The Role of Garlic Extract on the Effect of Organic Pesticides on Murine Liver Cells

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ABSTRACT:

BACK GROUND:

Organic phosphorus pesticides are most widely preferred pesticides, and know to have detrimental effect on various living organisms.

METHODS:

Twenty four male Balb/c mice were used. These were divided into four group (1,2,3,4) . Chlorpyriphos was given orally on alternate days by garage (20mg/kg body weight), aqueous garlic alone was given orally daily by garage (200mg/kg body weight, while garlic extract followed by chlorphriphos was also given.

RESULTS:

Lipid peroxidation (LPO) was measured in liver homogenate . The extent of LPO was significantly increased in animals treated with pesticide while non-significant result were obtained with garlic extract alone . Pretreatment with garlic followed by pesticide showed similar values. The levels of glutathione and total thiol was significantly decreased in animals treated with pesticide and non-significant with garlic . pretreatment with garlic followed by pesticide showed similar value. The free radical scavenging enzyme like SOD , as well as GSH- dependent enzyme (GPx, GST and GR) show significant decrease in animals treated with pesticide and non-significant with garlic. Pretreatment with garlic followed by pesticide showed similar to that of control.

CONCLUSION:

Pesticide exposure could be prevented by Co-administration of garlic extract.

KEY WORDS: Chlorpyriphos , Garlic extract , LPO, Glutathione.

INTRODUCTION:

Organophosphorus pesticides are most widely preferred pesticides owing to their efficiency, and known to have detrimental effects on various living organisms act by the inhibition of neuronal transmission[1]. Pesticide poisoning is an important cause of morbidity and mortality in many countries[2] .The dependence on pesticides to increase agricultural yield is a steadily augmenting phenomenon and is the single most instrumental factor in causing accidental exposure as also the degradation of soil nutrients [3]. Chlorpyriphos causes degradation of hepatocytes and renal cells[4]. Moreover, liver and Kidney are the primary organs affected by pesticide poisoning[5]. Oxygen free radical (OFR) enzymatic scavenger such as SOD, GST, GR and GPx have all been observed to play a pivotal role in protection of the cellular system from various deleterious effect of free radicals induced by pesticides [6-8]. Antigenotoxic and anticarcinogenic compounds are known to be present in commonly consumed vegetables, fruits, spices, nuts, vegetable oil, tea[9]. Garlic play an important role in dietary and medicinal, and recognized as a flavoring agent for food.

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The pharmacological effect of garlic are hypolipidemic, hypoglycemic, anticoagulant, antimicrobial, anteicancer and antitumor, as an antidote for heavy metal, poisoning, hepatoprotective and immunomodulator[10]. *In vitro*, kidney and liver cells of garlic-fed rats have been recogenized to exert inhibiting effects on free radical generation[11,12]. In the present report, *in vivo* study was carried out to understand the effect of pretreatment with aqueous garlic extract on pesticide induced oxidative stress in Balb/c mice.

MATERIAL AND METHODS:

Balb/c mice (age 8-10 weeks) weighing between (30-32) gm were used for the present study. Chlorpyriphos was diluted with water in order to obtain an effective concentration of (20 mg/kg body weight), the dose was injected orally on alternate days for period of (3) weeks.

An aqueous extract of freshly peeled garlic cloves was homogenized in an appropriate volume of distal water to give a concentration of 200 mg/kg[12]. The animals were divided into four groups of six animals each, animals, in group(1) was given tap water only and served as control, in group(2) was given daily aqueous garlic extract alone at a concentration of 200 mg/kg body wt.

The third group was given chlorpyriphos pesticide (20 mg/kg body wt.), on alternate days while the fourth group was pretreated with garlic extract followed by pesticide daily. After 3 weeks, the animals were sacrificed by cervical dislocation. Liver was quickly dissected and placed in ice cold 0.9% NaCl solution. GSH was determined by the method of Ellman[13], GPx activity was assayed by the method[14], with modifications from[15]. The GST activity was determined by[16]. Estimafion of GR was performed by[17], while total thiols by[18]. Lipid peroxidation was estimated by the method of[19], the activities of SOD was measured by method of[20]. The protein estimated by the method[21]. Data are presented as mean ± SD statistical differences were calculated by student's "t" test.

RESULTS:

Lipid peroxidation (LPO) was measured in liver homogenate, the effect of pretreatment with garlic extract on pesticide- induced change in the antioxidants status in liver are shown in Fig.1.

The extent of LPO was significantly increased in animals treated with pesticide (group III) and non-significant with garlic extract alone(group II) compared to control (group I). Pretreatment with garlic extract follwed by pesticide(group IV) showing similar values to that of control (group I).

The effect of garlic extract on levels of cellular antioxidant reserves like GSH and total thiol (Fig.2.A,B). The levels of GSH and total thiols was significantly decreased in animals treated with pesticide (group III) and non- significant increased with garlic extract alone (group II) compared to control (group I). Pretreatment with garlic extract followed by pesticide restored the level of GSH and total thiol near the normal (group IV).

The free radical scavenging enzyme like SOD as well as the GSH –dependent enzyme (GPx, GST and GR) show a significant decrease in activity on exposure to pesticide (group III), and non - significant increased with garlic extract alone (group II), as compared to untreated control (group I). Pretreatment with garlic extract followed by pesticide (group IV) showing values similar to that of control (group I) (Fig.3),(Fig. 4.A,B,C).

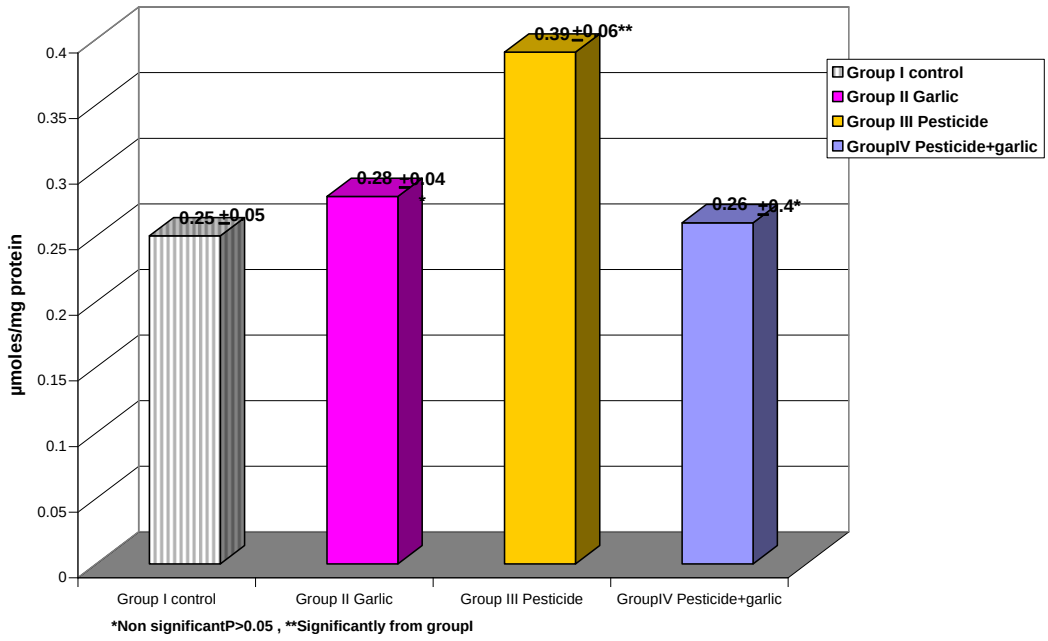

Fig.1.The effect of pretreatment with garlic extract on pesticide-induced lipid peroxidation (LPO) in liver of mice. Values are means (SD+,n=6)

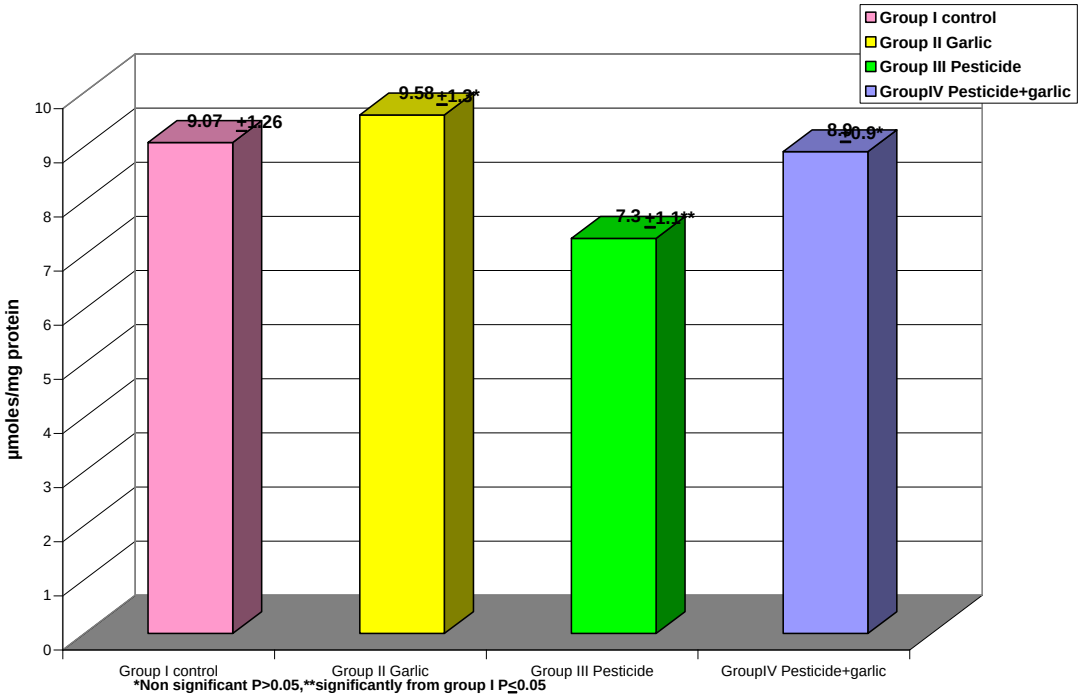

Fig.2.Protective effects of pretreatment of garlic and replenishment of cellular antioxidant reserves.values are mean(SD+,n=6) A-Glutathione(GSH)

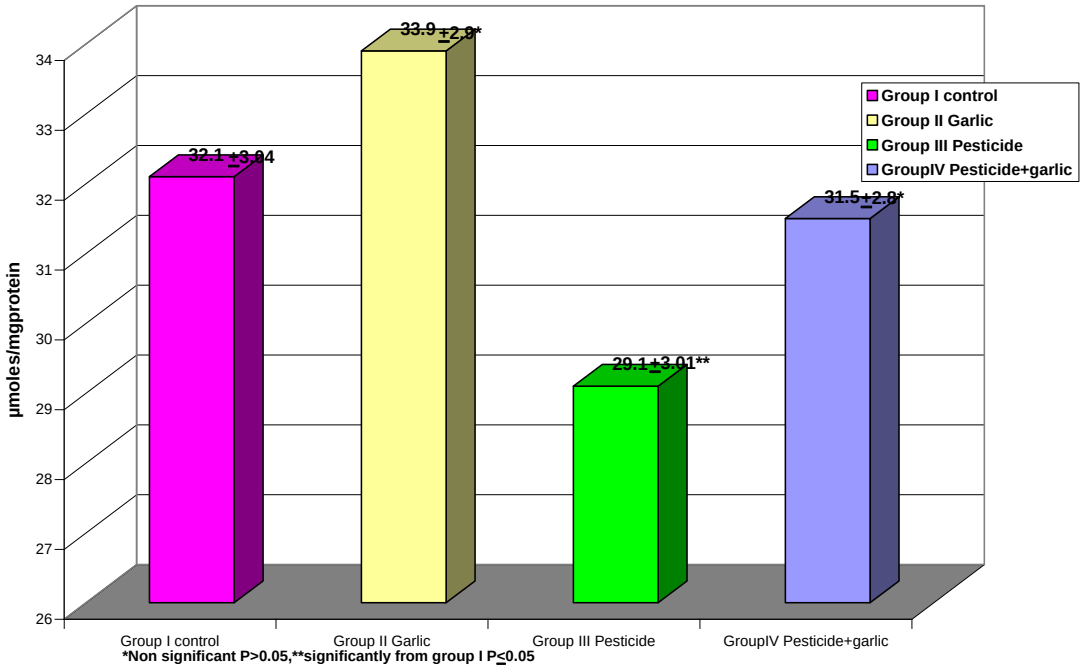

Fig.2.Protective effects of pretreatment of garlic and replenishment of cellular antioxidant reserves.values are mean(SD+,n=6) . B-Tolal thiol

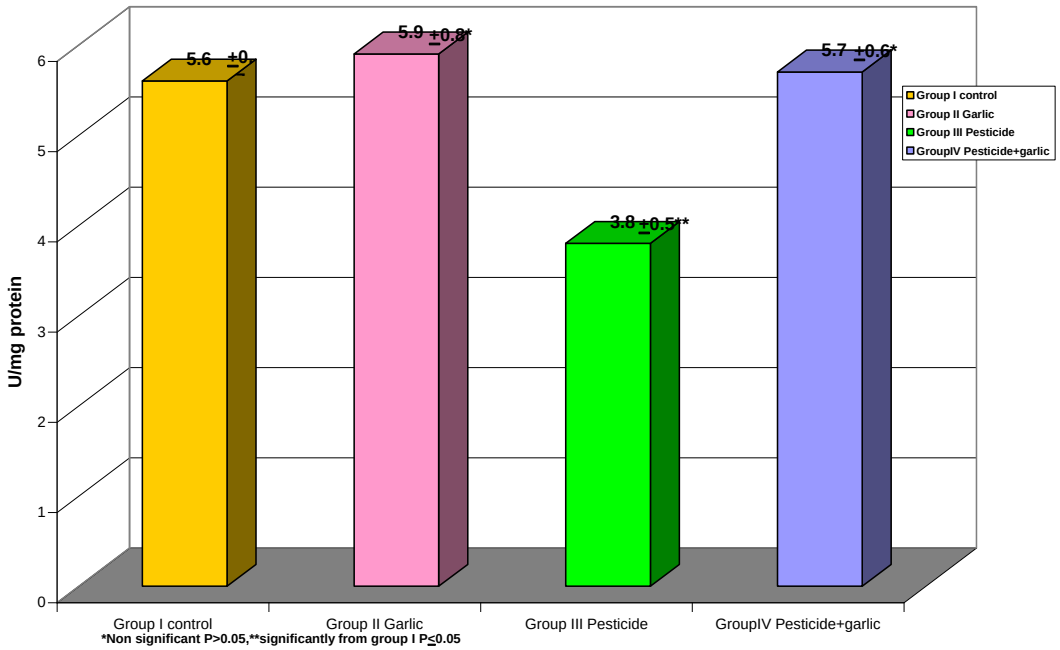

Fig.3.Effect of garlic extract on free radical scavenging enzymes SOD in liver of mice .(values are mean(SD+,n=6)

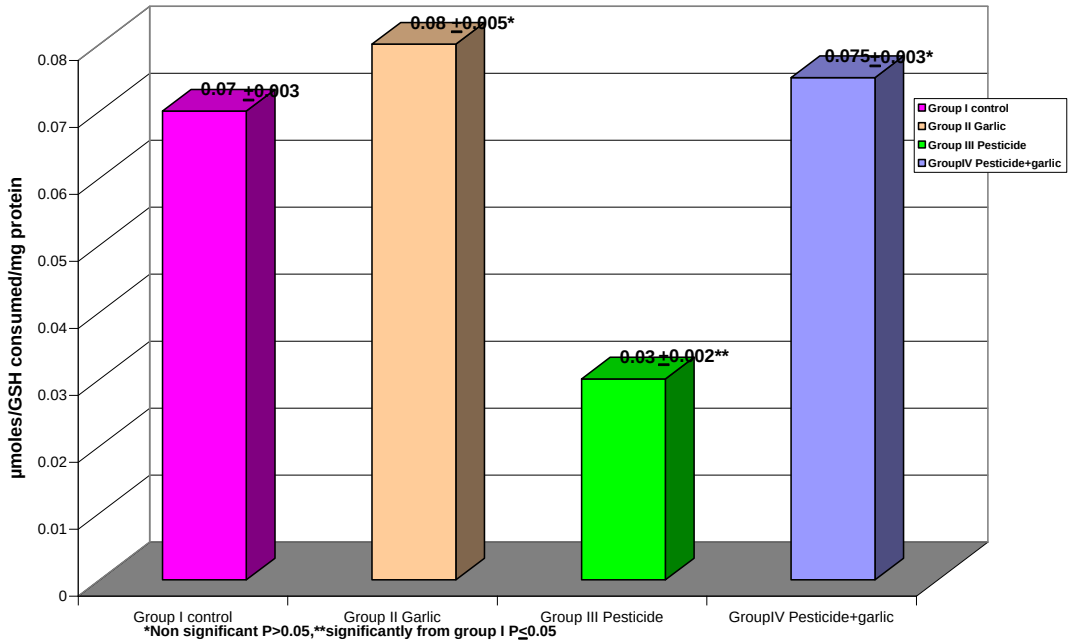

Fig.4. The influence of pretreatment with garlic extract on GSH-dependent antioxidant enzymes GPx, GR and GST in liver of mice. values are mean(SD+,n=6). A-GST

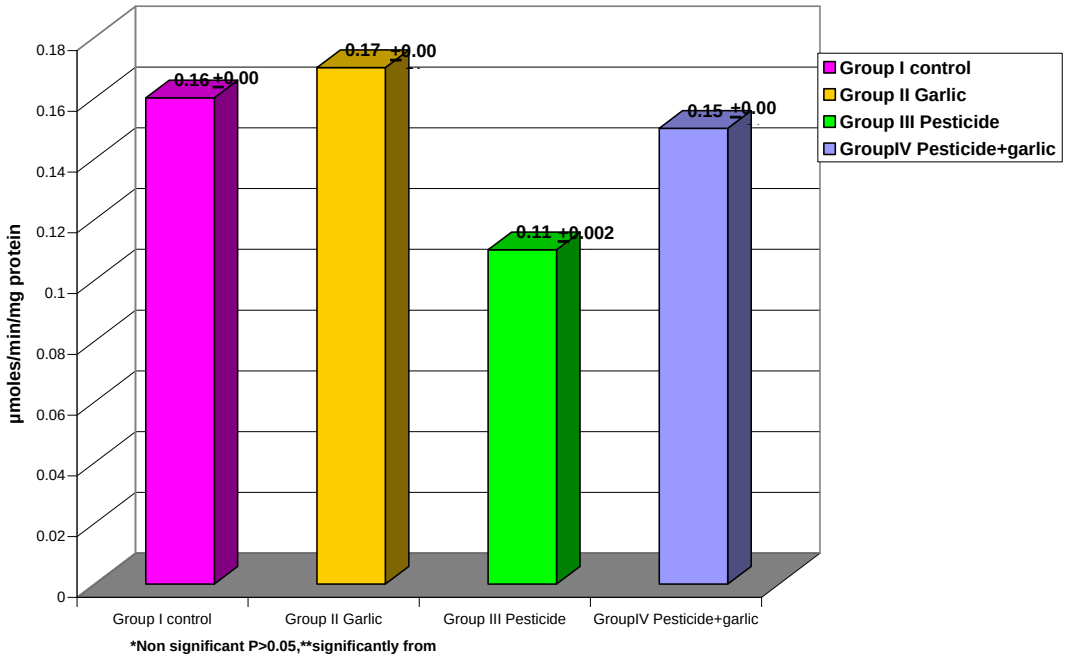

Fig.4. The influence of pretreatment with garlic extract on GSH-dependent antioxidant enzymes GPx, GR and GST in liver of mice. values are mean(SD+,n=6).B-GR

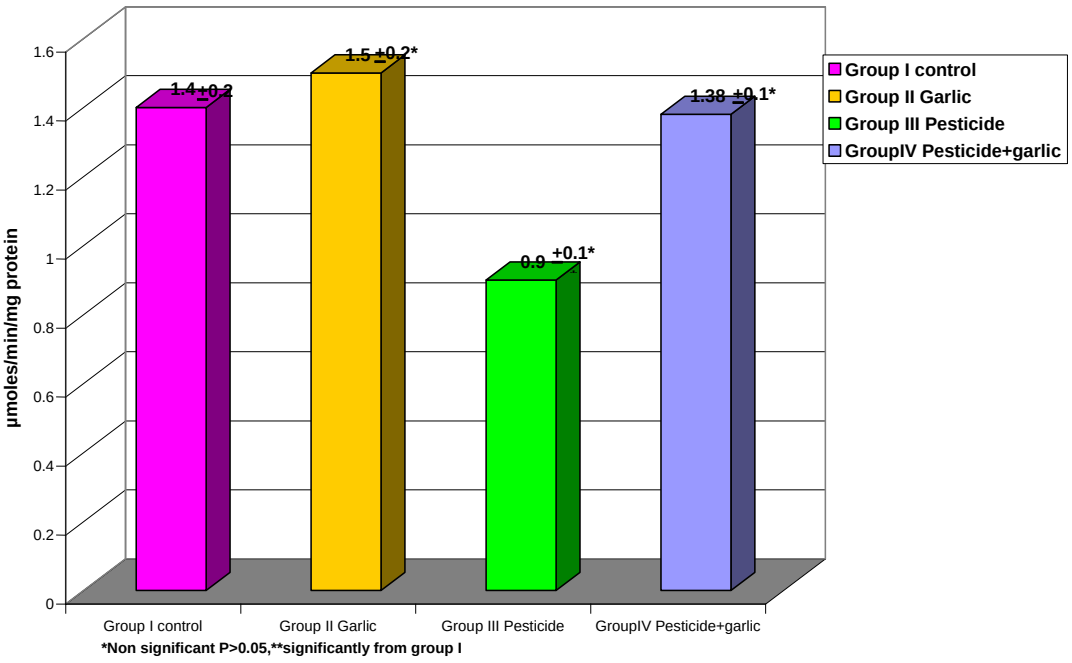

Fig.4. The influence of pretreatment with garlic extract on GSH-dependent antioxidant enzymes GPx, GR and GST in liver of mice. values are mean(SD+,n=6).C-GPx.

DISCUSSION:

Organphosphorus pesticides are most widely used in agriculture today, and have been known to cause symptomatic changes that arise through the inhibition of acetylcholine esterase, a key enzyme in neurotransmission[22]. These pesticides have a highly efficient rate of absorption and following absorption, are widely distributed among various tissues. Exposure of organphosphorus have postulated a putative role for the generation of free radical during the process [22,23]. The liver plays a central role in the homeostasis of GSH, a physiologically important non-protein thiol in conjunction with GPx and GST comprises the GSH redox cycle that maintains the redox staus of tissues and protects structural and regulatory proteins against Reactive oxygen species (ROS)-induced damage[24]. Organophosphorus pesticides are metabolized by liver microsomal enzymes to a variety of metabolites, which are susceptible to conjugation for proper elimination. Organophorus pesticide intoxication induces a derangement of antioxidant mechanisms of the liver cells, including decreased superoxide dismutase and catalase activities and alteration in reduced glutathione content leading to depressed GSH/GSSG ratios. Depletion of GSH and GSH-dependent enzymes may shift the physiological balance of the cellular redox status in pesticide-treated mice leading to adverse effects on crucial thiol group in biological proteins favoring oxidative damage[24]. Pretreatment with aqueous garlic extract effectively reduced the combination pesticide induced oxidative damage as shown by a decrease in lipid peroxidation and enhanced the antioxidant levels in liver of mice. The results of this study further substantiate and add to the extensive literature in support of the myriad physiologic effects of garlic[25]. Several plant products, including garlic allium components have been reported to modulate the levels of lipid peroxides (LPO) and antioxidants[26]. Raw garlic contains a number of potential antioxidant compounds including organo-sulfur compounds, allicin, as well as vitamin C and selenium that have anti-carcinogenic properties[27]. The enhancing effect of garlic on reduced glutathione (GSH) and phase II enzymes such as glutathione peroxidase (GPx) and glutathione-S-transferase (GST) may be considered as a generalized electrophilic counteractive response evoked by most chemo preventive agents. Modulation of LPO,enhancement of GSH and GPx together with elevation of GST are major mechanisms by which garlic exerts its chemo preventive effect[28].

Extensive evidence also point to the ability of ally sulfides from garlic to suppress tumor proliferation both *in vitro* and in vivo[29]. Our results are consistent with these findings. Moreover, our results suggest the pretreatment with garlic extract potentially reinforces the GSH/GST detoxification system and diminishes the oxidative stress induced by exposure to the combined dose of the pesticide.

CONCLUSION:

The results of the present study suggest that exposure to pesticide, chlorpyriphos, induces a substantial derangement in the cellular detoxification mechanisms in liver of mice and that pretreatment with aqueous garlic extract exerts significant protection against pesticide-induced oxidative stress.

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