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Kanika Kalai
PhD Scholar, Department of Veterinary Pathology, College of Veterinary Science, AAU, Khanapara, Assam, India

Tirtha Nath Upadhyaya
Professor, Department of Veterinary Pathology, College of Veterinary Science, AAU, Khanapara, Assam, India

Debesh Chandra Pathak
Professor, Department of Veterinary Pathology, College of Veterinary Science, AAU, Khanapara, Assam, India

Dulal Chandra Roy
Professor, Department of Veterinary Phamacology and Toxicology, College of Veterinary Science, AAU, Khanapara, Assam, India

Satya Sarma
Professor, Department of Veterinary Biochemistry, College of Veterinary Science, AAU, Khanapara, Assam, India

Girindra Kumar Saikia
Professor, Department of Veterinary Microbiology, College of Veterinary Science, AAU, Khanapara, Assam, India

Correspondence
Kanika Kalai
PhD Scholar, Department of Veterinary Pathology, College of Veterinary Science, AAU, Khanapara, Assam, India

Haemato-biochemical alterations in induced acute glyphosate ($C_3H_8NO_5P$) intoxication in Kuroiler™ birds

Kanika Kalai, Tirtha Nath Upadhyaya, Debesh Chandra Pathak, Dulal Chandra Roy, Satya Sarma and Girindra Kumar Saikia

Abstract
Present investigation was undertaken to analyse haemato-biochemical alterations of acute glyphosate toxicity in kuroiler birds which are at great risk to herbicide exposure. Total 60 birds were divided into two major groups (I and II). Group I was treated with LD_{50} of glyphosate orally for single dose. Group II served as control. Blood samples were assayed for haematological and biochemical changes. Haemogobin, Total Erythrocyte Count, Total Leucocyte Count, Packed Cell Volume increased significantly in group I. Mean Corpuscular Volume and Mean Corpuscular Haemoglobin decreased and Mean Corpuscular Haemoglobin Concentration increased in group I. Heterophil and thrombocyte count increased while lymphocyte decreased significantly in group I. Serum Alanine Aminotranferase, Aspertate transaminase, Gamma Glutamyl Transferase, Lactate Dehydrogenase and creatinine values increased. Acetyle Cholinesterase did not show any variation. Serum Total protein, albumin and globulin decreased. The results indicated that acute glyphosate intoxication produce changes in haematological and biochemical constituents in treated birds.

Keywords: Acute toxicity, biochemical, glyphosate, Hematology

Introduction
Glyphosate, named by IUPAC (International Union of Pure and Applied Chemistry) as N-(phosphonomethyl)-glycine, is one of the most widely used herbicides in agriculture, forestry, industrial weed control, lawn, garden and aquatic environment. It is used mostly as desiccant in cultivation of corn, soybean and pasture. It is also used in genetically engineered glyphosate tolerant soybeans, corn, cotton and canola. In India, it is registered to apply on various orchards including rubber, mango, orange, tea plantation etc.

Glyphosate inhibits 5-enolpyruvyl shikimate 3-phosphate synthase (EPSPS) enzyme of the shikimate pathway which governs the synthesis of aromatic amino compounds in higher plants, algae, bacteria and fungi and inhibit enzymes of the cytochrome P450 (Cyp450) family in vertebrates [1]. This might also act synergistically with disruption of the biosynthesis of aromatic amino acids by gut bacteria, as well as impairment of serum sulfate transport [2]. Glyphosate showed cytotoxic effects on different cells in vitro and might have contributed to the Parkinsonism [3].

As per reports, the consumption of glyphosate in India was 148 million tonnes in 2014-15, the highest for any herbicide.

Kuroiler (*Gallus gallus domesticus*) is crossbred backyard poultry bird developed in India mainly for egg purpose and popular as backyard poultry bird. In villages, most of the household is having 2 to 5 nos. of this dual purpose bird. There are all the chances to get exposure of these birds to the herbicide through contaminated field and soil as they are free ranging birds. In some commercialized farms, they may get it through feed and water. Reports on toxicological studies on glyphosate in India especially in birds could not be traced out in available literature. Therefore, the present study has been undertaken to study hematobiochemical alterations of acute glyphosate toxicity in kuroiler birds.

Materials and Methods
The experimental trial was approved by the Institutional Animal Ethics Committee (IAEC), Approval No. 770/ac/CPCSEA/FVSc/AAU/17-18/559 dated 09.08.2017 and conducted under its guidelines.

Total 60 kuroiler birds of 2-3 months old unsexed were procured from M/S SURABHI AGROVET ENTERPRISE, Garchuk, Guwahati 781035. Before starting of the experiment the birds were kept in the department of Pathology, College of Veterinary Science, AAU, Khanapara, Guwahati-781022 for 7 days for acclimatization and quarantine. Birds were provided with ad libitum clean drinking water and standard pellet diet. They were randomly distributed to 2 groups of 30 birds each, i. e., glyphosate treatment and control respectively.

Chemical (herbicide)

Commercial product of glyphosate (41%) mixed with POEA used in this study was procured from Insecticide India Ltd. Bharuch, Gujarat-392130, India.

Treatment groups

Total 60 nos. of birds were randomly segregated into 2 groups of 30 nos. in each and fasted for 6 hours prior to dosing. Following the period of fasting, the birds were weighed, and the doses were calculated according to the body weight. 2.5 gm/kg body weight glyphosate formulation was administered orally through gavage. The second group was given distilled water and served as control. The birds were closely observed for the reaction of the birds till death. All the treated birds died within 4-24 hours. Birds of control group were sacrificed after the end of the experiment.

Sampling

About 2 ml of blood from the birds of treated and control were asceptically collected from jugular vein/wing vein with a sterile 2 ml disposable syringe. Blood was collected from both the groups at every 2 hours interval up to 6 hours, then at 12 hour and subsequently at 12 hour interval till death. About 1 ml of blood was taken in a vial in eVac tube with K3EDTA @ 1mg/ml, for estimation of haematological parameters and remaining 1 ml of blood was collected in e Vac tube with clot activators for separation of serum.

Hematological assessment

Hb, TEC, TLC, PCV, MCV, MCH, MCHC, DLC, Thrombocyte were estimated with the help of automated hematology cell counter (Model-ms4e). DLC was analysed manually as per standard method.

Biochemical assessment

Serum was separated and used for analysis of ALT, AST, GGT, LDH, AChE, serum creatinine, total protein, albumin, globulin. Biochemical tests were performed using commercial kits on UV-spectrophotometer.

Statistical Analysis

All data were expressed as mean ± SE. The statistical significance of the differences of different parameters between control and treated were analysed by one-way ANOVA and in between groups were calculated by students t test. The vales of $p < 0.05$ and $P < 0.01$ were taken as cut off value to consider differences statically significant and highly significant respectively.

Results and Discussion

The acute toxicity group of birds developed signs of toxicity after 2 hours of dosing. The birds showed dullness, watery diarrhoea with white strands, coughing and excessive salivation, progressing to drooling, ruffled feathers, drooping of wings and closing of eyelids. Gradually the birds were unable to stand. The birds sat on their hock, with curled toe in some cases. Sitting was followed by tremor, convulsions, recumbence and death with frequent jerky movements of legs and rapid flapping of their wings indicating hyper excitability. The hyper excitability might be due to increase glutamate release in synaptic cleft that induced by glyphosate based herbicide [5].

The hematological values group I and II after the experiment were given in table no.1. The Hb, TEC, TLC, PCV, MCV, MCH, thrombocyte in group I showed increased values than group II while MCHC and lymphocyte count decreased than control group. The increased Hb, TEC and PCV recorded in the present study might be due to diarrhoea causing dehydration resulting in haemoconcentration. This observation was in agreement with earlier workers in birds [6-9]. The increased in TLC values might be due to heterophillia in intoxication [10] as well as dehydration caused by diarrhoea [8]. Increased haematocrit value was recorded in fatal glyphosate poisoning cases in human [11]. Significantly increased level of MCV and MCH were also observed in glyphosate treated rat [6] and mice [9]. The significant increase in heterophil percent and decrease in lymphocyte percent might be due to acute degenerative change in various organs as observed under microscope leading to increase heterophil population and resultant/ corresponding decrease in lymphocyte percent. The lymphopaenia observed in the present study might be due to systemic stress caused by glyphosate toxicity as almost all chemical toxicants increased glucocorticoid level in blood due to stress resulting heterophilia [12]. Increased platelet count in glyphosate toxicity study in rats recorded [6] in contrast decreased platelet count in glyphosate poisoning cases recorded in human [11].

The mean ALT, AST, LDH, GGT, AChE, Creatinie, TP, Albumin and Globulin have been presented in Table No. 2. Mean serum ALT, AST, LDH and GGT increased in group I. The increased level of AST and ALT might be due to degeneration and necrosis of hepatocytes which is correlated with Histopathological study of this experiment. Increase of plasma AST and ALT is the most specific indicator of muscle and liver cell damage and increased enzyme concentration observed due to recent organ damage rather than decreased organ function [13-15]. The mean values of AChE were similar between and the groups and within he groups between different time interval. In contrast, some researchers reported inhibition of AChE enzyme activity by glyphosate based herbicide in non mammalian model, fish [16, 17]. Increase LDH activity might be resulted from haemolysis, muscle damage or hepatocellular injury [18]. Serum creatinine increased significantly from 4 hours onwards post glyphosate feeding which indicated reduced kidney function in the acute experimental group due to tubular damage which could be observed microscopically. Serum total protein, albumin and globulin in acute experimental group decreased than control. Statistical analysis showed no significant variation of the mean values between acute experimental and control group. In contrast significantly higher level of albumin in wistar rats in glyphosate exposed group than control and similar serum protein values in both treatments and control animals [19]. The present findings might be due to degeneration of hepatocytes and renal damage.

Table 1: effect of acute glyphosate intoxication in kuroiler birds on hematology (Mean ± SE)

Haematology	Control					Acute glyphosate				
	Time of collection (hours)					Time of collection (hours)				
	0	2	4	6	12	0	2	4	6	12
Hb (g%)	A 16.26 ± 0.35 a	A 16.33± 0.35 a	A 16.47± 0.32 a	A 16.58± 0.21 a	A 16.95± 0.18 a	A16.23 ± 26a	A 16.85± 0.27 ab	B 17.55± 0.25 bc *	B 18.52± 0.28 c **	B 0.51± 0.84 d **
TEC (10^6/µl)	A 2.78 ± 0.07 a	A 2.86 ± 0.04 a	A 2.93 ± 0.06 ab	A 3.03 ± 0.06 bc	A 3.15 ± 0.05 c	A 2.84 ±0.06a	A 3.06±0.06 a **	B 3.45 ± 0.05 b **	B 3.47 ± 0.05 b **	B 3.50 ± 0.07 b *
TLC (10^3/µl)	A 21.44 ±0.33 a	A 21.46 ±0.25 a	A 21.55 ±0.23 a	A 21.79 ±0.23 a	A 21.88 ±0.25 a	A 21.49 ±0.2 3 a	A 22.05 ±0.27 a	B 22.71 ±0.29 a **	B 24.17 ±0.28 b **	B 25.52 ±0.20 c **
PCV (%)	A 39.35 ± 0.62 a	A 39.52 ± 0.64 a	A 39.88 ± 0.63 a	A 40.12 ± 0.64 a	A 40.25 ± 0.64 a	A 39.37 ±0.54a	A 39.75 ±0.51 a	A 40.86 ± 0.49 ab	B 42.62 ± 0.77 b *	B 46.37 ± 1.79 c **
MCV (fl)	A143.84 ±3.99 a	A 139.22 ± 3.19 ab	A 137.34 ±3.21 a	A 133.35 ±2.89 bc	A 128.47 ±2.59 c	A142.34 ±3.46 a	A 132.09 ±3.54 ab	B 119.41 ±2.48 b **	B 124.36 ±3.81 b *	B 132.27 ±2.82 ab *
MCH (pg)	A 59.77 ±0.06 a	A 57.49 ±0.05 a	A56.77 ±0.05 a	A 55.07 ±0.03 a	A 54.05 ±0.03 a	A58.09 ±0.05 a	A 55.70 ±0.05 a	B 51.20 ±0.04 a **	A 53.58 ±0.04 a	A 58.52 ±0.05 a
MCHC gm/dl)	A 41.59 ±1.06 a	A 41.60 ±1.06 a	A 41.57 ±0.98 a	A 41.58 ±0.72 a	A 42.34 ±0.66 a	A 41.37 ±0.74 a	A 42.55 ±0.78 a	A 43.05 ±0.63 a	A 43.59 ±0.74 a	A 44.24 ±0.90 a
Heterophil (%)	A 25.20 ±0.26 a	A 25.40 ±0.21 a	A 25.77 ±0.16 b	A 25.80 ±0.15 c	A 26.30 ±0.14 c	A25.13 ±0.30a	A 25.77 ±0.39 a	B 27.43 ±0.41 b **	B 27.76 ±0.53 c **	B 28.33 ±1.33 c **
Lymphocyte (%)	A 64.73 ±0.36 a	A 64.37 ±0.20 ab	A 63.87 ±0.18 bc	A 63.57 ±0.18 c	A 63.47 ±0.15 c	A 64.70 ±0.2 7 a	A 64.00 ±0.30 a	A 63.40 ±0.44 ab	B 62.06 ±0.50 b **	B 59.00 ±0.58 c **
Thrombocyte (%)	A 24.70 ±0.34 a	A 24.93 ±0.29 ab	A 25.10 ±0.22 ab	A 25.63 ±0.22 bc	A 26.00 ±0.25 c	A 24.80 ±0.33a	A 25.47 ±0.34 ab	B 26.10 ±0.29 abc **	B 26.88 ±0.28 bc **	B 27.33 ±0.88 c *

Mean carrying same superscripts don't differ significantly. Capital letters denote difference between groups and small letters denote difference amongst group. * <0.005, **< 0.001

Table 2: effect of acute glyphosate intoxication in kuroiler birds on hepato-renal enzymes (Mean ±SE)

Serum biochemical	Control					Acute glyphosate				
	Time of collection (hours)					Time of collection (hours)				
	0	2	4	6	12	0	2	4	6	12
ALT (IU/L)	A325.48 ± 0.14a	A325.56± 0.13ab	A326.34± 0.58ab	A326.65± 0.66ab	A 327.06± 0.66b	A325.61± 0.21a	B 334.90± 0.41b **	B 41.75± 0.7c **	B 350.99±1.14d **	B 360.7± 2.35e **
AST (IU/L)	A 348.28 ±0.33a	A348.31 ±0.29ab	A 348.65 ±0.26ab	A348.95 ±0.26ab	A349.19 ±0.24b	A345.21 ±0.55a	A 349.23±0.49b	B353.87 ±0.66c **	B 359.03 ±0.67d **	B 362.51 ±1.04e **
LDH (IU/L)	A 242.66 ±1.42 a	A 243.04 ±1.40 a	A 243.54 ±1.40 a	A 243.78 ±1.37 a	A 244.06 ±1.38 a	A 244.47 ±1.06a	A 246.47 ±1.08a	B249.53 ±1.06ab **	B 253.16 ±1.76b **	B 264.05 ±3.36c **
GGT (IU/L)	A 5.48 ± 0.14a	A 5.61 ± 0.14a	A 5.80 ± 0.13a	A 5.98 ± 0.13a	A 6.10 ± 0.13a	A 5.40 ±0.12a	B 6.09 ±0.12ab **	B 6.77 ± 0.12abc **	B 7.62 ± 0.16bc **	B 7.63 ± 0.22c *
AChE (IU/L)	A 462.87 ±1.57a	A 462.76 ±1.58a	A 462.74 ±1.57a	A462.70 ±1.57a	A462.65 ±1.58a	A462.92 ±1.00a	A 462.87±1.07a	A 462.71 ±1.07a	A 462.64 ±1.12a	A463.43 ±0.12a
Creatinine (IU/L)	A 348.28 ±0.33 a	A 348.30 ±0.34 a	A 348.47 ±0.41 a	A 348.12 ±0.50 a	A 348.16 ±0.49 a	A 347.46 ±0.42 a	A 349.54 ±0.55 a	B 352.27 ±0.65 b **	B 355.45 ±0.69 c **	B 359.09 ±2.80 d **
Serum Total Protein (gm/dl)	3.60 ±0.10 a	3.58 ±0.10 a	3.55 ±0.10 a	3.55 ±0.09 a	3.55 ±0.09 a	3.65 ±0.07 a	3.64 ±0.05 a	3.61 ±0.06 ab	3.58 ±0.07 ab	3.33 ±0.09 b
Albumin (gm/dl)	1.44 ±0.04 a	1.44 ±0.03. a	1.40 ±0.03 ab	1.35 ±0.03 ab	1.32 ±0.03 b	1.44 ±0.04 a	1.39 ±0.02 a	1.36 ±0.02 ab	1.28±0.02 ab	1.26 ±0.08 b
Globulin (gm/dl)	2.16 ±0.06 a	2.15 ±0.08 a	2.15 ±0.08 a	2.20 ±0.0715 a	2.22 ±0.08 a	2.21 ±0.07 a	2.24 ±0.06 a	2.26 ±0.06 a	2.37 ±0.09 a	2.07 ±0.06 a

Mean carrying same superscripts don't differ significantly. Capital letters denote difference between groups and small letters denote difference amongst group. * <0.005, **< 0.001

Conclusion

From the present investigation, it can be inferred that exposure to glyphosate produces haematological and biochemical alterations in kuroiler birds. However, further research is needed to know the exact mechanism that caused cell damage leading to haematological and biochemical alterations.

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References

1. Barry GF, Kishore GM, Padgette SR. Glyphosate tolerant 5-enolpyruvylshikimate-3-phosphate synthases. World Patent, WO 92/04449, 1992.
2. Samsel A, Seneff S. Glyphosate's Suppression of Cytochrome P450 Enzymes and Amino Acid Biosynthesis by the Gut Microbiome: Pathways to Modern Diseases. Entropy. 2013; 15:1417-1463.
3. Barbosa ER, Leiros da Costa MD, Bacheschi LA, Scaff M, Leite CC. Parkinsonism after glycine-derivative exposure. Mov Disord. 2001; 16:565-568.
4. Monsanto India. Annual Report 2007-08. Pesticide Briefing, Glyhosate and Roundup. Thanal, 2009, 2008. available at www.thanal.co.in
5. Cattani D, Cesconetto PA, Tavares MK, Parisotto EB, DeOliveira PA, Reig C *et al*. Developmental exposure to glyphosate based herbicide and depressive like behavior in adult offspring: implication of glutamate excitotoxicity and oxidative stress. Toxicology. 2017; 387:67-80.
6. Chan P, Mahler J. NTP Technical Report on Toxicit Sudies of Glyphosate administered in dosed feed to F44/N rats and B6C3F1 mice. National Toxicology Program. Toxicity Reports series. 1992; 16:19-23.
7. Malik G, Dhahiya JP, Sandeep G, Mishra SK. Clinico-pahological studies on chlorpyriphos intoxication in broiler chicken. Proceedings of Indian Association of Veterinary Pathologists. CSKHPKV, Palampur, Himachal Pradesh, India, 2002, 26-28.
8. Begum SA, Upadhyaya TN, Baruah GK, Rahman T, Pathak DC, Sarma K *et al*. Haemato-bochemical alterations of acute chlorpyriphos intoxication in indigenous chicken. Veterinary World. 2015; 8(6):750-754. Available at www.veterinaryworld.org/vol.8/june-2015/11.pdf
9. Jasper R, Locatelli GO, Pilati C, Locatelli C. Evaluation of biochemical, hematological and oxidative parameters in mice exposed to the herbicide glyphosate-Roundup®. Interdiscip Toxicol. 2012; 5(3):133-140. doi: 10.2478/v10102-012-0022-5
10. Benjamin MM. Outline of Veterinary Clinical Pathology. 3rd Edition, The Iowa State University Press, Ames, Iowa, USA, 1979.
11. Lee HL, Guo HR. The Haemodynamic Effects of the Formulations of Glyphosate Surfactant Herbicide, 2000. www.researchgate.net/publication/221910008
12. Tamuli SM. Pathology of experimental paraquat toxicity in calves. Ph.D. Thesis, Assam Agricultural University, Khanapara, Guwahati, 1998.
13. Lukaszewicz HA, Moniuszko JJ. A low dose of chlorfenvinphos affects hepatic enzymes in serum and antioxidant enzymes in the erythrocytes and liver of rats. Polish J. Environ. Stud. 2005; 14:199-202.
14. Ozer JM, Ratner M, Shaw W, Bailey, Schomaker S. The current state of serum biomarkers of hepatotoxicity. Toxicology. 2008; 245:194-205.
15. Lumeij JT. Avian Clinical Biochemistry. In: Clinical Biochemistry of Domestic Animals. Kneko, J. J.; Harvey, J. W. and Bruss, M. L. (eds.), 5th Edn, Academic Press, New York, 1997, 857-883.
16. Menendez –Helman RJ, Ferreyroa GV, Dos Santos Afonso M, Salibian A. Glyphosate as an Acetylcholinesterase inhibitor in Cnesterodon decemmaculatus. Bull Environ Contam Toxicol. 2012; 88(1):6-9.
17. Cattaneo R, Clasen B, Loro VL, de Menezes CC, Pretto A, Baldisserotto B *et al*. Toxicological responses of Cyprinus carpio exposed to a commercial formulation containing glyphosate. Bull. Environ. Contam. Toxicol. 2011; 87:597-602.
18. Latimer KS. Duncan and Prasses Veterinary Laboratory Medicine. Clinical Veterinary Pathology. 5th Edition, Wiley- Blackwell Publication. USA, 2015.
19. Tizhe EV, Ibrahim N, Fatihu MY, Igbokwe IO, George BDJ, Ambali SF *et al*. Serum biochemical assessment of hepatic and renal functions of rats during oral exposure to glyphosate with zinc. Comp Clin Pathol. 2014; 23:1043-1050. DOI 10.1007/s00580-013-1740-6.