

Toxicological Influences of Lambda Cyhalothrin and Evaluation of the Toxicity Ameliorative Effect of Pomegranate in Albino Rats

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Abstract: The present work aimed to investigate the ameliorative effect of pomegranate against the lambda cyhalothrin- induce toxicity in albino rats. The results showed that lambda cyhalothrin induced significant increases in liver, kidneys and brain weight. In addition, plasma ALT, AST and Alk-P activity was stimulated, fat LDH of plasma and brain activity was inhibited. On the other hands, plasma content of bilirubin, urea, uric acid, creatinine and glucose are increased significantly, but the plasma total protein and albumin was decreased. The lambda-cyhalothrin intoxicated rats induced significant increase in malondialdehyde and decrease in glutathione reductase and glutation, also, significantly inhibited the activities of glutathione reductase and glutathione-s-transferase of the liver. In contrast the activities of liver lysosomal enzymes (B-galactosidase, acid phosphatase and B-N-acetyl glucosaminidase) were stimulated by the insecticide intoxication in albino rats. These above disturbances, organs weight ratio, liver function, kidneys function, protein profile, peroxidation, flotation system and lysosomal enzymes were elevated and these parameters were improved by the four treatments of pomegranate (PP, PPE, PS and PSE). The hepatoprotective effects of pomegranate fractional in lambda- cyhalothrin intoxicated rats relative to health normal control were ordered as follows: pomegranate peels (PP) > pomegranate seeds > pomegranate peels > pomegranate seed extract.

Key words: Lambda • Cyhalothrin • Pomegranate • Liver function • Kidney function • Lysosome • Hepatoprotect • Glutathione

INTRODUCTION

Pesticides have been used in agriculture to enhance food production by eradicating unwanted insects and controlling disease vectors and in health care practices. Among common used pesticides are the organophosphorus and pyrethroids compounds. The first ones induced toxic influences causing damage to various membranous compounds of the cell. Pyrethroids are widely used in field pest control and household use also veterinary medicine and are among the most potent insecticides known [1]. The pyrethroid insecticides have low toxicity than organophosphorus ones. Lambda- cyhalothrin (LC) is a type II pyrethroid used predominantly on cattle and sheep and to a lesser extent in pigs and goats for control of a broad range of ectoparasites. It is non- systemic insecticide with contact and stomach action as well as repellent properties. L.C. knockdown and long residual activity and uses control of

a wide spectrum of insect pests eg. Aphids, Colorado beetles, Thrips, Lepidoptera larvae, Coleoptera larvae and adults, etc. in cereals, hops, ornamentals, potatoes, vegetables, cotton and other crops. Lambda- cyhalothrin provides good control of insect-borne plant viruses, also used for control of insect pests in public [2].

The extracts of St. Mary's thistle, milk thistle (*Silybum marianum*) have been used for centuries to treat liver and other organs disorders. Silymarin considered a remarkable protection of blood AST, ALT and LDH levels towards xenobiotic- induced hepatotoxicity [3].

Pomegranate (*Punicagranatum L*) has been used for centuries in ancient cultures for its medicinal purpose. Several reports observed that pomegranate used as antiviral antimicrobial and anticancer agent [4] but it is widely acknowledged for antioxidant properties, which are higher than most other fruit- related food items that were originally thought to contain the highest amounts of antioxidants [5].

Animal liver plays a major role in regulating various physiological and chemical function of animal bodies such as catabolic and anabolic processes as well as synthesis and secretion systems of xenobiotics. The hepatic parenchyma may prove delirious to these physiochemical function. Free radicals can cause oxidative damage to all biomolecules and initiate a chain reaction which results in physiological damage. This physiological damage can be repaired but may also accumulate over a period of time and cause many degenerative diseases [6].

Lambda cyhalothrin has been observed to exert significant genotoxic and cytotoxic effects on human lymphocytes cultured *in vitro*, a dose dependent chromosomal aberration in mice and changes in rabbit peripheral blood lymphocytes. Lambda cyhalothrin acts primarily on the central nervous system and may cause liver damage [7].

Thus the present research aims to study the biochemical damage caused by lambda-cyhalothrin at sub-lethal dose orally and evaluate *in vivo* hepatoprotective and curative effects of pomegranate fractions against the pesticide-induced hepatotoxicity in rats as a potential source of natural antioxidant.

MATERIALS AND METHODS

Lambda cyhalothrin is a synthetic pyrethroid insecticide ($C_{23}H_{19}ClF_3NO_3$). Its CAS chemical name is α -cyano-3-phenoxy-benzyl-3-(2-chloro-3,3,3-trifluoro-1-propenyl)-2,2-dimethyl cyclopropanecarboxylate which obtained from central Agriculture Pesticides Laboratory, Agriculture Research Center, Dokki-Giza-Egypt. All other chemicals were AR. LD₅₀ of Lambda cyhalothrin was found to be 79mg/kg.bw [2]. Pomegranate fruits were purchased from the local market and authenticated by the botanists in the faculty of Agriculture, Cairo University, Giza- Egypt.

Preparation of pomegranate fractions sample: pomegranate was fractionated to peels (PP) and seeds (PS), then were cut and dried in air oven at 50°C till complete dryness, the dried samples were ground to fine powder for their using in the experimental treatments.

Preparation of ethanolic extracts: The dried PP and PS were mixed with ethanol (80%) under shaking for 2 days. The resulting ethanolic extract was filtered and subsequently concentrated with rotary evaporator and dried in air oven at 50°C till complete dryness.

Experimental Animals: The sprague- Dawley albino male rats weighting around 90 ±10g were used for the present studies. The rats were obtained from National Research Center (NRC), Dokki-Giza - Egypt. The experimental rats were raised in the animal house and kept under normal laboratory conditions (temperature remain 25± 2°C) for one week before the initiation of experimental period and animals were allowed free access of water and basal diet which prepared according to the National Research council [8].

Dose; Rats was intoxicated orally with lambda-cyhalothrin and the dose was 1/20 of the LD₅₀ every 2 consecutive day intervals [9]. The pesticide was dissolved in corn oil before using.

Experiment: After the adaptation period, 42 of the male rats were divided into 7 groups (6 rats each) as follows.

First group (G1) was fed on the normal basal diet and ingested orally with corn oil (1.0ml every 2 consecutive days) which used as normal healthy control. The second group (G2) intoxicated rats were fed on the normal basal diet and ingested orally with 1/20 LD₅₀ of lambda cyhalothrin (1.0ml every 2 consecutive days Saturday, Monday, Wednesday) which used as the intoxicated control. The third group (G3), intoxicated rats treated orally with silymarin (0.2g/kg bw.) every 2 consecutive days Sunday, Tuesday, Thursday). Fourth group (G4), intoxicated rats were fed on dried semi- modified diet (5% PP in the normal diet). The fifth group (G5) intoxicated rats were fed on dried PS semi- modified diet (5% PS in the normal diet). Sixth group (G6) intoxicated rats were fed with normal diet and treated orally with PPE (0.3g/kg. bw in 1ml corn oil) every 2 consecutive days (Sunday, Tuesday, Thursday). Seventh group (G7) intoxicated rats fed with normal diet and treated orally with PPE (0.3g/kg. bw in 1ml corn oil every 2 consecutive days (Sunday, Tuesday and Thursday). At the end of 6 weeks, interval, rats were fasted overnight and then the animals were killed by decapitation. The blood samples were collected from each rat with heparin and subjected to centrifugation tube at 3000 xg to obtain the plasma fraction which was kept at 20°C for the subsequent investigation. Livers kidneys and brains were rapidly removed, washed with ice-cold 1.15% KCl saline and then weighed. Brains were rinsed in ice-cold phosphate buffer (pH 7.4) to remove blood and the homogenized in 5ml buffer containing 100 mM potassium phosphate (pH 7.0) and 2mM EDTA per one gram brain tissue. Centrifuge at 10.000 xg for 15 min at 4°C and the supernatant was kept on -20°C for LDH activity assay.

Livers were divided into two portions. The first portion was homogenized in phosphate buffer solution (pH 7.4) (1.5 tissues/ 10ml buffer). The homogenate was centrifuged at 3000 xg and the supernatant was used for determination of lipid peroxidation, GSH content as well as activities of glutathione reductase and glutathione -s- transferase. The second portion used to prepare lysosomal fraction which was isolated from liver according to the method of Tanaka and Iizuka [10] for the determination of the marker lysosomal enzymes.

For Biochemical Analysis: AST and ALT activities were determined according to the method of Reitman and Frankel [11]. Total bilirubin was determined according to Walter and Gerade [12]. Alk. P activity was determined by the method of Belfield and Goldberg [13]. LDH activity was determined by the method described by Tietz [14]. Urea was determined according Fawcett and Soctt method [15]. Uric acid was determined by the method of Barham and Trinder [16] and creatinine was determined by Schrimmeister *et al.* method, [17]. Total soluble protein was determined according to Bradford [18] method, but albumin was determined by the method described by Doumas *et al.* [19]. Plasma glucose content was determined according to the method of Trinder [20]. Lipid peroxides were determined in liver homogenate as TBARS level according to the method of Buege and Aust [21], GSH was determined by the method of Ellman [22]. The activities of glutathione reductase and glutathione -s- transferase were determined according to the method of Goldberg and Spooner [23] and Habig *et al.* [24] respectively. The three lysosomal hydrolases enzymes activities in livers homogenate (acid phosphate N- acetyl B-glucosaminidase and B-galactasidase) were determined by the method described by Hoof and Hers [25] which modified by Younan and Rosleff [26].

The obtained results were statistically analyzed using the method of Snedecor and Cochran [27] and LSD test was used to compare the significant differences between means of the treatment [28]. Values are expressed as mean \pm SD and significance at $p < 0.05$.

RESULTS AND DISCUSSION

The design of the present study was done as that the final body weight as well as liver, kidneys and brain weight also the ratio of organ weight / final body weight was determined of the experimental animals. The organs/body weight ratio of lambada cyhalothrin

intoxicated rats was significantly higher than those of control group Table (1). The intoxicated rats resulted in enlargement of liver and kidneys and slightly brain; also liver had pale reddish brown in color. The treated groups with semi- modified diets (PP and PS) as well as the ethanolic extracts (PPE and PSE) and silymarin showed organs size around to those found in the normal health control but lower than that of the intoxicated group. Also, a significant restoration in organ weight was obtained in intoxicated groups treated with pomegranate fractions or their extracts but still higher than normal control. A study reported that lambda cyhalothrin ingestion resulted in enlargement of the liver and liver weight to body weight ratio were increased at control [7], which in agreement with our work. Also another study pointed out some marked increases in the liver, kidneys, heart and spleen weight in intoxicated animal, with pesticides which in agreement with our results [29].

Effects of lambda cyhalothrin ingestion on liver function in male albino rats and the treatments with pomegranate fractions and their ethanolic extracts are summarized in Table (2). The lambda cyhalothrin toxicity significantly stimulated AST and ALT activities and increased plasma bilirubin content relative to control; these are in agreement with a study showed significant stimulation in plasma AST and ALT activities under the effect of lambda cyhalothrin toxicity [7]. A similar trend was observed in a study they found that the plasma bilirubin content and transaminases activity were elevated by pesticides ingestion [29]. The treatments with PP, PS, PPE and PSE as well as silymarin alleviated and improved the harmful effects on the liver function which produced by lambda cyhalothrin toxicity. In the case of kidneys function, the ingestion of lambda cyhalothrin produced significant increases in urea, uric acid and creatinine contents of intoxicated rat' plasma and renal function was disturbed under the pesticide intoxication (Table 2). These findings are in agreement with a study reported that pesticides induction is one of the primary causes of its nephrotoxic effects and produced oxidative stress and then damage [30]. The treatments with the present pomegranate fractions (peels and seeds) as well as their extraction and also silymarin (standard drug) ameliorated the lambda cyhalothrin toxicity and improved kidneys function of intoxicated rats.

The improvement effects of pomegranate fraction of liver and kidneys functions. Was due to a significant improvement in the both organs against the toxicity of pesticides ingestion [31, 32].

Table 1: Organs weight and its ratio per 100gm b.w of experimental albino rats

Treatment	Liver				Kidney				Brain			
	Body weight (g)	Weight (g)	Ratio 100/b.w	Ratio %	Weight (g)	Ratio 100/b.w	Ratio %	Weight (g)	Ratio 100/b.w	Ratio %	Weight (g)	Ratio 100/b.w
Healthy control	250±12	14.12±1.21 b	5.65	100	2.50±0.17 c	1.00	100	1.21±0.11 b	0.48	100		
Intoxicated control	191±13	15.94±1.72 a	8.35	148	3.80±0.20 a	1.99	199	1.30±0.10 a	0.68	142		
Semi-modified diet with pomegranate peels(PP)	236±14	14.00±0.99 b	5.93	105	3.00±0.19 b	1.27	127	1.18±0.08 b	0.50	104		
Semi-modified diet with pomegranate seeds(PS)	234±12	14.61±1.31 b	6.24	110	3.01±0.18 b	1.29	129	1.21±0.09 b	0.52	108		
pomegranate peels extract(PPE)	237±13	14.11±1.11 b	5.95	105	2.98±0.14 b	1.26	126	1.30±0.10 b	0.55	115		
pomegranate seeds extract (PSE)	238±14	14.12±1.24 b	5.93	105	3.00±0.16 b	1.26	126	1.28±0.11 b	0.54	113		
Silymarin (S)	235±15	13.98±1.21 b	5.95	105	2.88±0.18 b	1.23	123	1.22±0.11 b	0.52	108		

% Relative to control. Each value represented the mean of 6 rats (mean±SD). The same lower letters in each column represent insignificant difference at $p<0.05$

Table 2: Liver and kidney function of experimental albino rats

Treatment	Liver function						ALT activity					
	AST activity		ALT activity		Total bilirubin		Urea		Uric acid		Creatinine	
	U/L	%	U/L	%	mg/dL	%	mg/dL	%	mg/dL	%	mg/dL	%
Healthy control	60.11±4.41 d	100	31.31±1.79 d	100	0.32±0.02 e	100	24.01±1.54 d	100	0.50±0.03 d	100	0.45±0.27 d	100
Intoxicated control	124.21±7.83 a	207	63.01±4.01 a	201	5.00±0.41 a	1563	40.11±2.01 a	167	0.79±0.04 a	158	0.71±0.41 a	158
Semi-modified diet with pomegranate peels(PP)	72.07±4.12 c	120	44.12±2.79 c	141	1.11±0.06 d	347	26.73±2.00 c	111	0.57±0.51 c	114	0.51±0.31 c	113
Semi-modified diet with pomegranate seeds(PS)	79.12±5.01 b	132	47.73±3.02 b-c	152	1.32±0.07 c	413	28.12±1.67 c	117	0.61±0.40 c	122	0.57±0.42 b	127
pomegranate peels extract(PPE)	82.13±6.01 b	137	51.11±3.13 b	163	1.45±0.08 b	453	30.61±1.76 b	127	0.62±0.36 c	124	0.60±0.34 b	133
pomegranate seeds extract (PSE)	90.00±7.24 b	150	53.12±2.97 b	170	1.54±0.08 b	481	31.11±1.83 b	130	0.73±0.46 b	146	0.62±0.37 b	138
Silymarin (S)	73.00±4.12 c	121	50.01±2.59 b	160	1.31±0.07 c	409	27.00±1.46 c	112	0.61±0.39 c	122	0.58±0.41 b	129

% Relative to control. Each value represented the mean of 6 rats (mean±SD). The same lower letters in each column represent insignificant difference at $p<0.05$

Table 3: Plasma glucose content as well as Alkaline phosphatase, Lactate dehydrogenase activities of experimental albino rats

Treatment	Plasma glucose				Alkaline plasma phosphatase				Lactate dehydrogenase			
	Plasma glucose		Alkaline plasma phosphatase		Plasma		Brain		Plasma		Brain	
	mg/dl	%	IU/L	%	U/L	%	U/g	%	U/L	%	U/g	%
Healthy control	96.03±5.41 c	100	123.07±7.23 c	100	222.01±13.41 c	100	30.87±1.74 a	100				
Intoxicated control	169.21±9.11 a	176	261.21±14.12 a	212	400.21±24.21 a	180	19.71±1.01	64				
Semi-modified diet with pomegranate peels(PP)	100.23±6.21 c	104	160.71±9.91 b	131	285.52±17.11 b	129	25.21±1.33 c	82				
Semi-modified diet with pomegranate seeds(PS)	103.71±5.96 b	108	171.11±8.73 b	139	296.02±18.16 b	133	23.71±1.12 b	77				
pomegranate peels extract(PPE)	110.24±6.66 b	115	176.71±9.02 b	144	300.00±18.83 b	135	22.01±1.07 b-c	71				
pomegranate seeds extract (PSE)	109.01±6.03 b	114	177.00±8.87 b	144	306.11±19.01 b	138	21.00±1.32 b-c	68				
Silymarin (S)	110.00±7.02 b	115	167.77±9.24 b	136	291.21±19.32 b	131	24.11±1.41 b	78				

% Relative to control. Each value represented the mean of 6 rats (mean±SD). The same lower letters in each column represent insignificant difference at $p<0.05$

Table (3) shows that lambda cyhalothrin ingestion increased significantly blood glucose levels in short-term experimental period relative to control animals. The data in the same table demonstrated that plasma alkaline phosphate and lactate dehydrogenase activities were stimulated under the ingestion of the present pesticide conditions. In contrast, lactate dehydrogenase activity of brain tissues was significantly inhibited in lambda cyhalothrin - intoxicated rats compared to control group. Some study reported a decreased LDH activity in animal tissue including brain under various pesticide toxicity conditions [7,33], which are in agreement with our work. On the other hands, the treatments with PP, PS, PPE and PSE silymarin into lambda cyhalothrin- intoxicated rats ameliorated the harmful effects of the pesticide toxicity

and improved the above parameters compared to intoxicated control, but the values were still varied than those of healthy normal control rats. The alleviated influences of pomegranate fraction against lambda cyhalothrin- toxicity [31,32].

The changes of total soluble protein, albumin and globulin levels of experimental animal plasma showed decreases in the three plasma protein profile under the ingestion of lambda cyhalothrin toxicity. These decreases in total soluble protein content percent were about the same with albumin content, but for globulin were lower than the both fractions [34]. In treated animals with PP, PS, PPE and PSE against lambda cyhalothrin toxicity, a significant improvement in the plasma protein profile and the toxicity of the pesticide ingestion was ameliorated but lower than control [31].

The present experimental results (Table 5) showed significant decreases in the GSH content of liver tissues under the lambda cyhalothrin toxication condition. Contrary, liver lipid peroxidation was significantly increased. The results pointed out high elevation in lipid peroxide in liver homogenate. In addition, the same table reported harmful influences by lambda cyhalothrin ingestion on GSH- reductase and GSH-S- tranferase activities. These activities were significantly inhibited by the pesticide induction. The disturbances in lipid peroxidation, GSH, GSH-reductase and GSH-S-transferase by the harmful effects of lambada-cyhalothrin were alleviated and their values were improved by PP, PS, PPE, PSE and silymarin treatment in the intoxicated animals as compared with normal health control and intoxicated control.

In the case of lysosomal acid hydrolases enzymes, the lambda cyhalothrin ingestion (Table 5) showed stimulation in the activities of acid phosphatase, N-acetyl-B-glucosaminidase and B-galactosidaseactivities of liver tissues. These stimulations were highly significant relative to those of normal health controls. The treatment with PP, PS, PPE and PSE as well as silymarin ameliorated these toxic effects on lysosomal marker enzymes of liver, but the values were still more than those of normal health control.

The present results showed that animals exposed to lambda cyhalothrin insecticide (pyrethroid type2) induced marked significant increase in liver, kidneys and brain weight as well as in the ratio between the organ weight and 100g final body weight, these in spite of their collagen accumulation in liver and organs such as stress condition of pesticide intoxicated [35] also may be due to the tumefaction or enlargement effect on intoxicated animal [36]. These values begin to alleviate in treating intoxicated animals with pomegranate fractions and their extracts but still significantly higher than those of normal health control rats. These may be due to the nutrients and polyphenols as well as other antioxidants presented in pomegranate which detoxified the liver and other organs and improved the body weight and also organs weight. Lambda cyhalothrin is induced hepatotoxins and chronic induction of the present insecticides produced liver cirrhosis in rats [7]. Pesticides, due to their hydrophobic nature were accumulated in the biological membrane, especially in the phospholipid bilayers and in lipid rich internal tissues including body fat, liver, kidneys, ovaries and elements of the central and peripheral nervous system [37]. The Liver was the major site of pyrethroid

metabolism which accumulated greet concentrate of its metabolites [38]. Their toxic effects occurred through generation of reactive oxygen species and lambda cyhalothrin is accumulated in hepatic parenchymal cells and metabolically activated by cytochrome-P-450 dependent monooxygenases to form free radical [7] with attack polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides [34,38]. Lambda cyhalothrin- intoxicated rats significantly developed hepatic damage as shown stimulations in plasma activities of ALT and AST as well as increases in plasma bilirubin content. This pesticide ingestion increased permeability, damage and or necrosis of hepatocytes. The harmful in liver function and kidney function also in blood glucose may explain membrane break down and inducing hepatic damage [39]. The stimulation of plasma Alk. phosphatase in the intoxicated rats improved the previous suggestions. The stimulation of LDH activity in plasma (marker hemolysis) suggested anemia signs are related to intravascular hemolysis [1, 7]. This enzyme (LDH) is recognized as a marker for assessing the toxicity of xenobiotics. The inhibition of LDH activity in brain tissues of intoxicated rats may be due to the reduction in glycolytic process due to the lower metabolic rate under the lambda cyhalothrin toxicity likes several pesticide- intoxication [33].

The present results (Table 4) showed decreases in plasma protein profile (total soluble protein, albumin and globulin) as well as the albumin/globulin ratio in lambda cyhalothrin- intoxicated rats. These may be due to the harmful influences of the insecticide on hepatocytes where albumen is synthesized. The catabolism of lambda cyhalothrin in the liver may be produced electrophilic metabolites which bind with liver molecules (lipids and proteins) which are essentials for the hepatocyte life and necrosis [40].

Oxygen free radical induced lipid peroxidation, which damage to cell membranes and developed tissue injury [41]. Lambda cyhalothrin ingestion elevated MDA and reduced GSH contents in the liver also inhibited glutathion-s- transferase and glutathion reductase activity of liver tissue. The decreases of GSH in tissue produced an enhancement of lipid peroxidation which considers antioxidant defense. GSH, GS- reductase and GS- transferase are used in the cell as a antioxidant defense mechanism. Glutathion reductase reduced oxidized glutathion (GSSG) to reduced one (GSH) using NADPH.H produced from oxidative shunt of hexose monophosphate shunt that maintains adequate levels of

Table 4: Plasma protein profile of experimental albino rats

Treatment	Total soluble protein		Albumin		Globulin		Ratio of Albumin/Globulin	
	g/dL	100	g/dL	100	g/dL	100	Ratio	%
Healthy control	7.22±0.41 a	100	4.51±0.27 a	100	2.71 ±0.62 a	100	1.66 a	100
Intoxicated control	4.24±0.030 c	59	2.30±0.14 c	51	1.94±0.47 b	72	1.19 b	72
Semi-modified diet with pomegranate peels(PP)	5.51±0.31 b	76	3.54±0.19 b	78	1.97±0.61 b	73	1.80 a	108
Semi-modified diet with pomegranate seeds(PS)	5.12±0.27 b	71	3.20±0.18 b	71	1.92±0.62 b	71	1.67 a	101
pomegranate peels extract(PPE)	5.23±0.29 b	72	3.17±0.17 b	70	2.06±0.53 b	76	1.54 a	93
pomegranate seeds extract (PSE)	5.00±0.26 b	69	3.01±0.17 b	67	1.99±0.42 b	73	1.51 a	91
Silymarin (S)	5.34±0.28 b	74	3.41±0.16 b	67	1.93±0.48 b	71	1.77 a	107

% Relative to control. Each value represented the mean of 6 rats (mean±SD). The same lower letters in each column represent insignificant difference at $p<0.05$

Table 5: Lipid peroxidation and reduced glutathione content as well as the activities of glutathione reductase glutathione-s-transferase, acid phosphatase , N-acetyl-B-glucosaminidase and B-galactosidase of experimental albino rat

Treatment	Homogenate		Lysosomal acid hydrolase (Marker enzymes)									
	MDA lipid peroxide		GSH		GSH-reductase		GSH-S-Transferase		Acid phosphatase		Glucosaminidase	
	nmol/mg protein	%	Mg/mg protein	%	U/L	%	U/min/mg protein	%	nmol/ml/hr	%	nmol/ml/hr	%
Healthy control	1.76±0.91 d	100	40.12±3.00 a	100	250±27 a	100	1.61±0.07 a	100	1300±74 d	100	556±34 c	100
Intoxicated control	10.11±0.90 a	578	21.15±1.73 c	53	149±15 c	60	1.01±0.06 b	63	3511±82 a	270	1311±71 b	236
Semi-modified diet with pomegranate peels(PP)	2.00±0.11 c	114	35.12±1.61 b	88	230±19 a	92	1.51±0.07 a	94	2002±161 c	154	737±42 b	133
Semi-modified diet with pomegranate seeds(PS)	2.04±0.07 c	116	30.00±1.72 b	75	215±14 a-b	86	1.41±0.08 a	88	2431±149 b	187	796±40 b	143
pomegranate peels extract(PPE)	2.16±0.12 c	123	29.98±2.00 b	75	210±17 a-b	84	1.40±0.07 a-b	87	2467±181 b	197	841±51 b	151
pomegranate seeds extract (PSE)	2.22±0.12 c	126	29.00±1.87 b	72	201±16 b	80	1.38±0.06 a-b	87	26178±163 b	201	850±48 b	153
Silymarin (S)	3.02±0.24 b	176	30.71±1.91 b	76	219±14 a-b	88	1.44±0.08 a	89	2111±199 c	162	800±49 b	144

% Relative to control. Each value represented the mean of 6 rats (mean±SD). The same lower letters in each column represent insignificant difference at $p<0.05$

reduced cellular GSH. Reduced glutathione serves as an antioxidant with free radicals and organic peroxides, in amino acid transport and as a substrate for the glutathione peroxidases and glutathione-S-transfer in detoxification of organic peroxide and metabolism of xenobiotics, respectively [42].

The stimulated activities of lysosomal enzymes (Table 5) of liver and plasma transaminases confirmed each other and may be induced damage to hepatocyte involving lysosomes resulted from lambda cyhalothrin-ingestion. Lysosome a bag of acid hydrolyses which catabolites or breakdown protein, carbohydrate nucleic acid and lipid macromolecules. The phospholipids – rich lysosomal membrane is a potential site of free radical attack subsequently causing loss of membrane stability [43]. These stimulation in the enzyme's activity may be explained with necrotic and apoptotic death of hepatocytes. The harmful of lambda cyhalothrin may be due the destructive effects on cell membrane by lipid peroxide which disturbed the membrane function of integrity with cytotoxicity in hepatocytes [44].

Vegetables, fruits, herbs and spices used in folk and traditional medicine have been accepted currently as one of the main sources of chemo- preventive drug discovery and development [45]. It has been observed that many plant polyphenols, such as ellagic acid, catechins as well as chlorogenic, caffeic and ferulic acids act as potent

antioxidant, antimutagenic and anticarcinogenic agents [46]. A study reported that pomegranate peel contained ellagic acid ellagitannins and gallic acids [47]. The phytochemical analysis of ethanolic extract from pomegranate seeds or peels revealed the presence of a wide variety of constituents such as flavonoids, glycosides, tannins, anthocyanins, vitamins (B1, B2 and C) [31], it can be suggested that the antioxidant activity of pomegranate in the present work may due to the presence of these compounds. The results showed that PP, PS, PPE, PSE and silymarin significantly alleviated the harmful toxicity of lambda cyhalothrin ingestion and improved the disturbed values in the pesticide-intoxicated rats. It appears that silymarin and pomegranate fraction attenuated the insecticide toxicity through its antioxidant effects such as liver function, kidneys function, organ 100g body weight ratio, blood glucose, lactate dehydrogenase activity in plasma and brain and plasma protein profile. The treatments with silymarin or pomegranate fractions in intoxicated rats increased plasma total soluble protein and albumin. These alleviative actions may be due to the elevation of protein biosynthesis and improved the body function as well as antioxidative activity [40]. Also, these antioxidant treatments including silymarin were protective cellular membrane integrity due to stimulate GS-reductase activity which increased GSH values and important peroxide levels

through their antioxidant influences [42]. The present antioxidant treatments related to scavenging free radicals, elevate GSH content in the cell and normalizing the membrane effects by protected the lipid constituent of cell membrane of intoxicated rats [48]. Also, silymarin and pomegranate treatments into lambda cyhalothrin intoxicated rats stimulated the antioxidant enzymes activity (SOD, GS- reductase and GS- transferase) with inhibited lipid peroxidation [7].

CONCLUSION

The present results demonstrated that pomegranate fraction were able to reverse the pathological parameters of chronic liver damage induced by lambda cyhalothrin. The high content of antioxidant ingredients in pomegranate contributed free radical scavenging. Although, it seems difficult to entirely recuperate the chronic hepatic damage- induced by lambda cyhalothrin through the supplement of phytochemicals. Pomegranate indeed retarded the liver injury by blocking the oxidative stress. Therefore, pomegranate fraction may be useful as hepatoprotective agents against chemical- induced chronic liver fibrosis *in vivo*. It can be used pomegranate as adjuvant drug for hepatoprotection against organs damages which caused by xenobiotics.

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