



# Triazinone herbicide metribuzin induced acute liver injury: A study of animal model

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## ABSTRACT

**Objective:** To evaluate the acute toxicity effect of metribuzin at low dose on liver of mother rabbits and its fetus. **Methods:** Pregnant female rabbits *Oryctolagus cuniculus* were divided into three groups ( $n=5$ ). The first group of non-treated pregnancy rabbits served as control; the second group of pregnancy rabbits were treated with 1/100 LD<sub>50</sub> of metribuzin and the third group of pregnancy rabbits were treated with 1/50 LD<sub>50</sub> of metribuzin. Metribuzin was added in their drinking water for 60 d before and during pregnancy. Levels of liver malondialdehyde, liver glutathione S transferase, serum glutamic oxaloacetic transaminase and serum glutamic-pyruvic transaminase were determined. Liver reduced glutathione level was also determined by a colorimetric method. And hepatic homogenate was analyzed by HPLC analysis to determine the existence of traces of metribuzin. **Results:** Results revealed a significant increase in level of liver malondialdehyde, glutathione S transferase, serum glutamic oxalacetic transaminase and serum glutamic-pyruvic transaminase activities in mother and fetuses rabbits of both metribuzin treatment groups as compared to the control group. However the level of reduced glutathione was decreased in mother and fetuses rabbits of both groups treated with metribuzin compared to control group. Also, the results obtained by HPLC technique showed the presence of trace metribuzin in liver cells of mothers and fetuses rabbits of the both metribuzin treated groups. **Conclusions:** In conclusion, this study shows that exposure to metribuzin at low concentrations causes a acute toxicity in liver of mother rabbits and its fetus, also the trace of the metribuzin detected in the liver is the origin of possible malformation of the fetuses or abortion of the rabbits.

## 1. Introduction

Agricultural pesticides contribute to increasing agricultural productivity but at the same time pose potential risks to human health and the environment[1]. Algeria is ranked among the countries that use the largest quantities of pesticides, including the Algerian Association for the Protection of the Environment. Algeria is a major consumer of pesticides, 30 000 tons are applied every year[2]. Each food is likely to contain different pesticide

residues[3]. Sanitary control of food products is an important part of the program of activities of the Public Health Committee of the Partial Agreement in the Social and Public Health Field[4]. The limits for pesticide residues in food should be contingent on control and should take into account the maximum international limits recommended for pesticide residues developed by the Codex

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Alimentarius Commission[5]. Pesticides can be very harmful and are suspected to pose a risk to human health and the environment by accumulating in ecosystems[6]. They are frequently implicated in the degradation of the quality of fresh and coastal waters, air and soil, in the reduction of terrestrial biodiversity found in agricultural areas and in contaminated natural environments or Even in cases of excess mortality of bees and decreased production of hives[7]. The access of pesticides to living structures is either voluntary (experimental toxicology, suicide) or involuntary (accidental exposure). There are generally three main modes of access: inhalation (vapors, aerosols), contact (topical application) (skin, mucous membranes) and oral ingestion (spontaneous or forced, including criminal acts) [8]. Metribuzin [4-amino-6-tert-butyl-3- (methylthio)-1,2,4-triazin-5-one] is used worldwide as a pre- and post-emergence selective herbicide on grasses and Broadleaf weeds. It is applied to various crops, including alfalfa, asparagus, corn, potatoes and tomatoes[9]. Metribuzin has moderate acute oral toxicity ( $LD_{50}$  322 mg/kg bw) and low dermal and inhalation toxicity ( $LD_{50}$  > 5 000 mg/kg bw and  $LC_{50}$  > 2.0 mg/L, respectively). It is neither an irritant to the skin and eyes nor a skin sensitizer[10]. The aim of this study is to estimate the toxicity effect of pesticides Metribuzin on liver in mother rabbits and its fetus .

## 2. Materials and methods

### 2.1. Animals

In our study we used female rabbits *Oryctolagus cuniculus* with initial weight between 1 240-1 776 g. They were placed in three groups of 5 rabbits in each and kept in animal's house of Department of cellular and molecular biology, University of El Oued, Algeria. The animals are carried in a laboratory place for adaptation with conditions of temperature ( $18.08 \pm 0.62$  °C, humidity ( $64.59 \pm 1.14$ )% and photoperiod (12 hours of light/12 hours of black). Access to Standard diet and water is free for animals ad libitum during the experiments. The realization of the experimental part is respect to the ethical approval.

### 2.2. Experimental design

Coupling method was assisted by placing the individual females overnight in the home cage of a singly-housed male of the same stock. Positively pregnant females were only chosen and randomly divided into the following three groups (5 rabbits):

- Group I: the female rabbits were treated with deionized water only from 1 to 20 day of pregnancy (control group);
- Group II (M 1/100  $LD_{50}$ ): the female rabbits were given 3.22 mg/kg (1/100  $LD_{50}$ ) of metribuzin in drinking water from 60 days before and during pregnancy;

- Group III (M 1/50  $LD_{50}$ ): the female rabbits were given 6.44 mg/kg (1/50  $LD_{50}$ ) of metribuzin in drinking water from 60 days before and during pregnancy.

Metribuzin solution was prepared by dissolving in distilled water in concentrations of 3.22 mg/kg and 6.44 mg/kg of metribuzin respectively. The metribuzin solution was prepared every 2 days to minimize the metribuzin precipitates. Evaluate the body weight and food intake were controlled during the experiment.

### 2.3. Blood collection and tissue preparation

After of 8 weeks of metribuzin treatment and on day 20 of gestation, rabbits were fasted for 16 h, then sacrificed, the blood was collected in tubes without anticoagulants. The serum was obtained by centrifuging the blood at 3 000 rpm for 10 min and then stored at 20 °C, and used for transaminases activities assay. Then, the abdominal cavity was opened and the fetuses were gently removed and weighted separately (Figure 1). The liver of mother rabbits and fetuses of different groups was rapidly excised, weighed and stored at -20 °C until use for HPLC analysis and oxidative stress evaluation.



Figure 1. Fetus rabbits in amniotic sac.

### 2.4. Determination of transaminases activities

Measurement of transaminase, glutamic-pyruvic transaminase (GPT) and glutamic oxalacetic transaminase (GOT) activities were carried out by commercial kits from Spinreact (Girona, Spain) (ref: GPT-1001171 and GOT-1001161).

### 2.5. Antioxidants measurement

#### 2.5.1. Preparation of homogenates

One gram of liver from each mother rabbit and fetuses of the different experiment groups was used. The tissues were milled and homogenized in 9 mL of buffer solution of TBS (50 mM Tris, 150 mM NaCl, pH 7.4). The tissue suspension was centrifuged at 9 000 rpm for 15 min at 4 °C, the supernatant obtained was stored at -20 °C until use for the oxidative stress marker assay.

#### 2.5.2. Determination of lipid peroxidation

Liver lipid peroxidation levels was measured as malondialdehyde (MDA) which measured according to the technique of Sastre *et*

al.[11]. The method is based on the reaction between the carbonyl compounds of malondialdehyde with thiobarbituric acid to give absorbent pink chromophores at 532 nm. MDA level was expressed as nmol of MDA/mg prot.

### 2.5.3. Level of reduced glutathione (GSH) assay

Liver GSH level was determined by a colorimetric method according to the technique described by Ellman[12], the measurement of optical density results from the formation of thionitrobenzoic acid from the reduction of 5,5'-dithiodis-2-nitrobenzoic acid, which is called the Ellman reagent with the SH groups exist in GSH, which has an absorbance at 412 nm. Total GSH level was expressed as nmol GSH/mg prot.

### 2.5.4. Activity of glutathione-S-transferase (GST) assay

The activity of GST in Liver was measured spectrophotometrically by the method of Habig *et al*[13]. based on the formation kinetics of a complex between a GST substrate: 1-chloro-2-4-dinitrobenzene (CDNB) and GSH. The complex formed can be visualized by increasing the optical density at a 340 nm. The GST activity was expressed as nmol CDNB /min/mg prot.

## 2.6. HPLC analysis

The hepatic homogenates were filtered before injection. An HPLC system was used with a detector at  $\lambda = 238$  nm, the column used was of length 25 cm and diameter 4.6 mm, the stationary phase C18 and the mobile phase acetonitrile/water (60:40; v/v). Metribuzin was evaluated under the following conditions:

Debit: 1 mL/ min; injection volume: 20  $\mu$ L; temperature at: 25 °C; pressure 8.5-8.6; with acquisition time 20 min .The standard peak of metribuzin (Figure 1) was made by the pesticide (Vapcor©) (70% metribuzin) by comparing the retention times according to method of Johnson And Pepperman[14] and Elsayed And Prasher[15].

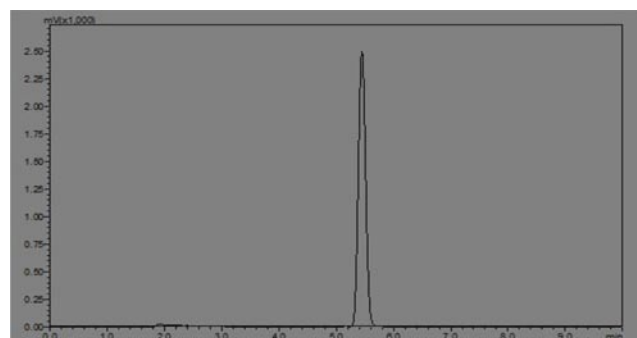


Figure 2. Chromatogram of metribuzin.

## 2.7. Statistical analysis

The statistical evaluation was carried out by the student's t test using Minitab 17.1 statistical package and the Excel 16.0 (Microsoft). The values were given as mean $\pm$ standard deviations (SD) for three groups of 5 rabbits each. Statistical significance was defined as  $P < 0.05$ .

## 3. Results

### 3.1. Initial body weight, body weight gain, relative liver weight and food intake

The results in the Table 1 showed that the body weight gain and food intake were significantly decreased ( $P < 0.05$  and  $P < 0.001$ ) in M 1/100 LD<sub>50</sub> and M 1/50 LD<sub>50</sub> groups compared to the control respectively. In another side, the results also showed that the relative liver weight was significantly increased ( $P < 0.01$ ) in animals contaminated with 1/100 LD<sub>50</sub> and 1/50 LD<sub>50</sub> of metribuzin compared to the control group.

### 3.2. Transaminases activities

The results in Figure 2 showed that the activities of the transaminases were significantly increased ( $P < 0.01$  and  $P < 0.001$ ) in the M 1/100 LD<sub>50</sub> and M 1/50 LD<sub>50</sub> groups for GOT activity respectively and highly significant increased ( $P < 0.001$ ) in the two groups treated with metribuzin for GPT activity compared to the control group.

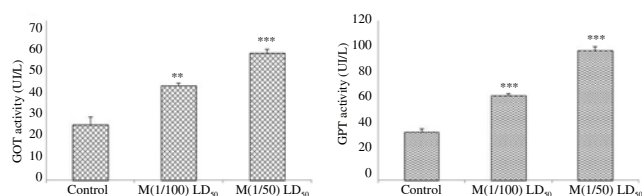
Table 1

Initial body weight, body weight gain, relative liver weight and food intake of control and experimental animals.

Parameters	Initial body weight (g)	Gain body weight (g/day/rab.)	Relative liver weight (g/100 g b.w)	Food intake (g)
Control	1 764.0 $\pm$ 12.8	5.88 $\pm$ 0.82	2.360 $\pm$ 0.189	390.30 $\pm$ 3.96
M (1/100) LD <sub>50</sub>	1 680.0 $\pm$ 10.2	5.14 $\pm$ 1.63*	2.945 $\pm$ 0.151**	379.47 $\pm$ 3.51*
M (1/50) LD <sub>50</sub>	1 308.5 $\pm$ 28.5	2.18 $\pm$ 0.19***	2.832 $\pm$ 0.323**	346.15 $\pm$ 8.22***

Means $\pm$  SE from 5 animals in each group.

Significance compared with control: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

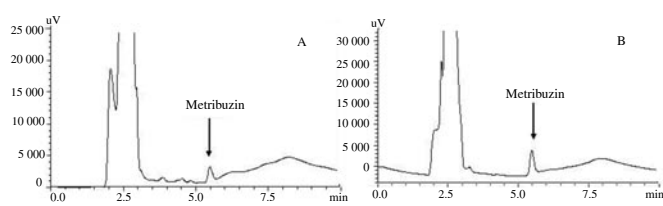


**Figure 3.** Transaminases (GOT and GPT) activities in control and experimental animals.

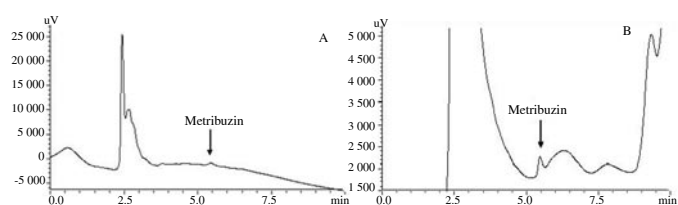
Means  $\pm$  SE from 5 animals in each group. Significance compared with control: \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

### 3.3. HPLC analysis

HPLC Analysis of the hepatic homogenate showed clearly the existence of traces of metribuzin in liver of all mother rabbits treated for 2 months with 1/100 LD<sub>50</sub> and 1/50 LD<sub>50</sub> of metribuzin (Figure 4). Result also showed that the technique of HPLC detected a trace of metribuzin in the fetal liver with different concentration for both groups of the metribuzin (Figure 5).



**Figure 4.** Chromatogram of metribuzin in liver rabbit (A) mother rabbit of group treated with (1/100) LD<sub>50</sub> of metribuzin (B) mother rabbit of group treated with (1/50) LD<sub>50</sub> of metribuzin.



**Figure 5.** Chromatogram of metribuzin in fetal liver of rabbits.

(A) fetal of rabbit treated with (1/100) LD<sub>50</sub> of metribuzin (B) fetal of rabbit treated with (1/50) LD<sub>50</sub> of metribuzin.

### 3.4. Oxidative stress parameters

As regards the results of oxidative stress, the Table 2 showed that the liver concentration of MDA and GST activity were significantly increased ( $P < 0.01$  and  $P < 0.001$ ) and the level of GSH was significantly decreased ( $P < 0.05$ ) in liver of mother rabbit group exposed to metribuzin at dose 1/100 LD<sub>50</sub>. MDA and GST activity were significantly increased ( $P < 0.01$  and  $P < 0.001$ ) and the level of GSH was significantly decreased ( $P < 0.01$ ) in liver of mother rabbit group exposed to metribuzin at the dose 1/50 LD<sub>50</sub> compared to the control rabbit.

**Table 2**

Liver oxidative stress parameters of mother rabbits.

Parameters	MDA ( $\mu\text{mol/mg prot}$ )	GSH ( $\text{nmol/mg prot}$ )	GST (nmol/min/ mg prot)
Control	0.065 $\pm$ 0.005	28.50 $\pm$ 5.38	0.120 $\pm$ 0.090
M (1/100) LD <sub>50</sub>	0.108 $\pm$ 0.012**	24.27 $\pm$ 3.84*	0.210 $\pm$ 0.020***
M (1/50) LD <sub>50</sub>	0.136 $\pm$ 0.024**	20.01 $\pm$ 2.7**	0.230 $\pm$ 0.012***

Means  $\pm$  SE from 5 animals in each group.

Significance compared with control: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

As shown in Table 3, concentration of MDA and GST activity were significantly increased ( $P < 0.01$  and  $P < 0.001$ ) and the level of GSH was significantly decreased ( $P < 0.01$ ) in liver fetus of rabbit exposed to metribuzin at dose 1/100 LD<sub>50</sub>. MDA and GST activity were significantly increased ( $P < 0.01$  and  $P < 0.001$ ) and the level of GSH was significantly decreased ( $P < 0.01$ ) in liver fetus of rabbit exposed to metribuzin at the dose 1/50 LD<sub>50</sub> compared to the control rabbit.

**Table 3**

Liver oxidative stress parameters of fetal rabbits.

Parameters	MDA ( $\mu\text{mol/mg prot}$ )	GSH ( $\text{nmol/mg prot}$ )	GST (nmol/min/ mg prot)
Control	0.033 $\pm$ 0.004	6.20 $\pm$ 0.32	0.002 $\pm$ 0.100
M (1/100) LD <sub>50</sub>	0.046 $\pm$ 0.009*	5.80 $\pm$ 0.86**	0.006 $\pm$ 0.270***
M (1/50) LD <sub>50</sub>	0.052 $\pm$ 0.010**	4.72 $\pm$ 0.57**	0.005 $\pm$ 0.340***

Means  $\pm$  SE from 5 animals in each group.

Significance compared with control: \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

## 4. Discussion

Metribuzin is a selective thiazine herbicide used mostly on vegetable crops. There is evidence from animal studies that metribuzin may cause adverse health effects, such as liver enzyme activities, endocrine modifications and fetus toxicity [16]. The objective of our study is to evaluate the acute toxicity of metribuzin to liver of mother rabbit and its fetus. The results of the effect of metribuzin on the body weight of rabbits show that there is a significant decrease, probably due to reduced food intake during the experiment. Results are consistent with the study of MORGAN, which used rabbits treated with metribuzin for 6-18 d. This study reported a correlation between decreased food intake, along with losing body weight of female rabbits during the period of treatment [17]. However, this weight loss is probably due to anorexia induced by the ingestion of this xenobiotic following continuous exposure over a long period [18]. The elevation of the relative weight of the liver of the M 1/100LD<sub>50</sub> and M 1/50 LD<sub>50</sub> groups indicated in our study may be due to liver necrosis due to the accumulation of lipid and metribuzin in hepatic cells [19]. This is in line with the presence of liver metribuzin reported in our results. Moreover, our results also show that there is an increase in the transaminases activities

(GOT, GPT) in the two groups treated with metribuzin compared to the control group. These results are consistent with the study by Merzouk *et al.* which showed an increase in plasma transaminases activities (GOT and GPT) in rats exposed to low dose metribuzin[20]. These results can probably be explained by the acute cytotoxic effect of metribuzin on liver cells. Transaminases are essential enzymes of cytolysis[21], they are active in the liver, heart and muscles. They pass into the serum in case of hepatic or muscular cytolysis. An important increase is observed in the cytolysis of toxic hepatitis[22]. The results of hepatic HPLC analysis show that there is a trace of metribuzin in the livers of treated rabbits and shows that hepatic cells are targets of metribuzin which is the pathogen responsible for Toxicity and hepatic lysis confirmed by the elevation of serum transaminases. Our results are in agreement with the study of EFSA Scientific Report, which shows that the liver is the important organ targeted by metribuzin[10]. The presence of metribuzin peak in the fetal liver also shows that this pesticide can cross the blood-brain barrier and rejoin the fetal bloodstream, which is metabolized in the liver, causing several pathologies or fetal-maternal complications induced by metribuzin. The results of the effect of metribuzin on the oxidative stress parameters show an increase in the concentration of hepatic MDA in all the groups studied including the mother rabbits exposed to metribuzin and its fetuses. These results are in agreement with the study of Kadeche *et al.* which shows an increase in tissue MDA concentration in rats exposed to low dose of metribuzin[23]. Metribuzin is capable of inducing intracellular oxidative stress[24]. Oxidative stress at the cellular level reflects an increase in MDA, a product of lipid peroxidation of membranes[25], which occurs at the level of membrane structures in cells (rich in phospholipids containing Unsaturated fatty acids) which are responsible to be altered by the radical forms. In the presence of free radicals and oxygen derivatives, the unsaturated fatty acids undergo oxidative degradation[26]. The free radicals that leads to lipid peroxidation are just inflammation, macrophages and neutrophils can produce very high amounts of reactive oxygen species, which produces via membrane NADPH oxidases[27]. The results of the effect of metribuzin on antioxidant parameters also show an increase in GSH level and GST activity in the liver. GSH is a non-enzymatic antioxidant that contributes to the defense system in the body against oxidative stress induced by reactive oxygen species[28]. The decrease in GSH is probably due to the appearance of a large amount of peroxides under the influence of metribuzin. The ability of glutathione to reduce The hydroperoxides formed during the metribuzin metabolism under the action of glutathione peroxidase lead to the massive oxidation of glutathione to oxidized glutathione leading to an imbalance in the GSH/GSSG ratio[29]. This leads to the consumption of GSH which is the reason for the significant decrease in its level. The involvement of antioxidant enzymes such as GST as protective factors of cells and organs against toxic agents and oxidative stress[30]. The enzyme that catalyzes GSH conjugation to a

wide variety of endogenous and exogenous electrophilic compounds is GST, which possesses the detoxification capability where their role in the cellular protection of oxidative stress appears[31]. The conjugation of metribuzin metabolites with GSH followed by the conversion of mercapturic acid derivatives appears to play a major role in detoxification and excretion[32].

The present study exhibits the toxic effects of metribuzin on liver by induced oxidative stress in mother rabbits and its fetus. From the study, it can be further concluded that the trace of the metribuzin detected in the liver is the origin of possible malformation of the fetuses or abortion of the rabbits.

### Conflict of interest statement

We declare that we have no conflict of interest.

### References

- [1] Popp J, Pet K, Nagy. Pesticide productivity and food security. *J Agron Sustain Dev* 2013; **33**: 243. doi:10.1007/s13593-012-0105-x.
- [2] Chiali FZ, Merzouk H, Merzouk SA, Medjdoub A, Narce M. Chronic low level metribuzin exposure induces metabolic alterations in rats. *Pest. Biochem Physiol* 2013; **106**: 38–44.
- [3] Fan AM, Jackson RJ. Pesticides and food safety. *Regulatory Toxicol Pharmacol* 1989; **9**(2): 158-174.
- [4] Mara D, Lane J, Scott B, Trouba D. Sanitation and Health. *PLoS Med* 2010; **7**(11): e1000363. doi:10.1371/journal.pmed.1000363.
- [5] Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Determination, interpretation and application of residue limits. *Food Control* 1997; **8**: 55-62.
- [6] Wasim MA, Dwaipayan S, Chowdhury A. Impact of pesticides use in agriculture: Their benefits and hazards. *Interdiscip Toxicol* 2009; **2**: 1-12.
- [7] Christos AD, Eleftherohorinos IG. Pesticide exposure, safety issues, and risk assessment indicators. *Int J Environ Res Public Health* 2011; **8**: 1402–1419.
- [8] Christos AD, Spyridon DK. Farmers' exposure to pesticides: Toxicity types and ways of prevention. *Toxics* 2016; **4**: 1. doi:10.3390/toxics4010001
- [9] Velisek J, Svobodova Z, Piackova V, Novotny L, Blahova J, Sudova E, et al. Effects of metribuzin on rainbow trout (*Oncorhynchus mykiss*). *Vet Med* 2008; **6**: 324-332.
- [10] EFSA scientific report. Conclusion regarding the peer review of the pesticide risk assessment of the active substance metribuzin. *EFSA Sci Rep* 2006; **88**: 1-74.
- [11] Sastre J, Pallardo FV, Asuncion J, Vina J. Mitochondria, oxidative stress and aging. *Free Radic Res* 2000; **32**: 189-198.
- [12] Weckbercker G, Cory JG. Ribonucleotide reductase activity and growth of glutathione-depleted mouse leukemia L1210 cells *in vitro*. *Cancer Lett* 1988; **40**(3): 257-264.



- [13]Habig WH, Pabst MJ, Jakoby WB. Glutathione S-transferase. The first enzymatic step in mercapturic acid formation, *J Biol Chem* 1974; **249**: 7130-7139.
- [14]Johnson RM, Pepperman AB. Analysis of metribuzin and associated metabolites in soil and water samples by solid phase extraction and reversed phase thin layer chromatography. *J Liq Chromatogr Relat Technol* 1995; **18**: 739-753.
- [15]Elsayed EM, Prasher SO. Effect of the presence of nonionic surfactant Brij35 on the mobility of metribuzin in soil. *Appl Sci* 2013; **3**: 469-489.
- [16]Surajudeen AY, Kadiri RS, Olatunbosun GA. Haematological and immunological indices in Nigerian farmworkers occupationally exposed to organophosphate pesticides. *Eur J Gen Med* 2014; **2**: 109-114.
- [17]Morgan J. *Evidence on the developmental and reproductive toxicity of metribuzin*. California: DRAFT, Ed; 2001.
- [18]Zaheer A, Iqbal MZ, Shoro AA. Lead-induced reduction in body and kidney weight of Wistar albino rats ameliorated by *Ginkgo biloba* extract (EGb 761). *Biochem Physiol* 2013; **2**: 1-4.
- [19]Marwa AA, Hassanein MAK. Cardio protective effects of *Nigella sativa* oil on lead induced cardio toxicity: Anti-inflammatory and antioxidant mechanism. *J Physiol Pathophysiol* 2013; **4**: 72-80.
- [20]Merzouk H, Chiali FZ, Merzouk SA, Medjdoub A, Narce M. Chronic low level Metribuzin exposure induces metabolic alterations in rats. *Pest Biochem Physiol* 2013; **106**: 38-44.
- [21]Clerc-Renaud P, Souillet G, Lahet C, Sotta C, Mathieu M, Mousson B. Serum 5'-nucleotidase and alkaline phosphatase activities after high dose chemotherapy and bone marrow transplantation in cases of malignancy in children. *Ann Biol Clin* 1995; **53**: 125-130.
- [22]McMillan HJ, Gregas M, Darras BT, Kang PB. Serum transaminase levels in boys with Duchenne and Becker muscular dystrophy. *Pediatrics* 2011; **127**: 132-136.
- [23]Kadeche L, Bourogaa E, Saoudi M, Boumendjel A, Djeflal A, El feki A, et al. Ameliorative effects of vanillin against metribuzin-induced oxidative stress and toxicity in rats. *Int J Pharm Pharm Sci* 2017; **9**: 56-62.
- [24]Medjdoub A, Merzouk SA, Merzouk H, Chiali FZ, Narce M. Effects of Mancozeb and Metribuzin on in vitro proliferative responses and oxidative stress of human and rat spleen lymphocytes stimulated by mitogens. *Pest Biochem Physiol* 2011; **101**: 27-33.
- [25]Djouadi A, Derouiche S. Study of fluoride-induced haematological alterations and liver oxidative stress in rats. *World J Pharm Pharm Sci* 2017; **6**: 211-221.
- [26]Jian-Ming L, Peter HL, Qizhi Y, Changyi C. Chemical and molecular mechanisms of antioxidants: experimental approaches and model systems. *J Cell Mol Med* 2010; **14**: 840-860.
- [27]Mittal M, Siddiqui MR, Tran K, Reddy SP, Malik AB. Reactive oxygen species in inflammation and tissue injury. *Antioxid Redox Signal* 2014; **20**: 1126-1167.
- [28]Ergul BK. The importance of antioxidants which play the role in cellular response against oxidative/nitrosative stress: current state. *Nutr J* 2016; **15**: 71. doi: 10.1186/s12937-016-0186-5
- [29]Aouacheri W, Saka S, Djafer R. The toxic effect of an insecticide (alphamethrin) on the activity of detoxifying glutathione enzymatic system. *Ann Toxicol Anal* 2009; **21**: 125-129.
- [30]Derouiche S, Djouadi A. An evaluation of stress oxidative and serum electrolytes in female hypothyroid patients. *Int J Biol Med Res* 2017; **8**: 5861-5865.
- [31]Ramsay EE, Dilda PJ. Glutathione S-conjugates as prodrugs to target drug-resistant tumors. *Front Pharmacol* 2014; **5**: 181. doi:10.3389/fphar.2014.00181.
- [32]Monks TJ, Anders MW, Dekant W, Stevens JL, Lau SS, van Bladeren PJ. Glutathione conjugate mediated toxicities. *Toxicol Appl Pharmacol* 1990; **106**: 1-19.