



Protective effects of propolis against metaflumizone induced cardiotoxicity through modulation of oxidative stress, inflammation, and the PI3K/Akt/mTOR pathway

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Abstract

This study aimed to evaluate the cardiotoxic effects of metaflumizone (MTF), a commonly used pesticide, and the potential protective role of propolis (PROP) against MTF-induced cardiac damage. Twenty-eight male Wistar albino rats were randomly divided into four groups: Control, PROP (200 mg/kg), MTF (500 mg/kg), and MTF+PROP. All treatments were administered orally for 21 days. Biochemical, molecular (RT-qPCR), histopathological, and UHPLC-Orbitrap®-HRMS analyses were performed to assess the outcomes. MTF administration significantly increased malondialdehyde (MDA) levels in whole blood and decreased glutathione (GSH) levels, indicating elevated oxidative stress. Additionally, superoxide dismutase (SOD) and catalase (CAT) activities were reduced in erythrocyte packs, further confirming systemic oxidative imbalance. At the molecular level, MTF suppressed the activities of PI3K, Akt, and mTOR in cardiac tissue and significantly upregulated the mRNA expression of TNF- α , IL-1 β , IL-6, NF- κ B, and Cyt-c. Histopathological evaluation revealed pronounced myocardial degeneration in the MTF group. In contrast, PROP supplementation effectively reversed these pathological alterations by restoring PI3K/Akt/mTOR pathway activity, attenuating oxidative and inflammatory responses, and preserving histological integrity. Collectively, the findings suggest that propolis exerts significant cardio-protective effects against MTF induced toxicity by modulating oxidative stress, inflammation, and apoptosis. These results provide the first in vivo evidence that propolis may mitigate MTF induced cardiotoxicity through regulation of oxidative stress, inflammation, and apoptosis.

Keywords Apoptosis · Cardiac · Inflammation · Metaflumizone · Propolis extract · UHPLC-Orbitrap®-HRMS

Introduction

The widespread use of pesticides in agriculture and their impact on public health continue to increase today (Mossa et al. 2018). The world uses about two million tons of pesticides annually (Sharma et al. 2019). The type of pesticides used against insects, each of which is effective against specific pests, is referred to in the literature as insecticides (Akande et al. 2024). Insecticides are widely used in our country and around the world to minimize damage to agricultural areas in line with increasing food needs and population growth (Mossa et al. 2018). Although insecticides have lethal properties because they block sodium channels in target organisms, they can also cause negative effects on non-target organisms when used unconsciously and in high doses (Hempel et al. 2007). The unconscious, widespread, and long-term use of synthetic insecticides has led to their

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accumulation in food, milk, and other environmental components, particularly soil and water (Mossa et al. 2018). Indeed, many studies have reported that this situation has negative impacts on humans and ecosystems, leading to health problems (Farag et al. 2003; Ngoula et al. 2007). Metaflumizone (MTF), which belongs to the semicarbazone class, is an insecticide that blocks voltage-dependent sodium channels in insects' nervous systems, causing paralysis (Wu et al. 2023). Hempel et al. (2007) reported that MTF is distributed to all organs and tissues via systemic circulation. MTF is an insecticide that acts by blocking voltage-dependent sodium channels and shares a structural similarity with indoxacarb. While indoxacarb poisoning has been associated with methemoglobinemia in humans, the toxicological effects of MTF in humans remain largely unclear. However, a clinical case reported a significant elevation of methemoglobin levels (up to 27.8%) following the ingestion of a high dose of MTF, along with glyphosate and alcohol (Oh and Choi 2014). In animal studies, oral administration of MTF at a dose of 300 mg/kg in rats has been shown to cause reduced food intake and mild histopathological alterations in hepatic tissue (Hempel et al. 2007). These findings collectively suggest that high-dose exposure to MTF may lead to both systemic and organ-specific toxic effects. Although the toxicological effects of MTF on hepatic and renal systems have been partially investigated, its potential cardiotoxic effects remain largely unexplored.

In recent years, the increasing prevalence of pesticide exposure and its associated health risks have prompted a growing interest in identifying natural agents with protective potential against pesticide-induced toxicity. Propolis, a resinous substance produced by bees, has attracted considerable scientific attention due to its well-documented antioxidant, anti-inflammatory, and cytoprotective properties. In this context, the present study represents one of the first experimental investigations into the potential cardioprotective effects of propolis against MTF-induced cardiac toxicity. Given the growing global concern over pesticide-related cardiovascular risks and the limited availability of effective protective strategies, this study aims to provide novel and comprehensive insights into the therapeutic potential of propolis. By combining biochemical, molecular, and histopathological assessments, we sought not only to quantify the extent of cardiac damage induced by MTF but also to elucidate the mechanisms through which propolis exerts its protective effects. This integrative approach revealed that propolis effectively mitigates oxidative stress and inflammatory responses, thereby underscoring its potential as a natural, safe, and effective cardioprotective agent against pesticide-induced cardiac injury.

Materials and methods

Chemical

In this present study, Alverde® (BASF, Tuerk Kimya San. ve Tic. Ltd. Sti, Istanbul, Turkey) was used as MTF (CAS no: 139968-49-3). Propolis was collected from beekeepers in the Solhan district of Bingöl province by special collector traps placed directly on the frames, under the roof, and on the sides of the hives without contamination and stored at -80°C until extraction. All additional chemicals and reagents utilized in this study were of analytical reagent grade, ensuring high purity and reliability for experimental procedures. These materials were obtained from reliable commercial suppliers to maintain consistency and accuracy in the analyses.

Ethanol extraction of propolis

Propolis samples were freshly purchased from beekeepers in the Solhan region of Bingöl Province and stored at -80°C for extraction purposes. The process of preparing the propolis ethanol extract involved freezing the propolis at -80°C ; then, it was ground into powder using a laboratory grinder (Waring 8010G). Subsequently, 500 g of propolis was placed in a 2.5-liter amber glass bottle, and 2 L of HPLC-grade ethanol was added. The mixture was kept in an ultrasonic bath for 60 min, and then it was stirred with a magnetic stirrer at 500 rpm in the dark at room temperature for 4 days. The mixture was filtered first through coarse filter paper and then through blue tape filter paper. The extract was evaporated under vacuum at 40°C using a rotary evaporator (IKA) to remove the ethanol. The obtained propolis extract was stored in amber glass containers at $+4^{\circ}\text{C}$ in the dark.

Experimental protocol

Animals and experimental models

The study was carried out at the appreciation of the Pamukkale University Animal Experiments Local Ethics Committee reference number PAUHDEK-2023/30. The study included 28 male Wistar albino rats, 3–4 months age and weighing 220–300 g, divided into four groups consisting of 7 animals each. The study was conducted at the Experimental Surgery Application and Research Center of Pamukkale University under controlled environmental conditions consisting of a 12-hour light/12-hour dark cycle, a temperature of $23 \pm 1^{\circ}\text{C}$, and relative humidity of 50–55%. The experiment lasted for 21 days, during which the animals were grouped as outlined in Table 1. All experimental procedures

Table 1 The procedure process and experimental groups

Groups	During the 21-day period	Termination of the experiment
Control group	Ethanol (0.5 mL) was administered via gastric gavage for a period of 21 days in this study.	On the 22nd day of the experiment, all animals were euthanized in accordance with ethical guidelines to obtain tissue and blood samples.
Propolis 200 mg/kg group (PROP)	Propolis extract was administered by gastric gavage at 200 mg/kg for 21 days.	
Metaflumizon 500 mg/kg group (MTF)	MTF was administered by gastric gavage at a dose of 500 mg/kg for 21 days.	
MTF + PROP	MTF at a dose of 500 mg/kg and propolis at a dose of 200 mg/kg were administered by gastric gavage for 21 days.	

were conducted in accordance with the ARRIVE guidelines and the European Directive 2010/63/EU for animal experiments.

Throughout the experimental period, all rats were provided with clean drinking water and standard rat chow ad libitum. The control group received 0.5 mL of ethanol daily via oral gavage for 21 consecutive days. MTF was used in the form of Alverde® (BASF, Tuerk Kimya San. ve Tic. Ltd. Şti., Istanbul, Turkey), and was administered to the respective groups at a dose of 500 mg/kg/day, which corresponds to 1/10 of the LD₅₀ value (5000 mg/kg) via intragastric gavage for 21 days (Rust et al. 2007; Demirel et al. 2025). The dose of propolis was selected based on a previous study reporting beneficial biological effects following oral administration in ethanol solution (Ngozi Ugbaja et al. 2021). Accordingly, propolis was administered at a dose of 200 mg/kg/day via oral gavage for 21 days. Both propolis and MTF were administered in accordance with the dose ranges specified in Table 1.

Collection of blood and tissue samples

The study groups were fasted overnight before blood and tissue sampling. On the following day, corresponding to day 22 after the completion of experimental procedures, appropriate anesthesia was induced in all groups via intraperitoneal administration of xylazine HCl (13 mg/kg) and ketamine HCl (87 mg/kg). Following anesthesia, cardiac blood samples were obtained via direct puncture and transferred into both heparinized and non-heparinized collection tubes. The samples collected were used for biochemical analyses. Following blood collection, the animals in each group were sacrificed in accordance with ethical guidelines, and cardiac tissues were carefully dissected and harvested for histopathological and molecular examinations.

Preparation of erythrocytes and tissue homogenates

Immediately after blood collection, the tubes were centrifuged at 3000 rpm for 15 min at 4 °C to separate the erythrocytes. Plasma and serum samples were carefully collected. The erythrocyte pellet remaining at the bottom of the heparinized tubes was washed three times with physiological saline, and the supernatant was discarded. Subsequently, equal volumes of isotonic saline and erythrocytes were transferred into Eppendorf tubes and stored at −20 °C until analysis. Before biochemical analysis, the samples were thawed at room temperature, and the erythrocyte suspensions were prepared by adding cold deionized water. All measurements were conducted within the first three days following thawing (Acaroz et al. 2018). Cardiac tissue samples were immediately harvested for clinical biochemistry and immunohistochemical evaluations. The tissues were rinsed with ice-cold isotonic saline to remove blood residues and surface contaminants. For biochemical analysis, 10% (w/v) cardiac tissue homogenates were prepared in Tris-HCl buffer (Ince et al. 2014). The homogenates were centrifuged at 3500 rpm for 10 min at 4 °C, and the resulting supernatants were collected and stored at −20 °C until further analysis.

Biochemical analysis

Analysis of oxidative stress parameters

Malondialdehyde (MDA), a well-known biomarker of oxidative stress, was quantified in whole blood using the thiobarbituric acid reactive substances (TBARS) assay as described by Ohkawa et al. (1979). Reduced glutathione (GSH) levels in whole blood were measured by the colorimetric method of Beutler et al. (1963). Superoxide dismutase (SOD) activity was assessed in erythrocyte hemolysates according to the method of Sun et al. (1988). Catalase (CAT) enzyme activity was determined spectrophotometrically from erythrocyte hemolysates using the protocol described by Luck (1965). Hemoglobin (Hb) levels were determined calorimetrically using the cyanmethemoglobin method according to Drabkin and Austin (1935). All spectrophotometric measurements were performed using a Shimadzu 1601 UV–vis spectrophotometer (Tokyo, Japan).

Determination of PI3K/Akt/mTOR signaling pathway activities in cardiac tissue using the ELISA technique

The concentrations of mammalian target of rapamycin (mTOR), phosphoinositide 3-kinase (PI3K), and protein kinase B (Akt) in cardiac tissue supernatants were quantified using commercially available rat-specific ELISA kits

(SunRed, Shanghai, China), following the manufacturer's protocols. Before analysis, the tissue supernatants were thawed and equilibrated to room temperature. The results were expressed as nanograms per gram of tissue (ng/g tissue). Absorbance was measured at 450 nm after the completion of the colorimetric reaction, and the resulting optical density values were used to calculate the analyte concentrations based on standard curves.

Preparation of cardiac tissues for metaflumizone analysis

Approximately 1 gram of cardiac tissue was placed into 2 mL lock-cap Eppendorf tubes, followed by the addition of 500 μ L phosphate-buffered saline (PBS, pH 7.4) and 500 μ L methanol. The tissues were homogenized for 15 min using a stainless-steel bead in a Tissue Lyser II device (Qia-gen, Germany). The homogenates were then centrifuged at 15,000 rpm for 15 min at 4 °C. The resulting supernatants were filtered through a 0.22 μ m pore-size, 25 mm diameter PTFE syringe filter (Marz Schuller) using a 10 mL syringe. The filtrates were subsequently analyzed using an ultra-high-performance liquid chromatography system coupled with Orbitrap[®] high-resolution mass spectrometry (UHPLC-Orbitrap[®]-HRMS).

Ultra-high-performance liquid chromatography - Orbitrap[®] - high resolution mass spectrometry (UHPLC-Orbitrap[®] - HRMS) analysis

All analyses were carried out using an Exactive Plus Orbitrap[®] high-resolution mass spectrometer (Thermo Fisher Scientific, USA) coupled with an ultra-high-performance liquid chromatography system (UHPLC-Orbitrap[®]-HRMS). Chromatographic separation was performed using the DIONEX Ultimate 3000 RS system, which included a binary pump, an autosampler, and a column oven (Thermo Fisher Scientific, USA).

Chromatographic conditions for metaflumizone analysis of cardiac tissues

Chromatographic separation was carried out using a DIONEX Ultimate 3000 RS UHPLC system (Thermo Fisher Scientific, USA), comprising a binary pump, autosampler, and column oven. Separation was performed on an SVEA[™] Core Shell C18 column (100 mm $\times$ 2.1 mm, 3 μ m) maintained at 30 °C. The mobile phase consisted of solvent A (0.5% acetic acid in water) and solvent B (LC-grade methanol), delivered at a flow rate of 0.7 mL/min. The gradient started at 0–1 min with 100% A and 0% B, increased to 0% A and 100% B from 1 to 1.1 min, maintained at 0% A and 100% B from 1.1 to 16 min, and returned to the initial

gradient of 100% A and 0% B at 16–16.1 min. The flow continued at 100% A and 0% B from 16.1 to 19 min. The total duration of the method was 20 min. A sample volume of 20 μ L was injected into the analytical column for analysis.

Chromatographic conditions for phenolic compound profile analysis of propolis ethanol extract by UHPLC-Orbitrap[®]-HRMS

Chromatographic separation was performed using a Merck Purospher STAR RP-18 end capped Hibar HR model UHPLC column (100 mm $\times$ 2.1 mm, 3 μ m particle size) maintained at 30 °C. The mobile phase flow rate was set to 0.3 mL/min. Mobile phase A consisted of 0.5% acetic acid in water, and mobile phase B was LC-grade methanol. The gradient elution was as follows: from 0 to 2 min, 100% A and 0% B; from 2 to 13 min, a ramp to 0% A and 98% B; from 13 to 15.9 min, 98% B; from 15.9 to 16 min, a return to 100% A and 0% B; and from 16.1 to 19 min, continuing at 100% A and 0% B. The total method duration was 20 min, and the injection volume was 20 μ L (Dursun et al. 2025; Gelen et al. 2025 et al. 2025).

Phenolic compound profile of propolis ethanol extract and metaflumizone analysis in cardiac tissues using Orbitrap[®] - HRMS conditions

The same instrumental conditions were applied for the analysis of both the phenolic compound profile in the ethanol extract of propolis and the quantification of MTF in cardiac tissue samples using Orbitrap[®]-HRMS. Mass spectrometric detection was carried out using an Exactive Plus high-resolution mass spectrometer (Thermo Fisher Scientific Inc., Waltham, MA, USA), equipped with a Heated Electrospray Ionization (HESI-II) source. The instrument operated in negative electrospray ionization (–ESI) mode, with a scan range of m/z 100–800. The following source parameters were used: spray voltage at 3.5 kV, sheath gas flow rate of 35 (arbitrary units), auxiliary gas flow rate of 7, sweep gas flow rate of 0, capillary temperature of 350 °C, auxiliary gas heater temperature of 350 °C, S-lens RF level at 50, and maximum injection time (IT) set to 2 ms. Mass spectra were obtained using two alternative acquisition modes: (1) Full MS mode, without fragmentation and with the high-energy collisional dissociation (HCD) cell closed; (2) All Ion Fragmentation (AIF) mode, with fragmentation performed via MS/MS, and HCD open (collision energy=25 eV). Automatic Gain Control (AGC) was set to 3×10^6 . The resolution was set to 17,500 for both Full MS and AIF modes. Data were collected and recorded using TraceFinder 3.2 (Thermo Scientific) software on the system computer that enabled the simultaneous operation of the UHPLC and

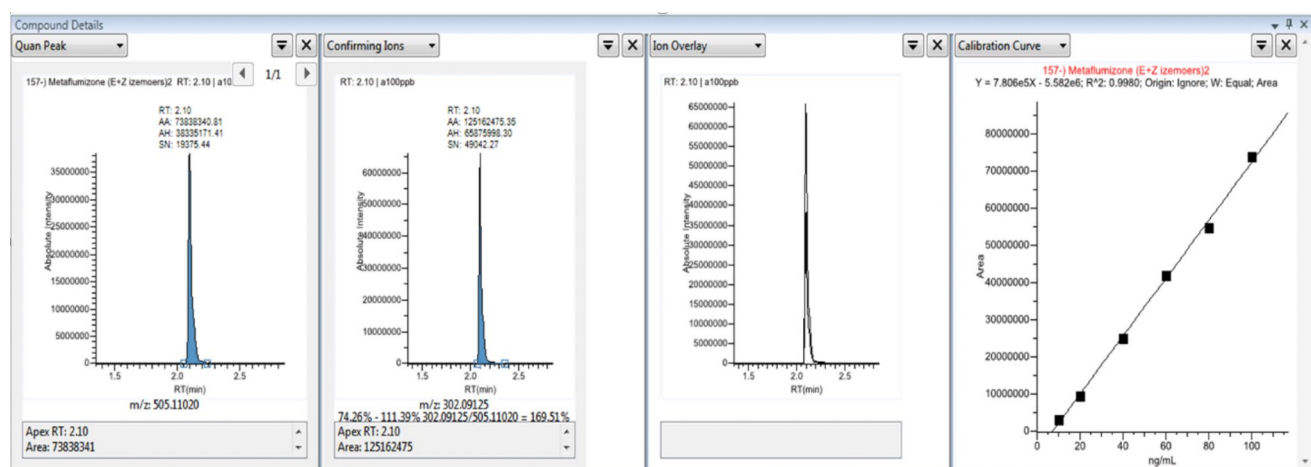


Fig. 1 UHPLC-Orbitrap®-HRMS **a** Quan Peak (Parent Ion), **b** Fragment Ions (m/z) (Confirming Ion) chromatograms and **c** calibration curves

Table 2 Analytical parameters of metaflumizone for UHPLC-Orbitrap®-HRMS analysis

Compound	RT	Quan peak (m/z)	Confirming ion (m/z)	Lineer range (ng/mL)	% Recov- ery ($n=3$)	% Recov- ery RSD ($n=3$)	LOD ($\mu\text{g/kg}$) ($n=3$)	LOQ ($\mu\text{g/kg}$) ($n=3$)	R^2
		Parent ion (m/z)	Fragment ions (m/z)						
Metaflumizone	2.1	505.11020	302.09125	10–100	96,88	1,88	0,55	1,82	0,9980

RT, Retention time; RSD, Relative standard deviation; LOD, Limit of detection; LOQ; Limit of quantification. R^2 , Correlation coefficient. Quan Peak: Parent Ion (m/z) of MTF compound standards, Confirming Ions: Fragment ions (m/z) of MTF compound standards.

Orbitrap⁶-HRMS components, and the data were processed with Xcalibur software version 2.1.0.1140 (Thermo Fisher Scientific). Identifications were based on determining the molecular weight with a deviation of less than 5 ppm and the retention time of the molecular ion. Fragment identification was performed within a mass tolerance of 5 ppm and with a minimum intensity threshold below 1000.

Method validation for metaflumizone analysis

The quantification of MTF in the samples was carried out using an ultra-high-performance liquid chromatography system coupled with Orbitrap[®] high-resolution mass spectrometry (UHPLC-Orbitrap[®]-HRMS). A standard stock solution of MTF was prepared at a concentration of 100 ng/mL in methanol. Working standard solutions were obtained by serial dilution of the stock solution in methanol to achieve final concentrations of 10, 20, 40, 60, 80, and 100 ng/mL. An external calibration curve was constructed using these standards, as presented in Fig. 1.

The quantification peak (parent ion) and confirming fragment ions of MTF, along with retention time (RT), concentration ranges, the determination coefficient (R^2) of the calibration curve, limit of detection (LOD, $\mu\text{g/kg}$), and limit of quantification (LOQ, $\mu\text{g/kg}$), were evaluated by spiking three blank control samples (free of the analyte) with a MTF concentration of 10 $\mu\text{g/kg}$. Furthermore, recovery (%) and

recovery relative standard deviation (RSD, %) values were determined and are summarized in Table 2.

Gene expression analysis

The mRNA expression levels of TNF- α , IL-1 β , IL-6, NF- κ B, and cytochrome-c in the harvested cardiac tissues were quantified using the real-time PCR (RT-qPCR) method and compared between the experimental groups. The method is briefly described as follows:

Real-time PCR analysis

Quantitative determination of mRNA expression by real-time PCR Cardiac tissues were homogenized in liquid nitrogen using a Tissue Lyser II (Qiagen, Germany). Total RNA extraction was then performed automatically using the QIAcube RNA isolation system (Qiagen) according to the manufacturer's instructions. Both RNA isolation and cDNA synthesis were carried out as previously described in our earlier studies (Cinar et al. 2022). Quantitative real-time PCR (RT-qPCR) was used to assess the mRNA expression levels of TNF- α , IL-6, IL-1 β , NF- κ B, and cytochrome-c, with β -actin serving as the internal housekeeping gene. Comparative expression analyses were conducted across all experimental groups. Results were normalized to the con-

Table 3 Rat primers used for RT-PCR

Genes	Forward primer	Reverse primer
TNF- α	CCAGGAGAAAGTCAG CCTCCT	TCATACCAGGG CTTGAGCTCA
IL-6	TCCTACCCCAACTTCC AATGCTC	TTGGATGGTCTT GGTCCTTAGCC
IL-1 β	CACCTCTCAAGCAGA GCACAG	GGGTTCATGG TGAAGTCAAC
NF-kB	ATCATCAACATGAGAA ACGATCTGTA	CAGCGGTCCAG AAGACTCAG
Cytochrome-c	CTTGGGCTAGAGAGC GGGA	GGTATCCTCTCC CCAGGTGAT
β -actin	TGTTACAGGAAGTCC CTTGCC	AATGCTATCACC TCCCCTGTG

trol group and expressed as fold changes using the $2^{-\Delta\Delta C_t}$ method (Table 3).

Histopathological analysis and evaluation

At the end of the experiment, the cardiac tissues of the sacrificed rats were placed in a 10% neutral formaldehyde solution and fixed for 72 h. It was then passed through a graded alcohol series and kept in xylol. They were then embedded in paraffin blocks, and 5 μ -thick sections were taken with a microtome device (Leica RM2125 RTS) for histopathologic evaluations. The sections obtained were stained with Mallory's Triple Stain as modified by Crossman for histopathologic examinations, and tissue damage assessments were performed by a histologist. A trinocular microscope with computer and camera attachment (Zeiss AXIO Scope A1, German) was used for microscopic examination. Ten visual fields were randomly chosen for each group and scored on a four-point scale for muscle fiber degeneration, necrosis, bleeding, congestion, and inflammatory cell infiltration. The ratings include: (–) no degeneration or necrosis; (+) mild degeneration or necrosis, scattered bleeding, and mild inflammation; (++) moderate degeneration or necrosis, several areas of bleeding, and widespread inflammation; and (+++) severe damage with combined necrosis, widespread inflammation, and bleeding at many sites (Al-Taher et al. 2020).

Statistical analysis

Statistical analyses were performed using SPSS version 27.0 (SPSS Inc., Chicago, IL, USA), and graphical illustrations were created using GraphPad Prism version 9.05 (GraphPad Software, San Diego, CA, USA). All data were expressed as mean $\pm$ standard error of the mean (SEM). The normality of the data distribution was assessed using the Shapiro–Wilk test, and homogeneity of variances was confirmed using Levene's test. For comparisons among multiple groups, one-way analysis of variance (ANOVA) followed by Duncan's post-hoc test was applied. A p -value < 0.05 was considered statistically significant.

Results

Effect of propolis on metaflumizone-induced blood oxidative stress

As shown in Table 4, MTF administration resulted in a significant increase in MDA levels in the blood compared to the control group ($p < 0.05$). Co-administration of propolis significantly reduced MDA levels in the MTF + PROP group ($p < 0.05$). MTF also led to a significant decrease in reduced GSH levels compared to the control ($p < 0.05$), whereas propolis treatment markedly increased GSH concentrations in the MTF + PROP group ($p < 0.05$). Similarly, a significant reduction in SOD activity was observed in the MTF group compared to the other experimental groups ($p < 0.05$). Propolis supplementation significantly elevated SOD activity in both the PROP and MTF + PROP groups ($p < 0.05$). In addition, MTF significantly decreased CAT activity relative to the control ($p < 0.05$), while propolis treatment notably restored CAT activity in the MTF + PROP group ($p < 0.05$).

Effect of propolis on metaflumizone-induced PI3K/Akt/mTOR signaling pathways in cardiac tissue

It is well established that the PI3K/Akt/mTOR signaling pathway is an important regulator of both autophagy and apoptosis (Peng et al. 2022). ELISA analyses revealed that MTF administration significantly suppressed PI3K, Akt,

Table 4 Effects of Metaflumizon and propolis on MDA and GSH levels in the blood and SOD and CAT activities in the erythrocytes of rats

Groups	MDA (nmol/ml)	GSH (nmol/ml)	SOD (U/mg Hb)	CAT (U/mg Hb)
CONTROL	5.42 $\pm$ 0.29 ^{bc}	40.19 $\pm$ 0.86 ^a	8.37 $\pm$ 0.67 ^b	5.65 $\pm$ 0.72 ^a
PROP	4.35 $\pm$ 0.16 ^c	38.56 $\pm$ 0.71 ^a	11.46 $\pm$ 1.23 ^a	5.87 $\pm$ 0.51 ^a
MTF	7.61 $\pm$ 0.52 ^a	29.15 $\pm$ 0.44 ^b	6.34 $\pm$ 0.45 ^c	3.05 $\pm$ 0.22 ^b
MTF + PROP	5.97 $\pm$ 0.35 ^b	37.43 $\pm$ 0.93 ^a	9.57 $\pm$ 0.95 ^a	5.32 $\pm$ 0.45 ^a

Values are expressed as mean $\pm$ standard error of the mean (SEM) ($n = 7$)

^{a,b,c} Different superscript letters within the same column indicate statistically significant differences ($p < 0.05$)

MDA, Malondialdehyde; GSH, Glutathione; SOD, Superoxide dismutase; CAT, Catalase

and mTOR activities in cardiac tissue ($p < 0.05$). However, co-treatment with propolis markedly restored the levels of these signaling molecules, with significant improvements compared to the MTF group alone ($p < 0.05$), indicating a protective effect of propolis on the PI3K/Akt/mTOR pathway (Fig. 2).

Effect of propolis on TNF- α , IL-6, IL-1 β , NF- κ B, and Cyt-c mRNA expression levels in the cardiac tissue of metaflumizone-induced rats

As shown in Fig. 3, the mRNA expression levels of TNF- α , IL-1 β , IL-6, NF- κ B, and cytochrome c (Cyt-c) were significantly elevated in the MTF group compared to both the control and PROP groups ($p < 0.05$). Co-administration of propolis (MTF+PROP group) markedly reduced the expression levels of these pro-inflammatory and pro-apoptotic markers when compared to the MTF group ($p < 0.05$), indicating the anti-inflammatory and anti-apoptotic potential of propolis against MTF-induced cardiac toxicity.

Results of metaflumizone analysis in cardiac tissues using UHPLC-Orbitrap®-HRMS

Metaflumizone concentrations in cardiac tissue were quantified using UHPLC-Orbitrap®-HRMS, and the results are expressed in Fig. 4 as nanograms per gram (ng/g) of cardiac tissue. Quantitative analysis revealed that MTF was undetectable in both the control and PROP-only groups, while it accumulated significantly in the cardiac tissue of rats treated with MTF ($p < 0.05$). In the MTF group, the concentration of MTF exceeded 2200 ng/g, indicating substantial tissue accumulation. Notably, co-administration of propolis (MTF+PROP group) 253.12 ng/g resulted in a statistically significant reduction in MTF accumulation compared to the MTF-only group ($p < 0.05$), suggesting that propolis may exert a modulatory effect on the bioavailability or metabolic processing of MTF.

Phenolic profile of propolis ethanolic extract determined by UHPLC-Orbitrap®-HRMS

UHPLC-Orbitrap®-HRMS analyses were conducted to characterize the profiles of 49 phenolic compounds present in the ethanolic extract of propolis. For each phenolic compound analyzed, the following parameters are provided in Table 5: retention time (RT), parent ions (m/z), fragment ions (m/z), ionization mode (polarity), correlation coefficient (R^2) of the calibration curves, and linear range ($\mu\text{g/L}$). The total ion chromatogram (TIC) representing the phenolic compound profile of the propolis ethanol extract is presented in Fig. 5.

A total of 32 phenolic compounds were identified in the ethanolic extract of propolis through UHPLC-Orbitrap®-HRMS analysis. These included benzoic acid, 4-hydroxybenzoic acid, protocatechuic acid, gallic acid, 3,4-dihydroxybenzaldehyde, vanillic acid, vanillin, trans-cinnamic acid, p-coumaric acid, caffeic acid, caffeic acid phenethyl ester (CAPE), ferulic acid, chlorogenic acid, quinic acid, 3-(4-hydroxyphenyl)propionic acid, chrysin, apigenin, acacetin, genkwanin, rutin, luteolin, diosmetin, galangin, quercetin, narcissin, isorhamnetin, leucoside, naringenin, sakuranetin, formononetin, ellagic acid, and glabridin.

The predominant phenolic compounds identified in the ethanolic extract of propolis were chrysin (5052.0 $\mu\text{g/g}$), naringenin (4437.5 $\mu\text{g/g}$), caffeic acid (3803.5 $\mu\text{g/g}$), benzoic acid (2798.0 $\mu\text{g/g}$), galangin (2581.6 $\mu\text{g/g}$), caffeic acid phenethyl ester (CAPE; 2134.9 $\mu\text{g/g}$), p-coumaric acid (1850.8 $\mu\text{g/g}$), apigenin (1394.5 $\mu\text{g/g}$), diosmetin (1305.0 $\mu\text{g/g}$), quercetin (983.2 $\mu\text{g/g}$), 3-(4-hydroxyphenyl)propionic acid (923.9 $\mu\text{g/g}$), isorhamnetin (887.5 $\mu\text{g/g}$), luteolin (750.5 $\mu\text{g/g}$), and ferulic acid (507.3 $\mu\text{g/g}$).

Histopathological findings

Histopathological alterations in the cardiac tissues of rats across experimental groups are detailed and illustrated in Fig. 6. In the control group (Fig. 6A), cardiac sections exhibited normally arranged myocardial fibers with longitudinal and transverse orientations, acidophilic cytoplasm, and centrally located oval vesicular nuclei. These fibers appeared branched, aligned in multiple directions, and interconnected, reflecting typical cardiac architecture. In the PROP group (Fig. 6B), the structural organization of cardiac muscle fibers remained largely unaltered, similar to the control group. Conversely, cardiac sections from the MTF-treated group (Fig. 6C) displayed marked pathological changes, including irregular and widened inter-fiber spaces and prominent cellular infiltration. Additionally, extensive degenerative muscle fibers were observed. In the MTF+PROP group (Fig. 6D), cardiac muscle fibers largely preserved their normal histological architecture, although occasional degenerative changes were still present. All histopathological scores and grading assessments are summarized in Table 6.

Discussion

In recent decades, advancements in agricultural technologies have been paralleled by a substantial increase in pesticide usage. These chemical agents, while effective in pest control, often disperse into the environment, leading to the

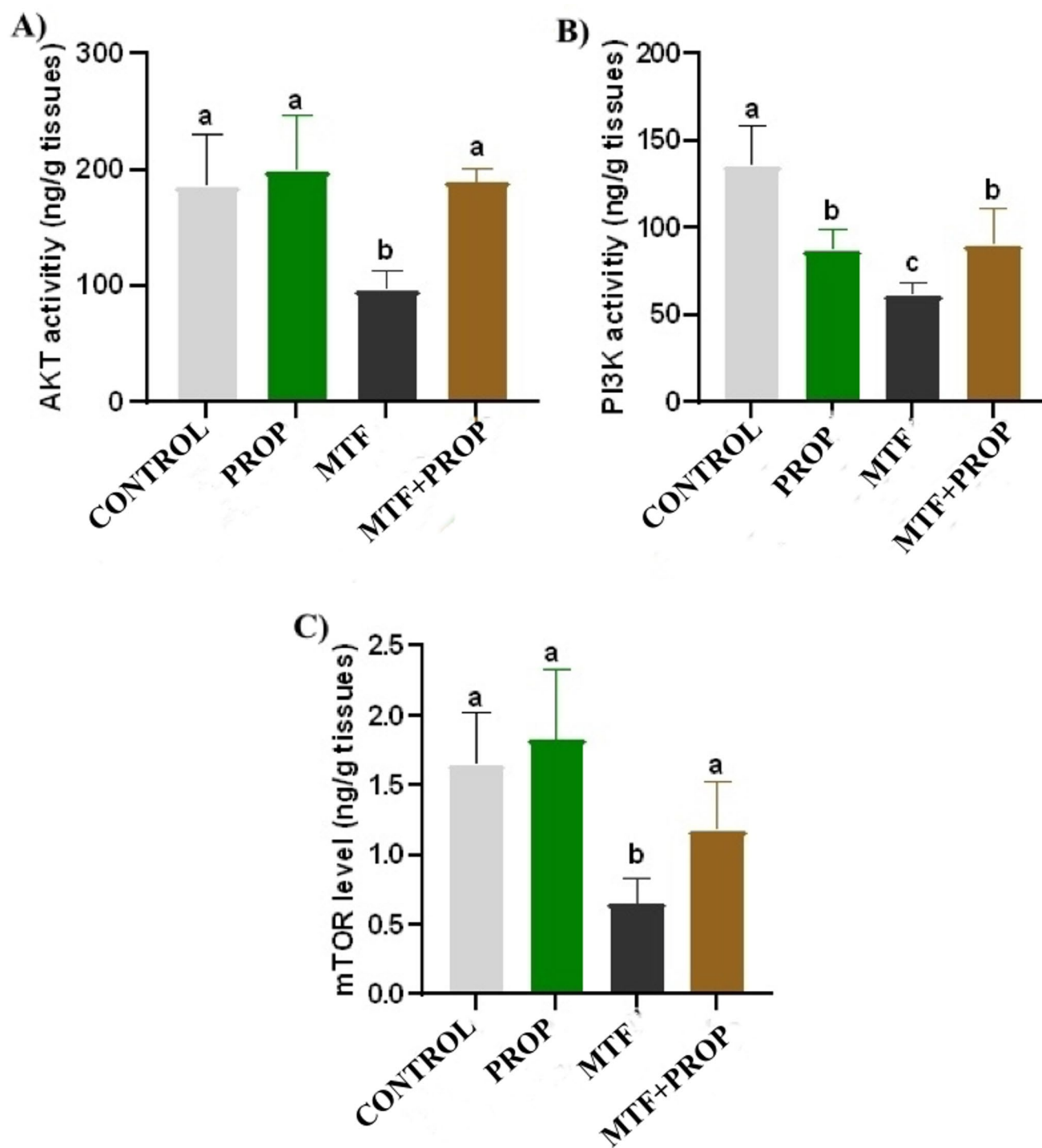


Fig. 2 Effect of propolis (PROP) on AKT (A), PI3K (B), and mTOR (C) activities in MTF induced cardiac toxicity in rats. Data are presented as mean $\pm$ SEM ($n=7$ per group). Different superscript letters (a, b, c) indicate statistically significant differences between groups ($p < 0.05$)

contamination of water sources and agricultural products. Consequently, non-target organisms, including humans and animals, are increasingly exposed to pesticide residues through the consumption of contaminated food and water

(Shadnia et al. 2005; Pietrzak et al. 2019). A growing body of evidence suggests that pesticides exert toxic effects not only on target pests but also on non-target biological systems. Such exposure has been linked to a wide range of

Fig. 3 Effect of propolis on MTF-induced changes in the relative mRNA expression levels of cardiac inflammatory and apoptotic markers: (A) TNF- α , (B) IL-1 β , (C) IL-6, (D) NF- κ B, and (E) Cyt-c. Data are presented as mean $\pm$ SEM. Different letters (a, b, c) indicate statistically significant differences between groups ($p < 0.05$). Statistical analysis was performed using one-way ANOVA followed by Duncan's multiple range test

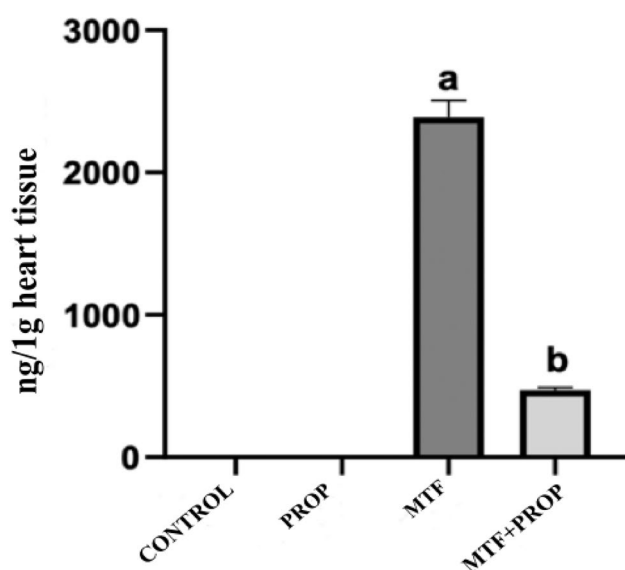
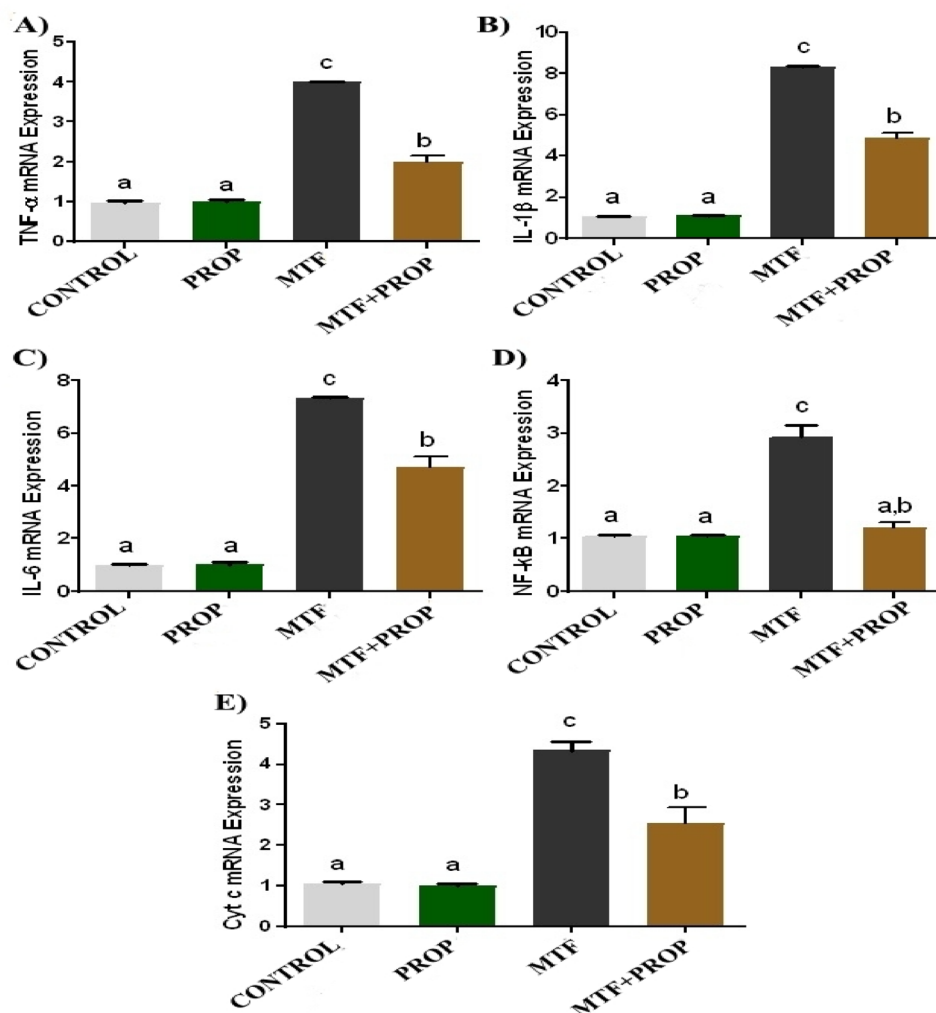


Fig. 4 The levels in the cardiac tissues of rats treated with MTF and PROP. Values are provided as the mean $\pm$ SEM. Different letters (a, b) suggest that there is a statistically significant difference between the groups ($p < 0.05$)

adverse outcomes, including acute or chronic metabolic disturbances, organ dysfunction, and increased risk of morbidity and mortality (Shekhar et al. 2024). Within this context, the present study was conducted to elucidate the cardiotoxic effects of MTF, a semicarbazone-class insecticide, and to evaluate the potential cardioprotective role of PROP, a natural bioactive compound known for its antioxidant and anti-inflammatory properties. The findings demonstrated that MTF significantly accumulates in cardiac tissue, disrupts redox homeostasis, suppresses the PI3K/AKT/mTOR signaling pathway, and induces marked inflammatory responses. In contrast, PROP administration either alone or in combination with MTF effectively ameliorated these pathological alterations, thereby indicating its promising cardioprotective capacity. In the present study, MTF exposure significantly disrupted redox balance in cardiac tissue, as evidenced by elevated levels of MDA and marked reductions in GSH, along with decreased activities of the antioxidant enzymes SOD and CAT. These findings suggest that MTF induces substantial oxidative damage in myocardial tissue, likely through enhanced lipid peroxidation and

Table 5 Parameters for UHPLC-Orbitrap®-HRMS analysis of phytochemicals and the phenolic compound profile in propolis extract

No	Target Compounds	RT	Parent Ions (m/z)	Fragment Ions (m/z)	Ion Mode	R^2	Liner range ($\mu\text{g/kg}$)	$\mu\text{g/g}$ propolis extract
1	Benzoic acid	8.93	121.02950	121.02950	–	0.9939	100–400	2798.0
2	4-Hydroxybenzoic acid	6.15	137.02442	93.03471	–	0.9921	10–200	47.8
3	Salicylic acid	9.9	137.02442	93.03468	–	0.9912	10–500	N.D.
4	3-hydroxybenzoic acid	6.93	137.02442	93.03471	–	0.9980	10–500	N.D.
5	Protocatechuic acid	8.96	153.01933	109.02949	–	0.9989	10–500	35.7
6	Gallic acid	0.79	169.01425	125.02461	–	0.9974	10–300	51.2
7	Protocatechuic acid ethyl ester	9.14	181.05063	108.02187	–	0.9923	10–200	N.D.
8	3,4-dihydroxybenzaldehyde	5.62	137.02442	136.01671	–	0.9960	10–500	140.0
9	2,4-dihydroxybenzoic acid	7.12	153.01933	67.01888	–	0.9905	10–500	N.D.
10	Vanillic acid	7.09	167.03498	108.02173	–	0.9904	10–100	56.3
11	Vanillin	7.60	151.04007	108.02178	–	0.9952	10–500	88.1
12	Gentisic acid	6.45	153.01933	153.01933	–	0.9980	10–500	N.D.
13	trans Cinnamic acid	10.28	147.04515	147.04515	–	0.9939	10–400	362.2
14	Coumaric acid	8.26	163.04007	119.05027	–	0.9925	10–300	1850.8
15	Caffeic acid	7.19	179.03498	135.04509	–	0.9978	10–500	3803.5
16	Caffeic acid phenhyl ester	11.99	283.09758	135.04526	–	0.9902	10–300	2134.9
17	Ferulic acid	8.52	193.05063	134.03751	–	0.9923	10–100	507.3
18	Sinapic acid	8.54	223.06120	193.01436	–	0.9940	10–100	N.D.
19	Chlorogenic acid	7.03	353.08781	191.05624	–	0.9901	10–500	21.6
20	Quinic acid	1.19	191.05611	85.02962	–	0.9946	10–100	179.5
21	3-(4-Hydroxyphenyl) propionic acid	7.6	165.05572	108.02195	–	0.9927	10–100	923.9
22	Catechin	6.21	289.07176	109.02975	–	0.9955	10–300	N.D.
23	Chrysin	12.20	253.05063	253.05063	–	0.9942	10–1000	5052.0
24	Apigenin	11.28	269.04555	117.03464	–	0.9904	10–400	1394.5
25	Acacetin	7.21	283.06120	268.03717	–	0.9944	10–80	315.1
26	Vicenin 2	7.84	593.15119	473.10941	–	0.9949	10–100	N.D.
27	Apiin	9.46	563.14063	269.04498	–	0.9955	10–100	N.D.
28	Vitexin	8.71	431.09837	311.05603	–	0.9984	10–500	N.D.
29	Schaftoside	8.33	563.14063	443.09949	–	0.9926	10–100	N.D.
30	Rutin	9.23	609.14611	300.02777	–	0.9922	10–500	36.2
31	Luteolin	9.83	285.04046	175.04039	–	0.9963	10–500	750.5
32	Diosmetin	11.32	299.05611	122.90711	–	0.9946	10–100	1305.0
33	Galangin	12.32	269.04555	269.04555	–	0.9912	10–100	2581.6
34	Quercetin	10.47	301.03538	151.00342	–	0.9963	10–400	983.2
35	Narcissin	9.80	623.16176	315.05139	–	0.9910	10–100	23.2
36	Isorhamnetin	11.25	315.05103	300.02780	–	0.9909	10–300	887.5
37	Afzelin	10.28	431.09837	285.04028	–	0.9926	10–500	N.D.
38	Kaempferide	12.35	299.05611	284.03265	–	0.9918	10–100	N.D.
39	Leucoside	9.82	287.05536	151.00372	–	0.9903	10–500	98.6
40	Fisetin hydrate	9.88	285.04046	135.00900	–	0.9921	10–80	N.D.
41	Naringenin	10.47	271.06120	119.05035	–	0.9932	10–100	4437.5
42	Sakuranetin	11.71	285.07685	119.05032	–	0.9957	10–200	397.2
43	Narirutin	8.83	579.17193	271.06107	–	0.9975	10–500	N.D.
44	Liquiritigenin	9.85	255.06628	255.06592	–	0.9969	10–80	N.D.
45	Daidzin	8.05	415.10346	253.05191	–	0.9932	10–100	N.D.
46	Formononetin	11.38	267.06628	252.04263	–	0.9932	10–100	17.0
47	Ellagic acid	9.43	300.99899	300.99872	–	0.9973	40–200	57.7
48	Glabridin	12.72	323.12888	135.04533	–	0.9904	10–100	11.2
49	Arbutin	0.79	271.08233	108.02180	–	0.9977	10–500	N.D.

N.D., Not detected

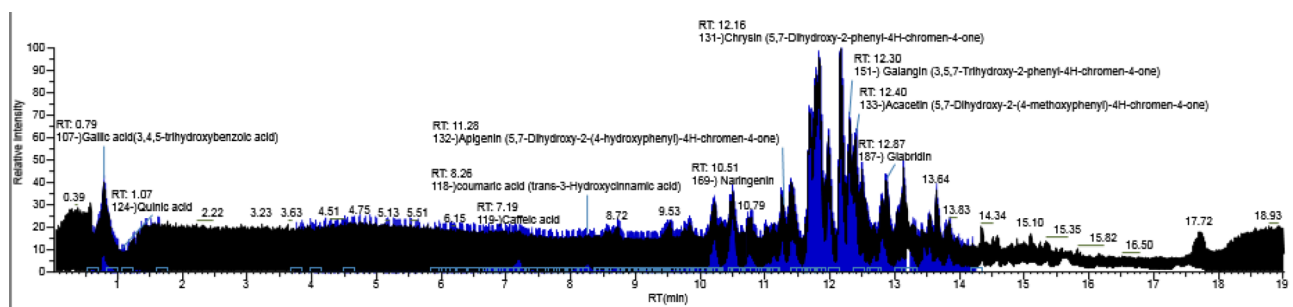


Fig. 5 Total ion chromatogram (TIC) representing the phenolic compound profile of the ethanol extract of propolis

Fig. 6 m; myocytes, asterisk; irregular spaces between myocytes, curved arrow; degeneration of myocytes, arrow; inflammatory cell infiltration

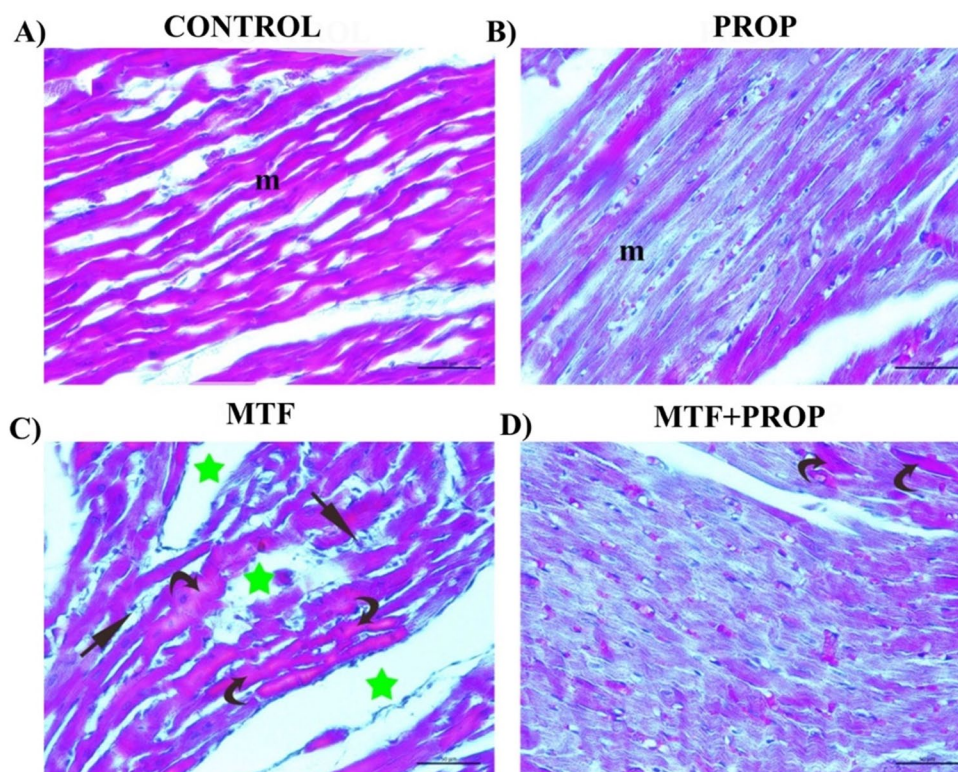


Table 6 Effects of metaflumizone and propolis on histopathological scores of rat cardiac tissue

Groups	Degeneration/necrosis	Bleeding/obstruction	Inflammatory cell infiltration
Control	–	–	–
PROP	–	–	–
MTF	+++	+	++
MTF + PROP	+	–	+

suppression of endogenous antioxidant defense mechanisms. Although (Demirel et al. 2025) did not directly evaluate MDA levels, they assessed malate dehydrogenase-1 (MDH) activity as an alternative oxidative stress biomarker in hepatic and renal tissues. Their results revealed a comparable redox disturbance following MTF administration, with significantly increased MDH activity and concomitant reductions in GSH, SOD, and CAT levels. These findings are consistent with our observations and indicate that MTF induced oxidative stress is not confined to cardiac tissue but

may contribute to broader systemic toxicity through mitochondrial dysfunction and impaired antioxidant capacity. In addition, findings from (Minassa et al. 2022) further support the centrality of oxidative stress in pesticide-induced cardiotoxicity. Their study demonstrated that intermittent exposure to chlorpyrifos, an organophosphate pesticide, induced significant cardiac remodeling in rats, characterized by hypertrophy and elevated oxidative stress markers such as NADPH oxidase (NOX₂), SOD1, CAT, and thio-barbituric acid reactive substances (TBARS) in heart tissue.

These findings confirm that chronic or repeated exposure to pesticides can overwhelm the antioxidant defense system, leading to structural and functional cardiac impairments. Notably, PROP supplementation in our study effectively reversed the oxidative alterations induced by MTF. PROP co-treatment significantly decreased MDA levels while restoring GSH, SOD, and CAT to near control values. The potent antioxidant action of propolis may be attributed to its rich content of polyphenolic and flavonoid compounds, including caffeic acid phenethyl ester (CAPE), galangin, chrysin (Russo et al. 2002), and quercetin (Kurek-Górecka et al. 2014), all of which have demonstrated free radical scavenging activity and the ability to upregulate endogenous antioxidant enzymes. Taken together, these findings highlight oxidative stress as a key pathophysiological mechanism in MTF induced cardiotoxicity and underscore the therapeutic potential of propolis as a natural antioxidant agent. Furthermore, the consistency of our results with both hepatic/renal models (Demirel et al. 2025) and chlorpyrifos induced cardiotoxicity models (Minassa et al. 2022) strengthens the translational relevance of targeting redox imbalance in pesticide-related cardiac injury. Indeed, this study constitutes one of the first experimental reports to demonstrate the ameliorative effects of propolis on MTF-induced cardiac injury, thereby highlighting its potential therapeutic value as a natural antioxidant agent in the management of pesticide-related cardiotoxicity.

The PI3K/Akt/mTOR signaling pathway plays a central role in the regulation of cellular growth, metabolism, proliferation, and anti-apoptotic mechanisms (Omolekan et al. 2024), and has been implicated in the pathogenesis of various diseases, including cardiovascular disorders (Aoyagi and Matsui 2011). Disruption or inhibition of the PI3K/Akt/mTOR signaling axis may exacerbate oxidative stress and promote apoptosis, thereby contributing to structural and functional deterioration in cardiac tissue under pathological conditions (Shackebaei et al. 2025). In the present study, MTF administration was found to significantly suppress the activities of PI3K, Akt, and mTOR in cardiac tissue supernatants. This finding suggests that MTF may induce apoptosis by inhibiting these signaling pathways, thereby contributing to cardiac cellular injury. Similarly, previous studies involving pesticide exposure (Jalouli et al. 2022) and other toxic agents (NIE et al. 2021; Xiang et al. 2025) have reported that suppression of the PI3K/Akt/mTOR signaling pathway is associated with impaired physiological function. In contrast, PROP supplementation in our study significantly increased the activity of the PI3K, Akt, and mTOR signaling pathway in the MTF+PROP group, indicating a reactivation of this critical cell survival mechanism. These findings are consistent with previous research demonstrating that propolis enhances cellular resilience under

oxidative stress conditions by modulating the PI3K/Akt/mTOR axis (Chang et al. 2018; Gelen et al. 2025). Accordingly, our results strongly support the notion that the cardioprotective effect of propolis against MTF-induced toxicity is mediated through the restoration and stabilization of PI3K/Akt/mTOR-dependent cell survival signaling. Indeed, this study is the first to demonstrate that propolis confers cardioprotective effects in an MTF-induced cardiotoxicity model by reactivating the PI3K/Akt/mTOR signaling pathway. These findings underscore the therapeutic potential of propolis in pesticide-related cardiac injury and provide a novel contribution to the biomedical literature on natural compound-based interventions.

In the present study, MTF exposure led to a significant upregulation of key inflammatory and apoptotic mediators in cardiac tissue. Specifically, the mRNA expression levels of TNF- α , IL-1 β , IL-6, NF- κ B, and cytochrome c (Cyt-c) were markedly increased in MTF-treated rats, indicating an enhanced inflammatory response and activation of mitochondria-mediated apoptotic pathways. These findings are consistent with earlier studies demonstrating that synthetic insecticides can induce systemic inflammation and apoptosis via oxidative stress-mediated activation of NF- κ B and related pathways (Gargouri et al. 2020; Hasanen et al. 2022). Propolis supplementation significantly attenuated these pathological molecular alterations. PROP treatment effectively downregulated the elevated expression levels of TNF- α , IL-1 β , IL-6, NF- κ B, and Cyt-c, highlighting its potent anti-inflammatory and anti-apoptotic properties. These effects are likely attributable to the phenolic and flavonoid content of propolis such as CAPE and quercetin which are known to inhibit NF- κ B activation and suppress pro-inflammatory cytokine production (Ansorge et al. 2003; Araujo et al. 2012). Similarly, (Demirel et al. 2025) reported that MTF administration significantly increased the expression of BAX, Caspase-3, NF- κ B, and TNF- α , while reducing BCL-2 expression in hepatic and renal tissues. In their study, taurine administration reversed these effects by downregulating pro-apoptotic and pro-inflammatory genes and restoring BCL-2 levels, indicating its cytoprotective potential. While both studies demonstrate that MTF toxicity involves inflammatory and apoptotic mechanisms, the present study is the first to document that propolis mitigates these responses specifically in cardiac tissue, suggesting its efficacy as a multi-targeted natural therapeutic agent in pesticide-induced cardiotoxicity. These findings align with previous evidence supporting the anti-apoptotic and anti-inflammatory efficacy of propolis in other models of organ injury (Mohamed et al. 2022; Gelen et al. 2025). These findings position propolis as a promising natural therapeutic agent with multi-targeted protective effects against pesticide-induced cardiotoxicity.

Indeed, this study is the first to demonstrate that PROP exerts cardioprotective effects in a MTF induced cardiotoxicity model. The findings clearly show that MTF leads to both structural and molecular damage in cardiac tissue by increasing oxidative stress, inflammation, and apoptosis. In contrast, PROP supplementation suppressed these detrimental effects, restored redox balance, modulated key intracellular signaling pathways, and contributed to the preservation of histological integrity. These results suggest that PROP may serve as a natural and effective therapeutic agent against pesticide-induced cardiac toxicity and provide a significant and original contribution to existing literature. However, some limitations of this study should be acknowledged. Specifically, the use of a fixed dose level and a single exposure duration may limit the generalizability of the findings across different toxicological profiles. Therefore, future studies should investigate dose-dependent effects of metaflumizone, assess different exposure durations, perform dose–response analyses, evaluate tissue-specific responses, and consider differences in bioavailability. Moreover, advanced molecular studies incorporating simultaneous analysis of multiple signaling pathways will be necessary to elucidate the detailed mechanisms underlying the cardioprotective effects of PROP. Such comprehensive investigations would further clarify the mechanisms of action and strengthen the scientific foundation for the broader biomedical application of PROP as a safe, natural, and effective therapeutic agent.

In conclusion, propolis significantly attenuated MTF induced cardiotoxicity by restoring redox balance, activating PI3K/Akt/mTOR signaling, and suppressing inflammatory and apoptotic responses. These findings highlight propolis as a promising natural cardioprotective agent against pesticide-induced oxidative damage.

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Data availability All data generated or analyzed during this study are included in the manuscript.
Declarations.

Declarations

Conflict of interest The authors declare no conflicts of interest.

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