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Chemical toxicity assessment and Physiological investigation in rats exposed to pyrethroid insecticide type 1 and possible mitigation of propolis

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Abstract

The current investigation aims to study the potential protective effects of propolis methanolic extract (100 mg/kg BW) on the systemic toxic effects after dietary exposure concentration (1/100 LD50 for 30 days) of permethrin (PM) administered in experimental rats. In this experiment, we added propolis four weeks after PM -administration to examining the medicinal effects. Therapeutic use of propolis mitigated PM -induced deterioration of liver and kidney functions and myocardial damage measured by cardiac enzymes lactate dehydrogenase (LDH) and creatine kinase MB (CK-MB) in serum. In addition, propolis treatment (prophylactic and therapeutic) prevented PM-induced apoptosis index, including B-cell lymphoma protein 2 (BCL-2)-associated X (BAX) protein activates, and lipid peroxide (LP). The results showed propolis induced a significant decrease in serum levels of thyroid hormones (T3 and T4), proinflammatory cytokines tumor necrosis factor-alpha (TNF-α), interferon-gamma (INF-γ), interleukin one beta (IL-1β), interleukin 12 (IL-12), and interleukin 6 (IL-6). Besides, nuclear factor-kappa B (NF-kB), acetylcholine esterase (AChE), and hematological constituents. Cardiac biomarkers, liver, and kidney functions were substantially lower in propolis treatment. High-performance liquid chromatography (HPLC) and Gas chromatography–mass spectrometry (GC- MS) of the propolis-MeOH extract showed valuable antioxidant phenolics and flavonoids capable of alleviating oxidative stress through the free-radical scavenging efficacy and regulating signaling pathways of proinflammatory cytokines.

Keywords: pyrethroid insecticides, propolis, antioxidants, apoptosis, oxidative stress

Introduction

Chemical toxicity is becoming a growing problem day by day, heavy metals gained increasing importance due to their health risk (1). Elimination of these metals from wastewater is required to produce a clean environment through sustainable innovative techniques (2–4), followed by a more complicated step to stabilize the hazardous solid residues (5–7). The insecticide is an important source of chemical toxicity that is widely used to control insects attacking crops, as well as combat household insects, which transmit human and animal diseases (8). Insecticides are categorized into two main types: first, inorganic insecticides that are being used to control insects or cutworms, sucking and piercing insects; second, natural organic insecticides with bioactivity on insects, plants, or pathogenic microorganisms, which are responsible for increasing plant production rates (9). However, the repeated, excessive, and incorrect use of these pesticides resulted in several problems because they are of a broad spectrum and very toxic to many insect species, which led to killing parasites and predators (vital enemies) and weakening their role in the process (10). These pesticides disturbed the natural control of insects caused a a significant imbalance in the environmental balance, besides causing significant health damage to non-target organisms such as pets, birds, bees, and humans (11).

PM, also identified as [(3-phenoxyphenyl) methyl 3-(2, 2-dichloroethenyl)-2,2-di-

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methylcyclopropane-1-carboxylate]. Type I pyrethroids are without an alpha-position cyanometry (i.e., permethrin, PM) and cause poisoning. Permethrin affects the neuronal membrane by blocking sodium ion movements from external to the neuronal membrane and disturbing leads to delayed repolarization and eventual paralysis of the nervous system (12). Pyrethroids seem to stabilize sodium channel gating particles, which slows down the mobility of the gates of activation and inactivation and shifts the gate voltage reliance in the hyperpolarizing direction (13). Pesticides' poisonous properties have recently prompted researchers to investigate their effects on oxidative stress and antioxidants in mammals (14). Toxic pesticides penetrate the skin upon contact, or enter the digestive system through contaminated vegetables and fruits that carry the remaining effects of these toxins, then reach the blood and all the body. Finally, settle in it and cause many serious diseases, including liver diseases, kidney failure, infertility, and cancer (15).

Natural products are an important means of discovering and creating new medicinal products for treating disorders (16). Propolis is a resinous natural product produced by honeybees. It has a good cytoprotective effect against different exogenous toxic agents (17).

Propolis is 50% resins, 30% wax, 10% essential oils, 5% pollen, and 5% different organic components, including flavonoids, mineral products, terpenes, and organic compounds, such as phenolic acids and their esters, fatty acids, aldehydes, stilbenes, and steroids. Propolis has antifungal, antibacterial, antipyretic, antiproliferation, and anti-inflammatory biological properties, which has been used in folk medicines (18).

Recent scientists have shown that propolis has a cytoprotective function because of its antioxidant property due to phenol compound ingredients (19,20). The antioxidant mechanism of the propolis is caused by phenolic compounds that donate hydrogen ions to free radicals to prevent oxidation and toxicity (21). Propolis can suppress free radicals, which are the leading cause of oxidation of lipid substances, nucleic acids, and proteins (22). Previous research has found that propolis can help prevent oxidative stress-induced hepatic and renal damage (23).

Kaempferol, catechin, quercetin, rutin, caffeic acid, coumaric acid, ferulic acid, caffeic acid phenethyl ester, or caffeic acid cinnamyl ester are all phenolic chemicals found in poplar-type propolis samples. They inhibit lipid peroxidation and protect cells from free radical damage and an excess of inflammation. Following the geographical origin, botanical source, race, sample season, and climate of the region, the chemical makeup of the propolis, and thus their biological activity, depends on (24). Several researchers have explored the influence on the chemical composition of the propolis of many components. As a result, several types of propolis may be found around the world, each of which is chemically distinct and has unique qualities and applications. Several investigations have shown that different propolis extracts from various sources have antibacterial (25,26), anti-inflammatory (27), cytotoxic (28,29), and immunomodulatory (30,31) properties.

This study's primary aim is to discover a possible and safe treatment to alleviate the oxidative stress induced by the insecticide in laboratory rats. Very little information is available from the previous studies, especially those related to bee products, protection, and treatment from the effects of toxins. Our promising results can be used and refined for the human situation in the future.

Materials and Methods

Chemicals

PM: (3-phenoxyphenyl) methyl 3-(2,2-dichloroethenyl)-2, 2-dimethylcyclopropane-1-carboxylate, Corn oil.

Propolis

A sample of Egyptian propolis was collected from Aga, Dakahlia, in the Center of Egyptian Delta (Agricultural Research Center), in 2019 and stored for five months at less than 20°C.

Measurement of Total Flavonoids (TFs) Content

The number of flavonoids was calculated following the Zhishen et al. (32), measured depending on the aluminum chloride ($AlCl_3$) compound. One milliliter of propolis extract, 4 ml of distilled water, and 0.3 ml of 5% $NaNO_2$ were mixed thoroughly. A 0.30 ml of 10% $AlCl_3$ was added after five-minute incubation at room temperature. In the sixth minute of incubation, 2 ml of 1M NaOH was added, and the mixture was formulated in distilled water with a total volume of up to 10 ml and mixed well. Using a Unicam UV-300 spectrophotometer at 510 nm, the absorbance was determined. TFs have been documented as dry weight (QE/g) mg quercetin/g, and findings were represented as mean values (Mn) ± standard deviation (SD).

HPLC Examination of Composites for Phenolics and Flavonoids

Using the slightly changed method adopted by a study of (33), the phenolic compounds and flavonoids present in propolis extract were analyzed. One gram of propolis was dissolved in 10 ml of ethanolic alcohol at 99% concentration for an hour at room temperature. The mixture was ultrasonicated for half an hour and then centrifuged at 10000 x g for 5 min. Propolis was filtered, left to evaporate, dried, and then dissolved in 1 ml of deionized water. Before injecting it into the HPLC, the collected extraction supernatant was filtered through a 0.45 µm WhaPMan® GF/F membrane. LC-1110 pump (GBC Scientific Equipment, Australia) and Kromasil column (Eka Chemicals Inc., Bohus, Sweden) were used to conduct chromatographic separation. Liquid chromatographic testing was conducted using water-acetic acid as mobile phase A at a concentration of 99:1 (volume/volume) and water-acetonitrile acetic acid as mobile phase B at a concentration of 67:32:1 (volume/volume/volume) in slope mode (0–10 minutes: 90% A and 10% B; 10–16 minutes: 80% A and 20% B; 16-20 minutes: 60% A and 10% B; 10–16 minutes: 80% A and 20% B; 16–20 minutes: 60% A). Notably, the flow rate was 0.8 and 1 ml/min, respectively, for

flavonoids and phenols. The injected sample volume was 20 µl, and the separation was done at 25°C. Then, at 280 nm, the phenols were detected while the flavonoids were tested with the GBC-UV/Vis reagent at 356 nm. Examining their reservation or retention periods with those of absolute norms, we evaluated the description of phenolics and flavonoids.

Chromatogram peaks were investigated using the chromatographic program Winchrom V1.3. Flavonoid and phenolic acid quantities were represented as microgram per gram (µg/g DW).

Antioxidant Assays in Vitro

DPPH ((2, 2-diphenyl-1-picrylhydrazyl) Radical Scavenging Activity

The strategy portrayed by (34) was implemented to survey the DPPH activities of the fluid concentrate of propolis. Summarily, 1 ml of each concentrate was added to 0.5 ml of 100 µM DPPH˙ arrangement (broke up in methanol). The blend was stirred well to be homogeneous and permitted to settle at room temperature within half an hour. The absorbance was estimated at 515 nm, using Unicam UV-300 UV/Vis spectrophotometer. Butylated hydroxytoluene (BHT) and Trolox were used as a reference antioxidant used to determine the DPPH· free-radical-scavenging activity. Data was expressed as DPPH˙ radical scavenging activity % and calculated as follows:

DPPH˙ radical repression activity (inhibition %) = [(Ac – As/Ac) × 100], where AC is the absorbance of the control reaction and As is the absorbance of the seaweed extract. All assessments were performed in three replicates. The lower the absorption of the reaction mixture, the higher the antiradical efficiency.

Radical Curbing Activity of ABTS+[2,2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)]

The test was conducted by (35). In brief, ABTS radical cations ($ABTS^{\cdot+}$) were created by responding to the solid oxidizing specialist potassium persulfate (2.6 mM) with ABTS salt (7.4 mM). The reaction was made by combining equivalent volumes (5 ml) of the two reagents and kept in dimness for 12–16 hours at room temperature. After that, the solution was diluted by combining 60 ml methanol and 1 ml of $ABTS^{\cdot+}$ radical cations; an absorbance estimation of 1.1 ± 0.02 at 734 nm was acquired by the Unicam UV-300 UV/Vis spectrophotometer. Approximately 150 µl of each examined substance was permitted to respond to 2850 µl of the newly arranged ABTS˙ radicals for two hours in obscurity and at room temperature. The absorbance was estimated at 734 nm. Trolox was utilized as a positive control. Data were communicated as $ABTS^{\cdot+}$ % and defined as follows:

$ABTS^{\cdot+}$ activity (inhibition %) = [(Ac – As/Ac) × 100], where AC is the absorbance of the control and AS is the absorbance of the seaweed extract.

Ferric-Reducing Antioxidant Power (FRAP)

The reducing capacity of bee venom and aqueous propolis in the extract was calculated using the (36). A 1 ml of propolis diluted specimen was blended completely with 2.5 ml of 50 mM phosphate support (pH 6.6) and 2.5 ml of 1% potassium ferricyanide. Then, the blend was hatched at 50°C for 20 min. Subsequently, a solution of about 2.5 ml of 10% trichloroacetic acid was added, and this mixture was centrifuged at 3000 × g for 10 minutes. The top layer was collected at 1.25 ml and mixed in 1.25 ml of deionized H_2O_2 and 0.25 ml of 0.1% $FeCl_3$, and the absorbance was assessed at 700 nm using a Unicam UV-300 UV/Vis spectrophotometer. The test was completed, its precision was established by performing it in triplicate, and the results were inferred from the Mn (µg/ml) ± SD.

GC-MS Analysis

The quantification of the propolis sample used in this study was carried out through gas chromatography, which is a chemical analysis device to separate and distinguish the chemicals in the samples. GC-MS was used in this study (Trace GC-ISQ; Thermo Scientific, Austin, USA). The stuffed injection port allows for a volume of the injected sample to be inserted into a TG-5MS high-capacity capillary (30 m x 0.25 mm x 0.25 micron film thickness) (37). Increasing the oven temperature leads to shortening the retention time, so the appropriate temperature must be determined for the required separation, and the reaction time should be limited. The column oven temperature was initially stabilized at 50°C and then increased 5°C/min until it reached 200° C for 2 minutes. Moreover, the thermal increase was continued until reaching the final temperature of 300°C at 25°C/min for 2 min. The temperatures of the injector and the MS transmission line were maintained at 270 and 260°C, respectively. The carrier gas used is helium gas with a continuous flow of 1 ml/min: the function of the carrier gas is to carry the sample through the system. Dilute 1 µl samples were injected automatically with the AS1300, and EI mass spectra were collected at 70 eV of ionization voltage over the range of m/z 50–650 complete scan technique. The ion source temperature has been set at 250°C.

The deconvolution of the essential oil's chemical constituents was conducted using AMDIS software, www.amdis.net, identified by their retention indices (relative to n-alkanes C8-C22) and mass spectrum matching to WILEY 09 and NIST 1 mass spectral databases (authentic standard).

Animals

Adult albino rats of both sexes, whose weights range between 160 and 180 g, were purchased from the Animal Husbandry House of the Nuclear Research Centre, Cairo, Egypt, and kept in the laboratory animal room where the temperature is 24 ± 3°C. Rats were subjected to a standard diet of pellet and ad libitum water and were acclimatized for one week before the trials began.

Analysis of Acute Toxicity of propolis

The experiment was carried out following the guidelines set out in Organization for Economic Co-operation and Development (OECD) (38).

Study Design

Forty rats were divided by stratified randomization into four groups (ten in each group, five males and five females): Group 1, control (no exposure to pesticides). A group II, rats were exposed to PM, which was dissolved in corn oil by intragastric tube at a dose of 15 mg/kg for 30 days ≈ 1/100 LD50 (39), the selected pesticide. A group III, normal rats, were given 100 mg/kg of propolis for three weeks. Finally, in group IV, rats were received PM (25mg/kg) by intragastric tube for 30 days and then received the propolis-MeOH extract (100 mg/kg b.wt.) for four weeks daily after 24 h of the last dose of PM.

All treated and control groups were kept in the same housing room (3 × 4 × 3.5 m) under identical conditions. After the final exposure, the rats were weighed, and then blood samples and various body tissues were taken under anaesthesia with Na-pentobarbital (60 mg/kg I.P.). Furthermore, the sera were isolated and used for biochemical measurements identified in this study. Liver, kidney, heart, lung, brain, spleen, pancreas, testes, small intestine, and ovarian tissues of all of the rats were removed for histopathological examination.

Collecting the Blood Samples

Blood samples were obtained under anesthesia from the orbital venous plexus in dry, sealed tubes. The sera were separated by centrifugation at 3000 x g for 15 min, divided into aliquots, and then stored at −20°C for further biochemical assays. Another set of blood samples was obtained in non-anticoagulant test tubes, allowed coagulation for 15 min at room temperature.

Measurement of Biochemical Parameters

Liver and Kidney Functions

Serum ALT, AST, ALP, and albumin were determined using a Biomerieux kit (France), total bilirubin using a Biocon kit (Biocon Diagnostik, Germany), and GGT (Fortress Diagnostics, Ltd., UK). Calculating total protein concentration (40) was conducted using Stanbio Laboratory kits. Creatinine, BUN, and kidney injury molecule-1 (KIM-1) concentrations were measured using Rat KIM-1 ELISA Kit (ab223858).

Heart markers, lipid peroxidation, and total antioxidant capacity

Serum CK-MB activity was assessed with the immune-inhibition method developed by Wurzburg et al. (41). LDH activity was determined enzymatically according to the method developed by Buhl and Jackson (42). The lipid peroxidation (TBARS) assay was calculated using the (43) process. The total antioxidant capacity (TAC) of serum was evaluated according to the design of Benzie and Strain (44).

Detection of Cytokines

Serum cytokine levels such as interleukins 6, 12 (IL-6, IL-12), and tumor necrosis factor-alpha (TNF-α) were appraised by ELISA using commercially prepared kits (R&D Systems, USA) according to the manufacturer's guidance. The cytokine concentrations were then calculated from the standard cytokine curve incorporated into the same sample plate.

Checks of Programmed Cell Death

The expressions of apoptotic indices, caspase-3, BAX, and BCL-2, were evaluated using the ELISA kit according to the manufacturer's instructions.

Detection of Rat Nuclear Factor-Kappa (NF-kB)

Plasma from all experimental animal groups was used to determine NF-kB activity, using a commercially available nuclear extraction kit from (Wuhan Fine Biotech Co., Ltd., Catalogue No.: ER1186), sensitivity: 0.094ng/ml.

Determination of T3, T4, and Acetylcholinesterase

Acetylcholinesterase (AChE) activities were determined by Ellman's method (45) using Química Clínica Aplicada kits.

Hematological Parameters

Total white and red blood cells, hemoglobin concentration (Hb), platelet count (PLT), and PCV percentage) were defined using Sysmex (Automated Hematology Analyzer Kx-2IN, Sysmex Corporation, Kobe, Japan). It applies a white blood cell (WBC) indicator obstacle and a WBC/HGB lyse reagent to estimate the WBC count and Hb concentration. Blood was assembled into microfuge tubes, which comprised EDTA whole blood, 44.7 pl, for electronic analyses, was aspirated into a calibrated pipette, and suspended in 10 ml Isotone I1 medium (Unopette Microcollection System, Becton Dickinson, Rutherford, NJ, USA). Extra 5 pl of blood was diluted in 1 ml red blood cell (RBC) suspension media for morphologic tests, whereas the remainder was aspirated into a thin 12 cm long polyethylene capillary tube (key 19-0040-0 1, Pharmacia, Uppsala, Sweden). The capillary tube was sealed at one end by considerately heating it above a flame. Each group of tubes had been carefully calibrated by weighing the water included through a definite length. The tubes with blood were centrifuged at 1500 x g for 15 min (4°C) maintained by a firm handle. The microhematocrit was recorded and 25.0 pl of compact RBC was separated from the midsection of the RBC column and suspended in 50 ml of buffer to generate a hematocrit of 0.05%.

Histological Examinations

Visceral samples of the liver, kidneys, small intestine, and spleen and tissues of the lungs, heart, pancreas, testes, ovary, and brain were taken from the control and experimental groups. Then a 10% formalin fixation was produced for approximately 24 hours. After various steps, according to (46), paraffin sections were cut at a thickness of 4 μm. These sections were stained with hematoxylin-eosin and examined with light microscopy.

Statistical Analysis

The parameter preferences were all revealed as the mean ± SEM. Significant differences among the groups were determined by a two-way analysis of variance (ANOVA) accompanied by Tukey's multiple comparison analyses utilizing the

SPSS 23.0 software packaging program. The results were recognized as notable if $P < 0.05$.

The variation in levels of biochemical parameters due to insecticide and propolis regimens can be assessed by estimating the percentage change in the insecticide-treated groups compared to normal values. To find out if the variation from normal values is due to insecticides, we used the following formula:

$$\% \text{ Change} = \frac{\text{Treatment Value - Control Value}}{\text{Control Value}} \times 100$$

Results

In Vitro Analysis

From the analysis of HPLC/MS, the biochemical evaluation of propolis and bee venom showed that the most abundant compounds in propolis were gallic acid, catechin, chlorogenic acid, and cinnamic acid in this extract (Table 1a and Figs. 1A &B). The results indicated that the concentration of total phenolic of propolis was 140.45 ± 0.61 mg Gallic acid/g DW, whereas that of flavonoids was 91.92 ± 0.70 mg QE/g DW. Moreover, the ferric-reducing antioxidant ability (FRAP) with an EC50 for propolis was 3.172 ± 0.259 µg/ml. Furthermore, the TAC of propolis was 4.00± 0.049 mg Gallic acid/gm and DPPH scavenging activity for propolis had an IC50 value of 0.320 ± 0.02. The radical scavenging activities of DPPH and ABTS, its FRAP, and the antioxidant properties of propolis-MeOH have been assessed. DPPH scavenging activity with the regular antioxidant BHT was shown by the propolis-MeOH extract to have an IC50 value of 10.45 ± 0.08 µg/ml. The propolis- MeOH extract exhibited an IC50 value of 170 ± 1.66 µg/ml, concerning the ABTS·+ quenching activity, but the regular Trolox had an IC50 concentration of 12.25 ± 1.31 µg/ml.

Phenolic and flavonoid compounds identified in the propolis-MeOH extract, as obtained by HPLC, are listed in Table 1a. Identification of these antioxidant compounds was primarily based on a comparison of their relative retention times with those obtained from the different available standard compounds.

The structures (Fig. 1C) illustrated the peaks, which were recognized by computer explorations in the reference libraries. A full summary of the library search results with the Wiley Registry and National Institution of Standards and Technology (NIST) mass- spectral library (Table 1b).

In Vivo Analysis

Body Weight

Two-way ANOVA showed that treatments have a significant effect on the body weight (BW) at the end of experiment, $F2 (1, 18) = 44.39$, $p < 0.05$, but the differences were nonsignificant for sex $F1 (1, 18) = 0.579$ and the interaction between sex and treatment, $F1*F2 (1, 18) = 0.424$, $p > 0.05$. On the other hand, the rats from the group exposed to insecticides, which were under study a week after exposure, had a significant decrease of the BW in males and females (Figs. 2A & B, respectively.).

Table 1a. HPLC analysis of phenolic and flavonoid compounds of BV and propolis whereas P<0.05, concentration (µg/g DW).

Compound	Propolis
P Coumaric acid	2322.83±1.02
Gallic acid	48.10±0.80
Chlorogenic acid	39.14±1.02
Cinnamic acid	73.79±0.65
Ferulic acid	2267.51±1.21
Rosamiric acid	589.78±0.92
Caffeic acid	278.90±0.55
Catechin	2540.02±1.55
Hesperidin	43.21±0.07
Kaempferol	178.65±0.81
Quercetin	1269.0±1.44
Rutin	433.36±0.60

As illustrated in Fig 2, rats were given PM showed a remarkable decrease in the average BW by 30.70% and 25.20% in males and females, respectively, compared to the control rats at the end of the experiment. However, the BW of rats given propolis-MeOH extract increased by 29.27% and 35.43% in males and females, respectively.

Liver and Kidney Functions

Liver functions represented by serum ALT, AST, bilirubin, and ALP activities besides, total bilirubin concentration were remarkably increased because of PM administration. AST was significantly ($p < 0.05$) increased in serums of the rats given PM with average values of 69.51 ± 3.42 U/l (131.47%) and 56.30 ± 1.33 U/l (116.46%) in males and females, respectively, compared to 30.03 ± 2.46 U/l and 26.01 ± 1.07 U/l in the control group. Also, administration of PM-induced a significant increase of ALT in males and females (287.75% and 169.90%, resp.). The total protein, albumin, and globulin levels were increased in a dose-dependent manner (Tables 2a & b). Interestingly, the proinflammatory GGT showed a significant ($p < 0.05$) concentration in animals given insecticide PM in males (0.05 ± 0.002 U/l; 85.29%) and (0.023 ± 0.002 U/l; 91.79%) in females much lower than that of the controls. There was a significant improvement for these biomarkers in all rats given propolis-MeOH.

Two-way ANOVA indicated that the serum creatinine, BUN, and KIM-1 levels were significantly increased due to insecticide administered to rats (Table 2c). The percent changes in these parameters were creatinine, 317.65% in males and 331.71% in females; BUN, 284.41% in males and 163.84% in females; KIM-1, 203.07% in males and 225.22% in females, compared to controls. However, the kidney functions were significantly ($p < 0.05$) improved after the propolis-MeOH treatments.

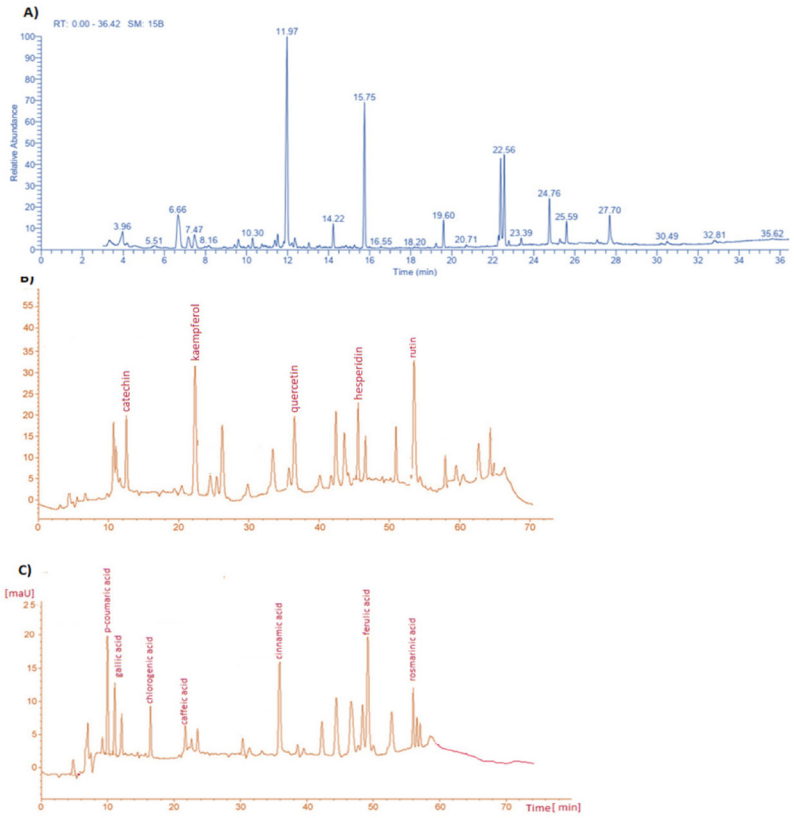

Figure 1. A) HPLC technique for the analysis of flavonoids from propolis; B) HPLC technique for the analysis of phenols from propolis; C) GC. MS analysis for the selected insecticides, the peaks which were recognized by computer explorations in the reference libraries.

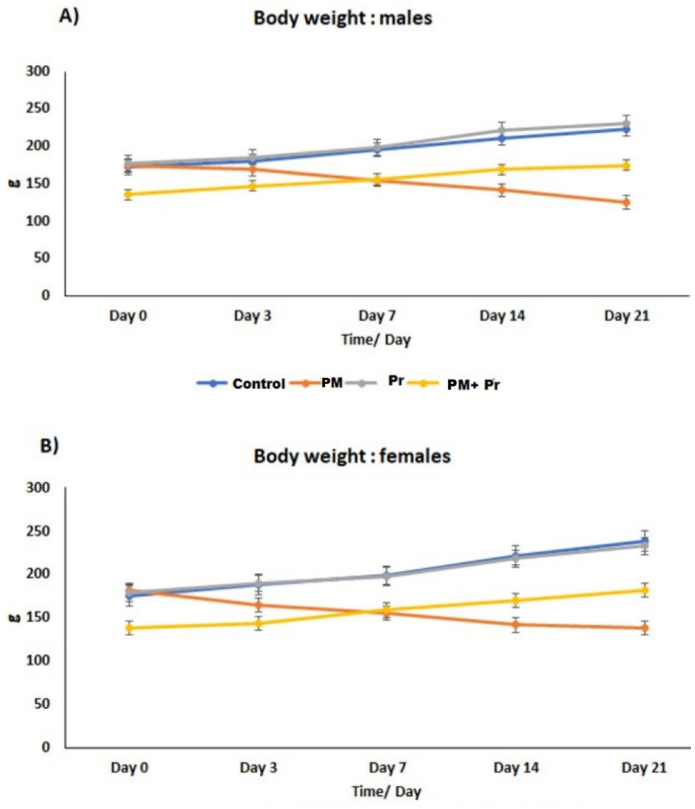

Figure 2. Effect of PM and treatment of propolis 100 mg/kg before and after insecticide exposure on body weight A) males and B) females.

Table 1b. Indicates the GC MS results of Propolis, the Retention time, name of the probable compound and percentage peak values of each peak.

RT	Name of the compound	Formula	MW	Peak area %
3.97	1,2-Benzenedicarboxylic acid, bis(8-methyl nonyl) ester	$C_{22}H_{34}O_4$	126	1.72
6.66	6-Octen-1OL,3,7-Dimethyl	$C_{10}H_{20}O$	156	7.18
7.17	2,6-Octadien-1-OL, 3,7-Dimethyl-, E-Geraniol	$C_{10}H_{18}O$	154	1.85
7.46	6-Octen-1-OL, 3,7-Dimethyl-, Formate	$C_{12}H_{22}O_2$	198	2.23
9.60	Cyclobuta [1,2:3,4] dicyclopentene	$C_{15}H_{24}$	204	1.07
10.29	Bicyclo [7.2.0] Undec-4-Ene, 4,11,11-Trimethyl-8-Methyle NE-, [1R-(1R*,4E,9S*)]-Caryophyllene	$C_{15}H_{24}$	204	1.18
11.38	7-Tetradecene	$C_{14}H_{28}$	196	0.94
11.52	1,6-Cyclohexadiene,1-Methyl-5-Methylene-8-(1Methylethyl)-,[S-(E, E)]-	$C_{15}H_{24}$	204	1.38
11.98	1-Dodecanamine, N, N-dimethyl-	$C_{14}H_{31}N$	213	28.20
12.25	2',6'-Dihydroxy-4'-methoxychalcone	$C_{16}H_{14}O_4$	204	1.28
13.04	Butanoic acid,3,7-dimethyl-2,6-Octadienyl ester, (E)-	$C_{14}H_{24}O_2$	224	0.61
14.22	3 –phenylchromen- 4-one (Isoflavone)	$C_{15}H_{10}O_2$	222	2.58
15.75	1-Tetradecanamine, N, N-dimethyl-	$C_{16}H_{35}N$	241	15.83
19.24	5-hydroxy-7-methoxy-2-phenylchromen-4-one (Tectochrysin)	$C_{16}H_{12}O_4$	268	24.34
19.60	Hexadecenoic acid, methyl ester	$C_{17}H_{34}O_2$	270	2.74
22.27	8,11-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	0.81
22.38	9-Octadecenoic acid (Z)-, Methyl ester	$C_{19}H_{36}O_2$	296	8.34
22.56	1-(3-(N-Benzyl-N-methylamino)-1,2-propanediol	$C_{11}H_{17}NO_2$	195	9.21
22.78	Heptadecanoic acid, 16-Methyl-, methyl ester	$C_{19}H_{38}O_2$	298	0.51
23.39	Ethyl Oleate	$C_{20}H_{38}O_2$	310	0.75
24.76	Eicosen-1-ol, cis-9-	$C_{20}H_{40}O$	296	4.66
25.27	Hexadecenoic acid,2,3-dihydroxypropyl ester	$C_{19}H_{38}O_4$	330	0.53
25.59	N-Methyl-N-benzyltetradecanamine	$C_{22}H_{39}N$	317	2.36
27.70	Glycidyl oleate	$C_{21}H_{38}O_3$	338	3.56

Heart Markers, LP, and Total Antioxidants

Two-way ANOVA revealed that the effects of insecticide toxicity on the LDH, CK-MB, LP, and total antioxidants (TA) activities were increased in a dose-dependent manner in males and females (Table 3). LDH showed significant ($p < 0.05$) increases in the rats given PM with average values of 363.97 ± 24.12 U/l (173.64%) in males and 315.50 ± 17.43 U/l (148.43%) in females, compared to the control (133.01 ± 8.93 and 127.0 ± 10.33 U/l, respectively). Moreover, CK-MB was significantly ($p < 0.05$) increased after insecticide administration with average values of 723.75 ± 18.23 U/l (241.63%) in males and 809.75 ± 22.17 U/l (258.30%) in females, compared to the controls. LP levels were also significantly ($p < 0.05$) increased after PM-administration to be 46.75 ± 2.51 (401.07%) g/dl in males and 52.10 ± 2.45 (523.95%) g/dl in females, compared to 9.33 ± 0.41 and 8.35 ± 0.66 g/dl, respectively, in the controls. On the other hand, a significant decline was observed in TA in rats given PM with average values of 0.24 ± 0.002% (82.98%) in males and 0.07 ± 0.004% (95.48%) in females, compared to the controls. However, cardiac levels, LP, and TA were significantly ($p < 0.05$) improved after the propolis-MeOH treatments.

Proinflammatory Cytokines

The cytokine concentrations of control and treatment groups are shown in Table 4. A significant increase of proinflammatory cytokines was seen after PM administration, which indicates the rapid severity of the inflammatory activity. Regarding TNF-α, after the exposure to PM, the TNF-α concentration increased by 152.51% and 166.02% in males and females, respectively, compared to the control. A notable increment of IFN-gamma was observed after the exposure to PM in males and females, 194.14% and 171.32%, respectively, compared to the control. Similarly, there was a significant increase of IL-6 in males and females after the exposure to PM, 889.90% and 1053.42%, respectively, compared to the control.

Compared to normal rats, a significant increase of IL-12 was observed in males and females after exposure to PM by 190.15% and 180.65%, respectively. However, the levels of these cytokines were significantly ($p < 0.05$) decreased in all the treated groups, using the propolis-MeOH extract.

Table 2a. Effect of insecticides on the liver functions

Groups	AST (U/l)		ALT (U/l)		GGT (U/l)		Bilirubin (U/ l)	
	Males	Females	Males	Females	Males	Females	Males	Females
GI	30.03±2.46^{b}	26.01±1.07^{b}	16.25±1.63^{c}	20.75±0.95^{c}	0.34±0.02^{a}	0.28±0.01^{a}	0.32±0.04^{c}	0.29±0.01^{c}
GII	123.75±10.42^{a}	56.30±1.33^{a}	63.01±2.94^{a}	56.00±1.82^{a}	0.05±0.01^{c}	0.023±0.002^{c}	1.47±0.02^{a}	1.16±0.02^{a}
GIII	35.76±211.44^{b}	31.87±1.33^{b}	22.81±1.54^{b}	28.00±1.28^{b}	0.31±0.01^{c}	0.30±0.002^{c}	0.37±0.02^{bc}	0.31±0.02^{bc}
GIV	34.70±1.47^{b}	29.20±1.20^{b}	22.70±1.30^{bc}	24.03±0.95^{bc}	0.22±0.004^{d}	0.21±0.002^{d}	0.41±0.03^{b}	0.33±0.02^{b}
F1	17.35		199.19		84.57		112.31	
P-value	0.000		0.000		0.000		0.000	
F2	2.11		328.60		5.84		65.11	
P-value	0.16		0.000		0.024		0.000	
F3	0.98		12.43		0.751		20.48	
P-value	0.42		0.000		0.53		0.000	

Values are expressed as means ± SD. Different superscript letters in the same row indicate significant differences at p< 0.05.

Table 2b. Effect of insecticides on the liver functions

Groups	ALP (U/l)		Total protein (g/dl)		Albumin (g/dl)		Globulin (g/ dl)	
	Males	Females	Males	Females	Males	Females	Males	Females
GI	133.01±8.93^{c}	127.00±10.33^{c}	5.22±0.37^{a}	5.26±0.53^{a}	4.28±0.28^{a}	4.68±0.47^{a}	0.91±0.09^{ab}	0.53±0.06^{ab}
GII	363.97±24.12^{a}	315.50±17.43^{a}	3.53±0.04^{c}	3.10±0.07^{c}	2.57±0.02^{b}	3.77±0.06^{b}	0.85±0.02^{bc}	0.71±0.01^{bc}
GIII	137.21±13.12^{b}	134.56±10.43^{b}	5.10±0.41^{c}	5.22±0.08^{c}	4.74±0.21^{b}	4.40±0.06^{b}	0.74±0.20^{bc}	0.58±0.02^{bc}
GIV	138.25±11.83^{b}	132.01±11.09^{b}	4.74±0.29^{c}	4.12±0.40^{c}	4.19±0.25^{b}	3.68±0.30^{b}	1.01±0.04^{c}	1.54±0.10^{c}
F1	261.94		36.74		23.54		4.70	
P-value	0.000		0.000		0.000		0.010	
F2	150.04		2.18		3.48		0.09	
P-value	0.000		0.15		0.07		0.76	
F3	94.70		1.42		6.43		2.26	
P-value	0.000		0.26		0.002		0.11	

Values are expressed as means ± SD. Different superscript letters in the same row indicate significant differences at p< 0.05. GI: Control, GII: TM, GIII: Pr, GIV: TM+ Pr.

Table 2c. Effect of insecticides on the kidney functions

Groups	BUN (U/l)		Creatinine (g/dl)		KIM-1 (pg/ml)	
	Males	Females	Males	Females	Males	Females
GI	27.19±1.40^{d}	31.25±1.26^{d}	0.34±0.04^{b}	0.41± 0.06^{b}	322.50±15.71^{c}	345.92±20.55^{c}
GII	104.52±9.54^{b}	82.45±3.72^{b}	1.42±0.07^{a}	1.77±0.11^{a}	977.39±26.71^{a}	1125±22.65^{a}
GIII	31.98±3.54^{b}	38.46±2.72^{b}	0.39±0.07^{b}	0.33±0.11^{b}	328.11±26.71^{bc}	351.02±22.65^{bc}
GIV	40.26±5.70^{a}	38.28±4.12^{a}	0.27±0.05^{b}	0.31±0.05^{b}	330.41±20.00^{b}	371.34±25.30^{b}
F1	147.61		137.38		55.87	
P-value	0.000		0.000		0.000	
F2	17.09		11.95		67.12	
P-value	0.004		0.003		0.000	
F3	22.23		28.99		22.34	
P-value	0.000		0.000		0.000	

Values are expressed as means ± SD. Different superscript letters in the same row indicate significant differences at p< 0.05. GI: Control, GII: TM, GIII: Pr, GIV: TM+ Pr.

Table 3. Effect of insecticides on the LDH, CK-MB, LP, and TA

Groups	LDH (U/l)		CK-MB (U/l)		LP (g/dl)		TA (%)	
	Males	Females	Males	Females	Males	Females	Males	Females
GI	225.50±12.23^{c}	321.0±9.81^{c}	211.86±14.16^{c}	226.0±13.80^{c}	9.33±0.41^{a}	8.35±0.66^{a}	1.41±0.004^{a}	1.55±0.02^{a}
GII	909.50±15.31^{a}	889.50±22.14^{a}	723.75±118.23^{a}	809.75±22.17^{a}	46.75±2.51^{c}	52.10±2.45^{c}	0.24±0.02^{c}	0.07±0.004^{c}
GIII	229.70±15.31^{a}	327.75±22.14^{a}	208.12±118.23^{a}	233.80±14.17^{a}	15.65±2.51^{c}	17.23±1.45^{c}	1.50±0.02^{ab}	1.34±0.004^{ab}
GIV	232.80±11.21^{a}	327.30±23.15^{a}	210.30±25.12^{a}	231.40±30.44^{a}	12.01±1.41^{d}	11.41±1.34^{d}	1.33±0.01^{b}	1.38±0.003^{b}
F1	241.50		235.44		190.66		138.31	
P-value	0.000		0.000		0.000		0.000	
F2	290.46		124.09		105.011		7.67	
P-value	0.000		0.000		0.000		0.01	
F3	54.41		19.52		42.80		11.85	
P-value	0.000		0.000		0.000		0.000	

Values are expressed as means ± SD. Different superscript letters in the same row indicate significant differences at $p< 0.05$. GI: Control, GII: TM, GIII: Pr, GIV: TM+ Pr.

Table 4. Effect of insecticides on cytokines in the experimental rats

Groups	TNF- α (pg/mg)		INF-γ (pg/ml)		IL-6 (pg/mg)		IL-12 (pg/ ml)	
	Males	Females	Males	Females	Males	Females	Males	Females
GI	37.50±2.08^{b}	29.70±1.23^{b}	209.68±18.16^{b}	252.02±21.07^{b}	30.66±2.10^{c}	39.50±2.03^{c}	72.38±4.05^{b}	83.50±9.08^{b}
GII	94.69±6.80^{a}	79.01±3.46^{a}	617.14±12.66^{a}	683.78±20.13^{a}	303.5±15.56^{a}	455.60±12.89^{a}	210.01±11.21^{a}	234.34±20.16^{a}
GIII	39.20±3.80^{b}	35.16±1.41^{b}	214.15±10.01^{b}	258.08±15.19^{b}	30.25±3.33^{c}	37.51±4.82^{c}	80.33±6.25^{b}	86.81±7.06^{b}
GIV	44.20±2.10^{b}	34.60±1.24^{b}	217.42±18.31^{b}	273.13±21.34^{b}	36.48±2.50^{b}	43.03±5.10^{b}	76.54±8.39^{b}	88.48±7.26^{b}
F1	170.46		33.20		9.98		67.51	
P-value	0.000		0.000		0.000		0.000	
F2	23.31		43.21		55.34		26.53	
P-value	0.000		0.000		0.000		0.000	
F3	1.62		18.49		91.21		4.27	
P-value	0.211		0.000		0.000		0.019	

Values are expressed as means ± SD. Different superscript letters in the same row indicate significant differences at $p< 0.05$.

Effect of Selected Pesticides on BAX, BCL2, and NF-KB

In this current study, the extent of the adverse effect of PM-administration was observed on BAX and BCL2, and NF-KB. PM induced a significant increase ($p < 0.05$) in both BAX and NF-KB over 100% in males and females, whereas the opposite resulted in BCL2 (>95%) in males and females (Fig. 3). On the other hand, the levels of these parameters were significantly improved in all the treated groups, using the propolis-MeOH extract to concentrations much closer to the control.

Effect of Pesticides on T3, T4, and AChE

Figure 4 A-C shows the effect of PM on T3, T4 (Figs. 4A &B respectively), and AChE (Fig. 4C). There was a significant increase ($p<0.05$) in T4, whereas there was a significant decrease ($p<0.05$) in both T3 and AChE. However, the levels of these parameters were significantly improved in all the treated groups with the propolis-MeOH extract to levels much closer to the control.

Hematological Counts

It was also found that the hematological counts RBCs, PCV, Hb, and WBCs decreased in rats given PM in males (37.28%, 66%, 48.65%, and 68.57%, respectively) and females (54.20%, 64.2%, 25.57%, and 44%), $p<0.05$, compared to controls (Table 5). The MCH% was significantly increased because of the toxic PM, where the percentages were 42.75% in males and 78.90% in females (Table 5). Interestingly, insecticide-induced stressed rats treated with the propolis-MeOH extract exhibited improvement in the hematological counts.

Histopathological Findings

Normal histopathological structure of the liver, kidney, lung, heart, and brain was present in the nontreated control group

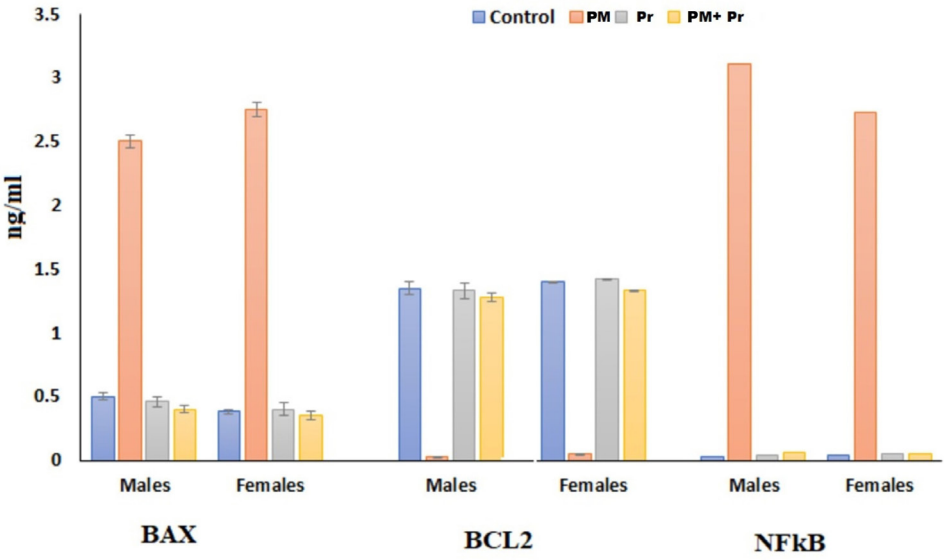

Figure 3. Effect of PM and treatment of propolis 100 mg/kg after insecticide exposure on BAX, BCL2 and NFKB in male and female rats.

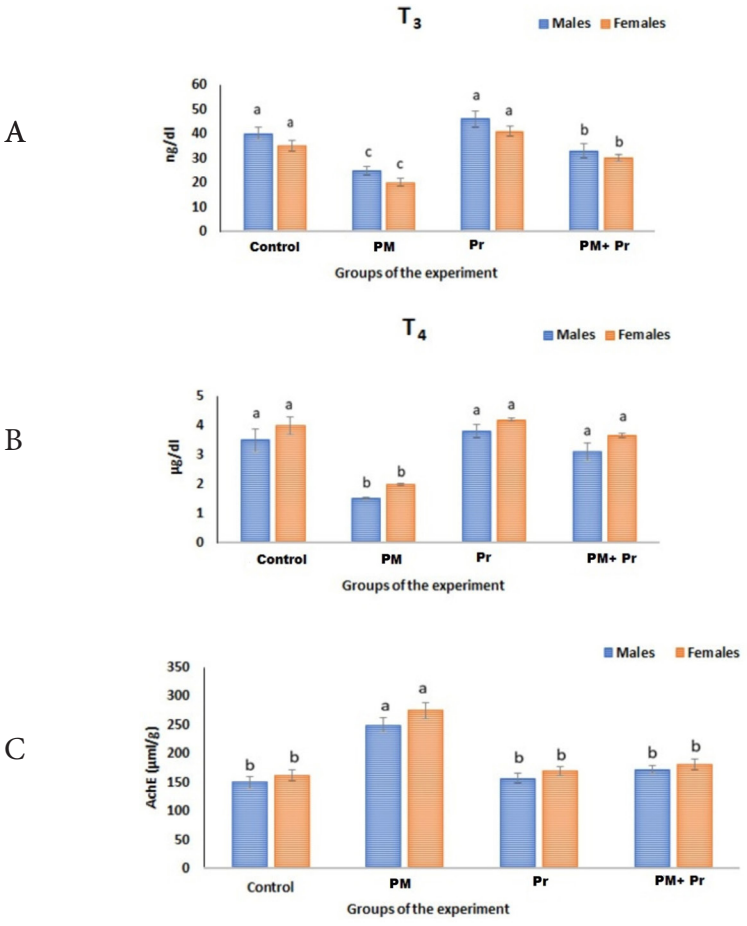

Figure 4. Effect of PM and treatment of propolis 100 mg/kg after insecticide exposure on A) T3 and B) T4 & C) AChE in male and female rats.

Table 5. Effect of insecticides on the hematological constituents

Groups	RBCs (g/dl)		PCV (%)		MCH (%)		Hb		WBCs (x 106/mm³)	
	Mªles	Femªles	Mªles	Femªles	Mªles	Femªles	Mªles	Femªles	Mªles	Femªles
GI	11.56± 0.75^{a}	12.01± 74^{a}	44.63± 4.60^{b}	41.15 ± 2.14^{b}	29.43±68^{c}	25.18± 1.35^{c}	12.19±1.05^{a}	13.10±0.61^{a}	8.05±0.36^{a}	7.32±0.50^{a}
GII	7.25± 0.35^{b}	5.50 ± 0.81^{b}	15.18± 1.27^{c}	14.56± 1.28^{c}	42.01± 1.82^{a}	45.33± 3.32^{a}	6.26±0.23^{c}	9.75±0.74^{c}	2.53±0.29^{b}	4.10±0.04^{b}
GIII	12.14± 0.31^{a}	11.85± 0.56^{a}	50.75± 1.42^{a}	47.35± 1.31^{a}	34.50± 1.38^{b}	31.61± 2.92^{b}	11.60±0.44^{a}	12.56±0.52^{a}	7.55±0.06^{a}	7.70±0.08^{a}
GIV	11.23± 0.52^{a}	10.89± 0.73^{a}	39.25± 5.12^{b}	38.85 ±3.29^{b}	25.00± 1.91^{c}	30.30± 0.63^{c}	10.88±0.31^{b}	10.30±0.62^{b}	7.08±0.04^{a}	6.70±0.05^{a}
F1	191.17		112.97		234.21		50.31		67.51	
P-value	0.000		0.000		0.000		0.000		0.000	
F2	6.40		0.03		0.451		23.43		0.74	
P-value	0.019		0.87		0.51		0.000		0.40	
F3	5.76		3.18		40.86		5.96		4.79	
P-value	0.004		0.042		0.000		0.004		0.02	

Values are expressed as means ± SD. Different superscript letters in the same row indicate significant differences at $p< 0.05$. GI: Control, GII: TM, GIII: Pr, GIV: TM+ Pr.

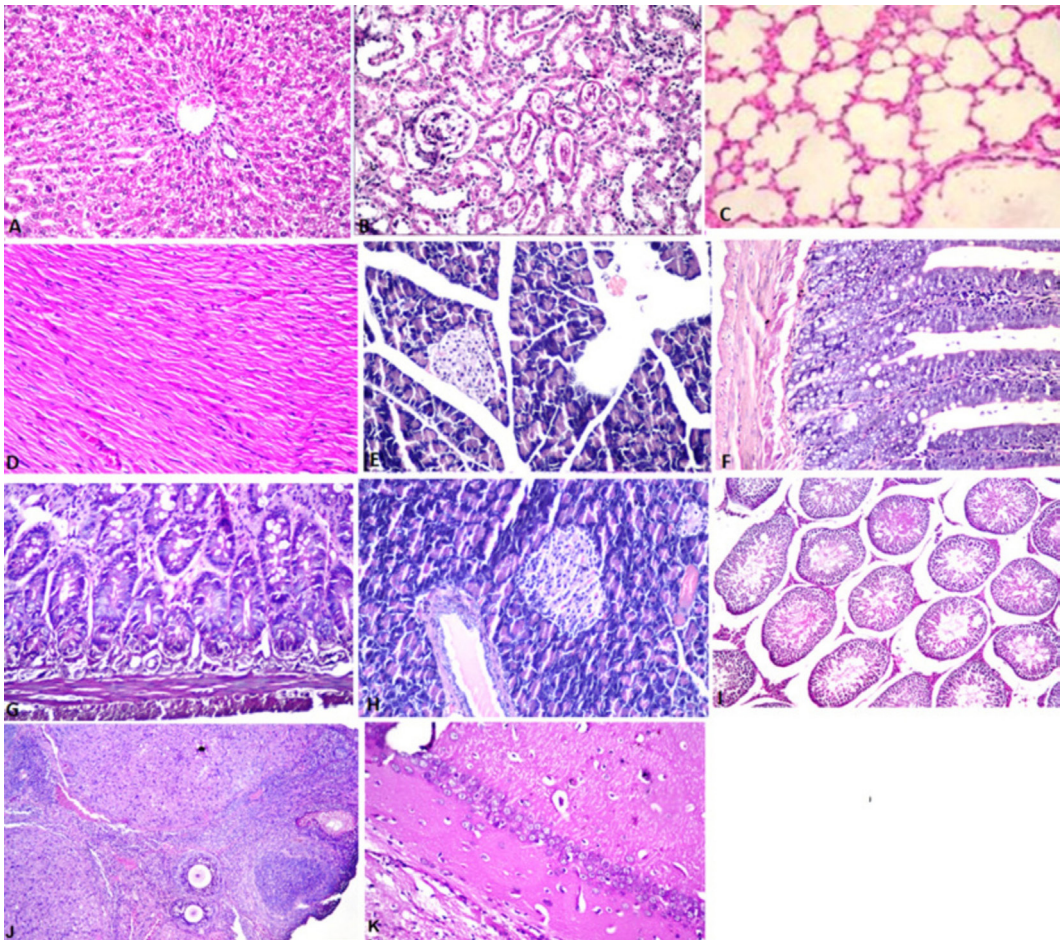

Figure 5. Histological examinations in control rats A) Control liver, B) Control kidney, C) Control lung. D) Control heart, E) Control spleen, F) Control stomach, G) Control small intestine, H) Control pancreas, I) Control testes, J) Control ovary, K) Control brain.

(Figs. 5A-K) and also rats were given the propolis-MeOH extract.

Microscopic examination of the livers of rats poisoned with PM - showed devastating effects compared to the control group (6A &B), where the liver showed congestion in the central and pyloric veins and hepatic sinuses with the activation of the inflammatory liver cells, fibrosis in the entrance area, and the appearance of the hepatic parenchyma and focal necrosis.

In the kidneys of rats administered PM, perivascular and periglomerular inflammatory cells infiltration with dilatation in the blood vessels was observed. Moreover, degeneration in the tubular lining epithelium was detected at the cortex of the kidneys in the rats fed R (Fig. 6C).

The effect of the pesticide used in this study on the lungs was also observed (Fig. 6D). There was bronchiolar epithelial hyperplasia with peribronchiolar fibrosis and lymphoid hyperplasia and collapse with emphysema in the air alveoli. There was also focal bleeding between the myocardial bundles due to PM- administration (Fig. 6E).

In the spleen in the rats administered PM, there was a lymphoid depletion in the white pulps with ischemia in the red pulps and sinusoids (Fig. 6F).

The mucosal layer of the stomach showed focal inflammatory cell aggregation, and a massive amount of focal inflammatory cell infiltration was observed in rats treated with PM (Fig. 6G).

In addition, in the small intestine in rats administered PM, inflammatory cell infiltration was detected in the lamina propria of the villi (Fig. 6H).

There were histopathological alterations in the pancreas in rats administered with PM on circumscribed around anaplastic cells proliferation in the islet of Langerhans cells with atrophy in the surrounding acini (adenoma) (Fig. 6I).

PM led to a loss of the spermatogenic sets in the lumen of the seminiferous tubules with edema in the interstitial Leydig cells (Fig. 6J). Sever congestion was observed in the medullary blood vessels with mature Graafian follicles and corpus luteum (Fig. 6 K).

The brain has been affected by the abuse of this pesticide (Fig. 6 L-N). Nuclear pyknosis and degeneration were detected in the neurons of the cerebral cortex (Fig.6L) and the neurons of the fascia dentate in the hippocampus (Fig. 6M). The striatum showed diffuse gliosis with congestion in the blood vessels (Fig. 6N).

In contrast, the administration of the propolis-MeOH extract revealed a more normal histological picture in the structure of the studied organs compared to the PM - group (Figs. 7 A-L).

Discussion

We have shown here that propolis MeOH extracts can effectively protect against PM-induced cytotoxicity. Different chemical combinations are required for propolis samples to have biological action. With the identification of their anticancer (24) and antioxidant properties (25), these chemicals isolated from propolis have sparked a lot of attention. Phenolic composites (flavonoids and phenolic compounds) are the pharmacologically active ingredients in propolis.

Interestingly, propolis demonstrated powerful antioxidant activities in vitro (DPPH and FRAP scavenging activities), in addition to having high phenolic and flavonoid contents (47). The observed significant antioxidant activities may be attributable to the gallic acid derivatives, coumaric acid derivatives, caffeic acid derivatives, ellagic acid, and resveratrol in Egyptian propolis. These findings are similar to those from previous studies, where propolis is from Malaysia (48).

The significant interest in these compounds extracted from propolis has arisen with the fulfillment of their antibacterial, anticancer, and antioxidant effects. The propolis-MeOH extract did not produce any indication of toxicity of up to 3,000 mg/kg BW under the protocol followed by this study. A similar study found that propolis extract in rats was safe for up to 2000 mg/kg at a dose level (49). Ramadan et al. (50), in also the subchronic study, indicated that the tested extracts did not produce any significant change in liver and kidney functions of rats, following oral administration for eight successive weeks at doses of 500 mg/kg. This study aimed to evaluate the therapeutic effect of propolis on nutritional damage and exposure to the toxic material PM. In this study, the animals treated with PM had stopped eating because of the bioaccumulation of PM inside their bodies, which confirms that the fat-soluble pesticides pose a health risk (51). Further investigations have determined that virulent chemicals build up quickly in the tissues, generating mortality and influencing growth in the animals (52). Pyrethroids are artificial chemical pesticides that have analytic compositions adapted from pyrethrin's chemical fabrications and serve analogously as pyrethrin (53). A study showed that the synthetic pyrethroid had a critical derma-toxicity of 330 mg/kg BW/day, which was significantly high in rats (54).

As illustrated in Figure 2, were given PM showed a remarkable decrease in the average BW by 30.70% and 25.20% in males and females, respectively, compared to the control rats at the end of the experiment. However, the BW of rats given propolis-MeOH extract increased by 29.27% and 35.43% in males and females, respectively.

The toxicity and bioaccumulation of PM in rats and their results suggested repression of some metabolic enzymes emanating from the gathering of the insecticide ingredients in the tissues. Thus, the accumulation of pesticides in these organs may indicate that the active ingredients are also being intercepted, leading to decreased enzyme synthesis, which should accelerate immediate metabolic processes, but are instead concentrated and predominant in organ tissues. Besides, the retention time may be slight within 10 minutes due to the ease of dissolution and low excretion rate leading to bioaccumulation (55).

The liver produces enzymes and structural proteins that aid in detoxification, and for these functions, liver cells use enzyme systems (56). The current study indicated an increase in liver enzymes and total bilirubin concentration, as well as ALP. A study previously revealed a significant increase in serum C-re-

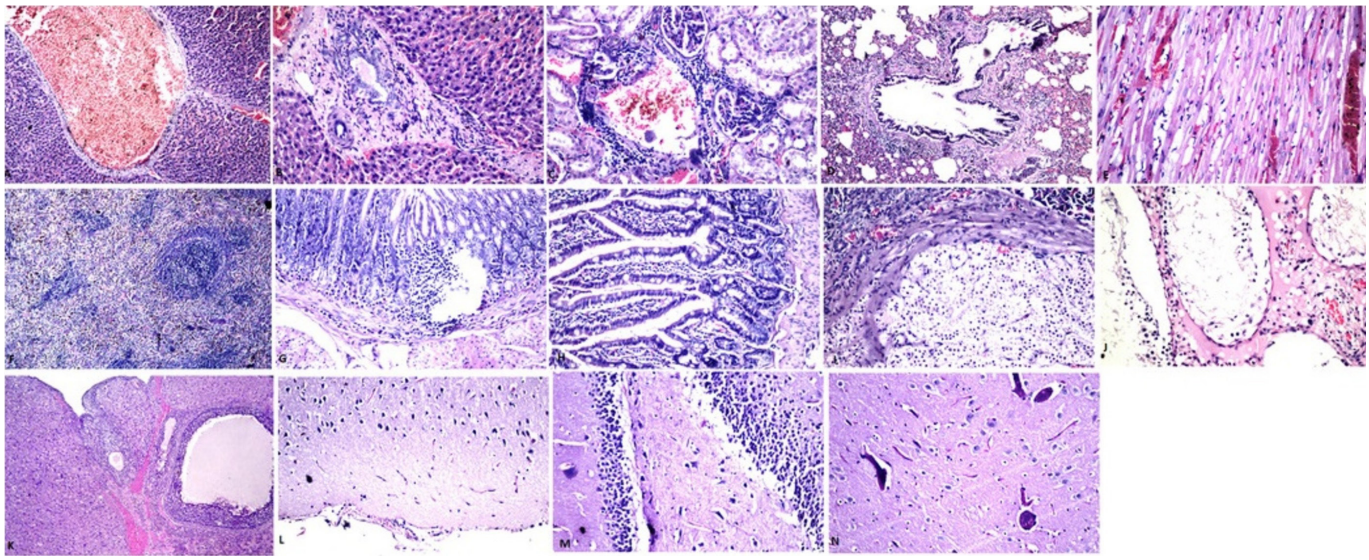

Figure 6. Effect of PM on the tissues of rats. A, B) Liver: Congestion was detected in the central and portal veins with fibrosis in the portal area. C) Perivascular and preglomerular inflammatory cells infiltration in kidney. D) There was bronchiolar epithelial hyperplasia with peribronchiolar fibrosis in the lung. E) Sever congestion was observed in the blood vessels and capillaries in the myocardium. F) There was lymphoid depletion in the white pulps in spleen. G) The mucosal layer showed focal inflammatory cells aggregation in the stomach. H) Inflammatory cell infiltration was detected in the lamina propria of the villi in the small intestine. I) histopathological alterations in Pancreas in rats administered of PM-on is stronger, circumscribed around anaplastic cells proliferation in the islet of Langerhans cells with atrophy in the surrounding acini (Adenoma). J) Insecticide PM- induced degeneration with the loss of spermatogenic series in the lumen of the seminiferous tubules associated with disintegration and oedema in the interstitial Leydig cells. K) Sever congestion was observed in the medullary blood vessels with presence of mature graffian follicles and corpus luteum. L) Nuclear pyknosis and degeneration were detected in the neurons of the cerebral cortex. M) Neurons of the fascia dentate in the hippocampus. N) Diffuse gliosis with congestion in the blood vessels of striatum.

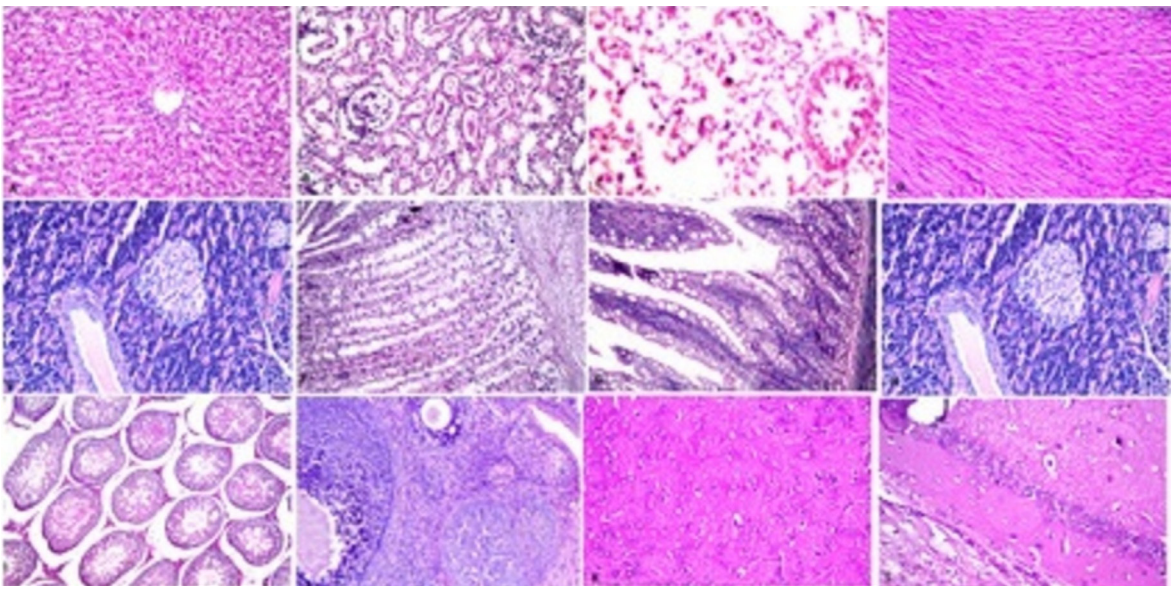

Figure 7. Effect of propolis-MeOH extract no histopathological alteration in the structure of the studied organs as compared to PM group A) Liver, B) Kidney, C) Lung, D) Heart, E) Spleen F) Stomach, G) Small intestine, H) Pancreas, I) Testes, J) Ovary, K & L) Brain.

active protein (CRP), the liver function determining enzymes, blood bilirubin, creatinine, blood glucose, and urea when compared to controls as a result of inhalation of pesticides (57).

In the current study, the activity of AST, ALT, and ALP among the exposed groups, whether males or females, increased significantly ($p < 0.05$, 0.5 folds), as compared to the control groups. There was a substantial reduction in total protein and albumin ($p < 0.05$). Several studies confirmed that pesticides led to an increase in ALP levels in workers who sprayed these pesticides. Increased blood AST, ALT, and ALP activity is a definite indication of biliary liver damage in humans and experimental animals (58). The increase in liver enzymes can be explained by inflammation and tissue degeneration occasioned by the prejudicial consequence of insecticides on hepatocytes. The cytotoxicity was enhanced depending on the dose after 30 days of susceptibility, and this indicated that PM could activate cell death in rats. The results of this current study indicate hepatoprotection induced by propolis. This protective effect may be due to the antioxidant effect of propolis. The treatment with propolis significantly prevented increasing transaminases and ameliorated proteins, suggesting its hepatoprotective potential. The induction of AST, ALT, and ALP reflects liver injury associated with necrosis, whereas GGT points to hepatic cholestasis (59).

Through some experimental and clinical evidence, several pesticides commonly used in many parts of the world have been identified as human nephrotoxins, a major cause of acute kidney injury (60). Therefore, it is worth noting that in this study, it was found that the insecticides used led to a remarkable increase in known kidney function such as urea and creatinine, and KIA. In our study, propolis (300 mg/kg) administered to rats before and after exposure to PM ameliorated kidney damage. It is well known that oxidative stress is increased by a system in which the rate of free-radical production is enhanced, and endogenous antioxidant mechanisms are impaired in renal injury.

Insecticides have a toxic effect on many body tissues, which may lead to cell death. In this study, it is evident that the use of insecticides leads to heart toxicity, as exposure to such toxic substances led to a significant increase in LDH and CK-MB. The propolis is characterized highly through the transmission of hydrogen atoms to the lipid peroxyl radicals. Propolis was promoted, likely to quench the free-radical process, limit the intracellular lipid peroxidation process, and lead to partial recovery of cardiac cells and consequently to a decrease in the amount of the antioxidants. Polyphenols, such as tannic acid, ascorbic acid, flavonoids, and reducing sugars, which have been confirmed to be present in the Egyptian propolis, are essential and indispensable antioxidants that may provide a safe effect on the leakage of these myocardium enzymes. However, the activities of all serum marker enzymes have been significantly improved before propolis treatment, indicating that propolis helps preserve membranous integrity and thus limits the leakage of enzymes (61).

A severe decline in antioxidants leads to toxicity and programmed cell death. In this current study, a significant decrease in TA was observed. Exposure to pesticides led to a considerable increase in LP. The degradation observed in many tissues studied may be attributed to the reduction in TA and rise in the LP. Post-treatment with propolis (100 mg/kg) reduced LP; this result can be attributed to flavonoids in propolis, which can scavenge LP products generated excessively by PM.

Evaluation of pro-inflammatory cytokines (such as IL-6, IL-12, IFN-gamma, and TNF-alpha) has been of interest, promoting PM and B lymphocyte proliferation, antibody synthesis, adhesion molecule production, and acute-phase proteins. They participate in specific and unspecified immune processes (62). The restoration of some functionally active lymphocytes caused one of the mechanisms for increased IFN-gamma in serum. Prolonged susceptibility to insecticides can lead to apparent disruption of various organs in the body, including the immune, nervous, and circulatory systems, such as the cardiovascular, respiratory, and other systems (62). Conversely, after treatments with propolis extract, levels of these pro-inflammatory markers decreased significantly ($p < 0.05$). Therefore, propolis supplementation has a positive effect on chronic inflammation (63).

The function of BAX and BCL-2 in apoptosis has been extensively studied in several laboratory and biological systems. In our current study, we observed an increase in NF-KB and BAX but a decrease in BCL-2 due to the abuse of insecticides. Propolis is a natural therapeutic factor that exhibits antioxidant activities that counter apoptosis triggered by exposure to the pesticide, resulting in increased BAX and observed deficiency of BCL2 (64).

In this study, exposure to insecticides led to a significant increase in T4 while decreasing T3. Our current results are consistent with those of Fillion et al. (65) showed a decrease in the T3 level in a group of people exposed to the pesticides (65). However, in all the treated groups, levels of thyroid hormones were improved with the propolis-MeOH extract to concentrations that were far closer to control.

The results also showed a significant decrease in AChE among the exposed group compared to the control group. According to (66), the pyrethroid caused a prolonged decline in AChE activity in rats after single-dose administration. Pesticides increase Na+ entrance into synaptic ends and produce a hypopolarized, which enhances the release of the neurotransmitter acetylcholine (67). The insecticides also increased the BAX level, inhibited BCL2 levels, and triggered NF-κB activity, which subsequently showed apoptosis via the pathway of these parameters. The inflammatory mediated by NF-βB is reportedly one of the targeted ROS pathways, acting as a crucial connection between oxidative stress and apoptosis. Consistent with the results of a previous report, propolis significantly lowers NF-ȚB anti-inflammatory effect in the tests (48).

The current study shows that administrating PM to albino rats (males and females) resulted in a substantial dose-dependent reduction in the number of red blood corpuscles, WBCs, PCV, and Hb content at medium and high doses. However,

mean cell hemoglobin (MCH) was significantly increased due to administering the used toxic materials in a dose-dependent mode. These effects were compatible with the findings of Achudume's study (55). Interestingly, the treatment with propolis extract showed much more positive amelioration in the hematological parameters. The remarkable increase in the hematological parameters after treatment with propolis is because it contains phenolic acids and flavonoids that could participate in its antioxidant activity (68).

Implementing PM had a significant impact on the histopathological structure of the studied organs, showing the harmful oxidative stress that resulted in these tissues. The rats treated with the propolis-MeOH extract showed more normal histological findings of these tissues than the nontreated one, suggesting the possible safety and curation induced by this extract.

Conclusion

The bioaccumulation of PM was determined by assessing the total nutritional exposure of the animal in an environment. Indicators of toxicity showed anemia with leukopenia and elevated liver and bilirubin enzymes, showing liver damage. Exposure to insecticides also injured many organs, such as the kidneys, lung, heart, small and large intestine, and spleen. We observed a significant increase in inflammatory and apoptosis markers in rats and a significant decrease in TA and BW.

These pesticides, warning of the dangers of these insecticides on human health and the environment, have influenced the histological study of different organs. The results confirmed the importance of minimizing exposure to such pesticides. Besides, the correct usage of the type of personal protective equipment is necessary when using these pesticides. The antioxidant activities and polyphenols found in propolis and their promising medicinal effects on oxidative damage resulting from exposure to the insecticide were documented in this study. As a result, it promotes health-beneficial outcomes, making it a potentially potent natural candidate for pharmaceutical companies, which warrants further investigation.

Declaration of Competing Interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethics approval and consent to participate

The Animal Care Committee and Experimental Work were referred to, as well as following the principles of laboratory animal care, in addition to specific national laws. These protocols that clarify animal performance follow the National Research Council (63) and the International Medical Association guide on animal welfare Lab and handle (64).

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Author Contributions

All authors conceived and designed the experiment performed all the in vivo experiments. AIH performed the in vivo experiments and data management and statistics. HMS, HAE, & IIB performed all the in vivo assays. HMS, IIB, KO & HAE contributed in writing and reviewing the manuscript.

Abbreviations

AKI: acute kidney injury; ALP: Alkaline phosphatase; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; BAX: homolog; BCL2: homolog; BUN: Blood urea nitrogen; CK-MB: Creatine kinase-MB; EDTA: Ethylenediaminetetraacetic acid; $FeCl_3$: Iron (III) chloride; FRAP: ferric reducing antioxidant power; G6P: Glucose 6- phosphate; GC-MS: Gas chromatograph-mass spectrometer; GGT or γGT: gamma-glutamyl transpeptidase; HGB or Hb: Haemoglobin; HCl: Hydrochloric acid; IFN-gamma: Interferon gamma; IL-12: Interleukin 6; IL-6: Interleukin 6; KIM-1: Kidney injury molecule-1; LDH: Lactate dehydrogenase; LP: lipid peroxide; NIST: National Institution of Standards and Technology; NF-Kb: Nuclear Factor kappa; PCV: packed cell volume; RBC: Red blood cell; T3: Triiodothyronine; T4: Thyroxin; TA: Total antioxidant; TBARS: Thiobarbituric acid reactive substances; TNF- α: Tumour necrosis factor alpha; TPTZ: Tripyridyltriazine; WBC: White blood cell; MCH: Mean corpuscular haemoglobin.

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