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BIOCHEMICAL AND CYTOTOXIC EFFECT METRIBUZIN HERBICIDE ON ALBINO RATS IN SHORT TERM OF TOXICITY

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ABSTRACT

Metribuzin considered as pre and post emergent herbicide used to remove a wide range of grass weeds. We investigate the sub-chronic cytotoxic effect of metribuzin in male albino rats that intubated orally with 0,110,220, and 440 mg/kg for three months twice per week. We founded that metribuzin containing herbicide Sencor 70% caused decrease in weight gain ratio of albino rats that was proportional with increase in dose level. Metribuzin increased liver enzymes (ALT and AST) that considered main target organ also significantly elevated LDH, urea and creatinine while decreased glucose, cholesterol, total protein and albumin level when compared to control group. The meterbuzin had histopathological changes in liver, kidney and spleen and testes. Also had significant effect on RBCs count causing normocytic normochromic anemia while showed significant increase in leukocytic count. Additionally, metribuzin induce anomalies in both head and tail of sperm that were dose dependent and significant increase in anomalies observed at 440 mg/kg. Notably, meterbuzin at dose of 440 mg/kg reduced level of il-2 expression in liver and level of globulin. While at low dose increase expression of il-2 and globulin level. On conclusion, meterbuzin at highest dose (440 mg/kg) had hepatorenal, reproductive toxicity and immunomodulation effect especially, reduction level of il-2 expression in liver.

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INTRODUCTION

Pesticide residues cause pollution of water and soil, many studies by the using of different bioassays demonstrated that there are robust cytotoxic and genotoxic effect of insecticides, herbicides, fungicides, etc. (Dimitrovk, 2006).Herbicide considered most detected pollutant chemical in water and besides synthetic fertilizers have leads to disturbance in the biodiversity of the ecosystem and its residues enter food chain and finally ingested by human .so environmental risk and protection has become as a global worry so testing of herbicides before registration must be carried on through several tests .The use of biological control agents and bio herbicides have play a great role to minimize the risk of herbicides on the ecosystem (Kortekamp,2011). etribuzine or 4-amino-6-{1,1-dimethylethyl}-3-{methylthio}- 1,2,4-triazin-5(4H)-one is considered as a potent pre- and post-emergent herbicide used to remove a wide range of grass weeds from soyabean ,potato, sugar cane,tomato and other crops with high water solubility 1.22 mg/ L and has low to moderate persistence in the soil (Chauhan and Kumari, 2007).Metribuzine act by inhibition of the photosynthesis by interference with the transport of electron in the Hill reaction of photosystem-II (bind to a plastoquinone binding site of photosystem II on the D1 protein complex founded in the chloroplast thylakoid membranes), result in destruction of the plant membranes and cell death (Stepanov *et al*., 2012). According to EFSA, (2006) Metribuzin has orally a moderate acute toxicity and nearly completely and rapidly absorbed orally after 36 hours of exposure, distributed widely with the heighest concentration in liver and kidney with half-lives nearly 18.4 hours , not accumulated in tissues and 95% of administered dose excreted within 72 hours of dosing through

urine (40.4%) and faces (51.8%) mainly in male while in female greater excretion through urine . Kimmerle *et al*. (1969) and James Morgan, (2001) found that LD50 of metribuzin in rats was 2200 mg/kg Bwt. Besides showing very slight irritation on exposure .Liver and kidney considered the main target organs in rats in acute toxicity and long term besides thyroid gland due to liver enzymes induction (EFSA, 2006). Metribuzin reduce body weight and increase thyroid and liver weight according to at dose level 1500 ppm in a two months study in albino rats according to Loser *et al*., (1969). also showed histopathological changes in liver and kidney and increase Glucose-6-phosphate dehydrogenase and lactate dehydrogenase as a marker of tissue and cell injury and decrease glucose , cholesterol ,total protein level and antioxidant enzymes as catalase and the acetyl choline esterase enzyme and increase in oxidative stress parameters as protein carbonyl , lipid hydro peroxides and thiol containing components in four week study in gold fish according to Husak *et al*., (2014).

Notably, Quercetin Attenuates Metribuzin-enhanced hematological and biochemical toxic effect in adult Rats. Metribuzin also induced oxidative stress in tissues based on increase levels of malondialdehyde, lower activities of glutathione peroxidase and decreased levels of reduced glutathione (Kadeche *et al*., 2016). Metribuzin increased the oxidative stress parameters as as TBRS (thiobarbituric acid reactive substances) while level of SOD and catalase was significantly decreased in 30 day study in cray fish at dose level 3.06 mg/l according to Koutnik *et al*., (2014).also by the way Sencor (metribuzin-containing herbicide) Increase SOD activity, but decrease catalase, GPx, and GR activities in liver and kidney and alter the liver metabolism and histology (Mosiichuk *et al*., 2015). The sub chronic toxicity of metribuzine on the developmental stages and larvae of common carp had been assessed for 30 days at concentration 0.9, 4, 14, and 32 mg/L, showed increased mortality at high concentrations besides with histopathological abnormalities in caudal kidney and liver (Stepanov *et al*., 2012). Metribuzin also caused a significant decrease in the feed consumption ,body and muscle weight in association with increase in plasma triglyceride ,glucose, creatinine, urea, AST and ALT levels with significant decrease in cholesterol at dose level 13 mg/kg besides Total lipid and protein contents were also altered where there is increase in VLDL and LDL protein while HDL protein is reduced with presence of oxidative stress as decrease GSH level, vitamin C and catalase while increase in hydro peroxides and carbonyl protein in a three months study in rats dosed 1.3 and 13 mg/kg in potato diet (Chiali *et al*. , 2013). The aim of this study to understand cytotoxic effect of metribuzin through evaluation biochemical parameters , hematology and histopathological changes

MATERIAL AND METHODS

Experimental animals and grouping

Male albino rats obtained from experimental unit, Faculty of pharmacy, Mansoura university; weighted from 95 to 115 gm. Animals were apparently healthy and housed in plastic cages contain wood shaving as a bedding material. Animals accommodated for 2 weeks before the experiment and maintained on a balanced ration also feed and water given ad libitium throughout the experiment.

Rats divided into four groups each one contains eight rats weighted 115±5 gm ; First three groups intubated with metribuzin orally dissolved in distilled water at dose level 1/5, 1/10 and 1/20 of the LD_{50} (Kimmerle *et al*. , 1969 and James Morgan , 2001) and fourth intubated with distilled water as control. Animals weighted twice per week before dosing to maintain constant dose throughout the experimental period. Note . our experiment was carried according animal ethics approved by the animal ethics committee of faculty of veterinary medicine

Body weight gain determination

The initial body weight was determined then the body weight calculated throughout the experiment before each administration; the body weight gain % calculated according to the following formula (Bhardwa *et al*., 2010).

$$\text{Body weight gain \%} = \frac{\text{Final body wt – initial body weight}}{\textit{initial body weight}} \times 100$$

Biochemical analysis

Serum of all rats were analyzed by spectrophotometer for ALT (Young, 1990), AST activity (Young, 2001), LDH (Ann, 1982), total protein (Tietz *et al*., 1999), total albumin level (Young, 1975), serum Glucose (Kaplan, 1984), urea (Burtis at al., 1999), serum creatinine (Young, 1990) and cholesterol levels (Naito and Kaplan, 1984). Additionally, Serum globulin can be calculated by subtracting the amount of serum albumin from the serum total protein.

Hematology

Blood taken from the apex of the left ventricle of the heart with a syringe then collected in gel tubes contain K3EDTA as anticoagulant and Blood sample analysis was carried out by hematological analyzer. where hemoglobin (HGB), red blood cell count (RBC), mean corpuscular volume (MCV), hematocrit (HCT), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) also white blood cell count (WBC), differential white blood cell count neutrophils, monocytes ,lymphocytes , basophils and eosinophils were evaluated; The mean value and the standard deviation calculated for each parameter according to (Agbasi *et al*., 2015).

Histopathological studies

Specimens from liver, spleen, kidney and testes were fixed in formalin 10% and section of 5µ thickness prepared from specimens and stained with hematoxylin and eosin (H&E) and examined under the microscope according to (Bancroff *et al*.,1990).

Immunohistochemistry

Immunocytochemistry. For immunocytochemistry, the following specific MAbs were used: (a) rabbit anti-mice IL-2 antibody (1:100) (h133-sc-7896), (ready for use dilutions) (DAKO system) and secondary antibody goat ant rabbit was used. The terminal reaction product was visualized using DAB (brownish-black signal).

Statistical analysis

Data statically analyzed by for variance by one way ANOVA and least standard difference LSD that as described by Snedecor and Cochran (1989) through using computerized SPSS program version 13.0.

RESULTS

Body weight gain % for Metribuzin

The results showed that there was a drastic decrease in body weight gain % in groups 1/20, 1/10 and 1/5 of LD_{50} (equivalent to 440 , 220 and 110 mg/kg) respectively in respect to control value (table 1).

Table 1. Showed mean initial and final weight and body weight gain % in rats exposed orally to Metribuzin twice per week for three months at dose level 1/20, 1/10 and 1/5 of LD_{50}

	Mean Initial body weight	Mean Final body weight	Body weight gain %G1
G1 Control	97.50±1.33	188.63±12.39	93.47
G 2(1/20 of LD 50%)	114.45±2.72	200.25±8.94	74.97
G3 (1/10 of LD 50%))	126.75±1.29	200.75±13.02	58.38
G4 (1/5 of LD 50%)	138.50±4.27	192.63±8.73	39.08

Biochemical changes ascribed to administration of metribuzin at dose level 1/20, 1/10 and 1/5 of the LD50 equivalent to 440, 220 and 110 mg/kg orally for 3 months twice per week

All doses of metribuzin (1/20, 1/10 and 1/5 of the LD50) showed significant increase in AST, LDH and creatinine level while the highest dose (1/5 of the LD50) showed significant increase in ALT, urea level in respect to control value after administration of metribuzin orally for 3 months twice per week at dose level (1/20, 1/10 and 1/5 of the LD50). On the other hand, All doses of metribuzin (1/20, 1/10 and 1/5 of the LD50) showed significant decrease in albumin, protein, Glucose and cholesterol after administration of metribuzin orally for 3 months twice per week at dose level (1/20, 1/10 and 1/5 of the LD50) (table 2).

Table 2. Showed the biochemical changes ascribed to administration of metribuzin at dose level 1/20, 1/10 and 1/5 of the LD50 equivalent to 440, 220 and 110 mg/kg orally for 3 months twice per week

	ALT (U/l)	AST (U/l)	Glucos (mg/dl)	Cholesterol (mg/dl)	Albumin (g/dl)	Protein (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)	LDH (U/L)	Globulin
G1 Control	22.40±0.78^{a}	33.50±1.17^{a}	150.91±4.41^{a}	119.30±3.95^{a}	5.52±0.31^{a}	8.80±0.21^{a}	61.53±1.46^{a}	0.43±0.01^{a}	555.62±60.74^{a}	3.88±0.2
G2	23.68±2.12^{a}	51.37±5.56^{b}	124.46±10.27^{b}	92.59±12.23^{b}	3.93±0.27^{b}	7.99±0.38^{b}	61.92±5.35^{a}	0.67±0.04^{c}	1529.53±115.16^{c}	4±0.3
G3	24.84±1.92^{a}	91.62±4.27^{c}	117.65±5.14^{b}	69.12±7.12^{b}	3.84±0.45^{b}	7.36±0.22^{bc}	71.42±5.41^{ab}	0.92±0.02^{b}	1797.38±177.16^{c}	3.53±0.32
G4	34.00±3.39^{a}	94.87±7.37^{c}	93.37±5.14^{c}	43.87.7.28^{c}	3±0.31^{c}	6.81±0.07^{c}	75.61±3.90^{a}	1.25±0.02^{a}	1855.51±186.79^{a}	3.3±.1

A, b, c, d: Different letters are significantly different between groups at P≤0.05

Hematological finding

The results showed that there was significant decrease in total erythrocytic count in group 1/5 of LD_{50} in respect to control value while there was no significant change in HGB, PCV, MCV, MCH and MCHC values than control " normocytic normochromic anemia"; on the other hand there was significant increase in total and differential leukocytic count in group 1/5 of LD_{50} in comparison to control (table 3).

Table 3. Showed the hematological parameters in rats exposed orally to metribuzin twice per week for three months.

	RBCs (mc/liter cells/ul.)	HGB (g/dl)	PCV (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)	TOTAL WBCs (1000 cells/ul)	LYMPHO (1000 cells/ul)	Neutrophil (1000 cells/ul)	MONO (1000 cells/ul)	ESINO (1000 cells/ul)	BASO (1000 cells/ul)
G1 CONTROL	8.65±0.3 a	15.35±0.20 a	44.60±0.67 a	55.68±2.01 a	19.17±0.74 a	34.42±0.23 a	4.70±0.09^{a}	4.47±0.08^{a}	1.05±0.10 a	0.62±0.11 a	0.10±0.00 a	0.0
G2	7.75±0.3 ab	14.87±0.23 ab	44.47±0.38 a	57.76±2.93 a	19.34±1.13 a	33.44±0.28 a	7.22±0.08^{b}	4.77±0.04^{a}	1.52±0.08 a	0.80±0.04 a	0.12±0.02 a	0.0
G3	7.47±0.1 ab	14.85±0.34 ab	44.05±0.42 a	58.96±2.46 a	19.87±0.51 a	33.73±0.98 a	7.37±0.04^{b}	4.95±0.05^{a}	1.52±0.10 a	0.75±0.02 a	0.12±0.02 a	0.0
G4	7.21±0.0 b	14.92±0.19 a	43.62±0.13 a	61.45±0.56 a	20.68±0.28 a	34.21±0.41 a	7.62±0.04^{b}	5.05±0.06^{a}	1.68±0.06 a	0.82±0.04 a	0.12±0.02 a	0.0

Sperm abnormalities detection for metribuzin

The result showed that metribuzin induce anomalies in both head and tail of sperm that were dose dependent and significant increase in anomalies observed at 1/5 of LD_{50} dose level in comparison with control ; sperm head abnormalities include hookless ,amorphous, double headed, detached headed, banana-shaped sperm on the other hand sperm tail anomalies include bent ,coiled and double-tailed sperm (table 4, 5, fig 1).

Table (4) showed number of normal and abnormal sperms and % of abnormality recovered from rats exposed to metribuzin orally at dose level 1/20, 1/10 and 1/5 of LD_{50} twice per week for 3 months

	Total no.of spermatozoa	Normal spermatozoa	Abnormal spermatozoa	% of abnormality
Control	1000	971	29	2.9%
Group 1/20	1000	913	87	8.7%
Group 1/10	1000	869	131	13.1%
Group1/5	1000	825	175	17.5%
Total chi-square =123.72		Degree of freedom= 3		Probability=0000

Table 5. Showed chi square analysis for normal and abnormal sperm recovered from rats exposed to metribuzin orally at dose level 1/20, 1/10 and 1/5 of LD_{50} twice per week for 3 months

	Control	G1/20	G1/10	G1/5
Control				
G1/20	29.73**			
G1/10	69.30**	9.52*		
G1/5	114.77**	33.24**	7.13*	

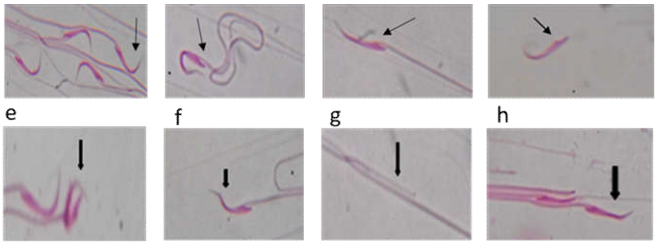

Fig (1) showed a) normal sperms b) coiled sperm c) hock less headed sperm d) detached headed sperm e) double headed sperm f) abnormal headed sperm h) double tailed sperm g) banana-shaped headed sperm recovered from rats exposed to metribuzin orally at dose level 1/20, 1/10 and 1/5 of LD_{50} twice per week for 3 months

Histopathological findings

Liver

Liver showed intralobular fibroblastic proliferation besides coagulative necrosis of the hepatocytes with leukocytic

infiltration and hyperplasia of interlobular ductules at dose level 1/5 of LD50 (equivalent to 440 mg/kg) while showed degenerative changes of the hepatocytes and severe congestion of the portal vein at dose level 1/10 and 1/20 of LD50 (equivalent to 220 and 110 mg/kg), results illustrated in fig (2a,b)

Kidney

Kidney showed fibrocollagenous proliferation besides coagulative necrosis of the renal tubules with leukocytic infiltration as mononuclear cells and dissolution of the renal glomeruli at dose level 1/5 of LD50 (equivalent to 440 mg/kg) while showed proliferation of the renal glomeruli with cloudy swelling of the renal tubular epithelium with interstitial lymphocytic infiltration at dose level 1/10 and 1/20 of LD50 (equivalent to 220 and 110 mg/kg), results illustrated in fig (2c,d)

Spleen

Spleen showed sever lymphoid tissue depletion with severe necrosis in the white pulp with hemosiderosis in the red pulp at dose level 1/5 of LD50 (equivalent to 440 mg/kg) while showed marked congestion of the splenic sinusoids with lymphoid tissue degeneration and depletion at dose level 1/10 and 1/20 of LD50 (equivalent to 220 and 110 mg/kg), results illustrated in fig (2e,f).

Testis

Testis showed degenerative changes of spermatocytes and spermatogonial cells that detached from the seminiferous tubules at dose level 1/5 of LD50 (equivalent to 440 mg/kg) while mild degenerative changes in the secondary spermatocytes at dose level 1/10 and 1/20 of LD50 (equivalent to 220 and 110 mg/kg), results illustrated in fig (2g, h)

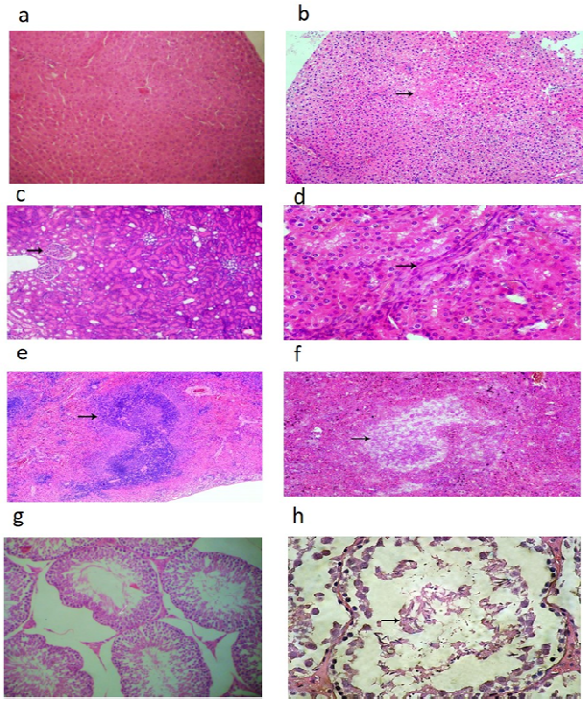

Fig. 2. Liver is showing coagulative necrosis of the hepatocytes (arrow). (HE, 100x) Kidney is showing fibrocollagenous proliferation (arrow). (HE, 400x) Spleen is showing severe necrosis of the lymphoid tissue. (HE, 100x)

Regarding immunomodulation of meterbuzin, at low dose of 110 mg/kg enhanced il-2 expression in liver and level of globulin while at higher dose 220 and 440 mg/kg reduced level of il-2 expression in liver and level of globulin (fig 3).

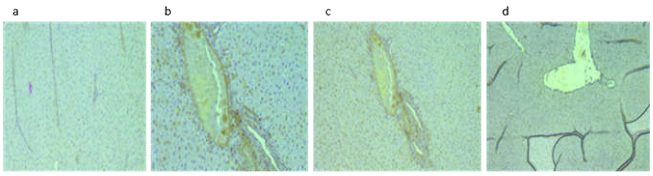

Fig (fig 3) show enhanced expression of il-2 in liver at dose of 110, 220 mg/kg of meterbuzin then reduced at highest dose of metribuzin treatment 440 mg/kg

DISCUSSION

Body weight gain % for Metribuzin

results showed that there was a drastic decrease in body weight gain % in groups 1/20, 1/10 and 1/5 of LD_{50} (equivalent to 110 , 220 and 440 mg/kg) respectively in respect to control value and such results agree with Chiali *et al*., (2013) who reported that In a chronic feeding study of a potato diet contain metribuzin at dose level 1.3 and 13 mg/kg for 3 months , the results showed a significant decrease in the feed consumption ,body and muscle weight and attributed according to Glusczak *et al*., (2006) that proposed that the usage of energy to detoxify compounds and alteration in protein and carbohydrate metabolism, and so less energy from food was available for promotion of growth also decrease in food consumption (table 1).

Biochemical changes

All doses of metribuzin (1/20, 1/10 and 1/5 of the LD50) showed significant increase in AST value while the highest dose (1/5 of the LD50) showed significant increase in ALT that considered according to Pereira *et al*., (2012) due to the leakage of transaminases from injured hepatocytes. ALT more specific for the hepatic injury whereas AST found in the liver , heart, kidney ,skeletal muscle and plasma; such result come in agreement with Chiali *et al*., (2013) who reported that In a chronic feeding study of a potato diet contain metribuzin at dose level 1.3 and 13 mg/kg for 3 months , the results showed a significant increase in plasma triglyceride ,glucose, creatinine, urea, AST and ALT levels that confirmed by the histopathological changes in liver that showed intralobular fibroblastic proliferation besides coagulative necrosis of the hepatocytes with leukocytic infiltration and hyperplasia of interlobular ductules.

All doses of metribuzin (1/20 ,1/10 and 1/5 of the LD50) showed significant increase in creatinine level and the highest dose 1/5 of the LD50 showed significant increase in urea level that used according to Chiali *et al*., (2013) to assess renal functions and that significant increase ensure renal failure also increase urea level may be due to increase protein catabolism ; such results come in agreement with Chiali *et al*., (2013) who reported that In a chronic feeding study of a potato diet contain metribuzin at dose level 1.3 and 13 mg/kg for 3

months ,the results showed that significant increase in plasma triglyceride ,glucose, creatinine, urea, AST and ALT levels and also confirmed with the histopathological changes in kidneys that showed that fibrocollagenous proliferation besides coagulative necrosis of the renal tubules with leukocytic infiltration as mononuclear cells and dissolution of the renal glomeruli. All doses of metribuzin (1/20 ,1/10 and 1/5 of the LD50) showed significant decrease in albumin, protein, cholesterol and Glucose after administration of metribuzin orally for 3 months twice per week that result attributed to Chiali *et al*., (2013) due decrease in cholesterol synthesis, decreased food intake and metabolic disruption with disturbances in protein , lipid and carbohydrates metabolism ; such results agree with Maksymiv *et al*., (2015) who reported that Acute toxicological study of metribuzine assessed after 96 h exposure in goldfish to 7.14, 35.7 and 71.4 mg/L and showed that decrease in glucose concentration in liver and plasma by 55% while increase in activity of alanine aminotransferase ,aspartate aminotransferase and lactate dehydrogenase ,also agree with Chiali *et al*., (2013) who founded that that In a chronic feeding study of a potato diet contain metribuzin at dose level 1.3 and 13 mg/kg for 3 months ,the results showed that significant decrease in cholesterol at dose level 13 mg/kg besides Total lipid and protein contents , also agree with Husak *et al*., (2014) who reported that Sencor (metribuzin-containing herbicide) toxicity in gold fish had been assessed in a 4 weeks study showed significant decrease in glucose , cholesterol ,total protein level and such results confirmed with decrease in the body weight gain ratio.

All doses of metribuzin (1/20 ,1/10 and 1/5 of the LD50) showed significant increase in LDH level that that attributed according to Tripathi and Shukla, (1990) to release of isozymes of LDH (five isoenzymes) from destroyed tissues of liver , muscles, lungs, reticuloendothelial system and heart; such results agree with Husak *et al*., (2014) who reported that Sencor (metribuzin-containing herbicide) toxicity in gold fish had been assessed in a 4 weeks study showed that significant increase in increase the enzyme activities in kidney as Glucose-6-phosphate dehydrogenase and lactate dehydrogenase as a marker of tissue and cell injury; also agree with Maksymiv *et al*., (2015) who founded that Acute toxicological study of metribuzine assessed after 96 h exposure in goldfish to 7.14, 35.7 and 71.4 mg/L and showed that decrease in glucose concentration in liver and plasma by 55% while increase in activity of alanine aminotransferase ,aspartate aminotransferase and lactate dehydrogenase and increase liver peroxides and such results confirmed with the destructive effect and histopathological changes of the kidney (table 2).

Hematological finding for metribuzin

Results showed that there was significant decrease in total erythrocytic count in group 1/5 of LD_{50} in respect to control value while there was no significant change in HGB, PCV, MCV, MCH and MCHC values than control " normocytic normochromic anemia" ; on the other hand there was significant increase in total and differential leukocytic count in group 1/5 of LD_{50} in comparison to control and such result agree with Velisek *et al*., (2008) the acute effect of metribuzin in rainbow trout was a significant lowering in erythrocyte count due to the effect of pesticides on organ forming blood represented by Bone marrow and also inhibition in many steps of the biosynthesis on the other hand increase in total and differential leukocytic count attributed according Velisek *et al*., (2008) to the attempt to fight against the and to promote the health status and such results agree with Chiali *et al*., (2013) In a chronic feeding study of a potato diet contain metribuzin at dose level 1.3 and 13 mg/kg for 3 months perivascular lymphocytic infiltration in the Centro lobular vein , inflammatory edema in hypertrophied muscle cells confirm the inflammatory response of the body to metribuzin (table 3).

Sperm abnormalities detection for metribuzin

The result showed that metribuzin induce anomalies in both head and tail of sperm that were dose dependent and significant increase in anomalies observed at 1/5 of LD_{50} dose level ; sperm head abnormalities include hookless ,amorphous, double headed, detached headed, banana-shaped sperm on the other hand sperm tail anomalies include bent ,coiled and double-tailed sperm and such results agree with (James Morgan,2001) who reported that in Reproductive study in beagle dogs show that reduced absolute testes weight besides causing immature testes and prostate at high concentration with systemic toxicity also (EPA,2015) proposed that Metribuzin was positive in steroidogenesis assay where it result in increase in estradiol production and reduce the aromatase activity in the aromatase assay also such results confirmed by histopathology of testes where Testis showed degenerative changes of spermatocytes and spermatogonial cells that detached from the seminiferous tubules at dose level 1/5 of LD50 **(**equivalent to 440 mg/kg) while mild degenerative changes in the secondary spermatocytes at dose level 1/10 and 1/20 of LD50 (equivalent to 220 and 110 mg/kg) (table 4,5 fig1).

Histopathological findings ascribed to metribuzin administration

Liver showed intralobular fibroblastic proliferation besides coagulative necrosis of the hepatocytes with leukocytic infiltration and hyperplasia of interlobular ductules at dose level 1/5 of LD50 (equivalent to 440 mg/kg)while showed degenerative changes of the hepatocytes and severe congestion of the portal vein at dose level 1/10 and 1/20 of LD50 (equivalent to 220 and 110 mg/kg)also Kidney showed fibrocollagenous proliferation besides coagulative necrosis of the renal tubules with leukocytic infiltration as mononuclear cells and dissolution of the renal glomeruli at dose level 1/5 of LD50 (equivalent to 440 mg/kg) while showed proliferation of the renal glomeruli with cloudy swelling of the renal tubular epithelium with interstitial lymphocytic infiltration at dose level 1/10 and 1/20 of LD50 (equivalent to 220 and 110 mg/kg) and such results agree with Chiali *et al*., (2013) who proposed that In a chronic feeding study of a potato diet contain metribuzin at dose level 1.3 and 13 mg/kg for 3 months liver show perivascular lymphocytic infiltration in the Centro lobular vein and degeneration in renal tubules also agree with Husak *et al*., (2014) who declared that Sencor (metribuzin-containing herbicide) toxicity in gold fish had been assessed in a 4 weeks study reviewed that histopathological changes in kidney and liver , as hypertrophy

of inter-tubular tissue and tubular epithelium show degeneration and necrosis further multiple hemorrhagic areas had been seen besides increase the enzyme activities in kidney also Maksymiv *et al*., (2015) founded that in Acute toxicological study of metribuzine assessed after 96 h exposure in goldfish to 7.14, 35.7 and 71.4 mg/L liver dilated sinusoids, diffuse hemorrhage and hepatic cell dystrophy, also results confirmed by Bleeke *et al*., (1985) who proposed that Acute toxicological study in mice exposed to 150 and 250 mg/kg of metribuzin intraperitoneally for 24 hour reveal that metribuzin is hepatotoxic hepatotoxicity and lethality of metribuzin due to reactive intermediate where detoxification of metribuzine by sulfoxidation result in electrophilic metabolites that deplete GSH then bind to liver protein produce hepatotoxicity also EFSA, (2006) stated that Liver and kidney considered the main target organs in rats in acute toxicity and long term (fig 2) .

Notably, meterbuzin at dose of 440 mg/kg reduced level of il-2 expression in liver and level of globulin. While at low dose increase expression of il-2 and globulin level. Similar studies, at high concentrations (25–100 μM) of metribuzin treated rat spleen lymphocytes stimulated by mitogens, metribuzin enhanced a dose-dependent inhibition of lymphocyte proliferation and cytokines (Medjdoub *et al*., 2011, Medjdoub *et al*.,2016) (fig 3). On conclusion, meterbuzin at highest dose (440 mg/kg) had hepatorenal, reproductive toxicity and immunomodulation effect especially, reduction level of il-2 expression in liver and globulin.

All authors had no conflict of interest

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