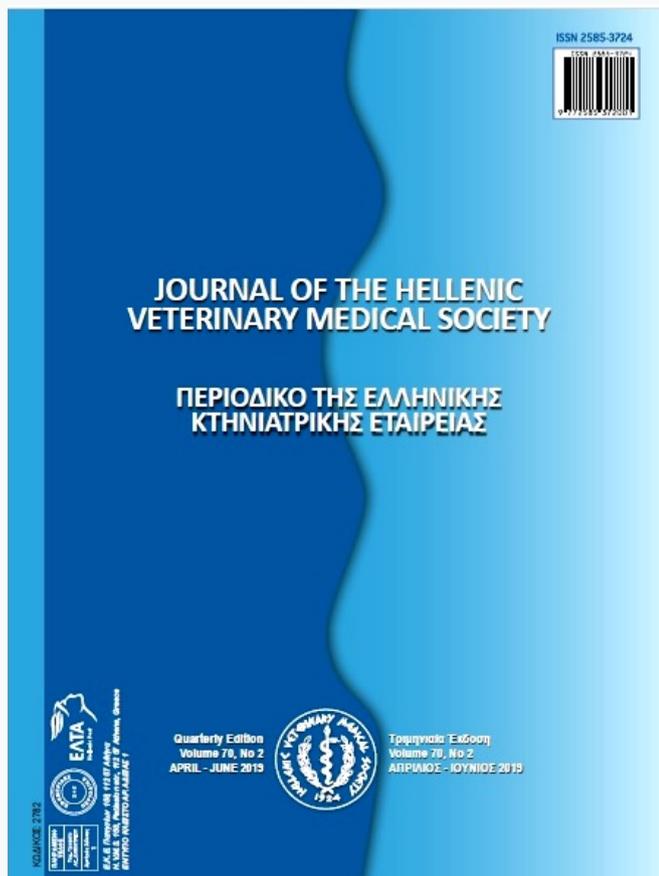


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## The effects of royal jelly on oxidative stress and toxicity in tissues induced by malathion, an organophosphate insecticide

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**ABSTRACT.** Royal jelly is a bee product frequently used in pharmaceutical, food and cosmetic industries due to its biological activities. The present study aimed to determine the effects of royal jelly on malathion-induced toxicity and biochemical changes. The rats that were used as experimental animals in the study were divided into 6 groups. Control group rats were administered nothing, while carrier chemicals (1% DMSO) were administered to sham group rats. Malathion group (MAL) rats were injected with 0.8 g/kg malathion in DMSO subcutaneously. Saline solution that included 100 mg/kg royal jelly was administered with gavage to the rats in the royal jelly group (RJ). 100 mg/kg royal jelly was administered to RJ+MAL group rats via gavage 1 hour before the injection of 0.8 g/kg malathion. 100 mg/kg royal jelly was administered to MAL+RJ group rats via gavage 1 hour after the injection of 0.8 g/kg malathion. After the experimental process (24 hours), blood samples were taken from the rats in each group under anesthesia (ketamine+xylazine). MDA, NO, GSH, GPx (glutathione peroxidase), CAT, SOD and AChE activities were determined in blood, liver, kidney and brain tissues. It was found that erythrocyte, liver, kidney and brain MDA (malondialdehyde) concentrations in MAL groups were statistically significantly higher when compared to the other groups ( $p < 0.05$ ). It was observed that GSH (glutathione) concentrations increased in the brain, while they decreased in erythrocyte, liver and kidney in the MAL group when compared to the control and sham groups. CAT (catalase) concentration significantly decreased in erythrocyte, liver, kidney and brain tissues in the MAL group when compared to the control and sham groups ( $p < 0.05$ ). SOD (superoxide dismutase) concentration in the MAL group decreased significantly ( $p < 0.05$ ) when compared to other groups, while SOD concentration increased significantly in the therapy and prevention groups ( $p < 0.05$ ) when compared to the others. It was found that serum acetylcholinesterase (AChE) concentration was significantly lower in the MAL group when compared to sham and control groups ( $p < 0.05$ ). Thus, it was concluded that malathion led to lipid peroxidation and oxidative stress in MDA and NO (nitric oxide) levels and toxicity in AChE activities. It was also determined that royal jelly could be effective against oxidative damage and toxicity. The findings suggested that the antioxidant effect of royal jelly could support the treatment of malathion, which is one of the insecticides that contain organophosphate and could lead to oxidative stress. It is considered that the prophylactic characteristics of royal jelly was more effective on malathion toxicity when compared to therapeutic properties.

**Keywords:** antioxidant, malathion, organophosphate, oxidative stress, royal jelly

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## INTRODUCTION

Royal jelly is a product secreted by the mandible and the pharyngeal gland of 5-15 days old worker bees to feed the young larvae (Khazaei et al., 2018). As soon as it is secreted, royal jelly is used directly to feed the larvae or the queen bee. The royal jelly is the primary nutrient for the queen bee, and it alters the morphology, longevity, and behavior of the queen bee during growth and development periods (Bucekova et al., 2017). Dry weight of the royal jelly includes sugar, protein, fat and amino acids. It also includes trace quantities of vitamins and minerals. Royal jelly contains all amino acids that are essential to humans. The most important amino acids among these are aspartic acid and glutamic acid. Among the enzymes found in the composition of royal jelly, glucose oxidase, phosphatase and cholinesterase are the most important. The most important fatty acid is 10-hydroxy-delta-(2)-decanoic acid (10-HDA), while the most important amino acids are glutamic acid and aspartic acid. It was suggested that the biological effects of the royal jelly were due to its royalactin, apicin and 10-hydroxy-delta-(2)-decanoic acid (10-HDA) content (Kim et al. 2010; Bincoletto et al., 2005). Several studies reported anti-tumor (Tamura et al., 1987), anti-diabetic and immune system stimulator (Heidrick et al. 1984), antiallergic (Okamoto et al., 2003), antibacterial and antiviral (Fontana et al., 2004), and anti-inflammatory (Majtan et al., 2010) properties of royal jelly.

Insecticides are the largest group of pesticides that are used to control insects which damage the agricultural crops. They affect the nervous system or destroy the biomolecules via reactive oxygen species. Since the nervous systems of the insects are highly developed and resemble those of mammals, the toxic effects of the insecticides and the organs they target are similar across the species (Costa et al., 2008). The insecticide dose, the intake method, the rate of metabolization, and the rate of exposure are significant. Globally, a large number of individuals die due to pesticide poisoning every year, and the majority of these cases are induced by insecticides (Jeyaratnam, 1990). Organophosphate compounds are not well soluble in water, however they are well soluble in oils and organic solvents. These insecticides are hydrolyzed in 2-4 weeks at the sites of administration or in solution form. Malathion [O,O-dimethyl-S-(1,2-dicarbethoxy-ethyl) phosphonodithioate], also known as Carbophos, Maldison and Mercaptothion, is an organophosphate insecticide with low toxicity in

mammals and used for insect control worldwide (Tósluty et al., 2003; Choudhary et al., 2008). Malathion is converted to more toxic malaoxon by cytochrome P450 enzymes in liver. Malaoxon is an inhibitor of the acetylcholinesterase enzyme (Aker et al., 2008). Carboxylesterase enzyme works faster in mammals when compared to insects, thus both malathion and malaoxon breaks down faster in mammals. Due to its high activity in humans, its toxic effects are scarcely observed. In insects, since this enzyme works slower, malaoxon accumulation leads to neural contractions and death (Timur et al., 2003).

Free oxygen radicals are molecules that can easily exchange electrons with other molecules due to the absence of electrons. Aerobic organisms are exposed to reactive oxygen species induced by molecular oxygen during their lifespan. The degradation of oxidant-antioxidant balance favoring the free radicals leads to various metabolic problems (Gutteridge and Mitchell, 1999). Certain studies demonstrated that antioxidant administration could reduce malathion induced oxidative stress (Kalender et al., 2010; Sodhi et al., 2008; John et al., 2001).

The present study was conducted to investigate oxidative stress that occurs due to organophosphate insecticides and malathion induced toxicity and the protective and therapeutic effects of royal jelly known to be effective against oxidative stress in rats. For this purpose, blood, liver, kidney and brain tissue MDA and NO concentrations, antioxidant levels (GSH, CAT, GPx, SOD) and AChE activities were investigated.

## MATERIAL AND METHOD

### Animals and Experimental Protocol

Ethics committee approval was obtained from Afyon Kocatepe University Animal Ethics Board Committee (AKÜHADYK-156-12). Forty-two 180-220 g Wistar-Albino rats were procured from Afyon Kocatepe University Experimental Animal Research and Application Center. The rats were kept in an environment with ideal light and temperature conditions (12 hours light/12 hours darkness) in polypropylene cages. The rats were fed ad libitum with standard mice food and water. The number of animals (n=7) was determined based on the requirements for a secure statistical analysis (Aksoy and Aslan, 2017). The rats were divided into 6 groups. No substances were administered/injected to the control group rats during the experiments. Injections were administered to the

sham group using DMSO (1%) solution subcutaneously on the loose skin behind the neck. MAL group rats were injected with 0.8 g/kg malathion in 1% DMSO subcutaneously (Moore et al. 2011; Aksoy et al., 2017). Royal jelly (RJ) group rats were administered 100 mg/kg/day royal jelly (Aksoy and Aslan, 2017). Royal jelly was dissolved in equal volumes of saline solution. MAL+ RJ group rats were first administered 100 mg/kg royal jelly in saline solution orally. After 1 hour, 0.8 g/kg malathion in 1% DMSO was injected subcutaneously. RJ+ MAL group was initially injected with 0.8 g/kg malathion in 1% DMSO subcutaneously. After 1 hour, 100 mg/kg royal jelly dissolved in saline solution was administered orally. All groups were fed on a regular basis. All rats were sacrificed, and blood samples were collected on the 24<sup>th</sup> hour of the study. Anesthesia was performed by intramuscular injection with ketamine+xylazine. Blood was collected and allowed to clot. Serum was separated at 3500 rpm for 15 minutes at 4°C, then used for the determination of serum AChE concentrations. Tissues were homogenized (1.40; w/v) in 0.1 M of phosphate buffer (pH 7.4) that contained 1mM ethylenediaminetetraacetic acid (EDTA). The homogenates were centrifuged at 18000×g for 15 min at 4°C to yield a clear supernatant fraction that was used for MDA, NO, GSH and CAT analyses. The erythrocytes were prepared with 5 min centrifuge at 3000 rpm and with 0.9% NaCl for 4000 rpm, after which the supernatants were discarded. Erythrocytes were hemolyzed with cold distilled water, and SOD and GPx values were measured.

### Biochemical Analysis

MDA concentration was determined by the Jain and Ohkawa method (Jain et al., 1989; Ohkawa et al., 1979), which is based on the measurement of the absorbance of the colored complex induced by TCA-TBA reaction at high temperatures at 535 nm. GSH concentration was determined by the method based on DTNB reduction (Beutler et al., 1963). CAT enzyme activity was measured by Aebi method (Aebi, 1974), based on the measurement of the reduction of the absorbance of H<sub>2</sub>O<sub>2</sub>, broken down by the enzyme at 240 nm. NOx levels were determined with the Griess method, where the absorbance of VCl<sub>3</sub> (Vanadium(III) chloride) and NEDD (N-1-naphthylethylenediamine dihydrochloride) formation with the addition of Somogyi reactive in the deproteinized tissues at 546 nm (Miranda et al., 2001). Serum AChE activity was measured with quantitative sandwich

EIA kits. In the measurement of SOD enzyme activity, the reaction catalyzed by xanthine oxidase from xanthine produces uric acid and superoxide radicals. The produced superoxide radical reacts with 2-(4-iodophenyl)-3-(4-nitro-phenyl-5-phenyltetrazolium chloride to form a red colored formazan compound. An assay kit was used to determine the SOD activity by the inhibition degree of this reaction. GSH was reduced by glutathione reductase in the presence of NADPH, during which NADPH (Nicotinamide adenine dinucleotide phosphate) is to NADP<sup>+</sup> (Oxidized nicotinamide adenine dinucleotide phosphate). The change in absorbance due to the decrease in reduced NADPH was measured spectrophotometrically at 340 nm and GPx enzyme activity was determined using the assay kit.

### Statistical Analysis

The data were expressed as the mean±standard deviation (SD) values. Statistical comparisons were conducted using ANOVA with Duncan post-hoc tests. Differences within p<0.05 were considered significant. The SPSS (12.0 Chicago, IL) for Windows software was used for statistical analyses.

## RESULTS AND DISCUSSION

Since OP (organophosphate) compounds are widely used, they lead to serious toxic events. The toxic effects of OP compounds occur by inhibition of the acetylcholinesterase enzyme. Another toxic effect of OP insecticide is through the production of reactive oxygen species (ROS) and facilitation of their adverse effects on biomolecules that are important for the organisms. It was emphasized that OPs are one of the causes of oxidative stress. It was demonstrated that OP compounds lead to intensive ROS production and oxidative destruction in biomolecules that are significant for the metabolism (Altuntas et al., 2003). In a previous study, it was demonstrated that malathion administration resulted in cytotoxic and genotoxic effects in bone marrow and liver cells. In the same study, it was stated that RJ was a potent antioxidant against cytotoxic and genotoxic effects on bone marrow and liver cells (Abd El-Monem, 2011). The present study was designed to determine the antioxidative effects of royal jelly on malathion induced toxicity/oxidative stress in various tissues.

Lipids are among the biomolecule groups that are destroyed by reactive oxygen species. Lipid peroxidation is among the most harmful reactions to metabolism since it proceeds as a self-sustaining chain

reaction and is irreversible. The most important product formed during the last stage of lipid peroxidation is MDA. It directly damages other cell components directly by destroying the membrane structure and indirectly by producing reactive aldehydes. These reactions lead to oxidative degradation in cellular membrane and severe tissue damage. MDA is frequently used to determine oxidative damage (Gaweł et al., 2004). The erythrocyte and liver, kidney and brain tissue MDA concentrations are presented in Table 1 for all groups. It was observed that there was a statistically significant difference between MDA levels of all MAL administered tissues and that of the other groups ( $p < 0.05$ ). The MAL group erythrocyte MDA concentration was statistically different when compared to the control and sham groups. This demon-

strated that OPs led to lipid peroxidation and played a role in oxidative stress. The high MDA in malathion administered group indicated lipid peroxidation. Pos-samai et al. (2007) analyzed oxidative damages in different tissues induced by acute and sub-chronic malathion exposure. It was observed that the present study provided a better biomarker of acute and sub-chronic malathion oxidative stress, and in particular demonstrated that lipoperoxidation was involved in OPs toxicity. The fact that MDA concentration was lower in royal jelly administered groups when compared to MAL groups ( $p < 0.05$ ) demonstrated that royal jelly reduced the damage induced by free radicals. It is suggested that it could be effective against oxidative stress by lowering the MDA when used as a preventive or therapeutic agent.

**Table 1.** Erythrocyte, liver, kidney and brain tissue MDA levels in malathion toxicity.

| Group    | Erythrocyte MDA (nmol/gHb) | Liver MDA (nmol/g protein) | Kidney MDA (nmol/g protein) | Brain MDA (nmol/g protein) |
|----------|----------------------------|----------------------------|-----------------------------|----------------------------|
| Control  | 30.59±1.87 <sup>a</sup>    | 56.40±3.80 <sup>a</sup>    | 40.17±4.73 <sup>b</sup>     | 37.26±2.98 <sup>a</sup>    |
| Sham     | 34.23±4.53 <sup>abc</sup>  | 68.74±2.64 <sup>b</sup>    | 46.70±4.14 <sup>c</sup>     | 90.38±19.78 <sup>c</sup>   |
| MAL      | 39.43±2.08 <sup>d</sup>    | 72.71±2.59 <sup>c</sup>    | 60.50±5.06 <sup>d</sup>     | 122.10±7.89 <sup>d</sup>   |
| RJ       | 32.65±4.55 <sup>ab</sup>   | 58.57±1.49 <sup>a</sup>    | 34.55±3.55 <sup>a</sup>     | 37.20±4.09 <sup>a</sup>    |
| RJ + MAL | 37.10±2.80 <sup>bcd</sup>  | 68.31±3.47 <sup>b</sup>    | 33.99±2.66 <sup>a</sup>     | 57.14±3.47 <sup>b</sup>    |
| MAL + RJ | 37.43±2.75 <sup>cd</sup>   | 67.15±3.14 <sup>b</sup>    | 32.35±4.18 <sup>a</sup>     | 51.57±9.71 <sup>b</sup>    |

Values are mean ± standard deviations (SD); n=7. a,b,c,d Different letters in the same column represent statistically significant differences ( $P < 0.05$ ). MAL, Malathion; RJ, Royal Jelly; MDA, Malondialdehyde; Hb, Hemoglobin.

NO, a free radical species produced by certain mammal cell types due to unpaired electrons, is synthesized mainly by Nitric Oxide Synthase (NOS) activity. NOS produces citrulline and NO from the L-arginine amino acid using the oxygen (Stuehr, 2004). As seen in Table 2, erythrocyte NO concentrations statistically significantly increased in malathion group when compared to the control ( $p < 0.05$ ). This suggested that malathion led to oxidative damage in erythrocytes. There was a statistically significant difference between liver NO concentration in the malathion group and control and sham groups ( $p < 0.05$ ). This finding demonstrated that malathion led to oxidative damage in liver. When kidney and brain NO concentrations were examined, it was determined that there was no statistical difference between control,

sham, and malathion groups. Although there was no statistically significant difference between liver NO concentrations in RJ + MAL and MAL + RJ groups, NO concentrations were lower in other groups when compared to the malathion group. No statistically significant differences were observed between kidney and brain NO concentrations in sham, control, malathion, RJ, RJ+MAL and MAL+RJ groups. A previous study investigated the effects of caffeic acid phenethyl ester (CAPE) and ellagic acid (EA) on nitric oxide (NO) activities in lung, liver and kidney tissues of rats exposed to acute malathion toxicity. NO levels significantly increased, however CAPE and EA decreased ( $p < 0.05$ ) NO levels due to severe tissue damage induced by MAL (Alp et al., 2011).

**Table 2.** Erythrocyte, liver, kidney and brain tissue NO levels in malathion toxicity.

| Group    | Erythrocyte NO<br>μmol (NOx)/g Hb | Liver NO<br>μmol(NOx)/g protein | Kidney NO<br>μmol(NOx)/g protein | Brain NO<br>μmol(NOx)/g protein |
|----------|-----------------------------------|---------------------------------|----------------------------------|---------------------------------|
| Control  | 102.87±6.48 <sup>a</sup>          | 95.43±7.31 <sup>a</sup>         | 75.54±5.68 <sup>ab</sup>         | 326.17±13.50 <sup>ab</sup>      |
| Sham     | 114.52±12.05 <sup>ab</sup>        | 105.44±2.88 <sup>b</sup>        | 59.18±4.22 <sup>a</sup>          | 335.19±19.33 <sup>a</sup>       |
| MAL      | 126.56±13.22 <sup>b</sup>         | 124.58±4.45 <sup>d</sup>        | 68.96±7.76 <sup>ab</sup>         | 330.30±30.80 <sup>ab</sup>      |
| RJ       | 115.86±11.59 <sup>ab</sup>        | 115.28±8.59 <sup>c</sup>        | 87.90±28.60 <sup>b</sup>         | 322.74±33.67 <sup>b</sup>       |
| RJ + MAL | 110.92±8.29 <sup>a</sup>          | 98.67±5.91 <sup>ab</sup>        | 79.73±4.70 <sup>ab</sup>         | 343.18±26.55 <sup>a</sup>       |
| MAL + RJ | 108.55±11.53 <sup>a</sup>         | 95.53±2.12 <sup>a</sup>         | 86.39±20.23 <sup>b</sup>         | 330.03±20.13 <sup>ab</sup>      |

Values are mean ± standard deviations (SD); n=7. a,b Different letters in the same column represent statistically significant differences (P<0.05). MAL, Malathion; RJ, Royal Jelly; NO, Nitric Oxide; Hb, Hemoglobin.

GSH is a tripeptide synthesized in liver. It is found at high levels in several tissues, especially in liver tissues. GSH is among the significant antioxidant cellular molecules. It reacts with free radicals and peroxides to protect the cell against oxidative damage. The cellular GSH concentration has a great impact on the antioxidant system. GSH can react with superoxide and hydroxyl radicals, thus directly exhibiting free radical scavenging properties. In particular, brain tissue is highly susceptible to lipid peroxidation due to its low GSH content, antioxidant defenses, and high polyunsaturated fatty acid content. (Barón and Muriel; 1999). As seen in Table 3, there was a statistically significant difference between the erythrocyte, liver, kidney and brain GSH levels in malathion group when compared to sham and control groups (p <0.05). The erythrocyte, liver and kidney GSH levels were lower in MAL group when compared to control and sham groups. When brain GSH levels were examined, it was determined that there was a statistically signifi-

cant increase (p <0.05) in the MAL group when compared to the control. Thus, it can be suggested that malathion administration increased toxicity in erythrocyte, liver and kidney tissues and GSH was utilized as an antioxidant defense agent. Salem et al. (2015) examined the biochemical changes that were induced by the administration of curcumin, royal jelly, and both against the oxidative stress induced by carbon tetrachloride (CCl<sub>4</sub>) in liver. It was demonstrated that curcumin, royal jelly and curcumin + royal jelly administration had significant hepatoprotective effects, preventing ROS scavenging activity and oxidative stress. The statistically significant differences (p <0.05) between erythrocyte and tissue GSH concentrations in both RJ+MAL and MAL+RJ groups when compared to MAL group suggested that royal jelly might act as protective and therapeutic agent through GSH, a significant part of the antioxidant system in malathion toxicity.

**Table 3.** Erythrocyte, liver, kidney and brain tissue GSH levels in malathion toxicity.

| Group    | Erythrocyte GSH<br>(nmol/gHb) | Liver GSH<br>(μmol/g protein) | Kidney GSH<br>(μmol/g protein) | Brain GSH<br>(μmol/g protein) |
|----------|-------------------------------|-------------------------------|--------------------------------|-------------------------------|
| Control  | 7.27±0.23 <sup>c</sup>        | 406.41±6.16 <sup>d</sup>      | 465.33±71.01 <sup>c</sup>      | 728.24±20.64 <sup>d</sup>     |
| Sham     | 6.77±0.44 <sup>b</sup>        | 384.75±6.75 <sup>b</sup>      | 346.00±45.70 <sup>ab</sup>     | 623.70±62.35 <sup>ab</sup>    |
| MAL      | 5.86±0.35 <sup>a</sup>        | 341.35±6.75 <sup>a</sup>      | 283.31±66.38 <sup>a</sup>      | 737.28±33.43 <sup>d</sup>     |
| RJ       | 7.37±0.23 <sup>c</sup>        | 340.42±6.53 <sup>a</sup>      | 361.46±40.30 <sup>b</sup>      | 693.11±10.63 <sup>cd</sup>    |
| RJ + MAL | 7.16±0.23 <sup>c</sup>        | 392.87±2.99 <sup>c</sup>      | 370.00±16.47 <sup>b</sup>      | 597.38±42.62 <sup>a</sup>     |
| MAL + RJ | 6.55±0.25 <sup>b</sup>        | 392.75±3.25 <sup>c</sup>      | 474.10±34.60 <sup>c</sup>      | 668.30±43.16 <sup>bc</sup>    |

Values are mean ± standard deviations (SD); n=7. a,b,c,d Different letters in the same column represent statistically significant differences (P<0.05). MAL, Malathion; RJ, Royal Jelly; GSH, Glutathione; Hb, Hemoglobin.

CAT is a hemoprotein that converts hydrogen peroxide into water and oxygen molecules. The amount of hydrogen peroxide in the medium is more active than catalase enzyme. When the hydrogen peroxide content is low, GPx steps in and removes H<sub>2</sub>O<sub>2</sub> from the medium (Gechev et al., 2002). Table 4 demonstrated that erythrocyte and liver, kidney and brain tissue CAT concentrations in the MAL group were statistically significantly lower when compared to the control group ( $p < 0.05$ ). It was suggested that CAT concentration decreased due to participation of catalase enzyme in antioxidative defense against oxidative damage induced by malathion administration to erythrocyte and the tissues. It was found that RJ +

MAL group erythrocyte and kidney and brain tissue CAT levels were statistically higher when compared to those of the MAL group. This suggested that royal jelly may be protective and supportive in MAL induced nephropathy and neuropathy, especially in kidney and brain tissues. In a study conducted with royal jelly, cytoprotective effects of nicotine on human umbilical vein endothelial cell (HUVECs) toxicity and catalase peroxidative functions were investigated. As a result of that study, it was concluded that hydrogen peroxide production during nicotine toxicity and vacuole-like structure formation would counteract the effect of nicotine through the RJ catalase activity (Supabphol and Supabphol, 2013).

**Table 4.** Erythrocyte, liver, kidney and brain tissue CAT levels in malathion toxicity.

| Group    | Erythrocyte CAT (k/gHb)  | Liver CAT (k/g protein) | Kidney CAT (k/g protein) | Brain CAT (k/g protein) |
|----------|--------------------------|-------------------------|--------------------------|-------------------------|
| Control  | 3.36±0.97 <sup>cd</sup>  | 26.75±4.30 <sup>b</sup> | 1.76±0.62 <sup>b</sup>   | 3.64±0.35 <sup>d</sup>  |
| Sham     | 2.06±0.29 <sup>ab</sup>  | 21.10±1.56 <sup>a</sup> | 1.53±0.30 <sup>c</sup>   | 2.27±0.27 <sup>b</sup>  |
| MAL      | 1.88±0.41 <sup>a</sup>   | 16.90±3.77 <sup>a</sup> | 1.27±0.32 <sup>a</sup>   | 1.70±0.45 <sup>a</sup>  |
| RJ       | 3.87±0.55 <sup>d</sup>   | 20.37±3.57 <sup>a</sup> | 1.89±0.35 <sup>b</sup>   | 3.20±0.17 <sup>c</sup>  |
| RJ + MAL | 2.92±0.68 <sup>bc</sup>  | 17.05±2.95 <sup>a</sup> | 1.67±0.32 <sup>c</sup>   | 2.89±0.21 <sup>c</sup>  |
| MAL + RJ | 2.74±0.68 <sup>abc</sup> | 19.27±3.43 <sup>a</sup> | 1.42±0.28 <sup>c</sup>   | 2.13±0.20 <sup>b</sup>  |

Values are mean ± standard deviations (SD); n=7. a,b,c,d Different letters in the same column represent statistically significant differences ( $P < 0.05$ ). MAL, Malathion; RJ, Royal Jelly; CAT, Catalase; Hb, Hemoglobin.

AChE activity is used as biomarker in determination of organophosphate insecticide contamination. OP compounds lead to toxic effects in humans and animals by inhibiting the acetylcholinesterase enzyme. To exhibit toxic action, OP should be oxidized to "P = O" or oxon metabolites. If it contains sulfur atoms (P = S), it does not exhibit inhibitory properties. The oxon intra-metabolites that are formed as a result of activation are hydrolyzed by the enzymes. These enzymes are present in mammal cells, however most insects lack these enzymes. Thus, insects are more susceptible to OP insecticides. Studies on organophosphate pesticides demonstrated that AChE inhibition varies based on the dose and duration of the administration, while inhibition rate varies based on the species and tissue. A 20% inhibition in acetylcholinesterase activity is an indication of the impact of organophosphorus pesticides. An inhibition of 50% or higher indicates a life-threatening condition (Worek et al., 1997). In an

experimental study conducted to investigate the effect of malathion toxicity on acetylcholinesterase activity in mice, mature mice were exposed to malathion at different doses at different times. It was found that malathion inhibited acetylcholinesterase activity in liver (Wankhade et al., 2009). It was observed that the acetylcholinesterase activity in the MAL group was statistically significantly lower when compared to all other groups ( $p < 0.05$ ) as demonstrated in Table 5. This suggested that malathion administration inhibited the enzyme. Furthermore, it is noteworthy that there was no statistically significant difference between the enzyme activities in control group and the therapeutic group.

SOD- is a metalloenzyme that protects the organism against toxic reactive oxygen derivatives by catalyzing the conversion of the superoxide radical to hydrogen peroxide and molecular oxygen. The physiological function of SOD is to protect oxygen-metab-

olizing cells against the adverse effects of superoxide free radicals such as lipid peroxidation (Culotta et al., 2006). As seen in Table 5, it was found that erythrocyte SOD activity in malathion group was statistically significantly lower when compared to all groups ( $p < 0.05$ ). It was also found that SOD enzyme activities in protective and therapeutic groups were statistically significantly higher when compared to the control group. The SOD activity findings reflected the presence of malathion induced toxicity and the defense by royal jelly due to its SOD content. Cihan et al. (2013) conducted a study to investigate the effects of bee spring on oxidative damage due to gamma-radiation in liver and lung tissues. In all exposed rats, lung and liver MDA concentrations were higher and GSH-Px, CAT and SOD were lower when compared to the control ( $p < 0.001$ ). It was observed that the application of royal jelly led to a significant decrease in oxidative stress parameters and an increase in antioxidant concentrations.

GPx- is a selenoenzyme that is responsible for the removal of cellular hydroperoxides and prevents cellular damage. The tissues with the highest enzyme activity are erythrocytes and liver tissues. GPx is the most important enzyme that protects lipids against peroxidation at the intracellular level. Therefore, this enzyme, located in the cellular cytosolic compartment, protects the cellular structure and functions (Cheeseman and Slater, 1993). As seen in Table 5, it was observed that the erythrocyte GPx activity in the malathion group was statistically significantly lower when compared to the control group ( $p < 0.05$ ). The decrease observed in the MAL group indicated that the GPx enzyme was responsible for preventing/inhibiting lipid peroxidation and hydroperoxides. The GPx activities in therapeutic and RJ groups were not statistically significantly different when compared to the control group ( $p > 0.05$ ). It was observed that royal jelly supported GPx activity in malathion induced toxicity.

**Table 5.** Serum acetylcholinesterase, erythrocyte superoxide dismutase and glutathione peroxidase enzyme activities.

| Group    | Serum AChE (U/L)           | Erythrocyte SOD (U/g Hb)    | Erythrocyte GPx (U/g Hb)  |
|----------|----------------------------|-----------------------------|---------------------------|
| Control  | 165.59±17.31 <sup>c</sup>  | 1029.17±106.48 <sup>c</sup> | 60.96±8.86 <sup>c</sup>   |
| Sham     | 145.48±23.44 <sup>bc</sup> | 904.82±159.66 <sup>b</sup>  | 55.67±7.51 <sup>abc</sup> |
| MAL      | 94.54±7.38 <sup>a</sup>    | 722.03±51.24 <sup>a</sup>   | 45.77±8.01 <sup>ab</sup>  |
| RJ       | 135.01±21.90 <sup>b</sup>  | 1144.44±70.15 <sup>c</sup>  | 65.30±10.95 <sup>c</sup>  |
| RJ + MAL | 127.72±11.14 <sup>b</sup>  | 1974.74±67.98 <sup>d</sup>  | 43.60±4.78 <sup>a</sup>   |
| MAL + RJ | 168.76±19.00 <sup>c</sup>  | 2010.83±42.87 <sup>d</sup>  | 57.15±11.46 <sup>bc</sup> |

Values are mean ± standard deviations (SD); n=7. a,b,c,d Different letters in the same column represent statistically significant differences ( $P < 0.05$ ). MAL, Malathion; RJ, Royal Jelly; AChE, acetylcholine esterase; SOD, superoxide dismutase; GPx, glutathione peroxidase; Hb, Hemoglobin.

## CONCLUSION

In conclusion, organophosphate toxicity affects several individuals, especially as a result of the contamination of nutrients. In the present study, it was observed via acetylcholinesterase concentration that malathion led to toxicity. Blood and tissue MDA levels demonstrated that malathion administration induced lipid peroxidation and NO levels demonstrated free radical formation in blood and liver tissues. The protective/preventive effects of royal jelly against lipid peroxidation were also observed. GSH concentrations in liver and kidney tissue, CAT concentrations in brain and kidney tissues and erythrocyte SOD and

GPx concentrations demonstrated that royal jelly supported the antioxidant defense against oxidative damage in these tissues. It can be suggested that royal jelly has particularly preventative effects against toxicity.

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## CONFLICT OF INTEREST

The authors declare no conflict of interest

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