Protective antioxidant effect of garlic against cypermethrin induced lung toxicity in adult male mice: Biochemical and Histopathological studies

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Abstract In the present study, the protective effect of garlic against cypermethrin -induced lung toxicity was studied. Adult male mice (N=20) with average weight 18- 20 g were used in the study. Animals were divided into four groups of 5 each: group I control received corn oil; group II received cypermethrin (2.8 mg/kg BW) in corn oil. Group III received garlic (500 mg/kg BW); group IV received both cypermethrin and garlic. All treatments were given by oral gavage for 14 days. The results showed that cypermethrin increased thiobarbituric acid-reactive substances (TBARS) and decreased activities of the antioxidant enzymes (GST: glutathione S – transferase; -SH group; SOD: superoxide dismutase). Lung injury was confirmed by histopathological changes. Animals treated with garlic and cypermethrin together showed that lung TBARS returned to the control level which indicating a protective effect of garlic. Also, garlic was able to increase the reduced activities of the antioxidant enzymes induced by cypermethrin. In addition, garlic protected the lung from histological changes induced by cypermethrin. In conclusion, garlic was found to provide protective effect against and cypermethrin induced damage in mice alveoli and bronchioles with the attenuation of the oxidative stress and the preservation in antioxidant enzymes. It could be advised as an as Therefore this effective dietary supplements in developing countries where pesticide pollution is high.
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1. Introduction

The potency of garlic (G) (*Allium sativum*) has been acknowledged for 5000 years. In ancient times, the Babylonians, Egyptians, Phoenicians, Vikings, Chinese, Greeks, Romans and Hindus used garlic frequently as a remedy for many diseases (Block, 1985). Garlic, one of the best-researched herbal remedies, holds a unique position in history, traditionally employed to treat infection, colds, diabetes, heart disease, and it has been evaluated for lowering blood pressure, cholesterol, and glucose concentration, as well as for the prevention of arteriosclerosis and cancer, garlic consumption inversely correlates with the risk of oral, stomach, esophageal, colon, and prostate cancers (Tsai *et al.,* 2012). In the recent time, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, due to their natural origin, cost effectiveness and lesser side effects (Naik *et al.,* 2003; Sharma *et al.,* 2010). Studies carried out on garlic constituents have reported the presence of two main classes of antioxidant components, namely flavonoids and sulfur-containing compounds (diallyl sulfide, trisulfide and allyl-cysteine) (Kodera *et al.,* 2002; Bozin *et al.,* 2008). In addition to sulfur-containing compound, garlic is also rich in trace elements and zinc, manganese, copper, selenium, and iodine are 556.1, 446.9, 143.3, 5.5 and 2.5 µg/ 100 g fresh garlic respectively (Gorinstein *et al.,* 2005). Over 70 fatty acids have been determined, with linoleic (46 -53%), palmitic (20 - 23%), oleic (4 -13%), and alinolenic (3-7 %) acids being most abundant, accounting for 80% of the total lipids (Tsiaganis *et al.,* 2006).The protein content of raw garlic ranges from 2.6% to 3.0%, depending on the variety of garlic. The average content of free amino acids is 2.13%. Concentrations of dietary fiber and total tocopherols in raw garlic are 2310 and 103.1 mg/100 g fresh weight, respectively. Ascorbic and total polyphenols levels are 73.6 and 1.9 mg in 100 g dry weight (Gorinstein *et al.,* 2008).

Garlic extract boosts some immune functions and selectively kills cancer cells by apoptosis (Ban *et al.,* 2007;.Shukla and Kalra, 2007) Also garlic prevents many types of cancer by disabling free radicals (Ide and Lau, 1997; Das and Saha, 2009). Free radicals are defined as species having one or more unpaired electrons which make them unstable and highly reactive. Among the most common oxygen free radicals are the superoxide anion ($O_2^{\cdot -}$), the hydroxyl radical ($^{\cdot}OH$) and peroxyl radicals ($ROO^{\cdot}$). Other kinds of ROS are not free radicals, the most important one being hydrogen peroxide (H_2O_2). The sources of the $O_2^{\cdot -}$ are dioxygen-reducing enzymes such as NADPH oxidases, xanthine oxidase, monoamine oxidase, prostaglandin synthases (Peyrot and Ducrocq, 2008). To neutralize free radicals and counteract the detrimental effect of ROS, cells

express a wide array of endogenous antioxidant enzymes. These include "direct antioxidants," such as superoxide dismutases (SODs), catalase, and glutathione peroxidase, as well as "indirect" antioxidant enzymes, such as glutathione S-transferases (GSTs), metallothioneins, and NADPH:quinine oxidoreductase (Cho *et al.,* 2006 ; Wesselkamper *et al.,* 2006). Proper regulation of these antioxidant enzymes is essential for mammals to maintain balances between oxidants and antioxidants (Gong *et al.,* 2009).

The lung is an organ susceptible to oxidative stresses that are derived from oxygen or inflammatory responses (Chow *et al.,* 2003; Nakamura *et al.,* 2005). The imbalance of oxidants and antioxidants plays an important role in the development of various pulmonary diseases, such as acute respiratory distress syndrome and chronic obstructive pulmonary disease (Christofidou-Solomidou and Muzykantov, 2006; Rahman, 2008; Gong *et al.,* 2009).

The widespread use of pesticides in public health protection and agricultural programs has caused severe environmental pollution and health hazards, particularly in developing countries, including cases of severe acute and chronic human and animal poisoning as well as damage to other non-targeted organisms (Assayed *et al.,* 2010 a). Cypermethrin is a type II pyrethroid compound classified as a toxicity class III chemical (Borges *et al.,* 2007). Cypermethrin is one of the most common contaminants in the ecosystem. Recently, there are many studies concerning the role of cypermethrin exposure on the induction of oxidative stress (Jin *et al.,* 2011; Hussiena *et al.,* 2011). The toxic effects of oxygen free radicals and reactive oxygen compounds can lead to a wide variety of health hazards, including aging, cancer, atherosclerosis, viral infections, stroke, myocardial infarction and arthritis (Ames *et al.,* 1993). During pyrethroid metabolism, reactive oxygen species (ROS) are generated and result in oxidative stress in intoxicated animals (Kale *et al.,* 1999). Toxicants may produce oxidative stress by direct biotransformation to electrophilic or free radical intermediates, inducing or altering enzymatic system within the cell such as cytochrome P-450 within the peroxisomes and mitochondria, generating free radicals and depleting or inhibiting normal enzymatic and nonenzymatic antioxidant systems which scavenge the free radicals and protect the cell or overburden the repair mechanisms within the cell (Datta *et al.,* 1992 ; Retter, 1993 ; Stephen *et al.,* 1997 ; Salah *et al.,* 2009). Cells have enzymatic and non-enzymatic scavenger systems against these free radicals. Nevertheless, if free radical production and scavenger systems somehow become unbalanced, cells are exposed to oxidative damage resulting in cell injury (Parinandi *et al.,* 1990; Griesmacher *et al.,* 1995).

This study aimed to evaluate (1) the influence of cypermethrin on antioxidant enzymes and hist pathological changes in lung tissue in male mice, (2) the protective role of garlic in alleviating the deteriorating effect of cypermethrin on lung function and structure.

2. Material and methods

2.1. Cypermethrin

Cypermethrin was obtained from one of the stores that sell pesticides in Jeddah, one of the import of the Saudi Company delta Chemical Industries. It is used in controlling insects, ticks, beetles, butterflies and larvae and is also used in crops, cotton, grain, ornamentals, potatoes and other vegetables, and appreciates the active ingredient pesticide by 10%.

2.2. Garlic

Garlic extraction was performed according to the earlier described methods (Batirel *et al.,* 1996; Saner *et al.,* 2003). Briefly, garlic bulbs (30 g) were crushed in distilled water (60 ml) and squeezed through a double cheese cloth and aliquots were stored at −20 ◦C till further use. Each ml of the extracted aliquot was equivalent to about 500 mg of garlic (Flora *et al.,* 2009).

2.3. Experimental design

In this study 20 adult males of albino mice (MF1) were used. Animals were purchased from the King Fahd Center for Medical Research, King Abdulaziz University in Jeddah. They were nearly of equal average weights (31± 2 g). The animals were acclimatized for 7 days prior to their use in experiments and housed in stainless steel cages in an air-conditioned room maintained at 25±2 ◦C and 12 h with alternate day and night cycles. Mice were then divided into four groups of five mice each and were treated as below for the period of 2 weeks:

Group I: control (treatment corn oil, drinking water).

Group II: Cypermethrin (CYP) the animals were orally gavaged 2.8 mg / kg of cypermethrin dissolved in corn oil for the duration of the experiment by oral tube feeding (Coombs *et al.,* 1976).

Group III: Aqueous extract of garlic, 500 mg/kg, orally, once daily (Flora *et al.,* 2009).

Group IV: CYP (as in group II) + garlic extract, 500 mg/kg, orally, once daily.

The aqueous extract of garlic was administered 1h prior to the cypermethrin dose. The doses of the garlic were selected based on previously reported work (El- Demerdash *et al.,* 2005; Eidi *et al.,*

2006). After the administration of the last dose, the animals were provided 24 h rest then the following were carried out:

a- Blood collection and biochemical studies:

Under ether anesthesia, blood was collected for serum separation and kept at -20 C till used. Animals were then sacrificed, the thorax was opened and lungs were removed, parts were washed with cold saline buffer. Washed tissues were immediately stored at - 80 ° C. To obtain the enzymatic extract, tissues were homogenized in ice-cold 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 m Methylene diaminetetraacetic acid (EDTA) to yield 10% (W/V) homogenate. The homogenates were then centrifuged at 1000 rpm for 10 min at 4° C. The concentration of measured as thiobarbituric acid reactive substances (TBARS) in the tissues of mice was assayed by the method of Ohkawa *et al.* (1979). Superoxide dismutase (SOD) activity was determined by the epinephrine method (Misra and Fridovich, 1972). Glutathione S- transferase (GST) activity toward 1-chloro-2,4- dinitrobenzene as a substrate was determined according to Habig *et al.* (1974). Sulphydryl groups (SH groups) were measured in tissue homogenates after reaction with 5,50-dithiobis-(2-nitrobenzoic acid) using the method of (Ellman, 1959).

b. Histopathological studies:

1. Light microscopic studies

For light microscopic examinations, the lung tissues were dissected, and the tissue samples were fixed in 10% formalin, processed in a series of graded ethanol solutions, and embedded in paraffin. Paraffin sections were cut with a microtome to a 5 µm thickness and stained with hematoxylin and eosin for light microscopic examination. The sections were viewed and photographed on a light microscope (Olympus BX51, Tokyo, Japan) with an attached camera (Olympus C-5050, Olympus Optical Co. Ltd., Japan).

2. Transmission electron microscopy studies

For electron microscopic examinations, primary fixation of small pieces from right lobes was done in 3% glutaraldehyde in sodium phosphate buffer (200 m M, pH 7.2) for 3 h at 4 ◦C. lung tissues were washed with the same buffer and postfixed in 1% osmium tetroxide (Agar Sci. Ltd.) in sodium phosphate buffer, pH 7.2, for 1 h at 4 ◦C. Tissue samples were washed with the same buffer for 3 hrs at 4 ◦C and then embedded in Araldite (Agar Sci. Ltd.). Thin sections were cut with a Leica EM UC6 (Leica Co., Austria) ultramicrotome. Samples were stained with 2% uranyl acetate and lead citrate. The sectionswere viewed and photographed on a Jeol 100 CXII transmission electron microscope (TEM) (Jeol Ltd., Japan) at 80 kV.

2.5. Statistical analysis

The program used was Statistical Packagee For Social Science (SPSS 15). Student s t-test was used to study the difference in concentration thiobarbituric acid-reactive substances TBARS and antioxidant enzymes in lung. One – way random statistical analysis was used and the results were written as Stander Erro ± Mean.

3. Results

3.1. Effects of cypermethrin and garlic treatment on lung TBARS and antioxidant enzymes.

The results summarized in (Table 1) indicated that treatment with cypermethrin alone significantly increased ($P < 0.05$) the level of TBARS and decreased GST, SH group and SOD comparing to control group. While the treatment of cypermethrin with garlic significantly ($P < 0.05$) decreased the levels of TBARS, increased the levels of the activities of the enzymes GST, SH and SOD in lung, compared to cypermethrin group alone.

Table 1: Means of TBARS, GST, SH and SOD levels in lung of male mice treated with cypermethrin (CYP), garlic (GAR) and cypermethrin (CYP) + garlic (GAR).

Experimental groups / Parameter	Control	CYP	GAR	CYP+ GAR
TBARS	13.50 ± 0.07 [b]	24.70 ± 0.20 [a]	13.14 ± 0.05 [c]	15.50 ± 0.10 [b]
GST	0.642 ± 0.007 [a]	0.604 ±0.002 [c]	0.642 ± 0.007 [a]	0.632 ± 0.010 [b]
-SH group	1.33 ± 0.007 [a]	0.009± 1.07 [c]	1.33± 0.006 [a]	1.28 ±0.004 [b]
SOD	12.50 ± 0.07 [a]	8.81 ± 0.07 [c]	12.51±0.06 [a]	11.70±0.18 [b]

Values were expressed as means ± SE; n = 5 for each treatment group. Mean values within a row not sharing a common superscript letters (a, b, c, d) were significantly different, $p < 0.05$; TBARS: thiobarbituric acid reactive substances (nmol/g tissue); GST: glutathione S-transferase (µmol/h/mg protein); -SH group (mmol/g);SOD: superoxide dismutase (U/mg protein).

3.2. Histopathology.

3.2.1. Light microscopic study.

Fig. (1) Shows the normal structure of control mice lung parenchyma including bronchioles, alveolar spaces and related blood vessels. Alveolar spaces and bronchioles are lined by normal epithelium reported in literatures (Fig. I). In cypermtherin group there was the bleeding and inflammatory cells within alveolar spaces and inside the cavity of bronchioles (Fig II). In cypermtherin + garlic group the alveolar spaces and bronchioles are normal except of few bronchioles that still showed congestion of their accompanied blood vessels (Fig IV). The garlic group did not perform pathological changes in the structure of alveolar disuse (Fig III).

3.2.2. Transmission electron microscopy.

Fig. (2) Fine structure alveolar spacesshowed that they is lined by pneumocytes type 2 and pneumocytes type 1. capillary- alveolar barrier was observed to be of normal thickness. Capillaries were identified by presence of red blood corpuscles Fig (I). Cypermtherin group exhibited a decrease in alveolar spaces with inflammatory cell infiltrate. Capillary congestion was also observed there was an increase in the number of pneumocytes type 2 and increased thickness alveolar septa between alveolar spaces (Fig II). No pathological changes in the structure of alveolar spaces in garlic group (Fig III). Cypermtherin + garlic group showed marked decrease in alveolar spaces inflammatory infiltrate, which which are lined by nearly normal pneumocytes type 2 which showed have microvilli and mitochondria, pneumocytes type 1 were also of normal appearance septal capillaries showed no signs of congestion observed in cypermtherin group (Fig IV).

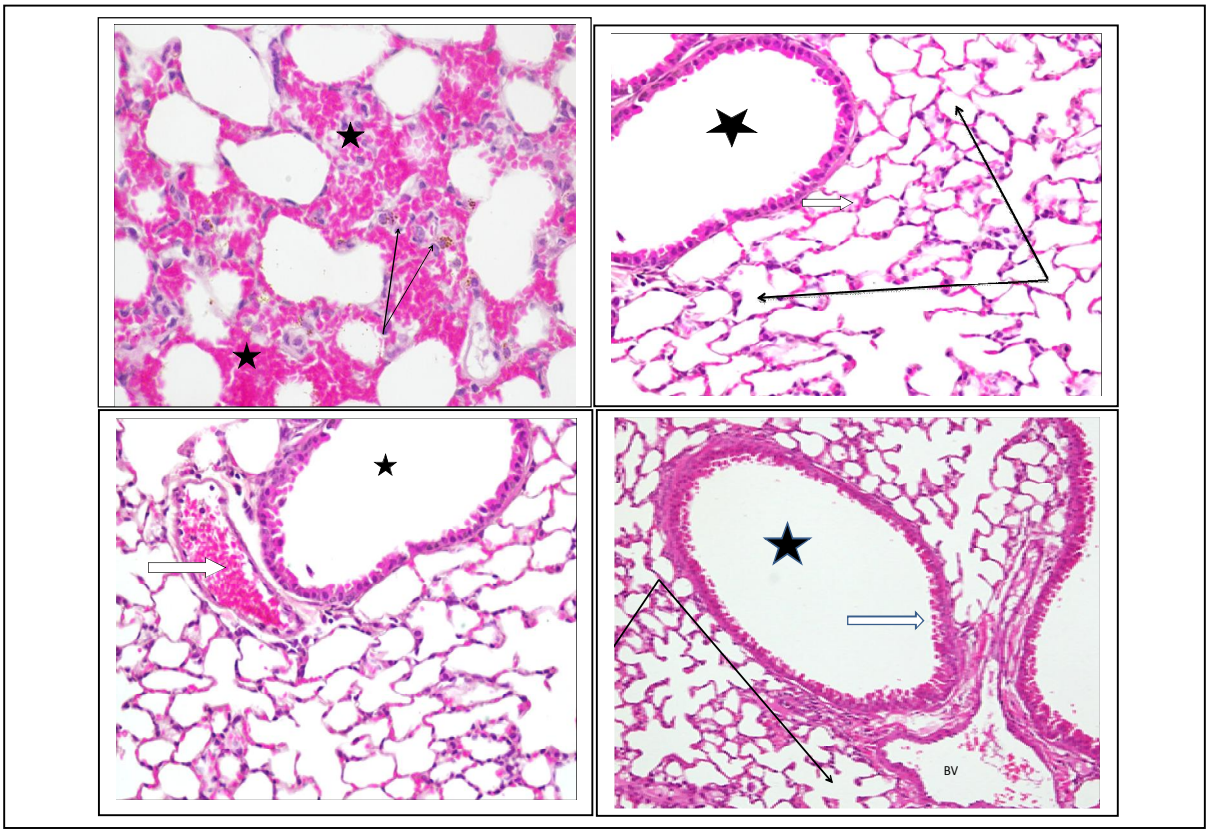

Fig. 1. Photomicrograph of mice lung stained with H&E. (I) Lung section of control group showing normal bronchioles (stars *) with normal epithelium (White arrow), alveolar spaces (thin black arrows), blood vessels (BV). (II) Cypermethrin group showed marked decrease in alveolar spaces. Most showed bleeding and inflammatory cell infiltrate (stars *). (III) The garlic group: note the normal structure of alveolar spaces (thin black arrows), bronchiole cavity free of any secretion (star *) and has normal epithelial lining (white arrows). (IV) Cypermtherin + garlic group: notice that alveolar spaces and are similar to control group of bronchiolar blood vessels (white arrow) were observed. (I) X 400, (II) X 400, (III) X400, (IV) X400.

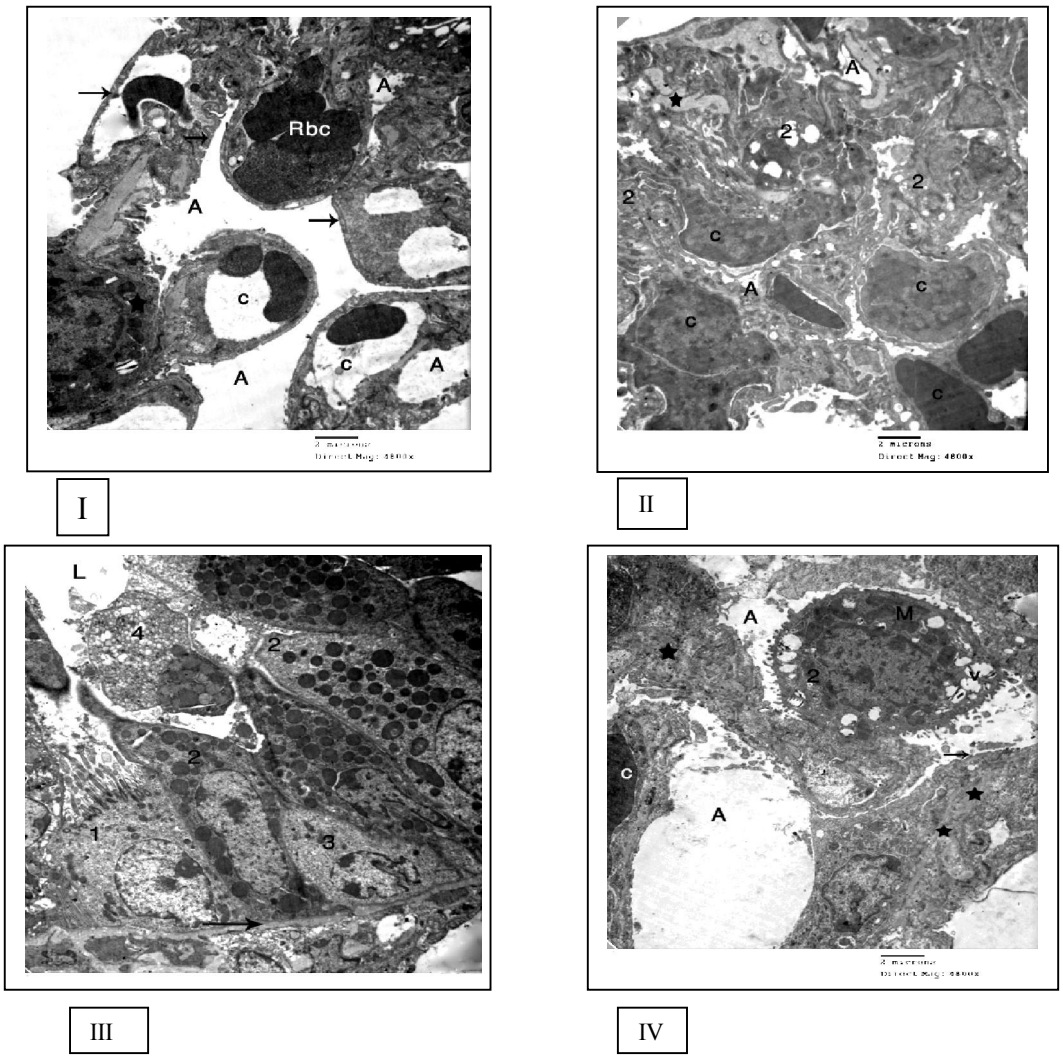

Fig. 2 Electron micrograph of lung in mice. (I) control group: showing the structure of alveolar spaces cells (A) pneumocytes type 2 (*) and pneumocytes type 1 (arrow) and also showing the capillary-alveolar membrane barrier (2) capillary contains red blood corpuscles (rbc). (II) Cypermtherin group showing a decrease in the alveolar space cavity (A) and an increase in the number of pneumocytes type 2 (2) and increased thickness of septal tissue between alveolar spaces (*). (III) No pathological changes in the structure of alveolar tissue in garlic group. (IV) Cypermtherin + garlic group showed an improvement in the inflammatory infiltrate of alveolar spaces, which contain pneumocytes type 2 (2) with microvilli (V) and mitochondria (M), it also shows the pneumocytes type 1 (arrow), alveolar space cavities (A) and shows the basement membrane of sepal capillaries (*). (I) X 4800, (II) X 4800, (III) X 3800, (IV) X4800.

4. Discussion

Epidemiological and experimental evidences of reported in past literature suggested that the use of synthetic chemicals is a major environmental carcinogenic enhancers via generation of free radicals such as reactive oxygen species (ROS) inducing oxidative stress and cellular injury (El-Wakf *et al.,* 2009; Shaarawy *et al.,* 2009; Hassan *et al.,* 2010). Free radicals are known to play an important role in the toxicity induced by pesticides and environmental chemicals. Changes in the status of the antioxidant, oxygen free radicals, the scavenging enzyme system, and lipid peroxidation (Etemadi-Aleagha *et al.,* 2002; Muthuviveganandavel *et al.,* 2011) induced plays an important role in the pathogenesis of various pulmonary diseases. Much work has been done to investigate the protective role of antioxidant enzymes, including GST, glutathione peroxidase, metallothionein, SOD, and catalase, in the lung (Gong *et al.,* 2009). Cypermethrin has also been shown to induce oxidative stress and generation of reactive oxygen species (ROS) in experimental systems

(Hussiena *et al.,* 2011). Gabbianelli *et al.* (2004) suggested that superoxide anion and hydrogen peroxide are the main source of cypermethrin-induced free radical production. Therefore, examining the change in activity of antioxidant enzymes such as SOD, CAT, and GPX is considered as an effective method of denoting oxidative stress. More recently, these enzymes have been used to detect biological toxicity and/or to monitor the impact of chemical pollutants (Xu *et al.,* 1997).

In the current study the cypermtherin group has significantly increased (P <0.05) the concentration of TBARS and antioxidant enzymes GST and SH and SOD in the lung compared with the control group (Table 1). These findings are in agreement with the study of (Abdollahi *et al.,* 2004), who noted that high levels of TBARS in plasma and various tissues is an evidence of a significant increase in the oxidation of cell membranes by free radicals that result in the emergence of oxidative stress. These findings are in agreement with the study of Cochrane (1991), who suggested that when antioxidants in the body is unable to dominate the free radicals production, oxidative stress occurs with subsequent cellular damage. Also, cypermethrin was reported to cause biochemical and haematological changes in rabbit. (Zatta *et al.,* 1998). The lung sections of the cypermethrin group under the light microscope showed pathological changes in thein the form of alveolar exudate and congestion and increased bronchiolar secretion. While under the electron microscopy the cypermtherin group showed a decrease in alveolar cavities as a result of capillary congestion, an increase in the number of pneumocyte type 2 and increased thickness of the basement membrane barriers between alveolar spaces and capillaries were observed. These findings are in agreement with the study of (Ulaiwi, 2011), who fed 24 rabbits cypermtherin for 21 days,and his results showed histopathological alteration such as engorgement of pulmonary capillary and congestion of pulmonary alveoli, thickening and congestion of the alveolar septa and swollen and tortuous of smaller veins, similar findings were reported by Nagarjuna and Jacob (2009) after mice exposure to sublethal doses (41 mg/kg bw) of cypermethrin as single dose, double dose and multiple dose. Cypermethrin administered at repeated oral doses of 5 and 20 mg/kg/day for 30 days produced thickening of alveolar septa in lungs (Grewal *et al.,* 2010). Sayim *et al.* (2005) and Hussiena *et al.* (2011) reported that Cypermethrin caused different histopathological changes in rat organs including ischemia and pyknosis of the cytoplasm of the neurons in the brain tissue A Study done by Al-Azizz (2012) demonstrated also lung toxicity in pigeonsupon exposure to different doses of cypermethrin. The pathological changes found by the author in the lungs with low, intermediate and high dose are emphysema and congestion, and some with foamy alveolar macrophages.

Biochemical assays done in the present study showed that administration of garlic with cypermethrin result in improvement of antioxidants enzymes levels with reduced TBARS levels. This could be attributed to the role of garlic as an antioxidant that preventing the accumulation of toxins within the cell and promote the production of energy. These findings are in agreement with study of (El-Banna *et al.,* 2009) who found that feeding garlic with chlorpyrifos (CPF) the most widely used organophosphate insecticides has led to a decrease in concentration of TBARS and an increase in the levels of antioxidant enzymes in the liver. However, the administration of garlic can modulate the oxidative stress and improve the antioxidant system through the direct cytoprotective effect of garlic oil constituents; diallyldisulphide (DADS, 60%), allyl propyl disulphide (6%), allicin (0.3–0.5%) and diallyltrisulphide (DATS) (Anwar and Meki, 2003; Sener *et al.,* 2003; Pari *et al.,* 2007; Hassan *et al.,* 2010). The results suggested by (Mirunalini *et al.,* 2004) that garlic oil may exert its chemopreventive effects by modulating lipid peroxidation and enhancing antioxidant status in the liver and blood during buccal pouch carcinogenesis induced by 7,12-dimethylbenz [a] anthracene (DMBA) in male Syrian hamsters. Another study done by Zare *et al.* (2008), who investigated the effect of 1, 2, and 3 times intraperitoneal injections of aged garlic extract on an established allergic airway inflammation in murine model (BALB/c mice). Their results indicated that three-time intra peritoneal injections of the aged garlic extract caused a significant decrease in the hallmark criteria of allergic airway inflammation levels.

Histopathological studies of the present study confirmed the protective role of garlic and the improvement of biochemical parameters. Animals fed garlic with cypermtherin showed return the alveolar tissue and bronchioles to their normal form except of few bronchioles that showed congestion of accompanied blood vessels. These findings are in agreement with the study of Assayed *et al.* (2010b), who suggested that garlic and ascorbic acid dampen the reproductive toxicity and/or teratogenicity of cypermethrin toxicity in rats. With the study of (Nwokocha *et al.,* 2012), garlic can be used in the reduction of some metal accumulation in the liver. (Ziu *et al.,* 1994) reported that mice injected with ascites tumor cells that were pretreated with an

extract of garlic developed strong immunity against the same type of tumor cells.

5. Conclusion

The present study concluded that, cypermethrin caused significant increase in the concentration TBARS and decreased antioxidant enzymes and this coincide with pathological changes seen in lung parenchyma under either by light or electron microscope. Moreover, using garlic in combination with cypermethrin minimized its toxic effects on most of the tested parameters and this may be attributed to the vital role of garlic as antioxidant that could be beneficial in alleviating cypermethrin toxicity. Consequently, exposure to cypermethrin should be reduced and attention must be paid to take care when dealing with cypermethrin sources.

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