International Journal of General Engineering and Technology (IJGET)
ISSN(P): 2278–9928; ISSN(E): 2278–9936
Vol. 8, Issue 6, Oct–Nov 2019; 1–10

International Academy of Science, Engineering and Technology
Connecting Researchers; Nurturing Innovations

HEMATOLOGICAL PARAMETERS INFLUENCED BY THE HERBICIDE ATRAZINE (AS AN ENVIRONMENTAL POLLUTANT) USING (WISTAR RATS) AS AN EXPERIMENTAL ANIMALS

Abdelgadir M. I[1], Dradkeh S. M[2] & Dradkeh M. S[3]

[1,2]Research Scholar, Department of Nursing, Alghad International Colleges for Applied Health Sciences, Qassim, Saudi Arabia

[3]Research Scholar, Jordan University of Science and Technology, Jordan

ABSTRACT

Twenty rats of different sex, were randomly divided into 4 groups (5 rats/ each). Three groups were subjected to different doses (27.3, 38.5, and 42.0 mg/kg bw) of the herbicide atrazine, through a duration of 40 days (initial, 10, 20, 30 and 40th time intervals). Complete blood count was made for all animals. All animals showed significantly decreased (P≤ 0.05): WBCs, Hb, RBCs, MCV, HCT, MCHC, and PLT count, at all doses, through increased time intervals, compared to control, remarkably at the dose 27.3 mg/kg bw by the 40th day of the experiment. Observed results may reflect no inflammatory indications (decreased WBCs count), but highly reflection of anemic impacts due to oral administration of the mentioned herbicide.This herbicide is heavily used as a weed killer in sugar – cane, and other commercial crop farms, that may lead to deleterious expected health effects on exposed humans and the environment, especially water systems and the aquatic biota.

KEYWORDS: *Herbicides, Atrazine, Environment, Pollution, Bio Magnification, Chronic Exposure, Engineered Pesticide-Resistant, LC_{50}*

Article History

***Received:* 23 Oct 2019 | *Revised:* 04 Nov 2019 | *Accepted:* 16 Nov 2019**

INTRODUCTION

The need for protection against pests has its roots in antiquity, when both organic and chemical substances were applied as pesticides Panagiotakopulu*et al.,* (1995). Since then, numerous chemical pesticides have been produced, and multinational agrochemical companies, apply new chemical substances with pesticide properties and implement biotechnological advances, thus diverging from traditional agricultural methods. Furthermore, current agricultural practices are based on the wide use of chemical pesticides that have been associated with negative impacts on human health, wildlife, and natural environment Goulson (2014), Goulson (2013). Current agriculture deals with population growth, food security, health risks from chemical pesticides, pesticide resistance, degradation of the natural environment, and climate change Hemingway J, and Ranson H (2000). In recent years, some new concepts regarding agriculture and food production have appeared, such as climate-smart agriculture that seeks solutions in the new context of climate change Steen werth KL *et al.,* (2014). Another major ongoing controversy exists between the advocates and the opponents of genetically engineered pesticide-resistant plants, regarding not only their safety, Séralini GE *et al.,* (2014) but also their impact on pesticide use Benbrook

CM (2012).Furthermore, the real-life chronic exposure to mixture of pesticides with possible additive or synergistic effects requires an in depth research. The underlying scientific uncertainty, the exposure of vulnerable groups and the fact that there are numerous possible mixtures reveal the real complex character of the problem Sexton K, (2012). The combination of substances with probably carcinogenic or endocrine-disrupting effects may produce unknown adverse health effects. Therefore, the determination of "safe" levels of exposure to single pesticides may underestimate the real health effects, ignoring also the chronic exposure to multiple chemical substances. Many of the pesticides have been associated with health and environmental issues World Health Organization (1990), Alewu B, and Nosiri C. (2011), and the agricultural use of certain pesticides has been abandoned Alewu B, and Nosiri C.(2011). Exposure to pesticides can be through contact with the skin, ingestion, or inhalation. The type of pesticide, the duration and route of exposure, and the individual health status are determining factors in the possible health outcome. Within a human or animal body, pesticides may be metabolized, excreted, stored, or bio accumulated in body fat Alewu B, and Nosiri C.(2011), Pirsaheb M, *et al.,* (2015). The numerous negative health effects that have been associated with chemical pesticides include, among other effects, dermatological, gastrointestinal, neurological, carcinogenic, respiratory, reproductive, and endocrine effects Alewu B, and Nosiri C.(2011), Thakur DS *et al.,* (2014). Furthermore, high occupational, accidental, or intentional exposure to pesticides can result in hospitalization and death Gunnell D *et al.,* (2007). Puigdoller*et al.,* (2007) reported that fish Atlantic salmon exposed to 100 gl -1 ATR had reduced food consumption after 10 and 15 days of exposure. Hussein *et al.* (1996) suggested that these behavioral changes were the result of decreased acetyl cholinesterase activity.Atrazine (ATZ) metabolism by human liver microsomes (HLM), cytochrome P450 (CYP) isoforms, and human liver (HL) S9 fractions, was investigated using HPLC/PDA and LC/MS/MS. CYP-dependent metabolites from pooled HLM are desethylatrazine (DEA), desisopropylatrazine (DIA), 1-hydroxyisopropylatrazine (HIATZ), and 2-hydroxyethyl atrazine (HEATZ). DEA and DIA were major metabolites in pooled HLM. CYP1A2 and 2C19, respectively, were major isoforms for DEA and DIA production. CYP3A4, while less active, is generally at high concentrations, produces both DEA and DIA and is significant. The percent total normalized rates (%TNR) for CYP1A2 and 3A4 in pooled HLM were 63% and 24% for DEA, and 35% and 56% for DIA production. Single donor HLM samples, showed correlations for CYP1A2 (r = 0.92) and 3A4 (r = 0.81) for DEA and DIA production, while variations in production of DEA and DIA were 8.5- and 6.0-fold, respectively. Pooled S9 fractions also mediate glutathione conjugation of atrazine, DEA and DIA. Hyun, (2010).Among all forms of chemicals, atrazine is considered to be the most hazardous with respect to environmental pollution, since it is very persistent, non-biodegradable and capable of bio-magnification as it moves up in the food chain. The study therefore investigated the effects of atrazine on some selected blood parameters of *Wistar rats* under laboratory conditions.

MATERIAL AND METHODS

Experimental Location and Source of Experimental Animals

The experiment was carried out at the Toxicity laboratory in "Meck–Nimir" Regional Research Centre (MRRC), Khartoum State, Sudan. Twenty *Wistar rats* of different age and sex were encaged in this center. Animals were put in quarantine for seven days. Animals were regularly fed on basal diet, and freely accessed to tab water. Cages were cleaned every 24 hours.

Procurement and Preparation of Test Solution

A commonly herbicide with brand name "Atrazine" was purchased in one kilogram bags and provided as 80% purity (WP=80) in powder form, dilutive in water. The solution of the chemical in water was prepared according to mg/kg bw of

the animals under test.

Experimental Design and Procedure

The experimental design was a completely randomized design (CRD) with three treatments (27.3, 38.5 and 42.0 mg/kg bw) levels and a control with each level having three replicates. Experimental room temperature was kept on Twenty (20) *C.*

Blood Sampling and Analysis

Blood sampling was conducted at the expiration of 10 days. Blood samples were collected from all animals with glass capillary tubes and preserved in disodium salt of ethylenediaminetetraacetic acid (EDTA) bottles for analysis. A complete blood count was done according to Buttarello, M; and Plebani, M (2008).

Statistical Analysis

Data obtained from the experiments were collated and subjected to ANOVA using Statistical Package for the social Sciences, (SPSS) version 10, differences among means were separated by IBM SPSS, One – Sample T Test, and were charted through Quality Control Pareto Charts.

RESULTS AND DISCUSSIONS

Over the course of the post-exposure periods, all rats were examined for any signs of hematological parameters. Study results are illustrated in Tables () and Figures (). The results showed decreased values of all CBC parameters (WBCs, Hb, RBCs, MCV, MCH, MCHC, and Platelet counts), as confirmed by Jana B. *et al.*,(2014)Who stated that acute exposure to atrazine resulted in significant changes in almost all haematological indices, especially in the groups exposed to the highest concentrations of atrazine (20 and 30 $mg \cdot L^{-1}$). A nonsignificant decrease in erythrocyte count in the group exposed to 30 $mg \cdot L^{-1}$ subsequently led to a significant decrease ($P < 0.05$) in haemoglobin concentration and haematocrit value in this group compared to the control. A significant decrease ($P < 0.05$) in white blood cells was observed in all experimental groups exposed to atrazine for 96 h. Significantly lower ($P < 0.05$) leukocyte count was also recorded in experimental groups exposed to 5 and 15 $mg \cdot L^{-1}$ of atrazine after seven days of recovery period in pure water compared to the control group. Acute exposure to atrazine resulted in an intensive, significant ($P < 0.05$) alteration of differential leukocyte counts compared to the control group; no changes were noticed during the recovery period. A several fold significant decrease ($P < 0.05$) in lymphocytes was found in experimental groups (15, 20, and 30 $mg \cdot L^{-1}$) during 96 h toxicity test. Blood offers important profile to study the toxicological impact on animal tissues. Different blood parameters are often subjected to change depending upon stress condition and various other environmental factors. Decrease or increase in certain blood parameters can be associated with the nature of species and the toxicants in different studies, stated Golam M.M *et al.*, (2015).

CBC: WBCs

White blood cells (WBCs) count was significantly decreased ($P \leq 0.05$), as indicated in table 5), in all doses (27.3, 38.5 and 42.0 mg/kg bw) through different time intervals (initial, 10, 20, 30 and 40 days), compared to control (Fig. 1& Table. 1). These results may indicate no inflammatory responses of rats upon atrazine. These results are confirmed by a study on fish (Acipensernudiventris), where WBC level changed at different concentrations of atrazine. Fish were survived at concentration of mf/L for 96 hours Naji M *et al.*, (2019). Also Ramesh M. *et al.*, (2009), Stated that white blood cells (WBCs) count was enhanced significantly ($p < 0.05$), may be used as an important tool for the assessment of pathological

conditions. Study results again were confirmed by Jana B. *et al.*, (2014) by observing a significant decrease ($p < 0.05$) in white blood cells in all experimental groups exposed to atrazine for 96 h. Significantly lower ($p < 0.05$) leukocyte, lymphocytes count was also recorded in experimental groups exposed to 5 and 15 mg·L−1 of atrazine after seven days of recovery period in pure water compared to the control group. Acute exposure to atrazine resulted in an intensive, significant ($p < 0.05$) alteration of differential leukocyte counts compared to the control group; no changes were noticed during the recovery period. A several fold significant decrease ($p < 0.05$) in lymphocytes was found in experimental groups (15, 20, and 30 mg·L−1) during 96 h toxicity test.

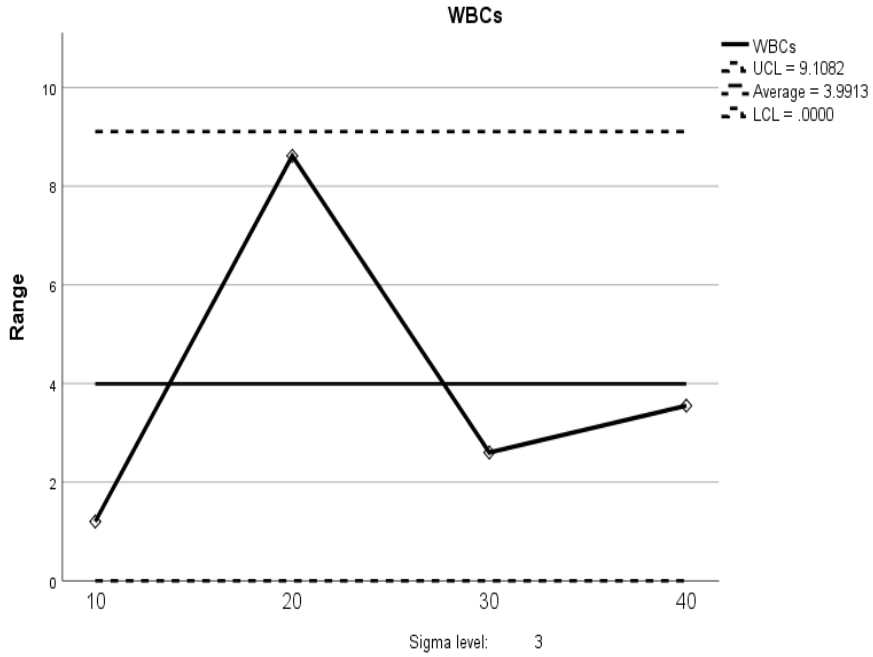

Figure 1

Table 1: Bootstrap for One-Sample Test

	Mean Difference	Bootstrap[a]				
		Bias	Std. Error	Sig. (2-Tailed)	95% Confidence Interval	
					Lower	Upper
WBCs	7.14481	.00043	.54095	.001	5.98277	8.13956
a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples						

CBC: Hb

Hemoglobin (Hb) values were significantly decreased ($P \leq 0.05$), as indicated in table 6), in all doses (27.3, 38.5 and 42.0 mg/kg bw) through different time intervals (initial, 10, 20, 30 and 40 days), compared to control (Fig. 2 and table 2). These results may indicate no inflammatory responses of rats upon atrazine. These results are confirmed by (Jana B. et al., 2014), who stated a non-significant decrease in erythrocyte count in a group of fish exposed to 30 mg·L−1 of atrazine, subsequently led to a significant decrease ($P \leq 0.05$) in haemoglobin concentration and haematocrit value in this group compared to the control. Hemoglobin (Hb) level changes at different concentrations of atrazine in “Acipensernudiventris” fish were survived at concentration 25 mf/L for 96 hours Naji M et al., (2019). Study results are credited by

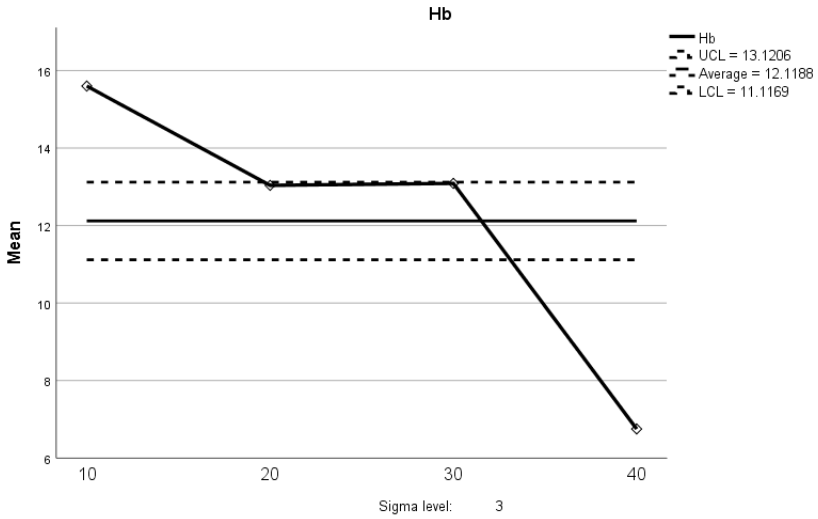

Figure 2

Table 2: Bootstrap for One-Sample Test

		Bootstrap[a]				
					95% Confidence Interval	
	Mean Difference	Bias	Std. Error	Sig. (2-Tailed)	Lower	Upper
Hb	14.07500	.00007	.32650	.001	13.43133	14.70922
a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples						

Ramesh M. *et al.,* (2009), who stated that acute toxicity of atrazine on fish, (Cyprinuscarpio), resulted in significantly decreased Hbvaluesc ($P \leq 0.05$).Reduction in Hb content of treated silver barb may be an indication of decline in Hb synthesis. Significant decrease ($p < 0.05$) in values of erythrocyte count, hematocrit, and Hb content compared to the control groups were observed in catfish at acute exposure to atrazine, as stated Adedeji (2010). Decreased values of hematological parameters of treated fish indicated that the physiological activities of the treated fish were affected.

CBC; RBCs

The decrease in hematological variables (PCV, Hb, and RBC) of the exposed fish may be due to haemolysis and shrinkage of RBC by QP leading to significant decrease in hematocrit value which results in fish anemia. The increase rate of the breakdown of RBC or reduction rate of formation of RBCs might also be responsible for reduction in RBC count. Similar observations were reported for *Clariasgariepinus* treated with endosulfan pesticides. Reduction in hematological indices may also be due to an appreciable decline in the hematopoiesis. Similar reduction in RBC was reported for cypermethrin treated *Labeorohita*, African cat fish (*C. gariepinus*) treated with diazinon, and freshwater common carp (*Cyprinuscarpio*) treated with atrazine Golam M.M *et al.,* (2015). Red blood cells (RBCs) counts were significantly decreased ($P \leq 0.05$), as indicated in (Fig.3 & Table. 3), in all doses (27.3, 38.5 and 42.0 mg/kg bw) through different time intervals (initial, 10, 20, 30 and 40 days), compared to control, remarkably at the dose (27.3 mg/kg bw) through the last timeinterval of the experiment. Study results were confirmed by Naji M *et al.,* (2019) who stated that hematocrit increased meaningfully according to increased atrazine concentration, and the red blood cells (RBC) decreased meaningfully in different treatments. Due to fish exposure to 24 h LC 50 of atrazine for 24 h, red blood cells (RBCs) count was significantly (($P \leq 0.05$) decreased as stared by Ramesh M. and Saravanan M. (2009). Farrell (2011) showed that fish, RBCs are elliptical or ovoid, but species have changes in RBC dimensions and length from 10– 20 mm and 6–10 mm in width.

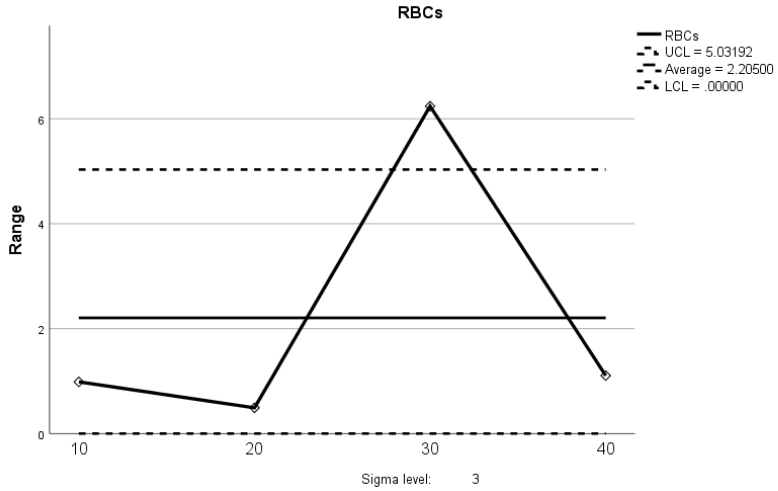

Figure 3

Table 3: Bootstrap for One-Sample Test

	Mean Difference	Bootstrap[a]				
		Bias	Std. Error	Sig. (2-Tailed)	95% Confidence Interval	
					Lower	Upper
RBCs	6.878125	.000486	.409492	.001	5.978220	7.578721
a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples						

CBC: MCV

Mean Corpuscular Volume (MCV) was significantly decreased ($P \leq 0.05$), as indicated in (Fig 4 and table 4), in all doses (27.3, 38.5 and 42.0 mg/kg bw) through different time intervals (initial, 10, 20, 30 and 40 days), compared to control, remarkably at the dose (27.3 mg/kg bw) through the second time interval of the experiment (20^{th} day). The result is in accordance with Ramesh et al. (2009) who assessed the effects of selecte d herbicides on haematology profile of fish blood. The values of MCHC, MCV were marginally decreased across the treatment exposed to atrazine.

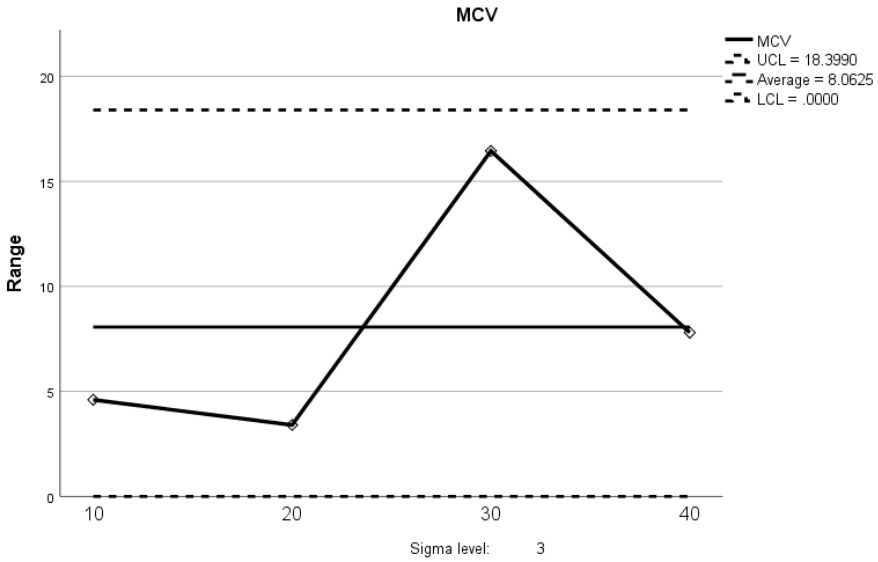

Figure 4

Table 4: Bootstrap for One-Sample Test

	Mean Difference	Bootstrap[a]				
		Bias	Std. Error	Sig. (2-Tailed)	95% Confidence Interval	
					Lower	Upper
MCV	73.71437	.00046	1.06804	.001	71.90010	76.06109
a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples						

CBC: MCH

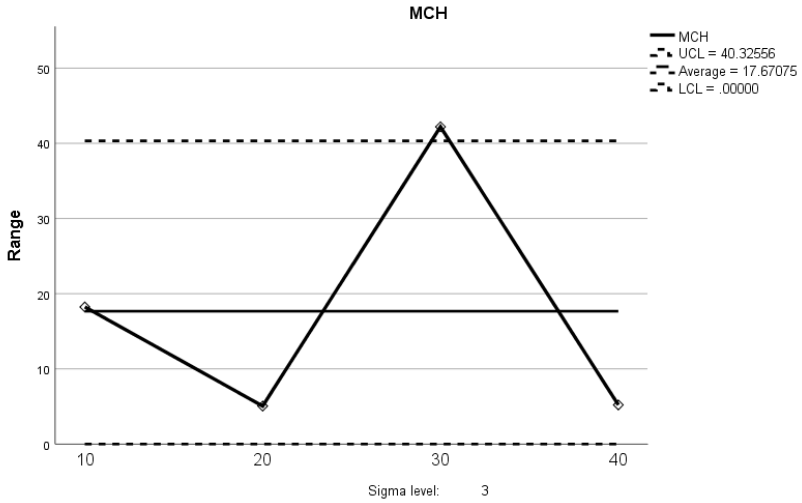

Figure 5.

Mean Corpuscular Hemoglobin (MCH) was significantly decreased ($P \leq 0.05$), as indicated in (Fig 5 and table 5), in all doses (27.3, 38.5 and 42.0 mg/kg bw) through different time intervals (initial, 10, 20, 30 and 40 days), compared to control, remarkably at the dose (27.3 mg/kg bw) through the second time interval of the experiment (20th day).

Table 5: Bootstrap for One-Sample Test

	Mean Difference	Bootstrap[a]				
		Bias	Std. Error	Sig. (2-Tailed)	95% Confidence Interval	
					Lower	Upper
MCH	50.658313	-.012159	3.025208	.001	44.347272	56.218556
a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples						

CBC: MCHC

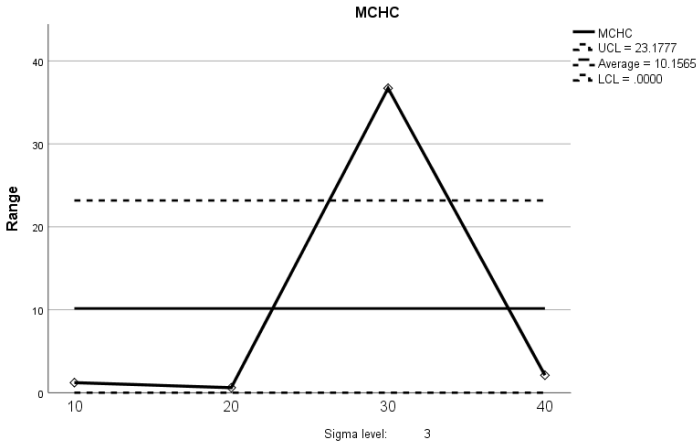

Figure 6

Table 6: Bootstrap for One-Sample Test

	Mean Difference	Bootstrap[a]				
		Bias	Std. Error	Sig. (2-Tailed)	95% Confidence Interval	
					Lower	Upper
MCHC	29.68013	.01126	2.25391	.001	26.43472	34.55601
a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples						

Mean Cell Hemoglobin Concentration (MCHC) was significantly decreased ($P \leq 0.05$), as indicated in (Figure 6 and table 6), in all doses (27.3, 38.5 and 42.0 mg/kg bw) through different time intervals (initial, 10, 20, 30 and 40 days), compared to control, remarkably at the dose (27.3 mg/kg bw) through the second time interval of the experiment (20th day). The result is in accordance with Ramesh et al. (2009) who reported the effects of selected herbicides on haematology profile of fish blood. The values of MCHC, MCV were marginally decreased across the treatment exposed to atrazine.

CBC: PLT

Platelets count (PLT) was significantly decreased ($P \leq 0.05$), as indicated in Fig. 7 and table 7), in all doses (27.3, 38.5 and 42.0 mg/kg bw) through different time intervals (initial, 10, 20, 30 and 40 days), compared to control, remarkably at the dose (27.3 mg/kg bw) through the last time interval of the experiment (40th day). Study results are confirmed by Varol*et al.*, (2014) who stated that in a group of farm workers, Platelet count was significantly lower in farm workers than those of controls ($p < 0.001$).

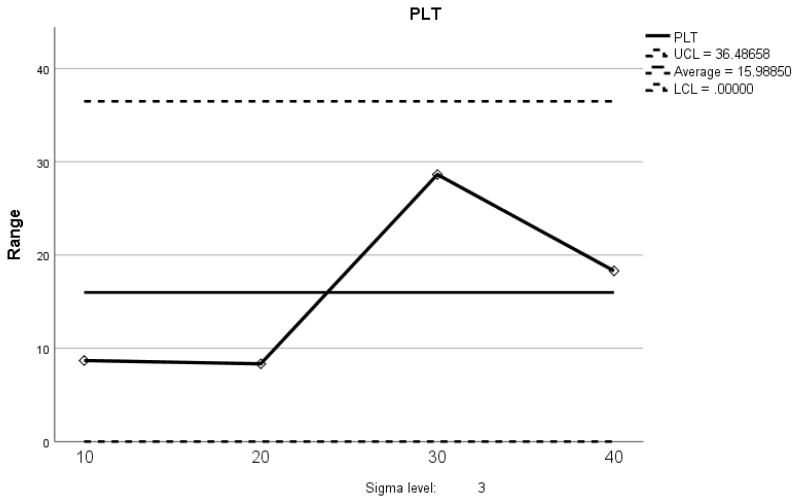

Figure 7.

Table 7: Bootstrap for One-Sample Test

	Mean Difference	Bootstrap[a]				
		Bias	Std. Error	Sig. (2-Tailed)	95% Confidence Interval	
					Lower	Upper
PLT	42.925625	-.261053	7.864966	.001	27.267812	58.544631
a. Unless otherwise noted, bootstrap results are based on 1000 bootstrap samples						

CONCLUSION AND RECOMMENDATIONS

The result obtained in this study revealed that atrazine/metalochlor is highly toxic. Toxicity of atrazine /metalochlor on *wistar rats* increased with increasing concentration of the pesticides. Due to that this herbicide almost reaches aquatic environment then aquatic biota and ends up in humans via food – chain, it is highly recommended that usage should be restricted.

ACKNOWLEDGEMENTS

Authors are extremely thanking "Meck – Nimir" Regional Research Centre (MRRC), Khartoum State, Sudan, for valuable experiment guidance.

REFERENCES

1. *Adedeji O. B. (2010). Acute effect of diazinon on blood plasma biochemistry in the African catfish (Clariasgariepinus) Journal of Clinical Medicine and Research. ;2(1):1–6.*
2. *Alewu B, and Nosiri C. (2011). Pesticides and human health. In: Stoytcheva M, editor. , editor. Pesticides in the Modern World – Effects of Pesticides Exposure. InTech; p. 231–50.*
3. *Benbrook CM. (2012). Impacts of genetically engineered crops on pesticide use in the U.S. – the first sixteen years. Environ SciEur 24:24.10.1186/2190-4715-24-24.*
4. *Buttarello, M; and Plebani, M (2008). "Automated blood cell counts: state of the art". American Journal of Clinical Pathology. 130 (1): 104–16.*
5. *Golam M.M , Mahbuba M.M , and M. S.I (2015). Alteration of Blood Parameters and Histoarchitecture of Liver and Kidney of Silver Barb after Chronic Exposure to Quinalphos. J Toxicol. 2015; 2015: 415984.*
6. *Gunnell D, Eddleston M, Phillips MR, and Konradsen F. (2007). The global distribution of fatal pesticide self-poisoning: systematic review. BMC Public Health 7:357.10.1186/1471-2458-7-357.*
7. *Goulson D. (2013). An overview of the environmental risks posed by neonicotinoid insecticides. J ApplEcol 50:977–87.10.1111/1365-2664.12111*
8. *Hemingway J, and Ranson H. (2000). Insecticide resistance in insect vectors of human disease. Annu Rev Entomol 45:371.*
9. *Hussein SY, El-Nasser MA, and Ahmed SM (1996). Comparative studies on the effects of herbicide atrazine on freshwater fish Oreochromisniloticus and ChrysichthyesauratusatAssiut, Egypt. Bull. Environ. Contam.Toxicol. 57: 503-510.*
10. *Hyun J. Kyoungiu Ch. And Ernest H. (2010). Human metabolism of atrazine Pesticide Biochemistry and Physiology98, (1): 73-79.*
11. *Jana B. Helena M. Marie S. Petr M. Lenka Z. Misa Sk. and Zdenka S. (2014). Evaluation of Biochemical, Haematological, and Histopathological Responses and Recovery Ability of Common Carp (Cyprinuscarpio L.) after Acute Exposure to Atrazine Herbicide BioMed Research International Volume 2014, Article ID 980948, 8 pages. http://dx.doi.org/10.1155/2014/980948.*
12. *Naji M ; YousefiJourdehi Y. and Hosseinzadeh S. H. (2019). Impacts of Atrazine on some blood and biochemical indices in farmed Acipensernudiventris. Journal of Survey in Fisheries Sciences 5(2) 19-27.*
13. *Panagiotakopulu E, Buckland PC, Day PM, Sarpaki AA, and Doumas C. (1995). Natural insecticides and insect repellents in antiquity: a review of the evidence. J ArchaeolSci 22:705–10.10.1016/S0305-4403(95)80156-1.*

14. *Pirsaheb M, Limoee M, Namdari F, and Khamutian R. (2015). Organochlorine pesticides residue in breast milk: a systematic review. Med J Islam Repub Iran 29:228.*

15. *Puigdoller KN, Bjornsson BT, and McCormick SD (2007). Effects of hexazinone and atrazine on the physiology and endocrinology of smolt development in Atlantic salmon. Aquat. Toxicol. 84: 27-37.*

16. *Ramesh M. Srinivasan R. and Saravanan M. (2009). Effect of atrazine herbicide on blood parameters of common carp (Cyprinuscarpio). African Journal of Environmental Science and Technology. 3 (12), 453-458.*

17. *Séralini GE, Clair E, Mesnage R, Gress S, Defarge N, and Malatesta M, (2014). Republished study: long-term toxicity of a Roundup herbicide and a Roundup-tolerant genetically modified maize. Environ SciEur 26:14.10.1186/s12302-014-0014-5.*

18. *Steenwerth KL, Hodson AK, Bloom AJ, Carter MR, Cattaneo A, and Chartres CJ, (2014). Climate-smart agriculture global research agenda: scientific basis for action. Agr Food Secur 3:11.10.1186/2048-7010.*

19. *Sexton K. (2012). Cumulative risk assessment: an overview of methodological approaches for evaluating combined health effects from exposure to multiple environmental stressors. Int J Environ Res Public Health 9:370–90.10.3390/ijerph9020370.*

20. *Thakur DS, Khot R, Joshi PP, Pandharipande M, and Nagpure K. (2014). Glyphosate poisoning with acute pulmonary edema. ToxicolInt 21:328–30.10.4103/0971-6580.155389.*

21. *Varol E, Ogut S, and Gultekin F (2014). Effect of pesticide exposure on platelet indices in farm workers. ToxicolInd Health. (7):630-4. doi: 10.1177/0748233712462477.*

22. *World Health Organization(1990). Public Health Impact of Pesticides Used in Agriculture. England: World Health Organization.*