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Research Article
Risk of Metribuzin (Triazinone Herbicide) on Hematological and Renal Structure and Function of Pregnant Rabbits

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Abstract

Background and Objective: Metribuzin (Triazinone herbicide) is used worldwide as an ecotoxic herbicide and is the most widely used in Algeria and in random ways without respect for the doses approved. The objective of this study was to investigate the effects of pesticide on hematological and renal structure and function in rabbits. **Materials and Methods:** About 15 pregnant female rabbits Oryctolagus cuniculus divided into three groups (n = 5), the first group of non-treated pregnant rabbits serve as control, the second group of pregnant rabbits treated with 3.22 mg kg^{-1} b.wt., of Metribuzin and the third group of pregnant rabbits treated with 6.44 mg kg^{-1} b.wt., of Metribuzin. Metribuzin added in their drinking water for 60 days before and during pregnancy. **Results:** Results revealed a significant increase ($p<0.05$) in level of serum glucose, urea, creatinine, kidney MDA and kidney GST activity, white blood cell (WBC), lymphocyte and Platelets (PLT) and decrease in GSH level, red blood cells (RBC) and hemoglobin (HGB) concentration in pregnant rabbits of both Metribuzin treatment groups as compared to the control. Also, the results clearly showed that Metribuzin causes alterations of glomerular and tubular structure in comparison with controls. **Conclusion:** It is concluded that the pesticide-Metribuzin caused severe toxicity with tissue modification of the kidney which limits renal function and threatens fetal development in rabbits.

Key words: Ecotoxic herbicide, metribuzin, fetal development, hematological, pregnant rabbits, pesticide toxicity, triazinone herbicide

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Food production capacity is faced with an ever-growing number of challenges, including a world population expected to grow to nearly 9.2 billion by 2050 and a falling ratio of arable land to population[1]. This explosive increase in world population is mostly in developing countries and this is where the need for food is greatest and starvation threatens human life[2] as FAO estimates that 800 million are already undernourished (especially children)[3]. Each food is likely to contain different pesticide residues[4]. 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[5]. 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 Aliment Arius Commission[5]. Pesticides are an undeniable part of modern life, used to protect everything from flower gardens to agricultural crops against specific pests. Pesticides have made a significant contribution to improving the quality of life through increased yields of crop production to ensure global food security[6]. Pesticides are biologically active chemicals, which have been thoroughly tested for safety and usefulness before they are released for agricultural use[7]. The use of pesticides in agriculture has increased over the last decade. Their widespread, often uncontrolled use causes thousands of people to be daily exposed to environmental agricultural chemicals, resulting in acute and chronic health effects[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 (LD50 322 mg kg^{-1} b.wt.) and low dermal and inhalation toxicity (LD50> 5000 mg kg^{-1} b.wt. and LC_{50}> 2.0 mg L^{-1}, respectively). It is neither an irritant to the skin and eyes nor a skin sensitizer[10]. In Algeria region there are a lot of renal insufficiency, fetal malformation and many other diseases in areas that know the great use of these pesticides for this and as a goal of this study was to estimate the toxicity effect of pesticides Metribuzin (Triazinone herbicide) at a low doses on hematological markers and kidney structure and function in pregnant rabbits.

MATERIALS AND METHODS

Ethical approval: The experimental procedures were carried out according to the national institute of health guidelines for animal care and approved by the ethics committee (n°: BCM 022/2016) of author's institution.

Animals: In this study females rabbits Oryctolagus cuniculus are used with initial weight between 1240 et 1776 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±0.62°C), humidity (64.59±1.14%) and photoperiod (12 h of light /12 h of black). Access to standard diet and water is free for animals *ad libitum* during the experiments.

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:** Female rabbits were treated with deionized water only from 1-20 day of pregnant (control group)
- **Group II (group M1):** Female rabbits were given 3.22 mg kg^{-1} b.wt. (1/100 LD_{50}) of Metribuzin in drinking water from 60 days before and during pregnancy
- **Group III (group M2):** Female rabbits were given 6.44 mg kg^{-1} b.wt., (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^{-1} b.wt. and 6.44 mg kg^{-1} b.wt., 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.

Blood collection and tissue preparation: After of 8 weeks of Metribuzin exposed 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 3000 rpm for 10 min and then stored at -20°C and used for transaminases activities assay. The kidney of rabbits of different groups was rapidly excised, weighed and stored at -20°C until use for oxidative stress evaluation.

Plasma biochemical and hematological parameters: Measurement of urea and creatinine level were carried out by commercial kits from Spinreact (Girona, Spain) (ref: urea, ref. 1001332, creatinine, ref. 1001113). Hematologic analysis (NFS) is performed by the auto analyzer (mythic 18 Orpheus).

Antioxidants measurement

Preparation of homogenates: One gram of kidney from each mother rabbit of the different experiment groups was used. After milling and homogenizing the tissues in 9 mL of buffer solution of TBS [50 mM Tris, 150 mM NaCl, pH 7.4]. The tissue suspension was centrifuged at 9000 rpm for 15 min at 4°C, the supernatant obtained was stored at -20°C until use for the oxidative stress marker assay.

Determination of lipid peroxidation: kidney 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 (TBA) to give absorbent pink chromophores at 532 nm. The MDA level was expressed as nmol of MDA/mg prot.

Reduced glutathione (GSH) assay: kidney Reduced Glutathione (GSH) level was determined by a colorimetric method according to the technique described by Derouiche *et al.*[12], the measurement of optical density results from the formation of thionitrobenzoic acid (TNB) from the reduction of the 5,5'dithiodis-2-nitrobenzoic acid (DTNB), 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.

Glutathione-S-transferase (GST) activity assay: The activity of Glutathione-S-transferase (GST) in kidney 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 reduced Glutathione (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^{-1} mg^{-1} prot.

Histopathological analysis: The kidney from each rat was removed immediately and conserved in a sample flask containing 10% formalin solution, kidney was processed by the paraffin technique. Sections of 5 µm thickness were cut and stained by hematoxylin and eosin for histological examination.

Statistical analysis: The statistical evaluation is carried out by the student's t-test using Minitab 17.1 statistical package and the Excel 16.0 (Microsoft). The values are given as mean and standard deviations [ES] for three groups of 5 rabbits each. Statistical significance was defined as $p<0.05$.

RESULTS

Initial body weight, body weight gain, relative kidney weight and food intake: The results obtained in the Table 1 show that the body weight gain and food intake were significantly decreased ($p<0.05$ and $p<0.001$) in M1 and M2 groups compared to the control, respectively. In another side, the results showed also that the relative kidney weight was significantly increased ($p<0.05$ and $p<0.01$) in animals contaminated with 3.22 and 6.44 mg kg^{-1} of Metribuzin compared to the control group, respectively.

Biochemical parameters: The results obtained in Table 2 show that the blood glucose level was significantly increased ($p<0.01$) in both M1 and M2 groups compared to control group. The results also showed renal dysfunction shown by the significant increase in serum urea ($p<0.05$ and $p<0.01$) and serum creatinine ($p<0.05$) in the two groups treated with metribuzin compared to the control rabbits.

Hematological parameters: It was seen from Table 3, Metribuzin at a dose 3.22 mg kg^{-1} b.wt. (M1) and 6.44 mg kg^{-1} b.wt. (M2) induced a significant increase in white blood cell (WBC) ($p<0.05$ and $p<0.01$), Lymphocyte (LYM) ($p<0.01$ and $p<0.01$) and Platelets (PLT) ($p<0.01$ and $p<0.05$) in pregnant rabbits compared to the control group, respectively. On the other hand, the results showed a significant decrease ($p<0.05$) in number of red blood cells (RBC) and in hemoglobin (HGB) concentration in the M1 and M2 groups compared to the controls.

Oxidative stress parameters: As regarded the results of oxidative stress, the Table 4 shows that the kidney concentration of MDA and GST activity were significantly increased ($p<0.05$ and $p<0.01$) and the level of GSH was significantly decreased ($p<0.05$) in kidney of pregnant rabbits group exposed to Metribuzin at dose 3.22 mg kg^{-1} b.wt., also, MDA and GST activity are significantly increased ($p<0.01$ and $p<0.001$) and the level of GSH is significantly decreased ($p<0.001$) in kidney of pregnant rabbits group exposed to Metribuzin at the dose 6.22 mg kg^{-1} b.wt., compared to the control pregnant rabbits.

Histopathological studies: Microscopic observation of histological sections of kidney from the control rat showed normal renal parenchyma with glomeruli, tubules and normal cortical and medullar areas (Fig. 1a). However, histological sections of the Metribuzin-exposed rat kidney revealed

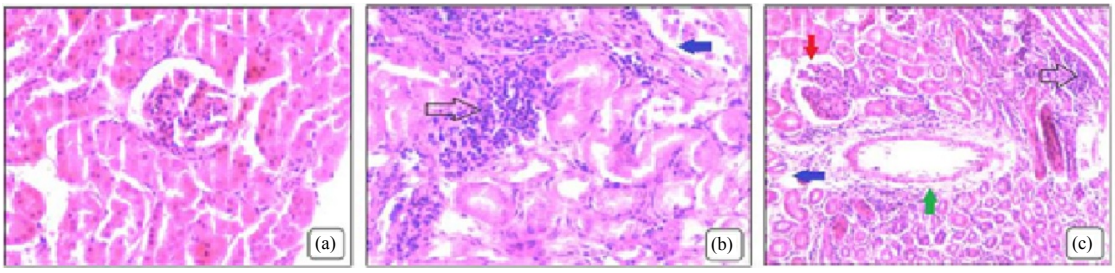

Fig. 1(a-c): Histological examination of kidney from a control pregnant rabbits showing renal tissue in the normal state (a) ×200, Metribuzin treated (group M1) in pregnant rabbits kidney showing focus of interstitial inflammation (black arrow) and tubular inflammation, (b) (blue arrow) ×200, kidney sections of the animals of group M2 and (c) ×200 showed focus of interstitial inflammation (black arrow)
Glomerular necrosis (green arrow), tubular inflammation (blue arrow) and large space of Bowman (red arrow)

Table 1: Metribuzin toxicity on the body weight gain, relative kidney weight and food intake of pregnant rabbits of control and experimental

Parameters	Control	Group M1	Group M2
Initial body weight (g)	1764.00±12.80	1680.00±10.2	1308.50±28.5
Gain body weight (g/day/rab.)	5.88±0.817	5.14±1.63*	2.18±0.19***
Relative kidney weight (g/100 g b.wt.)	0.20±0.004	0.26±0.038*	0.29±0.024**
Food intake (g)	390.30±3.960	379.47±3.51*	346.15±8.22***

Means±SE from 5 animals in each group. Significance from control, *p<0.05,**p<0.01, ***p<0.001

Table 2. Biochemical parameters level in serum of pregnant rabbits of control and experimental animals

Parameters	Control	Group M1	Group M2
Blood glucose (mg/100 mL)	109.000±2.30	146.000±8.8**	135.600±6.8**
Serum urea (mg/100 mL)	39.300±3.50	43.000±3.2*	53.600±0.6**
Serum creatinine (mg mL^{-1})	8.245±0.950	8.833±0.882*	8.910±0.445*

Means±SE from 5 animals in each group. Significance from control, *p<0.05, **p<0.01

Table 3: Haematological parameters in pregnant rabbits of control and experimental

Parameters	Control	Group M1	Group M2
WBC (10^3 μL^{-1})	10.767±0.26	18.760±2.68*	18.45±1.18**
LYM (U L^{-1})	7.467±0.485	8.933±0.238**	9.60±0.23**
RBC (^{106} μL^{-1})	5.400±1.10	5.200±1.6*	5.06± 4.9*
HGB (g dL^{-1})	11.533±0.21	11.233±0.30*	11.00±0.92*
PLT (10^3 μL^{-1})	313.330±32.51	383.330±27.73**	531.50±11.51*

Means±SE from 5 animals in each group. Significance from control, *p<0.05,**p<0.01

Table 4: Oxidative stress parameters in kidney of pregnant rabbits of control and experimental animals

Parameters	Control	Group M1	Group M2
MDA (μmol mg^{-1} prot)	0.089±0.009	0.133±0.026**	0.124±0.010**
GSH (nmol mg^{-1} prot)	27.060±2.34	25.250±3.47*	15.360±0.96***
GST (nmol min^{-1} mg^{-1} prot)	367.400±8.20	399.000±12.5*	459.400±11.5***

Means±SE from 5 animals in each group. Significance from control, *p<0.05, **p<0.01, ***p<0.001

degeneration represented by necrosis and glomerular atrophy apparent to their architecture with tubular necrosis dilatation and tabular vacuolation. In addition haemorrhage foci of inflammation and large space of Bowman was observed in the kidney sections of group 2 and 3 (Fig. 1b and c).

DISCUSSION

The results of the effect of Metribuzin on the body weight of rabbits showed that there was a significant decrease. Probably due to reduced food intake during the experiment. Results is consistent with the study of MORGAN. 2001 which used rabbits treated with Metribuzin for 6-18 days. This study reported a correlation between decreased food intake along with losing body weight of female rabbits during the period of treatment[14]. However, this weight loss probably due also to anorexia induced by the ingestion of this xenobiotic following continuous exposure over a long period[15]. The elevation of the relative kidney weight of the M 3.22 and M 6.44 mg kg^{-1} groups indicated in the current

study may be due to kidney necrosis due to the accumulation of Metribuzin in renal cells. This is in line with the presence of necrosis and inflammation in kidney reported in histopathology results. In both groups treated with Metribuzin (M 3.22 and M 6.44 mg kg^{-1} b.wt.) showed a significant increase in level of blood glucose, urea and creatinine compared to the control group, respectively. These results are in agreement with those previously obtained by Kadeche *et al.*[16] they found that urea and creatinine concentration were increased in experimental animals after exposure to Metribuzin pesticides (133.33 mg kg^{-1} b.wt.). These results may be attributed to reduction in glomerular filtration in the kidney and also reflect dysfunction of the kidney tubules in pregnant rabbits[17]. The results obtained show that there is a significant increase in number of WBC. Lymphocyte and PLT cells under the effect of the Metribuzin compared with the control group. Obtained results are consistent with the study of Medjdoub *et al.*[18] which showed that Metribuzin exposer leads to the activation and stimulation of lymphocyte proliferation and cytokine production in human spleen cells and rat. Leukocytes or white blood cells contribute to inflammation which is essential for the success of the immune response and secrete signaling proteins that activate and recruit of other cells to counter the invader[19]. On the other hand, a decrease in the number of red blood cells and hemoglobin concentration under the effect of Metribuzin compared to the control group. These results are in disagreement with the study of Velisek *et al.*[9], which has been exposed to SENCOR (70% active substance Metribuzin) in rats, its shows that there was a significant increase in hemoglobin level compared to the control group. Hemoglobin is a protein with the property of fixing transporting and delivering oxygen. A decrease in the hemoglobin and the number of red blood cells in pregnant rabbits direct the diagnosis towards anemia[20]. The current results showed also that a significant increase in the concentration of kidney MDA in all the groups studied compared to control. These results are in agreement with the study of Kadeche *et al.*[21], which shows an increase in tissue MDA concentration in rats exposed to low dose of Metribuzin[21]. There is increasing evidence that organophosphate (OP) and carbamate induced oxidative stress through the generation of free oxygen radicals, leading to lipid peroxidation[22]. Metribuzin is capable of inducing intracellular oxidative stress[19]. Oxidative stress at the cellular level reflects an increase in Malondialdehyde (MDA), a product of lipid peroxidation of membranes[23]. The free radicals that leads to lipid peroxidation are just inflammation, macrophages and neutrophils can produce very high amounts of reactive oxygen species (ERO) which produces via membrane NADPH oxidases[24]. The results also showed an increase in reduced glutathione (GSH) level and glutathione-S-transferase (GST) activity in the kidney. Glutathione (GSH) is a non-enzymatic antioxidant that contributes to the defense system in the body against oxidative stress induced by reactive oxygen species[25]. The decreased in GSH was 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[26]. This leads to the consumption of GSH which is the reason for the significant decreased in its level. The involvement of antioxidant enzymes such as GST as protective factors of cells and organs against toxic agents and oxidative stress[27]. 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[28]. The results of the histological analysis performed on the kidneys indicate the presence of necrosis and local inflammation in the Metribuzin-treated groups confirmed by the results of biochemical and the oxidative stress parameters. In the course of inflammatory phenomena there is a release of mediators derived from oxygen and toxic free radicals by phagocytic cells[29]. These mediators are probably responsible for the glomerulus or tubule lesion.

CONCLUSION

The present study exhibited the toxic effects of Metribuzin on kidney by induced oxidative stress in pregnant rabbits. From this study it can be further concluded that the Metribuzin is the origin of kidney damage and of possible abortion of the rabbits.

SIGNIFICANCE STATEMENT

This study discover the doses below the reference limit dose determined by international organism such as WHO and FAO for Metribuzin that exposed to the living being for food or water or air presents danger to the health especially in kidney of pregnant animals. That can be beneficial for people to be cautious in dealing with the agricultural products treated with Metribuzin. This study will help the researcher to

uncover the critical areas of toxic doses of Metribuzin during pregnancy many researchers were not able to explore. Thus a new theory on relationship of toxic dose of Metribuzin to pregnancy may be arrived at.

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