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Protective effect of quercetin liposome on acute low dose diazinon-induced oxidative stress and neurobehavioral disorders by affecting serotonin metabolite in mature male rats

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Abstract

Diazinon (DZN) is a widely used organophosphate. We studied the effect of quercetin pegylated liposome (QPEGL) on acute low dose DZN-induced oxidative stress and behavioral disorders through monitoring brain serotonin metabolite 5-hydroxyindoleacetic acid (5-HIAA) in mature male rats. Animals were treated in two control groups that received a single dose of normal saline and dimethyl sulfoxide, and four groups that received a single dose of DZN 10.00 mg kg^{-1} (DZN), DZN 10.00 mg kg + quercetin 20.00 mg kg^{-1}, DZN 10.00 mg kg^{-1} + PEGL 20.00 mg kg^{-1}, DZN 10.00 mg kg^{-1} + QPEGL 20.00 mg kg^{-1} (QPEGL), respectively. Performances of the rats were investigated by the open field and elevated plus maze tests. Twenty-four hr after the treatments, animals' brains were harvested and frozen at – 80.00 ˚C. Brain tissues 5-HIAA level was determined by the enzyme-linked immunosorbent assay. Furthermore, malondialdehyde (MDA), superoxide dismutase (SOD), and glutathione peroxidase (GPx) levels were determined for oxidative stress analysis. The motor activity was significantly reduced in the DZN group compared to the control group following increased anxiety-like behavior and ameliorated by QPEGL. Moreover, 5-HIAA and MDA levels notably increased in the DZN group compared to the control group and significantly decreased in the QPEGL group compared to the DZN group. The SOD and GPx contents were not significantly changed in the DZN group compared to the control; although, these parameters improved after treatment with QPEGL. Acute low dose DZN exposure resulted in lipid peroxidation and elevated levels of the serotonin metabolite (5-HIAA), leading to neurobehavioral disorders, such as anxiety-like behavior and impaired motor activity, which were alleviated by QPEGL.

Introduction

Diazinon (DZN) is one of the most commonly used organophosphates (OPs) and has been shown to have the greatest effect on the nervous system of living organisms, including humans, leading to neurobehavioral impairments. Several studies related to oxidative damage in acute exposure to DZN have been done in various systems, including the nervous system, at high doses. Observations of behavioral changes following low dose DZN exposure have been noted; therefore, it seems necessary to study the effect of acute low dose DZN exposure on the central nervous system oxidative stress and its possible role in neurobehavioral impairments. The impact of exposure to low doses of OPs on the neurobehavioral properties of motor activity and gait changes in rats has been specified previously.[1]

Studies have shown relations between acute and low dose DZN exposure and neuropsychological deficiencies, such as mental disorders, depression, and decreased motor activity in humans and animals.[2,3] It has been revealed that subacute DZN exposures could induce organ and cell damages by oxidative stress induction.[4-6]

The 5-hydroxyindoleacetic acid (5-HIAA), a subsequent metabolite of serotonin, is known as a potential chemical biomarker for neurological and psychiatric disorders, such as depression, and it has a direct link with oxidative stress in brain tissue *via* monoamine oxidase

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activity.[7,8] It has been indicated that changes in the quantity and quality of motor activity are known as one of the main symptoms of neurobehavioral deficiencies, being associated with the 5-HIAA level.[9]

The effects of acute low dose DZN (13.00 - 39.00 mg kg^{-1}) and OPs exposures on the serotonergic system and 5-hydroxytryptamine levels in mature rats have been investigated formerly.[10] It was found that a low level exposure to DZN (1.00 mg kg^{-1}) over five days altered the expression of the serotonin transporter, subsequently affecting behavior in rats.[11]

Quercetin is one of the most important polyphenolic bioflavonoids and can be found in many plants food sources; it is also known as a powerful anti-oxidant.[12] Quercetin exerts a protective effect against OP insecticides, such as chlorpyrifos, and improves behavioral deficiencies through reduction of serotonin metabolism and 5-HIAA level in an animal model of Huntington's disease.[13]

The anti- and pro-oxidant effects of quercetin (2.00 - 20.00 mg kg^{-1}) at a chronic administration and a significant reduction of malondialdehyde (MDA) in the liver compared to the rats fed a diet without vitamin E have been reported. In addition, quercetin at a dose of 25.00 mg kg^{-1} exhibited protective effects against oxidative stress induced by chronic exposure to DZN at a dose of 10.00 mg kg^{-1} in rats.[14,15] Moreover, nano-quercetin reduced oxidative stress in a Parkinson's disease model and nano-encapsulated quercetin protected different brain regions against ischemia-reperfusion-induced neuronal damage in rats.[16,17] Also, the neuroprotective role of quercetin has been shown in many studies.[18]

Accordingly, this investigation aimed to evaluate possible oxidative neurotoxicity with low dose DZN exposure and its effects on the serotonin metabolite (5-HIAA) and neurobehavior in the short term, as well as the protective effect of quercetin pegylated liposome (QPEGL).

Materials and Methods

Chemicals. Chemicals, including DZN, dimethyl sulfoxide (DMSO), quercetin, soy lecithin, cholesterol, polyethylene glycol (PEG) 4000, and thiobarbituric acid (TBA), were purchased from Sigma (St. Louis, USA), kits for glutathione peroxidase (GPx) and superoxide dismutase (SOD) assays were provided from Randox Co. (Crumlin, UK), and kit for 5-HIAA level determination was prepared from Abnova Co. (Heidelberg, Germany).

Preparation of QPEGL. The QPEGLs were prepared in the Research Laboratory of Nano-material, Faculty of Chemistry, University of Tabriz, Iran. In brief, a mixture of lecithin/cholesterol/quercetin/PEG 4000 (13:4:6:1 by weight ratios) was dissolved in a chloroform/methanol (3:1, v/v) solution and then, evaporated until being dried in a rotary evaporator under reduced pressure. The dried lipid film was sonicated in a 5.00% glucose solution and then, lyophilized under the vacuum. The empty PEGLs were prepared the same way as the QPEGL without quercetin. This product can be used directly or dissolved in saline intraperitoneally (IP).[19]

Characterization of liposomes. The particle size, polydispersity index (PDI), and zeta potential of liposome suspensions were measured using a Zetasizer Nano-ZS (Malvern Instruments Ltd., Malvern, UK). For this purpose, QPEGL suspensions were diluted (1:10) with deionized water to prevent the effects of multiple scattering.

Encapsulation efficiency (EE) and drug load capacity (DL) of QPEGL. The EE and DL of QPEGLs were assessed employing ultra-filtration and ultra-violet (UV) spectrometry with a UV-visible spectrophotometer (T60; PG Instruments Ltd., Leicestershire, UK). The regression curve of quercetin concentration *versus* absorbance at 370 nm was established. The QPEGLs were placed in ultra-filtration centrifuge tubes and then, centrifuged to separate unentrapped quercetin from QPEGLs (5,000 rpm; 15 min). Next, 1.00 mL of the free quercetin solution and 2.00 mL of methanol were combined in a new centrifuge tube. Then, the quercetin concentration was determined by the previously established regression curve, and EE and DL of QPEGLs were calculated using the following formulas:[20]

$$EE\ (\%) = (W_T - W_F) / W_T \times 100$$

$$DL\ (\%) = (W_T - W_F) / W_{L+PEG} \times 100$$

where, W_T presents the weight of quercetin in the dispersions, W_F presents free quercetin weight in the supernatant, and $W_{L+\ PEG}$ presents the total weight of the lipid ingredients and PEG 4,000.

Scanning electron microscopy (SEM). The liposomes size and morphology were observed and characterized utilizing SEM (MIRA3 FEG-SEM; Tescan, Brno, Czech Republic).

Fourier-transform infrared (FTIR) spectroscopy. To determine any interactions between bioactive functional groups in QPEGLs and the mixture of quercetin with lipid ingredients with PEG, FTIR spectroscopy was used.[21] The infrared spectra were scanned by the FTIR spectrophotometer in the frequency range between 4,000 and 400 cm^{-1} (Vertex 70, FTIR Spectrometer; Bruker, Rheinstetten, Germany) using the KBr pellet method with a 10:100 sample ratio.

Animals. A total number of 36 mature male Wistar rats (six-eight weeks), weighing 200 - 250 g, were provided from the Pasteur Institute, Karaj, Iran. The animals were kept under standard laboratory conditions with a 12-hr light: 12-hr dark cycle, at 22.00 ± 2.00 ˚C. They were fed with standard chow and tap water *ad libitum* during acclimatization and the experiments. The animal experiments were approved by the Animal Care and Use Committee of the University of Tabriz, Tabriz, Iran (Approval ID: IR.TABRIZU.REC.1400.067). After 7 days of acclimatization, animals were randomly divided into six

groups (n = 6) as follows: Normal saline group (NS) as a control group, vehicle control group that received DMSO, DZN (10.00 mg kg^{-1}), DZN (10.00 mg kg^{-1}) + quercetin (20.00 mg kg^{-1}), DZN (10.00 mg kg^{-1}) + PEGL (20.00 mg kg^{-1}), and DZN (10.00 mg kg^{-1}) + QPEGL (20.00 mg kg^{-1}). Stock solution (50.00 mM) of DZN was made in DMSO (final DMSO concentration: 0.01%) and then, diluted in normal saline to 10.00 mg kg^{-1}. Quercetin was freshly prepared in a minimal amount of DMSO (20.00 mg kg^{-1}) and the vehicle control group received an equal amount of DMSO in saline. The PEGLs and QPEGLs were diluted in normal saline to 20.00 mg kg^{-1}. Rats were treated with a single dose of normal saline and DMSO in NS and DMSO groups, and also a single dose of DZN (10.00 mg kg^{-1}; IP) in four DZN-treated groups. After 20 min of toxicity induction by DZN, animals in quercetin, PEGL, and QPEGL groups received quercetin (20.00 mg kg^{-1}; IP), PEGL (20.00 mg kg^{-1}; IP), and QPEGL (20.00 mg kg^{-1}; IP), respectively; all treatments were carried out in 1 day. Twenty-four hr after treatment, behavioral tests were performed. Animals were euthanized, and brains were instantly removed and stored at – 80.00 ˚C until use.

Open field test. The open field test was used to evaluate motor activity. The rats were placed in the center of the field, and their behavior was recorded for 10 min employing a video tracking system (Borj Sanat Co., Tehran, Iran). The motor activity of animals was determined by measuring the total distance traveled and velocity the animals moved in the apparatus during the 10 min; the field was cleaned with alcohol after each recording.

Elevated plus maze (EPM) test. Assessment of anxiety was performed employing EPM experiment. Rats at the beginning of the test were placed in a square section facing the open arm. Then, the rats were allowed to move in open and closed arms for 5 min. The EPM arms were cleaned with alcohol after each recording.

Brain tissue preparation. Brain tissues were homogenized in a 1.15% potassium chloride solution (Sigma) utilizing a glass mechanical homogenizer, transferred to the micro-tubules, and centrifuged at 4,000 rpm for 5 min. The total protein in the supernatant was determined by the Bradford method.[22] Then, the supernatant was used for oxidative parameters and 5-HIAA level analyses.

Glutathione peroxidase and SOD levels determination. Glutathione peroxidase activity was measured using the kit from Randox Company. Brain tissues SOD level was determined by a spectrophotometric method based on the inhibition of a superoxide-induced reduced nicotinamide adenine dinucleotide oxidation.[23,24]

Malondialdehyde level measurement. The brain tissue's MDA levels were determined using the TBA reactive substances method. Fifty µL of serum sample was added into a tube containing l.00 mL of distilled water. Then, 1.00 mL of a solution containing 29.00 mmol L^{-1} TBA in acetic acid was added (8.75 mmol L^{-1}; pH: 2.40 - 2.60) and mixed; samples were placed in the water bath and heated for 1 hr at 95.00 - l00 ˚C. The samples were then cooled under running cold water, 25.00 µL of hydrochloric acid (5.00 mol L^{-1}) was added, and the reaction mixture was extracted for agitation with 3.50 mL of n-butanol for 5 min. After centrifugation, the butanol phase was separated, and the butanol extract's fluorescence was measured by spectrophotometry (T60; PG Instruments Ltd., Leicestershire, UK) using 525 nm for excitation and 547 nm for emission.

Brain 5-HIAA level quantification. Brain 5-HIAA level was quantified by enzyme-linked immunosorbent assay utilizing Abnova kit. In brief, 25.00 µL of the controls, methylated standards, and samples were pipetted into the appropriate wells of the 5-HIAA µL strips, 50.00 µL of the 5-HIAA anti-serum was pipetted into the all wells, and the plate was covered by adhesive aluminum foil and incubated for 1 hr at room temperature (20.00 - 25.00 ˚C) on a shaker (≈ 600 rpm). The aluminum foil was removed and the content of the wells was discarded or aspirated. Then, the plate was washed four times by adding 300 µL of washing buffer, discarding the content, and blotting dry each time by tapping the inverted plate on the absorbent material. In the next step, 100 µL of the enzyme conjugate was pipetted into the all wells, and the plate was covered with adhesive aluminum foil and incubated for 1 hr at room temperature (20.00 - 25.00 ˚C) on a shaker (≈ 600 rpm). The foil was removed, the content of the wells was discarded or aspirated, and the plate was washed four times by adding 300.00 µL of washing buffer, discarding the content, and blotting dry each time by tapping the inverted plate on the absorbent material. In the next step, 100.00 µL of the substrate was pipetted into the all wells and incubated for 20 - 30 min at room temperature (20.00 - 25.00 ˚C) on a shaker (≈ 600 rpm). Then, 100 µL of the stop solution was added to each well and it was shaken to ensure a homogeneous distribution of the solution. Finally, the absorbance of the solution was read in the wells within 10 min, using a microplate reader set to 450 nm.

Statistical analysis. Results were expressed as mean ± standard deviation and comparison of different groups was analyzed by one-way analysis of variance with subsequent Tukey's *post hoc* tests. GraphPad Prism Software (version 9.4.0; GraphPad Inc., San Diego, USA) was used to perform all the analyses and calculations. The value of $p < 0.05$ was considered statistically significant.

Results

Characterization of QPEGLs. The SEM observation showed that final PEGLs and QPEGLs were small unilamellar liposomes with a size range of about 75.00 ± 25.00 nm, PDI value of 0.50 ± 0.20, and zeta potential of 38.50 mV. Quercetin EE and DL were 80.20 ± 9.30% and 60.00 ± 5.00%, respectively (Fig. 1).

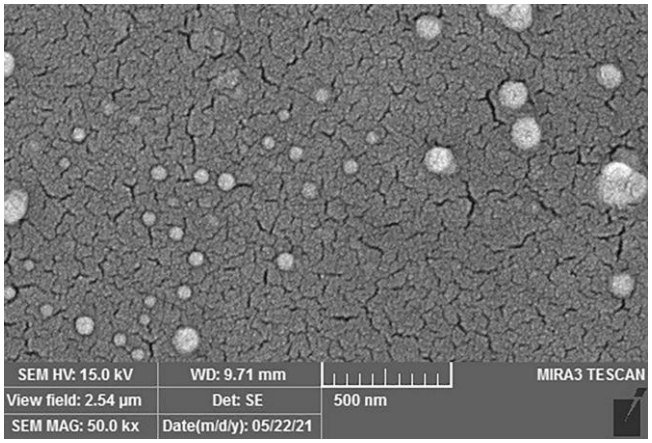

Fig. 1. Scanning electron microscopy image of quercetin pegylated liposomes.

Fourier-transform infrared spectroscopy. The FTIR spectra of QPEGLs and the mixture of liposome ingredients for functional groups determination were obtained as follows: The characteristic peaks were at 3388 cm^{-1} because of the presence of O–H, at 2924 cm^{-1} because of –CH, at 1740 cm^{-1} because of the C=O ester stretching ester, and at 1066 cm^{-1} because of the C–O ester stretching (Fig. 2).

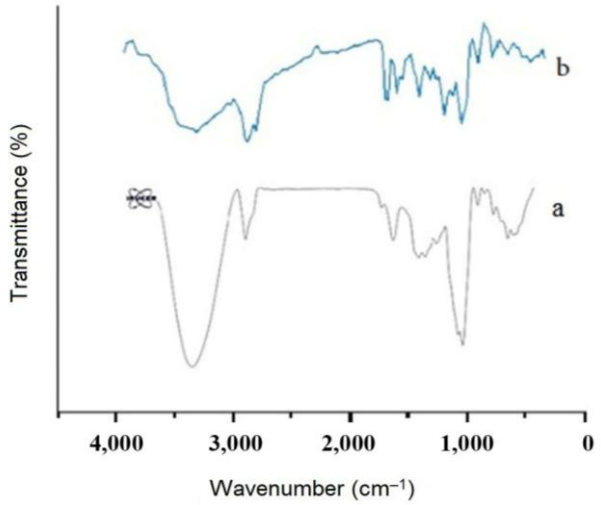

Fig. 2. The Fourier transform infrared spectroscopy spectra of quercetin pegylated liposome **(a)**, and mixture of quercetin with liposome ingredients **(b)**.

Open field findings. Variables, such as distance moved and mean velocity were considered for motor activity evaluation. The results showed that after 24 hr of exposure to DZN and subsequent treatment with quercetin, PEGL, and QPEGL distance moved and mean velocity in the DZN and PEGL groups had a significant decrease compared to the control group ($p = 0.0001$ and $p < 0.0001$, respectively); these parameters increased in the quercetin and QPEGL groups compared to the DZN group ($p > 0.05$ and $p < 0.0001$, respectively). Also, there was a significant difference between the QPEGL and quercetin groups ($p = 0.0001$ and $p = 0.0023$, respectively). There was no significant difference ($p > 0.05$) between the DZN and PEGL groups (Fig. 3).

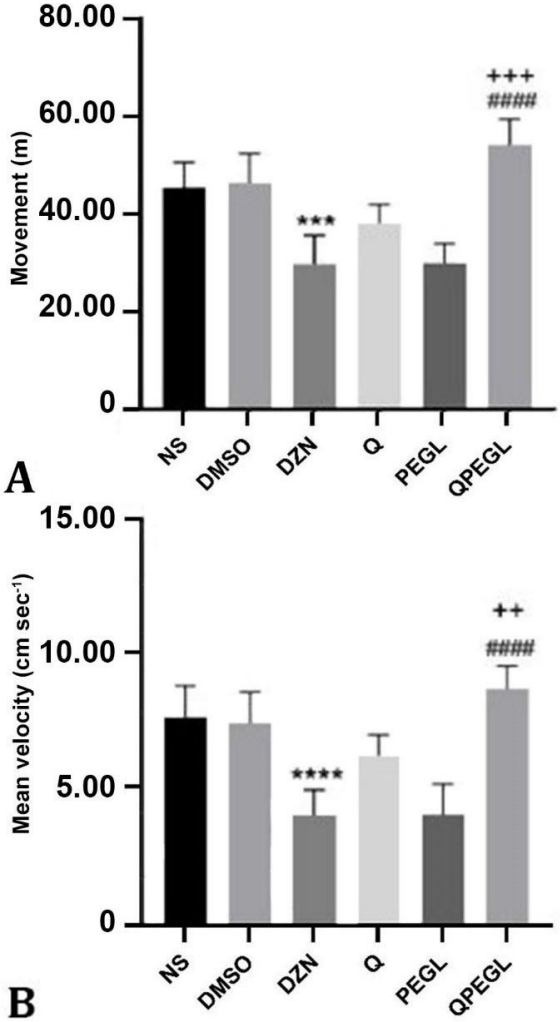

Fig. 3. A) Total movement and **B)** Mean velocity 24 hr after exposure to diazinon (DZN; 10.00 mg kg^{-1}) and treatment with quercetin (Q)/ quercetin pegylated liposome (QPEGL; 20.00 mg kg^{-1}, intra-peritoneally) in the open field test. *** $p = 0.0001$ and **** $p < 0.0001$ as compared to the control group, $^{####}$ $p < 0.0001$ as compared to the DZN group, and $^{+++}$ $p = 0.0001$ and $^{++}$ $p = 0.0023$ as compared to the Q group. NS: Normal saline; DMSO: Dimethyl sulfoxide; PEGL: Pegylated liposome (20.00 mg kg^{-1}).

Elevated plus maze findings. The percentage of total open arm entries and percent of time spent in open arms were measured as indices of anxiety. The data obtained from this experiment showed that after 24-hr exposure to DZN and treatment with quercetin, PEGL, and QPEGL, the percent of open arm entries in the DZN group significantly decreased compared to the control group ($p < 0.0001$). These parameters increased significantly in the quercetin and QPEGL groups ($p < 0.0001$) compared to the DZN group; in the QPEGL group, they increased differently

compared to the quercetin group ($p < 0.05$). The percent of total time spent in open arms was reduced notably in the DZN and PEGL groups compared to the control group ($p < 0.0001$); this parameter significantly elevated in the quercetin and QPEGL groups compared to the DZN group ($p < 0.0001$). Also, in the QPEGL group, it was significantly increased compared to the quercetin group ($p = 0.0086$). There was no significant difference ($p > 0.05$) between the DZN and PEGL groups (Fig. 4).

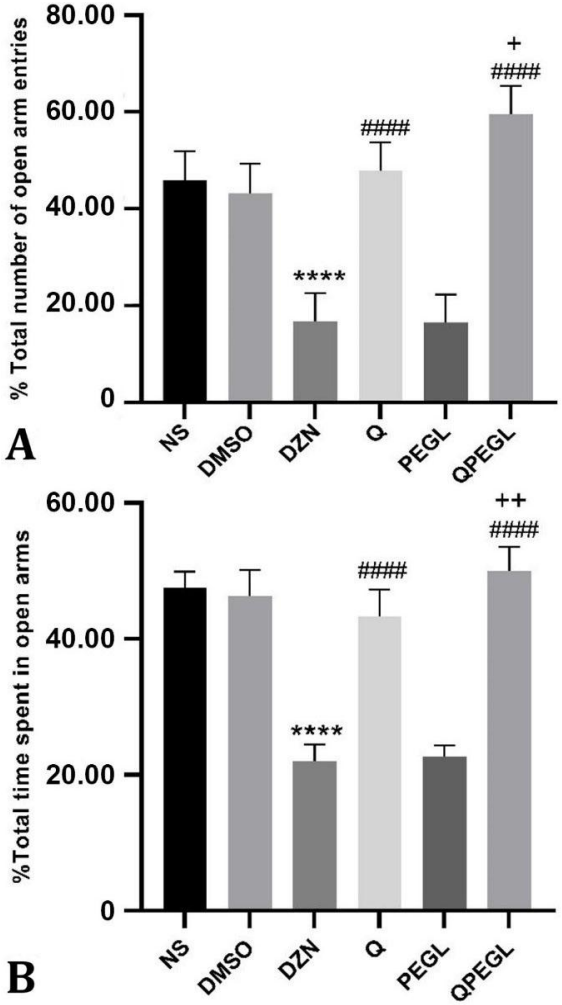

Fig. 4. The percent of open arm entries **(A)**, and percent of the time spent in open arms **(B)** 24 hr after exposure to diazinon (DZN; 10.00 mg kg^{-1}) and treatment with quercetin (Q)/ quercetin pegylated liposome (QPEGL; 20.00 mg kg^{-1}, intraperitoneally) in the elevated plus maze test. **** $p < 0.0001$ as compared to the control group, #### $p < 0.0001$ as compared to the DZN group, and + $p < 0.05$, and ++ $p = 0.0023$ as compared to the Q group. NS: Normal saline; DMSO: Dimethyl sulfoxide; PEGL: Pegylated liposome (20.00 mg kg^{-1}).

Glutathione peroxidase and SOD levels. The content of GPx and SOD in the brain tissue after 24 hr DZN exposure and treatment with PEGL and quercetin/QPEGL was as follows: GPx content in the DZN group did not differently change compared to the control group ($p > 0.05$); however, in the QPEGL group, it was significantly upgraded compared to the DZN group ($p < 0.05$). The SOD level in the DZN and QPEGL groups had no significant difference compared to the control and DZN groups, respectively ($p > 0.05$). These parameters had no significant difference ($p > 0.05$) among the DZN, PEGL, and quercetin groups (Fig. 5).

Malondialdehyde level. The MDA level of the brain tissue significantly increased in the DZN group compared to the control group ($p = 0.0001$) after 24 hr of exposure to DZN and treatment with quercetin, PEGL, and QPEGL; this oxidative stress parameter significantly decreased in the quercetin and QPEGL groups compared to the DZN group ($p = 0.0001$ and $p < 0.0001$, respectively). No significant difference ($p > 0.05$) was observed between the DZN and PEGL groups (Fig. 6A).

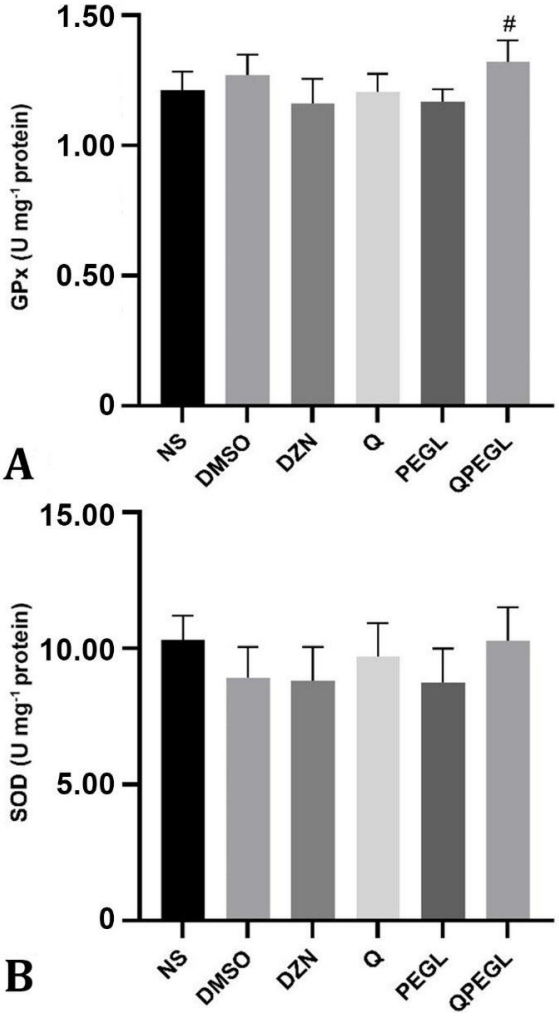

Fig. 5. The effect of quercetin (Q)/ quercetin pegylated liposome (QPEGL; 20.00 mg kg^{-1}) on glutathione peroxidase (GPx; **A**) and superoxide dismutase (SOD; **B**) levels of the brain after 24 hr exposure to diazinon (DZN; 10.00 mg kg^{-1}) and treatment with Q/QPEGL. # $p < 0.05$ as compared to the DZN group. NS: Normal saline; DMSO: Dimethyl sulfoxide; PEGL: Pegylated liposome (20.00 mg kg^{-1}).

5-hydroxyindoleacetic acid level. The content of 5-HIAA in the brain tissue in DZN group was notably raised *versus* the control group ($p < 0.0001$), and in quercetin and QPEGL groups significantly decreased compared to DZN group ($p = 0.0002$ and $p < 0.0001$, respectively) after 24 hr of DZN exposure and treatment with PEGL, quercetin, and QPEGL. There was no significant difference ($p > 0.05$) between the DZN and PEGL groups (Fig. 6B).

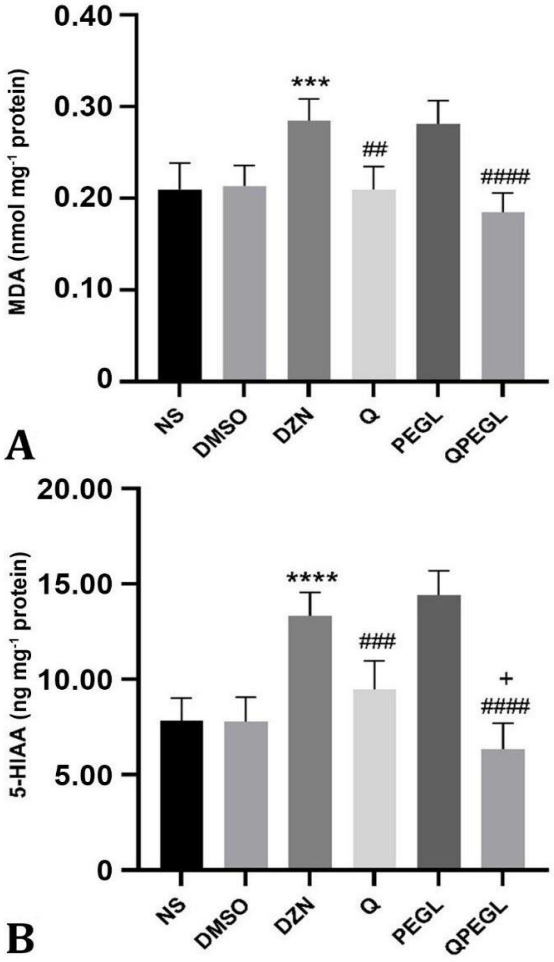

Fig. 6. The effect of quercetin (Q)/ quercetin pegylated liposome (QPEGL; 20.00 mg/kg) on **A)** malondialdehyde (MDA) content and **B)** 5-hydroxyindoleacetic acid (5-HIAA) level of the brain after 24 hr exposure to diazinon (DZN; 10.00 mg kg^{-1}) and treatment with Q/QPEGL. *** $p = 0.0001$ as compared to the control group, **** $p < 0.0001$ as compared to the control group, ## $p = 0.0001$ as compared to the DZN group, ###$p = 0.0002$ as compared to the DZN group, #### $p < 0.0001$ as compared to the DZN group, ### $p < 0.0001$ as compared to the DZN group, and + $p < 0.05$ as compared to the Q group.NS: Normal saline; DMSO: Dimethyl sulfoxide; PEGL: Pegylated liposome (20.00 mg kg^{-1}).

Discussion

This study aimed to investigate the protective effect of QPEGL against oxidative stress and behavioral disorders induced by low dose DZN exposure.

The results revealed four key findings, which are discussed below: First, behavioral parameters, including distance moved and mean velocity, being significantly reduced in the open field test in the DZN group compared to the control group. This finding confirms a reduction in general motor activity. In addition, the EPM analysis indicated that the percentages of open arm entries and time spent in the open arms significantly decreased in the DZN group compared to the control group at low doses. These observations highlight the behavioral effects of acute low dose DZN exposure.

Previous studies have established a link between DZN exposure and behavioral deficits. Psychiatric disorders, including mental health issues, depression, and decreased motor activity, have been reported among sheep farmers and in a zebrafish model exposed to low levels of OPs.[1,3,25]

Second, the results demonstrated markedly elevated MDA levels in the DZN group compared to the control group. However, there was no significant difference regarding the content of anti-oxidative capacity markers, such as GPx and SOD, between the DZN and control groups. Our observations indicated an increase in lipid peroxidation in the brain without a corresponding change in the anti-oxidant enzyme levels in the short term. This suggests that compensatory mechanisms may be at play to maintain anti-oxidant capacity during acute DZN exposure, even at low doses. The heightened sensitivity of the brain to increased lipid peroxidation acts as an urgent alarm mechanism to preserve its full anti-oxidant potential. Previous research has also shown compensatory mechanisms for anti-oxidant enzymes during acute exposure to a high dose of DZN (335 mg kg^{-1}).[26]

It has been reported that chronic, sub-chronic, and acute exposures to DZN induce oxidative stress in various tissues and cells, including the liver, sperm, heart, skeletal muscle, and kidney.[5,6,27]

Third, in DZN group, the serotonin metabolite 5-HIAA was significantly elevated compared to the control group, indicating neurobehavioral disorders observed during the initial phase of the study. The levels of 5-HIAA reflect serotonin levels, playing a crucial role in behavioral changes.

The mechanism of acute low dose DZN exposure effect on the serotonergic system in mature rats has been investigated.[10] Reportedly, it has been shown that low doses of DZN can reduce the expression of the serotonin receptor 5-HT1A, leading to cognitive and emotional deficiencies, such as depression.[28]

Fourth, in our study, QPEGL significantly improved behavioral, oxidative stress, and biochemical parameters, confirming the effective role of anti-oxidants in reducing monoamine oxidase activity. This reduction led to decreased levels of 5-HIAA and improved neurobehavioral disorders. Because of its pegylated nano-liposome structure, QPEGL exhibited a greater anti-oxidant effect than free quercetin at the same dosage. Quercetin has been

shown to alleviate neurobehavioral deficiencies, such as anxiety, *via* reducing serotonin metabolism in a rat model of Huntington's disease.[12]

Quercetin possesses high anti-oxidant potential compared to other flavonoids, attributed to its multiple hydroxyl groups that can bind to reactive oxygen species.[29] The anti-oxidant properties of quercetin were further demonstrated in studies investigating its effects alongside *ginkgo biloba* on hydrogen peroxide-induced oxidative stress in neurons.[30] Additionally, it has been observed that quercetin reduces oxidative metabolism in a dose-dependent manner, with decreases in catalase and SOD enzymes levels following administration.[31]

The neuroprotective effect of quercetin on the cognitive impairment induced by d-galactose has been shown and it has been revealed that quercetin elevates the GAP43 mRNA expression in the brain to regenerate the normal function of neurons against the cellular injury caused by d-galactose.[32] Recent findings also indicate that quercetin exhibits a synergistic effect with regular exercise in improving spatial memory in an animal model of Alzheimer's disease.[33]

It has been reported that quercetin inhibits the monoamine oxidase activity because of its strong anti-oxidant potential. The monoamine oxidase forms 5-HIAA, being responsible for monoamine deamination as serotonin. Clinical data show that the major depression symptoms of patients are reflected in changes in brain monoamine neurotransmitters, particularly serotonin.[34] Due to the quercetin poor solubility, low bioavailability, and limited ability to cross the blood-brain barrier, researchers have developed quercetin nano-particles, including liposome formulations. The effects of active herbal ingredients formulated as nano-particles on the blood-brain barrier have been studied.[35] Pegylation of liposomes is a key method used to improve the solubility of quercetin liposomes, taking advantage of the hydrophilic properties of PEG. This process also prolongs the half-life of liposomes in the bloodstream, facilitating a slower release and increasing the chances of accumulation in target tissues. Moreover, PEG is an inert and biocompatible polymer as confirmed in this study.[36] In conclusion, our research showed that low levels of DZN can acutely lead to lipid peroxidation in the brain and raise 5-HIAA levels without significant changes in the anti-oxidative content. This indicates that the brain employs compensatory mechanisms to maintain its anti-oxidative capacity, underscoring the high sensitivity of the serotonergic system to lipid peroxidation during acute exposure. These changes immediately impact the serotonin metabolite (5-HIAA) and may lead to sub-sequent neurobehavioral disorders. Furthermore, it was found that QPEGL demonstrated superior anti-oxidant properties compared to the free quercetin at the same dose, due to its more effective role in drug delivery.

Acknowledgments

Not applicable.

Conflict of interest

There is not any conflict of interest for authors in this study.

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