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Protective activity of L-carnitine and quercetin against atrazine-induced hematological toxicity in adult male rats

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Abstract

Atrazine (ATZ) is one of the most extensively used herbicides globally. It is classified as an endocrine disruptor chemical (EDC), interfering with several physiological functions. L-carnitine (LC) and quercetin (QT) have been shown to have significant antioxidant actions. Therefore, this study targeted to inspect the beneficial effects of LC and QT to defend againstameliorate ATZ toxicity through studying their effects on body weight, body weight gain, and hemogram in adult male rats. In light of this, four groups of seven adult male albino rats were established. The groups were control, ATZ, ATZ + LC and ATZ + QT and the experiment lasted for 56 days. Results showed no significant impacts on body weight ($P > 0.05$) in all groups. Additionally, compared to control group, ATZ dramatically decreased body weight gain (BWG) ($P < 0.001$). Moreover, rats that got ATZ plus the other treatments also showed a reduced BWG ($P < 0.001$). Moreover, ATZ intensely decreased the proportion and concentration of Hb, RBCs, WBCs and platelets counts ($P < 0.001$). Notably, administering LC restored Hb concentration and percentage, RBCs, WBCs and platelets counts to control levels ($P < 0.01$). Furthermore, Hb concentration and percentage were enhancedincreased numerically with QT. While ATZ reduced RBC, WBC, and platelet counts, QT markedly increased those counts ($P < 0.01$), though it did not bring them back to control levels. There were no discernible changes ($P > 0.05$) between the groups for the other hemogram parameters. Thus, LC and QT could be considered excellent candidates to abate the adverse effects of ATZ on hemogram.

1. Introduction

It has been reported recently that there are certain exogenous substances known as endocrine disruptors (EDs) that have negative impacts on reproduction in both humans and animals by interrupting the biosynthesis, release, transporting activity, binding, action, or removal of endogenous hormones (1). (ATZ) is one of the most common EDs and it was noticed to be found in the final category of chemicals in the initial Tier (1) screening in the EPA's Endocrine Disruptor Screening Program (EDSP) in 2009 (2,3).

ATZ, one of the world's most commonly used herbicides, routinely contaminates both human and animal drinking water (4). It exerts its effects on metabolism and reproduction, upsetting plant metabolic processes (5), and it is a strong endocrine disruptor that makes vertebrates, including humans and animals, have hormonal imbalances (6). Little doses may have no noticeable effects, but somewhat greater exposures may disrupt certain physiological processes, such as elevated activity of detoxifying enzymes, elevated stress hormones, or behavioral changes (7).

It has been demonstrated that ATZ can impair immune system activity, raising the risk of cancer and infectious diseases. use of ATZ lowered interleukin synthesis in human blood cells, as well as interferon and tumor necrosis factor (8). The use of ATZ , in rats for 24 consecutive weeks, caused severe lymphopenia (both T cells and B cells) (9). Hematological measurements including hematocrit, red blood cell counts, and hemoglobin levels were all observed to be significantly decreased by ATZ (10).

Naturally occurring as a tiny, water-soluble molecule, L-carnitine (3-hydroxy-4-N-trimethylaminobutyrate) is an amino acid found in meat and dairy products (11). During the beta-oxidation process in skeletal muscles, LC is crucial for moving free fatty acid to the mitochondrial matrix (12,13).

It has been noticed that the RBCs and its indicators were markedly enhanced by LC. Hemoglobin concentration, mean cell volume (MCV), mean cell hemoglobin (MCH), and mean cell hemoglobin concentration (MCHC) are all improved after receiving LC treatment (14). Erythrocyte stability, erythropoiesis, and hyperlipidemia are all positively impacted by LC (15). It has been determined that the presence of LC

corrected anomalies in RBC deformability, which in turn caused the hematocrit to rise (16). It has been noted that LC plays a significant protective role against oxidative damage caused by elevating H2O2. This function counteracts the detrimental effects of oxidative stress on the red bone marrow and erythropoiesis (17). Consumption of LC in food has a major effect on the control of erythropoiesis as well as inflammatory and immune cells (18).

(QT),, a bioflavonoid found in many fruits, vegetables, and drinks, has been extensively investigated in various scientific studies to display high antioxidant activity as well as favorable effects on cellular apoptosis and inflammation (19). QT is recognized as one of the excellent options in the amelioration of environmental contaminants-induced diverse unfavorable effects, which is accounted for its biological capabilities against oxidative stress, inflammation, and cancer (20,21). Dietary treatment with QT enhanced the haemato-biochemical changes and relieved oxidative stress brought on by exposure to abamectin (22). Treatment with QT was shown to reduce the harmful effects of Cd, including thyroid gland activity and hematological damage (23).

However, the information regarding the beneficial roles of LC and QT against ATZ toxicity on hematological parameters is still scarce. Therefore, the current study was conducted to evaluate the physiological effects of LC and QT in combating ATZ toxicity in adult male rats by assessing hematological parameters, body weight, and body weight gain (BWG).

2. Materials and methods

2.1. Chemicals

The toxicity experiments were conducted using ATZ (organo-chlorine herbicide) with 80% purity. ATZ powder (80% W/W) was bought from Syngenta Company and imported by Elnasr Pharmaceutical Co. in Egypt. ATZ powder was suspended in sterile distilled water to produce a stock suspension that achieved the recommended dose of 120 mg/kg BW. It was freshly prepared every day. The supplier of LC was MEPACO-MEDIFOOD Company for Pharmaceuticals and Medicinal Plants, Sharkeya, Egypt. The capsules had a dose of 350 mg per capsule and came in a package of 20. Capsules were opened and dissolved in distilled water (to get the required dose of 200 mg/kg BW), and the solution was prepared every 2 weeks. QT dihydrate powder was purchased from S. D. Fine Chem Ltd. company, Purity. ≥95% (HPLC), Mumbai-India. It is water suspended, and the suspension was prepared by suspending it in sterile distilled water (to get the appropriate dose of 25 mg/kg BW) to make a stock every 2 weeks.

2.2. Animals and experimental protocol

This study was conducted using 28 adult albino rats (Sprague Dawley) weighing between 150 and 180 gm BW. They were obtained from Minia University, Faculty of Medicine's animal house. All of the animals were kept under sanitary conditions. The animals were allowed unlimited access to standard food and tap water. They were housed in ventilated polypropylene cages with stainless steel mesh covers and bedding manufactured from clean wood shavings. The temperature was maintained at 22°C. They were allowed 15 days to acclimate to the lab environment before starting the experiment. All experiments were under the guidance of the rules of the Institutional Review Board of the Ethical Committee of Animal Care and Use in Scientific Research of the Faculty of Veterinary Medicine, Minia University (IRB-FVM-MU) with approval number: IRB-FVM-MU-2024-108.

The animals were divided into four groups randomly (7 animals per group) as follows:

1) Rats in the control negative group (control group, n=7) were gavaged 1.5 ml of distilled water.

2) Each rat in the second group (the ATZ group, or "control positive") was given 120 mg/Kg BW of ATZ solution in accordance with Abarikwu & Farombi (24).

3) The rats in the third group (ATZ+ LC) received LC (200 mg/Kg BW) one hour before gavage by 120 mg/Kg BW of ATZ solution following Bazotte & Lopes-Bertolini (25).

4) Similarly, rats in the fourth group (ATZ+QT) received QT (25mg/Kg BW) one hour before gavage by 120 mg/Kg BW of ATZ solution according to Jahan (26).

All treatments including the control were carried out using stomach tube gavage once daily for consecutive 56 days (27).

2.3. Body Weight and Body Weight Gain

Starting body weight for each rat was recorded at the beginning of the study and the rats were weighed again at the end of the study period (56 days) to calculate BWG. Furthermore, the total weight was calculated using the final body weights that were reported to each rat at the conclusion of the study.

2.4. Blood Sample Collection:

At the end of the study, each rat was anesthetized using Ketamine HCL 90mg/kg. bwt. and Xylazine HCL 5mg/kg. bwt. according to Hohlbaum et al. (28), then separate samples of blood were collected from each rat individually, from the retro-orbital venous plexus according to the previously described method (29), in vacuum EDTA tubes and stored at 4 C° for further analysis.

2.5. Assessment of hematological parameters:

An automatic CBC analyzer (MS4Se® VET hematology cell counter from msla Austria) was used to determine the assessment of hematological parameters, The fully automated hematology analyzer MS4Se® provides the following parameters (Leucocytes, Lymphocytes, Monocytes, Granulocytes, Erythrocytes, MCV, HCT, MCH, MCHC, RDW, Hb, PLT, MPV, PCT, PDW.)) with a 3-part differentiation of the leukocytes (lymphocytes, monocytes, granulocytes). The sample volume is 12µl whole blood (capillary or venous).

2.6. Statistical analysis:

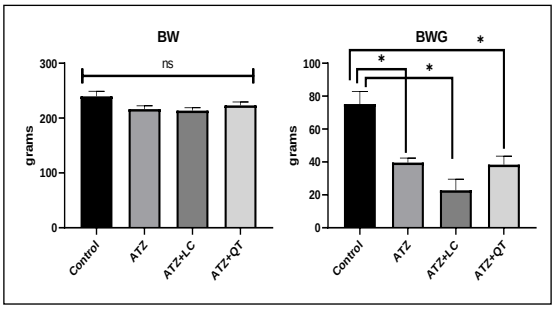
The SPSS program, version 20 Inc., Chicago, IL, USA, was used to conduct the statistical analysis. Standards errors of mean (SEM) ± means were used to report the results. Test groups and control group means were compared using one-way ANOVA,

and Tukey's test was used to assess post-hoc comparisons. It was decided to use a P-value "0.05" as a significance threshold.

3. Results

3.1. Body weight and body weight gain in control and treated animals.

The findings of body weight and BWG were demonstrated in Fig. 1. It has been noted that ATZ has no significant effects on body weight compared to all other groups ($P > 0.05$). Furthermore, in comparison to the rats in the control group, ATZ reduced BWG significantly ($P < 0.001$). In addition, BWG was found to be lower when rats were co-administered ATZ with LC or QT ($P < 0.001$) in comparison to the control group.



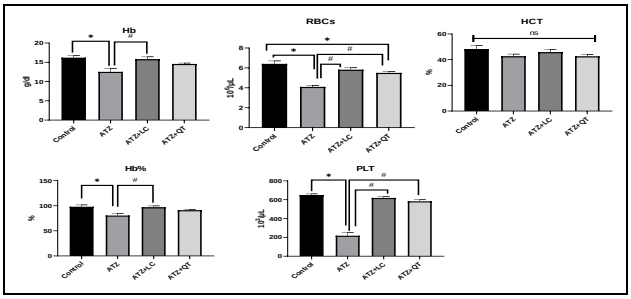
Figure(1): Body weight (BW), body weight gain (BWG) in control and treated animals.
ATZ: Atrazine, LC: L-carnitine, QT: Quercetin.
Data are expressed as mean ± standard error of the mean.
* $P < 0.001$ versus the control group.
ns: Values are not significantly different from one another ($P > 0.05$).

3.2. Complete blood picture characteristics in control and treated animals.

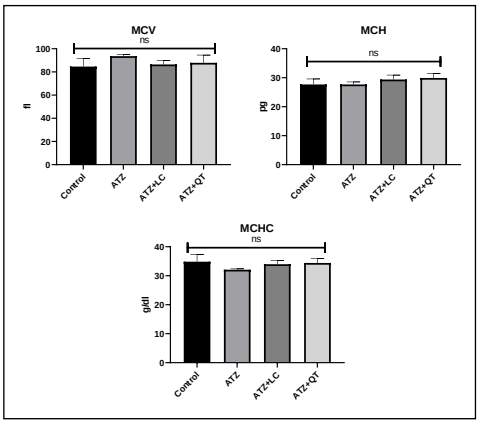
As shown in Fig. 2, Hb concentration and percentage as well as RBCs and platelets counts were significantly reduced with ATZ compared to the control group ($P < 0.001$). Interestingly, treating animals with LC significantly reduced the negative effects of ATZ and returned concentration and percentage of Hb, RBCs and platelets counts to control levels ($P < 0.01$). Furthermore, QT was also effective in significantly enhancing the RBCs and platelets counts that were reduced by ATZ ($P < 0.01$) but did not restore RBCs to control levels. Additionally, QT improved Hb concentration and percentage, but this improvement was not statistically significant ($P > 0.05$). Furthermore, Fig. 2 demonstrated that there were no differences among treated groups for HCT values ($P > 0.05$).

The findings in Fig. 3 demonstrated that the RBCs indices including MCV, MCH, and MCHC didn't show significant differences among all groups ($P > 0.05$). Moreover, as shown in Fig. 4, mean values of WBCs count were significantly lowered by ATZ ($P < 0.001$) and retrieved markedly with either LC or QT treatment ($P < 0.001$). The percentages of neutrophils, lymphocytes, monocytes, eosinophils, and basophils were observed to be comparable ($P > 0.05$) in all groups (Fig. 4).

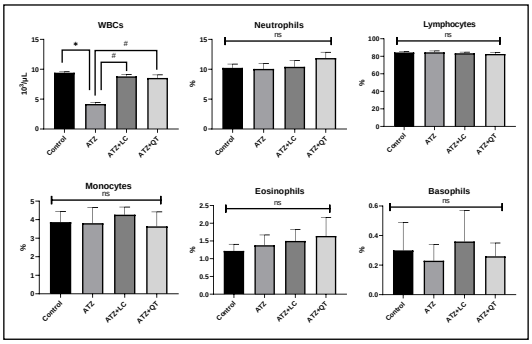
The recovery percentages of LC and QT for Hb concentration and percentage were calculated (125.68 and 120.83 vs. 120.37 and 118.07, respectively). In addition, the recovery percentages of LC and QT for RBC count, WBC and PLT count were also estimated (141.95, 210.77 and 284.56 vs. 134.15, 204.31 and 267.92, respectively). Based on these findings, we concluded that the favorable effects of LC upon these parameters were somewhat greater than those of QT.



Figure(2): Mean values of Hb, RBCs, HCT, Hb% and platelets in control and treated animals.
ATZ: Atrazine, LC: L-carnitine, QT: Quercetin, Hb: hemoglobin, RBCs: Red blood cells count, HCT: hematocrit, PLT: platelets.
*$P < 0.001$ versus control group, #$P < 0.01$ versus ATZ group.
ns: Values are not significantly different from one another ($P > 0.05$).



Figure(3): Mean values of MCV, MCH and MCHC in control and treated animals.
ATZ: Atrazine, LC: L-carnitine, QT: Quercetin, MCV: mean cell volume, MCH: mean cell hemoglobin, MCHC: mean cell hemoglobin concentration.
ns: Values are not significantly different from one another ($P > 0.05$).



Figure(4): Mean values of WBCs, neutrophils, lymphocytes, monocytes, eosinophils and basophils in control and treated animals.
ATZ: Atrazine, LC: L-carnitine, QT: Quercetin, WBCs: white blood cells count.
*$P < 0.001$ versus control group, #$P < 0.001$ versus ATZ group.
ns: Values are not significantly different from one another ($P > 0.05$).

4. Discussion

ATZ poses a major risk to the health of humans and animals. It is one of the most extensively used herbicides in the world, frequently contaminating animal and human drinking water (4). It is a strong endocrine disruptor that makes humans and animals suffer from hormonal imbalances (6). Thus, the current work aimed to evaluate the possibility of using the beneficial features of LC and QT to counteract ATZ toxicity in adult male rats.

The effects of ATZ on body weight and BWG are further explored in this study. It was obvious that the body weight mean values of animals in all groups were almost similar, with a non-significant drop in the ultimate weights of ATZ-treated rats compared to those in the control group. Moreover, BWG was reduced significantly with ATZ. Furthermore, BWG was observed to be significantly lower in rats in the groups that received co-treatments (ATZ+LC and ATZ+QT). Consistent with our results, supra-environmental ATZ exposure was found to either have no impact or cause rats and mice's body weight to drop (30–32) The previous studies generally agree that ATZ medication at doses more than 50 mg/kg resulted in a decrease in body weight (33–37).

The stress caused by atrazine appears to have led to a general increase in the utilization of energy reserves, explaining the reduced weight gain associated with atrazine exposure (43). This depletion of energy reserves included lower levels of glycogen, muscle protein (44), muscle lipids, and both muscle and hepatopancreatic proteins (45). It is widely recognized that energy reserves such as carbohydrates, lipids, and proteins are utilized to cope with exposure to various stressors, resulting in decreased growth rate and increased utilization of energy reserves (46). In other studies, ATZ was found to raise body weight. In this case, regardless of water and food intake, a brief exposure to a modest dose of ATZ caused a considerable gain in body weight. Curiously, neither the mean total weight gain nor the cumulative weight gain changed with a 10-fold increase in ATZ dosage (47). Rats treated with ATZ experienced a small (5.5%) rise in body weight without any changes to food consumption or physical activity, albeit this increase was not statistically significant (48). As a result, we may postulate that the variation in body weight findings with ATZ in these studies is due to the dose employed, species, and exposure length.

The reduction in body weight gain seen in our study with LC could be interpreted in light of the findings of Talenezhad (49), who found that LC when added to the diet of obese individuals promotes a considerable reduction in fat mass and thus body weight. In this context, LC was observed to induce anti-obesity activities especially when accompanied with certain lifestyle modifications (50). The reducing effect of LC on body weight could also be attributed to its significant role in β-oxidation of very long-chain fatty acids and also in the α-oxidation of phytanic acid (51,52). Furthermore, LC may enhance the expression of peroxisome proliferator-activated receptors (PPAR-γ) at both the mRNA and protein levels in the liver of cachectic mice, inhibiting the synthesis of fatty acids (53,54). All of the aforementioned investigations validated our findings about LC's lowering effect on BWG.

The study conducted by H. Jiang (55) may help to explain the reduction in BWG associated with QT. They demonstrated that QT and its glycosides were crucial in regulating AMP-activated protein kinase-driven pathways, which is vital in preventing hyperglycemia and obesity. In obese cases, adding QT to the diet was useful in lowering body fat depots (56). Supplementing the diet with QT led to a reduction in adipocyte size, which in turn reduced lipid accumulation and, ultimately, body weight (57), confirming our findings. Furthermore, QT's ability to regulate the signaling pathways for adenine monophosphate- and mitogen-activated protein kinase may potentially contribute to its anti-obesity effects (58).

In the current study, rats in the group treated with ATZ alone revealed reduced Hb concentration and percentage along with lowering of RBCs, platelets, and WBCs counts. However, there were no changes in the values of HCT, MCV, MCH, MCHC and differential leukocytic count. In this sense, it was shown that ATZ decreased hematological metrics, as evidenced by decreased hematocrit, hemoglobin, WBC, RBC, MCH, MCHC, monocytes, and lymphocytes, but noticeably increased HCT and platelet levels (59). ATZ against some blood parameters (WBC, RBC, Hb, PCV, MCV, MCH, MCHC, and PLT) was evaluated in rats (60), and was found to cause a non-significant alteration in all hematological parameters when compared to control. Ramesh (61) found that ATZ exposure resulted in a decline in RBCs and Hb, but an increase in WBC in common carp fish.

It has been found that ATZ negatively affects hematological parameters in juvenile through decreasing of HCT, Hb, RBCs and platelet count (62). ATZ was found to have detrimental effects on hemogram in rats represented by a reduction in the HCT, Hb concentration and RBCs count along with an increase in leukocyte count (63). It has been proved in the rat model that ATZ exposure has unfavorable effects on Hb concentration (64). Akhtar (65) found that snow trout () when treated with ATZ showed significantly greater values of platelets but much lower values of hematocrit, hemoglobin, WBC, RBC, MCH, MCHC, monocytes, and lymphocytes.

Oxidative stress and alterations in erythrocyte membranes brought on by ATZ may be the cause of the harmful effects on hematological parameters. In this respect, the sensitivity of erythrocytes to exposure to ATZ was examined (66), and the findings suggest that exposure to ATZ significantly induced oxidative stress as indicated by elevated levels of malondialdehyde (MDA).

In the current study, although co-administration of LC and QT with ATZ produced similar results in terms of some hematological parameters such as HCT, MCV, MCH, MCHC and differential leukocytic count, the percentage of Hb, RBCs, platelets and WBCs counts interestingly showed significant improvement. The improving effects were greater with LC than with QT. In this regard, LC exhibited potential protective effects against cefquinome sulfate (CS)-induced alteration in hematological items (67). LC was effective in raising Hb and reticulocyte count in anemic patients with chronic renal disease, which is what drives it to alleviate such anemia (68). Additionally, After LC treatment in rats, it has been noted that the hematological parameters, including the differential leukocytic count, Hb concentration, platelets and RBC counts were greatly improved (69).

Furthermore, in instances of acute Cd poisoning, QT showed beneficial effects on certain hematological traits (70). Furthermore, administering lead acetate to rats reduces Hb levels and RBC counts while boosting WBCs. These variables were interestingly switched to normal levels by co-administering lead acetate with QT (71).

The high antioxidant activity of LC and QT may explain their protective effects against ATZ-induced hematological changes in our investigation, which has been previously proven (72,73). In this respect, LC (10 and 30 mM) was observed to shield RBCs from the oxidative stage during long-period storage by enhancing the antioxidant enzymes and counteracting lipid peroxidation (74,75). QT was helpful in mitigating the oxidative stress caused by eccentric exercise in red blood cells by lowering erythrocyte lipid peroxidation levels and reducing their vulnerability to hemolysis caused by free radicals (76). QT has been observed to be successful choice in the treatment of sickle hemoglobinopathies as it reduces the RBCs oxidative stress and platelet surface P-selectin (77).

Conclusion

In conclusion, LC and QT were found to be viable therapies for improving hemogram that could be impaired by exposure to environmental pollutants such as ATZ. However, we advise additional research into the actual molecular mechanisms via which LC or QT exert their positive effects.

References

[1] L Brevini TA, Zanetto SB, Cillo F. Effects of endocrine disruptors on developmental and reproductive functions. Current Drug Targets-Immune, Endocrine & Metabolic Disorders. 2005;5(1):1–10.

[2] Park HO, Bae J. Disturbed relaxin signaling pathway and testicular dysfunction in mouse offspring upon maternal exposure to simazine. 2012; e44856-e44856.

[3] Park S, Kim S, Jin H, Lee K, Bae J. Impaired development of female mouse offspring maternally exposed to simazine. Environ Toxicol Pharmacol. 2014;38(3):845–51.

[4] Cook LE, Finger BJ, Green MP, Pask AJ. Exposure to atrazine during puberty reduces sperm viability, increases weight gain and alters the expression of key metabolic genes in the liver of male mice. Reprod Fertil Dev. 2019;31(5):920–31.

[5] Cook LE, Finger BJ, Green MP, Pask AJ. Exposure to atrazine during puberty reduces sperm viability, increases weight gain and alters the expression of key metabolic genes in the liver of male mice. Reprod Fertil Dev. 2019;31(5):920–31.

[6] Morgan AM, Ibrahim MA, Hussien AM. Glycyrrhizic acid modulates the atrazine-induced apoptosis in rabbit spleen. Environmental Science and Pollution Research. 2019 Dec 1;26(34):34924–30.

[7] Smith PN, Armbrust KL, Brain RA, Chen W, Galic N, Ghebremichael L, et al. Assessment of risks to listed species from the use of atrazine in the USA: a perspective. J Toxicol Environ Health B Crit Rev. 2021;24(6):223–306.

[8] Hooghe RJ, Devos S, Hooghe-Peters EL. Effects of selected herbicides on cytokine production in vitro. Life Sci. 2000;66(26):2519–25.

[9] Vos JG, Krajnc EI, Beekhof PK, Van Logten MJ. Methods for testing immune effects of toxic chemicals: evaluation of the immunotoxicity of various pesticides in the rat. In: Mode of Action, Metabolism and Toxicology. Elsevier; 1983. p. 497–504.9.Ge J, Liu J, Wang T, Huang D, Li J, Zhang S, et al. Prolonged exposure to the herbicide atrazine suppresses immune cell functions by inducing spleen cell apoptosis in rats. Ecotoxicol Environ Saf. 2021;220:112386.

[10] Gammon DW, Aldous CN, Carr WC, Sanborn JR, Pfeifer KF. A risk assessment of atrazine use in California: Human health and ecological aspects. In: Pest Management Science. 2005. p. 331–55.

[11] Le Borgne F, Ravaut G, Bernard A, Demarquoy J. L-carnitine protects C2C12 cells against mitochondrial superoxide overproduction and cell death. World J Biol Chem. 2017;8(1):86.

[12] Dehghani F, Hassanpour A, Poost-Pasand A, Noorafshan A, Karbalay-Doust S. Protective effects of L-carnitine and homogenized testis tissue on the testis and sperm parameters of busulfan-induced infertile male rats. Iran J Reprod Med. 2013;11(9):693.

[13] Fielding R, Riede L, Lugo JP, Bellamine A. L-carnitine supplementation in recovery after exercise. Nutrients. 2018;10(3):349.

[14] Bebekah RJ, Saleh MIA, Mohammed A, Tanko Y. Modulatory Effect of L-carnitine on Red Blood Cell and Indices in Testicular Ischaemic-Reperfusion in Wistar Rats. Communication in Physical Sciences. 2023;10(2).

[15] Uluisik D, Keskin E. The effects of L-carnitine on some hematological parameters in rats fed a cholesterol-rich diet. Biotechnic & Histochemistry [Internet]. 2014 Jul 1;89(5):393–7. Available from: https://doi.org/10.3109/10520295.2014.892153

[16] Nikolaos S, George A, Telemachos T, Maria S, Yannis M, Konstantinos M. EFFECT OF L-CARNITINE SUPPLEMENTATION ON RED BLOOD CELLS DEFORMABILITY IN HEMODIALYSIS PATIENTS. Ren Fail [Internet]. 2000 Jan 1;22(1):73–80. Available from: https://doi.org/10.1081/JDI-100100853

[17] Aghaa OB, Hamad HT. THE CORRELATION BETWEEN L-CARNITINE UPTAKE AND SOME HEMATOLOGICAL PARAMETERS IN OXIDATIVE STRESSED RATS. Military Medical Science Letters/Vojenské Zdravotnické Listy. 2022;91(4).

[18] Karadeniz A, Simsek N, Cakir S. Haematological effects of dietary L-carnitine supplementation in broiler chickens. Revue Méd Vét. 2008;159(8–9):437–44.

[19] Adedara IA, Subair TI, Ego VC, Oyediran O, Farombi EO. Chemoprotective role of quercetin in manganese-induced toxicity along the brain-pituitary-testicular axis in rats. Chem Biol Interact. 2017 Feb 1;263:88–98.

[20] Cornard JP, Merlin JC. Spectroscopic and structural study of complexes of quercetin with Al (III). J Inorg Biochem. 2002;92(1):19–27.

[21] Murakami A, Ashida H, Terao J. Multitargeted cancer prevention by quercetin. Cancer Lett. 2008;269(2):315–25.

[22] Mansour AT, Mahboub HH, Amen RM, El-Beltagy MA, Ramah A, Abdelfattah AM, et al. Ameliorative Effect of Quercetin against Abamectin-Induced Hemato-Biochemical Alterations and Hepatorenal Oxidative Damage in Nile Tilapia, Oreochromis niloticus. Animals. 2022;12(23):3429.

[23] Badr GM, Elsawy H, Sedky A, Eid R, Ali A, Abdallah BM, et al. Protective effects of quercetin supplementation against short-term toxicity of cadmium-induced hematological impairment, hypothyroidism, and testicular disturbances in albino rats. Environmental Science and Pollution Research. 2019;26:8202–11.

[24] Abarikwu SO, Farombi EO. Quercetin ameliorates atrazine-induced changes in the testicular function of rats. Toxicol Ind Health. 2016;32(7):1278–85.

[25] Bazotte RB, Lopes-Bertolini G. Effects of oral L-carnitine and DL-carnitine supplementation on alloxan-diabetic rats. Brazilian archives of biology and technology. 2012;55:81–8.

[26] Jahan S, Iftikhar N, Ullah H, Rukh G, Hussain I. Alleviative effect of quercetin on rat testis against arsenic: a histological and biochemical study. Syst Biol Reprod Med. 2015;61(2):89–95.

[27] Abdel Aziz RL, Abdel-Wahab A, Abo El-Ela FI, Hassan NEHY, El-Nahass ES, Ibrahim MA, et al. Dose- dependent ameliorative effects of quercetin and L-Carnitine against atrazine- induced reproductive toxicity in adult male Albino rats. Biomedicine and Pharmacotherapy. 2018 Jun 1;102:855–64.

[28] Hohlbaum K, Bert B, Dietze S, Palme R, Fink H, Thöne-Reineke C. Impact of repeated anesthesia with ketamine and xylazine on the well-being of C57BL/6JRj mice. PLoS One. 2018;13(9):e0203559.

[29] Van Herck H, Baumans V, Brandt C, Boere HAG, Hesp APM, Van Lith HA, et al. Blood sampling from the retro-orbital plexus, the saphenous vein and the tail vein in rats: comparative effects on selected behavioural and blood variables. Lab Anim. 2001;35(2):131–9.

[30] Riffle BW, Klinefelter GR, Cooper RL, Winnik WM, Swank A, Jayaraman S, et al. Novel molecular events associated with altered steroidogenesis induced by exposure to atrazine in the intact and castrate male rat. Reproductive toxicology. 2014;47:59–69.

[31] Stanko JP, Enoch RR, Rayner JL, Davis CC, Wolf DC, Malarkey DE, et al. Effects of prenatal exposure to a low dose atrazine metabolite mixture on pubertal timing and prostate development of male Long-Evans rats. Reproductive toxicology. 2010;30(4):540–9.

[32] Victor-Costa AB, Bandeira SMC, Oliveira AG, Mahecha GAB, Oliveira CA. Changes in testicular morphology and steroidogenesis in adult rats exposed to Atrazine. Reproductive toxicology. 2010;29(3):323–31.

[33] Abarikwu SO, Adesiyan AC, Oyeloja TO, Oyeyemi MO, Farombi EO. Changes in sperm characteristics and induction of oxidative stress in the testis and epididymis of experimental rats by a herbicide, atrazine. Arch Environ Contam Toxicol. 2010;58:874–82.

[34] Friedmann AS. Atrazine inhibition of testosterone production in rat males following peripubertal exposure. Reproductive toxicology. 2002;16(3):275–9.

[35] Kniewald J, Jakominić M, Tomljenović A, Šimić B, Romac P, Vranešić Đ, et al. Disorders of male rat reproductive tract under the influence of atrazine. Journal of Applied Toxicology: An International Journal. 2000;20(1):61–8.

[36] Stoker TE, Laws SC, Guidici DL, Cooper RL. The effect of atrazine on puberty in male Wistar rats: an evaluation in the protocol for the assessment of pubertal development and thyroid function. Toxicological Sciences. 2000;58(1):50–9.

[37] Trentacoste S V, Friedmann AS, Youker RT, Breckenridge CB, Zirkin BR. Atrazine effects on testosterone levels and androgen-dependent reproductive organs in peripubertal male rats. J Androl. 2001;22(1):142–8.

[38] Chang J, Liang C, Wang W, Yong L, Mao W, Yang H, et al. Toxic effects of atrazine on immune function in BALB/c mice. Environmental Science and Pollution Research. 2021;28:37978–94.

[39] KHOZIMY A, El-Danasoury H, Abuzeid M. Biochemical effects of treatments with herbicide atrazine in male albino rats. Journal of the Advances in Agricultural Researches. 2022;27(1):43–57.

[40] KHOZIMY A, El-Danasoury H, Abuzeid M. Biochemical effects of treatments with herbicide atrazine in male albino rats. Journal of the Advances in Agricultural Researches. 2022;27(1):43–57.

[41] Laws SC, Ferrell JM, Stoker TE, Schmid J, Cooper RL. The effects of atrazine on female Wistar rats: an evaluation of the protocol for assessing pubertal development and thyroid function. Toxicological sciences. 2000;58(2):366–76.

[42] Fukamachi K, Han BS, Kim CK, Takasuka N, Matsuoka Y, Matsuda E, et al. Possible enhancing effects of atrazine and nonylphenol on 7, 12-dimethylbenz [a] anthracene-induced mammary tumor development in human c-Ha-ras proto-oncogene transgenic rats. Cancer Sci. 2004;95(5):404–10.

[43] Mac Loughlin C, Canosa IS, Silveyra GR, Greco LSL, Rodríguez EM. Effects of atrazine on growth and sex differentiation, in juveniles of the freshwater crayfish Cherax quadricarinatus. Ecotoxicol Environ Saf. 2016;131:96–103.

[44] Frontera JL, Vatnick I, Chaulet A, Rodríguez EM. Effects of glyphosate and polyoxyethylenamine on growth and energetic reserves in the freshwater crayfish Cherax quadricarinatus (Decapoda, Parastacidae). Arch Environ

[45] Avigliano L, Fassiano AV, Medesani DA, Ríos de Molina M del C, Rodriguez EM. Effects of glyphosate on growth rate, metabolic rate and energy reserves of early juvenile crayfish, Cherax quadricarinatus M. Bull Environ Contam Toxicol. 2014;92:631–5.

[46] Stumpf L, Tropea C, Greco LSL. Recovery growth of Cherax quadricarinatus juveniles fed on two high-protein diets: Effect of daily feeding following a cyclic feeding period on growth, biochemical composition and activity of digestive enzymes. Aquaculture. 2014;433:404–10.

[47] Cook LE, Finger BJ, Green MP, Pask AJ. Exposure to atrazine during puberty reduces sperm viability, increases weight gain and alters the expression of key metabolic genes in the liver of male mice. Reprod Fertil Dev. 2019;31(5):920–31.

[48] Lim S, Ahn SY, Song IC, Chung MH, Jang HC, Park KS, et al. Chronic exposure to the herbicide, atrazine, causes mitochondrial dysfunction and insulin resistance. PLoS One. 2009;4(4):e5186.

[49] Talenezhad N, Mohammadi M, Ramezani-Jolfaie N, Mozaffari-Khosravi H, Salehi-Abargouei A. Effects of l-carnitine supplementation on weight loss and body composition: A systematic review and meta-analysis of 37 randomized controlled clinical trials with dose-response analysis. Clin Nutr ESPEN. 2020;37:9–23.

[50] Askarpour M, Hadi A, Miraghajani M, Symonds ME, Sheikhi A, Ghaedi E. Beneficial effects of l-carnitine supplementation for weight management in overweight and obese adults: An updated systematic review and dose-response meta-analysis of randomized controlled trials. Pharmacol Res. 2020;151:104554.

[51] Adeva-Andany MM, Calvo-Castro I, Fernández-Fernández C, Donapetry-García C, Pedre-Piñeiro AM. Significance of l-carnitine for human health. IUBMB Life. 2017;69(8):578–94.

[52] Ramsay RR. The carnitine acyltransferases: modulators of acyl-CoA-dependent reactions. Biochem Soc Trans. 2000;28(2):182–6.

[53] Batista ML, Neves RX das, Peres SB, Yamashita AS, Shida CS, Farmer SR, et al. Heterogeneous time-dependent response of adipose tissue during the development of cancer cachexia. Journal of Endocrinology. 2012;215(3):363–73.

[54] Jiang F, Zhang Z, Zhang Y, Pan X, Yu L, Liu S. L-Carnitine ameliorates cancer cachexia in mice partly via the carnitine palmitoyltransferase-associated PPAR-γ signaling pathway. Oncol Res Treat. 2015;38(10):511–6.

[55] Jiang H, Horiuchi Y, Hironao KY, Kitakaze T, Yamashita Y, Ashida H. Prevention effect of quercetin and its glycosides on obesity

and hyperglycemia through activating AMPKα in high-fat diet-fed ICR mice. J Clin Biochem Nutr. 2020;67(1):75–83.

[56] Aguirre L, Arias N, Teresa Macarulla M, Gracia A, P Portillo M. Beneficial effects of quercetin on obesity and diabetes. Open Nutraceuticals J. 2011;4(1).

[57] Aguirre L, Arias N, Teresa Macarulla M, Gracia A, P Portillo M. Beneficial effects of quercetin on obesity and diabetes. Open Nutraceuticals J. 2011;4(1).

[58] Nabavi SF, Russo GL, Daglia M, Nabavi SM. Role of quercetin as an alternative for obesity treatment: you are what you eat! Food Chem. 2015;179:305–10.

[59] Akhtar N, Khan MF, Tabassum S, Zahran E. Adverse effects of atrazine on blood parameters, biochemical profile and genotoxicity of snow trout (Schizothorax plagiostomus). Saudi J Biol Sci. 2021;28(3):1999–2003.

[60] Mahmood NMS, Hamad KR. Effect of Fenugreek Seed Extract on Some Haematological and Biochemical Parameters in Atrazine Treated Male Rats. Zanco J Pure Appl Sci. 2017;28:113–26.

[61] Ramesh M, Srinivasan R, Saravanan M. Effect of atrazine (Herbicide) on blood parameters of common carp Cyprinus carpio (Actinopterygii: Cypriniformes). Afr J Environ Sci Tech. 2009;3(12).

[62] Kanu KC, Okoboshi AC, Otitoloju AA. Haematological and biochemical toxicity in freshwater fish Clarias gariepinus and Oreochromis niloticus following pulse exposure to atrazine, mancozeb, chlorpyrifos, lambda-cyhalothrin, and their combination. Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology. 2023;270:109643.

[63] Ezenwaji NE, Nelo WC, Oluah NS. Effect of oral administration of sublethal concentration of atrazine on the haematological profile of albino rat. Animal Research International. 2012;9(2).

[64]Dooley GP, Prenni JE, Prentiss PL, Cranmer BK, Andersen ME, Tessari JD. Identification of a novel hemoglobin adduct in Sprague Dawley rats exposed to atrazine. Chem Res Toxicol. 2006;19(5):692–700.

[65]Akhtar N, Khan MF, Tabassum S, Zahran E. Adverse effects of atrazine on blood parameters, biochemical profile and genotoxicity of snow trout (Schizothorax plagiostomus). Saudi J Biol Sci. 2021;28(3):1999–2003.

[66] Singh M, Sandhir R, Kiran R. Atrazine-induced alterations in rat erythrocyte membranes: Ameliorating effect of vitamin E. J Biochem Mol Toxicol. 2008;22(5):363–9.

[67] El-Sawy AESF, El-Maddawy ZK, Elsaady MK, Ibrahim HS. Ameliorative Effect of L-Carnitine against Hematological and Hepatorenal Alterations Induced by Cefquinome Sulfate in Male Albino Rats. J Adv Vet Res. 2023;13(5):730–6.

[68] Singh H, Jain D, Bhaduri G, Gupta N, Sangwan R. Study on effects of L-carnitine supplementation on anaemia with erythropoietin hyporesponsiveness and lipid profile in chronic kidney disease patients on maintenance haemodialysis. Indian J Basic Appl Med Res. 2020;9:224–32.

[69] Chidiebere U, Ambali SF, Ayo JO, Eseivo KAN. Acetyl-L-carnitine attenuates haemotoxicity induced by subacute chlorpyrifos exposure in Wistar rats. 2011;292-303.

[70] Donmez HH, Donmez N, Kısadere I, Undag I. Protective effect of quercetin on some hematological parameters in rats exposed to cadmium. Biotechnic & Histochemistry. 2019;94(5):381–6.

[71] Al-Omair MA, Sedky A, Ali A, Elsawy H. Ameliorative potentials of quercetin against lead-induced hematological and testicular alterations in Albino rats. Chin J Physiol. 2017;60(1):54–61.

[72] Xu D, Hu MJ, Wang YQ, Cui YL. Antioxidant activities of quercetin and its complexes for medicinal application. Molecules. 2019;24(6):1123.

[73] Bayrak S, Aktaş S, Altun Z, Cakir Y, Tütüncü M, Kum Özşengezer S, et al. Antioxidant effect of acetyl-l-carnitine against cisplatin-induced cardiotoxicity. Journal of International Medical Research. 2020;48(8):0300060520951393.

[74] Masannagari P, Rajashekaraiah V. Attenuation of Oxidative Stress in Erythrocytes Stored with Vitamin C and l-Carnitine in Additive Solution-7. Biopreserv Biobank. 2024;10.1089.

[75] Ravikumar S, Prabhu S, Vani R. Effects of L-carnitine on the erythrocytes of stored human blood. Transfusion Medicine. 2020;30(3):215–25.

[76] Duranti G, Ceci R, Patrizio F, Sgrò P, Di Luigi L, Sabatini S, et al. Chronic consumption of quercetin reduces erythrocytes oxidative damage: Evaluation at resting and after eccentric exercise in humans. Nutrition Research. 2018;50:73–81.

[77] Lizarralde MA, Merriweather B, Conrey A, Saxena A, Shet AS. Effects of flavonoid quercetin on thrombo-inflammatory processes in patients with sickle cell disease. Blood. 2021;138:2020.