Research Article

Quercetin Attenuates Metribuzin-induced Biochemical and Hematological Toxicity in Adult Rats

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Accepted on: 16-06-2016; **Finalized on:** 31-08-2016.

ABSTRACT

The current study evaluates the protective effect of quercetin (Que) against metribuzin (Mtz) pesticide induced adverse effects on hematological and biochemical parameters as well as oxidative stress in various tissues of rats. Thirty-two rats were equally divided into four groups; the first (CT) served as a control, the remaining groups were respectively treated with Que (50 mg/kg b.w.), metribuzin (133.33 mg/kg b.w.) and a combination of Mtz and Que. After 21 days of treatment, a significant decrease in the levels of red blood cells, haemoglobin and hematocrit were noticed in the metribuzin-exposed group when compared to the control. Serum glucose levels were increased along with total cholesterol, triglycerides and bilirubin after Mtz exposure. Metribuzin caused also a significant induction of oxidative damage in tissues as evidenced by increased levels of malondialdehyde, decreased levels of reduced glutathione, and lower activities of glutathione peroxidase. However, quercetin supplementation has significantly reduced adverse effects of metribuzin. These findings suggest that Que may have protective effects by improving the antioxidant status in tissues and ameliorating the harmful effects induced by Mtz.

Keywords: Metribuzin, Quercetin, Antioxidants, Oxidative stress, Pesticides.

INTRODUCTION

Over the past few decades, the use of pesticides in agriculture to preserve crops for humans and animals has resulted in their undesirable accumulation in the environment[1]. Pesticides are xenobiotic, toxic and sometimes non-biodegradable and can cause serious problems to human health and environment[2].

Triazine herbicides constitute one of the largest groups of pesticides used throughout the world[3,4]. Triazinones, such as metribuzin (4-amino-6-tert-butyl-4,5-dihydro-3 methyltio-1,2,4-triazin-5-one), are used worldwide to control broadleaf weeds in crops such as potatoes, soybeans and other vegetable crops[5]. As a result of its widespread use, metribuzin paid more attention as it considered a potential environmental contaminant[6].

Metribuzin (Mtz) has been reported for its deleterious effects on human beings[7], fishes[8,9] and domestic animals[10]. In a study, the commercial formulation containing metribuzin was shown to cause changes in the metabolic parameters of male and female wistar rats. It has been reported that metribuzin was toxic to rats and the target organs were the liver, muscles, adipose tissue and intestine[11].

In addition, Medjdoub[7] demonstrated that metribuzin has potentially induced immunotoxicity *in vitro*.

Environmental exposure to pesticides may affect human health by increasing the incidence of certain disorders. Their toxic effects are clearly mediated by reactive oxygen species (ROS) which can react with biological molecules and initiate oxidative damage including protein oxidation, reduced glutathione (GSH) depletion and lipid peroxidation (LPO)[12].

Though, the organism has several biological defence mechanisms against intracellular oxidative stress[13], involving endogenous (enzymatic and non-enzymatic) antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione (GSH), and exogenous antioxidants such as vitamin C, vitamin E, carotenoids and polyphenols[14].

Thus, LPO rise, variations in the levels of GSH and antioxidant enzymes have been proposed as indicators of mediated oxidative stress[12]. Studies of metribuzin toxicity have showed an increased oxidative stress and altered antioxidants status *in vitro*[7] and *in vivo*[11].

To overcome oxidative stress, a positive correlation has been established between dietary supplementation with certain vegetables and plant products and the reduction of toxic effects of various environmental contaminants[13]. Studies on the antioxidative action of flavonoids, a group of compounds widespread throughout the plant kingdom, have been conducted very intensely in recent years[15,16].

Among these plant compounds, quercetin is being increasingly used in experimental studies[17].

Quercetin (3, 3′, 4′, 5, 7-pentahydroxyflavone) is a typical flavonoid, largely present in fruits and vegetables. It has attracted a great deal of attention as a potential antioxidant[18], where it has been shown that the majority of flavonoids like quercetin have five fold higher total antioxidant activities than vitamin E and C[15]. The

antioxidant properties of quercetin are mainly due to its ability to scavenge free radicals and reactive oxygen species (ROS) (superoxide anion, hydroxyl-radical), which lead to the inhibition of lipid peroxidation reactions and the elevation of antioxidant status[19].

There has been indication in some animal experimentation that quercetin can protect organisms against the toxicity of exogenous poisons, such as insecticides[20]. However, there are no reports regarding the role of quercetin (Que) against metribuzin induced oxidative stress. For this reason, an investigation concerning the possible protecting effect of quercetin against oxidative damage induced by metribuzin intoxicated male Wistar rats has been carried out.

MATERIALS AND METHODS

Chemicals

The commercial herbicide Metribuzin ($C_8H_{14}N_4OS$; CAS.Nos metribuzin 21087-64-9) used in this study was obtained from (INRAA, Algeria). It was tested in the form of Sencor WP 70 pesticide, of which the active substance was metribuzin in the amount of 70%. All other reagents used were of high quality and analytical grade.

Animals

A total of 32 male Wistar rats weighing approximately 200–250 g were used for this experiment. They were procured from the Pasteur Institute (Algiers, Algeria). Rats were maintained under standard conditions of temperature (22 ± 2 °C), humidity (40 %) and photoperiod. Food (Standard diet, supplied by the "ONAB, EL-Harrouch", Algeria) and water were available ad-libitum. After two weeks of acclimatization, rats were divided among the control and test groups. The study protocol was approved by the institutional Animal Ethics Committee constituted in accordance with the National Institute of Health Guide-lines for Animal Care, Algeria.

Experimental Design

Animals were randomly divided into 4 groups (8 rats in each).

1. Control group, where rats received drinking water;
2. quercetin-treated group (Que), animals received quercetin (Sigma-Aldrich Co., Steinheim, Germany) dissolved in 0.9% saline solution and administered by daily intraperitoneal injection at dose of 50 mg/kg body weight/day (in a volume of 1 ml/kg body weight);
3. metribuzin-treated group (Mtz), animals received through drinking water 133,33 mg/kg body weight of metribuzin. Rats in group (1) and (3) were daily given physiological saline (0.9% NaCl, 1ml/kg body weight) by intraperitoneal injection (i.p.) during the whole course of the experiment.
4. group (Mtz + Que) was treated daily with both metribuzin and quercetin as in group two and three.

The doses of Mtz and Que were selected on the basis of previous works of Xhaxhiu[21] and De Oliveira[22] respectively. The dose of Mtz used in this study represents 1/20 of LD_{50} (2345 mg/kg body weight) in rats.

The treatment was continued for a period of three consecutive weeks, and all individuals were weighed one every week. At the end of the experiment, total body weights were recorded and animals were sacrificed by decapitation without anesthesia to avoid animals stress. Different organs namely liver, kidney, brain and testis were removed and weighed in order to obtain the relative weight of each organ (%) which was calculated as g/100g body weight.

Blood Collection and Hematological Profile

At the time of sacrifice, blood samples for biochemical assays were collected in tubes without anticoagulant and serum was obtained by centrifugation of the samples for 15 min at (3000 rpm, 4 °C). Blood samples for hematological analysis were collected in tubes containing EDTA.

Hematological analysis was performed using an automatic hematological analyzer (Auto Hematology Analyz, MODEL PCE – 210N, Japan). Hematological parameters evaluated were total red blood cell (RBC), haemoglobin (Hb) concentration, hematocrit (HCT), mean corpuscular haemoglobin (MCH), mean corpuscular volume (MCV) and mean corpuscular haemoglobin concentration (MCHC).

Tissue Preparation

One gram of liver, kidney, brain and testis was homogenized in 2 ml of buffer solution of phosphate buffer saline 1:2 (w/v; 1g tissue with 2 ml TBS, pH 7.4), Homogenates were centrifuged at 10000 x g for 15 minutes at 4 °C, and the resultant supernatant was used for the determination of thiobarbituric acid reactive substances (TBARS), reduced glutathione and protein levels in one hand and the estimation of GSH-Px activity in the other hand.

Biochemical Profile

Biochemical analysis of serum samples was performed using commercial kits (Spinreact, Sant Esteve De Bas, Spain), according to the recommendations of the manufacturer. Biochemical parameters measured were glucose, triglycerides, cholesterol, urea, creatinine, bilirubin, total protein, alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT).

Determination of Lipid Peroxidation

The lipid peroxidation level in liver, kidney, brain and testis homogenates was measured as malondialdehyde (MDA) which is the end product of lipid peroxidation and reacts with thiobarbituric acid (TBA) as a TBARS to produce a red-colored complex with a peak absorbance

at 532 nm according to Buege and Aust[23]. Thus, 125 ml of supernatant were homogenized by sonication with 50 ml of PBS, 125 ml of trichloroacetic acid–butylhydroxytoluene in order to precipitate proteins, and then centrifuged (1000g, 10 min, 4 C°). Then, 200 ml of supernatant were mixed with 40 ml of HCl (0.6M) and 160 ml of TBA dissolved in Tris, and then the mixture was heated at 80 °C for 10 min. The absorbance of the resultant supernatant was obtained at 530 nm. The amount of TBARS was calculated using a molar extinction coefficient of 1.56 x 10^5 M/cm.

Reduced Glutathione (GSH) Levels

GSH level was estimated using a colorimetric technique, as mentioned by Ellman[24], modified by Jollow.[25] This assay is based on the development of yellow colour when DTNB (5, 5' dithiobis-(2-nitrobenzoic acid) is added to compounds containing sulfhydryl groups. In brief, 08 ml of liver supernatant was added to 0.3 ml of 0.25% sulfosalycylic acid, and then tubes were centrifuged at 2500 x g for 15 minutes. Supernatant (0.5 ml) was mixed with 0.025 ml of 0.01 M DTNB and 1 ml phosphate buffer (0.1 M, pH 7.4). The absorbance at 412 nm was recorded. Finally, total GSH content was expressed as nmol GSH/mg protein.

Glutathione Peroxidase (GPx) Activity

Glutathione peroxidase (GPx) (E.C. 1.1.1.1.9) activity was measured by the procedure of Flohe and Gunzler[26]. Supernatant obtained after centrifuging 5 % liver, kidney, brain and testis homogenate at 15000 x g for 10 min followed by 10.000 x g for 30 min at 4 C° was used for GPx assay. 1 ml of reaction mixture was prepared which contained 0.3 ml of phosphate buffer (0.1 M. pH 7.4). 0.2 ml of GSH (2mM), 0.1 ml of sodium azide (10mM), 0.1 H_2O_2 (1mM) and 0.3 ml of liver supernatant. After incubation at 37 °C for 15min, the reaction was terminated by addition of 0.5 ml 5% TCA.

Tubes were centrifuged at 1500 x g for 5 min and the supernatant was collected. 0.2 ml of phosphate buffer (0.1M pH 7.4) and 0.7 ml of DTNB (0.4 mg/ml) were added to 0.1 ml of reaction supernatant. After mixing, absorbance was recorded at 420 nm.

Protein Assay

The protein content of tissues samples was measured by the method of Bradford[27], using bovine serum albumin as a standard.

Statistical Analysis

Data were analyzed using the statistical software MINITAB 16. Statistical analysis between all groups was performed with one-way ANOVA followed by Fisher's LSD (Least Significant Difference) test to evaluate the significance of differences.

All experimental data were expressed as means ± standard deviation (SD). Means that do not share a letter are significantly different.

RESULTS

Effects of Treatments on Body, Absolute and Relative Weights

Table 1 shows body, absolute and relative organ weights (liver, kidney, brain and testis) of the control and experimental groups. The significant changes were occurred in the experimental groups when compared with the control.

The body weight has increased progressively throughout the study in all the groups associated with marked growth retardation of the rats traited by metribuzin, although there were no significant differences between them.

A significant increase of Mtz treated group in relative liver (+25.33%), kidney (+36.73%), brain (+12.19%) and testis (+9.43%) weights was also recorded, when compared with their respective control rats.

However, quercetin supplementation reserved these changes after 3 weeks of treatment.

Effects of Treatments on Plasma Hematological Parameters

The results of hematological profile in rats of control and treated group are presented in Table 2. Animals treated with metribuzin had significant lower RBC count (-17.16%), Hb (-20.38%), and HCT (-23.92%) compared to the control group.

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were not statically significant than that of the control. Whereas in rats co-treated with quercetin, there were no significant changes in hematological parameters when compared with the control values.

Effects of Treatments on Plasma Biochemical Parameters

Data represented in Table 3 show that oral treatment with metribuzin caused significant decrease (- 9.03 %) in serum total protein, whilst a significant increase was noted in the level of glucose, urea, creatinine, bilirubin, cholesterol and triglycerides by (6.76; 43.34; 73.47; 41.96; 29.17 and +17.89%), respectively compared to the control. In addition, the activities of AST, ALT and ALP were significantly increased (+14.13; +40.64 and +54.78%) in metribuzin group compared to the control. In contrast, co-administration of quercetin to metribuzin treated animals restored most of these biochemical parameters to nearly normal levels.

Effects of Treatments on Lipid Peroxidation

Results showed that metribuzin treatment caused significant increases of TBARS levels (Figure 1, 2, 3 and 4) in all tissue tested (liver, +19.31%), (kidney, +19.13%), (brain, +20%), (testis, +19.05%); when compared with their respective controls. Quercetin administered at 50 mg/kg to rats of (Mtz+ Que) group alleviated lipid

peroxidation and modulated significantly the levels of MDA in liver, kidney, brain and testis.

Effects of Treatments on Reduced Glutathione Contents

Data concerning liver, kidney, brain and testis reduced glutathione contents are presented in Figure 1, 2, 3 and 4. In Mtz group, liver, kidney, brain and testis reduced glutathione levels were decreased by (27.43, 17.7, 14.59 and -22.97%), respectively. Administration of quercetin improved liver, kidney, brain and testis reduced glutathione levels in (Mtz+ Que) group compared to those of Mtz group.

Effects of Treatments on Glutathione Peroxidase Activity

Data of glutathione peroxidase activity measured in liver, kidney, brain and testis are presented in Figure 1, 2, 3 and 4. In Mtz group, GPx activity in liver, kidney, brain and testis were decreased by (34.78, 18.75, 21.05 and -16.67%), respectively, compared to the control. However, administration of quercetin ameliorated GPx activity.

Table 1: Body weight, absolute and relative organ weights of control male rats, treated with quercetin, metribuzin and quercetin co-administrated with metribuzin, after 3 weeks of treatment.

Experimental Groups				
Parameters	Control	Que	Mtz	Mtz + Que
Initial body weight (g)	266.62 ± 33.36	269.5 ± 33.64	266.37 ± 31.07	269.62 ± 30.46
Final body weight (g)	303.75 ± 27.14^{a}	296.13 ± 31.04^{a}	275.88 ± 30.55^{a}	292.88 ± 28.23^{a}
Absolute liver weight (g)	9.09 ± 0.60^{c}	8.64 ± 0.63^{c}	10.60 ± 0.72^{a}	9.78 ± 0.39^{b}
Relative liver weight (g) (g/100 g b.w.)	3.00 ± 0.12^{c}	2.93 ± 0.14^{c}	3.76 ± 0.32^{a}	3.36 ± 0.26^{b}
Absolute kidney weight (g)	1.50 ± 0.18^{c}	1.54 ± 0.07^{bc}	1.83 ± 0.10^{a}	1.67 ± 0.09^{b}
Relative kidney weight (g/100 g b.w.)	0.49 ± 0.03^{c}	0.52 ± 0.03^{c}	0.67 ± 0.04^{a}	0.57 ± 0.03^{b}
Absolute brain weight (g)	1.23 ± 0.08^{a}	1.20 ± 0.06^{a}	1.26 ± 0.13^{a}	1.22 ± 0.05^{a}
Relative brain weight (g/100 g b.w.)	0.41 ± 0.05^{b}	0.41 ± 0.03^{b}	0.46 ± 0.08^{a}	0.42 ± 0.03^{ab}
Absolute testis weight (g)	3.22 ± 0.26^{a}	3.02 ± 0.34^{a}	3.28 ± 0.36^{a}	3.13 ± 0.31^{a}
Relative testis weight (g/100 g b.w.)	1.06 ± 0.08^{b}	1.03 ± 0.12^{b}	1.16 ± 0.11^{a}	1.07 ± 0.06^{ab}

Values are mean ± SD for groups of 8 animals each. Means that do not share a letter are significantly different.

Table 2: Hematological parameters of control male rats, treated with quercetin, metribuzin and quercetin co-administrated with metribuzin, after 3 weeks of treatment.

Experimental Groups				
Parameters	**Control**	**Que**	**Mtz**	**Mtz + Que**
RBC (× 10^{6}/ul)	9.15 ± 0.75^{a}	9.06 ± 0.74^{a}	7.58 ± 2.16^{b}	8.91 ± 1.12^{ab}
Hb (g/dl)	16.14 ± 1.00^{a}	15.44 ± 0.96^{a}	12.85 ± 3.95^{b}	14.81 ± 1.55^{ab}
HCT (%)	45.77 ± 3.42^{a}	43.51 ± 3.61^{a}	34.82 ± 9.65^{b}	41.15 ± 3.01^{a}
MCV (fl)	50.62 ± 4.56^{a}	48.25 ± 5.28^{a}	46.5 ± 1.93^{a}	47.12 ± 3.98^{a}
MCH (pg)	17.81 ± 1.11^{a}	16.96 ± 1.03^{a}	16.85 ±0.96^{a}	16.92 ± 1.06^{a}
MCHC (g/dl)	35.31 ± 1.75^{a}	35.66 ± 2.00^{a}	36.2 ± 2.60^{a}	36.05 ± 2.07^{a}

Values are mean ± SD for groups of 8 animals each. Means that do not share a letter are significantly different.

Table 3: Changes of biochemical parameters of control rats, treated with quercetin, metribuzin and quercetin co-administrated with metribuzin, after 3 weeks of treatment.

Experimental Groups				
Parameters	**Control**	**Que**	**Mtz**	**Mtz + Que**
Glucose (mg/dl)	107.25 ± 6.09^{b}	101.87 ± 4.29^{c}	114.5 ± 2.20^{a}	108.5 ± 5.10^{b}
Urea (mg/dl)	39.5 ± 6.70^{c}	36.62 ± 3.78^{c}	56.62 ± 7.59^{a}	45.87 ± 2.64^{b}
Creatinine (mg/dl)	0.49 ± 0.08^{bc}	0.42 ± 0.06^{c}	0.85 ± 0.10^{a}	0.57 ± 0.08^{b}
Cholesterol (g/l)	0.96 ± 0.13^{bc}	0.85 ± 0.07^{c}	1.24 ± 0.18^{a}	1.06 ± 0.12^{b}
Triglycerides (g/l)	0.95 ± 0.08^{bc}	0.93 ± 0.14^{c}	1.12 ± 0.09^{a}	1.03 ± 0.06^{ab}
Total protein (g/l)	71.75 ± 4.12^{a}	71.73 ± 4.34^{a}	65.27 ± 5.41^{b}	69.84 ± 3.08^{a}

Total bilirubin (mg/l)	5.10 ± 0.60^{bc}	4.76 ± 0.58^{c}	7.24 ± 0.91^{a}	5.73 ± 0.42^{b}
AST (U/L)	79.62 ± 5.83^{b}	83.37 ± 5.95^{b}	90.87 ± 4.85^{a}	84.37 ± 5.88^{b}
ALT (U/L)	31.37 ± 4.93^{b}	34.75 ± 4.77^{b}	44.12 ± 7.64^{a}	36.87 ± 5.25^{b}
ALP (U/L)	145.12 ± 9.33^{b}	142.25 ± 7.92^{b}	224.62 ± 49.14^{a}	156.75 ± 6.88^{b}

Values are mean ± SD for groups of 8 animals each. Means that do not share a letter are significantly different.

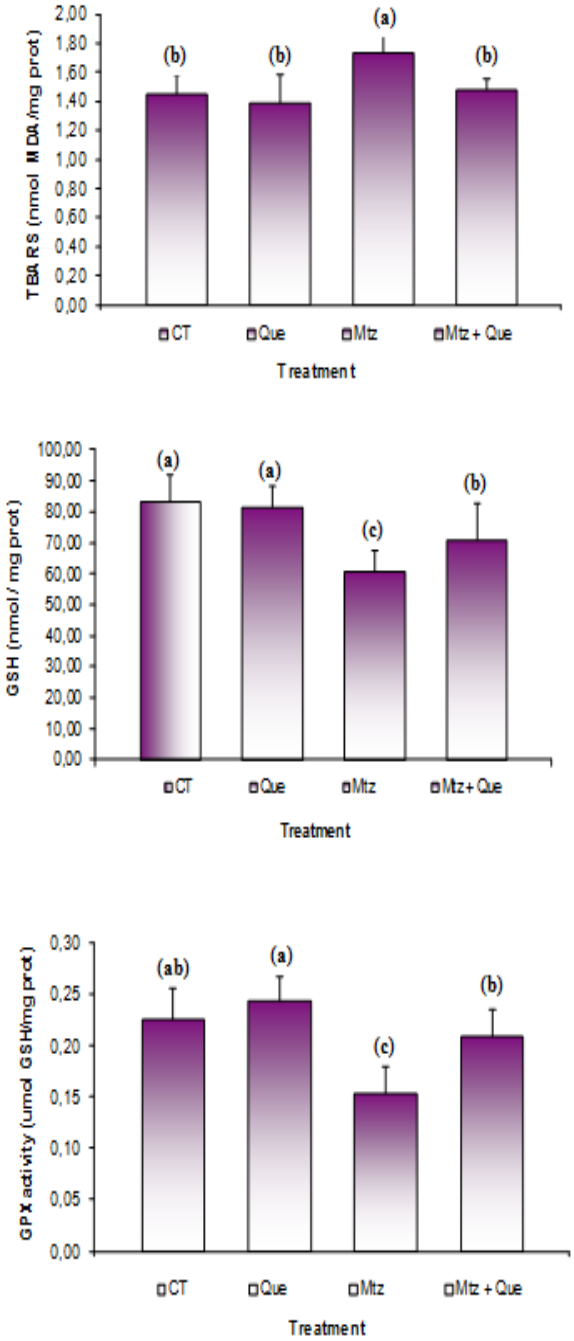

Figure 1: Thiobarbituric acid reactive substances (TBARS, nanomoles of MDA per milligram of protein), reduced glutathione (nanomoles per milligram of protein) and glutathione peroxidase activity in liver of control (CT) and rats treated with quercetin (Que), metribuzin (Mtz) and Que co-administrated with Mtz after 3 weeks of treatment. Values are given as mean ± SD for groups of 8 animals each. Means that do not share a letter are significantly different.

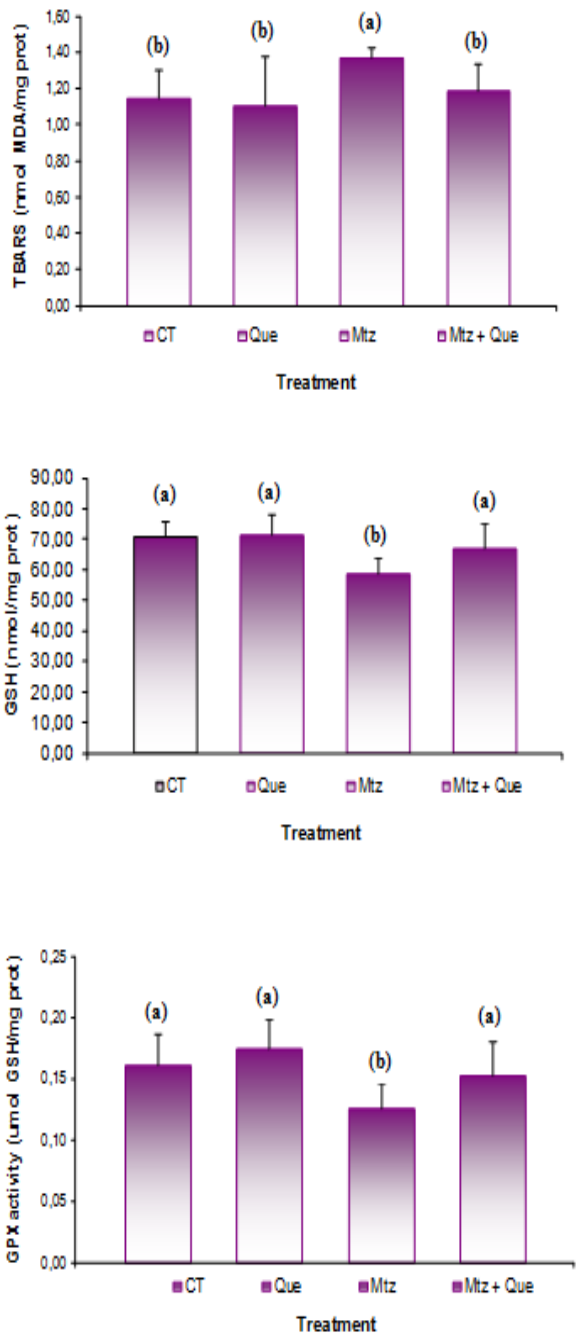

Figure 2: Thiobarbituric acid reactive substances (TBARS, nanomoles of MDA per milligram of protein), reduced glutathione (nanomoles per milligram of protein) and glutathione peroxidase activity in kidney of control (CT) and rats treated with quercetin (Que), metribuzin (Mtz) and Que co-administrated with Mtz after 3 weeks of treatment. Values are given as mean ± SD for groups of 8 animals each. Means that do not share a letter are significantly different.

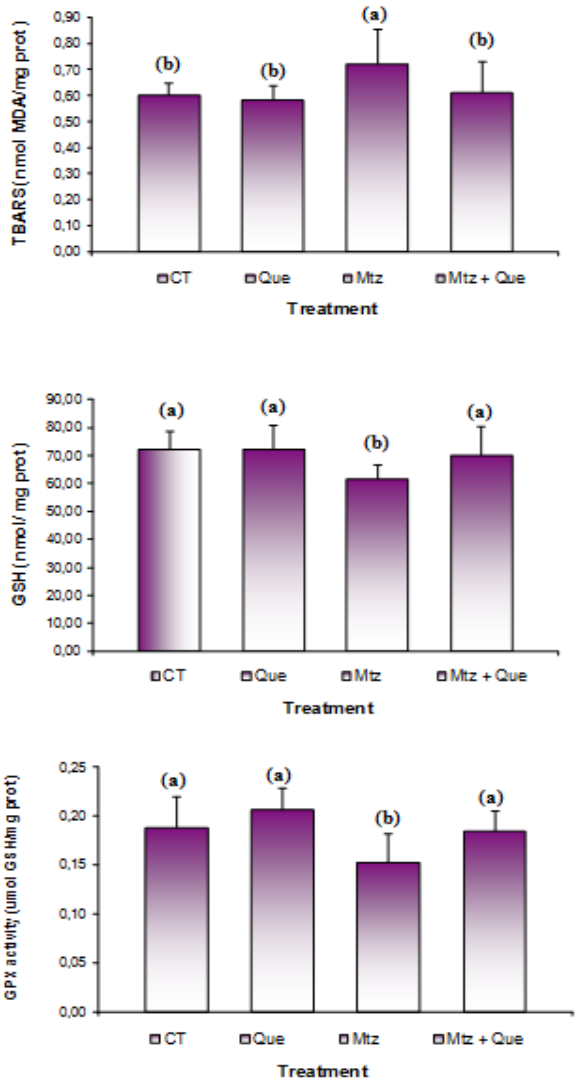

Figure 3: Thiobarbituric acid reactive substances (TBARS, nanomoles of MDA per milligram of protein), reduced glutathione (nanomoles per milligram of protein) and glutathione peroxidase activity in brain of control (CT) and rats treated with quercetin (Que), metribuzin (Mtz) and Que co-administrated with Mtz after 3 weeks of treatment. Values are given as mean ± SD for groups of 8 animals each. Means that do not share a letter are significantly different.

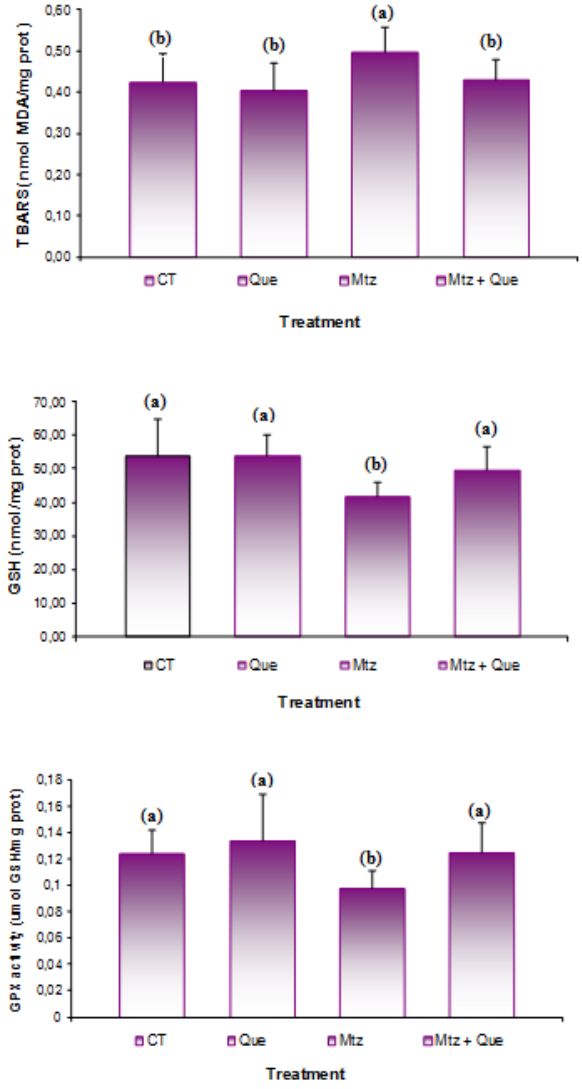

Figure 4: Thiobarbituric acid reactive substances (TBARS, nanomoles of MDA per milligram of protein), reduced glutathione (nanomoles per milligram of protein) and glutathione peroxidase activity in testis of control (CT) and rats treated with quercetin (Que), metribuzin (Mtz) and Que co-administrated with Mtz after 3 weeks of treatment. Values are given as mean ± SD for groups of 8 animals each. Means that do not share a letter are significantly different.

DISCUSSION

Free radicals have become an attractive means to explain the toxicity of numerous xenobiotics e.g. pesticides.[28]

In fact, certain pesticides may increase the production of (ROS) and, therefore, induce oxidative stress in non-target species.[29] However, antioxidants can play a crucial role in offering protection against pesticide induced oxidative damage.[30]

The findings of the present study clearly revealed the protective nature of quercetin against metribuzin-induced oxidative stress in rats.

Metribuzin exposure has changed absolute and relative weights of liver, kidney, brain and testis; leading to the alterations in organ-body weight ratio in this toxicity study. The significant increase in the absolute and relative weight of different vital organs may be an indication of adverse effects of subacute administration of metribuzin to experimental rats. Accordingly, all the morphological changes observed in metribuzin intoxicated rats were ameliorated by administering quercetin.

Further, the decrease in Hb concentration and RBCs count of Mtz-treated group might be due to the effect of pesticide on erythropiotic tissue. The poisoning by pesticide residues causes anemia which come from

reducing Hb biosynthesis and shortening the life span of circulating erythrocytes.[31] The reduced Hb synthesis may be responsible for decreased erythrocytic counts resulting in decline of heamtocrit values[32]. Our findings were supported with the data provided by Velisek[33] who noticed the decrease of RBC count, Hb concentration, and HCT % levels in fish after acute exposure to metribuzin. The lack of significant decrease of Hb, HCT and the concentration of RBCs recorded in-group co-treated with metribuzin and quercetin; when compared to the Mtz-treated group was an indication of the attenuation of Mtz-evoked anemia by the antioxidant quercetin. In fact, quercetin was able to protect red blood cells from oxidative damage[34], and its antioxidant effect on haematological parameters was documented *in vitro*[35-30], and *in vivo*[36] experimental studies.

The rise in blood glucose might be an indication of disrupted carbohydrate metabolism due to enhanced breakdown of liver glycogen, possibly mediated by the increase in adrenocorticotropic and glucagon hormones and/or reduced insulin activity[32]. Metribuzin-induced hyperglycemia has been also recorded in rats[11], and fish[33]. The improved change in blood glucose in the group given Que as a protective could be due to the fact that Que can stimulate glucose uptake in peripheral tissues, increase hepatic glucokinase, augmenting both oxidation and storage of glucose and reducing hepatic gluconeogenesis and glycogenolysis because of its antidiabetic effects.[37]

The elevation in serum urea and creatinine levels in Mtz-treated rats is considered as a significant marker of renal dysfunction[11], and it may be due to the effect of pesticides on liver function, as urea is the end product of protein catabolism[32]. While, the concentrations of serum creatinine and urea were reduced in rats that were administered a combination of (Mtz+ Que), which is an indication of renal protection by quercetin[38], that it was able to attenuate renal impairment[39].

An increase in the serum total cholesterol and triglycerides following exposure to metribuzin has been observed in this study. The elevation in the cholesterol level may be due to an increased cholesterol synthesis in the liver or it may be a sign of liver damage that can be attributed to the effect of pesticides on the permeability of liver cell membrane[40]. The elevation in serum triglycerides has been attributed to an inhibition of the lipase enzyme activity of both the hepatic triglycerides and plasma lipoproteins[40]. Similar hypercholesterolemia and hypertriglyceridemia have been observed in other studies of pesticide intoxication[31-32]. However, rats co-treated with quercetin along metribuzin reversed this condition; quercetin has been shown to have hypocholesterolaemic and hypotriacylglycerolaemic effects in animal studies[41].

Administration of metribuzin to rats has induced liver toxicity as reflected by the elevation of liver damage marker enzymes like AST, ALT and ALP. The elevations of these enzymes may be caused hepatocytes dysfunction with alteration of the liver membrane permeability[42]. The actual results are in resemblance with Chiali[11] who demonstrated that metribuzin administration can cause significant increases in hepatic transaminase activities associated with oxidative damage. Furthermore, the increase in serum total bilirubin of Mtz-treated rats may result from decreased liver uptake, conjugation or increased bilirubin production from hemolysis[40]. The decrease in the levels of total proteins might be due to changes in protein synthesis and/or metabolism[40].

On the other hand, the current results indicated that metribuzin co-treated by quercetin had no significant effects on serum biochemical parameters of rat liver, corroborating the findings of Krishnappa[43] which have indicated that quercetin has shown significant prophylactic effects by restoring rat liver function markers (AST, ALT, ALP, serum bilirubin, and total protein) after CCl_4 administration. In addition to its antioxidant action, quercetin seems to protect liver and ameliorate its hepatic function[39], as evidenced in other study[38], is concordant with the present report.

Lipid peroxidation has been suggested as one of the molecular mechanisms involved in pesticide-induced toxicity[28]. Malondialdehyde (MDA) level in Mtz treatment was significantly higher than that of the control. These indirectly suggest an increased production of oxygen free radicals in rat body. Highly reactive oxygen metabolites, especially hydroxyl radicals, act on membrane unsaturated fatty acids of phospholipid components to produce malondialdehyde, a lipid peroxidation product[28]. In other word, tissue MDA levels of all organs were decreased in-group treated by metribuzin plus quercetin, which may be related to the ability of quercetin to inhibit lipid peroxidation, because of its capacity to scavenge oxygen free radicals[19].

Subacute exposure to metribuzin (1/20 LD50) resulted in a decrease of glutathione levels and disturbance in the activities of GPx enzymes of liver, kidney, brain and testicular tissues. Decrease in GSH levels were also observed in the study of Chiali[11] who suggested that the reduced levels of GSH in treated rats could be the result of increased utilization of GSH for conjugation and/or its participation as an antioxidant against free radicals induced by metribuzin toxicity.

Additionally, the corresponding diminution in the activity of GPx is consistent with the data provided by Sharma[44] who indicated that the decrement in GPx level in rats treated by cypermethrin pesticide might come from the decrease in GSH level as the latter is used as substrate of GPx, therefore a decreased GSH levels showed in these results confirmed this theory. Moreover, a reduction in the oxidative damage was observed in metribuzin-quercetin group, with elevation in the level of GSH and enzymatic antioxidant (GPx), which may be due to the fact that quercetin can reduce the consumption of GSH and the enzymatic antioxidants under oxidative stress

condition.[30] In support of the present findings, other studies proved the protective effect of quercetin on enzymatic antioxidant system[43], and the nonenzymatic antioxidants as GSH[38].

In conclusion, the present study showed that Que has considerable cytoprotective outcome against Mtz induced oxidative damage. Such protection is likely attributed to its antioxidant properties by scavenging the toxic generated free radicals.

Acknowledgement: The present research was supported by the Algerian Ministry of Higher Education and Scientific Research, Directorate General for Scientific Research and Technological Development through the Research Laboratory "Laboratory of Biochemical and Environmental Toxicology" Faculty of Sciences, University of Badji Mokhtar, Annaba, Algeria.

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Source of Support: Nil, **Conflict of Interest:** None.