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Total Phenolic Contents and Antioxidant Activity of Pomegranate (*Punica Granatum L.*) Peel Extracts against Oxidative Stress Induced by Lambada Cyhalothrin Insecticides on Male Albino Rats

Awad R. Abd elfatah[1],Yasmin E. Abdel-Mobdy[2],Osama M.Abd elfatah[3], Emam A. Abdelrahim[3], Marwa E. Hassan[1*]

[1]*Pesticides Department, Research Institute of Medical Entomology, Giza, Egypt*
[2]*Entomology and Pesticide Department, Faculty of Agriculture, Cairo University, Cairo, Egypt*
[3]*Biochemistry department,Faculty of Agriculture, Cairo University, Cairo, Egypt*

Abstract
This study assess the pomegranate's antioxidant capacity and total phenolic content and its role as antioxidant against oxidative damages and alteration in biochemical, haematological and hormonal indices and renal and liver tissues after exposure to Lambda-cyhalothrin in experimental animals. The findings indicate that DPPH (1, 1diphenyl-2-picryl hydrazyl) and ABTS radical assays produced the strongest antioxidant activity, exceeding those of KMnO4. Pomegranate peels contain 77.09g/100g total carbohydrate, 17.08g/100g total soluble sugar, 2.27g/100g total phenols and 2.04 g/100g total flavonoids. Lambda-cyhalothrin had adverse effect on human and animal, since it modifies the activities of the kidneys and liver, hematological parameters and modify the organs' histological structure. Pomegranate peels improved toxicity induced by these insecticides because pomegranate peels contain high level of antioxidant compounds.
Keywords: antioxidant activity; Pomegranate peels; Lambda-cyhalothrin;antioxidant compounds.

1. Introduction

Lambda-cyhalothrin (LCT: α-cyano-3-phenoxybenzyl-3-(2-chloro-3,3,3-trifluoro-1 propenyl)-2,2- dimethylcyclopropane carboxylate), is a synthetic type II pyrethroid that is used as an acaricide, fungicide, and insecticide, and it is developed from cyhalothrin pyrethroid. [1, 2]. Insects that may serve as disease vectors can be controlled using lambda-cyhalothrin in pest management or public health applications [3, 4]. Lambda-cyhalothrin's lipophilic nature facilitates its absorption and accumulation in fatty tissues [5] and allows it to enter the nervous system through peripheral nerve sensory organs [6]. As a result, it is closely linked to toxicity in a variety of organs of non-target organisms, including aquatic and terrestrial animals [7]. In non-target animals, lambda-cyhalothrin is linked to hepatotoxicity [8], nephrotoxicity [9], neurotoxicity [10, 11] and reproductivetoxicity [12], pancreatic toxicity [13, 14], and embryonic toxicity [15]. By altering defensive mechanisms against free radicals and raising oxidation of lipids through the making of ROS in many organs of rats and rabbits, LCT caused cytotoxic oxidative stress [16, 17]. Nitric oxide generation and its genotoxic consequences may be other potential pathways of LCT cytotoxicity [6, 18].

In China, pomegranates (*Punica granatum L.*) are commonly utilized in traditional medicinal and food [19]. It has a lot of phytochemicals, like flavonoids, anthocyanins, polyphenols, gallic acid, ellagic acid, tannins, and catechins, which are strong antioxidants [20, 21]. These elements work by neutralizing reactive oxygen species, scavenging them, and improving oxidative biomarkers [22]. pomegranate have been demonstrated in numerous studies to have a broad variety of biological advantages like antibacterial, antioxidant, antiviral, anti-inflammatory, cancer prevention and cardioprotective properties [23]. According to certain research, *Punica granatum* methanolic extract prevents oxidative damage and histological modifications in the kidneys and liver [24, 25].

The current study's goal was to assess the pomegranate peel's total phenolic content and antioxidant activity, and its role as antioxidant against oxidative stress and alteration in biochemical, hematological and hormonal indices and liver and kidney tissues induced by LCT in experimental animals.

2. Material and methods

2.1. Chemicals and reagents

The supplier of 70% ethanol was Merck Chemical Company, located in Darmstadt, Germany. From Kafr El-Zayat for Pesticides and Chemical Company, located in Kafr El-Zayat City, Gharbia Governorate, Egypt, lambda-cyhalothrin (LCT

*Corresponding author email: m.marwa422@yahoo.com.; (Marwa Emam Hassan).
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98%) was acquired. Biodiagnostic Co. (Dokki, Cairo, Egypt) supplied the kits for glutathione transferase (GST), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA). Diagnostic Systems GmbH (Germany) supplied the kits for the biochemical analyses of alanine aminotransferases (ALT), uric acid, urea, alkaline phosphatase (ALK), triglycerides, albumin, aspartate aminotransferases (AST), creatinine, HDL, total protein, cholesterol, and LDL. White blood cell count (WBC), mean corpuscular volume (MCV), red blood cell count (RBC), mean corpuscular hemoglobin concentration (MCHC), hemoglobin concentration (Hb), hematocrit (HCT), , mean corpuscular hemoglobin (MCH), and platelet count were all measured using a Mindray BC-30 (Hamburg, Germany). A blood smear was obtained, fixed, and stained with Wright-Giemsa stain from Sigma-Aldrich Company (St. Louis, Missouri, US) in order to differentiate white blood cells. The ELISA Kit, which was obtained from the Bioassay Technology Laboratory in Shanghai, China, was used to measure testosterone.

2.2 *Punica Grantum* preparation

This study's pomegranate peel extract was made at the Medical Entomology Research Institute via soaking method [26, 27]

2.3**Antioxidant activity of *Punica Grantum* extract**

Antioxidant activity measured by 3 methods:-

1- DPPH (1, 1diphenyl-2-picryl hydrazyl) radical scavenging activity depending on the technique of Burits and Bucar [28]

2-ABTS radical cation scavenging assay depending on the technique of Pellegrini *et al*. [29]

3- Potassium permanganate method ($KMnO_4$) as a type of non-radical assay depending on the technique of Gaber *et al*. [30]

2.4. Quantification of total phenolic substances

Identification of phenolic chemicals was done by applying the technique outlined by Singleton and Rossi [31].

2.5. Quantification of Total Flavonoids

Flavonoid substances are identified by employing the technique outlined by Zhishen *et al*. [32].

2.6 Determination of carbohydrate

Determination of carbohydrate was determined using the technique outlined by Krishnaveni *et al*. [33].

2.7. Quantification of soluble sugars

The phenol-sulfuric acid method was utilized to determine the amount of soluble sugars,which was described by Nowotny[34]

2.8. Experimental animals

30 male albino rats (130 ± 5 g) were acquired from the Animal Breeding House of the research institute of medical entomology (RIME), Dokki, Giza, Egypt. A week before the trial began, the rats were acclimated. Under Ethical Review Committee number IME00066 (9/11/2022), they received humane treatment in compliance with the Guide for the Care and Use of Laboratory Animals in RI ME

2.9.Experimental protocol

6 groups of albino rat males are formed, with five rats in each group as follows:

Group 1:receivedcorn oil (1ml / rat) and considered as control(C)

Group 2:received extract of *Punica granatum* (PG) (400 mg/kg/day) which dissolve in distilled water for 3months[35]

Group 3: lambdacyhalothrin treated group (L1/10), the rats received dose of 7.9 mg/ kg/ b.w (1/10 of the LD50(79mg/kg))/ daily in distilled water[36].

Group 4: lambdacyhalothrin treated group (L1/30),the rats receiveddose of 2.6 mg/ kg/ b.w (1/30 of the LD50)/ daily in distilled water.

Group 5:the rats received*Punica granatum*then after 30 min lambdacyhalothrin 1/10 (L1/10+PG)

Group 6:the rats received*Punica granatum*then after 30 min lambdacyhalothrin 1/30 (L1/30+PG)

After 12 hours fasting, blood samples were collected from all groups into two types of vacutainer tubes; one of them contains EDTA (ethylenediaminetetraacetic acid) for assaying complete blood picture and the other without any additives for assaying biochemical, anti-inflammatory, and hormonal parameters. Rats killed via dislocation of the cervical spine after blood was extracted from the retero-orbital venous plexus using a fine, sterilized glass capillary. To extract the serum, samples of blood without any additives were centrifuged by a Heraeus Labofuge 400R (Kendro Laboratory Products GmbH, Germany) at 3000 rpm (600×g) for ten minutes at 4 °C after being allowed to clot in clean, dry tubes. Testosterone, albumin, creatinine, HDL , total protein, AST, urea, triglycerides,uric acid, cholesterol, triglycerides, LDL,ALT and ALK were all measured using serum.

2.10. Evaluation of antioxidant in tissues

Each group's rats' kidney and liver tissues were immediately taken out, and any clots or red blood cells were washed out using phosphate buffered saline. Using a high speed cooling centrifuge, the cold phosphate buffer (0.1MpH7.4) was used to homogenize the tissues, filtered, and centrifuged at 4000 rpm for fifteen minutes at 4 °C. For examination of antioxidants (MDA, CAT, GST, and SOD), the supernatant was extracted and kept at -80 °C.

2.11. The histological analysis

The kidney and liver were washed in xylene,embedded in paraffin wax, dehydrated using a graded series of alcohol, and fixed in 10% neutral formalin. For microscopic analysis, tissue slices (5 μm) were cut and stained with hematoxylin and eosin [37].

Statistical analysis

The data are analyzed by using SPSS for windows and expressed as mean ± SE paired samples. The control and treated groups are compared using one-way analysis of variance (ANOVA). According to Waller and Puncan [38], statistical analysis was employed to ascertain the differences between each treatment and the control as well as between the treatments themselves. $P < 0.05$ indicated that the differences were statistically significant.

3.Results and discussion

Determination of antioxidant activity (%) of different concentration of pomegranate peel extract by three methods (Table 1) showed that antioxidant activity was 45% and 95.9% at concentration of extract equal 12.5 ug/ml and 1000ug/ml respectively by DPPH method but it was 23.7% and 91.0 % at concentration of extract equal 12.5 ug/ml and 1000ug/ml respectively by ABTS method. In addition, antioxidant activity was 45.1% and 89.4 % at concentration of extract equal 12.5 ug/ml and 1000ug/ml respectively by KMno4 method. The findings are analogous to the report published by Singh *et al*.[39] who found that using DPPH model systems, the peels' extract demonstrated 81% antioxidant activity at 50 ug/ml.Elfalleh *et al*.[40] stated that the peel of pomegranates has more antioxidant properties than leaf, flower, and seed. Shadab *et al*. [41] discovered that PG's DPPH scavenging activity rose with concentration rose so DPPH· scavenging activity can used to determine activity of various samples since it is sensitive to low concentrations of natural active principles because a λmax of DPPH at 517 nm. This meaning that at 517 nm, low absorbance indicates high free radical scavenging activities of samples. According to the results, which are consistent with those of Gaber *et al*. [30], the strongest antioxidant activity were observed with the DPPH and ABTS radical assay more than KMnO4.

Table1: determination of antioxidant activity (%) of different concentrations of pomegranate peels extract by three different methods

Test	Conc. (ug/ml)						IC_{50} ug/ml
	12.5	25	50	100	200	1000	
DPPH	45	59	80.2	84.6	91	95.9	26.7
ABTS	23.7	47.0	64.8	79.0	87.5	91.0	32.8
KMnO4	45.1	46.82	72.35	79.3	82.32	89.4	30.86

Analysis of major component in *Punica Grantum* (Table 2) showed that pomegranate peels contain 77.09g/100g total carbohydrate, 17.08g/100g total soluble sugar, 2.27g/100g total phenols and 2.04 g/100g total flavonoids. Saxena *et al*. [42] and Kumar *et al*. [43] observed that values of phenols were 337 and 298 mg g−1 in pomegranate peel respectively. Hmid et al.[44] observed that flavonoid value was 56.98 mg ml−1,also, Efalleh *et al*. [40] observed that a peel contain 51.52 mg g^{-1} flavonoid .Nasser *et al*. [45] revealed that pomegranate contains high amount of carbohydrates (69.29 mg/100ml). In addition**,** Qamar *et al.* [46] found that pomegranate contains low quantity of fats while high carbohydrate content (26.44g/g). Melgarejo *et al*.[47] discovered that the pomegranate's total sugar content varied from 11.43 g/100 g to 13.5 g/100 g.

Table 2: analysis of major component in *Punica Grantum*

Test	Unit	Result
Total carbohydrates	g/100g	77.09
Total soluble sugar	g/100g	17.08
Total phenols	g/100g	2.27
Total flavonoids	g/100g	2.04

The effect of *Punica granatum* extract on biochemical variables (Table 3) revealed that when compared to the control group and the PG-treated group, L1/10 and 1/30 considerably raised the levels of ALP, creatinine, AST, uric acid, ALT and urea while dramatically lowering the levels of TP and Alb. Compared to the group that got L1/10 and 1/30 alone, the rats that given L1/10+PG or L1/30+PG had high levels of Alb and TP but significantly low levels of ALP, creatinine, AST, uric acid, ALT and urea. Compared to the L1/10+PG treated groups, the rats in the L1/30+PG group had slightly higher levels of ALT, urea, ALP, creatinine, AST and uric acid, but somewhat lower levels of TP and Alb. This results is analogy to studies of Waheed *et al*. [48], and Kobir *et al*. [49] who found that lambda-cyhalothrin increased level of liver and kidney functions, since lambda-cyhalothrin generate ROS, which lead to impairment in oxidation of protein and lipid, and harm to DNA and finally lead to oxidative damage in living organs and significant modifications to the liver and kidney's cellular and structural processes [50, 51]. Hossein *et al*.[52] stated that pomegranates have a number of anti-diabetic, anti-inflammatory, anti-hyperlipidemic, and antioxidant effects .Pomegranate administration improved liver and kidney functions in both humans and animals because it contains polyphenols and other biologically active compounds with strong antioxidant qualities, such as flavonols, gallicacid, flavanoids, ellagic acid, ellagitannins, anthocyanins, tannins, quercetin, and nitrate [53,54]. In addition, this study found that L1/10 or 1/30 significantly decreased the level of testosterone. Co-administration of PG with L1/10 or 1/30 improved the level of testosterone and this improvement was more observed in the group treated with L1/30. This results similar to studies by Li *et al*. [55], and Mustafa *et al*. [56] who observed that lambda-cyhalothrin effect on the hypothalamus's release of gonadotropin-releasing hormone which minimized secretion of luteinizing hormone from the anterior pituitary leading to decreased secretion of testosterone. Pomegranate peel neutralized free radical and inhibit lipid peroxidation so that

the lipid matrix in the spermatozoa's membranes remains structurally intact,and increase testosterone and motilityof sperm [57,58].

Table 3: Serum liver, kidney biomarkers and Testosterone of rats exposed to *Punica granatum* and lambdacyhalothrin

Groups / Parameter	C	PG	L1/10	L1/30	L1/10+PG	L1/30+PG
AST (u/l)	110.55 ± 0.80	117.43 ± 1.55a	295.07±1.15a,b	280.32 ± 2.80a,b,c	230.00 ± 1.14a,b,c,d	220.11±1.17a.b,c,d,e
ALT (u/l)	53.66 ± 0.82	59.00 ± 1.10a	87.76 ± 0.84a,b	85.11 ± 1.16a,b,c	73.22 ±1.17a,b,c,d	70.32±1.22a,b,c,d,e
ALP (u/l)	250.76 ± 1.55	255.96 ± 0.80a	380.60±1.23a,b	372.12 ± 1.35a,b,c	345.60 ± 1.40a,b,c,d	337.00±1.14a,b,c,d,e
Albumin (g/dl)	3.2 ± 0.12	3.5 ±0.09 a	1.8 ±0.09 a,b	2.3 ± 0.12 a,b,c	2.5 ±0.11a,b,c,d	2.8 ±0.10a,b,c,d,e
Total protein (g/dl)	5.4± 0.09	6.1±0.08 a	3.5±0.09 a,b	4.0± 0.11 a,b,c	4.3±0.08 a,b,c,d	4.8±0.15 a,b,c,d,e
Urea (mg/dl)	25.00 ± 1.15	30.32 ± 1.17 a	57.56 ± 2.3 a,b	54.30 ± 1.55 a,b,c	46.00 ±1.63 a,b,c,d	44.16 ±1.25 a,b,c,d,e
Uric acid (mg/dl)	2.7 ± 0.09	3.1 ±0.16 a	4.9±0.12 a,b	4.7± 0.11 a,b,c	4.3 ±0.14 a,b,c,d	4.0 ±0.15 a,b,c,d,e
Creatinine (mg/dl)	0.53 ± 0.009	0.55 ± 0.04a	0.90 ± 0.01 a,b	0.85 ± 0.03 a,b,c	0.71 ±0.04 a,b,c,d	0.68 ±0.02 a,b,c,d,e
Testosterone (ng/ mL)	2.25 ± 0.12	2.30 ± 0.55 a	0.94 ± 0.63 a,b	1.0 ± 0.82 a,b,c	1.60 ±0.75 a,b,c,d	1.75 ±0.78 a,b,c,d,e

According to the haematological parameter data (Table 4), the rats that received L 1/10 and 1/30 had significantly lower RBCs, MCHC, Hb , MCV, Hct , WBCs, monocytes, MCH, platelets, MCHC, lymphocytes, eosinophils, and basophils, but significantly higher neutrophils compared to the PG and control groups.The group of rats received L1/10+PG or L1/30+ PG showed that RBC's ,WBC's, Hb, MCV, Lymphocytes, MCH, ,Eosinophils, Hct, MCHC, Platelets,Monocytes, Basophils were significantly increased but Neutrophils was significantly decrease compared to L 1/10 and 1/30 treated group.Improvement in this parameters observed in L1/30+ PG treated group more than L1/10+ PG treated groups. Erythrocytes are especially susceptible to oxidative damage by ROS which produced by exposure to lambd-acyhalothrin because of the high levels of polyunsaturated fatty acids in their membranes and the high levels of hemoglobin and oxygen in their cells, so level of RBS and HB decreased in rats treated with lambda-cyhalothrin, also, in erythrocytes, lambda-cyhalothrin modifies the enzymatic defense mechanism [48]. Mani *et al*. [59] found that haematological parameters can be altered by the hydrophobic chemical lambda-cyhalothrin, which binds strongly to biological membranes, particularly phospholipid bilayers. It can also harm membranes by causing lipid peroxidation. Manthou *et al*.[60] found that pomegranates' high polyphenol content may make red blood cells more resistant to oxidative damage. As a result, it stopped RBC destruction since oxidative stress was decreased, which raised haematological parameters.

Table 4: blood profile of rats exposed to to *Punica granatum* and lambdacyhalothrin

Groups / Parameter	C	PG	L1/10	L1/30	L1/10+PG	L1/30+PG
WBC (10^3/cmm)	7.7± 0.10	7.9± 0.09 ^{a}	5.5±0.14 ^{a,b}	5.7±0.13 ^{a,b,c}	6.6±0.14 ^{a,b,c,d}	6.9±0.11 ^{a,b,c,d,e}
RBC (10^6/cmm)	6.15± 0.06	6.23± 0.01 ^{a,}	4.65±0.07 ^{a,b}	4.79±0.05 ^{a,b,c}	5. 30±0.03 ^{a,b,c,d}	5.45±0.02 ^{a,b,c,d,e}
HB (g/dl)	13.4±0.11	13.0± 0.16 ^{a,}	10.8±0.22 ^{a,b}	11.5±0.04 ^{a,b,c}	12.3±0.06 ^{a,b,c,d}	12.6±0.12 ^{a,b,c,d,e}
HCT (%)	37.2± 0.18	37.6± 0.13 ^{a,}	35.40±0.06 ^{a,b}	35.73±0.03 ^{a,b,c}	36.51±0.04 ^{a,b,c,d}	36.92±0.04 ^{a,b,c,d,}
MCV(FL)	54.2± 0.01	54.6± 0.03 ^{a,}	52.60±0.07 ^{a,b}	52.90±0.04 ^{a,b,c}	53.32±0.03 ^{a,b,c,d}	53.56±0.04 ^{a,b,c,d,}
MCH(PG)	20.2± 0.01	20.7± 0.07 ^{a,}	17.0±0.03 ^{a,b}	17.5±0.05 ^{a,b,c}	18.4±0.04 ^{a,b,c,d}	18.9±0.03 ^{a,b,c,d,e}
MCHC (%)	36.4± 0.05	37.0± 0.03 ^{a,}	34.2±0.02 ^{a,b}	34.7 ±0.04 ^{a,b,c}	35.5±0.02 ^{a,b,c,d}	35.9±0.03 ^{a,b,c,d,e}
Plateletcount (10^3/cmm)	582.0 ± 3.20	598.0 ± 2.5 ^{a,}	415.0 ±2.8 ^{a,b}	425.0 ±2.5 ^{a,b,c}	521.0±4.5 ^{a,b,c,d,}	530.0 ±2.7 ^{a,b,c,d,e}
Neutrophils (%)	10.9± 0.14	10.8±0.20	12.9±0.22 ^{a,b}	13.1±0.15 ^{a,b}	12.1±0.02 ^{a,b,c,d}	11.5±0.04 ^{a,b,c,d,e}
Lymphocytes (%)	75.2± 0.05	75.8± 0.03 ^{a,}	65.3±0.04 ^{a,b}	68.7±0.03 ^{a,b,c}	73.0±0.16 ^{a,b,c,d}	73.9±0.04 ^{a,b,c,d,e}
Monocytes (%)	10.2± 0.11	10.7± 0.15 ^{a,}	8.6±0.17 ^{a,b}	8.9±0.14 ^{a,b,c}	9.5±0.07 ^{a,b,c,d}	9.7±0.15 ^{a,b,c,d,e}
Eosinophils (%)	1.30 ± 0.06	1.5± 0.05 ^{a}	0.5±0.02 ^{a,b}	0.8±0.02 ^{a,b,c}	1.0 ±0.07 ^{a,b,c,d}	1.1 ±0.09 ^{a,b,c,d,e}
Basophils (%)	0	0	0	0	0	0

Punica granatum extract's effects on the lipid profile (Table 5) revealed that, in comparison to the control and PG-treated groups, L1/10 and 1/30 considerably raised the levels of LDL, triglycerides, and cholesterol while having a significant negative impact on HDL.The group of rats received L1/10+PG or L1/30+ PG showed a significantly decreased in level of LDL, cholesterol, and triglyceride, but significantly increased in level of HDLcompared to group received L 1/10 and 1/30 only. Improvement in this parameters observed in L1/30+ PG treated group more than L1/10+ PG treated groups. Several studies suggest that lambda-cyhalothrin blocks AMPK, which phosphorylates acetyl-CoA carboxylase to prevent synthesis of fatty acid and induce oxidation of fatty acid, so it causes fat accumulation, supports adipogenesis, and reduces liver enzymes. Additionally, by phosphorylating and inhibiting related transcription factors, lambda-cyhalothrin restricts the glycolytic and lipogenic transcriptional processes. Finally it manage lipid metabolism in cells [61, 62, 63]. Aviram *et al*.[64] found that Pomegranate contain high amount of polyphenols which neutralize free radicals and block oxidation of LDL both in vivo and in vitro, also it inhibit endogenous synthesis of lipids. In addition, Hossein *et al*.[65] reported that pomegranate has beneficial impact on inflammation and hyperlipidemia because it possess strong antioxidant compounds(polyphenols), so it can prevent lipid peroxidation and enhanced the levels of total antioxidant capacity (TAC) and reduced glutathione (GSH).

Table 5: lipid profile of rats exposed to to *Punica granatum* and Lambdacyhalothrin

Groups / Parameter	C	PG	L1/10	L1/30	L1/10+PG	L1/30+PG
Cholesterol (mg/dl)	76.30± 1.77	75.62± 0.85	125.0 ± 1.16 ^{a,b}	115.0 ± 2.09 ^{a,b,c}	95.33 ± 1.35 ^{a,b,c,d}	87.30± 2.83 ^{a,b,c,d,e}
Tri G (mg/dl)	55.0 ± 1.13	60.0 ±1.70 ^{a}	95.31± 1.82 ^{a,b}	83.0 ± 1.14 ^{a,b,c}	80.0 ±1.17 ^{a,b,c,d}	77.0 ±1.14 ^{a,b,c,d,e}
HDL (mg/dl)	64.0 ± 1.16	66.0 ± 1.17 ^{a}	38.0 ± 1.14 ^{a,b}	41.62± 1.35 ^{a,b,c}	52.0 ±1.12 ^{a,b,c,d}	57.0 ±1.16 ^{a,b,c,d,e}
LDL (mg/dl)	27.0 ± 1.15	30.20± 1.41	46.22± 1.40 ^{a,b}	43.0 ±1.14 ^{a,b,c}	38.00± 0.89 ^{a,b,c,d}	35.12±1.17 ^{a,b,c,d,e}

Rats given L1/10 or 1/30 in the current investigation had obvious high level of MDA in both the kidney and liver and obvious low levels of CAT, SOD, and GST compared to the control and PG groups (Table 6). Co-administration of PG with L1/10 or 1/30 improved the level of antioxidant enzymes and the level of MDA in both kidney and liver tissues compered to groups that received L1/10 or1/30 only. Improvement in this parameters observed in L1/30+ PG treated group more than L1/10+ PG treated groups. This results is similar to reports by El-Saad and Abdel-Wahab[8] and Xiaoqing *et al*.[66] who found that lambda-cyhlothrin damages cells by producing ROS, which raises the levels of protein carbonyl (PCO) and oxidation of lipid and lowers the levels of CAT, GPx, GST and SOD activities. It also raises the levels of malondialdehyde (MDA) in male rats. Pomegranates has high amount of polyphenols and other physiologically active substances that improve antioxidant status and lower oxidative stress indicators, such as low-density lipoprotein oxidations and lipid peroxidation, hence preventing oxidative stress [67].

Table 6: Antioxidant markers and MDA in tissue of rats exposed to *Punica granatum* and lambdacyhalothrin

parameter / Groups	Liver				Kidney			
	CAT (u/g)	**SOD (u/ml)**	**MDA (nmol/g)**	**GST (u/g)**	**CAT (u/g)**	**SOD (u/ml)**	**MDA (nmol/g)**	**GST (u/g)**
C	71.05±0.56	189.27 ± 2.46	30.52± 0.30	415. 22± 2.84	78.21 ± 0.41	205.25 ± 2.22	38.72± 0.62	385.13± 2.62
PG	73.20± 0.72 ^{a}	177.57 ± 2.11 ^{a}	34.49± 1.17 ^{a}	405.52± 2.80 ^{a}	82.30± 0.61 ^{a}	206.10± 0.91	40.40± 0.64 ^{a}	389.17± 2.12 ^{a}
L1/10	53.36 ±0.74 ^{a,b}	80.75± 0.92 ^{a,b}	57.15± 0.66 ^{a,b}	350.16 ± 1.87 ^{a,b}	53.12± 0.93 ^{a,b}	155.60± 1.42 ^{a,b}	65.30± 1.05 ^{a,b}	310.46± 1.50 ^{a,b}
L1/30	56.23 ±1.03 ^{a,b,c}	85.55 ± 1.14 ^{a,b,c}	50.26± 1.09 ^{a,b,c}	355.30± 1.73 ^{a,b,c}	57.50± 0.94 ^{a,b,c}	163.92± 1.44 ^{a,b,c}	60.20± 1.31 ^{a,b,c}	327.42± 1.47 ^{a,b,c}
L1/10+PG	59.19± 0.90 ^{a,b,c,d}	90.19 ± 1.13 ^{a,b,c,d}	47.32± 0.57 ^{a,b,c,d}	373.45± 1.82 ^{a,b,c,d}	64.29 ± 0.82 ^{a,b,c,d}	185.60± 1.38 ^{a,b,c,d}	52.22± 1.57 ^{a,b,c,d}	355.52± 1.09 ^{a,b,c,d}
L1/30+PG	62.95 ± 0.51 ^{a,b,c,d,e}	95.61 ± 1.11 ^{a,b,c,d,e}	40.14± 0.82 ^{a,b,c,d,e}	382.61± 3.28 ^{a,b,c,d,e}	69.10±0.51 ^{a,b,c,d,e}	190.72± 1.45 ^{a,b,c,d,e}	47.70± 0.62 ^{a,b,c,d,e}	360.25± 2.25 ^{a,b,c,d,e}

The control group's histological analysis of the liver revealed normal central vein (CV), blood sinusoids (S), nucleus (S), and hepatic architecture (Fig.1). Additionally, Figure 2 displays the liver of the PG therapy group with slightly dilated blood sinusoids (S), a central vein (Cv), and a nucleus (N) that are all almost normal, but figure 3 showing degeneration changes (arrowhead), with pyknotic nuclei (P) and mild dialled blood sinusoids (S) in liver of L 1/10 treated group and Figure 4 displays the liver of the L 1/30 treated group with notable degenerative alterations (arrowhead), pyknotic nuclei (P) and localized mononuclear cell infiltration (star) . The liver of the L1/10 + PG or L 1/30 + PG treatment groups showed improvement in histological structure with minor degenerative changes (arrowhead), slightly dilated blood sinusoids (S), and pyknotic nuclei (P) (Fig.5, 6). The control group's kidney photomicrograph (Fig. 7) displays the glomerulus (G), tubules (T),

and urine space (Us), in a normal configuration. The kidneys of the PG-treated group exhibit glomerulus (G), tubules (T), and urine space (us), with almost normal shape (Fig. 8). The kidney in the L 1/10 treated group exhibited moderate degeneration alterations, pyknotic nuclei (P), degeneration of tubular epithelial cell (arrowhead), and dilated urine space with slightly reduced glomeruli (G) (Fig.9). The kidney of the L 1/30 treated group exhibits clear signs of degeneration, including, tubular epithelial cell degeneration (arrowhead), pyknotic nuclei (P), and dilated urinary space with smaller glomeruli (G) (Fig. 10). Kidney of L 1/10 or L 1/30 with PG treatment groups exhibiting improvement in tubular with minor degeneration of some tubules (arrowhead), and glomeruli (G) with virtually normal structure (Fig.11, 12). These findings align with those of Waheed *et al*. [48], who discovered that exposure to lambda cyhalothrin enhanced reactive oxygen species (ROS),which causes reduction of antioxidants activity, and increased liver and kidney functions, also it causes histopthological changes in both of liver and kidney organs. These effects were mainly improved when treated animals with pomegranate extract since it suppressed NF-κB activation, and regulated apoptosis [68,69].

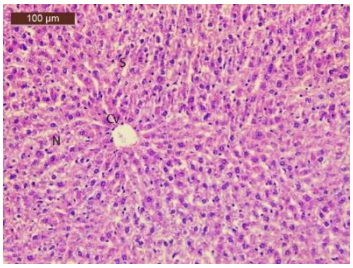

Fig. 1. A photomicrograph of liver of control group showing normal hepatic architecture, central vein (CV), blood sinusoids (S) and nucleus (S)

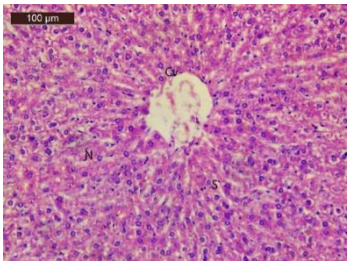

Fig. 2. A photomicrograph of liver of PG treatment group showingnearly normal hepatic structure, central vein (Cv), nucleus (N) and mild dilated blood sinusoids (S)

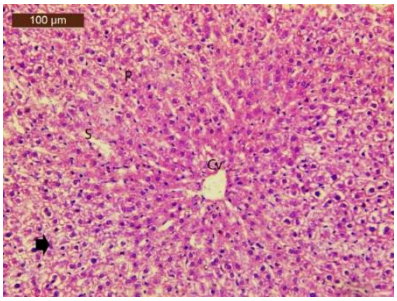

Fig. 4. A photomicrograph of liver of insecticides L 1/30 group showing remarkable degeneration changes (arrowhead), focal mononuclear cell infiltration (star) and pyknotic nuclei (P)

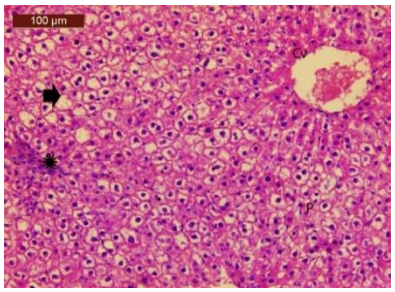

Fig. 3. A photomicrograph of liver of insecticides L 1/10 group showing degeneration changes (arrowhead), with pyknotic nuclei (P) and mild dialled blood sinusoids (S)

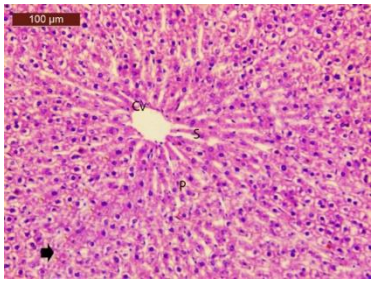

Fig. 5. A photomicrograph of liver of insecticides L1/10 and PG treatment group showing noticeable improvement of histological structure associated slight degeneration changes (arrowhead), slight dilated blood sinusoids (S) and pyknotic nuclei (P)

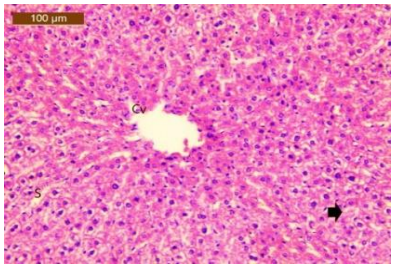

Fig. 6.A photomicrograph of rat liver of insecticides L 1/30 and PG treatment group showing obvious improvement of histological structure associated slight degeneration changes (arrowhead), and slight dilated blood sinusoids (S)

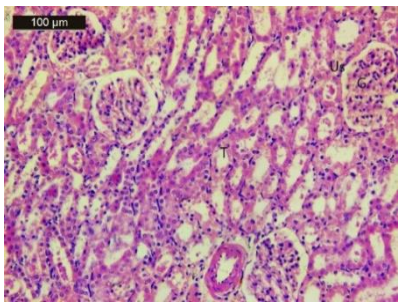

Fig. 7. A photomicrograph of kidney ofcontrol group showing normal structure of the glomerulus (G), urinary space (us) and tubules (T)

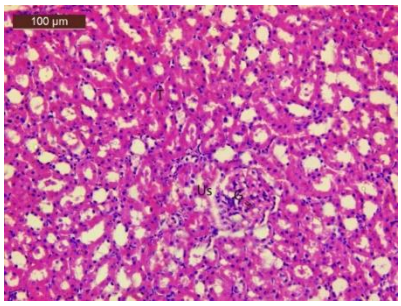

Fig. 8. A photomicrograph of kidney of PG treated group showing nearly normal structure of the glomerulus (G), urinary space (us) and tubules (T)

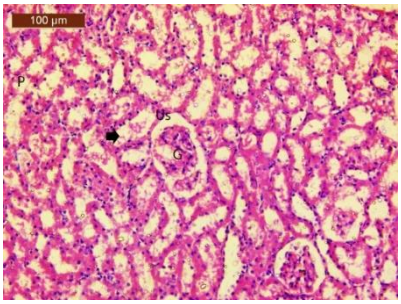

Fig.9.A photomicrograph of kidney of insecticides L 1/10 group showing moderated degeneration changes, slight shrunken glomeruli (G) with dilated urinary space (Us), tubular epithelial cell degeneration(arrowhead) and pyknotic nuclei (P)

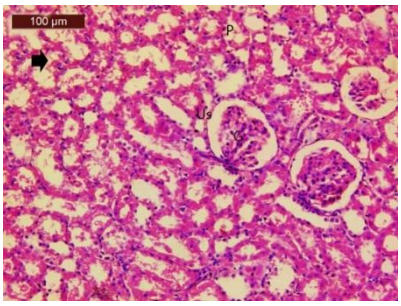

Fig. 10.A photomicrograph of kidney of insecticide L 1/30 group showing obvious degeneration changes, shrunken glomeruli (G) with dilated urinary space (Us), tubular epithelial cell degeneration(arrowhead) and pyknotic nuclei (P)

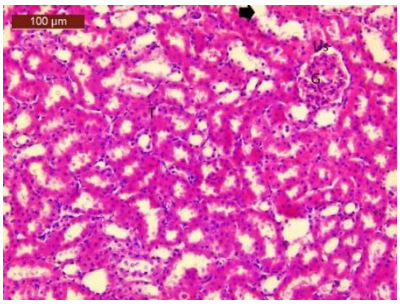

Fig.11.A photomicrograph of kidney of insecticides L1/10 and PG treatment group showing improvement with nearly normal structure of glomeruli (G), urinary space (Us), tubular with mild degeneration of some tubules (arrowhead)

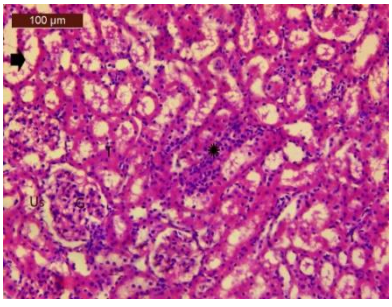

Fig. 12.A photomicrograph of kidney of insecticides L 1/30 and PG treatment group showing improvement with nearly normal structure of glomeruli (G), urinary space (Us), tubular with mild degeneration of some tubules (arrowhead), and minor interstitial inflammatory cells (star)

4. Conclusion

Lambda-cyhalothrin has negative effects on animals because it modifies the histological structure of organs, haematological parameters, and liver and renal functions. Pomegranate peels have been demonstrated to be useful in treating lambda cyhalothrin-induced toxicity. Given the high concentration of antioxidant chemicals in pomegranate peels, it demonstrated a notable reduction in tissue damage and haematological and biochemical changes carried by lambda-cyhalothrin. Pomegranate peels may be used in food preservation and medicine, and they may also have health benefits for people.

5. Conflict of interest

Absence of any conflicts of interest

6. References

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