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Evaluation of Bioelement Levels in Rat Tissues in Organophosphate Insecticide (Malathion) Toxicity: Effects of Caffeic Acid Phenethyl Ester (CAPE)

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Abstract

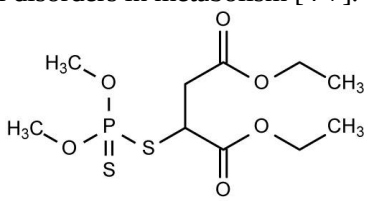
In this study, it is aimed to investigate the effects of caffeic acid phenethyl ester **(**CAPE) on bioelement levels of plasma, brain, kidney and liver tissues against malathion toxication in rats. The rats in Control group were fed with standard feed and water. Carrier chemicals given to rats were called Sham group. 0.8 gkg^{-1} malathion injected animals named MAL group. 10 μMkg^{-1} CAPE injection rats were in CAPE group. To the CAPE+MAL group, first 10 μMkg^{-1} CAPE was administered, after one hour, 0.8 gkg^{-1} malathion was injected. To the MAL+CAPE group, 0.8 gkg^{-1} malathion was injected, after an hour, 10 μMkg^{-1} CAPE was administered. Bioelement concentrations in blood and tissue samples were measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES). From the obtained data, Cu, Zn, Se, Pb, Fe, Mn level changes were remarkable. These elements are important because of their involvement in the antioxidant defense system and metallothioenin structure. The obtained bioelement concentrations show that malathion is an important factor for oxidative stress, and CAPE may an effective agent against lipid peroxidation induced oxidative damage in tissues.

Keywords: Malathion, CAPE, Bioelement, Antioxidant, ICP-OES.

Introduction

Malathion is a compound that includes organophosphate used as pesticide. Malathion that named O,O dimethyl-S-(1,2-diethyl-mercaptosuccinate) phosphorodithiodate is pale-yellow insecticide with a pungent garlic odor. Its chemical formula is $C_{10}H_{19}O_6PS_2$. Carboxyl esterase enzyme systems in mammals are faster than insects so that its toxical effect in mammals is low. Both malathion and malaoxon are rapidly metabolized and their toxicity reduced with this enzyme system. [1-3].

Bioelements have some extremely important physiological effects such as controlling of the metabolic and signaling pathways in biological processes, entering the structure of many enzyme and hormone systems. Malathion is a well known oxidant factor causes oxidative stress, considering the studies about it. It increases the formation of reactive oxygene species and repress radical scavenge of antioxidant system. Especially micro elements are involved in protection against reactive oxygen species (ROS). Both excess and lack of bioelements generates functional disorders in metabolism [4-7].



Structure of Malathion

Caffeic acid phenethyl ester (CAPE) is the most important active component of propolis. Also CAPE structure is similar to the flavonoids, is equipped with two ring structures. Two hydroxyl (OH) groups are connected to one of these rings [8]. Compounds that have antioxidant properties tend to make redox reaction. OH group containing CAPE also makes the redox reactions like antioxidant vitamins. Besides this functional group, it has a long hydrocarbon chain. Because of that it also indicates lipophilic properties. In this way, CAPE easily may pass cell membrane and comfortably reach the activity region [9, 10]. It has been reported that 10 µmol/L concentration of CAPE completely blocks ROS that are generated by neutrophills and xanthine dehydrogenase/ xanthine oxidase enzyme systems [11]. Studies have been carried out showing the antioxidative and antiinflammatory properties of CAPE against many pesticide toxication [12]. However there are no studies on the blood and tissue mineral levels against malathion toxication [13-14].

In this study, the effect of CAPE against malathion toxication, a pesticide containing organophosphate, is investigated. It is aimed to investigate how CAPE affects bioelement levels in plasma, kidney, liver and brain tissues damaged with toxicity.

Material and Methods

Animals and experimental design

Forty-two 3 months old, male wistar-albino rats with a weight of 180-240 g were used for the experiment. The study was approved by the ethics committee for animal experiments (Dated 14.06.2012, No: 180). The study was conducted in accordance with the guide for the care and use of laboratory. According to their weights, rats were divided into 6 equal groups (n=7). Any material wasn't injected to rats in the Control group (Group 1). Chemicals used as carrier (Methyl cellulose, dimethyl sulfoxide (DMSO), physiological saline) were administered to the rats in Sham group (Group 2). 0.8 gkg^{-1} malathion was injected to the rats in MAL group (Group 3). 10 μMkg^{-1} CAPE was administered to the rats in CAPE group (Group 4). 10 μMkg^{-1} CAPE was administered to the rats in CAPE+MAL group (Group 5). One hour later 0.8 gkg^{-1} malathion was injected to the same group. 0.8 gkg^{-1} malathion was injected intraperitoneally to the rats in MAL+CAPE group (Group 6) an hour later 10 μMkg^{-1} CAPE was administered injection. Twenty four hours after malathion and CAPE treatment, all rats were anesthetized with Ketamine HCl 50 mg/kg/im (Alfamin, Egevet, Istanbul, Turkey) and Xylazine HCl 7 mg/kg/im (Rampun, Bayer AG, Germany), then blood samples and required tissue samples (liver, brain, kidney) were taken.

Determination of plasma, brain, kidney, liver tissue bioelement levels

Pretreatment process was performed to degrade the organic compounds in plasma and tissue samples. Samples were placed in teflon vessels. Nitric acid (HNO_3), hydrogen peroxide (H_2O_2) and perchloric acid ($HClO_4$) were added to the samples. After the teflon vessels' lids were closed, they were placed in digestion bombs. Digestion bombs were heated in the microwave oven. The bombs were maintained 90^oC/15 min, 120^oC/15 min, 140^oC/60 min, 150^oC/60 min in microwave oven. They were allowed to cool from oven temperature to room temperature and samples in teflon vessels were transferred to flasks. Subsquently samples were diluted to 10 mL with 18.2 MΩ cm ultra distilled water. Bioelement concentrations in the samples taken to volumetric flasks were measured using inductively coupled plasma-optical emission spectroscopy (ICP-OES, Spectro Genesis, Germany).

Statistical analysis

The data obtained in the study results were expressed as mean ± standard deviation (SD). Statistical analysis of data were performed using a one-way analysis of variance (ANOVA) and Duncan posttest. Statistical analysis was performed using SPSS 15.0 software package. A value of $p<0.05$ was considered statistically significant.

Results and Discussion

Pesticides are commonly used for the protection of agricultural products from diseases, insects and harmful weeds. In this way, they help

to increase agricultural productivity and crop quality. Besides all of this, misuse of pesticides causes water, soil and air pollution. Furthermore, pesticides enter the food chain and affect animals and people. Acute pesticide poisoning ranks second after drug poisoning in all acute toxicities, therefore it is quite threat to public health [15]. Propolis is a substance collected by bees from plant extracts is used to close the holes and blocking the ingress of microorganisms and foreign matters [16]. Cinnamic acid and CAPE, are the most important active components of propolis [17, 18]. Minor elements act a vital role for antioxidant enzyme functions and stabilizations by joining their structures as metalloenzymes.

Plasma bioelement levels

Plasma bioelement levels are shown in Table 1, Al concentrations of MAL group were significantly different ($p<0.05$) from other groups. Al concentrations of CAPE group were significantly different from Control, CAPE+MAL and MAL+CAPE groups. Ba concentrations of MAL group were significantly different ($p<0.05$) from other groups. Ba concentrations of CAPE group were significantly different from Ba levels of Control, CAPE+MAL, MAL+CAPE groups. While there was no significant difference between Ca concentrations of CAPE+MAL group and MAL+CAPE group, Ca concentrations of all groups were significantly different ($p<0.05$) from each other. It was seen that Cu concentrations were significantly lower in MAL Group than other groups. Cu concentration levels of CAPE group were significantly different from the levels of Control, CAPE+MAL, MAL+CAPE groups. It was seen that Fe concentrations of CAPE group were statistically higher ($p<0.05$) than the other groups. Fe levels of MAL group were statistically lower than the other groups. Fe concentration levels of all groups were significantly different from each other, except MAL+CAPE groups Fe levels. K concentration levels of CAPE, CAPE+MAL and MAL+CAPE groups were significantly lower ($p<0.05$) than Sham and MAL groups' K levels. Li concentration levels of CAPE group were significantly different from Sham, Control, MAL and MAL+CAPE groups' Li levels. There was no significant difference between Sham and MAL Groups' Mg levels but the MAL groups' levels were significantly lower than the others. Na concentration levels of CAPE, CAPE+MAL, MAL+CAPE groups were significantly higher ($p<0.05$) than the Na levels of Sham, Control and MAL groups. There was no significant difference between Pb concentration levels of all groups. Se concentrations of MAL group were significantly lower ($p<0.05$) than Se levels of Sham, Control, CAPE groups. Se concentration levels couldn't be identified in CAPE+MAL and MAL+CAPE groups. Zn concentrations of Sham, Control, MAL, CAPE groups were significantly different ($p<0.05$) from each other.

Table 1. **Plasma trace and major element levels.**

mg/l	Control	Sham	MAL	CAPE	CAPE+MAL	MAL+CAPE
Al	4.67 ± 0.62^{a}	6.18 ± 1.59^{c}	9.96 ± 0.89^{d}	6.55 ± 0.38^{c}	4.42 ± 0.36^{ab}	3.35 ± 0.34^{b}
Ba	0.57 ± 0.11^{a}	1.48 ± 0.11^{d}	0.30 ± 0.06^{b}	1.50 ± 0.08^{d}	0.69 ± 0.05^{c}	0.54 ± 0.05^{a}
Ca	466.22 ± 18.44^{a}	373.83 ± 16.42^{b}	241.83 ± 19.96^{c}	318.77 ± 13.14^{d}	287.40 ± 6.59^{e}	273.68 ± 8.60^{e}
Cu	2.40 ± 0.09^{a}	1.86 ± 0.12^{b}	1.29 ± 0.16^{c}	1.74 ± 0.05^{b}	1.46 ± 0.08^{d}	1.59 ± 0.03^{d}
Fe	5.55 ± 0.41^{a}	4.34 ± 0.48^{b}	2.96 ± 0.61^{c}	6.87 ± 0.36^{e}	4.97 ± 0.19^{d}	4.80 ± 0.24^{bd}
K	1112.82 ± 90.22^{a}	906.09 ± 69.11^{b}	1036.53 ± 38.63^{a}	830.57 ± 35.94^{bc}	763.98 ± 49.85^{cd}	731.24 ± 56.28^{d}
Li	4.62 ± 0.18^{ab}	4.65 ± 0.16^{ab}	4.63 ± 0.44^{ab}	5.16 ± 0.36^{c}	5.05 ± 0.43^{bc}	4.55 ± 0.25^{a}
Mg	43.62 ± 3.25^{ad}	38.14 ± 2.30^{bc}	34.73 ± 3.63^{b}	40.65 ± 2.15^{ac}	47.13 ± 2.07^{d}	44.08 ± 1.94^{ad}
Na	384.74 ± 6.91^{a}	408.53 ± 6.34^{b}	377.94 ± 8.35^{a}	427.25 ± 15.37^{c}	441.80 ± 12.76^{d}	440.41 ± 7.06^{d}
Pb	16.21 ± 0.86^{a}	16.90 ± 0.36^{a}	17.10 ± 0.64^{a}	17.03 ± 0.54^{a}	16.60 ± 1.89^{a}	16.21 ± 0.72^{a}
Se	6.31 ± 0.54^{a}	6.06 ± 0.45^{b}	5.53 ± 0.50^{c}	6.21 ± 0.13^{a}	---	---
Zn	26.28 ± 2.47^{a}	23.64 ± 2.13^{b}	14.23 ± 0.99^{c}	18.16 ± 2.10^{d}	17.49 ± 2.07^{d}	12.78 ± 1.20^{c}

a, b, c, d, e: Differences between the groups that are coded with different letters in the same line are significant ($p<0.05$). MAL represents the group administered with malathion; CAPE represents the group solely administered with caffeic acid phenethyl ester; CAPE+MAL represents the group administered 10 μMkg^{-1} CAPE, after one hour, 0.8 gkg^{-1} malathion was injected; MAL+CAPE represents the group administered 0.8 gkg^{-1} malathion, after an hour, 10 μMkg^{-1} CAPE was administered.

Brain tissue bioelement levels

Brain tissue bioelement levels are shown in Table 2. When the Table 2 was examined, there was no significant difference between Al concentrations of all groups except CAPE+MAL and MAL+CAPE groups. In all groups, there was no significant difference between Ba and Fe levels. It was seen that Bi levels of MAL and CAPE groups were significantly different from ($p<0.05$) Control and Sham groups levels. Ca levels of Sham and MAL+CAPE groups were significantly higher ($p<0.05$) than the other groups Ca levels. Cu concentrations of MAL group were significantly different from MAL+CAPE and CAPE+MAL groups Cu levels. K levels of CAPE+MAL group were significantly higher ($p<0.05$) than Sham, Control and MAL groups K levels. Li levels of CAPE, CAPE+MAL and MAL+CAPE groups were significantly higher ($p<0.05$) than Li levels of Sham, Control and MAL groups. Mg levels of CAPE and MAL groups were found significantly lower ($p<0.05$) than Control groups levels. It was determined that Mn levels of Control group were significantly higher ($p<0.05$) than all groups. Na levels of CAPE, CAPE+MAL and MAL+CAPE were significantly higher than Na levels of Sham, Control and MAL groups. Pb levels of CAPE, CAPE+MAL and MAL+CAPE groups were significantly lower ($p<0.05$) than Pb levels of Control groups. Zn levels of MAL group were significantly higher ($p<0.05$) than Zn levels of all groups except CAPE+MAL groups.

Table 2. **Brain tissue trace and major element levels.**

ppm	Control	Sham	MAL	CAPE	CAPE+MAL	MAL+CAPE
Al	3.20 ± 0.19^{d}	2.55 ± 0.27^{c}	3.40 ± 0.27^{d}	1.90 ± 0.51^{a}	2.32 ± 0.42^{bc}	1.68 ± 0.26^{ab}
Ba	0.33 ± 0.17^{a}	0.29 ± 0.11^{a}	0.36 ± 0.10^{a}	0.36 ± 0.26^{a}	0.51 ± 0.25^{a}	0.43 ± 0.18^{a}
Bi	0.73 ± 0.09^{b}	0.43 ± 0.08^{a}	1.19 ± 0.08^{d}	0.97 ± 0.12^{c}	1.07 ± 0.11^{c}	0.78 ± 0.05^{b}
Ca	131.43 ± 7.99^{b}	102.65 ± 4.62^{a}	104.19 ± 3.00^{a}	94.20 ± 5.28^{a}	101.08 ± 9.14^{a}	126.73 ± 14.73^{b}
Cu	2.77 ± 0.32^{bc}	2.51 ± 0.16^{ab}	2.96 ± 0.12^{c}	2.62 ± 0.39^{abc}	2.54 ± 0.27^{ab}	2.39 ± 0.20^{a}
Fe	20.10 ± 3.59^{a}	17.35 ± 0.68^{a}	20.08 ± 2.39^{a}	18.92 ± 2.05^{a}	19.90 ± 1.84^{a}	18.24 ± 1.20^{a}
K	2126.58 ± 98.45^{ab}	2107.45 ± 15.31^{a}	2124.47 ± 42.62^{ab}	2248.87 ± 154.49^{bc}	2335.41 ± 53.64^{c}	2231.03 ± 98.83^{abc}
Li	0.51 ± 0.05^{b}	0.61 ± 0.05^{c}	0.05 ± 0.01^{a}	1.06 ± 0.08^{d}	0.96 ± 0.09^{d}	0.97 ± 0.05^{d}
Mg	170.87 ± 5.89^{c}	152.81 ± 5.27^{ab}	160.03 ± 4.56^{b}	151.83 ± 6.91^{ab}	172.35 ± 10.40^{c}	149.38 ± 6.68^{a}
Mn	0.40 ± 0.02^{b}	0.27 ± 0.01^{a}	0.34 ± 0.01^{c}	0.34 ± 0.03^{c}	0.29 ± 0.03^{a}	0.32 ± 0.03^{c}
Na	222.14 ± 7.59^{a}	224.99 ± 6.37^{a}	223.14 ± 6.34^{a}	253.87 ± 27.79^{b}	276.24 ± 18.59^{b}	267.72 ± 24.12^{b}
Pb	3.95 ± 0.08^{c}	3.75 ± 0.19^{bc}	3.75 ± 0.09^{bc}	3.45 ± 0.32^{a}	3.55 ± 0.17^{ab}	3.50 ± 0.11^{a}
Zn	15.51 ± 0.58^{a}	15.44 ± 0.82^{a}	17.53 ± 1.53^{ab}	21.61 ± 3.14^{c}	19.15 ± 3.50^{bc}	16.42 ± 3.46^{ab}

a, b, c, d : Differences between the groups that are coded with different letters in the same line are significant ($p<0.05$). MAL represents the group administered with malathion; CAPE represents the group solely administered with caffeic acid phenethyl ester; CAPE+MAL represents the group administered 10 μMkg^{-1} CAPE, after one hour, 0.8 gkg^{-1} malathion was injected; MAL+CAPE represents the group administered 0.8 gkg^{-1} malathion, after an hour, 10 μMkg^{-1} CAPE was administered.

Kidney tissue bioelement levels

Kidney tissue bioelement levels are shown in Table 3. When the Table 3 was examined, it was seen that Al concentrations of Sham group were significantly different ($p<0.05$) from CAPE group. Also Al concentrations of MAL+CAPE group were significantly different ($p<0.05$) from MAL and CAPE groups. When the Bi levels were examined, it was seen that only Bi levels of CAPE+MAL group and MAL group were different from each other. Ca concentrations of MAL+CAPE group were significantly different ($p<0.05$) from CAPE+MAL group. Ca concentrations of these two groups were significantly higher than the other groups. Cu concentrations of MAL+CAPE group were significantly different ($p<0.05$) from the levels of CAPE+MAL group. Fe concentrations of MAL group were significantly higher ($p<0.05$) than CAPE group. There was no significant difference between the K, Li, Mg, Pb levels of all groups in kidney tissue. Mn levels of CAPE+MAL group were significantly lower than all groups. Na levels of CAPE+MAL group were significantly higher ($p<0.05$) than all groups. Also Na levels of MAL+CAPE group were significantly higher ($p<0.05$) than Sham and Control groups. Se concentrations of MAL group were significantly lower ($p<0.05$) than Sham and Control groups. Zn levels of CAPE+MAL and MAL+CAPE groups were significantly higher ($p<0.05$) than the other groups.

Table 3. **Kidney tissue trace and major element levels.**

ppm	Control	Sham	MAL	CAPE	CAPE+MAL	MAL+CAPE
Al	1.57 ± 0.06^{abc}	1.48 ± 0.04^{ab}	1.66 ± 0.08^{bc}	1.78 ± 0.10^{c}	1.63 ± 0.14^{abc}	1.42 ± 0.33^{a}
Bi	1.58 ± 0.07^{ab}	1.49 ± 0.15^{ab}	1.39 ± 0.11^{a}	1.47 ± 0.23^{ab}	1.74 ± 0.32^{b}	1.65 ± 0.28^{ab}
Ca	73.65 ± 4.35^{a}	76.00 ± 5.25^{a}	64.12 ± 3.83^{a}	74.10 ± 5.05^{a}	130.72 ± 10.54^{b}	115.76 ± 18.62^{c}
Cu	5.85 ± 0.47^{ab}	5.75 ± 0.33^{ab}	5.69 ± 0.45^{ab}	5.95 ± 0.85^{ab}	5.04 ± 1.25^{a}	6.33 ± 0.43^{b}
Fe	86.71 ± 9.54^{bc}	65.42 ± 6.70^{a}	96.65 ± 3.70^{c}	79.32 ± 11.94^{b}	86.39 ± 10.88^{bc}	78.39 ± 10.25^{b}
K	1406.08 ± 55.22^{ab}	1401.96 ± 40.89^{ab}	1530.81 ± 77.82^{b}	1497.84 ± 146.22^{b}	1417.53 ± 92.71^{ab}	1292.57 ± 133.87^{ab}
Li	1.15 ± 0.09^{a}	1.20 ± 0.16^{a}	1.18 ± 0.07^{a}	1.10 ± 0.19^{a}	1.23 ± 0.11^{a}	1.15 ± 0.09^{a}
Mg	166.15 ± 17.11^{a}	173.33 ± 22.41^{a}	157.52 ± 22.30^{a}	172.35 ± 15.73^{a}	176.56 ± 13.69^{a}	174.55 ± 21.15^{a}
Mn	0.65 ± 0.02^{b}	0.67 ± 0.03^{b}	0.65 ± 0.06^{b}	0.66 ± 0.06^{b}	0.56 ± 0.03^{a}	0.62 ± 0.05^{b}
Na	299.38 ± 11.88^{a}	295.63 ± 11.65^{a}	313.79 ± 14.54^{ab}	309.93 ± 27.57^{ab}	373.86 ± 35.64^{c}	340.46 ± 24.14^{b}
Pb	3.58 ± 0.16^{a}	3.58 ± 0.12^{a}	3.59 ± 0.17^{a}	3.61 ± 0.14^{a}	3.52 ± 0.12^{a}	3.47 ± 0.12^{a}
Se	2.03 ± 0.12^{b}	2.57 ± 0.14^{c}	1.37 ± 0.20^{a}	2.11 ± 0.25^{b}	2.19 ± 0.24^{b}	1.93 ± 0.20^{b}
Zn	21.46 ± 1.73^{a}	21.83 ± 2.05^{a}	19.49 ± 1.90^{a}	19.55 ± 1.60^{a}	26.60 ± 3.22^{b}	25.90 ± 2.86^{b}

a, b, c, d: Differences between the groups that are coded with different letters in the same line are significant ($p<0.05$). MAL represents the group administered with malathion; CAPE represents the group solely administered with caffeic acid phenethyl ester; CAPE+MAL represents the group administered 10 μMkg^{-1} CAPE, after one hour, 0.8 gkg^{-1} malathion was injected; MAL+CAPE represents the group administered 0.8 gkg^{-1} malathion, after an hour, 10 μMkg^{-1} CAPE was administered.

Liver tissue bioelement levels

Liver tissue bioelement levels are shown in Table 4. When the Table 4 was examined, it was seen that Al concentrations of all groups were significantly different ($p<0.05$) from each other, except CAPE+MAL and MAL+CAPE groups. Ba levels of CAPE group were significantly higher than all groups. It was found that Bi concentrations of CAPE, CAPE+MAL and MAL+CAPE groups were significantly lower ($p<0.05$) than Control and Sham groups. Ca concentrations of CAPE, CAPE+MAL and MAL+CAPE groups were significantly different ($p<0.05$) from MAL, Control and Sham groups. Cu concentrations of MAL group were significantly lower ($p<0.05$) than the other groups. K concentrations of CAPE and CAPE+MAL groups were significantly higher than the other groups. Li concentrations of MAL+CAPE and CAPE+MAL groups were significantly lower ($p<0.05$) than the other groups. Just as Li concentrations, Mg concentrations of MAL+CAPE and CAPE+MAL groups were significantly lower ($p<0.05$) than the others. Mn levels of CAPE group were significantly different ($p<0.05$) from Control, MAL and CAPE+MAL groups. There was significant difference between Na levels of CAPE+MAL and Control groups. Pb levels of CAPE+MAL and MAL+CAPE groups were significantly lower ($p<0.05$) than MAL and CAPE groups. Se concentrations of MAL, CAPE and MAL+CAPE groups were significantly lower ($p<0.05$) than Sham group. Zn concentrations of CAPE+MAL and MAL+CAPE groups were significantly higher ($p<0.05$) than Control group. Also Zn levels of CAPE+MAL group were significantly higher ($p<0.05$) than CAPE group.

Table 4. **Liver tissue trace and major element levels.**

ppm	Control	Sham	MAL	CAPE	CAPE+MAL	MAL+CAPE
Al	1.49 ± 0.26^{b}	0.72 ± 0.17^{a}	2.58 ± 0.23^{c}	2.85 ± 0.05^{d}	1.33 ± 0.21^{b}	1.44 ± 0.05^{b}
Ba	0.22 ± 0.05^{ab}	0.39 ± 0.04^{c}	0.21 ± 0.03^{a}	0.52 ± 0.05^{d}	0.28 ± 0.07^{b}	0.21 ± 0.05^{a}
Bi	1.27 ± 0.05^{d}	1.31 ± 0.10^{d}	1.17 ± 0.13^{cd}	1.01 ± 0.13^{ab}	1.05 ± 0.15^{bc}	0.88 ± 0.08^{a}
Ca	78.70 ± 4.64^{b}	70.28 ± 9.69^{b}	68.48 ± 8.60^{b}	48.69 ± 7.11^{a}	129.72 ± 18.65^{c}	113.04 ± 7.78^{d}
Cu	4.34 ± 0.26^{ab}	3.96 ± 0.36^{a}	3.22 ± 0.24^{c}	4.24 ± 0.38^{ab}	4.54 ± 0.45^{b}	5.29 ± 0.42^{d}
Fe	294.56 ± 22.93^{bc}	240.81 ± 18.55^{a}	270.05 ± 24.54^{c}	320.28 ± 19.04^{b}	270.67 ± 18.62^{c}	283.57 ± 17.18^{c}
K	1951.57 ± 109.83^{a}	1911.13 ± 78.60^{a}	1995.18 ± 38.24^{ab}	2082.57 ± 33.84^{b}	2055.63 ± 87.36^{b}	1898.00 ± 24.94^{a}
Li	2.14 ± 0.05^{b}	1.97 ± 0.17^{b}	2.00 ± 0.08^{b}	1.98 ± 0.27^{b}	0.18 ± 0.14^{a}	0.18 ± 0.10^{a}
Mg	262.24 ± 11.32^{b}	267.69 ± 13.75^{b}	276.36 ± 9.78^{b}	267.04 ± 10.78^{b}	226.58 ± 20.21^{a}	218.12 ± 8.50^{a}
Mn	2.49 ± 0.19^{a}	2.23 ± 0.06^{b}	1.65 ± 0.12^{c}	2.09 ± 0.16^{b}	1.80 ± 0.07^{c}	2.18 ± 0.24^{b}
Na	211.11 ± 9.04^{ab}	228.67 ± 18.84^{b}	221.55 ± 17.17^{ab}	215.98 ± 17.11^{ab}	199.04 ± 17.54^{a}	215.36 ± 21.10^{ab}
Pb	3.40 ± 0.11^{abc}	3.37 ± 0.25^{abc}	3.56 ± 0.19^{c}	3.60 ± 0.12^{c}	3.23 ± 0.27^{a}	3.31 ± 0.15^{ab}
Se	2.05 ± 0.24^{a}	1.83 ± 0.10^{ab}	1.74 ± 0.07^{b}	1.67 ± 0.25^{b}	1.81 ± 0.06^{ab}	1.59 ± 0.28^{b}
Zn	30.50 ± 5.27^{a}	32.56 ± 5.76^{ac}	35.95 ± 4.81^{ac}	34.07 ± 5.83^{ac}	47.58 ± 11.23^{b}	40.80 ± 5.52^{bc}

a, b, c, d: Differences between the groups that are coded with different letters in the same line are significant ($p<0.05$). MAL represents the group administered with malathion; CAPE represents the group solely administered with caffeic acid phenethyl ester; CAPE+MAL represents the group administered 10 μMkg^{-1} CAPE, after one hour, 0.8 gkg^{-1} malathion was injected; MAL+CAPE represents the group administered 0.8 gkg^{-1} malathion, after an hour, 10 μMkg^{-1} CAPE was administered.

In this study, Se element concentrations in brain tissue, Mn and Bi element concentrations in plasma, Ba element concentrations in kidney tissue could not be measured because they were too low. Concentrations of these bioelements in the liver were measured. Among all study groups, there were no significant difference between Pb levels in plasma, Ba and Fe levels in brain tissue, K, Li, Mg and Pb levels in kidney, Li and Mg levels in liver.

Moreover, it was seen that Cu concentrations of MAL group in brain tissue was significantly higher than other groups, in plasma and liver tissue was significantly lower than the others. Cu is an essential minor element for the structure of enzymes such as superoxide dismutase (SOD). SOD is a well known antioxidant enzyme for intracellular and extracellular lipids. Cu ions also catalyze free radical reactions and their damages. Copper-induced oxidative reactions generally result with the formation of $OH^{\cdot}$ radical. $OH^{\cdot}$ as a high reactive metabolite leads lipid peroxidation induced tissue damage [19, 20]. In the study, the high Cu concentrations in brain tissue may be marker of brain tissue damage. It was seen that Zn concentrations of MAL+CAPE group in plasma were significantly lower than the other groups. Barany et al. have also referred to a positive correlation between Cu and Zn in whole blood and serum [21]. Zn's role for the activation of many enzymes in the organism is a well known data. Especially, Cu and Zn are equally found in the structure of the SOD. Plasma Zn is in free form. It is the cofactor of SOD and inhibits iron and copper's binding to biological molecules, thus prevents oxidative damage. Lower Zn concentrations of MAL groups may cause from the Zn content of SOD [22, 23].

Metallothionein is cysteine-rich, metal binding intracellular protein having a low molecular weight. Metallothionein acts in detoxification of heavy metals such as Cd, Hg and homeostasis of essential metals such as Zn, Cu. It has much affinity to the essential metals Zn and Cu. Due to the free radical scavenging effect, Metallothionein is an important protein in antioxidant defense against intracellular oxidative damage. Heavy metals, ionizing radiation and oxidative stress induced to metallothionein transcription [24].

Pb is a very famous toxic element, especially to the brain. It affects voltage-gated channels, the primary and secondary messengers reducing the regulatory effect of Ca. In this study, Pb levels of the treatment group was significantly lower than Control, Sham, MAL groups. This status can indicate the method we apply reduces the Pb toxicity. It was reported that, Pb damage can be decreased by the increase of Zn levels [25].

Se is an essential element for mammalian development. It is found in structure of many enzymes and structural proteins like selenocysteine. Glutathione Peroxidase (GSH-Px) is an antioxidant enzyme. This enzyme protects biomolecules such as DNA, lipids, lipoproteins from oxidative damage. It helps to maintain the integrity of cell membrane. GSH-Px levels in tissues and organs were directly related with Se levels [26, 27]. In this study, the reverse correlation between Pb and Se in liver is remarkable. A similar situation was observed in other studies [21]. SOD has three izoenzymes; Cu/Zn-SOD, Mn-SOD, Fe-SOD. Mn and Fe are neurotoxic agents also they show antioxidant effect joining the SOD structure. They can cause damage in dopaminergic neurons. This damage leads to ROS formation and dopamine auto-oxidation. It was noted that, high Mn concentration increases lipid peroxidation and OH radicals [28]. Mn accumulation also increases Fe levels. High Fe concentration increases lipid peroxidation by Fenton reaction. In this study, it was seen that there was no difference in brain Fe levels between groups. Mn concentrations were same in CAPE and MAL groups. It was seen that Fe concentrations of CAPE group were higher than the others; Mn concentrations of MAL group were lower than the others.

Conclusion

There is an antioxidant mechanism that protects tissues from the free radical induced damage. If free radicals that reactive products of the metabolism are not removed effectively, they cause tissue damage from peroxidation to cell deaths. In the antioxidant defense, bioelements of enzymes and hormones play an important protection role against ROS and oxidative stress

induced damages. Changes in trace element levels, reduce the effectiveness of antioxidant defense mechanisms, and increase the negative effects of oxygen free radicals on the cell membranes integrity. In this study, it is seen important changes in plasma, liver, brain and kidney tissue bioelement levels. The differences in Cu, Zn, Se, Pb, Fe, Mn concentrations are important because they are well known bioelements of the antioxidant enzymes SOD and GSH-Px and metal-binding proteins like Metallothionein. In conclusion, it can be noted that CAPE has positive effects on malathion origin damage when the bioelement concentrations are considered. For the better and net conclusions, further studies about oxidative damage and antioxidant enzyme concentrations are needed.

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