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ORIGINAL PAPER

Melatonin Protects Against Diazinon-Induced Neurobehavioral Changes in Rats

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Abstract Diazinon is an organophosphorous pesticide with a prominent toxicity on many body organs. Multiple mechanisms contribute to diazinon-induced deleterious effects. Inhibition of acetyl-cholinesterase, cholinergic hyperstimulation, and formation of reactive oxygen species may play a role. On the other hand, melatonin is a pineal hormone with a well-known potent antioxidant activity and a remarkable modulatory effect on many behavioral processes. The present study revealed that oral diazinon administration (25 mg/kg) increased anxiety behavior in rats subjected to elevated plus maze and open-field tests possibly via the induction of changes in brain monoamines levels (dopamine, norepinephrine, and serotonin). Additionally, brain lipid peroxides measured as malondialdehyde (MDA) and tumor necrosis factor alpha (TNF-α) levels were elevated, while the activity of brain glutathione peroxidase enzyme was reduced by diazinon. Co-administration of oral melatonin (10 mg/kg) significantly attenuated the anxiogenic activity of diazinon, rebalanced brain monoamines levels, decreased brain MDA and TNF-α levels, and increased the activity of brain glutathione peroxidase enzyme.

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Introduction

Anxiety-like behavior and emotional disturbances were commonly reported following exposure to organophosphorous compounds [1, 2]. It seems that the lipophilic character of organophosphorous pesticides facilitates their penetration through the cell membrane to induce alterations in membrane phospholipids, production of reactive oxygen species and generation of oxidative stress in different tissues [3–6].

Diazinon is a synthetic organophosphorous compound with a broad-spectrum insecticidal activity [3]. The main mechanism of action of diazinon is acetyl-cholinesterase enzyme inhibition [1], however, it may induce imbalance in the free radicals production/elimination processes with consequent induction of cellular damage [1–4]. Several experimental and clinical studies have reported diazinon-induced toxicities on several organs [3–6]. Emotional changes, aggression, cognitive impairment and neurotoxicity were observed following exposure to diazinon and other organophosphorous compounds [7–9].

Multiple approaches have been investigated in order to attenuate the toxicity of organophosphorous compounds, including antioxidants such as *N*-acetyl cysteine, selenium, vitamins C and E, in addition to natural compounds such as crocin and safranal [10–12]. Nevertheless, the need for a powerful intervention against toxicity by organophosphorous compounds remains persistent.

On the other hand, melatonin is a hormone biosynthesized from L-tryptophan and secreted from the pineal body

in the brain [13]. In addition to its pivotal role in regulation of circadian rhythm and sleep [14], melatonin is a well-known potent antioxidant [15]. Melatonin has the advantage of being soluble in lipids and water, a unique character that enhances its cellular distribution and enables it to cross the brain-blood barrier and enter the central nervous system [13]. In various neurological disorders, melatonin displayed psychotropic activities including sedative effects [16–18]. The ability of melatonin to reduce the deleterious effects of some neurotoxins such as acrylamide, D-amphetamine, and cisplatin, has been reported [19–21].

It seems that the possible efficacy of melatonin to attenuate diazinon-induced brain toxicity has not been studied yet. Accordingly, this study was performed in order to evaluate the anxiogenic activity of diazinon, and to elucidate the potential protective effects of melatonin against diazinon-induced behavioral, oxidative and neurochemical changes in rats.

Materials and Methods

Chemicals and Drugs

Melatonin and diazinon were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). All other chemicals were of the highest analytical grade.

Experimental Design and Preparation of Samples

Male Sprague–Dawley albino rats weighing 180–200 g were housed in plastic cages at a controlled temperature (25 ± 1 °C) with standard food pellets and water ad libitum. The animal experiments described later were performed in accordance with the ethical principles and guidelines for the care and use of laboratory animals adopted by the National Egyptian Community.

In order to achieve the aims of the study, rats were randomly distributed into four groups, each comprising ten rats. Rats were treated according to the following regimen; control group, in which rats received vehicle orally (rats were administered oral gavage twice, first with the vehicle for melatonin followed one hour later by the vehicle for diazinon). Rats of the second group were given oral diazinon as 25 mg/kg via oral gavage [22], whereas rats of the third group were administered oral melatonin in a dose of 10 mg/kg via oral gavage [23]. The last group of rats received melatonin followed by diazinon with a one-hour interval in-between. Treatments lasted for 15 consecutive days, whereas animals were challenged across the elevated plus maze and open-field behavioral tests at days 13 and 14 respectively. Following the last dose at day 15, animals were sacrificed by cervical dislocation; brains were quickly excised, immediately washed from blood in ice-cold saline, blotted on filter paper, and weighed. Specimens were stored at −80 °C until biochemical assays were performed.

Behavioral Tests for the Evaluation of Anxiety in Rats

Elevated Plus Maze Test

The elevated plus maze is widely used to test anxiety in animal models. Experiments were run based on the method described previously [24]. The apparatus consisted of two open arms (10 × 50) cm and two closed arms (10 × 50) cm extending from a common central platform (10 × 10) cm and at a height 50 cm apart from the floor. The brightly illuminated wooden maze had black colored walls and floor. Each individual rat was placed on the central platform at the start of the experiment with its head directed towards the open arm. Each rat was allowed 5 min to explore the apparatus freely. A video camera was used to record the behavior. An arm entry was defined by the entrance of all four legs of the animal into one of the arms. The variables measured include, latency to leave the central platform, the percentage time spent in the open arm and the percentage time spent in the closed arm.

Open-Field Test (Exploration of a Novel Mild Stressful Situation)

Open-field test, which represents a novel mild stressful condition, was used for detection of changes in exploratory behavior and emotionality [25]. The instrument comprised an open-topped box 80 cm (length) × 80 cm (width) × 40 cm (height). The sides were red in color while the floor was white colored and divided by black lines into 20 cm equal squares (4 × 4). Rats were placed individually at the center of the test box and observed carefully for a 3 min period. Behavioral parameters for exploratory activity (latency of the initial movement, ambulation frequency, the number of exploratory rearing) and emotionality (grooming frequency, urination occurrence, defecation indicated by the number of fecal pellets) were recorded for each rat.

Biochemical Tests

Determination of Brain Lipid Peroxides Level

Brain lipid peroxides were measured as malondialdehyde (MDA) level in the brain homogenate as described in previous studies [26]. The principle of the assay depends on the colorimetric determination of the pink-pigmented product resulting from the reaction of one molecule of MDA with two molecules of thiobarbituric acid at low pH (2–3) and 95 °C for 45 min. The resultant colored product

was extracted by *n*-butanol and measured at 535 nm by a spectrophotometer.

Determination of Brain Glutathione Peroxidase Activity

Glutathione peroxidase (GSH–PX) activity was measured by a ZeptoMetrix Corporation Kit, New York, USA according to the method described in previous studies [27]. Briefly, the enzymatic activity was measured in a medium containing NADPH, reduced glutathione, sodium azide, and glutathione reductase. The enzymatic reaction was initiated by the addition of hydrogen peroxide and the change in absorbance due to NADPH oxidation was monitored by a spectrophotometer at 340 nm.

Determination of Brain TNF-α Level

Tumor necrosis factor-α level (TNF-α) was determined by a rat specific ELISA kit (Thermo Scientific ELISA kit, Pierce Biotechnology, Rockford, USA) according to the method previously described in other studies [28]. Each sample was assayed in duplicate and the absorbance was calculated by subtracting the absorbance measured at 550 nm from that at 450 nm. Sample concentration of TNF-α was determined from a standard curve.

Determination of Brain Monoamines (Dopamine, Norepinephrine and Serotonin) Levels in Rats

Dopamine (DA), norepinephrine (NE), and serotonin (5HT) were estimated according to the method described in previous studies [29]. Briefly, brain tissues were weighed and homogenized in 75 % aqueous HPLC grade methanol 1:10 w/v. Homogenates were then centrifuged at 4,000×*g* for 10 min. In order to remove trace elements and lipid impurities, supernatants were immediately extracted by the use of a solid-phase extraction column (Chromabond NH2 phase, cat. No. 730031, Macherey–Nagel GmbH & Co., Germany). Prepared samples were then injected directly into a separating HPLC column (Aqua 5 µm C18, LC column 150 × 4.6 mm, Phenomenex, USA). The mobile phase was 97:3 potassium phosphate (20 mol/l, pH 3)/methanol and the flow rate was 1.5 ml/min. UV detection was performed at 270 nm. NE, DA and serotonin were separated after 12 min. The samples concentration of each monoamine was calculated by comparing the chromatogram of each sample to that of the standard.

Statistical Analysis

Values are expressed as mean ± SEM Comparisons between different groups were carried out using one way analysis of variance (ANOVA) followed by Tukey–Kramer multiple comparisons test for statistical evaluation of all data except urination occurrence percentage in the open-field behavior test, which was carried out using Chi square method. Graph-pad software Prism (v.3) was used to perform the statistical analysis.

Results

Effect of Diazinon, Melatonin, and Their Combination on the Behavioral Parameters in the Elevated Plus Maze Test in Rats

Whereas no statistically significant change in latency period between all groups were observed (Table 1), administration of diazinon (25 mg/kg) showed a significant decrease in the percentage time spent in the open arm by 57.7 %, while it significantly increased percentage time spent in the closed arm by 22.0 % as compared to the control group. Melatonin administration (10 mg/kg) significantly increased percentage time spent in the open arm by 54.7 and 266.1 % as compared to control and diazinon-treated groups respectively. On the other hand, melatonin-treated rats showed a significant decrease in the percentage time spent in the closed arm by 22.7 and 36.6 % as compared to control and diazinon-treated groups respectively (Table 1).

Co-administration of diazinon and melatonin significantly increased the percentage time spent in the open arm by 62.8 and 285.2 % as compared to control and to diazinon-treated groups respectively, while significantly decreased the percentage time spent in the closed arm by 21.3 and 35.5 % as compared to control and diazinon-treated groups respectively (Table 1).

Effect of Diazinon, Melatonin, and Their Combination on the Behavioral Parameters in the Open-Field Test in Rats

Administration of diazinon (25 mg/kg) increased rearing frequency in rats by 60.3 % as compared to control group and significantly elevated emotionality of rats in the open-field test as indicated by increased grooming frequency (150 %), defecation (no. of fecal pellets) (56.8 %) and percentage urination occurrence (50 %) versus control group (Table 2).

On the other hand, melatonin (10 mg/kg) significantly decreased ambulation and rearing frequencies of rats in the open-field test by 49.9 and 23.9 % respectively compared to control. Similarly, melatonin significantly reduced ambulation and rearing frequencies by 46.0 and 52.6 % respectively compared to diazinon-treated group (Table 2).

Table 1 Effect of diazinon (25 mg/kg, orally), melatonin (10 mg/kg, orally), and their combination on the behavioral parameters in the elevated plus maze test in rats

Groups	Latency time (s)	Percentage time spent in the open arm (%)	Percentage time spent in the closed arm (%)
Control (vehicle)	1.03 ± 0.06	14.82 ± 0.88	50.89 ± 0.94
Diazinon (25 mg/kg)	0.99 ± 0.07	6.26 ± 0.31*	62.10 ± 0.57*
Melatonin (10 mg/kg)	1.06 ± 0.08	22.93 ± 0.66*#	39.36 ± 0.48*#
Melatonin (10 mg/kg) + Diazinon (25 mg/kg)	1.06 ± 0.08	24.13 ± 0.59*#	40.06 ± 0.37*#

Data is represented as mean ± SEM (n = 10). ANOVA and Tukey's post hoc tests were used for statistical evaluation

* Significantly different from control group at $P < 0.05$

Significantly different from diazinon group at $P < 0.05$

Table 2 Effect of diazinon (25 mg/kg, orally), melatonin (10 mg/kg, orally), and their combination on the behavioral parameters in the open field test in rats

Groups	Latency time (s)	Ambulation frequency (counts/3 min)	Rearing frequency (counts/3 min)	Grooming frequency (counts/3 min)	Defecation (no. of pellets)	Urination occurrence (%)
Control (vehicle)	2.00 ± 0.21	39.10 ± 1.47	12.10 ± 0.28	2.20 ± 0.25	3.70 ± 0.37	60
Diazinon (25 mg/kg)	1.50 ± 0.17	36.30 ± 1.06	19.40 ± 0.45*	5.50 ± 0.34*	5.80 ± 0.29*	90*
Melatonin (10 mg/kg)	2.30 ± 0.26	19.60 ± 0.65*#	9.20 ± 0.39*#	2.80 ± 0.29#	3.60 ± 0.31#	50#
Melatonin (10 mg/kg) + Diazinon (25 mg/kg)	2.30 ± 0.21	15.90 ± 0.66*#	13.20 ± 0.33#	3.20 ± 0.32#	3.20 ± 0.36#	50#

Data is represented as mean ± SEM (n = 10). ANOVA and Tukey's post hoc tests were used for statistical evaluation of all data except for the urination occurrence percentage parameter, which was carried out using Chi square method

* Significantly different from control group at $P < 0.05$

Significantly different from diazinon group at $P < 0.05$

Administration of melatonin (10 mg/kg) significantly lowered the emotionality of rats in the open-field test indicated by reduction of grooming frequency (49.1 %), defecation (37.9 %) and percentage urination occurrence (44.4 %) in comparison to diazinon-treated group (Table 2).

Interestingly, co-administration of diazinon and melatonin reduced the exploratory activity of rats in the open-field test as evidenced by significant reduction of the ambulation frequency of rats by 59.3 and 56.2 % as compared to control and diazinon-treated groups respectively. Similarly, co-administration of diazinon and melatonin significantly lowered rearing frequency of rats by 31.9 % as compared to diazinon group (Table 2).

Emotionality of rats in the open-field test was significantly reduced by co-administration of melatonin and diazinon, where a significant decrease was observed in the grooming frequency (41.8 %), defecation (44.8 %) and percentage urination occurrence (44.4 %) as compared to diazinon-treated group (Table 2).

Effect of Diazinon, Melatonin, and Their Combination on Brain Malondialdehyde (MDA) Level, Glutathione Peroxidase Activity (GSH-PX), and Tumor Necrosis Factor Alpha (TNF-α) Level in Rats

Oral administration of diazinon (25 mg/kg) significantly increased brain lipid peroxides measured as MDA level and brain tumor necrosis factor alpha (TNF-α) levels by 107.8 and 158.2 % respectively as compared to control group. On the other hand, oral administration of melatonin (10 mg/kg) induced a significant decrease in brain MDA level observed as 20.6 and 61.8 % versus control and diazinon groups respectively, and a significant reduction (70.8 %) in brain TNF-α level in comparison to diazinon group. Administration of melatonin to diazinon-treated rats decreased brain MDA and TNF-α levels by a 43.5 and a 39.9 % respectively as compared to diazinon group (Figs. 1, 3). On the other hand, diazinon administration to rats inhibited brain glutathione peroxidase (GSH-PX) activity by 38.8 % as compared to control, while melatonin administration enhanced brain GSH-PX activity by 21.5 and 98.5 % as compared to

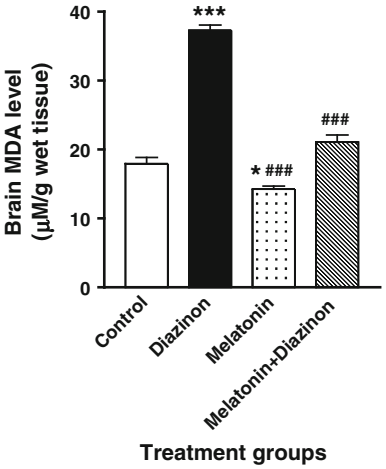

Fig. 1 Effect of diazinon, melatonin, and their combination on brain MDA level in rats. Rats were treated with diazinon (25 mg/kg, orally), melatonin (10 mg/kg, orally), or their combination for 15 successive days. Data is represented as mean ± SEM (n = 10). *, ***Significantly different from control group at $P < 0.05$ and $P < 0.001$ respectively. ###Significantly different from diazinon group at $P < 0.001$. ANOVA and Tukey's post hoc tests were used for statistical evaluation. Diazinon significantly increased brain MDA level as compared to control, whereas brain MDA level was significantly reduced by melatonin as compared to control or diazinon groups. Co-administration of melatonin and diazinon significantly reduced MDA level as compared to diazinon group

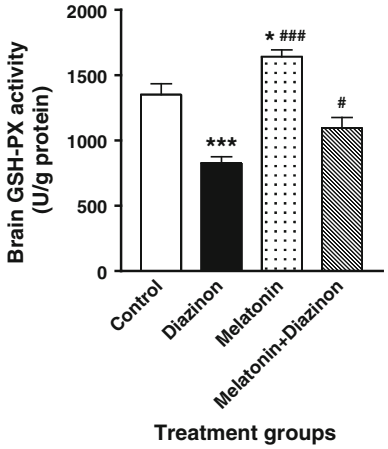

Fig. 2 Effect of diazinon, melatonin, and their combination on brain glutathione peroxidase activity (GSH-PX) in rats. Rats were treated with diazinon (25 mg/kg, orally), melatonin (10 mg/kg, orally), or their combination for 15 successive days. Data is represented as mean ± SEM (n = 10). *, ***Significantly different from control group at $P < 0.05$ and $P < 0.001$ respectively. #,###Significantly different from diazinon group at $P < 0.05$ and $P < 0.001$ respectively. ANOVA and Tukey's post hoc tests were used for statistical evaluation. Administration of diazinon significantly reduced brain GSH-PX activity in rats versus control, while melatonin administration significantly increased brain GSH-PX activity versus control or diazinon groups. Co-administration of diazinon and melatonin showed a significant increase in brain GSH-PX activity versus diazinon group

control and diazinon groups respectively. Co-administration of melatonin and diazinon enhanced brain GSH-PX activity by 32.2 % versus diazinon group (Fig. 2).

Effect of Diazinon, Melatonin, and Their Combination on Brain Dopamine, Norepinephrine and Serotonin Levels in Rats

Administration of diazinon (25 mg/kg) significantly increased brain dopamine and norepinephrine levels in rats by 11.6 and 14.7 % respectively while it significantly decreased brain serotonin level by 9.7 % as compared to control group. On the other hand, melatonin-treated group (10 mg/kg) showed a significant decrease in brain dopamine and nor-epinephrine levels by 14.6 and 14.8 % respectively and a significant elevation by 14.7 % in brain serotonin level in comparison to diazinon group. Co-administration of melatonin and diazinon showed significant reduction of brain dopamine and norepinephrine levels by 12.7 and 9.3 % respectively and a significant increase in brain serotonin level by 7.3 % as compared to diazinon group (Fig. 4).

Discussion

Diazinon, the organophosphorous pesticide, was reported to cause toxic effects in man [30, 31]. In the present study,

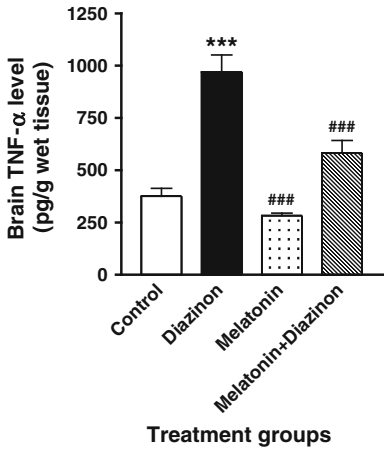

Fig. 3 Effect of diazinon, melatonin, and their combination on brain TNF-α level in rats. Rats were treated with diazinon (25 mg/kg, orally), melatonin (10 mg/kg, orally), or their combination for 15 successive days. Data is represented as mean ± SEM (n = 10). ***Significantly different from control group at $P < 0.001$. ###Significantly different from diazinon group at $P < 0.001$. ANOVA and Tukey's post hoc tests were used for statistical evaluation. A significant increase in brain TNF-α level was induced by diazinon as compared to control, whereas melatonin administration significantly reduced brain TNF-α level as compared to diazinon group. Co-administration of melatonin and diazinon significantly reduced TNF-α level as compared to diazinon group

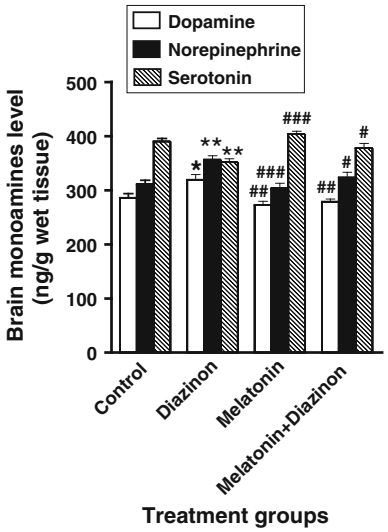

Fig. 4 Effect of diazinon, melatonin, and their combination on brain dopamine, norepinephrine, and serotonin levels in rats. Rats were treated with diazinon (25 mg/kg, orally), melatonin (10 mg/kg, orally), or their combination for 15 successive days. Data is represented as mean ± SEM (n = 10). *, **Significantly different from control group at $P < 0.05$ and $P < 0.01$ respectively. #, ##, ###Significantly different from diazinon group at $P < 0.05$, $P < 0.01$ and $P < 0.001$ respectively. ANOVA and Tukey's post hoc tests were used for statistical evaluation. Administration of diazinon to rats increased brain dopamine and norepinephrine levels while it decreased brain serotonin level as compared to control. On the other hand, melatonin administration significantly decreased brain dopamine and norepinephrine while it increased brain serotonin levels as compared to diazinon group. Co-administration of melatonin and diazinon to rats significantly reduced brain dopamine and norepinephrine and increased brain serotonin levels as compared to diazinon group

diazinon showed anxiogenic activity in rats accompanied with brain injury. Exposure to diazinon and other organophosphorous compounds was formerly reported to be associated with oxidative stress and disruption of the antioxidant defense mechanisms [32, 33]. Unfortunately, brain is highly sensitive to oxidative injury due to its high content of polyunsaturated fatty acids, which can be readily transformed into peroxides. Additionally, brain utilizes excessive amounts of oxygen and contains relatively low levels of antioxidant enzymes [34].

The ability of diazinon to induce anxiety in rats in this study was observed as increased time spent in the closed arm of the elevated plus maze, in addition to enhanced exploratory activity and elevated emotionality in the open-field test. These findings were parallel to previous reports about the association of organophosphorous pesticides exposure with psychological disturbances [35, 36]. In this study, co-administration of melatonin to diazinon-treated rats significantly reduced the observed anxiety behavior, evidenced by a significant increase in the time spent in the open arm of the elevated plus maze test and reduction in the exploratory activity and emotionality of rats in the open-field test. In the same sense, melatonin was reported to induce sedative effects against other anxiogenic compounds [18, 20].

The induced anxiety by organophosphorous pesticides was attributed to the inhibition of acetyl cholinesterase enzyme and accumulation of acetylcholine [33, 37]. Secondary neuronal damage and neuropsychiatric disorders such as anxiety and memory impairment may result from cholinergic neuronal overstimulation, generation of massive amounts of free radicals, cellular toxicity and inflammation [38]. Oxidative stress can produce neuronal injury either directly or via induction of excitatory amino acids release [39–41].

The observed elevation in brain MDA level and inhibition of brain glutathione peroxidase activity in rats treated with diazinon in the current study may be attributed to diazinon-induced oxidative stress. Free radicals were reported to cause lipid peroxidation and MDA production [42]. Cellular accumulation of lipid peroxides was believed to be a major cause of cell function loss under oxidative stress conditions. Therefore, it can be assumed that diazinon treatment may lead to peroxidation of polyunsaturated fatty acids, degradation of phospholipids and cellular deterioration [43]. Similarly, previous studies reported reduction in glutathione peroxidase activity and elevation in oxidative injury and MDA production in rats subjected to diazinon and other organophosphorous compounds [44–47]. Glutathione peroxidase helps in the maintenance of cell membrane integrity and prevents lipid peroxidation of cellular membranes by removing peroxides. This enzyme converts hydrogen peroxide into water using reduced glutathione (GSH). Oxidized glutathione (GSSG) is again converted into GSH by glutathione reductase enzyme and NADPH [48, 49]. Diazinon was reported to exert a direct inhibitory activity on glutathione peroxidase enzyme [50].

In the present study, administration of melatonin reversed the deleterious effects of diazinon on brain MDA level and glutathione peroxidase activity in rats. Melatonin is an endogenous neurohormone, which is appreciated for its powerful ability to scavenge free radicals and prevent tissue damage [51]. Previous studies showed that other antioxidants such as vitamins C and E could protect against the deleterious effects of organophosphorous compounds by scavenging free radicals [52, 53]. Interestingly, melatonin was found to possess higher antioxidant potency than the powerful natural antioxidants vitamin E and glutathione [54]. Melatonin was believed to exert its protective effects against neuronal degeneration by decreasing reactive oxygen species and lipid peroxides formation [55, 56]. It was added that melatonin might bind to, cause allosteric modulation of GABAA receptors, and enhance GABAergic inhibitory transmission [57].The antioxidant capacity of

melatonin was attributed to its ability to reduce the mRNA expression of various inflammatory cytokines involved in oxidative and nitrosative stress [54], in addition to the up-regulation of antioxidant enzymes expression [16].

In this study, diazinon administration elevated TNF-α brain level, which is a crucial marker of inflammation. Notably, diazinon and other organophosphorous pesticides could elicit inflammatory responses and increase TNF-α in the brain and other organs in experimental animals via induction of TNF-α mRNA expression [12, 58]. Though the inhibition of acetyl cholinesterase enzyme and over-stimulation of cholinergic receptors may lead to neurotoxicity and inflammation [38], diazinon-induced neuronal apoptosis and mortality were found to be independent from its anticholinesterase activity [59].

The reduction in brain TNF-α level observed in rats that received both melatonin and diazinon in this study, is in harmony with previously reported in vitro and in vivo abilities of antioxidants to reduce TNF-α [60–62]. Melatonin attenuated brain oxidative stress, TNF-α and apoptosis [51, 56, 63]. The inhibitory activity of melatonin on TNF-α may be mediated through its antioxidant potency [55], increase in mRNA expression of antioxidant enzymes or inhibition of TNF-α mRNA expression [16].

In the present study, diazinon elevated brain dopamine and norepinephrine levels in rats. Increases in the genes coding for monoamine synthesis and storage were previously observed in diazinon-administered rats without parallel increase in the activities of monoamines degradation enzymes [64]. Likewise, a significant increase in the locomotor activity of rats following cholinergic stimulation was observed and attributed to the stimulation of striatal dopamine release [65, 66].

Diazinon-induced changes in brain dopamine and norepinephrine levels in rats were reversed by melatonin. The present findings were in harmony with previous ones that revealed the ability of melatonin to reduce dopamine levels in multiple regions of rat brain [67, 68]. Melatonin and dopamine antagonized each other, whereas elevated levels of one induced low levels of the other [69]. Interestingly, melatonin enhanced the degeneration of dopaminergic neurons in a Parkinsonism model in rats and inhibited nicotine-induced dopamine release [70, 71].

The current study revealed that brain serotonin levels in rats were reduced by diazinon administration. Serotonin plays a crucial role as a melatonin precursor, and in the regulation of some mental functions, such as memory and learning [72]. Serotonin level in the brain is controlled by the cholinergic system, whereas inhibition of acetyl cholinesterase enzyme may affect the levels of serotonin and other neurotransmitters, in order to compensate or to adapt the increase in acetylcholine, leading finally to functional recovery of the animal [73]. However, the changes in neurotransmitters level following organophosphorous pesticides exposure may induce some behavioral changes [74]. Diazinon was reported to reduce serotonin receptors expression [2], while neonatal exposure to diazinon resulted in serotoninergic neuronal degeneration [75, 76]. Similarly, Organophosphorous compounds may decrease the synthesis and release of serotonin and its metabolites in multiple brain regions [76–78]. On the other hand, activation of brain serotoninergic system and depletion in serotonin levels were induced by acephate consequent to accumulation of acetylcholine and cholinergic excitation in rats [79, 80].

The increase in brain serotonin level observed on the administration of melatonin to diazinon-treated rats in the present study may be attributed to melatonin-induced modulation of serotonin synthesis, release and uptake [81, 82]. Interestingly, increased serotonin levels were correlated with decreased TNF-α production, as was observed with melatonin [83]. Likewise, melatonin was reported to attenuate amphetamine-induced oxidative stress and reduction in brain serotonin level in rats [84].

Conclusion and Recommendations

The present study revealed that diazinon administration induced anxiety in rats and elicited brain neurotoxicity marked by increase in brain lipid peroxides and TNF-α levels, reduction in brain glutathione peroxidase activity, and imbalance in brain monoamines levels. Melatonin, on the other hand, showed anxiolytic and neuroprotective activities against diazinon-induced deleterious changes by virtue of its potent antioxidant and anti-inflammatory properties, in addition to its ability to modulate the levels of brain monoamine neurotransmitters. Future studies are recommended to explore the clinical effectiveness of melatonin against diazinon-induced neurobehavioral toxicities in human.

Conflict of interest The authors declare that they have no conflict of interest and that they have contributed equally in every part of this work.

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