Hematological, Biochemical and Histopathological Alterations Induced by Lambda Cyhalothrin and The Mitigation Effect of Propolis (Bee Glue) in Male Albino Rats

Abdel-Razik, Reda K.* and Nadia A. Hamed

Department of Mammalian Toxicology, Central Agricultural Pesticide Lab,
Agricultural Research Center, Alexandria, Egypt.
*Corresponding author: re21699@yahoo.com

Abstract: Pyrethroid was used preferably due to their high effectiveness, low toxicity to non-target organisms and easy biodegrability. Lambda cyhalothrin (LCT) is a type II synthetic pyrethroid insecticide used for agricultural and public health applications. The present study aimed to investigate the adverse effects of repeated oral sublethal dose (1/50 LD_{50}) of LCT for four weeks (3doses / week) and two weeks after last treatment on some hematological and biochemical parameters as well as liver histopathological changes on male albino rats. Also, the role of propolis to mitigate the adverse effect of LCT was investigated. Rats were divided into four groups; group I (control), group II (propolis at 100 mg / kg bw), group III (LCT at 0.5 mg/kg bw) and group IV (100 mg/kg bw propolis plus 0.5 mg/kg bw LCT). Results showed significant decrease in the body weight and increase in the relative liver weight of group III animals. Also, significant decrease in the hematological parameters; red blood cells (RBC's) count, hemoglobin (Hb) concentration, hematocrit (Hct) value and increase white blood cells (WBC's), while there were no significant changes in the erythrocytes indices. Moreover, LCT reduced serum catalase (CAT), liver superoxide dismutase (SOD) activities and reduced glutathion (GSH) level in liver, while the lipid peroxidation level in serum was increased, jointly serum acetyl cholinesterase (AChE) activity showed no significant alteration. The effect of LCT on the previous parameters was confirmed by the histological changes of liver. In addition, propolis supplementation showed significant reduction of LCT adverse effect which reflects its protective role against LCT induced hepatotoxicity.

Keywords: Lambda cyhalothrin, Propolis, Relative organ weight, Hepatotoxicity, Hematological, Biochemical, Histopathological.

1. Introduction

Exposure to pesticides may involve large segments of population especially in agricultural work and public health causing outbreak of diseases among animals and human **(Casida and Quistad 2004).** LCT is widely used in Egypt and valued for its broad-spectrum control on a wide range of pests in a variety of applications such as the protection of cotton, cereals and vegetables as well as in public health application against insect; ticks and flies which may act as disease vectors **(Abdel Aziz and Abdel Rahem, 2010).** Consistent with its lipophilic nature **(Michelangeli *et al,* 1990),** LCT has been found to accumulate in biological membranes leading to oxidative damage. It was reported that LCT caused oxidative stress by altering antioxidant systems and increasing lipid peroxidation in mammals **(El-Demerdash, 2007 and Fetoui *et al,* 2009)**.

Animal liver plays a major role in regulating various physiological and chemical functions 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 functions. 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 **(Ames *et al,* 1993)**. LCT is rapidly metabolized in the liver via hydrolytic ester cleavage and oxidative pathways by the cytochrome P450 enzymes yield reactive oxygen species (ROS) **(Sankar *et al,* 2012).** The over production of ROS exceeds the capacity of the cell's endogenous systems to neutralize them results in oxidative stress. Cells had several biological defense mechanisms against intracellular oxidative stress includes antioxidant enzymes; superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione -S-transferase (GST) and non - enzymatic antioxidants; glutathione, caratenoids and vitamins C and E **(Halliwell, 2006)**. These mechanisms cooperate with each other to defend against reactive spe-

cies that induced cellular damage. Blood findings are important for the assessment of various systemic functions and health of animals under various environmental conditions and most importantly for diagnosis of drug or chemical induced hemolysis **(Atamanalp and Yanik, 2003)**. Minimum hematological package must include hematocrit (Hct) value, Hemoglobin (Hb) concentration and total erythrocyte counts (TEC); these parameters have been frequently included in toxicological studies **(Gad and Chengelis, 1988)**.

During the last decades, phenolic compounds have received considerable attention due to their antioxidant properties **(Gil -Izquierdo *et al,* 2001)**. Natural products are a promising source for the discovery of new pharmaceuticals. Several works dealing with propolis, which is a resinous hive product collected from plants by honey bees, showing a very complex chemical composition **(Bankova *et al,* 2000)**. Propolis has been used in folk medicine since ancient times, due to its many biological properties, such as antibacterial **(Sforcin *et al,* 2000),** antitumor **(Bazo *et al,* 2002)** and immunomodulatory **(Murad et al, 2002).** It contains more than 300 biochemical constituents **(Khalil 2006)**, Flavonoids and various phenolics are the most important pharmacologically active constituents in propolis capable of scavenging free radicals and thereby protecting lipids from being oxidized or destroyed during oxidative damage **(Nieva Moreno *et al,* 2000).** Propolis has gained popularity and used extensively in healthy drinks and foods to improve health and prevent diseases such as inflammation, heart disease, diabetes and even cancer **(Matsushige *et al,* 1995)**.

Therefore the present research aims to study the toxic effect of lambda cyhalothrin at sublethal dose and evaluate hepatoprotective effects of propolis as a potential source of natural antioxidant.

2. Materials and Methods

2.1. Chemicals

Lambda-cyhalothrin (Katron 5% EC) was obtained from National Agricultural Chemicals and Investment (El-Watania Co). Propolis (Bee Glue) was obtained from Natural Factors Coquitlam, Canada. All other chemicals used were of analytical grade purchased from Sigma – Aldrich Co.

2.2. Animals

Male albino rats weighing 150 -160 g were obtained from the animal house, Faculty of Medicine, Alexandria University. Animals were housed in groups each of five rats in stainless steel cages and kept under laboratory conditions; 25±°C, humidity (65 –75%) and light dark cycle (12 h light, 12 dark). Animals were given feed and water *ad libitum.* The animals were acclimatized to laboratory conditions for two weeks before being experimented. All procedures involving animals were performed in accordance with the guidelines of the standard procedures laid down by **OECD guideline (2008).**

2.3. Experimental design

Animals were divided into four groups and orally treated 3 doses /week for 4 weeks, then 2 weeks without treatment as a recovery period as the following: Group I: Rats were administered with water and served as control. Group II: Rats were administered with propolis at dose 100 mg/kg bw. Group III: Rats were administered with LCT at dose 0.5 mg/kg bw (1/50 LD_{50}; 23.4 mg/ kg bw which estimated by Department of Mammalian Toxicology, Pesticide Central Laboratory, Agriculture Research Center). Group IV: Rats were administered with 100 mg/ kg bw of propolis plus 0.5 mg/kg bw of LCT. The body weights of control and treated animals were recorded weekly. At the end of the experiment (6 weeks) the animals were sacrificed and dissected, then the liver was removed, rinsed in saline solution (0.9% NaCl), dried and weighted individually and the relative organ weight was calculated (organ weight / body weight) X 100. A specimen of the liver was fixed immediately in 10% buffered formalin for histological study.

2.4. Blood sample collection

Blood samples were collected from the animals under diethyl ether anaesthesia weekly all over the treatment period (4 weeks) from the retro-orbital plexus vein and at the end of experiment (after recovery period). Blood were placed immediately into two tubes; the first tube containing EDTA for hematological analysis and the second tube without anticoagulant for serum preparation. Non-coagulated blood samples were analyzed for peripheral blood cell indicators; white blood cells (WBC's) and red blood cells (RBC's) count, haemoglobin content (Hb), Hematocrite percentage (Hct) and total thromocyte (platelet) (PLT) count as described by **Dacie and Lewis (1991).** Erythrocyte indices including mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated. Blood samples which collected for serum preparation were allowed to stand for 30 min at room temperature till clotted and centrifuged at 4,000 g using Sigma 3K30 bench centrifuge for 15 min to separate the serum; then kept at -20 ^{o}C for the biochemical studies.

2.5. Biochemical studies

Livers were homogenized (10 % w/v) in 50 mM phosphate pH 7.5 and 1 mM EDTA using a glass Teflon homogenizer. The homogenate was centrifuged at 8,000 g using Sigma 3K30 bench centrifuge for 30 min at 4°C and the resultant supernatants were used for enzymes assay.

2.5.1. Reduced glutathione (GSH) content:

GSH content as a nonenzymatic antioxidant was determined in liver homogenate according to the method of **Beutler *et al,* 1963**. The method based on the reduction of 5, 5' dithiobis (2- nitrobenzoic acid) (DTNB) with glutathione to produce a yellow compound measured at 405 nm, the reduced chromogen directly proportional to GSH concentration. The amount of GSH was expressed as mg glutathione oxidized/g tissue

2.5.2. Lipid peroxidation (LPO):

LPO was carried out in serum following the procedure of **(Ohkawa *et al,* 1979)** using thiobarbituric acid (TBA). Malondialdehyde (MDA) formed as an end product of peroxidation of lipids, served as the index of the oxidative stress intensity. MDA reacts with TBA to generate a colored product that absorbs at 532 nm. The level of lipid peroxidation was expressed as nmoles of MDA / ml.

2.5.3. Superoxide dismutase (SOD) activity:

SOD as enzymatic antioxidant was measured in liver homogenate spectrophotometrically by the method of **(Nishikimi *et al,* 1972)** using nitroblue tetrazolium dye. The ability of the enzyme to inhibit the phenazine methosulphat - mediated reduction of nitroblue tetrazolium dye was monitored at 560 nm for 5 min at 25 °C (at least three volumes of the enzyme which produced between 30 to 60 % inhibition were used). One unit of the enzyme activity is defined as the amount which produced 50% inhibition of PMS under the standard assay conditions. SOD activities were expressed as Units / g tissue

2.5.4. Catalase (CAT) activity:

CAT activity was determined in serum according to the method of **Aebi (1984),** the rate of H_2O_2 decomposition was monitored at 510 nm. One unit of CAT activity is defined as the amount of enzymes required to decompose 1 µM of hydrogen peroxide/ min. CAT activity was expressed as U/ L.

2.5.5. Acetyl cholinesterase (AChE) activity:

AChE activity was determined in serum according to the method of **Ellman *et al,* (1961),** based on degradation of acetyl thiocholine iodide by AChE into a product which binds to 5,5-dithiobis-2-nitrobezoic acid (DTNB), forming yellow color. One unit of AChE activity was expressed as µmoles acetylcholine hydrolyzed/ min/ml.

2.6. Estimation of protein

Protein content was determined by the Biuret method, according to **Gornall *et al,* 1949.**

2.7. Histopathological studies

Liver samples were removed and fixed in 10 % formalin. Then samples were dehydrated by standard procedures and embedded in paraffin, sections approximately 5 µm thick were cut, stained with haematoxylin and eosin (H&E) stains and examined by light microscope, according to **Drury and Wallington (1980).**

2.8. Statistical analysis

All data were expressed as mean ± standard deviation (SD). Data were analyzed using one-way analysis of variance (ANOVA) followed by the Student–Newman –Keuls test to determine significance between different groups. The criterion for statistical significance was set at $p<0.05$

3. Results and Discussion

Lambda cyhalothrin (LCT), has been found to accumulate in biological membranes leading to oxidative damage due to its lipophilic nature **(Michelangeli *et al,* 1990).** The toxicity of LCT to mammals and its ability to induce oxidative stress *in vivo* and *in vitro* have been established from various reports **(El-Demerdash 2007 and Abdallah *et al,* 2012).**

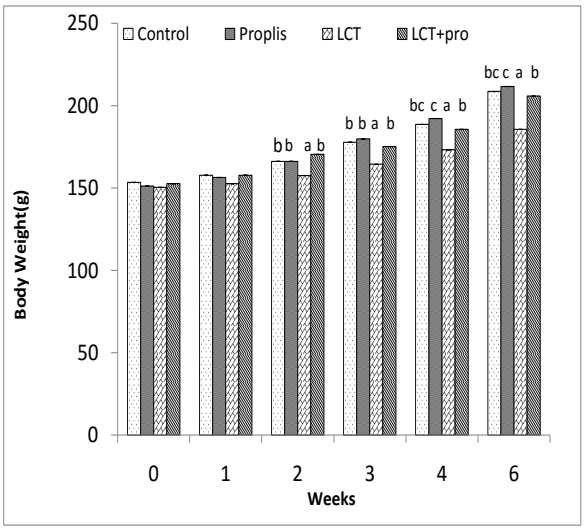
3.1. Body weight and relative organ weight

The physiological status of control and treated animals was noticed after 6 weeks as the change in the body weight gain and relative organ weight (Table 1 and Fig. 1). Rats orally administered with LCT showed a significant decrease ($p < 0.05$) in body weight and increase in the relative weights of liver in comparison to the control. The co-administration of propolis to animals intoxicated with LCT resulted in a body weight higher than the group III, treated with LCT only, while propolis alone did not cause any change in body weight. The effects of LCT, propolis and their combination on male albino rat body weights after different time intervals (0, 1, 2, 3& 4 weeks) of last treatment and two weeks for recovery are illustrated in (Fig. 1). The data showed that the body weights of all treated animals were increased in a time dependent manner.

The decrease in the body weight may be attributed to a decreased food intake (anorexia or food avoidance), poor food palatability or increased degradation of

lipids and protein due to treatment-related toxicity **(Mansour and Mossa 2010).** Similar results have been found in animals exposed to LCT **(El-Masry *et al,* 2014)** and different pyrethroid compounds **(Kilian *et al,* 2007).**

Fig. 1. Body Weight Pattern of Rats Orally Adminis trated to LCT (0.5 mg/kg bw), Propolis (100 mg/kg bw)And Their Combination.



Values in with different letters are significantly different at ($p \leq 0.05$).

Table 1. Body and Relative Liver Weights of Male Rats Administered with LCT (0.5 mg/kg bw), Propolis (100 mg/kg bw) And Their Combination At The End of Experiment.

Treatments	Body Weight (g)	Liver Weight (g)	Liver Relative Weight (%)	% Change[a]
Control	208.6 ± 2.70[bc]	6.82 ± 0.79[a]	3.27± 0.04[a]	0.0
Proplis	211.6 ± 4.39[c]	6.88 ± 0.36[a]	3.25± 0.05[a]	0.61
LCT	185.6 ± 1.14[a]	6.5 ± 0.39[a]	3.50± 0.03[c]	-7.03
LCT + Proplis	205.8 ± 2.77[b]	6.82 ± 0.54[a]	3.31± 0.04[ab]	-1.22

Values are expressed as means (5 rats) ± standard deviation (SD)
Values in column with different letters are significantly different at ($p \leq 0.05$).
[a] Percentage of increase (-) or decrease in treatment weights compared to control

3.2. Hematological studies

The results revealed a reduction in RBC count, Hb and in Hct value; while the WBC count was increased after one, two and three weeks of LCT treatment group, then significantly decreased at the end of the experiment period (Table 2). The MCV of group III showed significant reduction at the first two weeks and elevated significantly at the rest of experiment while MCH showed no significantly alteration at all tested time. Meanwhile MCHC was significantly increased at the second and the third weeks in groups III and IV treated rats then settle down by the end of experiment near to control (Table 3). These alterations were in line with previous studies and reports which demonstrated that pyrethroid insecticide exposure altered hematological parameters in rats and mice **(El-Masry *et al,* 2014 and Islam and Hoque 2015)**.

Collectively, the decrease in the erythrocyte count and haemoglobin content recorded in the present work indicated that LCT - treated rats were anaemic. Studies reported that alterations in hematological parameters were induced by LCT as an anaemic condition because of decreased synthesis of RBC which could be due to haemolysis as a result of type II pyrethroid which causes hemorrhages and reduced erythropoiesis **(El-Masry *et al,* 2014).** One of the molecular mechanisms of toxicity of some pesticides seems to be lipid peroxidation; as a consequence these compounds can disturb the biochemical and physiological functions of the RBC **(Akhgari *et al,* 2003).**

3.3. Biochemical studies:

Induction of oxidative stress is one of the main mechanisms of the action of many pesticides **(Amin and Hashem, 2012).** Oxidative stress induction involves an excessive production of reactive oxygen species (ROS or free radicals) resulting from impaired balance between the ROS generation and antioxidant defense capability. Antioxidant enzyme defense system includes SOD, CAT and others that may protect the system from deleterious effects of oxygen free radicals. LCT in certain doses and conditions is a potent inducer of oxidative stress **(Piner and Uner, 2012).** The present results in (Fig. 2 A) showed significant ($p < 0.05$) decrease in the GSH content (61%) of liver tissues under the lambda cyhalothrin (LCT) toxication condition. Co-administration of propolis with LCT modulated significantly ($p < 0.05$) the level of GSH but still less than control value. It is well established that GSH, the most important biomolecule protecting against chemically induced cytotoxicity, can participate in the elimination of reactive intermediates by conjugation or by direct free radical quenching. So the decrease in the levels of GSH in these tissues results in the accumulation of free radicals leading to increased rate of lipid peroxidation. These results were correlated with previous reports of **Kale *et al,* (1999)** who suggested that pyrethroid metabolism might generate reactive oxygen species, which in turn could lead to enhanced lipid peroxidation. While the Liver was the major site of pyrethroid metabolism which accumulated great concentration of its metabolites **(Giray *et al,* 2001),** and their toxic effects occurred through generation of reactive oxygen species. LCT was accumulated

Table 2. Effect Of Lambda Cyhalothrin, Propolis And Their Combination On The Peripheral Blood Cell Indicators Of Male Albino Rats.

Parameters	One week				Two Weeks				Three Weeks				Four Weeks				Six Weeks (Two Weeks Of Last Treatment)			
	C	P	LCT	LCT+P	C	P	LCT	LCT+P	C	P	LCT	LCT+P	C	P	LCT	LCT+P	C	P	LCT	LCT+P
WBC (10^3 / µl)	10.34^{a} ±0.39	10.72^{a} ±0.46	14.80^{c} ±0.64	12.03^{b} ±0.57	14.918^{a} ±0.57	14.864^{a} ±0.65	14.926^{a} ±1.01	18.11^{b} ±1.31	18.53^{b} ±1.08	18.50^{b} ±1.35	16.87^{b} ±1.10	14.77^{a} ±0.91	15.80^{b} ±0.29	16.82^{c} ±0.33	16.22^{b} ±0.50	14.59^{a} ±0.248	12.82^{b} ±0.62	12.73^{b} ±0.83	10.41^{a} ±0.63	12.15^{b} ±0.89
RBCs (10^6 / µl)	5.82^{b} ±0.34	5.08^{a} ±0.16	7.00^{c} ±0.46	7.13^{c} ±0.28	7.44^{b} ±0.39	6.26^{a} ±0.38	6.90^{b} ±0.48	6.99^{b} ±0.23	7.33^{a} ±0.17	6.80^{a} ±0.38	6.98^{a} ±0.14	7.29^{a} ±0.56	7.82^{b} ±0.23	7.69^{b} ±0.45	7.43^{ab} ±0.17	7.18^{a} ±0.22	8.05^{b} ±0.26	7.26^{a} ±0.29	7.72^{b} ± 0.46	8.05^{b} ±0.04
Hb (g/dl)	12.72^{b} ±0.38	11.50^{a} ±0.45	14.04^{c} ±0.69	14.52^{c} ±0.19	14.36^{c} ±0.74	11.66^{a} ±0.44	13.14^{b} ±0.82	12.94^{b} ±0.49	13.16^{a} ±0.65	12.42^{a} ±0.51	13.52^{a} ±0.69	12.72^{a} ±1.18	13.76^{a} ±0.32	13.50^{a} ±0.38	13.22^{a} ±0.49	13.36^{a} ±0.80	14.02^{a} ±0.72	13.46^{a} ±0.44	13.10^{a} ±0.82	13.20^{a} ±0.53
Hct (%)	41.58^{a} ±1.63	41.22^{a} ±1.33	48.82^{b} ±1.08	49.22^{b} ±2.28	49.14^{d} ±1.46	46.30^{c} ±1.68	36.00^{a} ±1.02	40.20^{b} ±1.59	46.08^{b} ±1.48	43.90^{ab} ±1.75	45.62^{ab} ±0.91	43.28^{a} ±1.67	45.90^{ab} ±0.90	45.06^{a} ±0.62	46.54^{b} ±0.45	46.58^{b} ±0.43	45.44^{a} ±2.36	46.00^{a} ±1.48	43.78^{a} ±1.56	43.18^{a} ±1.26
PLT (10^3/ µl)	991^{a} ±25.06	1023^{a} ±33.57	935^{a} ±28.01	948^{a} ±25.41	736.6^{d} ±34.60	578^{c} ±28.03	171.6^{a} ±19.20	247.8^{b} ±25.94	548.4^{a} ±46.17	540.6^{a} ±37.40	507.8^{a} ±60.61	466.4^{a} ±31.97	489.6^{b} ±49.54	453.8^{b} ±12.21	471.8^{b} ±23.26	398.4^{a} ±43.58	571.2^{c} ±22.87	659.0^{d} ±14.07	402.8^{a} ±41.53	499.0^{b} ±51.96

C: Control, P: Propolis (100 mg/kg b.w.), LCT: Lambda Cyhalothrin (0.5 mg/kg b.w.), LCT+P: their combination
Values are expressed as means (5 rats) ± standard deviation (SD).
Values in rows with different letters are significantly different at ($p \leq 0.05$).

Table 3. Effect Of Lambda Cyhalothrin, Propolis And Their Combination On The Erythrocytes Indices Of Male Albino Rats.

Parameters	One week				Two Weeks				Three Weeks				Four Weeks				Six Weeks (Two Weeks Of Last Treatment)			
	C	P	LCT	LCT+P	C	P	LCT	LCT+P	C	P	LCT	LCT+P	C	P	LCT	LCT+P	C	P	LCT	LCT+P
MCV (fL)	74.74^{c} ±2.28	75.7^{c} ±2.92	70.5^{b} ±3.41	66.7^{a} ±2.21	67.18^{c} ±4.95	69.02^{c} ±2.07	52.8^{a} ±2.2	58.1^{b} ±2.61	62.0^{a} ±1.34	65.6^{b} ±1.32	64.2^{ab} ±2.62	62.7^{a} ±1.53	59.40^{a} ±1.53	61.6^{bc} ±1.10	62.2^{c} ±1.46	60.0^{ab} ±1.23	56.5^{ab} ±1.01	56.2^{ab} ±2.06	57.8^{b} ±1.74	54.4^{a} ±2.08
MCH (Pg)	21.26^{a} ±1.63	20.4^{a} ±1.14	20.7^{a} ±1.16	20.5^{a} ±0.58	18.80^{a} ±0.88	18.68^{a} ±0.40	19.0^{a} ±0.46	18.7^{a} ±0.41	18.2^{a} ±0.79	18.3^{a} ±0.27	18.8^{a} ±0.57	18.1^{a} ±0.54	17.88^{a} ±0.54	17.7^{a} ±0.70	18.0^{a} ±0.76	17.7^{a} ±0.43	17.3^{b} ±0.40	17.1^{b} ±0.34	17.3^{b} ±0.24	16.3^{a} ±0.67
MCHC (g / dL)	29.88^{a} ±1.00	29.2^{a} ±2.09	30.3^{a} ±1.44	29.6^{a} ±1.69	28.94^{b} ±0.75	26.68^{a} ±0.87	36.0^{d} ±1.75	32.1^{c} ±0.99	28.1^{a} ±0.29	27.8^{a} ±0.45	29.3^{b} ±0.59	29.2^{b} ±0.59	29.86^{a} ±0.35	29.6^{a} ±0.43	29.4^{a} ±0.76	29.8^{a} ±0.71	30.6^{b} ±0.41	29.7^{a} ±0.26	29.9^{ab} ±0.86	30.0^{ab} ±0.13

C: Control, P: Propolis (100 mg/kg b.w.), LCT: Lambda Cyhalothrin (0.5 mg/kg b.w.), LCT+P: their combination
Values are expressed as means (5 rats) ± standard deviation (SD).
Values in rows with different letters are significantly different at ($p \leq 0.05$).

in hepatic parenchymal cells and metabolically activated by cytochrome-P-450 dependent monooxgenases to form free radical **(Abdel-Kawy *et al,* 2013)** with attack polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides **(Salah *et al,* 2010 and Giray *et al,* 2001)**. Oxygen free radical induced lipid peroxidation, which damaged to cell membranes and developed tissue injury **(Sener *et al,* 2005).**The present results pointed out high elevation of lipid peroxidation (about 2 fold) in liver homogenate, propolis treatment alleviated lipid peroxidation induced by LCT and modulated significantly ($p < 0.05$) the levels of MDA compared to control (Fig. 2 B). Propolis 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 treated rats **(Abdel- Mobdy and Abdel-Rahim 2015).** These data were in agreement with previous studies of **Sae -Yong *et al,* 2002**.

The antioxidant activities of propolis are related to its ability to scavenge free radicals; singlet oxygen, superoxide anions, proxy radicals, hydroxyl radicals and peroxynitrite that cause lipid peroxidation **(Ferrali *et al,* 1997)**. The other mechanism may comprise the inhibition of xanthine oxidase, which is known to cause free radicals to be generated **(Kanbura *et al,* 2009).**

Present data showed that, activity of SOD and CAT revealed a significant ($p \leq 0.05$) decrease in LCT group as compared with controls (Fig. 2 C and D).Under normal physiological conditions, a delicate balance exists between the rate of formation of H_2O_2 via dismutation of $O_2\cdot-$ by SOD activity and the rate of removal of H_2O_2 by CAT. Therefore, any impairment in this pathway will af-fect the activities of other enzymes in the cascade **(Kono and Fridovich, 1992).** For example, reduction in the ac-tivity of SOD will result in an increased level of $O_2\cdot-$, while a decrease in the activity of CAT will lead to accu-mulation of H_2O_2 in the cell, which leads to peroxidation of membrane lipids via Fenton-type reaction. The decline of SOD, CAT activities in this study, supported previously by **Mossa *et al,* 2013 and Sharma *et al,* 2014**.

Therefore, LCT caused oxidative stress and consequently decreased the activities of the antioxidant enzymes (SOD and CAT) and GSH content in contrast to marked increase in the MDA levels which in a line with **(Fetoui *et al,* 2009; 2010 and Ansari *et al,* 2012)**. By co-administration of propolis, the activities of these antioxidant enzymes were approached to their control values (Fig. 2), these data are in agreement with the results obtained by **Jasprica *et al,* (2007)** who reported that propolis caused reduction in the MDA level and increased the activities of the antioxidant enzymes (SOD and CAT) indicating that propolis tended to prevent the damage and suppressed the leakage of enzymes through cellular membranes.

Liver was known as the main organ that synthesized the Cholinesterase (ChE), pseudocholinesterase, in hepatocytes and secreted it into the blood stream **(Brown

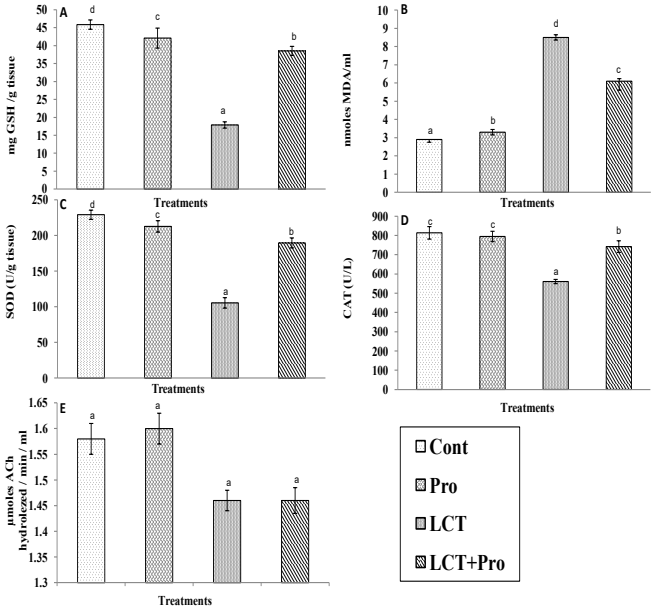


Fig. 2. GSH content (A), MDA level (B), SOD activity (C), CAT activity (D) and ChE activity (E) of male rats treated with LCT (0.5 mg/kg bw), propolis (100 mg / kg bw) and their combination after six weeks.

-Values are expressed as mean ± SD for five animals per group.

-Different superscript letters indicate statistical significant differences between groups ($P \leq 0.05$).

et al, **1981),** ChE activity is reduced in liver dysfunction due to reduced synthesis **(Moss and Henderson, 1999).** The present results in (Fig. 2 E) showed that there were non significant differences in serum AChE activities in all treated groups. The normal serum AChE levels in LCT exposed rats could be corresponded to the low sublethal dose-response. Some studies have found an association between exposure to pesticides and decreased ChE activity in farm workers **(Safi *et al,* 2005 and Remor *et al,* 2009)**. The present results are in agreement with those of **Sudjaroen, (2015)** who found that, mean AChE activities of both organophosphates (OPs) and carbamates (CB), sprayer groups were within the normal range and didn't exhibit any statistically significant difference.

3.4. Histopathological examination

Histological changes provide a rapid means for detecting the effects of pesticides in various animal tissues and organs. **(Khaldoun-Oularbi *et al,* 2013)** The light microscopic investigations showed many histological abnormalities in the liver of LCT exposed rats. Clinical chemistry and histopathological assessments are regularly utilized for identifying organ-specific effects related to chemical exposure **(Crissman *et al,* 2004).** Fig.(3) illustrated that liver of control (A and B) and propolis (C and D) groups showed normal architecture and pathological-free hepatic central vein (CV) and portal triad (Pt). The LCT treated rats showed degenerative changes in the hepa -tocytes in the form of sever inflammation within the por-

tal triad (E). Also the results appeared fibrosis: fibrous septae (Fs) and focus inflammation within the hepatocyte (F and G, respectively). Mild lymphocytic infiltrate (LI), hyperplastic bile duct, hepatic vein and hepatic artery within the portal triad (H) and focal hepatic degeneration (HD) were showed (I). Co-administration of propolis revealed that, Portal triad and central vein showing nominal histopathological changes denoting recovery (J and K), which means that propolis alleviated the lesions caused by LCT treatment in rats.

Previous studies mentioned that different pesticides as toxic materials reached to the liver via the gastro intestinal tract blood supply. **Abd-Allah, (1987)**, reported that inflammatory cells were aggregated in portal tracts and present as differential foci in the liver parenchyma act as a defense mechanism due to irritation of toxic material and for the same reason the kupffer cells were activated. Liver lesions were observed by many investigator **(Chu *et al,* 1986; El- bendary *et al,* 2014; Islam and Hoque, 2015)** which in line with the present results.

Conclusion

The present study demonstrated that sublethal oral administration of lambda cyhalothrin at 1/50 LD_{50} under previous experimental conduction caused cytotoxic changes in the hepatic biomarkers which reflects hazardous effects at various levels to non-target organisms. Propolis indeed retarded the LCT-induced liver injury by blocking the oxidative stress due to its high content of antioxidant ingredients. So that propolis can be used as adjuvant drug for hepatoprotection against liver damages caused by xenobiotics.

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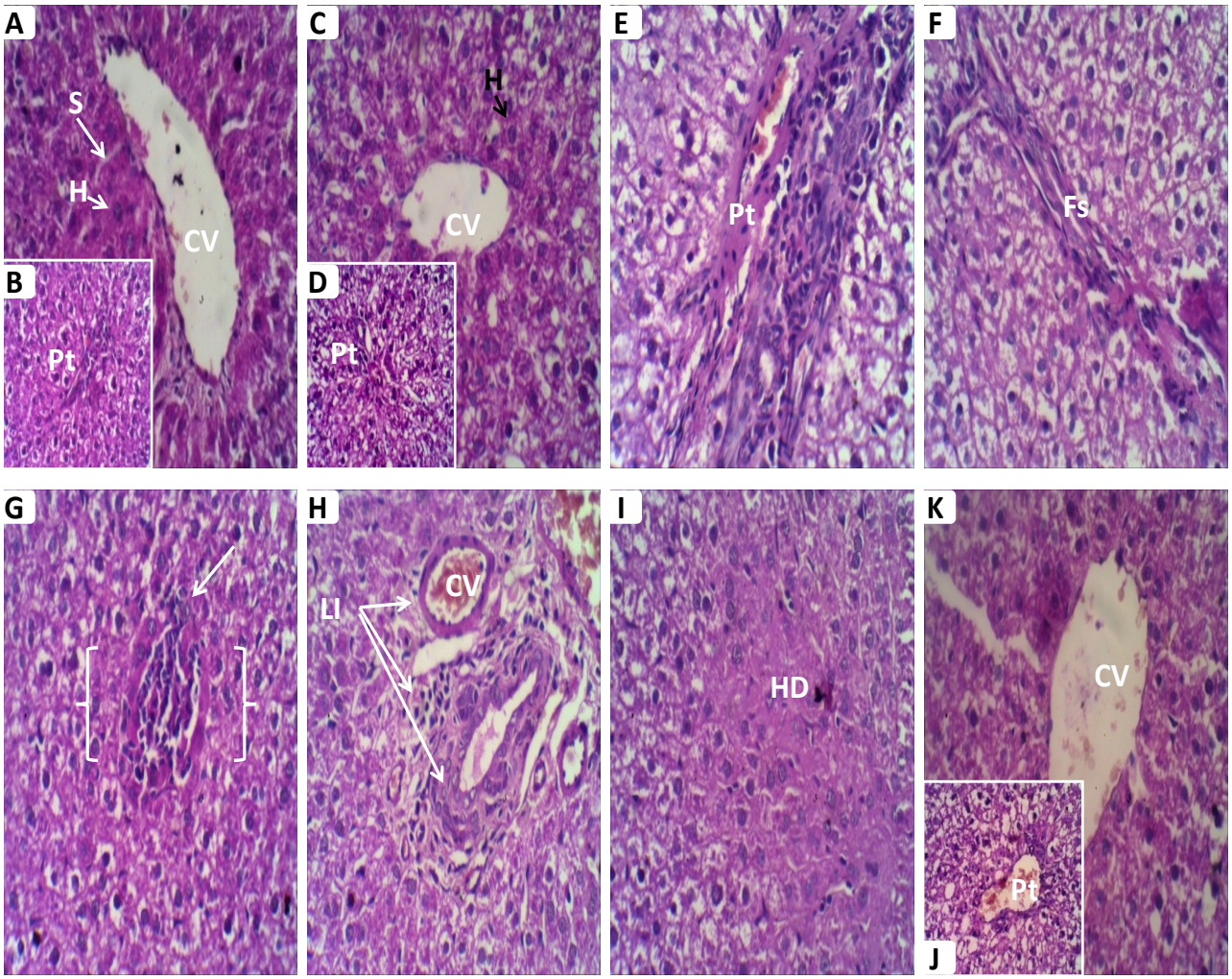

Fig. 3. Liver sections of male rats treated with LCT (0.5 mg/kg bw), propolis (100 mg /kg bw) and their combination using H & E stain X 400. Photomicrograph of the liver sections of control (A&B)and propolis groups (C&D) showing pathological free hepatic central vein (CV), portal triad (Pt), hepatocytes (H) and sinusoids (S) with normal architecture. Liver sections of LCT groups(E, F, G, H and I) revealed considerable degenerative damage in liver, with the appearance of (E) Inflammation within the portal triad, (F) Fibrosis: fibrous-septae (Fs), (G) focus inflammation within the hepatocyte, (H) mild lymphocytic infiltrate (LI), hyperplastic bile duct, hepatic vein and hepatic artery within the portal triad, (I) focal hepatic degeneration (HD). Liver sections of LCT plus propolis groups (J&K) showed hepatic central vein (CV) and portal triad (Pt) with nominal histopathological changes denoting recovery.

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التغيرات الدموية، الكيموحيوية والهستوباثولوجية المستحثة بواسطة لامبدا سيهالوثرين والتأثير التخفيفى للبروبوليس (صمغ النحل) في ذكور الجرذان البيضاء

رضا خميس عبد الرازق و نادية على حامد

قسم بحوث سمية المبيدات للثديات - المعمل المركزى للمبيدات - مركز البحوث الزراعية - الاسكندرية.

الملخص العربى:

تستخدم البيرثرويدات المخلقة صناعيا وخاصة مبيد اللامبدا سيهالوثرين على نطاق واسع لفاعليتها العالية وكذلك سميتها المنخفضة على الكائنات الغير مستهدفة.
تهدف الدراسة تقدير التأثيرات الضارة (هيماتولوجية – بيولوجية) لمبيد اللامبداسيهالوثرين على ذكور الجرذان المعاملة لمدة أربع اسابيع (ثلاث جرعات / اسبوع) بجرعة ٠,٥ ملجم / كم (١/٥٠ من الجرعة المميتة لنصف عدد الحيونات المعاملة). وكذلك دور البروبوليس فى حماية الحيونات من التأثير الضار للمبيد.
- تم تقسيم الحيونات لأربع مجاميع: الاولى: (المجموعة الضابطة)، الثانية: حيونات معاملة بالبروبوليس بجرعة ١٠٠ ملجم / كجم، الثالثة: حيونات معاملة بمبيد اللامبداسيهالوثرين بجرعة ٠,٥ ملجم / كم ، الرابعة: حيونات معاملة بالبروبوليس بجرعة ١٠٠ ملجم / كم + المبيد بجرعة ٠,٥ ملجم / كم .

أوضحت نتائج الدراسة أن مبيد اللامبدا السيهالوثرين أحدث التأثيرات التالية:-

- نقص وزن الحيوانات المعاملة و زيادة وزن الكبد النسبى.
- انخفاض معنوى فى كل من عدد كرات الدم الحمراء، مستوى الهيموجلوبين ،مستوى الهيماتوكريت، بينما زاد عدد كرات الدم البيضاء. وعلى العكس فإن ثوابت الدم لم تتغير عند كل الازمنه المختبرة (٠،١،٢،٣،٤)اسابيع وكذلك بعد اسبوعين من أخر جرعة معاملة بالمبيد).
- انخفاض فى مستوى الجلوتاثيون المختزل وكذلك نشاط انزيمى السوبر اوكسيد ديسميوتيز والكتاليز بينما حدثت زيادة ملحوظة فى مستوى اكسدة الدهون فى حين لم تظهر اى تأثيرات معنوية فى نشاط انزيم الأسيتيل كولين استيراز مقارنة بالمجموعة الضابطة.
- وتم تأكيد التأثيرات السابقة للمبيد على الحيوانات المعاملة بالتغيرات التشريحية فى الكبد للحيوانات المعاملة بالمبيد.
- وايضا أوضحت الدراسة أن البروبوليس بالجرعة المستخدمة تمكن من تقليل الضرر الناتج من المبيد على الحيوانات المعاملة مما يعكس الدور الفعال له ضد السمية الكبدية الناجمة عن اللامبداسيهالوثرين.