Bromuconazole caused genotoxicity, hepatic and renal failure via oxidative stress process in Wistar rats

Karima RJIBA (karimarjiba2@gmail.com)
University of Monastir Faculty of Dental Medicine of Monastir: Universite de Monastir Faculte de Medecine Dentaire de Monastir

Hiba Hamdi
Universite de Monastir Faculte de Medecine de Monastir

Asma M'nassri
UM FMDM: Universite de Monastir Faculte de Medecine Dentaire de Monastir

Yosra Guedri
Hôpital Universitaire Sahloul: Hopital Sahloul

Moncef Mokni
Centre Hospitalier Universitaire Farhat Hached de Sousse

Salwa Abid
Université de Monastir Faculté de Médecine Dentaire de Monastir: Universite de Monastir Faculte de Medecine Dentaire de Monastir

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Abstract

Bromuconazole is a triazole pesticide used to protect vegetables and fruits against diverse fungi pathologies. However, its utilization may be accompanied by diverse tissues injuries. For this, we tried to examine bromuconazole effects in liver and kidney tissues by the evaluation of biochemical and histopathological modifications also by genotoxic and oxidative stress analysis. Adult male Wistar rats were divided into four groups, each consisting of 6 animals. The control group received daily a corn oil (vehicle) orally. Three oral Bromuconazole doses were tested (1, 5 and 10 % of LD50) daily for 28 days. Bromuconazole increased the plasma activities of transaminases (AST, ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), creatinine and uric acid levels. histopathological check showed that Bromuconazole caused organs failure. This study make known that Bromuconazole caused conspicuous DNA damage either in hepatic and kidney tissues, with a significant increase in malondialdehyde and protein carbonyl levels followed by the increase in the enzymatic activity of catalase and superoxide dismutase in a dose dependent manner. Glutathione-S-transferase (GST) and peroxidase (GPx) activities were also recorded. Our results highlight that bromuconazole exposure induced genotoxic damage and organs failure that may be caused by the disturbances of oxidative stress statue in liver and kidney tissues.

1. Introduction

Fungicides are a pesticide group applied in agriculture production to protect against several fungal spores and fungi growth observed in vegetables, fruits, and tubers. It has been shown by Gupta and Aggarwal (2007) that, azole compounds play a crucial role either to prevent or to cure fungal infections observed in ornamental plants, turf grasses, standing crops and tress. While azole compounds are classified chemically into triazoles and imidazoles; Tomlin, (1997) then Roberts and Hutson (1999) reported that the antifungal activity of triazole class is due to the same molecular mechanism. Triazole fungicide act by blocking the synthesis of ergosterol, known as an essential membrane compound, leading to the disturbance of cell membrane assembly of yeast and fungi (Lamb et al., 2001). Bromuconazole is a triazole fungicide used for its preventive and curative action against ascomycetes, basidiomycetes, and deuteromycetes diseases (Menegola et al., 2005). Bromuconazole is used primarily in enclosed commercial greenhouses to control several vegetables and fruits diseases in bananas, cereals, grapes, rice and vegetables (Osman et al., 2001). Previous studies demonstrated that bromuconazole was a toxic agent in experimental animals, this toxicity was caused by trans-Bromuconazole. Studies carried out by Suzuki et al. (2000) and Sun et al. (2006) demonstrate that the toxic effect of this chiral triazole was due to the inhibition of cytochrome P450 activity of fungal. According to EPA, (1995) bromuconazole caused urinary bladder and renal damage (hydronephrosis and urothelial hyperplasia in renal pelvis) observed in rats, mice and dogs. Other researchers showed that bromuconazole increase the incidence of liver adenoma in mice (Juberg et al., 2006) and induced hyperplasia of thyroid follicular epithelial cells in rats (Noda et al., 2005). In spite of broad apply of bromuconazole as a fungicide only little details were available regarding the molecular mechanism

adopted by this triazole to cause nephrotoxicity and hepatotoxicity. In this current study, we aimed to check the mechanism adopted by Bromuconazole to cause liver and kidney failure, for this, assessment of oxidative damage and genotoxity was performed.

2. Materials And Methods

2.1. Chemicals

Bromuconazole (1-[[4-bromo-2-(2,4-dichlorophenyl) tetrahydro-2-furanyl] methyl]-1H-1,2,4-triazole); 2,4-Dinitro-phenylhydrazine (2,4-DNPH); 2,7-Dichlorofluoresce diacetate (DCFH-DA); Low melting point agarose (LMA) and normal melting point agarose (NMA) were purchased from Sigma (Steinheim, Germany).

2.2. Animal treatment

In the current study rats (160 ± 20 g) were acclimatized at room temperature 22 ± 2°C, 12-hlight/12-dark cycles and permitted access to food and water at libido. In this experimental design, rats were divided into four groups (six animals in each group). The control group received daily a corn oil (vehicle) orally. To test genotoxic damage, oxidative stress and organs failure induced by bromuconazole, three oral doses using stomach gavage were tested: 3.28, 16.4 and 32.8 mg/kg/j (dissolved in vehicle) corresponding respectively to 1, 5 and 10 % the oral LD50 (328 mg/kg b.w.) (EFSA; 2010). Body weight of rats was measured every week and continued mortality control during the time of experience. After 28 days, groups were sacrificed by decapitation and their liver and kidney were removed for analysis. blood sample was taken by cardiac puncture in heparin tubes.

2.3. Preparation of kidney and liver extracts

Organs were crushed with 10 mM of Tris-hydrochloric acid (Tris-HCl; pH 7.4) at 4°C. After centrifugation, protein concentrations were determined in homogenates using the protein Bio-Rad assay (Bradford et al., 1976).

2.4. Assessment of body and organs weight

During experiment, animal weight was measured every week, organs weight was measured immediately after the sacrifice.

Body weight gain (%) = (Final body weight-Initial body weight X 100)/Initial body weight Relative organ weight (%) = Organ weight X 100/Final body weight.

2.5. Examination of liver and kidney biochemical parameters

Plasma activities of alkaline phosphatase (ALP) was done according to Young et al. (1975), lactate dehydrogenase (LDH) was determined according to Vassault (1983), aspartate aminotransferase (AST)

and alanine aminotransferase (ALT) were evaluated according to the method of Reitman and Frankel (1957), creatinine and uric acid according to Tietz et al. (1994) and Tietz (1995).

2.6. Histopathological examination of kidney and liver tissue

At the end of the experimental period liver and kidneys were removed and were cut in a sagittal section into two halves, fixed in buffered formalin (15 %), dehydrated in alcohol concentrations (70 to 100 %) and impregnated with paraffin (56 to 57°C). Samples were cut using a microtome to make sections of 5 µm thickness and stained with hematoxylin-eosin for light microscopy examination. Slides were analyzed for the degree of liver and kidney abnormality using scores of none (−), slight (+), moderate (++), and severe (+++) damage scale.

2.7. Determination of lipid peroxidation level

Malondialdehyde (MDA) levels in organs were quantified by a spectrophotometric method according to Ohkawa et al. (1979). The optic density corresponding to the complex formed with the TBAMDA was proportional to the concentration of MDA and to the lipid peroxide. The concentration of millimoles of MDA/mg of proteins was calculated from the absorbance at 530 nm using the molar extinction coefficient of MDA 1.56 x 10^5mol/l/cm.

2.8. Protein carbonyl level in liver and kidney tissues

The level of protein carbonyl in hepatic and renal homogenates, was determined by a spectrophotometric method, as described by Mercier et al. (2004). Protein carbonyl concentration was determined from the absorbance at 370 nm, applying the molar extinction coefficient of 22.0 $mM^{-1}cm^{-1}$.

2.9. Determination of superoxide dismutase activity

The superoxide dismutase (SOD) activity assay was performed by analysis of the autoxidation of pyrogallol at 420 nm (Marklund and Marklund, 1974). One unit of SOD activity was calculated as the amount of protein that caused a 50% inhibition of pyrogallol autooxidation. The SOD activity is expressed in units per milligram of protein.

2.10. Determination of catalase activity

Catalase activity was measured by spectrophotometry method at 240 nm, 25°C according to Clairbone, (1985). Catalase activity was calculated using the molar extinction coefficient (0.04 mM/cm). Results were expressed as micromoles of H_2O_2 per minute per milligram of proteins.

2.11 Measurement of glutathione peroxidase activity

The activity of glutathione peroxidase (GPx) was determined according to the method of Flohe and Gunzler (1984) modified using H_2O_2 as a substrate. The GPx activity was determined by calculating the

decrease of reduced GSH level compared to the non-enzymatic “white” reaction. It was expressed in micromole of oxidized GSH per minute per milligram of protein.

2.12. Measurement of glutathione S-transferase activity

The enzymatic activity of glutathione S-transferase (GST) was measured according to the method used by Habig et al. (1974). The activity of this enzyme was determined via the formation of 1-glutathione-2,4-dinitrobenzene which serves as a chromophore at the wavelength of 340 nm (at 25°C) from 1-Cl-2,4-dinitrobenzene (CDNB). GST activity was expressed in micromole per milligram of protein.

2.13. Determination of DNA damage by the comet assay

Alkaline comet assay was conducted according to Tice et al. (2000) with minor modifications (Picada, 2003). 5 ml of hepatic and renal cell suspensions were embedded in 60 ml of 1% low-melting-point agarose and spread on agarose-precoated microscope slides. Slides were immersed lysis solution and left at 4°C overnight (Banath, 2002), placed in an electrophoresis alkaline buffer (pH > 13), and the embedded cells were exposed to this alkaline solution (20 min). Electrophoresis (20 min, 25 V, 300 mA). After electrophoresis, slides were neutralized with Tris (0.4M, pH 7.5), and DNA was stained with 50 ml of ethidium bromide (20 mg/ml). As described by Collins et al. (1996), a total of 100 comets on each slide were visually scored according to the intensity of fluorescence in the tail and classified by one of five classes. Total score was evaluated according to the following equation: (% of cells in class 0 X 0) + (% of cells in class 1 X 1) + (% of cells in class 2 X 2) + (% of cells in class 3 X 3) + (% of cells in class 4 X 4).

2.14. Statistical analysis

All data were expressed as means ± SD. Statistical significance of the differences among different groups was evaluated by one-way analysis of variance followed by Fisher multiple comparisons test as a post hoc test. Data were analyzed using SPSS statistical program (version 10.0 software, SPSS Inc., Chicago, Illinois, USA). Value of $p < 0.05$ was considered to be significant. (*) indicates significant difference from control. Each experiment was carried out separately at least three times.

3. Results

3.1. Body weight, absolute and relative organs weights

Throughout treatment period, as compared to the untreated group ($p < 0.05$), the body weight, absolute and relative weights of the liver and kidney of treated animals were increased (Table 1). In our experiment conditions, absence of mortality was observed in each group.

3.2. Serum biomarkers examination

Bromuconazole administration at the doses of 3.28, 16.4 and 32.8 mg/kg b.w. corresponding respectively to 1 % LD50, 5 % LD50 and 10 % LD50, enhanced significantly ($p < 0.05$) the level of biochemical markers in the liver (AST, ALT, ALP, and LDH) and in kidney (uric acid and creatinine) as compared to the control group (Table 2).

3.3. Histopathological observations

Histopathological examination and their score in liver and kidney of rats treated with Bromuconazole (1 %, 5 % and 10 % LD50) for 28 days was shown in Figs. 1a, 1b and Table 3 and organs failure was compared with control. Examination of liver tissue indicated degeneration of hepatocytes, dilation and congestion of central vein, necrosis, dilation and congestion of portal triad, lipid vacuolation, and infiltration of inflammatory leucocytes (Fig. 1a) (C–H). Kidney tissue examination at different doses showed atrophy of glomerulus and hypertrophy of glomerular chamber, infiltration of inflammatory leucocytes, atrophy of distal and proximal convoluted tubules and brush border and lipid vacuolation (Fig. 1b) (C–F). kidney examination of untreated sections revealed normal histo-architecture of renal parenchyma (Fig. 1b A, B). All histopathological alterations and their score were illustrated in Table 3.

3.4. Induction of lipid peroxidation

In order to estimate lipid peroxidation status, the MDA level was measured and the results were illustrated in Fig. 2. As compared with the control group bromuconazole administration at different doses induced a significant increase ($p < 0.05$) in MDA level in both kidney and liver tissues. Therefore, in hepatic tissue, the MDA level increased from a basal level of 7.25 ± 0.95 mmol/mg of protein to reach 64.65 ± 3.85 mmol/mg of protein in the bromuconazole-treated group (10 % LD50).

3.5. Protein carbonyl formation in kidney and liver extracts

As compared to the control group, protein carbonyl level in liver extracts increased significantly from the basal value of 21.22 ± 1.50 nmol/mg of protein in the control group to 84.12 ± 7.24 nmol/mg of protein in rats treated with bromuconazole at 10 % LD50. Figure 3 showed that carbonyl level in kidney extracts increased significantly from the basal value of 18.9 ± 1.72 nmol/mg of protein (control group) to 69.23 ± 6.33 nmol/mg of protein (the bromuconazole-treated group).

3.6. Evaluation of Catalase and SOD activities

Level of catalase can indicate the magnitude of oxidative stress that occurs. The effect of Bromuconazole on catalase activity was illustrated in Fig. 4. Our results showed that Bromuconazole induced a marked increase in catalase activity in both kidney and liver extracts. In liver extract, the increase in catalase activity in bromuconazole-treated (10% LD50) group was about 4 folds compared to untreated group. Moreover, we observed a decrease in catalase activity of rat treated with bromuconazole at the dose of 32.8 mg/kg b.w. Furthermore, rat exposed to triazole presented significant enhance in SOD activity. In renal extract enzymatic activity of SOD passed from the basal value of 5.2 ± 1.2 USOD/mg of proteins in the untreated group to 36.79 ± 4.31 USOD/mg of proteins in bromuconazole treated rats (10% of LD50) (Fig. 5).

3.7. Evaluation of GPx, and GST enzymes activities

As indicated in Fig. 6 (A and B), GPx and GST activities increased significantly ($p < 0.05$), as compared to the control group, either in kidney and livers tissues following bromuconazole administration. In the hepatic extract, GPx activity passed from 46.27 ± 3 to 129.84 ± 9.01 µmol of GSH oxidized/min/mg of proteins in bromuconazole treated group (5 % of LD50). In the renal extract, GST activity passed from 0.030 ± 0.004 to 0.178 ± 0.014 µmol/min/mg of proteins in bromuconazole treated group (5 % of LD50). Furthermore, we showed that GPx and GST activities decreased significantly at the highest dose's exposure (10 % LD50).

3.8. Genotoxic effect of bromuconazole assessed by comet assay

We observed a significant increase in the total DNA damage in rats treated with bromuconazole at different experimental doses in either hepatic and renal extracts. As compared to the control group, this increase reach to about 16 folds either in liver and kidney tissues at the highest dose of exposition (10 % LD50). The examination of the control group showed no specific DNA fragmentation. Figure 7 illustrated the results of the visual scoring of total basic DNA damage.

4. Discussion

Bromuconazole is a widely used triazole pesticide which is toxic not only to target fungi but also to animals and humans. It has high-affinity binding ability to aromatase cytochrome P450 enzyme and its encoding gene Cyp19A1, which converts androgens into the corresponding estrogens, therefore they can inhibit aromatase and block estradiol biosynthesis (Edwards and Godley, 2010; Sun et al., 2006; Tully et al., 2006). In the curent work, we looked to explore the mecanisme adopted by bromuconazole to cause hepatic and renal failure.

Primary, nephrotoxicity and hepatotoxicity caused by bromuconazole was gauged by body weight increase, kidney and liver relative and absolute weight gain and biochemical parameters changes with an increase in plasma level activities of hepatic enzymes: AST, ALT, LDH, ALP. An enhanced level of biochemical marker of kidney toxicity (uric acid and creatinine) was also shown. These results were in agreement with those of EFSA (2010), Osman et al. (2011) and Abdelhady et al. (2017) who indicated that bromuconazole exposition caused liver and kidney injuries marked by organs weight gains and significant enhancement of serum activities of urea, creatinine, ALP, AST, y-glutamyl transpeptidase, acid phosphatases.

Histopatological changes in liver tissue marked by hepatocytes degeneration, necrosis, dilation and congestion either of central vein and of portal triad and infiltration of inflammatory leucocytes was observed. Our results are in agreement with those of Abdelhady et al. (2017) showing necrobiotic changes in liver tissue with characteristic vacuolation after chronic bromuconazole exposure. Morever, Osman et al. (2011) reported that bromuconazole administration for 90 days caused multiple

microscopic foci of hepatocellular carcinoma. In the same context, severe histopathological bromuconazole administration caused severe modifications in kidney tissue including atrophy of glomerulus and hypertrophy of glomerular chamber, atrophy of distal and proximal convoluted tubules and lipid vacuolation. These results were in accord with those of Osman et al. 2011 observing glomerulonephritis and degeneration of tubular epithelial lining with intralumenal eosinophilic casts after bromuconazole exposition.

It has been shown that reactive oxygen species (ROS) have been proposed to be involved in a variety of human illnesses. For this, to determine the mechanism adopted by bromuconazole to cause liver and kidney failure oxidative stress was investigated. Oxidative stress defined as an imbalance between formation of reactive oxygen spieces and antioxidant defense mechanisms. Pilz et al. (2000) and Suttnar et al. (1997) demonstrated that ROS interact with double bonds of polyunsaturated fatty acids to produce lipid hydroperoxides. Malondialdehyde (MDA) is the product of peroxidized polyunsaturated fatty acids, that has been commonly used for the assessment of lipoperoxidation in biological and medical sciences (Suttnar et al., 2001, Bird., 1984). For this, to evaluate the oxidative damage induced by bromuconaole in hepatic and renal tissues, we looked at the level of MDA. In this issue, rat's exposure to bromuconaole at different doses corresponding to 1, 5 and 10 % of LD50 caused a significant enhance in MDA level either in hepatic and renal tissues. This is in agreement with previous work showing an enhanced MDA level in hepatic and renal tissues of rats exposed to bromuconaole (Osman et al., 2011; Abdelhady et al., 2017). To more evaluate the oxidant activity of triazole, we looked for protein carbonyl generation. In our study, bromuconaole administration caused a noticeable protein alteration assessed by an increase in protein carbonyl generation either in renal and hepatic extracts. This damage occurs in a dose dependent manner with a significant pronounced degree at a dose corresponding to 10 % of LD50. Our findings were in accordance with those of Bruno et al. (2009), showing that conazole fungicide exposition caused protein carbonyls generation in hepatic tissue of mouse.

In the other hand, oxidative lesions produced by bromuconazole was also explored through evaluating of endogenous defense system such as CAT, SOD, GPx, and GST. It has been known that CAT, an enzyme degrading hydrogen peroxide and SOD, a quite effective enzyme in dismutating superoxide anion, can be used as a therapeutic agent to reduce ROS generated under pathophysiological conditions (Nishikawa et al., 2009). Our study showed that bromuconazole enhanced CAT and SOD activity and this activation was more marked when rats were exposed to a dose corresponding to 32.8 mg/kg/j. It is commonly known that GST catalyze the reaction of the sulfydryl group of the tripeptide glutathione of various xenobiotics. In addition to this direct detoxication, GSTs catalyze the secondary metabolism of compounds oxidized by other enzymes (Parke and Piotrowski 1996; Malmezat et al., 2000). Our results obviously showed that bromuconaole exposure either at 1, 5 and 10 % of LD50 increased significantly the level of GST and GPX in kidney and liver tissues. According to these results, we can suggest that bromuconazole caused the alterations of antioxidant defense and consequently support the hypothesis that this triazole exercise its toxicity in liver and kidney tissues via an oxidative stress process.

Given that the oxidative stress can attack biomolecules, such as DNA, leading to genotoxic damage, potential genotoxic effect of bromuconazole in liver and kidney tissues was checked. In this study, by the use of the comet assay, we clearly showed that bromuconazole at different doses caused a significant increase in DNA fragmentation as compared to the control group.

According to our results, we can suppose that bromuconazole was hepatoxic and nephrotoxic triazole in wistar rats. We also showed that this fungicide was genotoxic in both liver and kidney tissues. This side effect was due to its oxidant potential.

Declarations

Funding information

This study was supported by The Tunisian Ministry of Higher Education, Scientific Research and Technology. Compliance with ethical standards

Ethical Approval and consent to participate

The experimental procedures were carried out according to the National Institute of Health Guidelines for Animal Care (Council of European Communities 1986) and approved by the local Ethics Committee of Faculty of Pharmacy of Monastir.

Consent to Publish

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Declaration of Conflicting Interests

The author(s) assure no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Availability of data and materials

The author (s) declare the availability of data and materials. The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Authors Contributions

Dr. Karima Rjiba Touati designed the study, conducted the study and wrote the manuscript. Mis. Hiba Hamdi supervised the study and conducted statistical analysis, Mis. Asma M’nassri conducted statistical analysis, Yosra Guedri and Moncef Mokni participated in the biochemical and histological studies. The authors are thankful to Pr. Salwa Abid for their laboratory supports.

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Tables

Table 1 : Body weight, absolute, and relative liver and kidney weights of control and treated rats with bromuconazole at differents doses. Values represent mean ± SD. *p < 0.05 versus control.

Groups	Body weight gain (g)	Kidney weight (g)	Liver weight (g)	Kidney weight/100g b.w.	Liver weight/100g b.w.
Controls	11.12 ± 0.75	1.15 ± 0.02	5.22 ± 0.15	0.63 ± 0.03	2.84 ± 0.1
1% of LD50	23.82 ± 1.83	1.54 ± 0.08	6.62 ± 0.26	0.70 ± 0.04	3.01 ±.0.12
5% of LD50	45.91± 3.22	1.92 ± 0.04	11.46 ± 0.38	0.74 ± 0.02	4.44 ±.0.17
10% of LD50	70.03 ± 4.06	2.60 ± 0.16	14.47 ± 0.26	0.92 ± 0.07	5.11 ±. 0.12

Table 2 : Plasma transaminases (AST, ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) activities, creatinine and BUN levels in liver (A) and kidney (B) of untreated group and rats exposed to bromuconazole. Values represent mean ± SD. *p < 0.05 vs. control.

	Controls	1% LD50	5% LD50	10% LD50
Liver tissue				
AST (UI/l)	80,5 ± 6,36	89 ± 2,9	107,5 ± 7,78	129 ± 15,66
ALT (UI/l)	33 ± 4,25	37 ± 1,42	48,5 ± 2,12	83 ± 14,25
ALP (UI/l)	95,37 ± 17,24	131,93 ± 15,048	142,5 ± 12,82	185,25 ± 17,43
LDH (UI/l)	156,5 ± 14,95	293 ± 21,22	358 ± 16,97	413 ± 29,9
Renal tissue				
Creatinine (µmol/l)	44 ± 2,83	69 ± 4,95	114,5 ± 4,95	236,5 ± 25,33
Uric acid (mmol/l)	33,50 ± 3,53	48,5 ± 7,78	75 ± 8,48	117,5 ± 13,53

Table 3 : Grading of the histopathological alterations in hepatic and renal tissues of rats, treated by different doses of Bromuconazole. (−) none, (+) mild, (++) moderate, (+++) severe

Histopathological alterations	Controls	1% of LD50	5% of LD50	10% of LD50
Hepatique alteration				
Degeneration of hepatocytes	-	+	++	+++
Necrosis	-	+	++	+++
Dilated sinusoid	-	+	++	+++
Lipid vacuolization	-	+	++	+++
Infiltration of inflammatory leucocytes	-	+	++	+++
Dilation and congestion of portal triad	-	+	++	+++
Dilation and congestion of central vein	-	+	++	+++
Kidney alteration				
Lipid vacuolization	-	+	++	+++
Infiltration of inflammatory leucocytes	-	+	++	+++
Atrophy of distal and proximal convoluted tubules and brush border	-	+	++	+++
Atrophy of glomerulus	-	+	++	+++
Hypertrophy of the glomerular chamber	-	+	++	+++

Figures

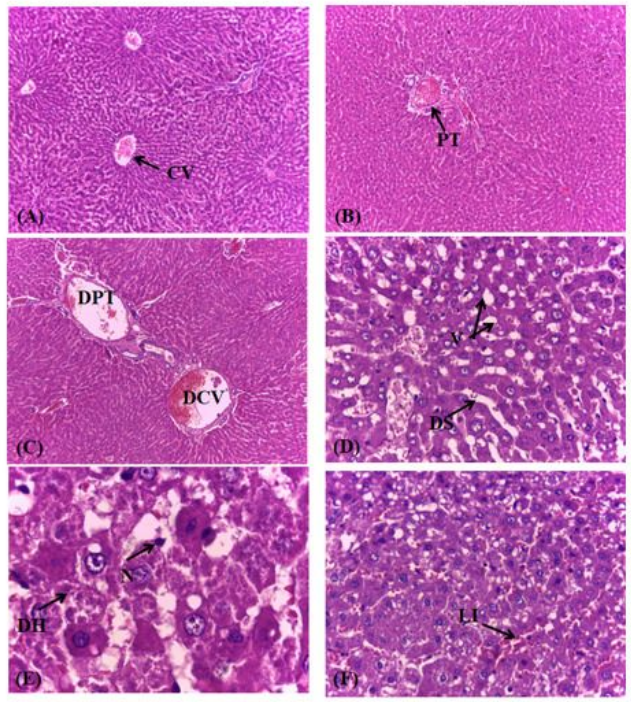

Figure 1a

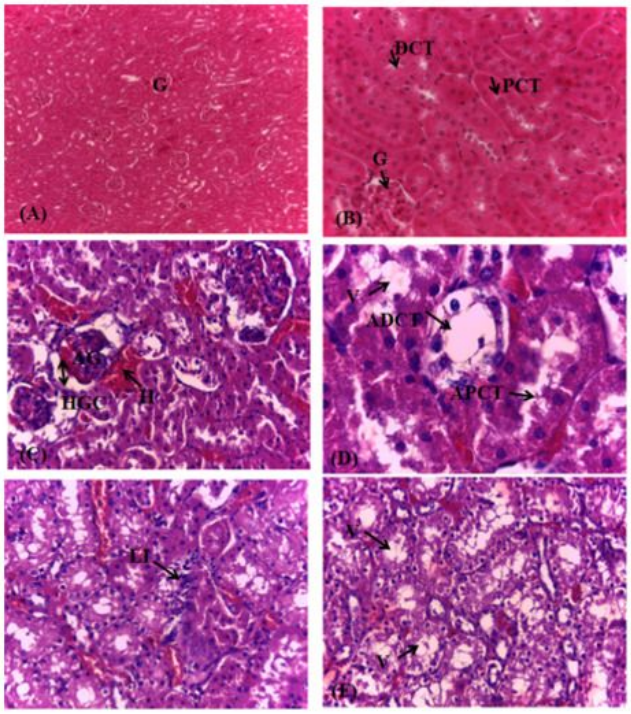

Figure 1b

Figure 1

a : Examination of liver tissue stained by H & E. Liver sections of the control group revealed normal preserved histoarchitecture (A, B). The damage in liver sections (C–F) induced by bromuconazole increases in a dose dependent manner. DS, dilated sinusoid; S, sinusoids; CV, central vein; V, vacuoles; DH, degenerated hepatocyte ; Nc, necrotic cell; DCV, dilated central vein; DPT, dilated portal triad; LI, inflammatory leucocyte infiltrations. b : Microscopic examination of kidney stained by H & E of. Kidney

sections of the control rats revealed normal preserved histoarchitecture (A, B). The damage in kidney sections (C–F) induced by bromuconazole increases in dose dependent manner. HGC, hypertrophy of glomerular chamber; V, vacuolation; ADCT and APCT, atrophy of distal and proximal convoluted tubules ; H, hemorrhage ; LI, lymphocyte infiltration ; AG, atrophy of glomerulus.

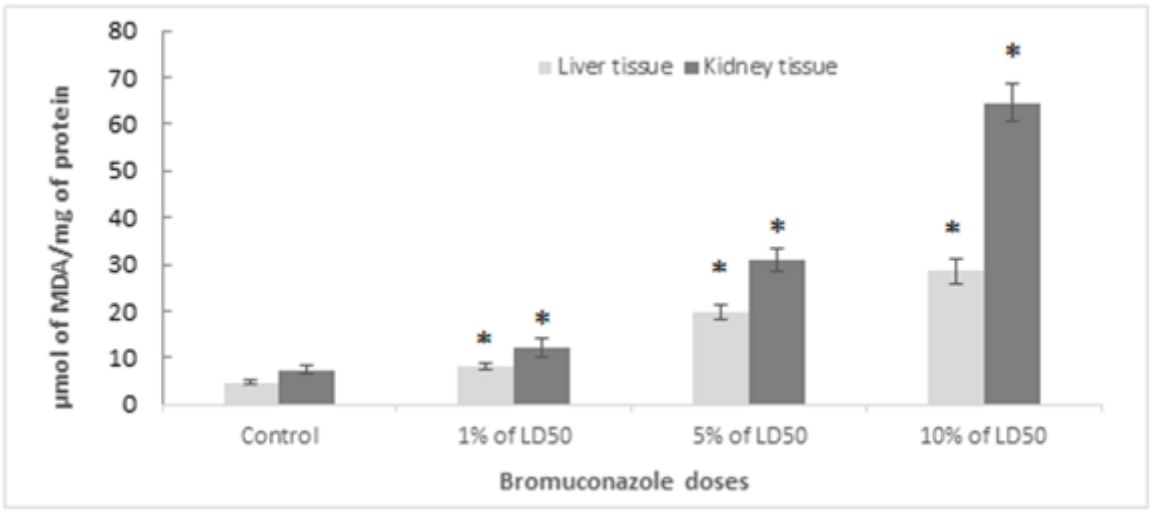

Figure 2

Lipid peroxidation as determined by MDA level in liver and kidney tissues of Wistar rat. bromuconazole was administered at 1%, 5% and 10% of LD50. *p < 0.05 versus control. MDA: malondialdehyde. Values represent mean ± SD. (*p < 0.05) vs. control.

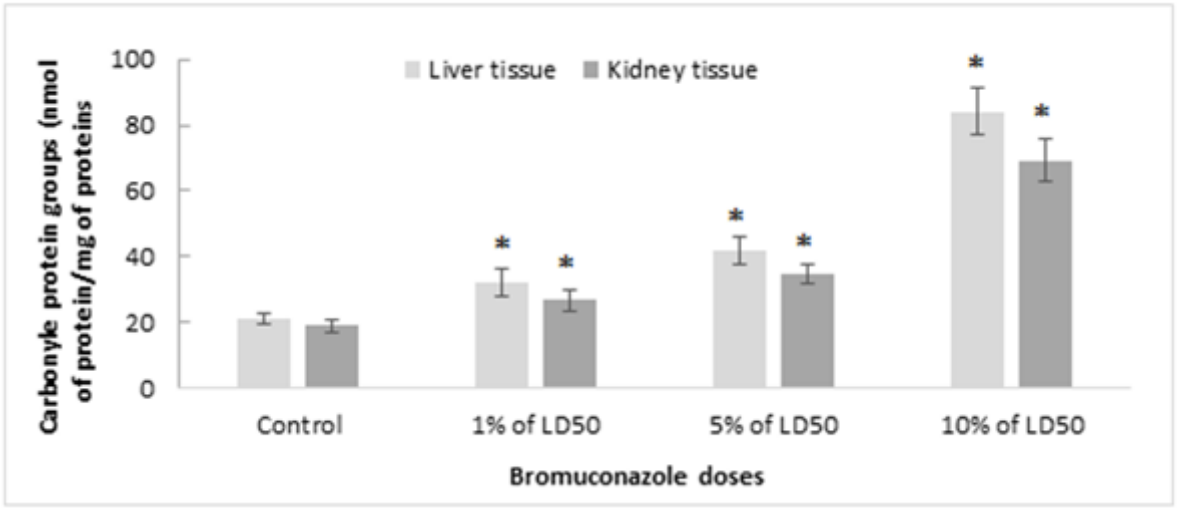

Figure 3

Effect of bromuconazole exposition on protein carbonyl levels in rat kidney and liver. *p < 0.05 versus control.

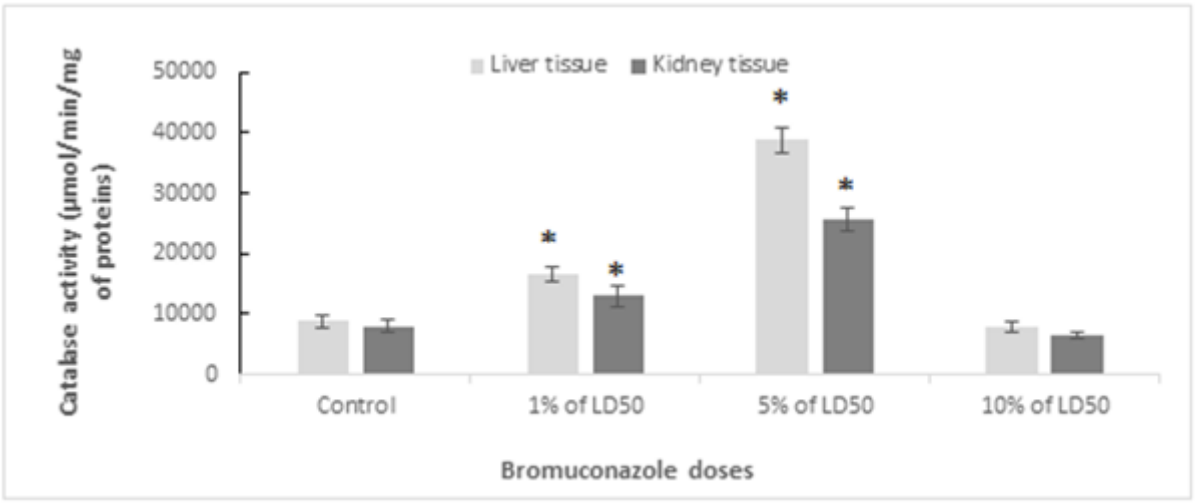

Figure 4

Evaluation of superoxide dismutase activity (SOD) in the liver (a) and kidney (b) after 28 days of exposure to bromuconazole at the indicated doses; 1%, 5% and 10% of LD50. Values represent mean ± SD. (*p < 0.05) vs. control.

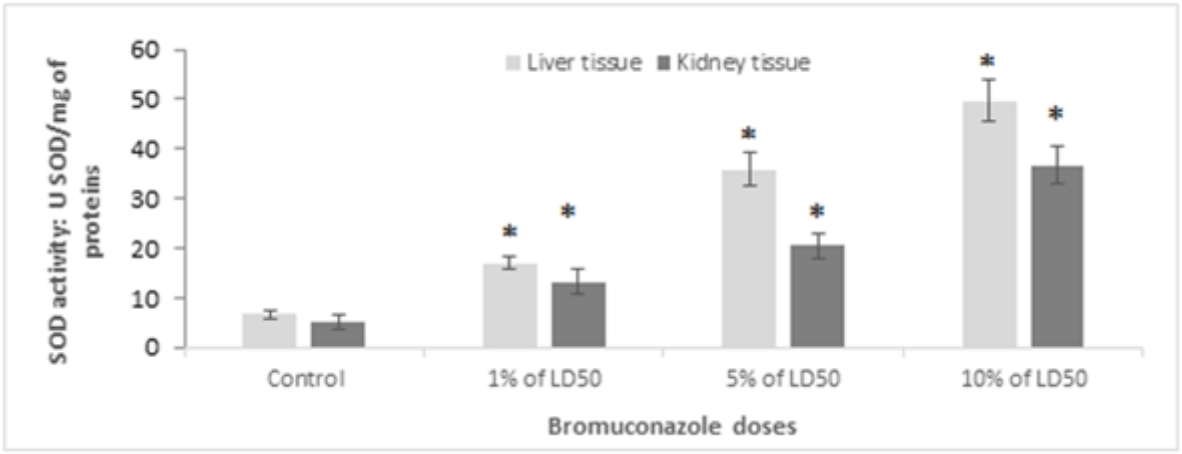

Figure 5

Evaluation of catalase activity (CAT) in the liver and kidney tissue after 28 days of exposure to bromuconazole at the indicated doses; 8, 24, 40, and 56 mg/kg bw representing, respectively, 1%, 5% and 10% of LD50.Values representmean ± SD. (*p < 0.05) vs. control.

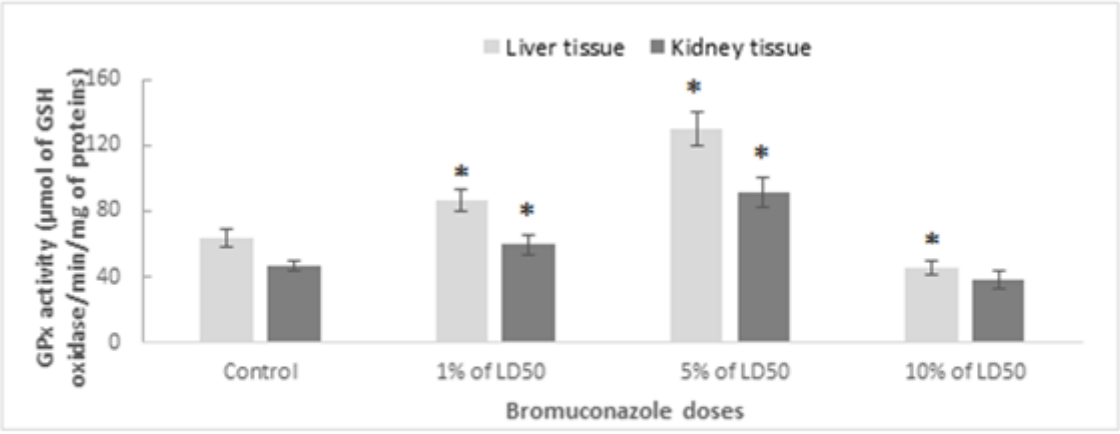

Figure 6A

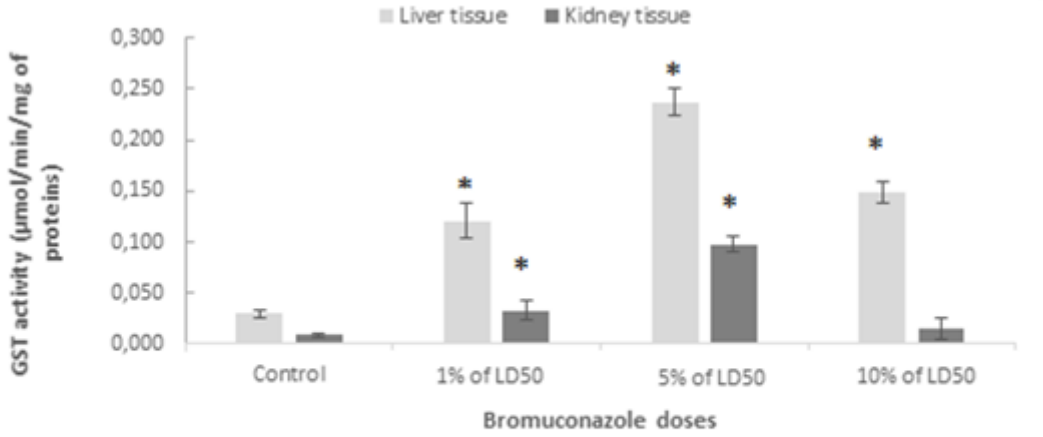

Figure 6B

Figure 6

(A) Measurement of glutathione peroxidase activity (GPx) in the liver (a) and kidney (b) after 28 days of exposure to bromuconazole at the indicated doses; 28, 16.4 and 32.8 mg/kg/j representing, respectively, 1%, 5% and 10% of LD50. Values represent mean ± SD. (*p < 0.05) vs. control. (B) Measurement of glutathione S-transferase activity (GST) in the liver (a) and kidney (b) after 28 days of exposure to bromuconazole at the indicated doses; 28, 16.4 and 32.8 mg/kg/j representing, respectively, 1%, 5% and 10% of LD50. Values represent mean ± SD. (*p < 0.05) vs. control.

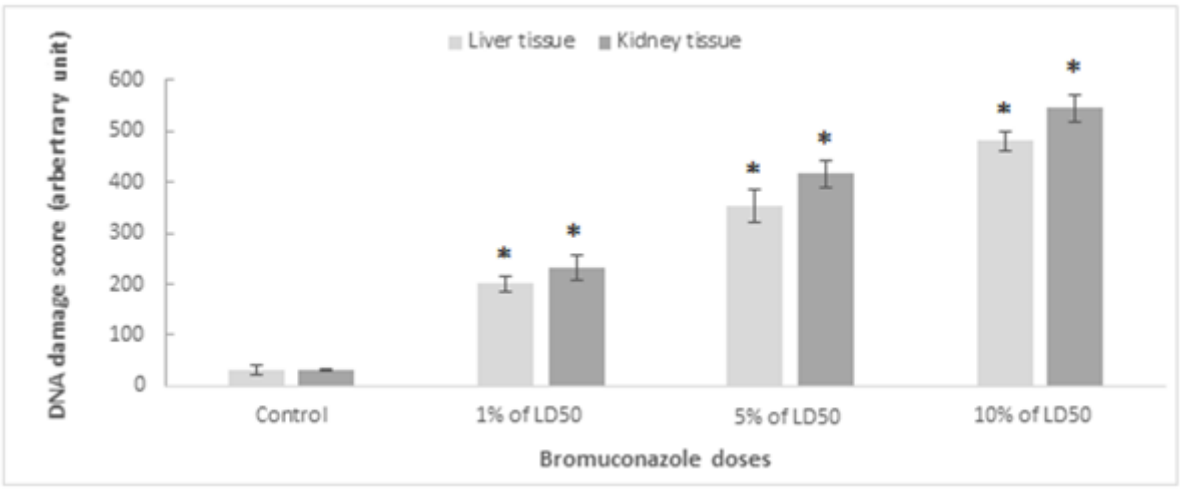

Figure 7

Total DNA damage measured by the alkaline comet assay in isolated cells of rat kidney (A) and liver (B). DNA fragmentation in renal and hepatic tissues of rats after a bromuconazole exposure. *p < 0.05 versus control.