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Research Article

Potential Role of Curcumin and Garlic Acid against Diazinon and Propoxur Hepatotoxicity

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ABSTRACT

Diazinon, an organophosphorus insecticide and propoxur, a carbamate insecticide have been used in agriculture and public health for several years. The aim of the present study was to evaluate the oxidative stress caused by the two insecticides and biochemical changes in adult male Wistar rats and to evaluate the protective effect of curcumin and garlic. Diazinon (10 mg/kg per day in corn oil), propoxur (10 mg/kg per day in corn oil) alone or with curcumin (100 mg/kg per day in corn oil) and/or garlic (20 mg/kg per day in distilled water) were given to rats (n=11) orally through gavage for four weeks. Biochemical parameters in serum [total protein, albumin, triglyceride (TG), cholesterol, uric acid, urea, creatinine, γ-glutamyl transpeptidase (γ-GT), glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), lactate dehydrogenase (LDH), catalase (CAT) and total antioxidant capacity (TAC)], superoxide dismutase (SOD) and glutathion reduced (GSH) were determined in liver homogenate and malondialdehyde (MAD) was determined in RBCs. All ultrastructural changes were investigated at the end of 4th week. Results obtained showed that albumin, total protein, GOT, GPT, LDH, creatinine, urea, GSH and SOD were statistically high significance ($p<0.001$), γ-GT, uric acid, CAT, TAC and cholesterol were statistically significance ($p<0.01$) when diazinon or propoxur-treated groups compared to control group. On the other hand, GOT, GPT, LDH, GSH and SOD were statistically high significance ($p<0.001$), albumin, total protein, γ-GT, urea, CAT and TAC were statistically significance ($p<0.01$) when curcumin and/or garlic + diazinon or propoxur-treated groups compared to diazinon or propoxur-treated groups. We conclude that curcumin and garlic decreases diazinon and propoxur oxidative toxic effects and hepatotoxicity.

Keywords: curcumin, garlic acid, diazinon, propoxur, hepatotoxicity

INTRODUCTION

The agricultural chemicals commonly labeled, as pesticides are perhaps the largest group of poisonous substances being disseminated throughout our environment[1]. The contamination of food, water and air with these pollutants has become imminent, and consequently adverse health effects are inevitable in humans, animals, wildlife and fish[2].

Carbamates compounds such as propoxur have a common mechanism of action toward insect pests and unintended toxicity to non target organisms including humans, that is, acetylcholinesterase (AChE) inhibition by carbamylating the serine hydroxyl group in the active site of the enzyme in the nervous system, leading to the persistent action of the neurotransmitter, acetylcholine, on cholinergic postsynaptic receptors[3-5]. Propoxur (2-isopropoxyphenyl N-methyl carbamate) is a widely used broad spectrum insecticide. In addition to the control of cockroaches, mosquitoes, bugs, fleas, ants, millipedes, this insecticide is also used against pests in food stores, open areas, and households. Propoxur exhibits a toxic effect characterized by the inhibition of the enzyme cholinesterase[6]. Although mildly toxic to humans and domestic animals (class II), propoxur is highly toxic to birds and fish and cannot be used in the later. Propoxur is highly toxic to honeybees[7]. Several cases of suicidal and occupational poisoning have also been reported[8]. Hence, propoxur has generated considerable concern regarding its subtle health effects. Possible effect of persistent exposure to propoxur on oxidative stress and functional integrity of the immune system has heightened interest in these parameters as additional indices to analyze potential long term health effects[9]. Recent studies on animals and human from our laboratory suggest that propoxur can cause oxidative stress and immunotoxicity[10].

Diazinon (*o,o* -diethyl-*o*-[2-isopropyl-6-methyl-4-pyrimidinyl] phosphoro-thioate) is an organophosphorus pesticide insecticide with a broad range of activities which inhibit acetyl-cholinesterase activity. It has been widely and effectively used throughout the world with applications in agriculture and horticulture for controlling insects in crops, ornamentals, lawns, fruit, vegetables and other food products[11]. Some reports have been published with respect to Diazinon and its effects on haematological and biochemical parameters of rat, rabbits and mice[12]. Many insecticides such as Diazinon are hydrophobic molecules, which bind extensively to biological membranes, especially to the phospholipids bilayers. Diazinon may interfere with lipid metabolism in mammalian animals that included the levels of total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides and phospholipids and its effect was dose-dependent[13]. Diazinon's mutagenicity studies, its ability to cause genetic damage, showed that Diazinon in fact can damage DNA in human blood cells, in cells from laboratory animals, and bacteria[14]. Diazinon exposure was found to increase the occurrence of a type of genetic damage called micronuclei. Micronuclei may be induced by strand breaks in DNA due to oxidative stress[15].

Plant products are known to exert their protective effects by scavenging free radicals and modulating antioxidant defense system. Curcumin (diferuloymethane), a yellow orange dye derived from the rhizomes of Curcuma , is used as a spice and food-coloring agent in cooking[16,17]. Curcumin represents a class of anti-inflammatory and antioxidants reported to be a potent inhibitor of reactive oxygen species formation[18,19]. Reddy and Lokesh indicated that Curcumin is a potent scavenger of a variety of reactive oxygen species including superoxide radicals and hydroxyl radicals[20].

Garlic, , is a member of the lily family that has been cultivated by humans as a food plant over 10,000 years. Ancient Egyptian records mentioned that use of garlic as a remedy for a variety of diseases[21]. Recently, it has been found that the sulfur-containing compounds of garlic have anti-mutagenesis and anti-carcinogenesis effects. In vivo studies show that garlic and its associated sulfur compound. So, the aim of this study was to evaluate the oxidative toxic effects of the organophosphorus pesticide diazinon and the carbamate pesticide propoxur and to investigate the protective effect of curcumin and garlic.

MATERIALS AND METHODS

Animals

Male Wistar albino rats, weighing 100-150 gm were obtained from the animal house of the Faculty of Pharmaceutical Science of Mansoura University. The animals were fed a standard laboratory diet and water . Rats were kept 12 h light 12 h dark cycles at a room temperature of 25˚C at least two days prior testing. All animal experiments were approved by the Animal House of Biochemistry branch of Chemistry Department of Faculty of Science of Mansoura University.

Chemicals

Animal Treatment Schedule

Rats were divided into 13 groups (n=11). The compounds were administered in the morning (between 10:00 and 12:00 AM) to non-fasted rats. All rats were treated for 4 weeks.

Group 1: control group

Rats fed a standard laboratory diet and tap water .

Group 2: corn oil group

Rats received corn oil through gavages daily.

Group 3: diazinon-treated group

Rats received Diazinon at dose of (10 mg/kg bw/day) in corn oil through gavages.

Group 4: diazinon+curcumin-treated group

Rats received Diazinon at dose of (10 mg/kg bw/day) in corn oil and Curcumin (100 mg/kg bw/day) in corn oil both through gavages.

Group 5: diazinon+garlic-treated group

Rats received Diazinon at dose of (10 mg/kg bw/day) in corn oil and Garlic (20 mg/kg bw/day) in distilled water both through gavages.

Group 6: diazinon+curcumin+garlic-treated group

Rats received Diazinon at dose of (10 mg/kg bw/day) in corn oil, Curcumin (100 mg/kg bw/day) in corn oil and Garlic (20 mg/kg bw/day) in distilled water all through gavages.

Group 7: propoxur-treated group

Rats received Propoxur at dose of (10 mg/kg bw/day) in corn oil through gavages.

Group 8: propoxur+curcumin-treated group

Rats received Propoxur at dose of (10 mg/kg bw/day) in corn oil and Curcumin (100 mg/kg bw/day) in corn oil both through gavages.

Group 9: propoxur+garlic-treated group

Rats received Propoxur at dose of (10 mg/kg bw/day) in corn oil and Garlic (20 mg/kg bw/day) in distilled water both through gavages.

Group 10: propoxur+curcumin+garlic-treated group

Rats received Garlic at dose of (20 mg/kg bw/day) in distilled water through gavages.

Group 11: garlic-treated group

Rats received Curcumin at dose of (100 mg/kg bw/day) in corn oil through gavages.

Group 12: curcumin+garlic-treated group

Rats received Curcumin at dose of (100 mg/kg bw/day) in corn oil and Garlic (20 mg/kg bw/day) in distilled water both through gavages.

Collection of Samples

Collection of Blood Samples

Blood samples were collected from all groups by cardiac puncture into heparin-coated and dry tubes.

The blood samples collected were centrifuged at 3000 rpm for 10 min for the separation of plasma and sera.

The lower erythrocyte layer in the heparinised tubes was washed three times with phosphate buffered saline and diluted with an equal volume of the indicated solution.

Next the erythrocytes were hemolysed with ice-cold distilled water (1:5).

Collection of Liver Samples

Livers were excised, washed with deionized water for the removal of blood, and later fatty parts were removed. Livers were rinsed with isotonic saline and dried by blotting between 2 pieces of filter paper. Livers were stored at -20°C in plastic vials containing a 0.5 ml of ice-cold sterile isotonic saline.

Preparation of Homogenates

An accurately weighed piece of liver tissue was homogenized in an ice-cold phosphate buffer solution with a PH value adjusted to 7.4 using a Teflon Pestle connected to a Braun Homogenizer motor (25 strokes per minute at 1000 rev/min), then the liver homogenate was diluted to yield a 5% (w/v) liver homogenate. The homogenate was centrifuged at 5000 rpm for 30 minutes at 4°C to remove cell debris and nuclei. The resulting supernatant was used for biochemical analysis.

Biochemical and Specific Biomarker Evaluation

Biodiagnostic label kits were used for the determination of serum total protein, albumin, triglyceride (TG), cholesterol, uric acid, urea, creatinine, γ-glutamyl transpeptidase (γ-GT), glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), lactate dehydrogenase (LDH), catalase (CAT) and total antioxidant capacity (TAC). Superoxide dismutase (SOD) and glutathion reduced (GSH) were determined in liver homogenate. Malondialdehyde (MAD) was determined in RBCs[22].

Statistical Analysis

The mean ± SEM were determined for each group. Data were analyzed by SPSS 19 to calculate the significance. $P<0.01$ value between study groups was taken as statistically significant and $P<0.001$ value was taken as statistically high significant.

RESULTS

Table 1 showed significant decreased levels of serum albumin, total protein and urea in rats treated with either diazinon or propoxur than that non-treated. However, levels increased significantly in rats treated with curcumin and/or garlic before pesticides treatment. On the other hand, serum γ-glutamyl transpeptidase, glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) and lactate dehydrogenase (LDH) levels showed significant increase in rats treated with either diazinon or propoxur than normal rats, but these levels became like normal by curcumin and/or garlic pretreatment. Uric acid and creatinine showed elevated levels in pesticides treated rats, but with the curcumin and/or garlic pretreatment the levels became near levels of normal rats.

Table 2 showed significant increased levels in serum triglycerides (TG) and cholesterol and RBCs malondialdehyde (MAD) in rats treated with pesticides only compared to normal rats, but these levels decreased in rats pretreated with curcumin and/or garlic compared to pesticides treated rats. On the other way, table 2 showed significant decreased levels of tissue glutathione (GSH) and superoxide dismutase and serum catalase (CAT) and total antioxidant capacity (TAC) in rats treated with diazinon or propoxur. These levels showed significant elevations in rats treated with curcumin and/or garlic before pesticides treatment.

Table 1: Effect of diazinon, diazinon + curcumin and/or garlic, propoxur and propoxur + curcumin and/or garlic on liver and kidney function enzymes.

Parameter	Albumin g/dl	Protein g/dl	γ-GT IU/L	GOT IU/L	GPT IU/L	LDH IU/L	Uric acid mg/dl	Creatinine mg/dl	Urea mg/dl
Control	3.0±0.1	7.2±0.2	38.9±3.8	18.1±0.4	15.0±0.6	272.2±14.3	3.9±0.4	0.7 ± 0.03	39.8 ± 2.04
Corn oil	2.9±0.2	7.2±0.3	29.9±3.1	32.8±0.9	26.0±0.5ii	272.0±12.1	4.1±0.5	0.7±0.03	33.0±4.05
Diazinon (DZN) 10mg/kg	1.9±0.2ii	5.2±0.3ii	70.1±7.3i	58.2±2.5ii	44.7±0.5ii	811.5±10.2ii	5.4±0.4i	1.1±0.07ii	16.0±1.9ii
Curcumin+DZN	2.7±0.2*	6.4±0.4*	34.9±6.3*	43.1±1.0ii**	33.9±1.2ii**	455.9±13.9ii**	4.2±0.4	0.9±0.1*	23.4±2.5ii**
Garlic+DZN	2.5±0.1i*	6.8±0.1**	45.9±2.2*	42.0±0.7ii**	34.0±1.0ii**	418.4±17.9ii**	4.2±0.5	0.9±0.1*	23.7±1.5ii*
Curcumin+garlic+DZN	2.2±0.1ii	7.0±0.4*	28.3±3.2*	42.4±1.1ii**	34.8±1.2ii**	457.7±18.6ii**	4.7±0.3	0.9±0.1	27.3±4.9i*
Propoxur (PPX) 10mg/kg	2.1±0.1ii	5.0±0.2ii	60.9±4.1i	75.1±1.2ii	53.0±0.6ii	918.9±20.9ii	5.7±0.5i	1.1±0.06ii	22.9±2.08ii
Curcumin+PPX	2.9±0.3*	6.5±0.3*	36.4±3.4**	45.8±0.4ii**	40.4±0.3ii**	647.4±17.8ii**	5.1±0.4i	0.9±0.1*	23.5±1.7ii
Garlic+PPX	3.0±0.2**	6.2±0.4i*	29.3±2.3**	45.0±1.1ii**	39.0±1.8ii**	589.6±19.2ii**	3.4±0.3*	1.03±0.1i	22.8±3.0ii
Curcumin+garlic+PPX	2.8±0.2*	4.9±0.5ii	34.3±3.1**	46.6±1.0ii**	41.2±1.5ii**	585.4±21.9ii**	4.1±0.4*	0.9±0.11i	21.9±3.8ii
Garlic	2.9±0.2	7.2±0.4	33.1±2.3	31.5±0.9i	23.2±0.8ii	597.6±15.6ii	4.2±0.2	0.8±0.04	24.7±1.7ii
Curcumin	2.8±0.2	5.3±0.3ii	26.1±0.95i	33.1±0.6i	28.0±0.8ii	628.0±15.4ii	4.4±0.3	0.8±0.02	23.8±1.8ii
Garlic + Curcumin	3.2±0.4	6.2±0.7	27.5±0.95i	42.9±0.6i	29.4±0.8ii	538.0±8.4ii	4.9±0.5	0.8±0.05	23.3±3.8i

Data showed as mean ± standard error of the mean (SEM), i= Significant and ii= highly significant compared with those of the control and *= Significant and **= highly significant compared with those of pesticide treated only.

Table 2: Effect of diazinon, diazinon + curcumin and/or garlic, propoxur and propoxur + curcumin and/or garlic on biochemical indices levels in rats.

Parameter	TG mg/dl	Cholesterol mg/dl	GSH mg/gm tissue	SOD IU/gm tissue	CAT IU/L	TAC mmol/L	MAD ($\times 10^{-5}$) mmol/1ml packed cells
Control	76.9±10.6	68.1 ± 5.67	68.4±3.7	682.7±11.0	686.9±83.3	0.54±0.02	0.68±0.16
Corn oil	66.7±7.6	76.9±11.91	64.1±5.5	691.0±13.0	629.9±87.1	0.53±0.02	0.48±0.08
Diazinon (DZN) 10mg/kg	90.6±7.0	94.0±7.0i	42.8±0.9ii	393.8±9.1ii	474.9±54.4i	0.30±0.09i	1.10±0.21
Curcumin+DZN	85.7±3.1	101.1±15.3	52.9±0.3ii**	630.7±7.0i**	789.0±68.8*	0.52±0.02*	0.45±0.11*
Garlic+DZN	85.5±6.8	75.2±9.04	58.2±0.4i**	611.3±4.9ii**	710.2±79.0*	0.52±0.03*	0.82±0.08
Curcumin+garlic+DZN	95.4±9.0	49.2±5.7**	52.3±0.3i**	576.5±8.6ii**	482.1±65.6	0.34±0.03ii	0.76±0.21
Propoxur (PPX) 10mg/kg	94.0±5.4	95.7±5.6i	35.0±0.8ii	448.3±4.9ii	488.0±53.7	0.22±0.05ii	1.08±0.22
Curcumin+PPX	85.5±3.7	83.8±7.3	52.4±0.6ii**	687.9±8.4**	688.3±75.9*	0.47±0.05*	0.54±0.07*
Garlic+PPX	89.7±2.6	89.7±7.4	56.1±0.5i**	618.8±7.3ii**	612.0±82.5	0.36±0.07i	0.22±0.04i*
Curcumin+garlic+PPX	89.2±9.0	70.8±7.8*	55.4±0.3i**	596.3±6.9ii**	535.6±76.7	0.38±0.06i	0.83±0.07
Garlic	92.3±2.9	99.3±6.0i	61.0±0.4i	721.8±6.7i	609.6±22.2	0.44±0.03i	0.52±0.08
Curcumin	105.8±4.5i	94.2±6.1i	59.7±0.6i	718.8±8.5i	411.9±49.9i	0.52±0.03	0.53±0.11
Garlic + Curcumin	90.4±6.1	94.2±6.1i	61.3±0.7	722.3±8.5i	617.8 ±53.0	0.50±0.03	0.72±0.06

Data showed as mean ± standard error of the mean (SEM), i= Significant and ii= highly significant compared with those of the control and *= Significant and **= highly significant compared with those of pesticide treated only.

DISCUSSION

At present there is considerable interest in free radical-mediated damage in biological systems following pesticide exposure.

However, there is no consensus with regard to the best quantitative indices of pesticide-induced oxidative stress and the effect of antioxidant interventions.

Diazinon not only used in control of vegetables and fruits but also in ectoparasiticide formulations for sheep and cattle and in collars and washes for external asitic control.

Diazinon not only has toxic effect orally, it also has toxic effects by dermal and inhalation way[11].

On the other hand, carbamate insecticides such as propoxur cause oxidative stress through the generation of free radicals and changes in antioxidant enzymes and oxygen-free radical scavengers. Lipid peroxidation is known to be one of the molecular mechanisms of carbamate-induced toxicity[10].

There is evidence that curcumin enhances liver detoxification by increasing the activity of glutathione S-transferase, an enzyme which conjugates glutathione with a wide variety of toxins to facilitate their removal from body[23]. It has the protective effect against pesticide induced biochemical alterations and oxidative damage in the various organs of rats. This protective effect is due to its free radical scavenging activity and increased antioxidant enzymes in rats[24]. Garlic has ability to reduce free radical-induced oxidative damage in the liver[25], garlic extract has been shown to decrease liver enzymes in serum and prevent liver damage of rats with liver fibrosis[26].

Treated rats with diazinon or propoxur showed significant increase in γGT, GOT, GPT and LDH and significant decrease in total protein and albumin. All these biochemical indices including γGT, GOT, GPT, LDH, total protein and albumin are mainly monitored for the elevation of liver damage. Albumin is the main protein in blood and is made by the liver. Changes in serum albumin content indicated either increased albumin synthesis by the tissues or decreased albumin degradation within each tissue. The probability of albumin synthesis by the various rat tissues contributing to an increase in serum albumin content can be considered as more realistic. Hepatotoxicity leads to decrease in albumin production[27]. Nevertheless, decreased content of protein in serum had also been reported[28]. The estimation of total proteins in the body is helpful in differentiating between a normal and damaged liver function as the majority of plasma proteins like albumins and globulins are produced in the liver[27]. γ-Glutamyl transferase (γGT) is an enzyme which is found in liver, kidney and pancreatic tissues, the enzyme concentration being low in liver as compared to kidney[29]. γ-Glutamyl transferase catalyzed the transfer of a glutamyl moiety between peptide donors and amino acid/peptide acceptors[30]. γ-Glutamyl transferase was also involved in the transfer of amino acid across the cell membrane. Further, γ-glutamyl transferase had a role in glutathione metabolism, transferring the glutamyl moiety to various acceptor molecule including water, L-amino acids and peptides. Such a process results in the retention of the cysteinyl glycine that was considered to preserve intracellular homoeostasis during oxidative stress[31]. Glutamic pyruvic transaminase (GPT) is an enzyme that helps metabolize protein. When the liver is damaged, GPT is increased in liver and released in the bloodstream, so high level of this enzyme is observed. The estimation of this enzyme is a more specific test for detecting liver abnormalities since it is primarily found in the liver[32-34], also this enzyme showed elevated levels during hepatocellular necrosis. Glutamic oxaloacetate transaminase (GOT) is another liver enzyme that aids in producing proteins. It catalyzes the reductive transfer of an amino group from aspartate to α-ketoglutarate to yield oxaloacetate and glutamate. Glutamic oxaloacetic transaminase is the mitochondrial enzyme, predominantly found in the liver, skeletal muscles and kidneys. Injury to any of these tissues can cause an elevated blood level[35]. It also helps in detecting hepatocellular necrosis but is considered a less specific biomarker enzyme for hepatocellular injury[29] as it can also signify abnormalities in heart, muscle, brain or kidney[33,34]. The ratio of serum GOT to GPT can be used to differentiate liver damage from other organ damage[35]. Lactate dehydrogenase (LDH) can be used as an indicator for cellular damage and cytotoxicity of toxic agents. In fact, elevation in lactate dehydrogenase (LDH) activity indicates cell lysis and death as well as the switching over of anaerobic glycolysis to aerobic respiration[36]. The change in lactate dehydrogenase (LDH) activity resulted from overproduction of superoxide anions and hydroxyl radicals, which cause oxidative damage to the cell membrane[37] and increase in membrane permeability[38]. Elevated level of this enzyme is released from damaged cells in many areas of the body, including the liver. It also helps in detecting hepatocellular necrosis[27].

Rats treated with either diazinon or propoxur showed slight increase of creatinine and uric acid in blood than normal rats. So the results of creatinine and uric acid showed no significantly disorder in kidney. On other hand, Urea levels were observed to be significantly decreased in rats treated with diazinon or propoxur than non-treated rats. Rats treated with curcumin and/or garlic before pesticides treatment show slight decrease in serum urea levels than normal rats. Low levels of urea in blood showed that there is disorder in urea cycle in which liver is responsible for. Low blood urea nitrogen levels are not usually a cause for concern. They may be seen in severe liver disease and over hydrated, but blood urea nitrogen levels is not usually used to diagnose for liver disease or over degredation. However, Rats treated with diazinon or propoxur had high levels of glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT), γ-Glutamyl transferase (γGT) and Lactate dehydrogenase (LDH) specific activities and low levels of albumin and total protein. These results emphasizes that the depletion of urea blood nitrogen levels in these rats is due to disorder in urea cycle, which in turn due to liver damage or liver dysfunction. Blood urea results of rats treated with curcumin and/or garlic indicate the protective effect of curcumin and garlic against toxic effects of diazinon and propoxur as organophophorus and carbamate pesticide respectively.

Superoxide dismutase (SOD) enzyme activity in liver tissue showed decreased levels in rats treated with diazinon or propoxur than non-treated rats. Also, liver glutathione reduced (GSH) showed the same decreased levels in pesticides treated rats. Catalase and total antioxidant capacity (TAC) showed decreased levels in rats treated with diazinon or propoxur than normal rats. All these results indicate the potential of diazinon and propoxur to induce oxidative stress. Oxidative stress can cause irreversible cellular damage because intracellular defense mechanisms are depleted and therefore cannot protect cells against reactive oxygen species (ROS), which are a group of highly reactive zmolecules that are produced through sequential reductions of O_2[39]. There is a balance between ROS generation and ROS degredation with antioxidants in the cells. Both excess of ROS or antioxidants can result in an abnormal oxidative stress state[40]. Most chemicals such as hydrogen peroxide (H_2O_2) damage liver cells mainly by including lipid peroxidation and oxidative stress in liver[41]. The cells have different mechanism to alleviate and repaired damaged macromolecules. The primary defense is offered by enzymatic and non enzymatic antioxidants which have been shown to scavenge free radicals and reactive oxygen species (ROS). The antioxidant enzymes, SOD and CAT have been shown to be significantly affected by pesticides[42]. Increased levels of serum GOT, GPT, γ-GT and LDH and decreased levels in albumin and total protein of treated rats with pesticides prove that the livers of rats are damaged. However, it is not evidence that liver damage is due to oxidative stress caused by pesticides. So, we needed other specific markers to prove the oxidative stress mechanism in liver damage after pesticides treatment. Hepatic levels GSH and SOD estimated in liver homogenate to confirm that the oxidative stress is the main cause of the liver dysfunction. If we looked to the rest of hepatic GSH and SOD results, we would find the role of the two used antioxidants, curcumin and garlic, in liver protection. All these results emphasize that diazinon and propoxur induce oxidative stress in rats' body. As the liver is the first defense organ in body which protects the body from toxicants through hepatic biotransformation, involve phase I and phase II, it is the first organ to be affected.

Result data showed increased levels of cholesterol and triglycerides in rats treated with diazinon or propoxur. Cholesterol and triglycerides levels were considered as valuable indicator of drug-induced disruption of lipid metabolism. Increase of cholesterol and triglycerides levels in rats suggest increased synthesis and accumulation of cholesterol and triglycerides. Accumulation of pesticides in the liver is reported to disrupt lipid metabolism and increase serum cholesterol and triglycerides[43]. This disruption may be due to decreased lipoprotein lipase activity in adipose tissue and increased the levels of total serum cholesterol and triglycerides in the affected rats[44].

Red blood cells are highly susceptible to oxidative damage due to the high cell concentration of oxygen and hemoglobin, a powerful promoter of the oxidative process. They are one of the first cells to be affected by adverse conditions. RBCs have a plasma membrane rich in polyunsaturated fatty acid chains. Thus they are highly susceptible to oxidation. Lipids are considered crucial in the maintenance of the red blood cells shape. Even minimal changes in the surface area may lead to morphological and functional abnormalities. Red cell membrane fluidity is also important for proper red cell function. Reactive oxygen species attack causes lipid peroxidation and formation of an array of unwanted products[45]. Malondialdehyde (MDA) is a major lipid peroxidation product. Several hypotheses in relation to the *in vivo* formation of MDA have been proposed. It was proposed that oxidized lipids are able to produce MDA as a decomposition product and the mechanism is thought to involve formation of prostaglandins, like endoperoxides from poly unsaturated fatty acid with two or more double bonds[46]. Also, it was suggested an alternative mechanism for the generation of MDA, based on successive hydroperoxide formation and β-cleavage of poly unsaturated fatty acid which is the main source of MDA generation *in vivo*[47].

However other minor sources of MDA formation also exist such as byproducts of free radical generation by ionizing radiation and biosynthesis of prostaglandins[48].

Curcumin and garlic results showed protective effect against pesticides oxidative stress. Curcumin was suggested to exert protective effect by modulating the biochemical marker enzymes, lipid peroxidation and augmenting antioxidant defense system[49]. A protective effect of curcumin has also been reported in *ex vivo* experiments against cadmium and lead induced lipid peroxidation in rat brain homogenates[50]. Aqueous garlic extract effectively reduces the combination pesticide induced oxidative damage as shown by a decrease in lipid peroxidation and enhanced the antioxidant levels in liver of mice[51]. Several plant products, including garlic *and its* components have been reported to modulate the levels of lipid peroxides and antioxidants[52].

CONCLUSION

From the present results, it can be concluded that exposure of animals to diazinon or propoxur are capable of including marked hazardous alterations. The generation of excessive levels of free radicals is one of the basic underlying mechanisms of these changes. Changes observed in oxidative stress markers further support this. The result also demonstrated the protective effect of curcumin and garlic against pesticide oxidative stress toxicity.

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