Indian Journal of Experimental Biology
Vol. 61, January 2023, pp. 51-58
DOI: 10.56042/ijeb.v61i01.49688

Subacute exposure to dimethoate induces hepatotoxic and nephrotoxic effects on male rats: Ameliorative effects of ferulic acid

Fatma Gökçe Apaydın*[1], Suna Kalender[2] & Yusuf Kalender[1]

[1]Department of Biology, Faculty of Science, Gazi University, 06500 Ankara, Turkey

[2]Department of Science, Faculty of Gazi Education, Gazi University, 06500 Ankara, Turkey

Received 08 May 2021; *revised 19 November 2022*

Dimethoate commonly used as environmental ares for control pests which is widely used throughout in the world and itcaused toxic effects on nontarget organisms especially mammalian. Ferulic acid is known to protective compound generally used in toxicology studies. Thus, inthis study, we investigatedthe protective role of ferulic acid against the possible toxic effects of low and high doses of dimethoate. Male rats were randomly divided into six groups: control; ferulic acid; low and high dose dimethoate; both ferulic acid and low dose dimethoate; both ferulic acid and high dose dimethoate. The dimethoate treatment to rats caused oxidative stress in liver and kidney tissue via increased malondialdehyde levels and changes in superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase activities. All dose dimethoate treatments also caused histopathological alterations and differences in activities in alanine aminotransferase, aspartate aminotransferase, total protein, albumin, lactate dehydrogenase, total cholesterol, urea, uric acid, and creatinine levels. The histopathological results verified the biochemical findings for both liver and kidney. Co-administration of ferulic acid with dimethoate improved antioxidative parameters and eased some biochemical parameters mentioned above. Ferulic acid was also seen to play a beneficial role in the histopathological effects of dimethoate for both liver and kidney.

Keywords: Histopathology**,** Kidney toxicity, Liver toxicity, Oxidative stress

Pesticides have been commonly used in agricultural areas to combat pests, to keep vectors under control and to enhance nutritional quality[1,2]. Pesticides have had toxicological effects in various ways to different organ systems, such as, hematological system[3], neurotoxicity[4,5], lung[6], liver[7,8] spleen[5] and cytotoxic effects[5,9] in experimental animals.

Among pesticides, dimethoate (DM) is an important organophosphorus compound (OP) and is used for pears, leaf miners and apples to protection against several pests[10.] Recently, some researchers have reported that DM causes important changes in glucose homeostasis and is related to toxicity in the pancreas in rats[11]. Dimethoate exposure causes tissue injury and is associated with this oxidative damage[12].Jallouli *et al*.[13] Investigated the oral administration of dimethoate, and they observed the inhibition of testicular acetylcholinesterase activities and a decrease in antioxidant activities. In particular, we compared the low and high dose dimethoate rates and investigate the ameliorative effect of ferulic acid. Phenolic compounds have been investigated in coffee, wheat, corn, rice, tomatoes and some fruits and vegetables[14-18].

Studies have indicated that polyphenols regulate enzyme activities[19], and protect cell membranes from lipid peroxidation (LPO), peroxyl radicals[20] and scavenges hydroxyl radicals[21]. Ferulic acid (FA) (4-hydroxy-3-methoxycinnamic acid) is a natural antioxidant phenolic compound, which is a phytochemical classified as a hydroxycinnamic acid, and has also been investigated in wheat, corn, rice, tomatoes, spinach, berries, peas, asparagus and cabbage[14,15]. Recently, Roumili *et al*.[22], who studied the antioxidant and anti-inflammatory activities of White Willow *Salyx alba* have shown FA to be one of major phytoconstituents. Studies have reported that dietary FA reduces the incidence of many diseases[23,24]. The antioxidant capacity of cells is associated with oxidative damage, and antioxidant enzyme levels in cells show different levels of oxidative stress[11]. Many antioxidant enzymes in cells, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), and glutathione (GSH)[25].Oxidative stress is associated with the amount of ROS, including free radicals[26-29].

*Corresponding author:
Phone: +90 312 2021506; Fax: +90 312 2122279
E-Mail: fguzun@gazi.edu.tr

In this study, we chose the liver and kidney because the liver is an organ involved in detoxification and homeostasis[30], and the kidney is an important organ that maintains the internal balance of the body[31]. This study determined histopathological, biochemical and anti-oxidative changes following subchronic exposure to DM in the kidney and liver of rats.

Materials and Methods

Reagents

Ferulic acid (purity >97%), dimethoate (purity >97%), reduced glutathione, nicotinamide adenine dinucleotide phosphate reduced form (NADPH) and thiobarbituric acid (TBA) were purchased from Sigma (St. Louis, MO, USA). All other chemicals were of analytical grade and were purchased from standard commercial suppliers.

Animals and treatment

Adult Wistar rats, weighing 200-250 g. were purchased from Gazi University Laboratory of Animals Raising and Experimental Research Center. The animals had free access to commercial food pellet diet and water *ad libitum* exposed to a 12 h light/dark cycle, and maintained at a laboratory temperature of 20±2°C. All the chemicals were applied by oral gavage. Our study was conducted the confirmation of the Gazi University Animal Experiments Local Ethics Committee (G.U. ET-17.004). Our treatment groups were as follows:Gr. I: Administered with distilled water (Control rats); Gr. II: Ferulic acid (FA) treated rats (30 mg/kg body wt.); Gr. III: Low dose-dimethoate treated rats (3 mg/kg body wt.), Gr. IV: High dose-dimethoate treated rats (30 mg/kg body wt.), Gr. V: Low dose-DM (3 mg/kg) plus FA (30 mg/kg body wt.) treated rats, and Gr. VI:High dose-DM plus FA both (30 mg/kg body wt.).At the end of the experimental period, animals of different groups were euthanized with the alphamine and alphazine combination. Blood was collected in EDTA tubes and centrifuged at 2200g for 10 min. Tissue samples were taken for microscopic and enzymatic investigations of both liver and kidney tissue.

Assay of antioxidant enzyme status

Superoxide dismutase level in the liver and kidney was estimated by the method of Marklung & Marklung[32], and its activity was expressed as units/mg protein. Catalase was assayed according to the method of Aebi[33] and expressed as nmol/mg protein of hydrogen peroxide unit's consumed/min/mg protein. Glutathione-S-tranferase enzyme activity was assayed by the method of Habig *et al*.[34] And expressed as µmol/mg protein of CDNB-GSH conjugate formed. Glutathione peroxidase was assayed by the method of Paglia and Valentine[35] and its activity was expressed as nmol/mg protein. Protein concentration in the kidney and liver was determined by the method of Lowry *et al*.[36] using bovine serum albumin (BSA) as the standard.

Lipid peroxidation

Lipid peroxidation was determined by malondialdehyde production and measured in liver and kidney homogenates according to the method of Ohkawa *et al.*[37] Based on TBARS formation, and it was expressed as MDA content.

Liver and kidney histopathology

At the end of the study, the rats were sacrificed and examined for tissue pathological abnormalities. After the tissues were obtained, they were taken into 10% formalin fixation and dehydrated in alcohol, and finally embedded in paraffin. Then, kidney and liver sections were cut with 6-7 µ thickness. The slides were then rehydrated and stained with hematoxylin and eosin. At least ten slides were observed with a microscope for both liver and kidney (Olympus, Tokyo, Japan). Each liver and kidney tissue were examined and assigned for the severity of changes using scores on the scale of none (-), mild (+), moderate (++), and severe (+++) damage.

Blood sampling and Biochemical markers in plasma

Blood samples were collected from the heart with gel blood tubes for liver and kidney biochemical analyses. Blood samples kept in non-anticoagulant tubes were centrifuged at 1000 g for 10 min, and the serum was used. The activities of plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein (TP), albumin, lactate dehydrogenase (LDH), and total cholesterol (TK), Creatinine, Blood urea nitrogen (BUN), and Uric acid were assayed with a commercial spectrophotometer-enzymatic kit and analyzed (Roche Cobas c501).

Statistical analysis

All data groups were analyzed with the use of SPSS for Windows (version 13.0). The results are presented as a mean value ± standard error of the mean (SEM) by ANOVA.

Results

Oxidative stress and antioxidant system parameters

Liver

Data presented in Table 1 demonstrated that HDM-treatment significantly increased MDA level compared to control and FA groups. A statistically

Table 1 — Effects of dimethoate on liver and kidney antioxidant enzymes

	SOD (U/mg protein)	CAT (mmol/mg protein)	GPx (nmol/mg protein)	GST (µmol/mg protein)	MDA (nmol/mg protein)
Liver					
Control	0,0280±0,0067	5449,17±688,50	0,0025±0,00041	8,0335±1,453	282,382±42,870
FA	0,0277±0,0092	5618±1165,46	0,0026±0,0017	6,4600±2,818	268,002±45,892
LDMT	0,0160±0,0033[a,b]	5312±756,70	0,0061±0,0031	17,631±3,132[a,b]	378,053±43,148
HDMT	0,0170±0,0055[a,b]	3655,24±345,30[a,b]	0,0099±0,0058[a,b]	19,366±4,530[a,b]	451,597±97,524[a,b]
FA+LDMT	0,0202±0,0039	4103,88±1434,55	0,0016±0,0006[d]	19,597±2,061[a,b]	320,315±52,229[d]
FA+HDMT	0,0187±0,00332	3331,73±1041,16[a,b,c]	0,0020±0,0003[d]	19,108±2,334[a,b]	304,923±86,415[d]
Kidney					
Control	0,0044±0,0007	6331,59±660,09	0,0002±0,00001	1,2235±0,117	208,87±71,055
FA	0,0040±0,0006	7072,56±952,43	0,0002±0,00004	1,0329±0,185	137±57±61,024
LDMT	0,0040±0,0004	7473,44±579,99	0,0003±0,00007[a,b]	1,3614±0,175	280,10±115,81
HDMT	0,0022±0,0002[a,b,c]	2867,50±1211,76[a,b,c]	0,0003±0,00003[a,b]	1,5951±0,295[a,b]	502,88±45,355[a,b,c]
FA+LDMT	0,0052±0,0009[d]	5869,76±958,56[c,d]	0,0002±0,00004[c,d]	1,2674±0,224	229,309±125,843[d]
FA+HDMT	0,0053±0,002[d]	7326,28±424,07[d]	0,0002±0,00012	1,3710±0,119	173,578±97,484[d]

[Values are mean ± SD of six rats in each group. Significance at $P<0.05$. [a] Control *vs*. other groups; [b] FA group *vs*. other groups; [c] LDMT group *vs*. other groups; and [d] HDMT group *vs*. other groups]

significant decrease was observed in the MDA levels in FA+LDM and FA+HDM-treated groups compared to the HDM-treated group. SOD activity statistically decreased in both LDM and HDM-treated groups compared with to the control group. We showed that the decrease in FA+LDM and FA+HDM groups was not statistically significant compared to the control and FA-treated groups. However, we showed that there was an increase in SOD activity in FA+LDM and FA+HDM groups, compared to LDM and HDM-treated groups, but not statistically. A significant decrease was observed in the activities of CAT in both the LDM and HDM groups. Additionally, a significant decrease was noticed in the FA+HDM treated group compared to other groups. No significant changes were observed in the other groups. We observed a significant increase in GPx activity in the LDM and HDM-treated group compared to the control group. There was a significant decrease in the GPx activity in the FALDM and FAHDM groups compared with all other groups. In the GST activity, we observed a statistically significant increase in the HDM, LDM, FALDM and FAHDM-treated groups compared to the control group (Table 1).

Kidney

In MDA levels, a statistically significant increase in the HDM-treated group was observed compared to control, FA and LDM-treated groups. We detected a statistically significant decrease in FA+LDM and FA+HDM-treated groups compared to the HDM group. In the SOD activity, a statistically significant decrease was shown in HDM compared to control, FA and LDM-treated groups. In FA+LDM and FA+HDM-treated groups, statistically significant increases were noticed compared to the HDM.A significant decrease was observed in the activities of CAT in the HDM-treated group compared to the control, FA and LDM-treated groups. A significant increase in the FA+LDM-treated group compared to LDM and HDM treated groups. Also, in FA+HDM-treated groups, we observed statistically significant increases compared to the HDM group. When we look at the GPx activity, it was statistically increased in the LDM and HDM-treated groups compared to control and ferulic acid-treated groups. There is a statistically significant decrease in FALDM compared to LDM and HDM-treated groups. GST activity was statistically increased in HDM compared to control and ferulic acid treated groups (Table 1).

Histopathological findings

Kidney

The normal histological structure was shown in the renal tissue of the control and ferulic acid treated groups (Fig. 1A). Tubular degeneration and inflammatory changes were observed in LDM-treated groups (Fig. 1B). Glomerular atrophy, dilatation in the bowman capsule and tubular degeneration were noticed in HDM-treated rats (Fig. 1C). Hemorrhage and tubular degeneration were observed in LDM plus FA-treated rats (Fig. 1D). Edema and hemorrhage were observed in the HDM plus FA-treated groups (Fig. 1E).

Liver

Light microscopic findings showed normal hepatic parenchyma in the control group (Fig. 2A). Several histopathological changes occurred in treated groups. Kuppfer cell proliferation, sinusoidal dilatation and hemorrhage were observed in the groups treated with low dose dimethoate (Fig. 2B). Inflammatory

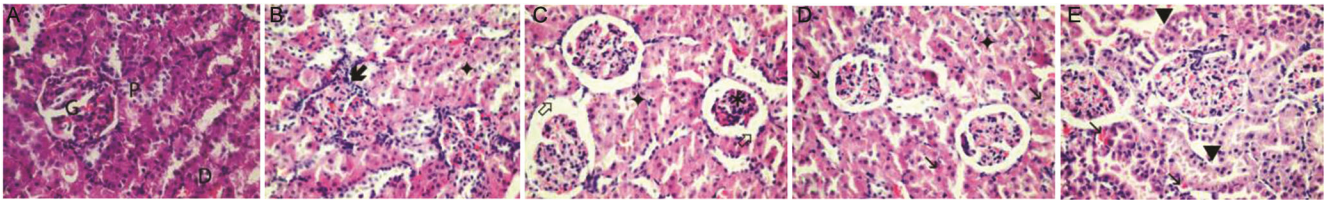

Fig. 1 — Kidney sections of (A) control group rats, (G) glomeruli, (B) bowman capsule, (P) proximal tubules as revealed by light microscopy, 200X;. (B) low dose dimethoate group showing tubular degeneration (✦) and inflammatory changes (⮝),200X; (C) high dose dimethoate group showing glomerular atrophy (✱), dilatation in bowman capsule (⇨) and tubular degeneration (✦),200X; (D) low dose dimethoate plus ferulic acid group showing hemorrhage (→) and tubular degeneration (✦),200X;and (E) high dose dimethoate plus ferulic acid group showingedema (▲) and hemorrhage (→),200X.

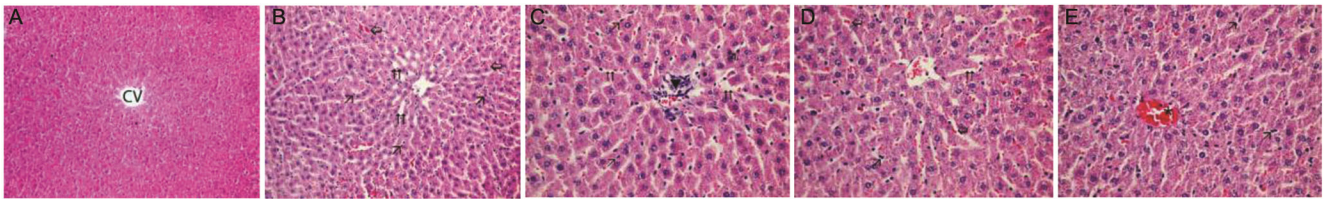

Fig. 2 — Liver sections of (A) control group rats, as revealed by light microscopy, 200X; (B) low dose dimethoate group showing kuppfer cell proliferation (↗), sinusoidal dilatation (⇉) and hemorrhage (⇨), 200X; (C) high dose dimethoate group inflammation (▼) and kuppfer cell proliferation (↗), sinusoidal dilatation (⇉),400X; (D) low dose dimethoate group plus ferulic acid hemorrhage (⇨) and sinusoidal dilatation (⇉), 400X; and (E) high dose dimethoate group plus ferulic acid, congestion (★), and kuppfer cell proliferation (↗), 400X.

Table 2 — Grading of the histopathological changes in the liver and kidney sections of dimethoate exposure to rats. Scoring was done as follows: none (-), mild (+), moderate (++) and severe (+++)

	Congestion	Kuppfer cell proliferation	Dilatation of sinusoids	Hemorrhage	Inflammation	
Liver						
Control	-	-	-	-	-	
FA	-	-	-	-	-	
LDM	-	++	++	++	-	
HDM	+	+++	+++	+	++	
FA+LDM	-	++	++	++	-	
FA+HDM	+	+++	+	+	+	
Kidney	Degeneration	Atrophy	Dilatation of bowman	Hemorrhage	Inflammation	Edema
Control	-	-	-	-	-	-
FA	-	-	-	-	-	-
LDM	++	-	-	-	++	-
HDM	+++	++	+++	-	+	+
FA+LDM	++	-	-	+	-	-
FA+HDM	+	-	-	+	-	+

changes, kuppfer cell proliferation and sinusoidal dilatation were reported in high dose dimethoate-treated groups (Fig. 2C). Less hemorrhage and sinusoidal dilatation was observed in LDM plus FA-treated rats (Fig. 2D). In the HDM plus FA-treated groups, we noticed congestion and kuppfer cell proliferation (Fig. 2E).

Biochemical findings

Effects of DM on the serum levels of liver biochemical enzymes

When we look at the hepatoprotective efficacy of ferulic acid, according to Table 2, ALT value is a statistically increased in the HDM group compared to the control and FA groups. In AST parameters, there was a statistically significant increase compared to the control, FA and LDM group. FALDM statistically decreased. When we look at TP, there is a statistically significant increase in HDM compared to the control and FA. In Albumin parameters, we observed a statistically significant increase in the HDM group compared to the control, FA and LDM-treated groups. There was a significant decrease in FALDM and FAHDM compared to HDM-treated groups. In LDH and TK parameters, there were no statistically significant differences between the groups (Table 2).

Effects of DM on the serum levels of kidney biochemical enzymes

Serum nephrotoxic markers are known as urea, uric acid, and creatinine. In creatinine, there was a statistically significant increase in LDM compared to

Table 3 — Effects of dimethoate on kidney and liver function test parameters

	Control	Ferulic acid	LowDM	High DM	FA+Low DM	FA+High DM
Liver						
ALT (U/L)	42,333±5,680	43,500±8,384	46,933±5,982	56,166±5,741[a,b]	51,500±5,009	47,166±6,852
AST (U/L)	184,17±14,716	192,66±10,269	202,16±18,946	248,00±11,627[a,b,c]	171,83±34,504	167,00±37,894
TP(g/dL)	5,185±0,099	5,220±0,069	5,753±0,309	6,385±0,392[a,b]	5,326±0,239[d]	4,771±0,239
Albumin (g/dL)	2,523±0,171	2,500±0,109	2,573±0,131	2,795±0,138[a,b,c]	0,548±0,037[d]	2,478±0,098[d]
LDH(U/L)	1034,66±148,98	1085,16±283,57	918,83±194,38	1135,83±114,45	1072,16±70,43	1060,83±114,38
TK (mg/dL)	47,60±6,15	49,10±4,32	47,66±6,43	50,66±4,36	45,15±3,70	41,71±3,22
Kidney						
Creatinine (mg/dL)	0,286±0,030	0,291±0,032	0,306±0,021[a]	0,350±0,037[a,b]	0,355[a,b]±0,035	0,338±0,020
BUN (mg/dL)	43,166±2,562	43,833±3,311	45,833±1,722	51,666±3,983[a]	43,333±5,715[d]	47,166±3,430
Uric acid (mg/dL)	0,5450±0,063	0,5983±0,030	0,6767±0,121	0,9233±0,264[a,b]	0,6867±0,071	0,8850±0,231[a]

[Values are mean ± SD of six rats in each group. Significance at $P< 0.05$. [a] Control *vs*. other groups; [b] FA group *vs*. other groups; [c] LDMT group *vs*. other groups; and [d] HDMT group *vs*. other groups]

control and ferulic acid. Significant increases in the HDM and FALDM-treated groups were noticed compared to control and FA-treated groups. In the BUN, there is a significant increase in the HDM-treated group compared to the control group. A significant decrease in the FALDM group compared to the HDM group. In uric acid, there is a significant increase in the HDM group compared to the control and FA. Similarly, there is a significant increase in the FAHDM group compared to the control group (Table 3).

Discussion

Organophosphate is the most used and studied groups among the pesticides. These environmental agents cause harm to humans through food[3]. Food supplements protective against many pests that are exposed through food in our daily lives have gained importance[38]. One of the markers of DM on liver and kidney systems is histopathological changes. We demonstrated several changes in each tissue when we applied DM to experimental animals. Cellular oxidative damage is thought to cause the correlation of biochemical, enzymatic and histopathological results and the tissue damage[39]. Light microscopic analysis is a critical indicator to show cellular damage. In this experimental study, dimethoate especially high dose dimethoate caused degeneration in the cell structure. We showed serious damage to the cells in light microscopic examinations in the dimethoate-treated group. These pathological changes in the kidney and liver could be due to the increased formation of ROS in cells and are related to LPO level. In this study, the light microscopic results are parallel with the biochemical assay findings. It has been reported that LD_{50} doses of dimethoate is 1350 mg/kg body wt. for male rats[40]. For comparison, two different doses were used in this study.

The most known antioxidants in mammals are SOD, catalase, GPX and glutathione[26,3]. When we look at the role of these antioxidants in the cell; GSH plays a role in the first defense line for scavenging of free radicals, detoxification of toxic substances and cofactor of GST and GPx. GST is responsible for catalyzing the conjugation of GSH electrophilic substrate to convert less toxic forms, and thereby LPO gets reduced. GPx protects the cells from the oxidative damage of LPO, and it converts H_2O_2 to water. SOD converts H_2O_2 to superoxide radicals, while CAT converts superoxide radicals to water and oxygen[41]. These enzymes are important in determining tissue damage[30,42,43]. The leakage of enzymes that must be present in the cell outside the cell may be an indication of damage to the cell membrane. However, the increase in the MDA level and changes in enzymatic antioxidants like SOD, GPx, CAT, GST show that cell injury is related to ROS production[44,45]. In this study, we showed that MDA levels increased in both liver and kidney tissue, and there were decreases and increases in enzyme activities compared to the control group. Some investigators have shown hepatic and nephrotic damage through oxidative stress and histopathology[44,45].

Our experimental study showed that subchronic administration of dimethoate causes hepatic and nephrotic damage in rats because of the increases in serum markers, such as ALT, AST, LDH, total cholesterol, total protein, BUN, uric acid, and creatinine. Increased free radicals may cause structural disruption by damaging the hepatocyte membranes, and AST, ALT, TP, albumin in cytosol were mixed with blood and increased compared to control. It has been reported that a higher concentration of bilirubin is associated with the reduced erythrocytes and shows hepatic disruption[41]. The increased total cholesterol level may be due to the toxicity of OPs on the hepatic membrane permeability[41].

The alteration in the total protein level might be due to changes in protein synthesis in the liver[46]. If there is damage to the cell membrane, enzymes that must be present in the cell leak out of the cell[39]. Hence, blood biochemical parameter changes provide information about the damage caused by toxic substances. The increase in these enzymes may be due to the liver dysfunction, and disturbance in the biosynthesis of these enzymes with alteration in the permeability of liver membrane occurs. Also, the increase in serum LDH activity may be due to the hepatocellular necrosis leading to leakage of the enzyme into the blood stream, and it is also an indicator of cellular damage[44]. Previous studies have shown that the enzymatic activities of AST, ALT, and LDH are sensitive to determinative liver toxicity[44].

Because of the damage to the nephron, urea, uric acid and creatinine can begin to accumulate in the blood, and an increase in serum is observed. This increase indicates that kidney function is impaired[45,47]. Similar to previous studies, the increase in plasma creatinine, uric acid and urea levels in this experiment may be due to kidney dysfunction as suggested by oxidative stress and pathological changes[48].Pesticides can also cause serious pathological effects on other organs[5,8,49].

Vitamin C, vitamin E, quercetin, catechin, hesperidin, morin, ferulic acid, etc. are the major exogenous radical scavenging agents that play a role in the prevention of oxidative injury[38,50]. There are many investigations on the free radical scavenging properties of phenolic compounds[51,52]. Many studies have shown the protective effects of ferulic acid on toxic substances. With its free radical activity scavenging properties, FA can be well applied to oxidative stress[15].

Conclusion

Possibly, its is the first study investigating the effectiveness of ferulic acid against different doses of dimethoate toxicity. Moreover, in toxicological studies, it is widely used as a biomarker in addition to cell oxidative parameters, histopathological lesions and serum biochemical levels, such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), urea and uric acid. According to the results of this study, high dose of HDMT is more effective than LDMT may be due to increased amounts of ROS cause membrane damage**.** The use of ferulic acid with DMT causes protection on the kidney and liver compared with individual use of these pesticides. The use of antioxidants like vitamins and flavonoids reduces pesticide damage, such as dimethoate.The data proved that ferulic acid exhibits promising hepatoprotective in some parameters and nephroprotective potential against the dimethoate-induced hepatotoxicity and nephrotoxicity.

Acknowledgment

Authors are thankful to the Gazi University for their support. This work is financially supported by the Gazi University Research Fund (Project No. 05/2017-19).

Conflict of Interest

Authors declare no competing interests.

References

1 Prakasam A, Sethupathy S & Lalitha S, Plasma and RBCs antioxidant status in occupatiponal male pesticide sprayers. *Clinica Chimica Acta*, 310 (2001) 107.

2 Farag AT, El-Aswad AF & Shaaban NA, Assessment of reproductive toxicity of orally administrated technica dimethoatein male mice. *Reprod Toxicol*, 23 (2007) 232.

3 Apaydın FG, Pandır D, Kalender S, Bas H & Kalender Y, Hametoprotective effect of vitamins C and E against subchronic toxicity of bendiocarb: Biochemical evidence. *J Food Biochem*, 42 (2018) 12659.

4 Ahmed T & Banerjee BD, Permethrin induced cytotoxicity of rat splenocytes: Protective effect of N-acetylcysteine. *Indian J Exp Biol*, 59 (2021) 11.

5 Amara IB, Soudani N, Hakim A, Troudi A, Zeghal KM, Boudawara T & Zeghal N, Selenium and vitamin E, natural antioxidants, protect rat cerebral cortex against dimethoate-induced neurotoxicity. *PesticBiochem Physiol*, 101 (2011a) 165.

6 Uzun FG, Demir F, Kalender S, Bas H & Kalender Y, Protective effect of catechin and quercetin on chlorpyrifos-induced lung toxicity in male rats. *Food ChemToxicol*, 45 (2010) 1714.

7 Sharma CD & Bansal G, Malathion alters biochemical parameters of liver in albino rats. *Indian J Exp Biol*, 60 (2022) 438.

8 Wang P, Xu MY, Liang YL, Wang HP, Sun YJ, Long DX & Wu YJ, Subchronic toxicity of low dose propoxur, permethrin, and their combination on the redox status of rat liver. *Chem Biol Interact*, 272 (2017) 21.

9 Ahmed T & Banerjee BD, Permethrin induced cytotoxicity of rat splenocytes: Protective effect of N-acetylcysteine. *Indian J Exp Biol*, 59 (2021) 11.

10 Hayes WJ & Laws ER, *Handbook of Pesticide Toxicology*. (Acedemic Press Inc., New York), 1991.

11 Kamath V, Joshi AKR & Rajini PS, Dimethoate induced biochemcal perturbations in rat pancreas and its attenuation by cashew nut skin extract. *Pestic Biochem Physiol*, 90 (2008) 58.

12 Amara IB, Soudani N, Troudi A, Bouaziz H, Boudawara T & Zeghal N, Antioxidant effect of vitamin E and selenium on

hepatotoxicity induced by dimethoate in female adult rats. *Ecotoxicol Environ Safe*, 74 (2011b) 811.

13 Jallouli M, Dhouib IE, Dhouib H, Gharbi N & El Fazaa S, Effects of dimethoate in male mice reproductive parameters. *Regul Toxicol Pharmacol*, 73 (2015) 853.

14 Anselmi C, Bernardi F, Centini M, Gaggelli E, Gaggelli N, Valensin D & Valensin G, Interaction of ferulic acid and derivatives with human erythrocytes monitored by pulse field gradient NMR diffusion and NMR relaxation studies. *Chem Phys Lipids*, 134 (2007) 109.

15 Amić A, Marković A, Marković JMD, Milenković D & Stepanić V, Antioxidative potential of ferulic acid phenoxyl radical. *Phytochemistry*, 170 (2020) 112218.

16 Özbucak TB, Karataş T & Ayvaz MÇ, Effects of different ecological and phenological factors on antioxidant activity and phenolic content of Ornithogalum sigmoideum Freyn & Sint. from Turkey. *Indian J Exp Biol*, 60 (2022) 322.

17 Stefanović O, Mladenović D, Mladenović K, Grujović M & Ivanović D, *Escherichia coli* biofilm formation and control by phenolic compounds from Salvia officinalis L. extracts. *Indian J Exp Biol*, 60 (2022) 771. DOI: 10.56042/ ijeb.v60i10.41435.

18 Albayrak S, Biological activities of essential oil and methanolic extract of endemic *Dorytoechas hastata* Boiss et Heldr. ex Bentham (Lamiaceae) from Turkey. *Indian J Exp Biol*, 59 (2021) 891

19 Roumili I, Mayouf N, Charef N, Arrar L & Baghiani A, HPLC analysis, acute toxicity and anti-inflammatory effects of *Salix alba* L. barks extracts on experimental animal models. *Indian J Exp Biol*, 60 (2022) 842. DOI: 10.56042/ijeb.v60i11.51723.

20 Uzunbayır H & Apaydın FG, Protective Role of Ferulic Acid on Testis-Histoarchitecture and Oxidative Damages Induced by Dimethoate in Rats. *Braz Arch Biol Technol*, 64 (2021) 21210300.

21 Trombino S, Serini, S & Di Nicuolo F, Antioxidant effect of ferulic acid in isolated membranes and intact cells: synergistic interactions with alpha-tocopherol, beta-carotene, and ascorbic acid. *J Agric Food Chem*, 52 (2004) 2411.

22 Ogiwara T, Satoh K, Kadoma Y, Murakami Y, Unten S, Atsumi T, Sakagami H & Fujisawa S, Radical scavenging activity and cytotoxicity of ferulic acid. *Anticancer Res*, 22 (2002) 2711.

23 Farinetti A, Zurlo V, Manenti A, Coppi F & Mattioli AV, Mediterranean diet and colorectal cancer: a systematic review. *Nutrition*, 83 (2017) 43.

24 Mendonça RD, Carvalho NC, Martin Morano JM, Pimenta AM, Lopez ACS, Gea A, Martinetz-Gonzalez MA & Bes-Rastrollo M, Total polyphenol intake, polyphenol subtypes and incidence of cardiovascular disease: the SUN cohort study. *Nutr Metab Cardiovasc Dis*, 29 (2019) 69.

25 Galal AAA, Ramadan RA, Metwally MMM & El-Sheiskh SMA, Protective effect of N-acethylcysteine on fenithrothion-induced toxicity: The antioxidant status and metabolizing enzymes expression in rats. *Ecotoxicol Environ Safe*, 171 (2019) 502.

26 Saha C, Das A, Das P &, Chakraborty A, Effect of Quercetin loaded Silver nanoparticles on Gram negative and Gram positive bacteria. *Indian J Exp Biol*, 59 (2021) 132.

27 Raeeszadeh M, Zobeiri A, Khademi N & Mortazavi P, Prominent nephroprotective effect of methanolic Broccoli (*Brassica olaracea* var. *italica*) extract on oxidative lead damage in mice kidney: Biochemical parameters and pathological changes.*Indian J Exp Biol*, 59 (2021) 626.

28 Majee C, Mazumde R & Choudhary AN, Hepatoprotective potential of isolated flavonoids from *Trapa natans* L. against the paracetamol induced oxidative stress in rats. *Indian J Exp Biol*, 60 (2022) 910. DOI: 10.56042/ijeb.v60i12.55735.

29 Uzun FG & Kalender Y, Protective effect of vitamins c and e on malathion-induced nephrotoxicity in male rats. *Gazi Univ J Sci*, 24 (2011) 193.

30 Hussain F, Malik A, Ayyaz U, Shafique H, Rana Z & Hussain Z, Efficient hepatoprotective activity of cranberry extract against CCl4-induced hepatotoxicity in Wistar albino rat model: Down-regulation of liver enzymes and strong antioxidant activity. *Asian Pac J Trop Med*, 10 (2017) 1054.

31 Liu C, Ma J & Suna Y, Quercetin protects the rat kidney against oxidative stress-mediated DNA damage and apoptosis induced by lead. *Environ Toxicol Pharmacol*,30 (2010) 264.

32 Marklund S & Marklund G, Involvement of the superoxide anion radical inthe autoxidation of pyrogallol and a convenient assay for superoxide dismutase. *FEBS J*, 47 (1974) 469.

33 Aebi H, Catalase *in vitro*. *Methods Enzymol*, 105 (1984).121.

34 Habig WH, Pabst MJ & Jakoby WB, Glutathione-S-transferases: the first enzymatic step in mercapturic acid formation. *J Biol Chem*, 249 (1974) 7130.

35 Paglia DE & Valentine WN, Studies on the quantative and qualitative characterization of erythrocytes glutathione peroxidase. *J Lab Med*, 70, (1967).

36 Lowry OH, Rosebrough NJ, Farr AL & Randall RJ, Protein measurement with the Folin phenol reagent. *J Biol Chem*, 19 (1951) 265.

37 Ohkawa H, Ohishi N & Yagi K, Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Anal Biochem*, 95 (1979) 351.

38 Amin I, Hussain I, Rehman MU, Mir BA, Ganaie SA, Ahmad SB, Mir MUR, Shanaz S, Muzamil S, Arafah A & Ahmad P, Zingerone prevents lead-induced toxicity in liver and kidney tissues by regulating the oxidative damage in Wistar rats. *J Food Biochem*, 45 (2021) 13241.

39 Uzun FG & Kalender Y, Chloropyrifos-induced hepatotoxicity and hematological changes in rats: the role of quercetin and catechin. *Food ChemToxicol*, 55 (2013) 549.

40 John S, Kale M, Rathore N & Bhatnagar D, Protective effect of vitamin E in dimethoate and malathion induced oxidative stress in rat erythrocytes. *J Nutr Biochem*, 12 (2001) 500.

41 Milosevic MD, Paunovic MG, Matic MM & Ognjanovic BI, The ameliorating efeects of selenium and vitamin C against fenithrothion-induced blood toxicity in Wistar rats. *Environ Toxicol Pharmacol*, 56 (2017) 204.

42 Bas H, Kalender S, Karaboduk H & Apaydın FG, The Effects on Antioxidant Enzyme Systems in Rat Brain Tissues of Lead Nitrate and Mercury Chloride. *Gazi Univ J Sci*, 28 (2015) 169.

43 Kalender S, Apaydın FG & Kalender Y, Testicular toxicity of orally administrated bisphenol A in rats and protective role of taurine and curcumin, *Pak J Pharm Sci*, 32 (2019) 1043.

44 Apaydin FG, Baş H, Kalender S & Kalender Y, Bendiocarb induced histopathological and biochemical alterations in rat

liver and preventive role of vitamins C and E. *Environ Toxicol Pharmacol*, 49 (2017) 148.

45 Adıguzel C & Kalender Y, Bendiocarb-induced nephrotoxicity in rats and the protective role of vitamins C and E. *Environ Sci Poll Res*, 27 (2020) 6449.

46 Abdel-Ghany R, Mohammed E, Anis S & Barakat W, Impact of exposure to fenithrothion on vital organs in rats. *J Toxicol*, 2016 (2016) 5609734.

47 Baş H &Kalender Y, Nephrotoxic effects of lead nitrate exposure in diabetic and nondiabetic rats: involvement of oxidative stress and the protective role of sodium selenite. *Environ Toxicol*, 31 (2016) 1229.

48 Apaydın FG, Bas H, Kalender S & Kalender Y, Subacute effects of low dose lead nitrate and mercury chloride exposure on kidney of rats. *Environ Toxicol Pharmacol*, 41, (2016) 219.

49 Kalender Y, Kalender S, Uzunhisarcikli M, Ogutcu A, Açikgoz F & Durak D, Effects of endosulfan on B cells of Langerhans islets in rat pancreas. *Toxicology*, 200, (2004) 205.

50 Jo E, Youn CK, Jun Y & Cho S, The protective role of ferulic acid against cisplatin-induced ototoxicity, *Int J Pediatr Otorhinolaryngol,* 120 (2019) 30.

51 Galano A & Alvarez-Idaboy JR, Computational strategies for predicting free radical scavengers' protection against oxidative stress: where are we and what might follow? *Int J Quantum Chem*, 119 (2019) 25665.

52 Kalender S, Apaydın FG, Bas H & Kalender Y, Protective effects of sodium selenite on lead nitrate-induced hepatotoxicity in diabetic and non-diabetic rats. *Environ Toxicol Pharmacol* 40 (2015) 568.