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Original Research

Efficacy of *Allium sativum* oil to alleviate tebuconazol-induced oxidative stress in the liver of adult rats

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Abstract: The present study focused on the protective efficacy of *Allium sativum* oil (ASO) against tebuconazol (TEB)-induced oxidative stress in the liver of adult rats. Thirty-two rats were randomly divided into four groups of eight each: group I served as control rats, group II was treated with TEB (100 mg/kg bw), group III received ASO (5ml/kg bw). The animals of group IV were treated with TEB and ASO, during 4 weeks. The obtained results showed that TEB induced a significant change of some hematological parameters, including red blood cells (RBC), haemoglobin content (Hb), haematocrit (Ht), white blood cells (WBC) and platelet (Plt) compared to the control group. Moreover, while the total cholesterol levels and the activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH) and γ-Glutamyltranspeptidase (γGT) significantly increased due to TEB administration, the concentrations of plasma total protein, albumin and triglyceride considerably decreased. Furthermore, the exposure to TEB significantly increased the malondialdehyde (MDA), protein carbonyl (PCO) and advanced oxidation protein products (AOPP) levels and decreased glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) activities in the hepatic tissues. The results were confirmed by the histological impairments. Besides, the co-administration of ASO improved the status of all studied parameters. Therefore, our investigation revealed that ASO had protective effects against TEB-induced liver injury, which could be attributed to its phenolic compounds.

Key words: Tebuconazole; *Allium sativum*; Hepatotoxicity; Oxidative stress.

Introduction

The use of pesticides in agriculture remains the most effective method for the protection of plants and animals from a large number of pests (1). Tebuconazole is a very effective fungicide used for the control of mildew and rust on wheat, barly, rice, fruits and vegetables (2). It belongs to the group of triazole fungicides, whose mode of action is by the inhibition of the activity of lanosterol 14α-demethylase (CYP51), resulting in the membrane disruption and subsequent inhibition of cell growth (2,3). The disadvantages of fungicides include their toxicity to humans, animals, useful plants and the persistence (long life) of some of these chemicals in the environment (4). TEB is a potent xenobiotic to which exposure can cause metabolic alterations and the death of different organisms (5). The exposure to TEB produces immunological neurobehavioral and neuropathological deficiency (6) and cause cancer, reproductive and development toxicity, as well as various other effects. The most sensitive endpoints used for risk assessment include the effects on the liver, spleen and the adrenal glands (7).

TEB could cause oxidative stress in many organisms, leading to the production of free radicals (8). Cells have enzymatic and non-enzymatic scavenger systems against these free radicals (9). The imbalance between defense and free radical production systems causes lesions at the level of the body cells (10).

To counteract oxidative stress, endogenous and exogenous antioxidants play a crucial role to remove ROS. They act as free radical scavengers preventing cells and tissue damage. Exogenous antioxidants obtained from natural sources are considered relatively safe and without undesirable side effects (11)

Many medicinal plants have interesting biological and pharmacological activities and are used as therapeutic agents (12). Garlic (*Allium sativum* L.) belongs to the Alliaceae family (13). It is a popular spice in cooking and it is widely used as a medicinal herb across the globe. Garlic and its components have a variety of beneficial biological activities. It has been proved to elicit antimicrobial, antihypertensive, hypolipidemic, hepatoprotective, antidiabetic, and insecticidal properties (14). The immunomodulation and antitumor activities of garlic have also been reported (15). Studies carried out on garlic have reported the presence of two main classes of antioxidant components, namely flavonoids and polyphenolics. These are likely to play an important role in the widely demonstrated biological effects of garlic (16).

Garlic oil behaves as a nutraceutical compound, with numerous applications in food and pharmaceutical industries such as the flavoring some cuisine such as salads, and sauces; reducing blood pressure, and preventing cancer and cardiovascular diseases through reducing serum LDL cholesterol and triglyceride (17).

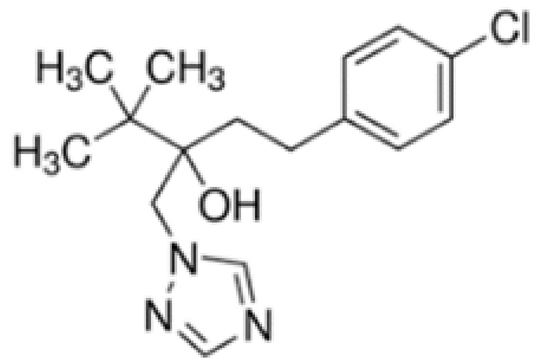

Figure 1. Chemical structure of tebuconazol.

In addition, this oil has divergent effects on the target organ and host tissues that reflect its modulatory role in cell proliferation. It has been reported to scavenger free radical species (18), and used to protect humans against oxidative stress. Garlic possesses potential health-promoting effects due to its high phenolic phytochemical content. It is also a source of natural antioxidants (19). The objective of this work is to study the protective effect of *Allium sativum* against tebuconazole-induced oxidative damage and hepatotoxicity in adult male rats.

Materials and Methods

Chemicals

Tebuconazole ($C_{16}H_{22}ClN_3O$) is a triazole fungicide (Figure 1); CAS chemical name: [(RS)-1-p-chlorophenyl]-4, 4-dimethyl-3-(1H-1,2,4-triazol-1-ylmethyl) pentan-3-ol]. The commercial formulation studied in the present work was Medalion®. The latter contained 430g/L of tebuconazole as the active ingredient, and was produced by Rotam Agrochemical (HK) Co. Ltd. All other chemical products used in this study were purchased from Sigma Chemical Co. (StLouis, France).

Plant material

A. Sativum oil was obtained from a local commercial market (it was produced by El Captain Company-CAP PHARM- grow and extracted in Egypt).

Total phenolic contents

The total phenolic content was measured with the Folin-Ciocalteu reagent according to the procedure described by Bouaziz et al (20). Gallic acid was used as a reference standard, and the results were expressed as milligram gallic acid equivalent (mg GAE/g ASO).

Total flavonoid contents

Flavonoid contents was determined by the method of Zhishen (21). Flavonoid content was estimated using catechin as a standard, analytical results are expressed in milligrams of catechin equivalents (mg CE/g ASO).

Determination of total condensed tannin contents

The total amount of condensed tannin was determined spectrophotometrcally according to Hagerman and Butler (22). The amount of condensed tannins was estimated using catechin as a standard, and the analytical results were expressed in milligrams catechin equivalents (mg CE/g ASO).

Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity assay

The antioxidant activity of ASO was firstly evaluated by monitoring its ability in quenching the stable free radical DPPH. The radical scavenging activity of ASO against DPPH free radicals was measured using the method of Bouaziz et al (23). Ascorbic acid was used as a positive control. The DPPH radical scavenging activity was calculated according to the following equation: PI (% inhibition) = 100 $(A_T - A_S)/ A_T$. Where A_T is the absorbance of the control (total) and A_S is the absorbance in the presence of the extract (sample).

Total antioxidant capacity assays

The total antioxidant capacity (TAC) was determined using the method of Prieto et al (24). The TAC was expressed as mg vitamin C equivalent (mg Vit C/g ASO).

The TAC of ASO was also estimated by ABTS method according to Turoly et al (25). The stock solution of the ABTS radical was prepared by dissolving 38.4mg of 2,2- azinobis (3-ethylbenzthiazoline-6-acid) (ABTS) in potassium persulfate solution (2.4mM). The working solution was obtained by diluting the stock solution of the ABTS radical cation with methanol to obtain an absorbance of 0.7±0.002 at 730 nm.

Animals and experimental procedure

Thirty-two Wistar male rats with body weight 290±20 g were used in this study. They were obtained from Pasteur institute (Algiers, Algeria), and acclimated for 2 weeks prior to experimentation. They were housed in cages at 25±2°C and provided with water and standard diet. The rats were randomly divided into four groups of eight each.

- Group I (C): served as controls.
- Group II (TEB): received, via force-feeding with TEB (100mg/kg bw).
- Group III (ASO): received, via force-feeding with ASO (5ml/kg bw).
- Group IV (TEB+ASO): received both TEB and ASO.

The dose of TEB used in this study represented 1/17 of LD50 (1700mg/kg bw). This dose was used by previous investigations since it is toxic but not lethal to rats (8). The dose of ASO used in our study and in other findings gave high protection against stress conditions in several tissues (18).

Samples preparation

Blood collection

At the end of the experiment, all rats were fasted overnight and sacrificed by cervical decapitation. Prior to sacrifice, blood was collected and divided into two portions. The first portion was transferred into tubes containing EDTA for the determination of hematological parameters. While the remaining blood was placed into tubes containing heparin and centrifuged at 3000 rpm for 15min to separate the plasma for biochemical analyses.

Preparation of liver homogenates

Livers were rapidly excised and washed in 0.9% NaCl solution, blotted with filter paper, and immediately homogenized in trisbuffer solution (TBS: 50mm Tris, 150mm NaCl, pH 7.4) under ice cold conditions. The liver homogenate was later centrifuged at 10.000rpm for 15 min at 4 °C and the resultant supernatant was stored at -20°C for the assay of oxidative stress related parameters.

Hematological parameters

Blood samples in EDTA tubes analyzed for hematological parameters (RBC, WBC, Hb, HT and PLT) were determined by electronic hematological counter (ERMA INC, model PCE-210N).

Biochemical parameters

The determination of protein, bilirubin, albumin and enzymes markers in plasma were measured using commercial colorimetric kits. The activities of transaminases (ALT and AST), ALP, γGT and LDH were assayed using commercial kits from Spinreact (SPINREACT.S.A/ SAU. Ctra. Santa Coloma, 7 E-17176SANT ESTEVE DE BAS (GI) SPAIN).

Protein assays

Protein content in liver was measured spectrophotometrically at 595 nm according to the method of Bradford (26), using bovine serum albumin as a standard.

Estimation of lipid peroxidation levels

The lipid peroxidation (LPO) activity was measured by the method of to Buege and Aust (27). The malondialdehyde (MDA) values were expressed as nomoles of MDA/mg protein.

Determination of advanced oxidation protein product levels

The liver levels of advanced oxidation protein products (AOPP) were determined according to the method of Kayali et al (28). The AOPP concentration in each sample was calculated using the extinction coefficient of 261 $cm^{-1}mM^{-1}$. The results were expressed as nmoles/ mg protein.

Determination of protein carbonyl levels

Protein carbonyl (PCO) was measured using the DNPH method according to Reznick and Packer (29). The PCO content was expressed as nmoles/mg protein. The results were measured spectrophotometrically at 370 nm.

Estimation of antioxidant enzymes activities

Glutathione peroxidase (GPx) activity was measured according to the procedure of Flohe and Gunzler (30). The enzyme activity was expressed as micromoles of GSH oxidized/min/mg protein.

The superoxide dismutase (SOD) activity was evaluated using the method of Asada et al (31). A unit of SOD is defined as the amount of enzyme that inhibits by 50% photoreduction of nitro bleu tetrazolium (NBT). Enzyme activity was expressed as U/mg of protein.

Catalase activity (CAT) was measured according to the method of Aebi (32). The change of H_2O_2 absorbance in 1min was measured at 240 nm. Catalase activity was calculated and expressed in µmol H_2O_2/min/mg protein.

Glutathione-S-transferase (GST) activity was measured according to Habig et al (33). The extinction coefficient used for GSH-CDNB was 9.6 mM.cm. The activity was expressed as nmol CDNB/min/mg protein.

Evaluation of reduced Glutathione levels

Reduced glutathione (GSH) activity was measured by the method of Weekbeker and Cory (34). The total GSH content was expressed as nanomoles GSH/mg of protein. The absorbance was recorded at 412 nm.

Measurement of vitamin C levels

The determination of vitamin C content in the liver tissue was performed as described by Jacques-Silva et al (35). The absorbance was measured at 540nm, and the results were expressed as micromoles/g tissue.

Determination of plasma nitric oxide levels

The nitric oxide (NO) level was determined by the method of Green et al (36). The nitrite concentration in the samples was determined with ELISA reader (nindray MR-96 A). The results were expressed as micromoles/mg protein.

Histopathological examination

Immediately after sacrifice, small pieces of liver from rats in all studied groups was fixed in formol solution, then passed through ethanol and xylene series kept in paraffin in a stove (Leica TP 1020) before being embedded in paraffin blocks (Leica EG 1160). Paraffin blocks were sliced at 3-4µm (Leica RM 2125 RTS), stained with hematoxyline and eosin and examined by light microscope (37).

Statistical analysis

The results are presented as the mean ±SD for five rats per group. The statistical significance of difference between groups was analyzed by Student's test (Microsoft® Office Excel® 2010). The level of significance was set at p≤0.05.

Results

Antioxidant activity of *Allium sativum* Oil

In this study, ASO was analyzed for its proximate composition and antioxidant activity (Table 1). The results showed that ASO contained 1.67mg of the total polyphenols, 1.61 mg of flavonoids and 0.95 mg of con-

Table 1. Amounts of total phenols content, total flavonoids and condensed tannins levels, antiradical DPPH, ABTS and TAC in *Allium sativum* oil.

Parameters	Contents
Total phenolic content (mg GAE/100 g of ASO)	1.67±0.05
Total flavonoid content (mg QAE/g ASO)	1.61±0.02
50 % scavenging concentration (mg/ml) on DPPH radical	72.50±2.42
Condensed tannins (mg CAE/g NSO)	0.95±0.07
ABTS [TEAC]	1.17±0.02
TAC (mg of ascorbic acid E/ g ASO)	1.50±0.16

GAE: gallic acid equivalent, QAE:quercetin acid equivalent, CAE: catechin acid equivalent.

Table 2. Initial and final body weights, absolute and relative liver weights. Food and water intake of control and rats treated with TEB, ASO, or their combination (TEB+ASO) during 4 weeks.

Parameters	Control	TEB	ASO	TEB+ASO
Initial body weight (g)	297.14±21.2	297.12±21.54	294.14±12.68	296.13±21.69
Final body weight (g)	331.6±12.3	288.8±12.39***	322.4±9.63	316.4±20.19##
Absolute liver weight (g)	7.73±0.47	11.02±1.32***	8.59±0.56	8.84±1.34##
Relative liver weight (g/100g bw)	2.33±0.18	3.83±0.57***	2.67±0.22	2.82±0.58*,##
Food intake (g/day/rat)	19.56±0.87	14.96±3.66*	17.18±4.25	16.81±1.74*
Water intake (mL/day/rat)	24.9±0.62	28.9±3**	21.45±2.01	28.66±5.87

Values are expressed as means±SD; n=8 for each treatment group. Significant difference: (*p<0.05, **p<0.01, ***p<0.001) compared with control group, (##p<0.01) compared with TEB+ASO.

densed tannins. The estimation of antioxidant activity DPPH revealed an IC_{50} value of 72.5 µg/ml. However, the value of ASO on ABTS radical-scavenging activity was 1.17mg with the total capacity antioxidant (TAC) equal to 1.5 mg.

Body, absolute and relative liver weight, food and water intakes

During the experimental period, no observed mortality was detected in any experimental group. Moreover, while the daily food intakes decreased in TEB-treated rats, the daily water consumption increased in rats treated with TEB compared to the control group. A significant decrease of body weight was observed in TEB-treated group. Absolute and relative liver weights increased in TEB-treated group compared to the control (Table 2).

Hematological estimations

Table 3 shows the hematological parameters under the different experimental procedures. The TEB treated rats showed lower RBC, HB and HT compared with control group. Although TEB significantly raised WBC, no significant change was observed in PLT when compared with the control. The co-administration of ASO restored the hematological parameters to near normal values when compared to TEB-treated groups.

Biochemical analysis

The toxicology results of TEB on biochemical parameters are presented in Table 4. The total cholesterol levels and the activities of AST, ALT, ALP, LDH and was increased in rats treated with TEB compared to normal group. Albumin, total protein and triglyceride concentrations decreased in TEB treated group compared to the control. Supplementation of ASO of the TEB-treated group produced recovery in the above mentioned biochemical variables.

NO levels

In this study, plasma nitric oxide significantly increased compared with the controls (Table 4). Interest-

Table 3. Change in hematological parameters of control and rats treated with TEB, ASO or their combination (TEB+ASO) after 4-week treatment.

Parameters	Control	TEB	ASO	TEB+ASO
RBC (10^6/µL)	10.48±0.75	9.26±0.93**	10.3±0.67	10.15±0.48#
WBC (10^3/µL)	7.66±1.00	12.6±1.21***	8.08±0.97	9.84±2.00*,##
Hb (g/dL)	21.12±1.74	17.26±1.89**	20.84±2.02	19.22±1.55*,#
HT (%)	50.48±4.34	42.58±4.47**	45.34±5.15	48.4±2.96#
PLT (10^3/µL)	555.4±59.12	515.6±33.93	592.6±58.09	551.8±33.16

RBC: red blood cell; WBC: white blood cell; Hb: Hemoglobin; Ht: Haematocrit; PLT: Platetet. Values are expressed as means±SD; n=8 for each treatment group. Significant difference: (*p<0.05, **p<0.01, ***p<0.001) compared with control group,(#p<0.05, ##p<0.01) compared with TEB+ASO.

Table 4. Serum markers of control and rats treated with TEB, ASO, or their combination (TEB+ASO) after 4-week treatment.

Parameters	Control	TEB	ASO	TEB+ASO
AST (U/L)	154.8±18.89	183.2±16.05**	132.4±9.44	152.2±27.95#
ALT (U/L)	54.6±7.86	83.2±8.37***	52.2±6.79	64.6±8.01*,##
ALP (U/L)	17.4±45.61	329.8±50.98***	157.6±54	230.4±69.76##
LDH (U/L)	847±185.20	1073.4±134.88*	890.4±17.70	855.2±158.51#
GGT (U/L)	2.8±2.28	6.6±2.07**	4.6±1.94	5.00±1.87##
Total bilirubin (mg/L)	0.46±0.54	1.6±0.89	0.4±0.54	1.00±0.1
Total protein (g/L)	84.4±3.28	76.6± 3.13**	82± 2.54	81.6±2.07##
Albumin (g/dL)	27.4±1.67	21±2***	27.2±0.83	25±1.22**,##
NO (µmoles/g protein)	1.05±0.34	1.84±0.09**	1.11±0.35	1.52±0.15*,##

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; LDH: lacatate dehydrogenase; ALP: alkaline phosphatase; γGT: γ-Glutamyltranspeptidase; NO: Nitric oxide. Values are expressed as means±SD; n=8 for each treatment group. Significant difference: (*p<0.05, **p<0.01, ***p<0.001) compared with control group, (#p<0.05, ##p<0.01) compared with TEB+ASO.

ingly, the co-administration of ASO modulated the NO levels in liver homogenate.

Antioxidant enzyme activities

GPx, SOD, CAT and GST activities were presented in Table 5. All the antioxidant enzyme activities decreased significantly in the TEB-treated group compared to the control. However, ASO conferred a protective effect because rats co-treated with ASO had significantly increased antioxidant enzyme activities when compared to the control.

MDA, AOPP, PCO, GSH and vitamin C levels

In the present study, the levels of MDA, AOPP and PCO were significantly elevated in TEB-treated group (Table 6). The administration of ASO ameliorated MDA, AOPP and PCO levels in (TEB+ASO) group compared to that of TEB. The levels of GSH and vitamin C decreased significantly in the TEB-treated group compared to the control. However, the supplementation of ASO has significantly restored GSH and vitamin C compared to the TEB-treated group (Table 6).

Histopathological results

Under light microscope, the histopathological analyses of liver tissue of rats exposed to TEB (Table 7 and Fig. 2) revealed an inflammatory cell infiltration (black arrow) with degenerative changes in hepatocytes (white arrow) and cell apoptotic (yellow arrow) (Fig.2B). Furthermore, the combined treatment of TEB+ASO showed inflammatory cell infiltration (black arrow) and normal cells morphology compared to TEB-treated animals (Fig. 2D). On the other hand, the liver of control (Fig. 2A) and liver of ASO-treated animals showed normal hepatic tissue (Fig.2C).

Discussion

The preliminary phytochemical analysis revealed that different active constituents are present in ASO, such as phenols, flavonoids and condensed tannin. Phe-

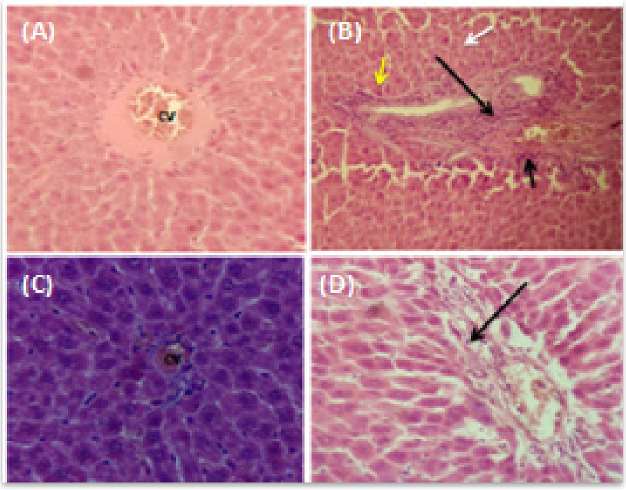

Figure 2. Histopathological changes in rat's liver in different groups. Liver section (×400) of rat treated with (A): control showing central vein surrounded by normal hepatocytes. (B): TEB (100 mg/kg) showing, inflammatory cells infiltration (black arrow), Degeneration of hepatocytes (white arrow) and apoptotic cells (yellow arrow). (C): ASO (5 ml/kg) showing normal appearance of hepatocytes. (D): TEB+ASO (100mg/kg; 5ml/kg) showing inflammatory cells infiltration (black arrow)

Table 5. Antioxidant enzymes activities in liver of control and rats treated with TEB, ASO, or their combination (TEB+ASO) after 4-week treatment.

Parameters	Control	TEB	ASO	TEB+ASO
GPx (µmoles GSH/min/mg prot.)	3.9±0.86	1.73±0.7***	4.42±1.8	2.82±0.62$^{*,\#\#}$
SOD (unit/mg prot.)	46.77±2.51	43.24±2.89*	45.91±3.95	45.71±3.95
Catalase (µmol/min/mg prot.)	76.26±4.13	67.68±3.97**	76.01±4.56	73.01±4.21$^{\#}$
GST (nmoles CDNB/min/mg prot.)	0.12±0.03	0.05±0.02*	0.1±0.01	0.09±0.01$^{\#}$

GPx: Glutathione peroxidase, SOD: superoxide dismutase, GST: Glutathione-S-transferase.

Table 6. Oxidative stress parameters in liver of control and rats treated with TEB, ASO, or their combination (TEB+ASO) after 4-week treatment.

Parameters and treatments	Control	TEB	ASO	TEB+ASO
MDA (nmol/mg prot)	8.72±2.19	15.45±3.8**	9.42±2.83	11.35±3.64$^{\#}$
AOPP (nmol/mg prot.)	7.28±1.22	11.87±4.49*	7.58±3.6	7.42±1.95$^{\#}$
PCO (nmol/mg protein)	30.98±9.69	44.32±5.18**	32.57±5.81	40.24±0.39$^{*,\#}$
GSH (nmol/mg prot)	2.17±0.25	1.16±0.41***	2.08±0.36	1.76±0.43$^{*,\#}$
Vitamin C (µmol/mg prot.)	0.17±0.004	0.13±0.01**	0.16±0.005	0.16±0.009$^{\#}$

MDA: malondialdehyde, AOPP: advanced oxidation protein products, PCO: protein carbonyls, GSH: reduced glutathione. Values are expressed as means±SD; n=8 for each treatment group. Significant difference: (*p<0.05, **p<0.01, ***p<0.001) compared with control group, ($^{\#}$p<0.05) compared with TEB+ASO.

Table 7. Histopathological examination of liver tissue of control and rats treated with TEB, ASO, or their combination (TEB+ASO) after 4-week treatment.

Parameters and treatments	Control	TEB	ASO	TEB+ASO
Degeneration of hepatocytes	-	++	-	-
Inflammatory cells infiltration	-	+++	-	+
Apoptotic cells	-	+	-	-

(−) indicates normal, (+) indicates mild, (++) indicates moderate and (+++) indicates severe.

nolic compounds, in general, and flavonoids, in particular, have the ability to provide protection against oxidative stress. However, the presence of flavonoids and phenolic compounds in ASO could be considered responsible for converting antioxidant ability. The stable DPPH radical is widely used to evaluate the free radical-scavenging activity in many plants (38). The antioxidant activities of *Allium sativum* were determined by ABTS and DPPH radical scavenging assays. Therefore, the protective effects of *Allium sativum* are due to their antioxidant and radical scavenging capacities (39-41).

The exposure to TEB caused a significant decrease in the body weight gain, which could be explained either by the decrease in food consumption and/or by the toxicity induced by this toxicant (42). However, we observed a significant increase in water consumption as shown in TEB-treated groups, which could be justified by the mechanism to counteract the toxicity induced by this fungicide (11). The additional effects of TEB treatment revealed an increase in the relative liver weight. This elevation might be due to the edema observed in the liver tissue and a marker of inflammation. In fact, an increase in the absolute liver weight and relative liver weight can be caused by some pesticides in experimental animals (43,44).

The hematological findings showed a significant decrease in RBC, Hb and Ht levels, indicating haemolysis and shrinkage of RBC by TEB, which results in rat anemia. Our findings suggest that the decrease in RBC counts occurred due to the excessive damage to the erythrocytes or inhibition of erythrocyte formation, which is in accordance with the findings reported by Kasmi et al (45), Hossen et al (46). Moreover, WBC count is a marker of systemic inflammation, whose level increased in the group treated with TEB, which might be an indicative of the animals defense immune system (47), It could also be due to the tissue damage and necrosis caused by the pesticide (48). Moreover, the treatment of the rats with ASO rescinded the induced anemia by TEB, which may be due to the antioxidant effect of ASO (49).

The measurement of plasma biochemical parameters showed that ALT, AST, γGT, LDH, and ALP increased in TEB-treated rats. The increase in the activities of these enzymes indicates a dysfunction of liver tissue. This elevation reflecting hepatocellular injury and necrosis led to the damage of hepatocytes (50).

Interestingly, the treatment with ASO reduced the activities of these enzymes. The antioxidants in ASO are able to counteract or minimize the undesirable effects induced by TEB (16). Furthermore, the albumin and total protein levels significantly decreased in TEB-treated rats. This reduction may be due to the decrease in the functional ability of liver cells under the effect of TEB. Moreover, the reduction of albumin means the low ability to synthesize protein in the liver (51).

In addition, TEB treatment caused significant increase in the total cholesterol contents. This increment may be based upon the effect of pesticides on the cell membrane permeability of liver. Therefore, the increment in total cholesterol contents may be due to the hepatic bile ducts blockage that stops or reduces the secretion of cholesterol into the duodenum portion of the small intestine. The enhanced levels of cholesterol may be a sign of hepatic damage. Increased cholesterol level has been recorded by Badgujar et al (52) in rats exposed to Bendiocarb an insecticide (53). However, a significant decrease in the concentration of triglyceride was observed in TEB- treated rats. Our study is in accordance with previous findings which denoted that rats exposed to an insecticide (54).

Oxidative stress induction involves an excessive production of reactive oxygen species (ROS), resulting from impaired balance between the ROS generation and antioxidant defense capability, which could affect lipid peroxidation and membrane integrity. Many pesticides were found to induce oxidative stress, leading to the generation of free radicals and alternation of antioxidant or oxygen free radical scavenging enzyme system (4). MDA levels are often measured to determine the degree of lipid peroxidation in the cell (55). In the present study, TEB induced lipid peroxidation in the liver of adult rats as revealed by a marked elevation in MDA. This might be the result of an increased generation of free radicals in the liver tissue of rats (56).

AOPP is another marker of oxidative stress and protein oxidation (8). Protein carbonylation is an indicator of the oxidative modification of proteins. Besides, ROS altered protein and led to the formation of carbonyl, which is non-reversible, causing conformational changes and decreases in the enzyme catalytic activities, resulting in the breakdown of proteins by proteases. The PCO levels were also found to increase in the TEB-treated rats. These results are in agreement with Chaâbane et al (11). In our study, TEB treatment produced the elevation in the levels of AOPP and PCO. This increase could be justified by the generation of the reactive species of oxygen, as reported by Ben Saad et al (6). ASO protected the hepatic cell from oxidative damage induced by TEB, which was demonstrated by inhibiting the elevation of MDA, AOPP and PCO levels in intoxicated groups. This might be attributed to the free radical scavenging property of the oil (57,58).

Antioxidant enzymes (CAT, SOD, GPx, and GST) are considered to be the first line of cellular defense against oxidative damage (59). A redox balance between prooxidants and antioxidants is essential for the normal cellular functioning (52). The present study has shown a decrease of antioxidant enzyme in TEB-treated rats. These decreased activities evidently indicate that TEB can increment free radicals produced in the oxidative stress process (53). GSH is an intracellular reductant that protects cells against free radicals, peroxides and other toxic compounds. In addition, GSH is central to the cellular antioxidant defenses, which acts as an essential cofactor for antioxidant enzymes including GPx and GST (60).

Moreover, GSH and vitamin C are an effective reductive non-enzymatic antioxidant that provides a secondary line of defense against intracellular harmful effects of free radicals (61). GSH is able to regenerate the most important antioxidants, vitamins C and E (62). Regarding vitamin C, a soluble vitamin with effective properties in scavenging free radicals (45). In the present study, TEB treatment caused a significant decrease in the level of GSH in liver tissues. The reduction in GSH is indicative of oxidative stress (63). The obtained results also exhibited a significant decrease in vitamin C level, which may be due to the depletion of GSH since it is directly involved in recycling vitamin C (56). The co-treatment with ASO enhanced the enzymatic and non-enzymatic antioxidant status of animals exposed to TEB. Polyphenol is a powerful antioxidant compound present in ASO. Mukthamba and Srinivasan (64) have demonstrated that ASO exerts an antioxidant action by scavenging ROS, indicating that this oil participated in the reduction of TEB toxicity.

Nitric oxide (NO), a potent vasodilator synthesized by the endothelium which plays a pivotal role in modulating endothelial function (65). However, in this work, TEB treatment increased plasma NO concentrations of the animals. The increment of this parameter could be explained by an increase in the level of free radicals chronic inflammation and stimulation of the immune system (66). In contrast, the co-treatment with ASO caused a significant decrease of NO level induced by TEB exposure. The obtained results were congruent with those found by Savas (57).

In this study, the hematological and biochemical findings were confirmed by the histopathological changes in the liver. The administration of TEB resulted in inflammatory cells infiltrates as well as degenerated hepatocytes, necrosis and the presence of apoptotic cells. These results are in accordance with those of previous studies Abdelhady et al (67). These alterations could result from ROS generation that interacted with biological target molecules, thus causing liver injury and TEB-induced membrane distribution (6).

The co-treatment of ASO lessened these histopathological alterations induced by TEB as evidenced by the restoration of the architecture of hepatic tissue almost similar to control liver, which could be related to the various natural antioxidant agents of the ASO (16,68).

In conclusion, the present study have demonstrated that tebuconazole intoxication induces oxidative stress in hepatic tissue via enhancing free radicals production. The treatments with ASO ameliorate this TEB-induced hepatotoxicity through improving the rat antioxidant status and modulating oxidative stress.

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Interest conflict

The authors reported no potential conflict of interest.

Author's contribution

All authors contributed equally to this work.

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