Histopathological, oxidative damage, biochemical and genotoxicity alterations in hepatic rats exposed to deltamethrin: modulatory effects of garlic (*Allium sativum*)

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Abstract

Deltamethrin is a pesticide widely used as a synthetic pyrethroid. The aim of this study was undertaken to investigate the effects of deltamethrin to induce oxidative stress and changes in biochemical parameters, hepatotoxicity and genotoxicity in female rats following a short-term (30 days) oral exposure and attenuation of these effects by *Allium sativum* extract. Indeed, *Allium sativum* is known to be a good antioxidant food resource which helps destroy free radicals particles. Our results showed that deltamethrin treatment caused an increase in liver enzyme activities of aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH); and hepatic lipid peroxidation (LPO) level. However, it induced a decrease in activities of hepatic catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) ($p < 0.01$). *Allium sativum* extract normalized significantly ($p < 0.01$) the mentioned parameters in deltamethrin treated rats. For genotoxic evaluation, deltamethrin treatment showed a significant increase in frequencies of micronucleus in bone-marrow cells. Micronucleus formation is an indicator of chromosomal damage which has been increasingly used to detect the genotoxic potential of environmental pests. The present study showed that *Allium sativum* diminished the adverse effects induced by this synthetic pyrethroid insecticide.

Key Words: Deltamethrin, *Allium sativum*, genotoxicity, oxidative stress

1. Introduction

Pesticides have become indispensable in modern agriculture but are also pollutants. The mastery of food resources (higher yields) and the improvement of public health (especially in the fight against insect vectors of diseases) are advantages of these products. However, it is apparent that these products are potential hazards to the environment, biodiversity and human health. Pyrethroid pesticides have emerged as a major class of highly active insecticides because of their high bio-efficacy and relatively low toxicity compared to organochlorine and organophosphorus pesticides (Abou Almagd et al. 2011). Pyrethroids are widely used in agriculture, forestry, and public health (Soderlund et al. 2002). Their use has increased several-fold in recent years due to their low mammalian toxicity and limited persistence in soil as compared to organochlorine insecticides. Deltamethrin, a synthetic pyrethroid type II, is highly effective against a broad spectrum of insects. Also, the oral route constitutes the main source of general population exposure to this pesticide which is ingested within food and water (Barlow et al. 2001). Several studies have shown that pyrethroids caused alterations in biochemical, hematologic and reproductive parameters (El-Demerdash et al. 2004; Yousef et al. 1998). Recent studies showed that the induction of oxidative stress is one of the main mechanisms of deltamethrin toxicity (Tuzmen et al. 2008; Yousef et al. 2006). However, several studies have demonstrated genotoxic and immunotoxic effects of deltamethrin in mammalian species (El-Gerbed 2012). Deltamethrin research has shown that oxidative stress is the main cause of genotoxicity in erythrocytes (Ansari et al. 2009). Because several studies have shown that exposure to pesticides may induce genotoxic effects in occupationally exposed human populations, the evaluation of the genotoxicity of pesticides in use is of immediate concern (Naravaneni and Jamil 2005). Recent studies indicate that deltamethrin may induce toxic manifestations by enhancing the production of free radicals and DNA

damage. The administration of medicinal plants (green tea) protected against deltamethrin-induced oxidative damage and DNA fragmentation (Ogaly et al. 2015).

On the other hand, the usage of natural and potential antioxidants is a strategy to prevent oxidative damage in many health disturbances that have oxidative stress as a factor in their pathophysiology (Pincemail et al. 2002). Garlic is an aromatic plant known since antiquity. Particularly today the medicinal use of garlic is widespread and growing. Garlic is regularly consumed and is known to have diverse biologic activities, particularly due to its antioxidant properties. Studies carried out on garlic (*Allium sativum*) have reported the presence of two main classes of antioxidant components, namely flavonoids (Bozin et al. 2008) and polyphenolics. These are likely to play an important role in the widely demonstrated biological effects of garlic.

The biological responses of garlic have been largely attributed to reduction of risk factors for cardiovascular diseases and cancer, stimulation of immune function, enhanced detoxification of foreign compounds, hepatoprotection, antimicrobial effects and antioxidant effects (Banerjee et al. 2002). However, Reports showed that higher concentrations of garlic extract by orally route may exert significant cellular damage, including gastrointestinal problems and to be clastogenic depending on the mode of administration (Amagase et al. 2000; Das et al. 1996). Banerjee et al. (2001) have reported that treatment of rats with higher doses (500 and 1000 mg/kg/day) by oral route of garlic caused greater oxidative stress. The vegetables preparations were commonly used as a folk medicine for the treatment of many diseases; These "natural products allegedly" safe "can be dangerous. The main problem with these "natural products" is the purity and quantity of a particular ingredient contained in the extracts. So it's very import to describe their active ingredients, the actual quantities and clarify the possible side effects (Tarantino et al. 2009). So, little information is available on

the antioxidant profile of garlic and its potential protective role against oxidative damage and genotoxicity induced by deltamethrin.

Therefore, the aim of this study was to evaluate the *in vivo* antioxidative capacity of *Allium sativum* extract against deltamethrin-induced oxidative damage and genotoxicity in adult female rats.

2. Materials and methods

2.1. Chemicals

Deltamethrin is a synthetic pyrethroid insecticide (C₂₂H₁₉Br₂NO₃). The CAS chemical name is (α-cyano-3-phenoxybenzyl (1R,3R)-3-(2,2-dibromovinyl)-2,2 dimethyl cyclopropanecarboxylate). It is available and used in experimentation in Tunisia. The name “deca-methrin” was originally proposed for this compound and was used in the literature, but was rejected because of a conflict with a trade mark. All other chemical products used in this study were purchased from Sigma Chemicals (Aldrich Chemical Company).

2.2. Preparation of plant extract

200 g of garlic cloves, medium dry, red, were subjected to delipidation with hexane, then macerated in 80% methanol for 3 days (dark). The hydroalcoholic filtrate obtained under evaporation and then dissolution in water. The aqueous phase thus obtained was subjected to a series of fractionations using solvents of methanol in hexane of increasing polarity. The fractions obtained were dried using a rotary evaporator and then weighed to calculate their yields. The extraction yield, determined as a percentage (%), was calculated by dividing the mass of the dry to fresh weight of plant material.

2.3. Animals

Adult female albino *Wistar* rats weighing 150-180 g were obtained from Central Pharmacy of Tunisia (SIPHAT, Tunisia). The animals were handled under standard laboratory conditions of a 12-h light/dark cycle in a temperature- and humidity-controlled room. The rats were fed with a commercial balanced diet (SNA, Sfax, Tunisia) and drinking water was offered *ad libitum*. All animal experiments were conducted without anesthesia and according to the ethical Committee Guidelines for the care and use of laboratory animals at our institution.

2.3.1. Experimental conditions

Rats were randomly divided into four groups of eight animals each (n=8).

Group (C) served as control and received distilled water *ad libitum*.

Group (AE) received *Allium sativum* extract (20 mg/kg BW) (Rafieian-Kopaei et al. 2013) by gavage for 4 weeks of treatment.

Groups (D) and (AE+D) received deltamethrin (7.2 mg/kg BW) (Catinot et al. 1989) dissolved in 1 ml of corn oil and deltamethrin with *Allium sativum* extract combination, respectively.

Rats were treated with repeated doses of *Allium sativum* extract and deltamethrin for 4 weeks by gavage administration.

After treatment, the animals were killed by cervical decapitation. Blood samples were collected, allowed to clot at room temperature and serum separated by centrifuging at 4000 rpm for 15 min for various biochemical parameters. The liver were quickly excised, minced with ice cold saline, blotted on filter paper and homogenized (Ultra Turrax T25, Germany) (1:2, w/v) in 50 mmol/l phosphate buffer (pH 7.4). The supernatant and serum were frozen at -20°C in aliquots until analysis.

2.3.2. Biochemical parameters

Serum samples were obtained by the centrifugation of blood at 4000 rpm for 15 min at 4°C, and were then divided into Eppendorf tubes. Isolated sera were stored at –20°C until they were used for the analyses. The levels of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and lactate dehydrogenase (LDH) were performed in Biochemistry Laboratory, CHU Habib-Bourguiba of Sfax.

2.3.3. Determination of protein carbonyl content

Method described previously (Ardestani and Yazdanparast 2007). For determination of protein carbonyl content in the samples, 1 ml of 10 mM 2,4-dinitrophenylhydrazine (DNPH) in 2M HCl was added to the samples (1 mg). Samples were incubated for 30 min at RT. Then, 1 ml of cold TCA (20%, w/v) was added to the mixture and centrifuged at 3000g for 10 min. The protein pellet was washed three times with 2 ml of ethanol/ethyl acetate (1:1, v/v) and dissolved in 1 ml of guanidine hydrochloride (6 M, pH 2.3). The absorbance of the sample was read at 370 nm. The carbonyl content was calculated based on the molar extinction coefficient of DNPH ($\epsilon = 2.2 \cdot 10^4 \text{ cm}^{-1} \text{ M}^{-1}$). The data were expressed as nmol/mg protein.

2.3.4. Determination of conjugated dienes

The conjugated dienes were measured in hepatic tissue slightly modified. The hepatic tissues were homogenized separately in ice-cold phosphate buffer (pH 7.4) at a tissue concentration of 50 mg/ml. The hepatic tissues were also homogenized in the same buffer at a concentration of 5 mg/ml. A 0.5-ml aliquot and a chloroform–methanol mixture (2:1) were taken in a centrifuge tube. This mixture was centrifuged at 1000×g for 5 min. Chloroform was evaporated after steaming at 50°C. The lipid residue was dissolved in 1.5 ml methanol. Readings were taken at 233 nm (Slater 1984).

2.3.5. Oxidative stress analysis

Lipid peroxidation (LPO) was measured following the method of Buege and Aust 1972 as thiobarbituric acid reactive substances (TBARS). Since malondialdehyde (MDA) is a degradation product of peroxidized lipids, the development of pink color with the absorption characteristics (absorption maxima at 532 nm) of TBA-MDA chromophore was taken as an index of LPO. TBARS values were expressed as nanomoles of MDA per milliliter.

In liver tissues, SOD activity was determined according to the colorimetric method of Beyer and Fridovich 1987 using the oxidizing reaction of nitroblue tetrazolium (NBT); CAT activity was measured by the UV colorimetric method of Aebi 1974 using H_2O_2 as substrate; glutathione peroxidase (GPx) activity was measured by a modification of the colorimetric method of Flohe and Günzler 1984 using H_2O_2 as substrate and the reduced GSH.

2.3.6. Histopathological examination

Pieces of liver tissues were excised, washed with normal saline and processed separately for histopathological observation. The liver tissues were fixed in Bouin's solution, dehydrated in graded (50-100%) alcohol and embedded in paraffin. Thin sections (4 - 5 μm) were cut and stained with routine hematoxylin-eosin (H&E). The sections were examined microscopically for histopathology changes, including cell necrosis, fatty change, and ballooning degeneration (Gabe 1968).

2.3.7. In vivo micronucleus assay

Femurs of rats were removed through the pelvic bone. The epiphyses were cut and the bone marrow was flushed out by gentle flushing and aspiration with foetal calf serum. The cell suspension was centrifuged and a small drop (3 μl) of the re-suspended cell pellet was spread on a microscope slide and stained with May-Grünwald/Giemsa. Three slides per animal were stained with acridine orange (AO) and washed twice with phosphate buffer (pH 6.8).

Micronuclei were scored with a Zeiss Axioskop microscope at 100X using previously proposed criteria (Heddle et al. 2011). Approximately 2000 erythrocytes were scored per animal to estimate the frequency of micronucleated erythrocytes.

2.3.8. Qualitative assay of DNA fragmentation by agarose gel electrophoresis

The DNA was extracted from rat liver using Wizard Ge-180 nomic DNA Purification Kit (Quick-gDNATM MiniPrep Catalog Nos. D3006, D3007, D3024, and D3025). DNA was then loaded onto agarose gel (0.3µg/lane). DNA laddering was determined by constant voltage mode electrophoresis (in a large submarine at 80 V, for 60 min) on a 1.7% agarose gel containing 0.5 µg/ml ethidium bromide (Miller et al. 1988). Gels were illuminated with 300 nm UV light and a photographic record was made.

3. Statistical analysis

Statistical analysis was performed using the SPSS version 17.0 software (SPSS Inc., Chicago, IL, USA). All values are expressed as mean ± SD. The results were analyzed by one-way analysis of variance (ANOVA) followed by Tukey test for multiple comparisons. Mann–Whitney U test was used for micronucleus frequencies comparisons. Differences were considered significant at $p < 0.05$.

4. Results

4.1. Serum biochemical parameters

The activities of various biochemical enzymes in normal, deltamethrin control and treated groups are presented in Table 1. The activities of AST, ALT, ALP and LDH were significantly increased in deltamethrin-treated rats compared to normal control. The levels of the above enzymes were significantly reversed on treatment with *Allium sativum* extract.

4.2. Protein carbonyl content and conjugated dienes

The changes in the levels of hepatic protein carbonyl content and conjugated dienes in control and experimental rats are shown in Table 2. The levels of protein carbonyl content and conjugated dienes were significantly increased (** $p < 0.01$; *** $p < 0.001$, respectively) in deltamethrin treated rats when compared with normal control rats. The administration of *Allium sativum* extract along with deltamethrin significantly lowered the levels of protein carbonyl content and conjugated dienes in the liver of rats when compared to deltamethrin treated rats.

4.3. Lipid peroxidation level and enzymatic antioxidant status in liver

TBARS level is widely used as a marker of free radical-mediated lipid peroxidation injury. The results of hepatic TBARS levels are shown in Figure 1. TBARS levels in the deltamethrin-treated group were significantly higher than in the control group. TBARS levels in the *Allium sativum* extract treated group were significantly lower than that in the deltamethrin-treated group ($p < 0.01$).

SOD, CAT and GPx activities were measured as an index of antioxidant status of tissues. Significantly lower hepatic SOD, CAT and GPx activities ($p < 0.01$; $p < 0.05$ and $p < 0.01$, respectively) were observed in rats from the deltamethrin-treated group compared to the normal control group (Table 3). There was a significant increase of SOD, CAT and GPx activities in *Allium sativum* extract-treated group compared to the deltamethrin-treated group. Treatment with deltamethrin significantly decreased the SOD, CAT and GPx levels in the liver (23.72 ± 2.64 Units/mg protein, 40.82 ± 2.31 $\mu\text{mol H}_2\text{O}_2$ /mg protein, 0.0027 ± 0.0005 $\mu\text{mol GSH/min/mg protein}$, respectively) compared to the normal control group (47.63 ± 3.86 Units/mg protein, 50.26 ± 5.93 $\mu\text{mol H}_2\text{O}_2$ /mg protein, 0.0043 ± 0.0012 $\mu\text{mol GSH/min/mg protein}$, respectively).

4.4. Pathological analysis

After decapitation of the rats, the liver of normal and treated animals was separated to observe the macroscopic changes. Liver tissue samples of the deltamethrin group had significant macroscopic steatosis and necrosis compared to the pretreatment and control groups (Figure 2). However, the liver tissue samples of rats treated with *Allium sativum* extract and the rats treated with the combination of *Allium sativum* extract and deltamethrin did not show marked macroscopic changes compared to normal control liver samples.

The liver histopathological changes are shown in Figure 3. The central vein, hepatocyte cords, hepatocytes and portal areas were observed to be normal in the control group. In the present study, deltamethrin application produced histopathological changes of severe liver damage including sinusoidal dilation, vacuolization, congestion and inflammatory cell infiltration. The above changes were reduced in the liver of rats treated with *Allium sativum* extract and deltamethrin. The histological pattern was almost normal in rats treated with *Allium sativum* extract alone.

4.5. Micronucleus frequencies in erythrocytes

Micronucleus frequencies in erythrocytes and representative photomicrographs indicating micronucleated cells of controls, rats treated with *Allium sativum* extract, rats treated with deltamethrin and rats treated with *Allium sativum* extract combined with deltamethrin are summarized in Table 4 and Figure 4. As can be seen from the table 4, the micronucleus frequencies in erythrocytes significantly increased following deltamethrin treatment ($p < 0.01$) compared to control animals. The frequency of micronuclei in deltamethrin-treated animals was $0.346 \pm 0.165 \%$ in bone marrow erythrocytes and $0.304 \pm 0.061 \%$ in erythrocytes from whole blood. In addition, deltamethrin group showed highest MN and

spherical nuclear fragments separated from the parent nucleus compared to the control normal group (Figure 4). However, treatment with *Allium sativum* extract combined with deltamethrin reduced significantly the micronucleus frequencies in peripheral blood erythrocytes and bone marrow (0.068 ± 0.013 ; 0.082 ± 0.007 , respectively; $p < 0.05$) compared to control animals. *Allium sativum* extract alone did not change significantly the micronucleus frequencies in blood erythrocytes, bone marrow and representative micronuclear cells compared to controls.

4.6. DNA fragmentation by agarose gel electrophoresis

Figure 5 shows the qualitative changes in the integrity of the liver genomic DNA. As compared to control group, the deltamethrin treatment group induced marked increases in the DNA fragmentation. On the other hand, simultaneous treatment of rats with deltamethrin and *Allium sativum* extract showed moderately improvement in the DNA damage. DNA isolated from control samples (lane 1) and *Allium sativum* extract (lane 2) alone exposed tissues showed no specific DNA fragments.

5. Discussion

Allium sativum contained a considerable amount of polyphenolic and flavonoid compounds. according to findings of Chowdhury et al. 2008 who reported that aqueous garlic extract (*Allium sativum* L.) was a good source of natural antioxidants such as flavonoids and polyphenols, and consumption of *Allium sativum* or its products may contribute substantial amounts of antioxidants to the diet. The *Allium sativum* extract was found to have strong superoxide radical scavenging activity, which may be due to the presence of polyphenolic compounds. Numerous evidences clearly demonstrated the importance of medicinal plants in the treatment of oxidative stress-induced cell death (Jung et al. 2006).

The present study was carried out to investigate the ameliorative effects of *Allium sativum* extract on deltamethrin-induced oxidative stress and genotoxicity in rats. In the deltamethrin-treated rats, the activities of AST, ALT, ALP and LDH were significantly increased as compared to controls, indicating liver cell damage. This was confirmed by histopathology. Many reports mentioned that high doses of insecticides may cause increase in serum AST, ALP and ALT activities (Kalender et al. 2005; Saoudi et al. 2011). The damage of liver cell membrane can cause an overall secretion into blood of several enzymes from the hepatocyte cytosol, including AST, ALP and ALT. In contrast, the pre-treatment with *Allium sativum* extract caused a significant restoration of liver AST, ALP, LDH and ALT activities induced by deltamethrin intoxication in rats treated with the combination of *Allium sativum* extract and deltamethrin. The results indicated that *Allium sativum* extract given orally for 4 weeks attenuated the extensive changes in hepatic biochemical parameters in deltamethrin-treated rats. These disorders in biochemical parameters induced by deltamethrin did not appear in AE rats orally given *Allium sativum* extract alone. The antioxidants in *Allium sativum* extract are likely able to counteract or to minimize the undesirable effects induced by deltamethrin. Similar results demonstrated that garlic supplementation reduced the toxicity of heavy metals (nickel II & chromium VI) in haematology and erythrocyte antioxidant defense systems in albino rats (Tikare et al. 2012).

In addition to lipid peroxidation, protein carbonyl levels also served as a marker for protein oxidation particularly for the proteins containing amino acid residues like lysine, arginine, proline, threonine and glutamic acid. The present investigation showed a significant increased level of protein carbonyl content and conjugated dienes in the liver of deltamethrin exposed rats which confirm the oxidative damage in hepatic tissues. The increase of protein oxidation and the protection by the medicinal plant *Allium sativum* is in accordance with the findings of (Ajiboye et al. 2014) which demonstrated that phenolic extract of *Parkia biglobosa* fruit pulp

decrease significantly the hepatic protein oxidation in the aflatoxin B treated rats. The increased levels of conjugated dienes (a mutagenic product of lipid peroxidation) in deltamethrin –treated rats indicate state of redox imbalance and could lead to oxidative stress. *Allium sativum* extract decrease significantly hepatic conjugated dienes in deltamethrin treated rats which prevent peroxidation of lipids. This could be due to the presence of the phenolic compounds in the extract.

Antioxidant enzymes are considered to be the first line of cellular defense against oxidative damage. Among them, superoxide dismutase (SOD) and catalase (CAT) mutually function in the elimination of radical oxygen species. In the current study, treatment with deltamethrin resulted in a significant decrease of antioxidant enzymes such as SOD, CAT and GPx activities compared to control animals. The reduction in SOD activity in deltamethrin-exposed animals may be due to the enhanced production of superoxide radical anions (Abdel-Daim et al. 2013). Catalase scavenges H_2O_2 that has been generated by free radicals or by SOD in removal of superoxide anions. The administration of *Allium sativum* extract restored the activities of antioxidant enzymes in liver. Polyphenolic compounds are present in *Allium sativum*, which have powerful antioxidant properties, i.e, free radical scavenging activity (Che Othman et al. 2011). Lipid peroxidation is one of the characteristic features of increased oxidative stress associated with deltamethrin toxicity (Scharma and Singh 2013). Lipid peroxidation is associated with a wide variety of toxicological effects, including decreased membrane fluidity and function and inhibition of enzymes. Assessment of TBARS is probably the most commonly applied method for the measurement of lipid peroxidation (Armutcu et al. 2005). Increased TBARS level is an index of enhanced lipid peroxidation (Devipriya et al. 2007). The oral administration of *Allium sativum* extract in combination with deltamethrin significantly lowered the enhanced TBARS level in hepatic tissues of deltamethrin-treated rats. *Allium sativum* extract may suppress lipid peroxidation through different chemical

mechanisms, including free radical quenching, electron transfer, radical addition, or radical recombination (Borek 2001). This observation directly demonstrates the anti-peroxidative and antioxidant effects of *Allium sativum* extract. Furthermore, these findings were confirmed by histopathological analysis. In our study, deltamethrin selectively induced toxicity in the liver of treated animals compared to controls. Also, the hepatic architecture of the deltamethrin-treated rats resulted in necrotic changes, inflammatory cell infiltration, fatty degeneration and vacuolization. Our results corroborated the findings of Kan et al. 2012 who demonstrated deltamethrin-induced liver histopathology in fish (*Oreochromis niloticus*). In our results, the administration of *Allium sativum* extract noticeably reduced the macroscopic changes and histological alterations induced by deltamethrin.

The MN test is widely employed to evaluate genotoxicity of chemical compounds after direct or indirect exposure *in vivo* (Cavas and Ergene-Gozukara 2003). In the present study, results showed a significant increase in the micronucleus frequencies in erythrocytes following deltamethrin treatment compared to controls. Similarly, Bhunya and Pati (1990) demonstrated a significant increase in micronuclei frequencies in bone marrow in addition to sperm abnormalities in mice-treated with deltamethrin. Also, Sharma et al. (2010) noted a significant clastogenic potential with cyhalothrin in rats after 30 days oral treatment, including chromosome and chromatid gaps, chromosome breaks, chromatid breaks and fragments. Our study is in accordance with previous findings which denoted that rats exposed to a systemic organophosphorus insecticide, the phorate at varying oral doses for 14 days, exhibited substantial oxidative stress, cellular DNA damage and activation of apoptosis-related p53, caspase 3 and 9 genes (Saqib et al. 2012). In contrast, this study demonstrates that oral administration of garlic had the ability to reduce the genotoxic effect of deltamethrin, as indicated by the significant reduction in the erythrocyte micronuclei frequencies. Recent findings indicate that pesticide exposure had elevated levels of oxidative DNA damage

(Koureas et al. 2014). Our results are consistent with those of Assayed et al. who demonstrated that rats given garlic in combination with cypermethrin insecticide showed a very low frequency of micronuclei in bone marrow cells *in vivo*, which did not differ significantly from control values in comparison with corresponding values in rats given the pesticide alone. The authors suggested that garlic extract reduced significantly the genotoxic potential of cypermethrin more efficiently than vitamin C. while the combination of both natural elements (garlic extract and vitamin C) produced, in most cases, a more pronounced protective effect than when each administered alone. The absorption and the nature of deltamethrin may be the main causative agent for its accumulation in body systems; free radicals production and DNA damage (Galal et al. 2014). The oxidative stress is also known to cause massive DNA fragmentation. The DNA fragments observed in the present study is the normal consequence of oxidative stress that was demonstrated after the enzymatic antioxidant status in liver. The deltamethrin has been shown to induce DNA fragmentation in rat brain (Galal et al. 2014). Our investigation showed that deltamethrin treatment induced DNA damage as compared to controls. The co-administration of *Allium sativum* extract moderately decreased the DNA fragmentation.

In conclusion, our results demonstrated that high doses of deltamethrin can cause a variety of liver toxicities. Implications for human exposures need to take into account the dose response. Garlic extract reduced significantly the liver damage and genotoxicity, suggesting that it could be a suitable agent for preventing the oxidative damage and the genotoxic potential of deltamethrin.

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Draft

Figure 1

Effects of deltamethrin (D), *Allium sativum* extract (AE) and their combinations (AE+D) on hepatic TBARS of control and experimental rats.

Values are mean $\pm$ SD for eight rats in each group. D, AE and AE+D treated groups vs control group: ** $p < 0.01$; D group vs (AE+D) group: ## $p < 0.01$.

Figure 2

Macroscopic changes of rat livers

(A) Control liver; (B) liver after pretreatment with *Allium sativum* extract (20 mg/kg BW); (C) liver of a rat intoxicated with deltamethrin (7.2 mg/kg BW) and (D) liver treated with deltamethrin combined to *Allium sativum* extract.

Figure 3

Representative photographs from the liver showing the protective effect of *Allium sativum* extract on deltamethrin-induced hepatic injury in rats. (A) Controls, (AE) rats treated with *Allium sativum* extract, (D) rats treated with deltamethrin and (AE+D) rats treated with *Allium sativum* extract combined to deltamethrin. Liver sections were stained using the hematoxylin-eosin method. Original magnifications: X400; cv: central vein in the liver * congestion; + inflammatory cell infiltration; $\times$ vacuolization.

Figure 4

Representative photomicrographs indicating micronucleated cells under acridine orange staining (400X) in rat erythrocytes of control and treated animals.

Legends:

(► normal nucleus, ➡ micronucleus).

(A) Controls, (AE) rats treated with *Allium sativum* extract, (D) rats treated with deltamethrin and (AE+D) rats treated with *Allium sativum* extract combined to deltamethrin.

Figure 5

Changes in genomic DNA degeneration of rat liver due to treatment with deltamethrin (D), *Allium sativum* extract (AE) and their combinations (AE+D). M: Marker (2 kb DNA ladder); lane 1 control; lane 2 *allium sativum* extract (AE); lane 3 deltamethrin (D) and lane 4 AE+D.

Draft

50x28mm (300 x 300 DPI)

25x26mm (300 x 300 DPI)

42x29mm (300 x 300 DPI)

48x51mm (300 x 300 DPI)

25x27mm (300 x 300 DPI)

Table 1

Hepatic markers in the serum of control and treated rats with deltamethrin (D), *Allium sativum* extract (AE) and their combinations (AE+D)

AST: aspartate aminotransferase; ALT: alanine aminotransfearse; ALP: alkaline phosphatse; LDH: lacatate dehydrogenase

Values are mean $\pm$ SD for eight rats in each group. D, AE and AE+D treated groups vs control group: * $p < 0.05$, ** $p < 0.01$; D group vs (AE+D) group: # $p < 0.05$, ## $p < 0.01$.

Parameters	Experimental groups			
	Control	AE	D	AE + D
AST (U/l)	220.5 $\pm$ 12.5	221.5 $\pm$ 24.5	239 $\pm$ 12 *	209 $\pm$ 24
ALT (U/l)	51.66 $\pm$ 8.53	49.33 $\pm$ 5.23	68.66 $\pm$ 22.77**	46 $\pm$ 1.52##
ALP (U/l)	249 $\pm$ 7	256 $\pm$ 11	332.5 $\pm$ 29.5**	303.5 $\pm$ 3.5#
LDH (U/l)	1538 $\pm$ 118	1533.33 $\pm$ 102.05	1723.5 $\pm$ 91.5*	1540 $\pm$ 168

Table 2

Hepatic protein carbonyl and conjugated diene levels of control and treated rats with deltamethrin (D), *Allium sativum* extract (AE) and their combinations (AE+D)

Values are mean $\pm$ SD for eight rats in each group. D, AE and AE+D treated groups vs control group: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; D group vs (AE+D) group: # $p < 0.05$.

Treatment and parameters	C	AE	D	AE+D
Protein carbonyls (nmol/mg protein)	0.62 $\pm$ 0.08	0.98 $\pm$ 0.42	1.26 $\pm$ 0.16***	1.12 $\pm$ 0.32
Conjugated dienes (nmol/mg protein)	8.81 $\pm$ 3.55	13.38 $\pm$ 5.3	18.5 $\pm$ 4.44**	14.29 $\pm$ 3.26 #

Table 3

Effects of deltamethrin (D), *Allium sativum* extract (AE) and their combinations (AE+D) on the activities of enzymatic antioxidants in liver of control and experimental rats

Values are mean $\pm$ SD for eight rats in each group. D, AE and AE+D treated groups vs control group: * $p < 0.05$, ** $p < 0.01$; D group vs (AE+D) group: # $p < 0.05$, ## $p < 0.01$.

Treatment and parameters	C	AE	D	AE+D
SOD (Units/mg protein)	47.63 $\pm$ 3.86	39.46 $\pm$ 2.29	23.72 $\pm$ 2.64**	25.26 $\pm$ 0.2
CAT (μ mol H ₂ O ₂ /mg protein)	50.26 $\pm$ 5.93	51.49 $\pm$ 3.74	40.82 $\pm$ 2.31*	82.15 $\pm$ 8.64##
GPx (μ mol GSH/min/mg protein)	0.0043 $\pm$ 0.0012	0.0032 $\pm$ 0.0006	0.0027 $\pm$ 0.0005**	0.0039 $\pm$ 0.00077#

Table 4

Effect of deltamethrin (D), *Allium sativum* extract (AE) and their combinations (AE+D) on micronucleus induction in rat peripheral blood erythrocytes and bone marrow

The counts were performed on 2000 cells per rat.

Values are mean $\pm$ SD for four rats in each group. D, AE and AE+D treated groups vs control group: ** $p < 0.01$; D group vs (AE+D) group: # $p < 0.05$.

Treatment and parameters	Groups			
	C	AE	D	AE+D
Peripheral blood erythrocytes	0.022 $\pm$ 0.004	0.029 $\pm$ 0.009	0.304 $\pm$ 0.061 **	0.068 $\pm$ 0.013 #
Bone marrow	0.028 $\pm$ 0.004	0.031 $\pm$ 0.010	0.346 $\pm$ 0.165 **	0.082 $\pm$ 0.007 #