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Investigation of the Protective Effects of Diosmin Against Emamectin Benzoate Induced Oxidative Damage in Rats*,**

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Abstract: This study was aimed to investigate the effects of diosmin (DIO) in rats exposed to emamectin benzoate (EB). For this purpose, a total of 60 Wistar Albino male rats, aged 6 to 8 weeks and weighing 180 to 250 g, were used, 10 in each group. The groups were determined, respectively, control, EB (10 mg kg^{-1}), DIO (50 mg kg^{-1}), DIO (100 mg kg^{-1}), EB (10 mg kg^{-1}) + DIO (50 mg kg^{-1}), EB (10 mg kg^{-1}) + DIO (100 mg kg^{-1}), and the indicated doses were applied by gavage for 21 days. At the end of the trial period, a heart puncture was performed under anesthesia and blood samples were taken into tubes with heparinized and anticoagulant-free properties. The liver, kidney, brain, testis, heart and lung tissues were removed after cervical dislocation. While some biochemical markers, such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), triglyceride, cholesterol, total protein, albumin, uric acid, blood urea nitrogen (BUN) and, creatinine were assessed in the serum, oxidative stress indicators, such as MDA, NO, SOD, CAT, GSH-Px, GR, GST, and GSH, were determined in tissue samples. At the end of the investigation, no changes between the parameters of the DIO groups and the control group were discovered. Comparing the EB group to the control group, it was found that the levels of MDA and NO in tissues/erythrocytes had significantly increased. The activity of the GSH, GR, GST, SOD, and CAT enzymes, as well as GSH levels, was found to have decreased. Furthermore, serum total protein and albumin levels were reduced, although AST, ALT, ALP, LDH, cholesterol, triglyceride, BUN, uric acid, and creatinine levels/activities were elevated. Depending on the dose, it was found that the values of the groups coadministered with EMB and DIO were close to those of the control group. In conclusion, it is proposed that DIO could provide protection against EMB-induced toxicity in rats.
Keywords: Emamectin benzoate, diosmin, oxidative damage, sıçan

Sıçanlarda Emamektin Benzoat Kaynaklı Oksidatif Hasara Karşı Diosminin Koruyucu Etkilerinin Araştırılması

Öz: Bu çalışma ile sıçanlarda emamektin benzoat (EB) maruziyetine karşı diosminin (DİO) etkilerinin araştırılması amaçlandı. Çalışmada her grupta 10 tane olacak şekilde toplam 60 adet 6-8 haftalık 180-250 g ağırlığında Wistar Albino ırkı erkek sıçan kullanıldı. Gruplar sırasıyla kontrol, EB (10 mg kg^{-1}), DİO (50 mg kg^{-1}), DİO (100 mg kg^{-1}), EB (10 mg kg^{-1}) + DİO (50 mg kg^{-1}), EB (10 mg kg^{-1}) + DİO (100 mg kg^{-1}) olarak belirlenerek belirtilen miktarlarda 21 gün boyunca gavajla uygulama yapıldı. Deneme süresinin sonunda anestezi altında kalbe punksiyon yapılarak heparinize ve antikoagulansız tüplere kan örneği alındı. Servikal dislokasyon sonrası sıçanların karaciğer, böbrek, beyin, testis, kalp ve akciğer dokuları çıkarıldı. Serumda bazı biyokimyasal parametreler (aspartat aminotransferaz (AST), alanin aminotransferaz (ALT), laktat dehidrogenaz (LDH), alkalin fosfataz (ALP), trigliserit, kolesterol, total protein, albümin, ürik asit, kan üre azotu (BUN) ve kreatinin) ölçülürken doku örneklerinde ise oksidatif stres parametreleri (MDA, NO, SOD, CAT, GSH-Px, GR, GST ve GSH) analiz edildi. Çalışma sonunda kontrol grubu ile karşılaştırıldığında, DİO uygulanan grubun parametrelerinde herhangi bir değişiklik tespit edilmedi. EMB uygulanan grupta kontrole kıyasla tüm dokularda MDA ile NO seviyelerinde anlamlı bir artış kaydedildi. GSH düzeyleri ile GSH, GR, GST, SOD ve CAT enzim aktivitelerinde ise azalma olduğu belirlendi. Ayrıca serum AST, ALT, ALP, LDH, kolesterol, trigliserit, BUN, ürik asit ve kreatinin düzey/aktivitelerinde artış görülürken serum total protein ve albümin düzeylerinde azalma görüldü. EB ile DİO'nun birlikte uygulandığı grupların değerlerinin doza bağlı olarak kısmen ya da tamamen kontrol grubunun değerlerine yaklaştığı tespit edildi. Sonuç olarak, DİO'nun sıçanlarda EMB kaynaklı toksisiteye karşı koruma sağlayabileceği düşünülmektedir.
Anahtar kelimeler: Emamektin benzoat, diosmin, oksidatif hasar, rat

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Introduction

Pesticides are chemical compounds that are commonly used in agriculture or public health protection programs to protect plants from pests, weeds, dis-

eases, livestock from pest infestations, and people from vector-borne diseases (e.g., Lyme disease, malaria, West Nile virus). Despite their beneficial effects, pesticides are not selective, and as a result, they pose severe risks to people, the environment, or non-target organisms such as beneficial soil bacteria, insects, plants, fish, and birds (Aktar et al., 2009).

Avermectins, a class of 16-membered macrocyclic lactones, were discovered at the Kitasato Institute in Japan in 1967 as a natural fermentation product of the soil actinomycete *Streptomyces avermitilis* (Bai and Ogbourne, 2016). Emamectin benzoate (EB), a semi-synthetic avermectin derivative, is used to treat sea lice in salmon and to control lepidopteran pests in leafy vegetables such as lettuce, broccoli, cabbage, and cauliflower (JECFA, 2013). EB, a neurotoxin, disrupts the normal function of gamma-aminobutyric acid (GABA) like other avermectins. The opening of GABA-gated chloride channels, triggered by EB, causes increased permeability of chloride ions, resulting in a signal blockade at neuromuscular junctions. Previous studies have demonstrated the toxic effects of EB on different organs, including the liver (Khaldoun Oularbi et al., 2017), kidney (Abou-Zeid et al., 2018), testis (Zhang et al., 2020), and brain (Madkour et al., 2021) due to oxidative stress.

Since ancient times, it has been common to use plants and plant-derived phytochemicals for the treatment or prevention of health issues (Patel et al., 2013). Diosmin (DIO) is a naturally occurring flavone glycoside that can be produced by dehydrating hesperidin, a flavanone glycoside, or by extraction from a variety of plant sources, including *Teucrium gnaphalodes*. DIO, which was isolated from *Scrophularia nodosa* in 1925, was introduced as a therapeutic agent in 1969 (Mustafa et al., 2022). Due to its vascular protective function, it is used to treat varicose veins, lymphedema, and chronic venous insufficiency. Studies have also reported that it has many beneficial effects, such as antioxidant, anti-apoptotic, anti-ulcer, hepatoprotective, and neuroprotective activity (Gerges et al., 2022).

A number of studies on the effectiveness of several natural products, including vitamin C (Khaldoun Oularbi et al., 2017), pumpkin seed oil (Abou-Zeid et al., 2018), and Nigella sativa oil (Madkour et al., 2021), against oxidative stress and lipid peroxidation caused by EB are available in the literature. It has been demonstrated that diosmin protects against a variety of substances, including methotrexate (Abdel-Daim ve ark., 2017), aflatoxin (Eraslan et al., 2017), cadmium (Ağır and Eraslan, 2019), lead (Bozdağ and Eraslan, 2020), and deltamethrin (Tekeli et al., 2021). However, there was no mention of any scientific report on the efficacy of DIO against oxidative stress and lipid peroxidation induced by EB. Therefore, the purpose of this investigation was to evaluate the beneficial role of DIO on lipid peroxidation and enzymatic/non-enzymatic antioxidant status in rats exposed to EB.

Materials and Methods

Animals and experimental design

A total of 60 Wistar albino male rats, weighing 180-250 g and aged 6-8 weeks, were utilized in the study. Throughout the experiment, water and commercial pellet feed (protein 24%, fats 5.09%, cellulose 3.2%, and a total of 3100 kcal/kg metabolic energy) were provided to all rats ad libitum in cages made of polyethylene, which were housed in accordance with standard laboratory conditions (12-hour light/dark cycle at 22±2 °C and relative humidity of 45–55%). Protocols for the experiments were approved by the ERU Animal Research Ethics Committee and conducted in compliance with global standards (Report No. 19/051). On the basis of prior research, the EB (Khaldoun-Oularbi et al., 2017) and DIO (Abdel-Daim et al., 2017) dose administered to rats were determined. Corn oil, a commercially available product that is not an analytical standard, was utilized as a vehicle substance. Rats were administered suspensions of EB and DIO in corn oil to construct identical circumstances. A total of six groups of ten rats each were designed at random. The first group was identified as a control, and 1 ml kg^{-1} body weight (BW) of the corn oil was administered orally for 21 days. The other groups received continuous oral administration of EB (10 mg kg^{-1}.BW day^{-1}), DIO (50 mg kg^{-1}.BW day^{-1}), DIO (100 mg kg^{-1}.BW day^{-1}), DIO (50 mg kg^{-1}.BW day^{-1}) with EB (10 mg kg^{-1}.BW day^{-1}), and DIO (100 mg kg^{-1}.BW day^{-1}) with EB (10 mg kg^{-1}.BW day^{-1}), respectively, for a total of 21 days.

Sampling and preparation for laboratory measurements

At the end of the trial period, the rats in all groups were anesthetized with a combination of ketamine-xylazine intraperitoneally under the same conditions. Subsequently, blood samples were taken into tubes with heparinized and anticoagulant-free properties by puncturing the heart. After that, cervical dislocation was applied to sacrifice. The serum samples were separated from the blood samples in the anticoagulant-free tubes by centrifugation at 3000 rpm for 10 minutes at 4°C and analyzed on the same day. The plasma-containing supernatant was carefully separated after centrifuging blood samples in heparinized test tubes at 3000 rpm for 10 minutes (min) at 4°C. The buffy coat was removed from the remaining phase, and the erythrocytes were washed three times in normal saline (0.9% NaCl). For this process, the mixture was centrifuged at 2000 g for 5 min, and the supernatant was discarded. The erythrocytes were

diluted with an equal volume of normal saline before being stored at -80°C. Before analysis, erythrocytes were hemolyzed 1:5 with ice-cold distilled water. The brain, heart, kidney, liver, lung, and testis tissues were quickly dissected, and blood clots were eliminated by washing the organs in 0.9% NaCl solution. Using a homogenizer (Silent Crusher M, Heidolph), tissues were homogenized 1:5 in cold phosphate buffer (pH 7.4) on ice before being centrifuged for 60 minutes at 10000 rpm at 4°C. In order to measure tissue enzymatic or non-enzymatic antioxidants and lipid peroxidation, supernatants were transferred to Eppendorf tubes and stored in a deep freezer (-80° C).

Measurement of serum biochemical parameters

A Roche Cobas C 8000 autoanalyzer and the same brand kits were used for the determination of serum triglyceride, cholesterol, albumin, total protein, BUN, uric acid and creatinine levels, and LDH, AST, ALT and ALP enzyme activities.

Measurement of lipid peroxidation and enzymatic/ non-enzymatic antioxidant parameters

The spectrophotometric method described by Lowry et al. (1951) was applied to measure the protein levels in tissue homogenates. According to the procedures of Habig et al. (1957), Paglia and Valentine (1967), and Carlberg and Mannervik (1985), glutathione S transferase (GST), glutathione peroxidase (GPx), and glutathione reductase (GR) activity in tissues/esrythrocyte was analyzed, respectively. In terms of nmol/min/g protein or mg hemoglobin, enzyme activity was expressed. By using the Sun et al. (1988) technique, the enzyme activity of superoxide dismutase (SOD) in tissues/esrythrocyte was quantified and expressed as U/g protein or mg hemoglobin. The Luck (1965) method was utilized to determine the catalase (CAT) enzyme activity in tissues/ esrythrocyte, and was presented as katal/g protein or mg hemoglobin. The amounts of reduced glutathione (GSH), malondialdehyde (MDA), and nitric oxide (NO) in tissues/esrythrocyte/plasma were tested by Sedlak and Lindsay (1968), Ohkawa et al. (1979), and Tracey et al. (1995), respectively. These data were stated in nmol per g protein or mg hemoglobin.

Statistical analysis

The research data were statistically analyzed utilizing SPSS 21.0 statistical program. The data were expressed as arithmetic mean and standard deviation. The conformity of the variables to the normal distribution was evaluated with the Kolmogrov-Smirnov test. The homogeneity of variances was assessed by Levene's test. One-way analysis of variance (one-way ANOVA) was utilized to assess the statistical difference. The Welch test was utilized to assess the difference between the groups in situations when the homogeneity of variance assumption was rejected. Statistical differences between the groups were evaluated with the Tukey test in situations where there was homogeneity of variance and with the Games Howell test in situations where the homogeneity of variance assumption was not maintained. Data were regarded significant at $P<0.05$.

Results

The biochemical and oxidative stress parameters assessed in all samples were compared between the control and diosmin-treated groups, but no statistically significant difference was found ($P>0.05$) (Table 1-3).

When compared to the control group, the group exposed to EB showed a significant reduction in tissue/ esrythrocyte GSH levels and GPx, GR, GST, SOD, and CAT enzyme activity, as well as a significant increase in plasma/tissue MDA and NO levels ($P<0.05$). In comparison to the control group, additionally, it was also observed that serum total protein and albumin levels were lower, but AST, ALT, ALP, LDH, cholesterol, triglycerides, BUN, uric acid, and creatinine activities/levels were higher ($P<0.05$) (Table 1-3).

It was discovered that the group co-administered with EMB and DIO (50 mg kg^{-1}) had lower plasma and tissue MDA levels than the group exposed to EB ($P<0.05$). Similarly, all tissues other than plasma showed a significant reduction in NO levels ($P<0.05$). The GSH level was higher in the other tissues than in the EB group, with the exception of the heart, lung, and plasma tissues ($P<0.05$). Compared to the EB group, a significant increase was observed in GPX activity in plasma and tissues other than heart tissue ($P<0.05$). Comparatively to the group exposed to EB, CAT and GR activity increased in all tissues ($P<0.05$). Except for the liver, all tissues displayed a considerable increase in GST activity ($P<0.05$). Except for cardiac tissue, SOD activity increased in tissues and erythrocytes ($P<0.05$). Serum total protein and albumin levels increased in comparison to the EB group, but AST, ALT, ALP, LDH, cholesterol, triglyceride, BUN, and uric acid levels and activities decreased ($P<0.05$). Along with this, it was determined that the CAT, SOD, GPX, and GR enzyme activities in liver tissue, the SOD and GR enzyme activities in kidney tissue, and the LDH, AST, and ALT enzyme activities in serum were close to those of the control group ($P>0.05$) (Table 1-3).

All oxidative stress and biochemical stress parameters altered by EB exposure were either completely or partially reversed in the group co-administered with EMB and DIO (50 mg kg^{-1}). Additionally, it was determined that the levels/activities of GSH, MDA, NO GPX, GR, GST, SOD, and CAT in liver tissue, the SOD MDA, NO, GPX, GR, and GST in kidney

tissue, NO, GSH, GPX, GST, and SOD in heart tissue, GSH, GR, and GST in testis tissue, NO, GST, and CAT in plasma/esrythrocyte and the albumin, creatinine, uric acid, LDH, AST, and ALT in serum were close to those of the control group (P>0.05) (Table 1-3).

The changes in all biochemical and oxidative stress parameters investigated in the combination groups were dose-dependent.

Table 1. Effects of DIO treatment on the levels/activities of AST, ALT, LDH, ALP, triglyceride, cholesterol, total protein, albumin, uric acid, BUN and, creatinine in serum of EB-exposed rats

Parameters	Groups Control	EB	DIO50	DIO100	EB+DIO50	EB+DIO100
BUN (mg/dl)	16.5±1.6^{a}	21.6±1.2^{d}	17.0±0.8^{ab}	16.5±0.9^{a}	19.5±1.0^{c}	18.1±1.5^{bc}
		P<0.001	P=0.898	P=1.000	P<0.001	P=0.031
Creatinine (mg/dl)	0.31±0.03^{ab}	0.37±0.03^{d}	0.31±0.02^{a}	0.31±0.01^{a}	0.34±0.02^{cd}	0.33±0.02^{ab}
		P<0.001	P=0.997	P=1.000	P<0.001	P<0.001
Uric Acid (mg/dl)	0.94±0.19^{a}	1.74±0.27^{c}	1.00±0.24^{ab}	1.22±0.25^{ab}	1.32±0.36^{b}	0.99±0.25^{ab}
		P<0.001	P=0.996	P=0.181	P=0.024	P=0.998
Triglyceride (mg/dl)	98.8±17.2^{a}	172.4±29.2^{d}	104.3±16.6^{ab}	117.9±29.9^{abc}	142.2±17.9^{c}	130.8±13.0^{bc}
		P<0.001	P=0.993	P=0.371	P=0.001	P=0.020
Cholesterol (mg/dl)	59.5±5.5^{a}	98.6±7.8^{d}	64.3±4.06^{a}	61.0±3.94^{a}	82.9±5.74^{c}	75.4±5.66^{b}
		P<0.001	P=0.404	P=0.991	P<0.001	P<0.001
LDH (U/L)	1514.8±115.9^{ab}	1966.9±98.7^{c}	1456.9±96.1^{a}	1441.2±100.8^{a}	1622.9±125.5^{b}	1574.2±127.2^{ab}
		P<0.001	P=0.853	P=0.680	P=0.269	P=0.839
AST (U/L)	106.8±6.5^{abc}	129.9±10.2^{d}	102.5±5.0^{ab}	99.3±9.3^{a}	116.1±8.2^{c}	111.8±9.8^{bc}
		P<0.001	P=0.860	P=0.356	P=0.149	P=0.766
ALT (U/L)	41.2±4.7^{ab}	52.0±3.0^{d}	39.4±2.8^{a}	40.4±3.5^{ab}	47.4±3.2^{b}	44.8±2.4^{bc}
		P<0.001	P=0.835	P=0.995	P=0.002	P=0.175
ALP (U/L)	253±13.4^{a}	346±22.5^{c}	264±19.1^{a}	257±17.5^{a}	297±22.6^{b}	290±13.4^{b}
		P<0.001	P=0.744	P=0.993	P<0.001	P=0.001
Total Protein (mg/dl)	7.38±0.15^{a}	5.67±0.10^{d}	7.36±0.27^{a}	7.19±0.12^{a}	6.30±0.10^{c}	6.55±0.19^{b}
		P<0.001	P=1.000	P=0.165	P<0.001	P<0.001
Albumin (mg/dl)	4.85±0.36^{ab}	3.67±0.20^{d}	4.95±0.30^{a}	5.02±0.25^{a}	4.30±0.27^{c}	4.45±0.42^{bc}
		P<0.001	P=0.972	P=0.808	P=0.003	P=0.060

The mean ± standard deviation is used to express the data. A statistically significant difference (P<0.05) between groups is indicated by different superscripts (a, b, and c) in the same row.
Groups: Control, corn oil; EB, emamectin benzoate (10 mg kg^{-1}.BW day^{-1}); DIO50, diosmin (50 mg kg^{-1}.BW day^{-1}); DIO100, diosmin (100 mg kg^{-1}.BW day^{-1}); EB+DIO50, emamectin benzoate plus diosmin (50 mg kg^{-1}.BW day^{-1}); EB+DIO100, emamectin benzoate plus diosmin (100 mg kg^{-1}.BW day^{-1}).

Table 2. Effects of DIO treatment on the levels/activities of MDA, NO, CAT, and SOD in liver, kidney, brain, testis, heart and lung tissues, and erythrocytes/plasma of EB-exposed rats

Sample	Groups	Parameters MDA nmol/mg P	NO nmol/mg P	SOD U/g P	CAT k/g P or mg Hb
Liver	Kontrol	2.04±0.31^{a}	3.54±0.53^{a}	0.14±0.02^{a}	601.6±24.5^{abc}
	EB	3.83±0.58^{c}	5.23±0.55^{c}	0.07±0.02^{b}	524.5±18.0^{d}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	2.09±0.41^{a}	3.48±0.61^{a}	0.12±0.05^{a}	611.0±29.8^{ab}
		P=1.000	P=1.000	P=0.223	P=0.923
	DIO100	1.99±0.52^{a}	3.40±0.53^{a}	0.13±0.02^{a}	616.2±18.9^{a}
		P=1.000	P=0.989	P=0.803	P=0.662
	EB+DIO50	2.83±0.55^{b}	4.31±0.45^{b}	0.11±0.01^{a}	575.3±14.7^{c}
		P=0.006	P=0.014	P=0.137	P=0.104
	EB+DIO100	2.45±0.43^{ab}	4.05±0.32^{ab}	0.11±0.02^{a}	584.2±24.2^{bc}
		P=0.380	P=0.234	P=0.155	P=0.496
Kidney	Kontrol	1.21±0.28^{a}	4.38±0.38^{ab}	0.24±0.02^{a}	730.7±90.6^{a}
	EB	2.40±0.31^{c}	6.04±0.55^{c}	0.19±0.02^{b}	316.6±41.6^{d}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	1.26±0.21^{a}	4.34±0.70^{ab}	0.25±0.03^{a}	712.6±118.9^{ab}
		P=0.999	P=1.000	P=1.000	P=0.998
	DIO100	1.17±0.27^{a}	4.26±0.37^{a}	0.24±0.02^{a}	752.3±97.1^{a}
		P=1.000	P=0.997	P=0.950	P=0.994
	EB+DIO50	1.76±0.31^{b}	5.10±0.76^{b}	0.22±0.02^{a}	538.6±88.8^{c}
		P<0.001	P=0.066	P=0.337	P<0.001
	EB+DIO100	1.48±0.25^{ab}	4.94±0.56^{ab}	0.23±0.02^{a}	600.9±76.9^{bc}
		P=0.219	P=0.260	P=0.588	P=0.022
Brain	Kontrol	1.67±0.20^{a}	2.14±0.29^{a}	0.29±0.02^{a}	355.1±51.3^{a}
	EB	3.72±0.16^{d}	3.75±0.32^{c}	0.13±0.03^{d}	142.3±33.8^{c}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	1.64±0.18^{a}	2.10±0.37^{a}	0.27±0.03^{ab}	361.0±51.9^{a}
		P=0.998	P=1.000	P=0.410	P=1.000
	DIO100	1.84±0.17^{a}	2.12±0.22^{a}	0.30±0.03^{a}	337.4±49.9^{a}
		P=0.397	P=1.000	P=1.000	P=0.938
	EB+DIO50	2.87±0.27^{c}	3.19±0.18^{b}	0.19±0.03^{c}	230.4±28.1^{b}
		P<0.001	P<0.001	P<0.001	P<0.001
	EB+DIO100	2.43±0.17^{b}	2.87±0.29^{b}	0.24±0.03^{b}	268.5±36.0^{b}
		P<0.001	P<0.001	P<0.001	P<0.001
Testis	Kontrol	3.45±0.24^{a}	2.18±0.36^{a}	0.11±0.03^{a}	116.7±12.9^{a}
	EB	5.25±0.32^{d}	3.56±0.15^{c}	0.06±0.02^{c}	60.6±11.8^{c}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	3.54±0.31^{a}	1.88±0.25^{a}	0.11±0.01^{a}	108.9±5.7^{a}
		P=0.988	P=0.052	P=0.998	P=0.705
	DIO100	3.72±0.38^{ab}	1.89±0.13^{a}	0.13±0.02^{a}	119.6±13.9^{a}
		P=0.452	P=0.078	P=0.682	P=0.994
	EB+DIO50	4.14±0.40^{bc}	3.29±0.17^{bc}	0.08±0.01^{b}	81.2±12.3^{b}
		P<0.001	P<0.001	P=0.003	P<0.001
	EB+DIO100	4.22±0.30^{c}	2.99±0.25^{b}	0.09±0.01^{b}	91.0±14.4^{b}
		P<0.001	P<0.001	P=0.011	P<0.001
Heart	Kontrol	2.17±0.28^{a}	3.11±0.21^{a}	0.17±0.02^{a}	338.2±32.0^{ab}
	EB	4.30±0.31^{c}	4.41±0.38^{c}	0.10±0.01^{d}	217.3±28.0^{d}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	2.27±0.31^{a}	3.21±0.40^{ab}	0.16±0.02^{a}	334.7±23.7^{ab}
		P=0.098	P=0.988	P=0.825	P=1.000
	DIO100	2.02±0.24^{a}	3.10±0.34^{a}	0.16±0.02^{ab}	351.2±43.6^{a}
		P=0.866	P=1.000	P=0.458	P=0.932
	EB+DIO50	3.21±0.36^{b}	3.63±0.25^{b}	0.12±0.02^{cd}	264.1±25.4^{c}
		P<0.001	P=0.010	P<0.001	P<0.001
	EB+DIO100	2.83±0.28^{b}	3.43±0.30^{ab}	0.13±0.02^{bc}	298.2±27.8^{bc}
		P<0.001	P=0.265	P<0.001	P=0.057
Lung	Kontrol	3.34±0.36^{a}	3.84±0.67^{a}	0.36±0.02^{a}	100.6±7.6^{a}
	EB	5.18±0.42^{d}	6.35±0.34^{c}	0.28±0.01^{c}	59.9±4.4^{c}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	3.49±0.39^{a}	3.81±0.39^{a}	0.35±0.01^{a}	97.2±8.7^{a}
		P=0.908	P=1.000	P=0.867	P=0.960
	DIO100	3.36±0.28^{a}	3.78±0.37^{a}	0.36±0.02^{a}	103.8±12.8^{a}
		P=1.000	P=0.999	P=0.999	P=0.971
	EB+DIO50	4.48±0.32^{c}	5.59±0.52^{b}	0.31±0.01^{b}	73.8±10.6^{b}
		P<0.001	P<0.001	P=0.001	P<0.001
	EB+DIO100	3.95±0.27^{b}	5.04±0.33^{b}	0.33±0.01^{b}	78.7±8.2^{b}
		P=0.003	P<0.001	P=0.027	P<0.001
Esryth rocyte s/ plas- ma	Kontrol	11.27±0.76^{a}	18.00±7.40^{a}	0.71±0.04^{a}	2039.4±139.7^{ab}
	EB	16.12±0.79^{c}	53.26±11.27^{c}	0.51±0.03^{d}	1469.8±145.9^{d}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	11.68±0.85^{a}	26.07±7.50^{ab}	0.72±0.03^{a}	2095.6±211.1^{a}
		P=0.851	P=0.200	P=0.778	P=0.969
	DIO100	11.12±0.80^{a}	19.71±3.73^{a}	0.72±0.04^{a}	1949.2±162.5^{ab}
		P=0.998	P=0.984	P=0.834	P=0.804
	EB+DIO50	14.01±0.76^{b}	37.81±13.16^{bc}	0.58±0.03^{c}	1715.6±107.3^{c}
		P<0.001	P=0.010	P<0.001	P<0.001
	EB+DIO100	13.65±0.66^{b}	34.64±14.14^{ab}	0.63±0.02^{b}	1850.6±173.1^{bc}
		P<0.001	P=0.050	P<0.001	P=0.104

The mean ± standard deviation is used to express the data. A statistically significant difference (P<0.05) between groups is indicated by different superscripts (a, b, and c) in the same column.

Groups: Control, corn oil; EB, emamectin benzoate (10 mg kg^{-1}.BW day^{-1}); DIO50, diosmin (50 mg kg^{-1}.BW day^{-1}); DIO100, diosmin (100 mg kg^{-1}.BW day^{-1}); EB+DIO50, emamectin benzoate plus diosmin (50 mg kg^{-1}.BW day^{-1}); EB+DIO100, emamectin benzoate plus diosmin (100 mg kg^{-1}.BW day^{-1}).

Table 3. Effects of DIO treatment on the levels/activities of GSH, GPx, GR, and GST in liver, kidney, brain, testis, heart and lung tissues, and erythrocytes/plasma of EB-exposed rats

Sample	Groups	Parameters			
		GSH nmol/mg P or	GPX nmol/min/g P	GR nmol/min/g P	GST nmol/min/g P
Liver	Kontrol	98.19±7.15^{a}	19.25±2.30^{a}	29.41±2.52^{a}	135.17±17.66^{ab}
	EB	73.88±8.18^{c}	14.67±1.32^{b}	21.08±4.06^{b}	96.97±14.88^{c}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	95.81±7.45^{a}	19.30±1.61^{a}	29.81±2.53^{a}	134.68±15.38^{ab}
		P=0.977	P=1.000	P=1.000	P=1.000
	DIO100	99.81±6.45^{a}	19.09±2.01^{a}	29.67±3.21^{a}	141.50±16.67^{a}
		P=0.996	P=1.000	P=1.000	P=0.952
	EB+DIO50	85.15±7.74^{b}	17.45±2.07^{a}	27.95±1.95^{a}	114.74±19.91^{bc}
		P=0.002	P=0.316	P=0.871	P=0.072
	EB+DIO100	91.31±6.20^{ab}	18.26±2.13^{a}	26.13±2.88^{a}	128.44±12.17^{ab}
		P=0.289	P=0.862	P=0.142	P=0.939
Kidney	Kontrol	90.70±7.38^{a}	41.54±2.93^{a}	43.15±2.65^{ab}	148.38±18.22^{a}
	EB	55.66±7.39^{c}	27.84±2.74^{c}	30.29±6.39^{c}	102.81±9.50^{c}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	91.86±7.94^{a}	39.57±5.74^{ab}	42.94±4.33^{ab}	144.24±13.81^{a}
		P=1.000	P=0.901	P=1.000	P=0.980
	DIO100	90.25±9.87^{a}	41.26±4.60^{a}	44.00±6.10^{a}	147.17±9.72^{a}
		P=1.000	P=1.000	P=0.999	P=1.000
	EB+DIO50	75.35±9.19^{b}	35.28±3.55^{b}	37.07±4.36^{b}	125.36±13.52^{b}
		P=0.001	P=0.019	P=0.068	P=0.003
	EB+DIO100	77.53±6.57^{b}	37.61±4.90^{ab}	40.88±3.97^{ab}	131.60±11.30^{ab}
		P=0.008	P=0.312	P=0.897	P=0.060
Brain	Kontrol	57.34±2.11^{a}	18.74±1.74^{a}	25.22±1.59^{a}	48.71±3.67^{a}
	EB	38.70±2.69^{d}	11.16±2.13^{d}	15.27±1.79^{c}	35.01±2.42^{c}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	56.30±3.33^{a}	17.39±0.63^{ab}	23.81±1.98^{a}	49.08±1.95^{a}
		P=0.960	P=0.444	P=0.464	P=1.000
	DIO100	58.52±3.11^{a}	18.17±1.44^{ab}	25.16±1.88^{a}	47.93±3.92^{a}
		P=0.930	P=0.970	P=1.000	P=0.992
	EB+DIO50	45.27±2.79^{c}	13.71±1.08^{c}	18.92±1.81^{b}	41.43±3.28^{b}
		P<0.001	P<0.001	P<0.001	P<0.001
	EB+DIO100	49.67±2.41^{b}	16.36±2.19^{b}	19.72±1.37^{b}	43.43±2.47^{b}
		P<0.001	P=0.023	P<0.001	P=0.004
Testis	Kontrol	38.43±4.51^{a}	9.94±1.00^{a}	12.14±1.64^{a}	318.87±23.67^{ab}
	EB	23.54±5.65^{c}	6.24±0.63^{c}	8.21±0.45^{c}	163.90±18.43^{d}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	37.20±2.00^{ab}	10.49±1.25^{a}	12.40±1.28^{a}	306.95±18.62^{ab}
		P=0.969	P=0.824	P=0.997	P=0.806
	DIO100	38.34±3.75^{a}	10.34±1.25^{a}	12.68±1.61^{a}	325.88±8.37^{a}
		P=1.000	P=0.945	P=0.922	P=0.943
	EB+DIO50	33.69±1.40^{b}	7.97±1.11^{b}	10.24±0.79^{b}	221.95±33.65^{c}
		P=0.042	P=0.001	P=0.011	P<0.001
	EB+DIO100	34.53±1.30^{ab}	8.17±0.48^{b}	11.15±1.04^{ab}	282.62±29.95^{b}
		P=0.147	P=0.003	P=0.459	P=0.072
Heart	Kontrol	72.68±11.01^{a}	15.41±1.45^{a}	7.12±0.86^{a}	24.93±2.24^{ab}
	EB	43.68±13.15^{c}	11.78±1.17^{c}	3.33±0.65^{c}	11.51±3.05^{d}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	67.07±12.63^{ab}	14.69±1.65^{ab}	7.34±0.43^{a}	25.06±4.14^{ab}
		P=0.860	P=0.797	P=0.984	P=1.000
	DIO100	71.54±9.10^{a}	15.51±1.27^{a}	7.04±0.79^{a}	26.89±3.58^{a}
		P=1.000	P=1.000	P=1.000	P=0.760
	EB+DIO50	54.81±7.99^{bc}	13.43±0.91^{bc}	4.78±0.65^{b}	17.84±3.86^{c}
		P=0.007	P=0.011	P<0.001	P<0.001
	EB+DIO100	61.28±10.90^{ab}	14.44±0.95^{ab}	5.32±0.87^{b}	21.08±2.01^{bc}
		P=0.201	P=0.529	P<0.001	P=0.102
Lung	Kontrol	103.72±11.25^{a}	28.48±1.31^{a}	37.52±1.42^{a}	89.84±7.65^{a}
	EB	72.38±7.47^{d}	20.97±1.68^{d}	25.21±2.16^{d}	53.43±4.53^{c}
		P<0.001	P<0.001	P<0.001	P<0.001
	DIO50	97.47±6.83^{ab}	27.77±1.53^{ab}	36.47±3.92^{ab}	83.30±4.58^{a}
		P=0.578	P=0.872	P=0.963	P=0.140
	DIO100	98.82±4.62^{a}	28.11±1.14^{ab}	37.03±3.35^{a}	86.98±5.33^{a}
		P=0.792	P=0.984	P=0.998	P=0.882
	EB+DIO50	83.30±9.84^{cd}	24.10±1.45^{c}	31.32±3.16^{c}	64.75±5.92^{b}
		P<0.001	P<0.001	P=0.001	P<0.001
	EB+DIO100	87.44±9.52^{bc}	25.48±2.27^{bc}	32.63±2.36^{bc}	71.25±6.39^{b}
		P=0.001	P=0.026	P=0.001	P<0.001
Esryth-rocytes/ plasma	Kontrol	39.18±3.46^{a}	31.62±1.60^{a}		32.56±2.43^{ab}
	EB	24.32±2.79^{c}	20.94±1.45^{c}		22.27±1.89^{d}
		P<0.001	P<0.001		P<0.001
	DIO50	37.74±2.37^{a}	32.01±1.52^{a}		34.03±2.66^{a}
		P=0.940	P=0.995		P=0.790
	DIO100	38.46±5.38^{a}	31.55±2.01^{a}		35.92±3.11^{a}
		P=0.997	P=1.000		P=0.052
	EB+DIO50	28.96±3.05^{bc}	25.69±1.93^{b}		27.40±2.40^{c}
		P<0.001	P<0.001		P<0.001
	EB+DIO100	31.68±3.23^{b}	26.42±1.52^{b}		29.47±2.68^{bc}
		P<0.001	P<0.001		P=0.091

The mean ± standard deviation is used to express the data. A statistically significant difference (P<0.05) between groups is indicated by different superscripts (a, b, and c) in the same column.

Groups: Control, corn oil; EB, emamectin benzoate (10 mg kg^{-1}.BW day^{-1}); DIO50, diosmin (50 mg kg^{-1}.BW day^{-1}); DIO100, diosmin (100 mg kg^{-1}.BW day^{-1}); EB+DIO50, emamectin benzoate plus diosmin (50 mg kg^{-1}.BW day^{-1}); EB+DIO100, emamectin benzoate plus diosmin (100 mg kg^{-1}.BW day^{-1}).

Discussion and Conclusion

One of the primary mechanisms by which pesticides can damage cells and tissues is through oxidative stress processes, which include the production of free radicals, lipid peroxides, oxidized proteins, and oxidized carbohydrates (Jabłońska–Trypuć and Wiater, 2022). As is well known, oxidative stress is described as an imbalance between the generation of free radicals and reactive metabolites known as oxidants or reactive oxygen species (ROS) and their elimination by defense systems known as antioxidants (Pizzino et al., 2017). ROS are generated by cells as a byproduct of their metabolic processes. When ROS levels are too high, they interact negatively with DNA, lipids, and proteins in the body (Birben et al., 2012). Excessive formation of hydroxyl radicals and peroxynitrite causes lipid peroxidation, which damages cell membranes and lipoproteins (Pizzino et al., 2017). MDA, a lipid peroxidation marker, is one of the secondary oxidation products of lipid peroxidation (LPO). NO, another lipid peroxidation marker and reactive nitrogen species (RNS), interacts with the superoxide (O_2^-) radical to form peroxynitrite ($ONOO^-$), which is responsible for its toxic effect (Birben et al., 2012). The increase in MDA and NO levels in all tissues of rats exposed to EB in the study indicates lipid peroxidation triggered by excessive ROS and RNS production. Enzymatic and non-enzymatic antioxidants are responsible for protecting the cell from ROS-induced damage (Lü et al., 2010). GSH is an essential part of metabolic defensive processes such as free radical quenching, hydroperoxide reduction, and xenobiotic detoxification. The GSH -dependent antioxidant system consists of GSH and enzymes with similar functions, including GST, GSH-Px, and GR.GSH-Px reduces hydroperoxides and H_2O_2, whereas GSH is oxidized to GSSG. Then, GR regenerates GSH from GSSG (Nimse and Pal, 2015). The study revealed that GSHPx activity and GSH levels were decreased in all tissues of rats exposed to EB. The reduction in GSH levels promotes lipid peroxidation and oxidative stress (Jabłońska-Trypuć, 2017). Due to the antioxidant role of GSH in inhibiting free radical reactions in the tissues, it is possible that the decrease in GPx activity seen in the study was caused by GSH depletion. The initial lines of defense against reactive intermediates are SOD and CAT. Superoxide radicals are scavenged by SOD, while hydrogen peroxide radicals are neutralized by CAT (Nimse and Pal, 2015). The reduction of CAT activity in all tissues in this study can be attributed to excessive production of H_2O_2, which indicates EB-induced oxidative stress. The decreased SOD activity of rats exposed to EB may be the result of its increased degradation and decreased production as a result of increased oxidative stress. The findings of our study are in parallel with those of previous studies. Similarly, Madkour et al. (2021) reported that EB exposure (orally 9 mg kg^{-1} BW for 6 weeks) increased MDA levels in the brain tissue of rats while decreasing GPx, CAT, and SOD activities. In the study published by El-Sheikh and Galal (2015), the researchers noted that liver tissue MDA dramatically increased while SOD activity decreased when EB (2.5 mg EB kg^{-1} BW) was administered orally for 28 days. Abou-Zeid et al. (2018) reported that 75 ppm EB in the diet of mice induces oxidative stress by increasing MDA levels and suppressing GSH, CAT, and SOD levels and activities in liver and kidney tissue, as well as inhibiting SOD activity and increasing MDA levels in brain tissue. In the testes of mice exposed to EB in their study, Zhang et al. (2020) found that GPx and SOD activity decreased and MDA levels elevated. In the liver and kidney tissues of rats exposed to abamectin, another avermectin derivative, Abdel-Daim and Abdellatief (2018) discovered an elevation in MDA concentrations and a reduction in GSH levels and antioxidant enzyme activity (GPX, CAT, and SOD).

Our results, which demonstrate decreased total protein and albumin, are in line with earlier research (El-Sheikh and Galal, 2015; Madkour et al., 2021). Their concentration may have decreased due to an imbalance between the rates of protein synthesis and degradation. The liver is the organ where albumin is intensively synthesized. Therefore, pesticide-induced hepatocyte damage may reduce the capacity for albumin synthesis. It is thought that liver damage due to the toxic effects of EB also causes a decrease in albumin levels. The serum AST, ALT, ALP, LDH, cholesterol, and triglyceride levels/activities that increased as a result of EB administration were observed in our findings to be consistent with other research (El-Sheikh and Galal, 2015; Abdel-Daim and Abdellatief, 2018; Khaldoun Oularbi et al., 2017). The enhanced plasma membrane permeability caused by EB toxicity in this study may be the cause of the elevated ALT and AST levels. The increase in serum total cholesterol level can be attributed to decreased secretion into the duodenum due to obstruction of the liver bile ducts. Triglyceride accumulation is the result of an imbalance between the synthesis and release of triglycerides into the systemic circulation by parenchymal cells. The increase in these parameters suggests potential liver tissue damage (Hamed and Abdel-Razik, 2015; Meligi and Hassan, 2017). In line with prior investigations, our study also found increased BUN, uric acid, and creatinine levels in serum (Meligi and Hassan, 2017; Abdel-Daim and Abdellatief, 2018; Madkour et al., 2021). The elevation in these measurements could be evidence of renal tissue damage associated with oxidative stress triggered by EB. Increased levels of uric acid and creatinine in the serum can be linked to decreased renal glomerular filtration and urine excretion capacity, which reflect renal tubular dysfunction (Madkour et

al., 2021). As the kidney is the organ that primarily secretes urea into the urine, a high uric acid level is an indicator of reduced renal function. Due to the fact that urea is the end product of protein catabolism, elevated creatinine levels are linked to increased protein catabolism. Our study, which states that EB decreases the total protein level due to increased protein catabolism, is compatible with the literature (Meligi and Hassan, 2017).

Investigating the ability of natural antioxidants to mitigate oxidative stress induced by pesticides is gaining more attention. Flavonoids, which are members of the polyphenol family, have strong antioxidant properties (Zeng et al., 2021). The main molecular modes of action of flavonoids include their capacity to chelate metals, inhibit different types of oxidases including lipoxygenases, and cyclooxygenases, and promote the activity of antioxidant enzymes including SOD, CAT, and GPX (Jabłońska–Trypuć and Wiater, 2022). Furthermore, they can reduce free radical levels in the cell by inhibiting the activities or expression of free radical-producing enzymes like NAD(P)H oxidase and xanthine oxidase (XO) (Lü et al., 2010). Due to the fact that diosmin is a flavonoid, it shares similar effects with other flavonoids. All tissues in the groups in the current study that only received DIO were similar to those in the control group; therefore, it can be concluded that DIO had no negative effects. Similar findings were reported in previous studies (Rehman et al., 2013; Eraslan et al., 2017; Ağır and Eraslan, 2019). Depending on the dose, it caused the effects of EB at both doses to be completely or almost completely reversed in the groups that received EB plus DIO. This effect may be due to either the inhibition of XO enzyme activity, which causes free radical formation, or the reduction of the cellular level of free radicals as a result of its radical scavenging activity. Previous investigations found similar results. Tekeli et al. (2021) reported that DIO can protect against deltamethrin in rats by increasing GSH levels and GPx, SOD, and CAT activities while decreasing MDA and NO levels in the liver, kidney, brain, testis, heart, and erythrocyte/plasma. According to Bozdağ and Eraslan (2020), DIO reverses the effects of lead by increasing total protein and albumin levels while decreasing AST, ALT, ALP, LDH, cholesterol, triglyceride, BUN, uric acid, and creatinine activity/levels in serum. Additionally, they demonstrated that decreasing MDA and NO levels and increasing GSH, GPX, SOD, and CAT levels/activities can alleviate lead-induced damage to the blood, liver, kidneys, testes, brain, and heart. As said by Abdel-Daim et al. (2017), the treatment of DIO (50 and 100 mg kg-1) either partially or completely restored dose-dependently the elevated AST, ALT, ALP, LDH, urea, and creatinine levels/activities in the serum in mice exposed to methotrexate. Moreover, depending on the dose, DIO treatment improved the levels and activities of MDA, NO, GSH, GST, GR, GPx, SOD, and CAT in the liver, kidney, and heart tissue closer to those of the control group.

In conclusion, it was demonstrated that EB administered orally might decrease the antioxidant defenses of rats and increase their susceptibility to oxidative stress. However, the severity of oxidative damage induced by EB regressed with DIO treatment in a dose-dependent manner, especially at a dose of 100 mg/kg. As a result of its antioxidant effect, it is expected that the aforementioned flavonoid may be used as a preservative and help to prevent pesticide toxicity. However, more investigation is required to completely understand the precise mechanisms of action and the application of DIO to mitigate pesticide -induced toxicities.

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