate (N-(phosphonomethyl) glycine) was purchased from market. Glyphosate was 48% formulation having 48% glyphosate IPA (isopropylammonium) salt contents (Glyphosate contents was 36.30%). Oral

LD₅₀ of glyphosate for rabbits is 5000 or 4000-6000mg/kg/bw [23].

Three concentrations 50mg/kg/bw, 100mg/kg/bw and 150mg/kg/bw from 36.30%w/v formulation of glyphosate for BC1, BC2 and BC3 groups were selected, respectively. All doses of herbicide were measured and mixed with few drops of water for dilution and given by needleless syringe 10cc into each animal's mouth of treated groups for 45 days according to their body weights except group C which was kept as control.

Blood sample collection

Blood collection site was Jugular vein. Animals was warmed up at neck region by pouring warm water and then held at the site edge of table forelimb extending downward and head upward, needle was inserted, and blood was taken from each group at day 15th, 30th and 45th.

Hematological tests

For CBC tests, 3ml blood was stored in CBC vials from each animal. Automated hematological analyzer made in Sweden (Medonic-M-series M32M) was used with hematological analysis by using commercial kits purchased from market (Merck Germany) [24]. Hematological analysis was done by coulter method [25].

Biochemical tests

For biochemical tests two gel tubes were used each time for individual animal having 2.5ml blood, respectively. For biochemical tests, blood samples were centrifuged at 3000rpm in centrifuge machine for 10 minutes [26] and then stored at 2-8 °C until analysis. Chemistry analyzer (Microlab 400 S.N 16-60018) was used for analysis by using diagnostic commercial kits purchased from market (Merck Germany) [24]. Bilirubin, AST, ALT, ALP levels were measured by method of [26, 27] urea level was measured by method [28] and creatinine level by Jeffe method [29].

Statistical analysis

Statistical analysis was done by using software (IBM SPSS V21.0.) One-way ANOVA was used followed by *post hoc* test Least Significant Difference test (LSD), whose results were mentioned by different letters at significant level ($p \leq 0.05$)

Results

Hematological parameters

For WBC at day 15th BC1 and BC2 (8.57 ± 0.00 , 9.20 ± 0.05) have no significant difference with control C (8.50 ± 0.57) but BC3 (10.40 ± 0.05) significantly increased WBCs with respect to control. At 30th and 45th day BC1 (9.78 ± 0.00 and 9.40 ± 0.11) have no difference with control C (9.40 ± 0.05 , 7.40 ± 0.11), respectively. BC2 and BC3 (10.50 ± 0.57 , 10.90 ± 0.05) and (11.03 ± 0.57 , 12.00 ± 1.15) have significant difference with control C. Overall WBC count increased with increasing concentration (Figure 1).

The RBC count at day 15th there was no significant difference of BC1, BC2, BC3 (6.40 ± 0.11 , 6.37 ± 0.01 , 6.26 ± 0.00) with control C (6.70 ± 0.57). At day 30th and 45th (5.81 ± 0.00 , 5.73 ± 0.01 , 5.60 ± 0.05) and (5.23 ± 0.00 , 5.01 ± 0.00 , 4.98 ± 0.00) have significant difference with control C (6.82 ± 0.05 , 6.60 ± 0.05), respectively. RBCs number significantly decreased with increasing concentration (Figure 2).

The hemoglobin at 15th day BC1 have no significant difference with control C (12.10 ± 0.05) but BC2, BC3 (11.80 ± 0.05 , 11.40 ± 0.05) significantly decreased with respect to control. At day 30th there was no significant difference of BC1, BC2 with control but BC3 (11.00 ± 1.15) significantly decreased hemoglobin level compared to control C (13.60 ± 0.11). At day 45th all BC1, BC2, BC3 (12.30 ± 0.05 , 11.70 ± 0.05 , 10.50 ± 0.05) significantly decreased in hemoglobin level with respect to control C (12.90 ± 0.05). Overall hemoglobin level decreased with increasing concentration (Figure 3).

The HCT level at day 15th BC1 have no significant difference with control but BC2, BC3 (39.10 ± 0.05 , 39.00 ± 0.57) had significant difference with control C (41.70 ± 0.05). At day 30th, 45th all BC1, BC2, BC3 (39.00 ± 1.15 , 38.30 ± 0.05 , 38.00 ± 0.57) and (38.80 ± 0.05 , 37.60 ± 0.57 , 36.70 ± 0.05) have significant decreased with respect to control C (43.60 ± 0.00 , 42.60 ± 0.05), respectively. HCT level decreased with increasing concentration (Figure 4).

The MCV level at day 15th BC1, BC2, BC3 (61.70 ± 0.11 , 61.40 ± 0.05 , 60.10 ± 0.05) all have significant difference with control C (62.20 ± 0.05). At day 30th and 45th (61.50 ± 0.05 , 61.30 ± 0.05 , 61.10 ± 0.57) and (62.30 ± 0.00 , 61.60 ± 0.05 , 61.30 ± 0.57) all have significant difference with control C (63.90 ± 0.57 , 64.50 ± 0.05), respectively (Figure 5).

The MCH level at day 15th and 30th day of BC1, BC2, BC3 (18.70 ± 0.05 , 18.50 ± 0.05 , 18.10 ± 0.57) and (19.70 ± 0.05 , 19.20 ± 0.05 , 19.10 ± 0.05) significantly decreased in MCH level compared to control C (19.80 ± 0.05 , 9.90 ± 0.05), respectively. At day 45th BC1, BC2 (20.10 ± 0.57 , 19.80 ± 0.05) have no significant difference with control C (20.50 ± 0.05) but BC3 (18.70 ± 0.11) have significant difference with control C. Overall decreased in MCH level was observed (Figure 6).

The MCHC level at day 15th BC1 (29.60 ± 0.05) had no significant difference with control C (30.00 ± 0.05) but BC2, BC3 (28.50 ± 0.05 , 28.00 ± 0.57) had significant difference by decreasing MCHC level with respect to control C. At day 30th and 45th BC1, BC2, BC3 (30.60 ± 0.05 , 29.80 ± 0.05 , 28.40 ± 0.05) and (30.30 ± 0.05 , 29.20 ± 0.05 , 27.03 ± 0.00) had significant difference with control C (31.10 ± 0.05 , 31.50 ± 0.05), respectively (Figure 7).

The number of platelets at 15th and 30th day BC1, BC2, BC3 (347.00 ± 1.15 , 395.00 ± 0.57 , 425.00 ± 1.15) and (352.00 ± 1.15 ,

406.00 ± 0.57 , 436.00 ± 0.57) all have significant difference with control C (341.00 ± 1.15 , 339.00 ± 0.57), respectively. At 45th day BC1 (343.00 ± 0.57) have no any significant difference with control C (340.00 ± 1.73) and BC2, BC3 (365.00 ± 0.57 , 453.00 ± 0.57) have significantly increased number of platelets compared to control C (Figure 8).

Biochemical parameters

The Bilirubin level of rabbits for glyphosate (B) at day 15th BC1, BC2, BC3 (0.90 ± 0.05 , 0.90 ± 0.05 , 0.90 ± 0.05), respectively have no significant difference with each other and with control C (0.80 ± 0.05). At day 30th BC1, BC2 (0.90 ± 0.05 , 0.90 ± 0.05), respectively had no significant difference with control C (0.80 ± 0.05) but BC3 (1.00 ± 0.05) had significantly increased in bilirubin level compared to control C. At 45th day BC2 and BC3 (1.00 ± 0.05 and 1.00 ± 0.05) had significantly increased in bilirubin level compared to control C (0.80 ± 0.05) BC1 (0.90 ± 0.00) had no significance difference with control C. Overall increase in level was observed as compared to control C (Figure 9).

The ALT level difference was observed of three concentrations of treatment B glyphosate at 15th, 30th and 45th day. At 15th day BC1, BC2 and BC3 (167.00 ± 0.57 , 178.00 ± 0.57 and 196.00 ± 0.57), respectively significantly increased ALT level with respect to control C (156.00 ± 1.15) and have difference with each other. At day 30th BC1 (179.60 ± 0.11) had no significant difference with control C (179.00 ± 1.73) but had significant difference with treated groups. BC2 and BC3 (203.00 ± 0.57 and 246.70 ± 0.05) had significant difference with each other and with control C by increasing ALT level. At day 45th BC1, BC2 and BC3 (212.00 ± 1.15 , 298.00 ± 0.57 and 313.60 ± 0.57), respectively had significant difference with each other and with control C (165.00 ± 1.15). Significant increase in ALT

level was observed with respect to control and with increasing concentration of herbicide (Figure 10).

The AST level for glyphosate (B) at 15th day for BC1 (149.70±0.05) had no significant difference with control C (148.00±1.15) but have significant difference with treated groups. But BC2, BC3 (157.90±0.05, 199.00±0.57) shows significant difference with control and with each other. At day 30 and 45 the BC1, BC2, BC3 (193.00±0.57, 207.70±0.05, 278.00±0.57) and (192.30±0.57, 335.00±1.15, 366.30±0.05) had significant differences with control C (184.00±0.57 and 167.00±0.57), respectively and with each other (Figure 11).

The Alkaline phosphatase (ALP) level for glyphosate (B) at 15th and 30th day BC1, BC2, BC3 (256.00±0.57, 277.00±0.57, 301.00±1.15) and (259.00±0.57, 279.00±0.57, 308.50±0.05) all had significant difference with control C (234.00±1.15 and 227.00±0.57), respectively and with each other. At 45th day BC1 (213.40±0.11) had no significant difference with control C (211.00±1.15) but BC2 and

BC3 (280.70±0.05, 312.00±1.15) have significant difference with each other and with control. It increased with increasing concentration (Figure 12).

The Urea level for glyphosate (B) in all 15th, 30th and 45th day for BC1, BC2, BC3 (47.00±1.73, 56.00±1.15, 67.00±1.15), (43.00±0.57, 66.00±2.30, 69.00±1.15) and (46.70±0.05, 67.00±0.57, 69.80±0.05) had significant difference compared to control C (37.00±1.15, 36.00±1.15 and 35.00±0.57), respectively. With the increase in concentration of glyphosate there was an increase in urea level (Figure 13).

The creatinine level at 15th day for BC1, BC2, BC3 (1.20±0.05, 1.20±0.05, 1.30±0.05) all had significant results and creatinine level increased with respect to control C (1.00±0.05) was detected. At 30th and 45th day BC2, BC3 (1.50±0.05, 1.60±0.05) and (1.60±0.05, 1.70±0.00) have significantly increased creatinine level compared to control C (1.00±0.05, 1.10±0.11), respectively but BC1 had no difference with control. Creatinine level increased with increasing concentration of glyphosate (Figure 14).

Figure 1. Number of white blood cells (WBCs) of rabbits in C (control) and different concentration groups BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) of treatment B (Glyphosate) at 15th, 30th and 45th day. Bars with different letters are significantly different ($p \leq 0.05$)

Figure 2. Total red blood cells (RBCs) of rabbits in C (control) and different concentration groups BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) of treatment B (Glyphosate) at 15th, 30th and 45th day. Bars with different letters are significantly different ($p \leq 0.05$)

Figure 3. Hemoglobin level of rabbits in C (control) and different concentration groups BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) of treatment B (Glyphosate) at 15th, 30th and 45th day. Bars with different letters are significantly different ($p \leq 0.05$)

Figure 4. Hematocrits (HCT) of rabbits in C (control) and different concentration groups BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) of treatment B (Glyphosate) at 15th, 30th and 45th day. Bars with different letters are significantly different ($p \leq 0.05$)

Figure 5. Mean corpuscular volume (MCV) of rabbits in C(control) and different concentration groups BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) of treatment B (Glyphosate) at 15th, 30th and 45th day. Bars with different letters are significantly different ($p \leq 0.05$)

Figure 6. Mean Corpuscular Hemoglobin (MCH) of rabbits in C(control) and different concentration groups BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) of treatment B (Glyphosate) at 15th, 30th and 45th day. Bars with different letters are significantly different ($p \leq 0.05$)

Figure 7. Mean Corpuscular Hemoglobin Concentration (MCHC) of rabbits in C(control) and different concentration groups BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) of treatment B (Glyphosate) at 15th, 30th and 45th day. Bars with different letters are significantly different ($p \leq 0.05$)

Figure 8. No of platelets of rabbits in C (control) and different concentration groups BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) of treatment B (Glyphosate) at 15th, 30th and 45th day. Bars with different letters are significantly different ($p \leq 0.05$)

Figure 9. Bilirubin level of rabbits in C (control) and different concentration groups BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) of treatment B (Glyphosate) at 15th, 30th and 45th day. Bars with different letters are significantly different ($p \leq 0.05$)

Figure 10. Alanine aminotransferase (ALT) level of rabbits in C (control) and different concentration groups BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) of treatment B (Glyphosate) at 15th, 30th and 45th day. Bars with different letters are significantly different ($p \leq 0.05$)

Figure 11. Aspartate aminotransferase (AST) level of rabbits in C (control) and different concentration groups BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) of treatment B (Glyphosate) at 15th, 30th and 45th day. Bars with different letters are significantly different ($p \leq 0.05$)

Figure 12. Alkaline phosphatase level of rabbits in C (control) and different concentration groups BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) of treatment B (Glyphosate) at 15th, 30th and 45th day. Bars with different letters are significantly different ($p \leq 0.05$)

Figure 13. Urea level of rabbits in C (control) and different concentration groups BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) of treatment B (Glyphosate) at 15th, 30th and 45th day. Bars with different letters are significantly different ($p \leq 0.05$)

Figure 14. Creatinine level of rabbits in C (control) and different concentration groups BC1 (50mg/kg/bw), BC2 (100mg/kg/bw) and BC3 (150mg/kg/bw) of treatment B (Glyphosate) at 15th, 30th and 45th day. Bars with different letters are significantly different ($p \leq 0.05$)

Discussion

For monitoring and diagnosing of any alteration in animal's body or blood damage investigation due to any toxicity or disease usually hematological parameters are considered. It refers to the study of numbers and morphology of the blood cells including red blood cells, white blood cells and platelets etc. [30-32]. These parameters are very important for diagnosing the physical condition of animals [33]. These parameters reflect the true picture of blood and blood forming organs [34, 35]. Blood is a good indicator of pathological condition of an animal that are exposed to any toxic compound [36].

In present study red blood cells, hematocrits, hemoglobin, mean corpuscle volume, mean corpuscle hemoglobin, mean corpuscle hemoglobin concentration were significantly decreased for all concentrations as compared to control and this decrease was directly related to the increasing concentrations of herbicide and time of exposure. White blood cells and platelets were increased as compared to control. It was due to oxidative

stress [37]. By exposure to any chemical there is erythroblast and resulting into decrease in HCT level. In anemic condition or hypoxia, decrease in hemoglobin level was observed in a study done by [38]. Immature neutrophils rise in blood was also experimented in rats. It was due to poisonousness strain in non-specific tissues follow-on production of free radicals and prostaglandins resulting inflammatory reaction by production of neutrophilia and lymphopenia [11]. According to a study [39] glyphosate and their metabolites cause hemolysis and hemoglobin oxidation and changing in erythrocytes increase with increasing concentrations. This change in erythrocytes was only due to the poisoning of chemicals. And cause genotoxicity in erythrocytes and gills cell in the fish [40]. According to a study [41] alanine aminotransferase (ALT or SGPT) and aspartate aminotransferase (AST or SGOT), which metabolize the amino acids vary from normal level and demonstrated as good pointers of hepatic toxicity.

In current study, significantly elevation in bilirubin, alanine aminotransferase, aspartate aminotransferase, ALP, urea and creatinine level in treated groups as compared to control was observed. A study [42] also demonstrated such types of results in which significantly increase in AST, ALT, urea and creatinine level due to toxicity was observed as compared to control in rabbits. In another study, [43] the toxicity test is evaluated of diminazene aceturate on rats and significant increase in alanine aminotransferase, creatinine and urea level compared to control due to haptic and renal toxicity were observed. Similarly, the study [24] revealed there was elevation in AST, ALT and ALP level in female rabbits and slightly increase in bilirubin level and significant increase in urea and creatinine level in male rabbit due to toxicity.

Conclusion

Glyphosate showed remarkable toxicity for hematological and biochemical parameters and toxicity was directly related to the increasing concentration of glyphosate and time of exposure. There is need of more investigation at molecular level to know the mechanism of entry of glyphosate into the cells and how it interacts with vital cellular molecules.

Authors' contributions

Conceived and designed the experiments: S Naz, R Iqbal & MF Malik, Performed the experiments: S Naz & R Yaqoob, Analyzed the data: S Naz & M Jabbar, Contributed materials/ analysis/ tools: M Saeed, A Hussain, T Aziz, SU Haider & A Razaq, Wrote the paper: S Naz & A Ahmed.

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