The potentially preventive role of erdosteine against subacute diazinon-induced oxidative stress and inflammation in rats

Yavuz Osman Birdane (ybirdane@aku.edu.tr)
Afyon Kocatepe University: Afyon Kocatepe Universitesi https://orcid.org/0000-0003-0754-7909
Gulcan Avci
Afyon Kocatepe University: Afyon Kocatepe Universitesi
Fatih Mehmet Birdane
Afyon Kocatepe University: Afyon Kocatepe Universitesi
Ruhi Turkmen
Afyon Kocatepe University: Afyon Kocatepe Universitesi
Orkun Atik
Afyon Kocatepe University: Afyon Kocatepe Universitesi
Hulya Atik
Afyon Kocatepe University: Afyon Kocatepe Universitesi

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Abstract

In today's world, pesticides are widely used with the aim of pest control and advanced agriculture. As an Organophosphorus Insecticide (OPI), Diazinon (DZN) is a commonly used substance. However, the wide usage of DZN increases the probability of incidence regarding possible toxication reported to be shaped not through the experienced cholinergic syndromes as a result of acetylcholinesterase inhibition – the primary effect of these cases-, but by altering the oxidative stress production and the inflammatory responses. In this study, erdosteine (ERDOS) implementation's protective impact on subacute DZN exposure have been investigated. A total of 24 albino rats (Wistar, male) were separated into 4 research groups (with 6 rats in each group); namely, control, DZN group (15 mg/kg/day), ERDOS group (10 mg/kg/day), and DZN + ERDOS group (15 mg/kg/day); these medications were given through an oval gavage for 28 days. With the blood samples taken from the rats, oxidant-antioxidant parameters and cytokine levels were measured. DZN had a negative effect on the oxidant-antioxidant parameters (MDA, GSH, SOD, CAT, GPx, NOx, AOA) ($p < 0.05$) and cytokine levels (IL-1β, IL-10, and TNF-α), when compared with control ($p < 0.05$). However, the ERDOS implementations were detected to ameliorate the harmful effects of DZN on the oxidant-antioxidant parameters and cytokine levels ($p < 0.05$). Conclusively, besides the known mucolytic efficiency of ERDOS, it can also be stated to display free radical scavenger, antioxidant, and anti-inflammatory characteristics. In addition, the ameliorating property of ERDOS can be benefited from in possible diazinon toxication cases.

1. Introduction

Recently, the use of pesticides have been common practice increase food production during every period of agricultural products with the aim of providing high efficiency and quality from these products. Environmental pollution emerging from the wide use of pesticides has negative effects, especially on mammal species. This exposure can especially become dangerous for people living in rural areas and engaging in agriculture (Clayton et al. 2003; McCauley et al. 2003; Abdollahi et al. 2004).

Organophosphorus pesticides (OPs) are a type of pesticide widely used throughout the world due to their features such as displaying low accumulation, availability, and persisting only for short periods in the environment. However, the extensive use of OPs also increases the prevalence of toxicity cases induced by them (Goozner et al. 2002; Jafari and Pourheidari 2006). Regarded as one of the most toxic pesticides for vertebrates, OPs cause constant stimulation by inhibiting acetylcholinesterase (AChE) enzymes through a covalent bond to its sarin residues (Abu-Qare and Abou-Donia 2001). Muscarinic receptors are stimulated based on OPs toxication; as a result, symptoms like stomachache, diarrhea, increased secretion and sweating (Büyükokuroğlu et al. 2008). In addition, they can cause involuntary twitching, weakness, and even paralysis by stimulating nicotinic receptors in neuromuscular junctions (Olson, 2018).

As an organophosphorus pesticide, Diazinon (DZN, O, O-diethyl O-[6-methyl-2-(1-methyethyl)-4pyrimidinyl] phosphorothioate) works as an anti-parasitic agent and is commonly used to fight external parasites like

acari and ticks in the field of veterinary medicine (Oñate et al. 2009). It is widely used in agriculture for pest control (Bailey et al. 2000). DZN residues on grown food products are regarded as a global problem. Agricultural products absorb DZN, and its harmful effects emerge when people consume these products (Boussabbeh et al. 2016). Therefore, 0.01 mg/kg has been determined to be the acceptable limit for the residue on vegetables or fruits (EU Pesticides Database 2021).

As is the case with other OPIs, the cholinergic receptors' hyperstimulation determine DZN toxicity's main mechanism, as a result of AChE inhibition. Nevertheless, DZN itself is not a strong AChE inhibitor. Even though DZN is eliminated primarily through kidneys from the body, its metabolites created by cytochrome P450 enzymes in the liver (diazoxon, hydroxydiazoxon, and hydroxydiazinon) are extremely strong AChE inhibitors and are responsible for the development of cholinergic syndrome (WHO, 1998). However, researchers state that this cholinergic hyperstimulability caused by DZN exposure is not the basis of all the harmful effects resulting from DZN (Jafari et al. 2012; Danaei and Karami 2017). Its function in the production of reactive oxygen species (ROS) is demonstrated to be another important reason behind these negative effects caused by DZN (Abdou and El Mazoudy 2010; Bhatti et al. 2010; Lukaszewicz-Hussain 2010). It has been put forward in numerous studies that acute or chronic DZN toxicity causes oxidative stress development (Shah and Iqbal 2010; Danaei et al. 2019; Yaghubi Beklar et al. 2020).

Erdosteine (ERDOS) molecule has mucolytic properties and is a thiol-based prodrug metabolized to ring-opened metabolite M1 (MET 1). As it is demonstrated in experimental research, ERDOS controls MET 1's ROS production and thereby eliminates or lowers lung tissue damage associated with oxidative stress (Boyaci et al. 2006; Demiralay et al. 2006; Erdem et al. 2017). In a recent meta-analysis study carried out to compare the mucolytic and antioxidant features of medications with the participation of 2753 individuals with a history of a chronic obstructive pulmonary disease (COPD), the general efficiency/reliability of ERDOS was demonstrated to be superior to those of carbocysteine and N-acetylcysteine (NAC) (Rogliani et al. 2019). Even though the protective effect of NAC, a mucolytic agent with antioxidant features, against pesticide toxication has been put forth (Abdel-Daim et al. 2019a; Turkmen et al. 2019), no such study for ERDOS has been seen yet.

An imbalance between oxidants and anti-oxidants, caused by the differences in the antioxidant defense mechanism and increased free-radical production characterize oxidative stress (Cemek et al. 2011). In this case, ROS reacts rapidly with proteins, carbohydrates, nucleic acids, and lipids, and thus, causes disorder within the structures and functions of cellular components. The thiobarbituric acid test (TBA) is used to test the malondialdehyde (MDA) formation; It is the most used lipid peroxidation index in studies conducted with animal (De Zwart et al. 1999). The antioxidant defense system primarily consists of antioxidant enzymes such as glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD). Alongside reduced glutathione (GSH), these enzymes contribute to free-radical cleaning, as well as defending cells from oxidative-stress damage caused by different xenobiotics (Banerjee et al. 1999; Valko et al. 2007). Total Antioxidant Capacity (AOA) is the measure of the sensitivity of a cell for oxidative stress. This analysis test has been found to be highly applicable in testing chemicals due to its ability to

provide integration with various defense mechanisms at the cellular level against ROS and quantitative measurement of the total oxidative buffer capacity of plasmas, tissues, and organs (Sies 2007).

Reactive oxygen species, in addition to causing oxidative damage, activate inflammatory pathways and, as a result, trigger inflammatory proteins to be expressed (Lee and Yang 2012). Therefore, the anti-inflammatory effects of ERDOS, besides its mucolytic and antioxidant efficiency, should also be studied. In recent studies, ERDOS has been demonstrated to decrease inflammation in experimental models composed of various chemicals (Park et al. 2016; Xi et al. 2020). Nevertheless, its anti-inflammatory effectiveness against pesticide toxication caused by DZN has not been ascertained.

In this study, the protective role of ERDOS, which has numerous advantages such as availability, affordability, and reliability (Rogliani et al. 2019), against the subacute exposure of DZN, among the most widely used pesticides on the globe, will be assessed in terms of oxidant-antioxidant parameters and some proinflammatory cytokines.

2. Materials And Methods

2.1. Chemicals

Diazinon was bought from Syngenta (Basudin 60 EM, 630 g/L diazinon, Izmir, Turkey). Moreover, ERDOS (SML1529) was bought from the company Sigma-Aldrich (Missouri, USA). Invitrogen (California, USA) was the provider for the Cytokine ELISA (IL-1β, IL-10, and TNF-α) kits while GPx ELISA kit was obtained from Cayman Chemical (Michigan, USA). All of the other reagents and chemicals used by the researchers in the experiments were of analytical quality.

2.2. Animals and Experimental Design

Healthy albino rats (Wistar, male, n = 24) were acquired from the Experimental Animal Research and Application Center's animal breeding laboratories located in Afyon, Turkey. Their average age was 10 weeks and they weighed 200–250 g. Approval for conducting the experiments was obtained from the Afyon Kocatepe University's Animal Experiments Local Ethics Committee (AKUHADYEK-49533702/390), and from the National Institute of Health's Guide to the Care and Use of Laboratory Animals. The rats were housed in a 12h light-dark period at room temperature (25 ± 2 ^{0}C) and 50–55% relative humidity with ad libitum access to regular rodent food and water. The test animals were allowed a minimum of 7 days to acclimate to the facility before the experiments began. They were fed a regular rodent diet for 7 days prior to the tests to help them transition to the laboratory conditions.

The animals were separated into 4 groups with 6 rats in each. Control group was provided with 0.5 ml Corn oil as a vehicle (Group I). DZN group was provided with 15 mg kg^{-1} DZN dissolved in corn oil at the same volume (Group II). ERDOS group was provided with 10 mg kg^{-1} ERDOS dissolved in distilled water at the same volume (Group III). The last group (DZN + ERDOS; Group IV) was provided with DZN and ERDOS with the same volume. The chemicals were administered once each for 28 days via a gastric

gavage. The animals were also administered ERDOS half an hour before DZN was given. The doses of ERDOS and DZN used in this study were chosen based on previous research on the subject (Fadillioglu et al. 2003; Shah and Iqbal 2010; Yaghubi Beklar et al. 2020).

2.3. Blood collection and the preparation of erythrocytes

Each group's blood samples were taken under light anesthesia by cardiopuncture inside heparinized and non-heparinized tubes at the end of 28 days. Centrifugation was used for precipitating the erythrocytes at 600g for 15 min at 4°C within 30 minutes after blood collection and the plasma and serum were extracted. The puffy coat was separated after washing the erythrocytes three times in isotonic saline. The same amounts of isotonic saline and erythrocyte were then introduced to vials and they were frozen at -20°C in a freezer. Five times the amount of cold deionized water was used as the osmotic pressure applied destroyed the erythrocyte suspensions. The erythrocyte lysate was stored at 4°C for three days prior to the measurements (Winterbourn et al. 1975).

2.4. Evaluation of the oxidative stress markers

2.4.1. Measurement of whole blood malondialdehyde (MDA)

Using the technique by Draper and Hadley (1990), as an LPO marker, MDA was found in the blood sample. This is a technique carried out by the spectrophotometric measurement of the purple color, which is formed when TBA interacts with MDA. With this aim, a 2.5 ml (10%) TCA solution was added into the blood samples placed in the tubes; The tubes were later placed for exactly 15 minutes in a bath of boiling water. The tubes were then centrifuged at 1000×g for 10 minutes after they cooled to room temperature, and 2 ml of each supernatant was moved to a test tube with 1 ml (0.67%) of TBA solution inside. Then, the tubes were immersed for 15 minutes in a boiling-water bath. The absorbance was measured at 532 nm using a spectrophotometer (Shimadzu UV-1601), after the test tubes cooled to room temperature. The MDA concentration was calculated according to the TBA–MDA complex's absorbance coefficient (ε = 1.56×105 cm/M). MDA concentration is measured in nmol ml^{-1} blood.

2.4.2. Measurement of the Plasma NOx (nitric oxide) levels

Inside aerated solutions, Nitric oxide (NOx) quickly decomposes and forms stable nitrite/nitrate products. In this study, the plasma nitrite/nitrate concentration was measured using a modified version of the Griess test, described by Miranda et al. in their study (2001). This test relies on detection by the acidic Griess reaction as well as the reduction of nitrate by vanadium. Prior to the test, the samples were deproteinized for a short amount of time. The serum was then added into the 96% cold ethanol at 1:2 (v/v) and later was vortexed for 5 minutes. After a 30-minute incubation at 4°C, it was centrifuged at 8000×g for 5 minutes and then the supernatants were used in the Griess test. The analysis was carried out on a microtiter plate. In the analysis, 100 µl of Vanadium (III) (VCl_3) was mixed with 100 µl filtrated plasma, then Griess reagents containing 50 microliters of sulfanilamide and 50 microliters of N-(1-naphthyl) ethylenediamine dihydrochloride (NEDD) added into the mixture. A Multiskan Spectrum brand

microplate reader (Thermo Labsystems, Finland) was used to test the absorbance at 540 nanometers. The concentration of nitrite/nitrate was measured using a sodium nitrite normal curve and noted in µM.

2.5. Evaluation of nonenzimatic and enzimatic antioxidan markers

2.5.1. Measurement of the GSH (reduced glutathione) levels in whole blood samples and plasma total AOA (antioxidant activity)

The blood sample's GSH concentration was measured using the technique by Beutler et al., mentioned in their study (1963). In a short period of time, 0.2 ml of the blood sample was added into 1.8 ml of distilled water. The 3 ml precipitating solution containing 30 g NaCl, 1.67 g metaphosphoric acid, and 0.2 g EDTA, in 100 ml distilled water was mixed with hemolysate. After a 5-minute rest, the mixture was filtered (Whatman No. 42), and 2 ml of the filtrate was transferred to a different container, after this, 8 ml of the phosphate solution (0.3 M disodium hydrogen phosphate) and 1 ml DTNB were added inside. Using 2 ml diluted precipitating solution (three parts purified water and two parts phosphate solution), 8 ml phosphate solution, and 1 ml DTNB reagent a blank was prepared. A standard 40 mg/100 ml GSH solution was prepared. Using the spectrophotometer, the optical density was measured at 412 nm.

The total AOA value was calculated using the technique Koracevic et al. described in their study (2001). This test calculates the inhibition capacity of the plasma for the production of TBARS (Thiobarbituric acid reactive substances) from benzoate, under the influence of the oxygen-free radicals resulting from Fenton’s reaction. At 532 nm, the reaction was spectrophotometrically measured. Antioxidants inside the sample inhibit the TBARS production; The suppression of color formation is named as AOA. As standard, a 1 mmol/l uric acid was used in the test.

2.5.2. Measurement of the activities of catalase (CAT), glutathione peroxidase (GPx), and superoxide dismutase (SOD) in erythrocyte lysate

The antioxidant enzyme activity of SOD in erythrocyte lysate was determined using the method described by Sun et al. in their study (1988). The reaction of xanthine with xanthine oxidase as a source of a substrate (superoxide) and reduced nitro blue tetrazolium (NBT) as a superoxide indicator was used to quantify SOD. In this procedure, xanthine-xanthine oxidase was used to create a superoxide flux. The absorbance produced by superoxide's reduction of NBT into blue formazan was measured spectrophotometrically at 560 nm. SOD activity was defined as U gHb^{-1} erythrocyte.

Aebi's (1984) techniques were used to identify CAT activity in erythrocyte lysate. The procedure was based on the catalase's decomposition of H_2O_2. The reaction mixture consisted of a 50 mM phosphate buffer with a pH of 7.0, 10 mM H_2O_2, and the research sample. At room temperature, the reduction rate of H_2O_2 was controlled for 45 seconds at 240 nm. The catalase activity (k; nmol min^{-1}) was explained in k gHb^{-1} erythrocytes; One unit of catalase is the volume of catalase that decomposes 1.0 mol of H_2O_2 per minute at pH 4.5 at 25°C.

The Cayman test kit (item No. 703102, Cayman Co, Michigan, USA) was used to measure GPx activity in erythrocyte lysate. Results are presented in nmol min ml^{-1}.

2.6. Measurement of the cytokine levels

The serum was used for the assays. Concentrations of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-10 (IL-10) were calculated with enzymatic methods using commercial kits according to manufacturer's instructions (BMS622 rat TNF-α kit, BMS630 rat IL-1β Elisa kit, and BMS629 rat IL-10 Elisa kit; Thermo Fisher Scientific, Massachusetts, USA). Cytokine levels were presented as pg/ml.

2.7. Measurement of Hemoglobin

Hemoglobin (Hb) was measured using the colorimetric cyanomethemoglobin method described by Drabkin and Austin (1935).

2.8. Statistical analysis

The data obtained from the experimental animals are expressed in the study as means and standard deviations (± SD) and they were analyzed with One-way ANOVA, followed by Duncan posthoc tests using SPSS v.20. A p-value < 0.05 was determined to be significant in the study.

3. Results

3.1. Effects of DZN and ERDOS on the MDA and NOx levels

MDA values are commonly used as a marker for LPO caused by free radicals. MDA levels in the blood samples of rats administered DZN were observed to increase significantly, compared to those in the control group ($p < 0.05$). On the other hand, MDA levels of the DZN + ERDOS group were observed to decrease significantly ($p < 0.05$), compared to the DZN group (Fig. 1 (a)).

The NOx levels of the plasma samples of rats in the DZN group were observed to increase more administered substantially, compared to the control group ($p < 0.05$). However, a more significant decrease was observed in the NOx levels of the DZN + ERDOS group, compared to the DZN group (Fig. 1 (b)).

3.2. Effects of DZN and ERDOS on the nonenzimatic antioxidan markers

GSH is a nonenzymatic antioxidant in the detoxification pathway and it reduces pesticides' toxic metabolites. GSH levels of blood samples of the DZN group were observed to be significantly lower than the control ($p < 0.05$) (Fig. 2 (a)).

Nevertheless, in the rat plasmas, AOA levels were determined as presented in Fig. 2 (b). AOA was observed to decrease in the DZN group's samples ($p < 0.05$). Following ERDOS treatment, plasma AOA

levels of the DZN + ERDOS group were observed to significantly increase, compared to the DZN group ($p < 0.05$).

3.3. Effects of DZN and ERDOS on the antioxidant enzymes

The activities of antioxidant enzymes SOD, CAT, and GPx in rats' erythrocytes are presented in Figs. 3 (a), (b), and (c), respectively. The animals given DZN were determined to have a substantial rise in erythrocyte SOD activities, compared to the control group ($p < 0.05$). The erythrocyte CAT activities of the experimental animal given DZN were also observed to increase significantly, compared to the control group ($p < 0.05$). On the other hand, the GPx activity in the animals' erythrocytes was observed to decrease, compared to the control group. DZN-induced changes in SOD, CAT, and GPx activities, on the other hand, reversed the effects of ERDOS administration. The administration of ERDOS, however, was reversed by the DZN-induced alteration of SOD, GPx, and CAT activities.

3.4. Effects of DZN and ERDOS on the IL-1β, IL-10, and TNF-α levels

In this study, the administration of DZN was determined to significantly ($p < 0.05$) increase the serum IL-1β, IL-10, and TNF-α levels of the DZN group, compared to the control (Figs. 4a, 4b, and 4c). In contrast, administration of ERDOS was observed to improve the DZN-induced cytokine levels of the experimental animals ($p < 0.05$).

4. Discussion

Oxidative stress, besides being one of the central factors that contribute to the toxicity of various environmental polluters such as diazinon, has also been argued to play a part in the activation of inflammatory processes (Toraih et al. 2018). Although it is currently forbidden, diazinon is still commonly used as a nematicide, acaricide, and/or insecticide in agriculture (Oropesa et al. 2014). Its extensive area of use affects biological diversity severely (Mahmood et al. 2016) and constitutes a toxicological risk for non-target organisms (Tsaboula et al. 2016). The utilization of various antioxidants to prevent pesticide-related (Turkmen et al. 2019a, b), especially subacute diazinon-induced oxidative incidents (Abdel-Daim et al. 2016, 2019b, 2020) has become the focal point for a lot of researchers. In such a case, the use of substances with exogen-related antioxidant features can be regarded as one of the rational treatment options.

That said, no data is available concerning a possible protective impact of ERDOS, whose antioxidant and anti-inflammatory are known and which is a reliable medication, against subacute diazinon toxicity. Thus, the current study aims to investigate the possible immune system-stimulation and antioxidant effects of ERDOS implementation on ameliorating the toxic effects of DZN in rat blood samples.

In this study conducted on rats, the subacute DZN exposure increased the MDA and nitric oxide (NOx) levels in rat blood samples. DZN have been reported to be able to improve the MDA development as a key product of lipid peroxidation in blood and various tissues in some in vivo, as well as in vitro studies

(Altuntas et al. 2004; Hernández-Moreno et al. 2018; Khazaie et al. 2019; Tatipamula and Kukavica 2020; Tahmasebi et al. 2020). In addition, our results have shown ERDOS to decrease the complete blood MDA levels of the rats. These results conform with the MDA results of another DZN toxication study in which NAC, which has antioxidant features similar to ERDOS, was implemented (Tahmasebi et al. 2020). Synthesized while being bound to nitric oxide synthase (iNOS) enzyme activity, NO is an active molecule having various effects on several physiologic and pathological incidents, and leads to the development of peroxynitrites ($ONOO^-$) that cause damage and eventually LPO development in cells by reacting with superoxide anion ($O_2^{-\bullet}$) in an aerobic atmosphere (Weinstein et al. 2000; Sayed-Ahmed et al. 2001). There are various studies in parallel with our study, demonstrating increased NOx levels after DZN implementation (Alp et al. 2011; Beydilli et al. 2015; Vahidirad et al. 2018), while ERDOS implementation has been contrarily observed to have a decreasing effect on NOx levels. This may have been caused by the fact that ERDOS hinders iNOS activity leading NOx production to decrease, and prevents LPO by directly scavenging primary radicals such as $ONOO^-$.

Reduced glutathione (GSH) is a thiol-containing non-enzymatic tripeptide which has a role in various operations necessary to have normal biological functions (e.g. DNA and protein synthesis). Along with other antioxidant enzymes (SOD, CAT, GPx), it also constitutes important biological defense systems for to defend tissues and cells from the adverse effects of oxidative stress. Also, GPx is an enzyme needed for GSH to reduce hydroperoxides, while SOD and CAT are enzymes responsible for the detoxification of O_2^- radical by converting it first to H_2O_2 and then water (Hassani et al. 2018). In light of all this information, enzymatic (SOD, CAT, and GPx) and non-enzymatic (GSH) antioxidant defense systems and even AOA (Lovásová and Sesztáková 2009) are thought to be important oxidative stress biomarkers used to measure the exposure level to varying xenobiotics (Hernández-Moreno et al. 2018). In our study, when checked against the control, the levels of GSH and GPx and AOA activities of the groups with DZN implementation were observed to decrease significantly. In their study on rabbits, Hernández-Moreno et al. (2018) observed that DZN's both low and high doses decreased the GSH levels, conforming to our results. However, Velki et al. (2018) observed that GSH levels of zebrafish larvae exposed to differing doses of DZN in differing amounts of time increased after one-hour of exposure. Additiaonally, increased GSH levels were found to be an indicator of oxidative stress, and the formed ROSs could already have been cleaned by increased antioxidant defense systems. The results in our study can be interpreted as the consumption of the GPx, AOA, and GSH levels in time to detoxify ROS molecules originating from DZN exposure. In a time-dependent mechanistic study done by Hassani et al. (2018), acute implementation of DZN was stated to disrupt the plasma antioxidant systems even in non-fatal doses. Also, in other diazinon exposure studies carried out on rats and mice, some contradictory statements expressing a decrease in antioxidant enzymes like SOD and CAT (El-Shenawy et al. 2010; El-Demerdash and Nasr 2014) and an increase (Akturk et al. 2006) have been observed. In this study, compared to the control, the erythrocyte SOD and CAT activities of the DZN group were observed to increase. The increased SOD and CAT activities in animals administered DZN may be a sign of their antioxidant features in dealing with the overproduction of ROSs. When the ERDOS + DZN group was compared to the DZN group, ERDOS was found to significantly ameliorate the enzymatic and non-enzymatic oxidative

stress biomarkers (SOD, CAT, GPx, AOA, and GSH). This can be expressed as another indicator that ERDOS is an agent that could be considered for cellular defense as it displays a strong antioxidant characteristic after its metabolization, besides its mucolytic efficiency.

In the present study, DZN was expressed to increase proinflammatory cytokine levels like IL-1β, IL-10, and TNF-α. This incidence, that occured after the DZN implementation, was found to conform to the findings of other research (Moallem et al. 2014; Danaei and Karami 2017; Abdel-Daim et al. 2019b). The reason for this can be that the oxidative stress formed by the DZN implementation activates the inflammatory response (Toraih et al. 2018) or that DZN directly causes the mRNA expression of proinflammatory cytokines to increase (Hariri et al. 2010). Upon comparing the ERDOS + DZN group with the DZN group, it was seen that ERDOS displayed its strong antioxidant effects, decreasing the proinflammatory cytokine levels such as IL-1β, IL-10, and TNF-α. In addition to this, the anti-inflammatory efficiency of ERDOS may result from its suppressive effect on the transcription factor NF-kB (nuclear factor kappa B), which is at the core of the cytokine synthesis process (Park et al. 2016).

5. Conclusion

This study demonstrates erdosteine implementation to be effective in preventing oxidative stress and inflammation caused by diazinon. It can be said that erdosteine displays this effect by increasing the antioxidant defense system activity, inhibiting lipid peroxidation, and decreasing proinflammatory cytokine levels. The lack of investigation regarding erdosteine's effect on blood glucose levels, which is one of the adverse effects of pesticides and AChE, constitutes the limitations of this study. It has been concluded that besides its antioxidant and anti-inflammatory efficiency, the antidotal efficiency of erdosteine should also be put forth with the help of biochemical, histopathological, and molecular studies carried out on tissues, along with two main limitations that could be approached in future studies.

Declarations

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Ethics

The Animal Experiments Local Ethics Board, Afyon Kocatepe University, Afyon, (Registration number: AKUHADYEK-49533702/390) has approved this work.

Consent to participate

Not applicable as the study did not include human subject

Consent to Publish

All co-authors agree to publish this manuscript in the Journal (Environmental pollution and research)

Authors' contributions:

The idea of the research was suggested by Y.O.B and the study design was also by Y.O.B.. Y.O.B., G.E.A., F.M.B., and R.T. performed the experimental work. Y.O.B., R.T., O.A. and H.A. wrote/drafted/edited the manuscript and interpreted the results. Y.O.B., G.E.A. F.M.B. and R.T. performed the laboratory analysis. All of the authors listed above were involved in editing this manuscript critically for important intellectual content and they all approved this final version to be published.

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Conflict of interest

The authors declare no conflict of interest.

Availability of data and materials

All research data are available from the corresponding author when needed.

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Figures

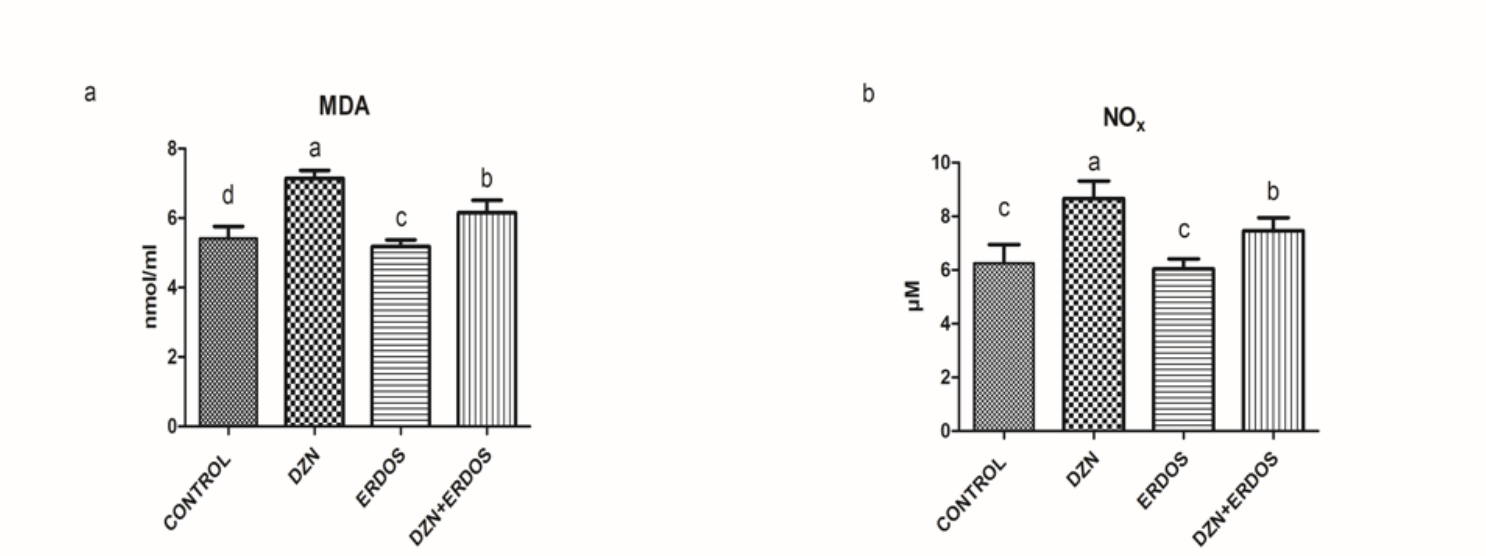

Figure 1

Effects of ERDOS on the MDA (a), and NOx (b) levels in diazinon-intoxicated rat. Data are presented as mean ±standard deviation (n= 7 per group). Values bearing different letters on the bars show statistically significant differences in the whole blood, and plasma $p < 0.05$). DZN: Diazinon; ERDOS: Erdosteine; MDA: Malondialdehyde; NOx: nitric oxide.

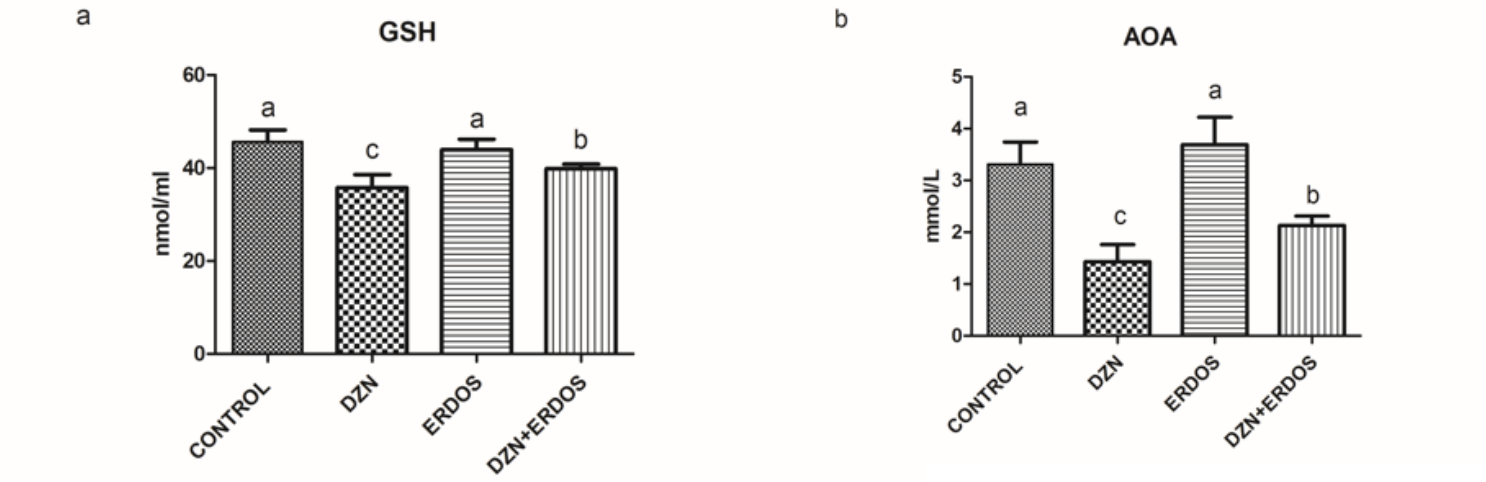

Figure 2

Effects of ERDOS on the GSH (a), and AOA (b) levels in diazinon-intoxicated rat. Data are presented as mean ±standard deviation (n= 7 per group). Values bearing different letters on the bars show statistically significant differences in the whole blood, and plasma p < 0.05). DZN: Diazinon; ERDOS: Erdosteine; GSH: Reduced glutathione; AOA: total antioxidant capacity.

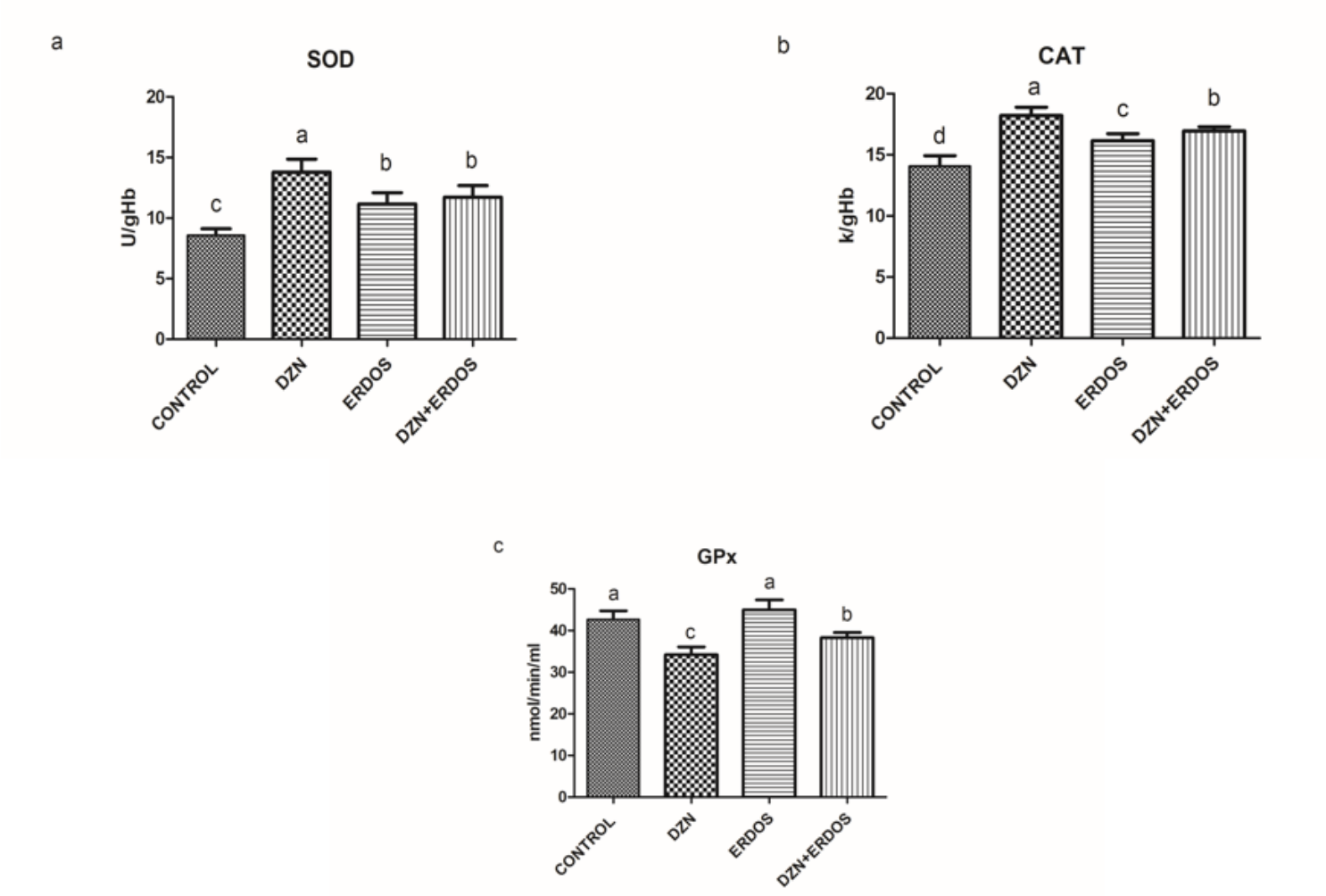

Figure 3

Effects of ERDOS on the SOD (a) CAT (b), and GPx (c) activities in diazinon-intoxicated rat. Data are presented as mean ±standard deviation (n= 7 per group). Values bearing different letters on the bars show statistically significant differences in the erythrocyte p < 0.05). DZN: Diazinon; ERDOS: Erdosteine; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase.

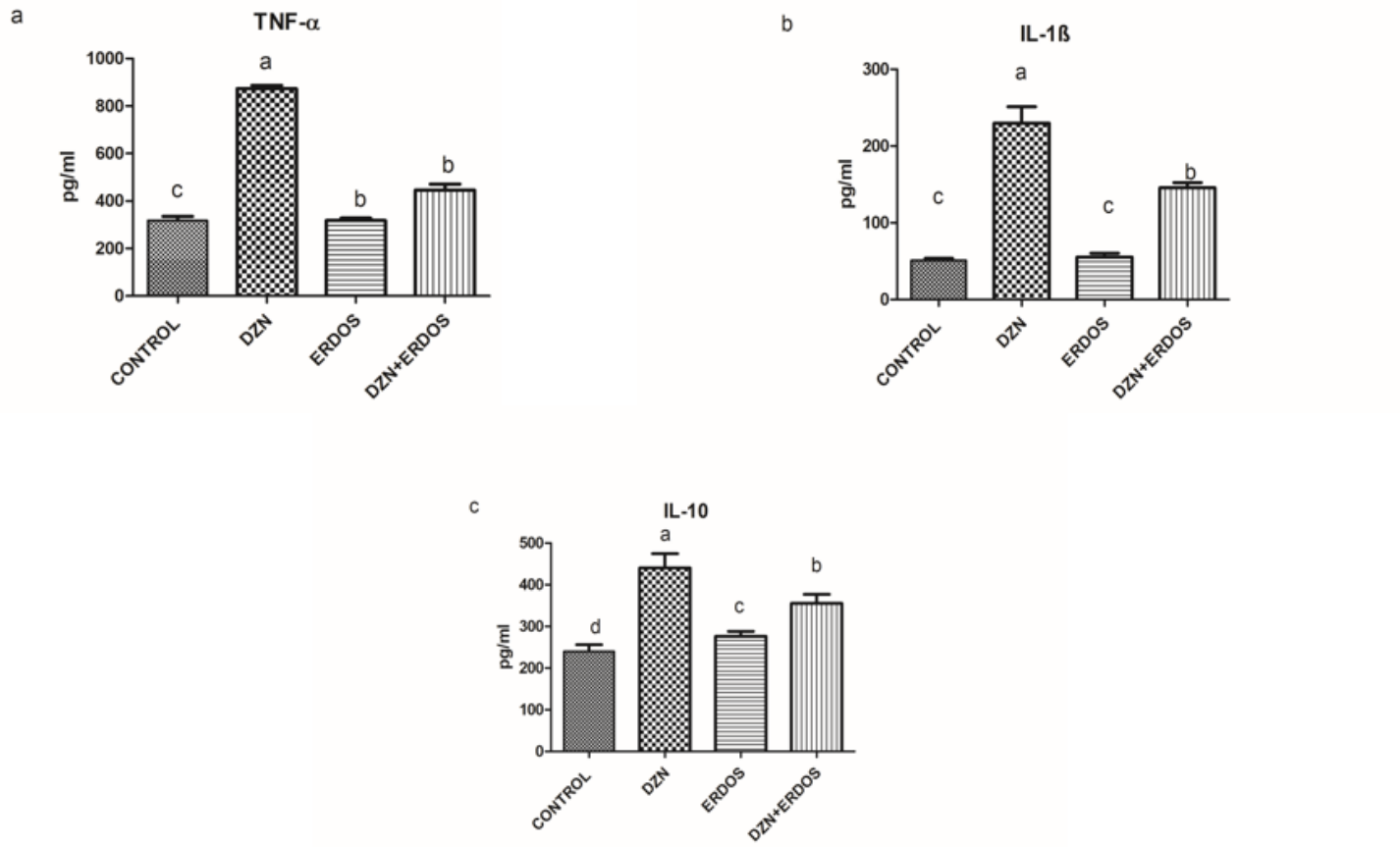

Figure 4

Effects of ERDOS on TNF-α (a), IL-1β (b) and IL-10 (c) levels in diazinon-intoxicated rat. Data are presented as mean ±standard deviation (n= 7 per group). Values bearing different letters on the bars show statistically significant differences in the serum $p < 0.05$). DZN: Diazinon; ERDOS: Erdosteine; TNF-α: Tumor necrosis factor-alpha; IL-1β: Interleukin 1 beta; IL-10: Interleukin 10.