Effect of cyanocobalamin (vitamin B12) on paraquat-induced brain injury in mice

Marzieh Jafari Fakhrabad 1, Mohammad Moshiri 2, 3, Fatemeh Ariakia 4, Vahid Reza Askari 5, 6, 7, Zahra Salmasi 8, 9, Leila Etemad 4, 10*

1 Department of Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
2 Medical Toxicology Research Center, School of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
3 Department of Clinical Toxicology, Imam Reza Hospital, Mashhad University of Medical Sciences, Mashhad, Iran
4 Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
5 Applied Biomedical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
6 Department of Pharmaceutical Sciences in Persian Medicine, School of Persian and Complementary Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
7 Department of Persian Medicine, School of Persian and Complementary Medicine, Mashhad University of Medical Sciences, Mashhad, Iran
8 Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
9 Department of Pharmaceutical Nanotechnology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
10 Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

Abstract
Objective(s): The goal of this study was to evaluate the neuroprotective effects of vit B12 on paraquat-induced neurotoxicity.

Materials and Methods: Thirty-six male mice were randomly divided into six groups. Three groups were treated intraperitoneally with paraquat (10 mg/kg) twice a week (with a 3-day interval) for 3 weeks. Normal saline, vit B12 (1 mg/kg), or vit C (50 mg/kg) was injected 30 min before paraquat administration. Other groups only received normal saline (control), vit B12, or vit C in the same protocol. Motor performance and coordination were assayed by challenging beam traversal, pole, open field, and rotorod tests. The hippocampus and serum samples were isolated to evaluate the oxidative stress (GSH and ROS), apoptosis (caspase 3), and inflammatory markers (TNF-α and IL-1β).

Results: Administration of paraquat leads to induction of motor deficits, which were improved by treatment with vit B12. In addition, vit B12 could prevent oxidative damage, apoptosis, and inflammation caused by paraquat.

Conclusion: It seems that vit B12 could be a novel therapeutic agent in the management of paraquat induced-neurotoxicity.

Introduction
Paraquat (PQ) dichloride (1,1-dimethyl-4,4-bipyridylium dichloride) is a herbicide with specific use in the control of weeds and grass(1). Accidental or intentional PQ poisoning is considered a major health problem (1, 2) ranging from impairment in the lung, gastrointestinal tract, pancreas, kidney, liver, and heart to the brain(1, 4-6). The lung is the main target organ in PQ poisoning, and respiratory failure is the most common cause of death. The clinical manifestation of PQ acute poisoning is usually dose-dependent, and classified into three stages: mild, moderate, and fulminant (1, 7-9). Epidemiological studies indicate that chronic exposure to PQ can be a risk factor for cognitive decline and to PQ (13, 17-19). PQ can gradually pass the blood-brain barrier (BBB) by neutral amino acid transporters and reach neurotoxic levels, which in turn promotes neurological complications (20). PQ induces excessive generation of reactive oxygen species (ROS) and nitric oxide, which can lead to cellular damage through activation of inflammatory factors and NF-kB, lipid peroxidation, mitochondrial dysfunction, and apoptosis (21, 22). Up to now, no medication has been approved by the U.S. Food and Drug Administration (FDA) for PQ poisoning and treatment.

Cobalamin (vit B12), plays a pivotal role in metabolic processes and is required for optimal function of the CNS and production of red blood cells (23). In mammalian cells, the activity of two important enzymes, methionine synthase (MS) and methyl malonyl CoA mutase (MCM) is dependent on vit B12. Vit B12 deficiency is associated with reduction of enzyme activity and therefore decreased synthesis of phospholipids and myelin and increased
neurological damage (24-28). Vit B12 deficiency along with raised homocysteine and methylmalonic acid levels have been observed in patients with Alzheimer’s and PD (26, 27, 29, 30). It reduces or inhibits the synthesis of inflammatory mediators such as TNF-α, IL-6, and hs-CRP and increases the antioxidant enzymes such as SOD (superoxide dismutase), CAT(catalase), and GPx (glutathione peroxidase)(31-34). Studies have shown that B vitamins can prevent the nervous system dysfunction through scavenging the ROS, especially superoxide radicals, and reduction of inflammatory markers (35, 36). Vit B12 can penetrate the BBB and exerts its neuroprotective effects in neurodegenerative diseases (37, 38). It has a functional role in the peripheral nervous system repairing and reducing the destruction of nerve cells as well as maintaining the balance between neurotoxic and neurotrophic factors in the region of the hippocampus and cerebral cortex (30, 39, 40). This study aimed to investigate the neuroprotective effects of vit B12 on PQ neurological complications by inspecting changes in behavioral responses, inflammatory mediators, the oxidant-antioxidant system as well as apoposis markers.

Materials and Methods

Reagents

PQ (active ingredient content of 200 g/L Arad chem, China), vit B12(Excir company, Iran), vit C (Latif Company, Iran), Ellman’s reagent (DTNB, Sigma Aldrich, German), 2,7-dichloro dihydro fluorescein diacetate (DCFH-DA, Sigma), TNF-α(mouse) ELISA kit (88-7324- Invitrogen, Inc), IL-1β(mouse) ELISA kit (88-7013-Invitrogen, Inc), caspase-3(Cell Signaling, #9665), horseradish-peroxidase conjugated anti-rabbit antibody (Cell Signaling, #7074).

Animal treatments

Male albino mice (aged 7 weeks, weighing 25-30 gr) were purchased from the animal room of the School of Pharmacy, Mashhad University of Medical Sciences (Mashhad, Iran). All animals were kept at constant room temperature (21±2 °C) under a 12/12 hr light/dark cycle at least 10 days prior to the test. 36 male mice were randomly divided into six groups of six each. Three groups were treated intraperitoneally with PQ (10 mg/kg) twice a week (with a 3-day interval) for 3 weeks (41). Normal saline, vit B12 (1 mg/kg), or vit C (50mg/kg) was injected 30 min before PQ administration. Other groups received normal saline alone (control) or plus vit B12 or vit C, in the same protocol.

The body weight changes of animals were evaluated every day of treatment during the experiment. Behavioral tests were performed three days after the last injection. At the end of the procedure, the animals were sacrificed and the midbrain was also removed and stored at -80 °C for further analysis.

Motor function assessment

Challenging beam traversal

Briefly, the apparatus was made of a Plexiglas beam consisting of four segments (25 cm each, 1 m total length), the width of each section was different from the widest to the narrowest section (3.5 cm to 0.5 cm by 1 cm increments). Animals were placed on the beam and allowed to traverse the beam from the widest to the narrowest end, which leads directly into the home cage. On the day of the test, a metal mesh grid (1 cm²) corresponding to width of each segment was placed on top of the beam. Animals of each group were individually videotaped while traversing the metal grid-surfaced beam for a total of 5 trials, the error rate per step, number of steps, and time to traverse the beam were assessed. The experiment was performed from 7:00 AM to 9:00 AM.

Pole test

The pole test is a simple behavior test used to assess motor performance and coordination. In summary, mice were placed head up on the top of a vertical wooden pole apparatus of 50 cm height (diameter: 1cm). Animals were habituated for 2 consecutive days before the experiment. The time to orient down (T-Turn), time for arriving at the 25 cm points (T-25cm), and climb down (T-Total) were recorded. The test was performed from 10:00 AM to 12:00 PM.

Open field test

The open-field test is used to measure locomotor activity, anxiety, and exploratory behavior. The open field apparatus consisted of white wood 60×60 cm with a 40 cm high wall, the floor was divided by blue lines into 25 squares. The mice were trained one day before the test for 5 min. Briefly, each mouse was individually placed in the center of the open field, and locomotor activities were evaluated for 5 min. The behavioral parameters registered were the total number of squares crossed (frequency with which the mouse crosses the squares with all four paws), the number of central squares crossed, and the number of outer squares crossed; the three measures were referred to as total, central, and peripheral locomotion, respectively. The floor was cleaned between each mouse with 20% ethanol. The test was performed from 1:00 PM to 3:00 PM.

Rotarod test

Motor coordination and balance were assessed using the rotarod test. The rotarod apparatus consists of a metal rod coated with rubber rotating on an axis. Briefly, mice were trained for one day on a rod rotating at the initial and final speeds of 10 and 20 rpm (the acceleration time was 20 sec). On the next day, mice were placed on the rotating rod for a maximum period of 300 sec. The time-to-fall off the Rotarod was recorded. The experiment was performed from 4:00 PM to 6:00 PM.

Oxidative stress assays

Glutathione assay

In order to measure the content of glutathione (GSH), the midbrain was removed and 10% tissue homogenates were mixed with an equal amount of 10% trichloroacetic acid. After centrifugation (5000 rpm, 4 °C, 10 min), Ellman’s regent in PBS (phosphate buffer saline, pH=8 ) was added to the supernatant. Finally, the change of absorbance was measured at 412 nm by a spectrophotometer. The total GSH content was estimated using the standard curve.

ROS assay

ROS production in the brain was assayed using the fluorogenic dye Dihydrodichlorofluorescein diacetate (H2DCFDA). H2DCFDA can penetrate the cell and convert to a non-fluorescent compound by intracellular esterase, and then to a highly fluorescent compound 2,7-Dichlorofluorescein (DCF) through oxidation. In this
method, briefly, midbrain samples were homogenized at room temperature (RT) in 2 ml phosphate buffer (PH: 7.4). After centrifugation (1000 rpm, 4 °C, 10 min), 15 µl of DCFH-DA regent (5µM) was added to the supernatant. The conversion of DCFH-DA to the fluorescent product DCF was measured using a spectrofluorimeter with excitation at 484 nm and emission at 530 nm. ROS formation was quantified using an H2O2 standard curve.

**Apoptotic assay**

Western blotting was carried out to assess the caspase-3 expression level. In summary, the midbrain samples were added to RIPA-lysis buffer (50 mM Tris-HCl (pH=7.4), 150 mM NaCl (1%), Triton X-100 (1%), Sodium Deoxycholate (0.1%), SDS, Sodium Orthovanadate, Sodium Fluoride, EDTA, 1 mM PMSF and protease inhibitor cocktail) on ice and mechanically homogenized. After centrifugation (10000 rpm, for 10 min, at 4 °C), the protein content was determined using the Bradford method with bovine serum albumin as standard. Equal amounts of protein extracts were loaded on 10% SDS-PAGE and separated by electrophoresis. In the next step, proteins were transferred onto the polyvinylidene difluoride (PVDF) membrane and blocked with 5% skim milk for 2 hr at RT. Then, the blots were incubated with specific primary antibody caspase-3 at 4 °C overnight. Each membrane was washed three times, and then the blots were subsequently incubated with secondary antibody HRP for 1.5 hr at 37 °C. Enhanced chemiluminescence (Pierce ECL western blotting substrate) and Alli gence gel doc (Alliance 4.7 Gel doc, UK) were used for detection of the protein bands.

**Inflammatory factors assay**

TNF-α and IL-1β are potent inflammatory cytokines produced by macrophages/monocytes in response to inflammation. Midbrain levels of TNF-α and IL-1β were measured using an ELISA kit in accordance with the manufacturer’s protocol. The absorbance was measured at 450 nm.

**Statistical analysis**

All data were expressed as mean±SD (n=6). The statistical significance of differences between the means was determined using one-way ANOVA, followed by Tukey–Kramer test using SPSS (ver. 16.0) software. The level of significance was set at P<0.05.

**Results**

**Body Weight**

The result showed that there was no difference in the weight changes of animals between different groups (data not shown). The rate of mortality was zero.

**Motor function analysis**

**Challenging beam traversal test**

In this study, for assessment of fine motor performance and coordination, animals underwent the challenging beam test. The results indicated that PQ-treated mice made significantly more errors per step compared with the control mice (P<0.001) (Figure 1A). Post hoc analysis also revealed that the PQ group took a significantly longer time to traverse the beam (P<0.001) (Figure 1B). While no difference was observed in the number of steps between groups (Figure 1C). Pretreatment with vit B12 reversed the toxic effects of PQ. The results showed that pretreatment with vit B12 significantly reduced the number of errors per step (P<0.001) and time to traverse the beam (P<0.001) compared with the PQ-treated group. In addition, vit B12 treatment significantly improved the errors per step (P<0.01) and time to traverse the beam (P<0.001) (in PQ-treated mice compared with the PQ+vit C group.

**Pole test**

Analysis of pole test performance demonstrated markedly increased T-Turn, T-25cm, and T-Total in the PQ group compared with the control group (P<0.001) (Figure 2). While pretreatment with vit B12 or vit C improved the
Cyanocobalamin on paraquat-induced Brain injury

Effect of PQ, vit B12, and vit C on motor coordination in mice. PQ, vit B12, and vit C were injected intraperitoneally (IP) at 10, 1, and 50 mg/kg, respectively. PQ was administered twice a week (with a 3-day interval) and vit B12, vit C, or normal saline was administered 30 min before PQ administration. Results were expressed as the mean±SD (n=6). ***P<0.001 versus control, **P<0.01 compared with PQ group, *P<0.05 and #P<0.001 PQ+vit B12 versus PQ+vit C. No significant (ns) difference between vit B12 and vit C treatment at P>0.05.

PQ-induced motor deficits. The data showed that treatment with vit B12 significantly decreased T-Turn, T-25cm, and T-Total as compared with the PQ group (P<0.001). No difference was observed between vit B12 and vit C treatment groups.

Open field test

The animals were assessed for motor behavioral activity after the last treatment using the open field test. Our findings suggested that PQ decreased the open field parameters. Post hoc analysis revealed a significant reduction in the total distance traveled, number of entries into the center zone, and peripheral locomotion by PQ-treated mice when compared with the control group (P<0.001). However, pretreatment with vit B12 markedly reversed the PQ-induced motor dysfunction (P<0.001) (Figure 3). Administration of vit C also significantly increased the total distance traveled and peripheral locomotion (P<0.001), while no difference was observed in the number of central locomotion as compared with the PQ group. Data analysis also revealed that vit B12 treatment increased the open field parameters (the total distance traveled and central locomotion) compared with the PQ+vit C group (respectively; P<0.01 and P<0.001).

Rota rod test

The results obtained showed that repeated administration of PQ decreased motion balance in the rotarod test. The results indicated that PQ-treated mice had markedly reduced latency to fall when compared with the control group (P<0.001). While pretreatment with vit B12 and vit C significantly increased the retention time on the rotating rod as compared with the PQ group (P<0.001). In addition, vit B12 treatment increased the latency of falls compared with the PQ+vit C group (P<0.001) (Figure 4).

Effects of Vit B12 on GSH levels in PQ-intoxicated mice

Based on the results of the present study, PQ significantly reduced levels of GSH in the midbrain as compared with the control group (P<0.001). However treatment with vit B12 (1 mg/kg) or vit C (50 mg/kg), significantly increased the GSH levels within the midbrain as compared with the PQ-treated mice (respectively; P<0.001, P<0.01), which means that vit B12 and vit C could have a protective effect against oxidative stress caused by PQ (Figure 5). In addition, vit B12 treatment significantly increased the GSH levels in PQ-treated mice compared with the PQ+vit C group (P<0.01).

Effect of Vit B12 on PQ-induced oxidative stress in the midbrain

To determine the role of ROS in PQ-induced oxidative stress and nerve damage, the levels of ROS were evaluated using DCFH-DA fluorescence staining in the midbrain. The results of the current study indicated that the levels of ROS in the midbrain PQ-treated mice were significantly enhanced compared with the control group (P<0.01) (Figure 6). However, pretreatment with vit B12 (1 mg/kg) significantly decreased the level of ROS compared with PQ treated group (P<0.01). Analysis of the results showed that vit B12 could reduce ROS levels and had a protective effect against PQ-induced oxidative stress, while no difference in vit C was observed in the ROS levels compared with the PQ.
Effect of Vit B12 on PQ-induced inflammation

Analysis of the results revealed that administration of PQ led to a significant increase in the concentration of TNF-α and IL-1β in PQ-intoxicated mice. PQ, vit B12, and vit C were injected intraperitoneally (IP) at 10, 1, and 50 mg/kg, respectively. PQ was administered twice a week (with a 3-day interval) and vit B12, vit C, or normal saline was administered 30 min before PQ administration. The results showed that vit B12 could prevent increased PQ-induced ROS. Results were expressed as the mean±SD (n=6). **P<0.01 versus control, ***P<0.001 compared with PQ group and $$$P<0.001 versus PQ+vit C.

**Figure 6.** Effect of B12 treatment on PQ-induced ROS generation in the midbrain. PQ, vit B12, and vit C were injected intraperitoneally (IP) at 10, 1, and 50 mg/kg, respectively. PQ was administered twice a week (with a 3-day interval) and vit B12, vit C, or normal saline was administered 30 min before PQ administration. The results showed that vit B12 could prevent increased PQ-induced ROS. Results were expressed as the mean±SD (n=6). **P<0.01 versus control, ***P<0.001 compared with PQ group and $$$P<0.001 versus PQ+vit C.

Effect of Vit B12 on PQ-induced apoptosis

PQ exposure up-regulated expression of caspase-3 protein level in the midbrain of intoxicated mice compared with the control (P<0.001). Although vit B12 and vit C treatment resulted in decreased apoptosis marker level in comparison with the PQ group (P<0.001), only vit B12 could return it to the normal level. A significant difference was observed between PQ+ vit B12 and PQ+ vit C groups (P<0.05) (Figures 7 A and B).

**Effect of Vit B12 on PQ-induced inflammation**

**Figure 7.** Effect of vit B12 on the level of caspase 3 in the midbrain of PQ-intoxicated mice. PQ, vit B12, and vit C were injected intraperitoneally (IP) at 10, 1, and 50 mg/kg, respectively. PQ was administered twice a week (with a 3-day interval) and vit B12, vit C, or normal saline was administered 30 min before PQ administration. (A) Representative photograph of the western blot analysis. (B) Densitometric data of protein analysis. Values were presented as the mean±SD (n=6). **P<0.01 compared with PQ group, and $$$P<0.001 versus PQ+vit C.

Effect of Vit B12 on PQ-induced inflammation

Analysis of the results revealed that administration of PQ led to a significant increase in the concentration of TNF-α and IL-1β in PQ-intoxicated mice. PQ, vit B12, and vit C were injected intraperitoneally (IP) at 10, 1, and 50 mg/kg, respectively. PQ was administered twice a week (with a 3-day interval) and vit B12, vit C, or normal saline was administered 30 min before PQ administration. The results showed that vit B12 could prevent increased PQ-induced ROS. Results were expressed as the mean±SD (n=6). **P<0.01 versus control, ***P<0.001 compared with PQ group and $$$P<0.001 versus PQ+vit C.

**Figure 8.** Effect of vit B12 on the midbrain level of TNF-α and IL-1β in PQ-intoxicated mice. PQ, vit B12, and vit C were injected intraperitoneally (IP) at 10, 1, and 50 mg/kg, respectively. PQ was administered twice a week (with a 3-day interval) and vit B12, vit C, or normal saline was administered 30 min before PQ administration. Values were presented as the mean±SD (n=6). **P<0.01 versus PQ group and $$$P<0.001 versus PQ+vit C.

**Discussion**

The results of the present study revealed that vit B12 can decrease the PQ-induced behavioral and neurochemical alterations. PQ is an herbicide for the control of weeds with a broad spectrum of toxicological properties for humans. Epidemiological studies indicated that chronic exposure to PQ can be a risk factor for neurodegenerative diseases such as Parkinson’s, Alzheimer’s, and Huntington’s (5, 18, 42-45). Studies with rodent models have suggested that intraperitoneal (IP) administration of PQ induced motor impairment as well as loss of hippocampal neurons, leading to learning and memory deficits (27). The results of this study showed that repeated injection of PQ (10 mg/kg) induced motor impairment and incoordination in pole, challenging beam traversal, and rotorod tests as well as a decrease in spontaneous motor activity in the open field test. In this study, administration of PQ increased the time to orient down (T-Turn) and climb down (T-Total), as a measure of motor impairments, which was in agreement with the results of previous studies (46-48). In vivo studies showed that chronic exposure to PQ induced significant impairments in balance and vertical limb movements through prolonged latency of T25cm, T-turn, and T-total in the pole test (41, 49, 50). The effect of PQ on motor performance and coordination was also assessed in the challenging beam traversal test. Our findings were in line with the study conducted by Fernagut et al. PQ administration (10 mg/kg, IP) prolonged the time to traverse the beam and increased the number of errors in challenging beam traversal tests as an indicator for motor deficits and muscle incoordination (51). PQ administration also remarkably reduced the spontaneous motor activity and the parameters evaluated in the open field and confirmed the neurobehavioral disorders.
Cyanocobalamin (CNCbl, vit B12) is a crucial micronutrient in numerous biological processes with the optimal function of the neural system (61). It has been reported that vitamin B inhibits the release of excessive glutamate and excitotoxic neurotransmitters in the brain (62, 63). Vit B12 can penetrate the BBB, which is evidence of support for its neuroprotective effects in neurodegenerative diseases (37, 38). It showed a neuroprotective effect in the tibial nerve-damaged model in rats through improvement of motor function and attenuation of the neuron cell degeneration (64). Moreover, vit B12 on sciatic nerve damage reduces the destruction process and improves its regeneration (37).

In line with our results, vit B12 (1 mg/kg) reduced motor impairment in a dose-dependent manner in a rat model of multiple sclerosis and ameliorated the open-field behavior (65). Vit B12 deficiency in rat diet also led to reduction of line distance traveled and motor activity as well as motor balance impairment and a decline in the latency of falls on the open field and rotarod tests (66, 67). It was documented that dietary supplementation containing vit B12 and folate, in a model of ischemic injury in mice, could improve the motor activity in the rotarod test (68). Vitamin C also known as ascorbic acid (AA) is a water-soluble vitamin that plays a vital role as an antioxidant in reducing oxidative stress and inflammation and as a co-factor in many major biological processes (69, 70). Although vit C is not able to directly penetrate the BBB, its oxidized form (dehydroascorbic acid) easily enters the brain via glucose transporters, which is evidence of amplification of its antioxidant potential in CNS (71, 72). In an experimental sepsis model in rats, vit C administration at a high dose (200 mg/kg, IP) effectively improved cognitive impairment in the Morris Water Maze test (73). Another study showed vit C deficiency in mice diet also led to behavioral disorders and reduced locomotor activity that was alleviated by vit C treatment (74). In a recent study, the results of pole, open field, and rotarod tests showed the protective effect of vit C against PQ-induced behavioral alterations. However, challenging beam traversal test results did not confirm it. Indeed, it was indicated that vit B12 exerted its neuroprotective effect stronger than vit C.

Different methods such as inflammatory and oxidative stress have been proposed in PQ neurotoxicity up to now (75, 76). In the present study, repeated administration of PQ also impaired prooxidative/antioxidative homeostasis through excessive generation of ROS and reduction in GSH content in the midbrain. In vivo and in vitro studies confirmed that PQ induced neurotoxicity and oxidative stress via overproduction of ROS and decrease in GSH and antioxidant enzyme levels (77-79). Another study revealed frequent injections twice a week for 6 weeks of PQ (10 mg/kg) and maneb (MB) (30 mg/kg) considerably activated ROS production and decreased GSH levels in the midbrain of mice. It was also documented that administration of PQ significantly reduced total GSH content in three regions of the brain: striatum, hippocampus, and cortex in mice (80). PQ-induced decreased GSH content and increased ROS levels were also reported in the substantia nigra pars compacta (SNpc) and midbrain regions of the mice (81-82). Excessive accumulation of ROS in the brain can damage a wide range of cellular compounds including DNA, proteins, and lipids, and has been implicated as a major underlying cause of neuropathy and various disorders including neurodegenerative diseases such as PD (21, 83-85). The result of this study clearly showed excess ROS generation and reduced GSH content by PQ administration in the midbrain that was returned to the normal rates by vit B12 treatment. Research has shown that vit B12 plays a protective role against elevated superoxide levels and preserves GSH content in the reduced state (86, 87). In our previous study, vit B12 administration could prevent the neurotoxicity of methamphetamine in the striatum and cortex regions of the mouse brain via an increase in GSH level and decreased apoptotic index (88). Moreover, vit B12 therapy after brain ischemia significantly increased the expression of the anti-oxidant enzyme (GSH and SOD) and inhibited lipid peroxidation in the brain of ischemic rats which was in harmony with our study (89). In the recent study, the protective effect of vit C against PQ-induced oxidative stress was only shown in increased GSH content and not in ROS levels. The antioxidant effect of vit c through decreasing the ROS level and increasing the GSH level has been shown in numerous studies (90). In line with our study, subchronic administration of PQ treatment in PQ-induced lung toxicity can either accelerate the HO production or exacerbate oxidative stress (91).

In our study, the significant elevation of midbrain TNF-α and IL-1β levels in mice treated with PQ alone was observed, which was significantly attenuated by vit B12 administration.

Inflammatory processes have been confirmed to play a vital role in neurodegenerative disorders such as Alzheimer’s and PD (92). It has been reported that frequent exposure to PQ caused an induction in proinflammatory cytokine gene expression which led to increased inflammatory-related genes such as IL-6, IL-1β, and TNF-α in three regions of the brain: SN, hippocampus, and frontal cortex (17, 93-95). In line with our study, subchronic administration of PQ significantly elevated the amount of TNF-α in the midbrain and induced parkinsonism in mice (96, 97). However, vit B12 pretreatment attenuated PQ-induced inflammatory response by reducing TNF-α and IL-1β levels. The result of clinical study of intensive care unit patients also indicated that high-dose parenteral vit B12 could modulate systemic inflammation (98). It seems that vit B12 can be useful as treatment for a broad spectrum of
inflammatory diseases associated with oxidative stress (99-101). Human studies reported a considerable imbalance between serum vit B12 and inflammatory mediator (TNF-α and IL-1β) levels, which could recover by vit B12 treatments (27, 102). In an experimental allergic encephalomyelitis model in rats, co-administration of bee venom and vit B12 reduced TNF-α level, gliosis, and NO generation (106). An in vitro study conducted by Li et al. showed that vit C treatment significantly inhibited the expression of inflammatory factors (IL-1β, and IL-6) caused by hypoxia in rat intestinal epithelial cell line (IEC-6) (104). In contrast, a clinical study in 25 subjects with metabolic syndrome showed intravenous vit C administration increased the expression of inflammatory-related genes such as TNF-α, Interleukin 4 (IL-4), and Interferon-gamma (IFN-γ) in the mononuclear cells (105). Our results showed that vit C treatment significantly reduced midbrain inflammatory factors (TNF-α, IL-1β) which was consistent with previous studies (106-108). However, the results revealed that vit B12 is more effective than vit C in reduction of PQ-induced inflammatory cytokines.

We also examined the effects of vit B12 on the expression of apoptosis markers in the midbrain of mice treated with PQ. In accordance with previous studies, the apoptosis process was considered a cause of PQ toxicity (109). It has been reported that acute exposure to PQ (40 mg/kg, twice daily) significantly increased the expression of caspase 3 and apoptotic cells in the brain rat (110). Another study indicated that PQ administration significantly elevated caspase 3 levels and cell apoptosis in the hippocampus and midbrain sections of intoxicated mice (111, 112). Vit B12 prevented the apoptosis induced by PQ with strong down-regulation of caspase 3 in the midbrain, which often serves as a trigger for apoptosis cascades. In a pneumoencephalitis rat model, administration of vit B12 could modulate caspase 3 activity and apoptosis in the hippocampal dentate gyrus (113). The findings of animal studies showed that treatment with a B12 supplement effectively inhibits the process of apoptosis via decreased mRNA expression levels of caspase 3 and in the abdominal aorta or brain in rats (47, 114). It was also reported that pretreatment of vit C (250 mg/kg) significantly reduced caspase-3 activity and prevented apoptotic neurodegeneration caused by pentylenetetrazol (PTZ) in rat brains (115). In the present study, vit B12 significantly reduced the PQ-induced caspase 3 levels in the midbrain, in comparison with vit C.

Conclusion

Finally, the present results indicate that vit B12 had potential preventive and therapeutic effects on PQ-induced neurotoxicity and locomotor activity impairment in mice. One explanation could be that vit B12 exerted direct antioxidant and free radical scavenging activities. Vit B12 also possessed noteworthy anti-inflammatory properties and decreased the level of IL-1β and TNF-α. Vit B12’s protective effect resulted in attenuation of apoptosis cell death through inhibiting expression of caspase 3. These findings suggest that Vit B12 can be a potentially novel therapeutic strategy for prevention of PQ-induced neurotoxicity.

Acknowledgment

The results presented here are from a Master’s thesis. This study was financially supported by the Research Council of Mashhad University of Medical Sciences, National Institute for Medical Research and development (981355), Iran.

Authors’ Contributions

LE and MJF conceived the original idea. MJF and FA performed experiments. LE and VRA supervised the research. LE, MJF, ZS, VRA, and MM analyzed the data. MJF, ZS, LE, and FA prepared the original draft. MJF, ZS, FA, and LE helped in writing, reviewing, and editing.

Conflicts of Interest

The authors declare that they have no conflicts of interest to disclose.

References

1. Sittipunt C. Paraquat poisoning. Respir Care 2005; 50:383-385.
2. Zyoud SeH. Investigating global trends in paraquat intoxication research from 1962 to 2015 using bibliometric analysis. Am J Ind Med 2018; 61:462-470.
3. Dawson AH, Eddleston M, Senarathna L, Mohamed F, Gawarammana I, Bowe SJ, et al. Acute human lethal toxicity of agricultural pesticides: a prospective cohort study. PLoS Med 2010; 7:e1000357.
4. Suntes ZE. Role of antioxidants in paraquat toxicity. Toxicology 2002; 180:65-77.
5. FAO/WHO. (2022). Pesticide residues in food-Report 2021 - Joint FAO/WHO Meeting on Pesticide Residues. Rome: FAO/WHO.
6. Banday T, Bashir S, Bhat S, Ashwin K, Praveen JS. Manifestation and management of paraquat intoxication. A deadly poison. IOSR J Dent Med Sci 2013; 12:74-76.
7. Vale J, Meredith T, Buckley B. Paraquat poisoning: clinical features and immediate general management. Hum Toxicol 1987; 6:41-47.
8. Dinis-Oliveira RJ, Sarmento A, Reis P, Amaro A, Remião F, Bastos ML, et al. Acute paraquat poisoning: report of a survival case following intake of a potential lethal dose. Pediatr Emerg Care 2006; 22:537-540.
9. Agarwal R, Srinivas R, Aggarwal A, Gupta D. Experience with paraquat poisoning in a respiratory intensive care unit in North India. Singapore Med J 2006; 47:1033-1037.
10. Chen L, Yoo S-E, Na R, Liu Y, Ran Q. Cognitive impairment and increased Aβ levels induced by paraquat exposure are attenuated by enhanced removal of mitochondrial H2O2. Neurobiol Aging 2012; 33:432, e415-432, e426.
11. Baltazar MT, Dinis-Oliveira RJ, de Lourdes Bastos M, Tsatsakis AM, Duarte JA, Carvalho F. Pesticides exposure as etiological factors of Parkinson’s disease and other neurodegenerative diseases—a mechanistic approach. Toxicol Lett 2014; 230:85-103.
12. Miranda-Contreras L, Dávila-Ovalles R, Benítez-Díaz P, Peña-Contreras Z, Palacios-Prü E. Effects of prenatal paraquat and mancozeb exposure on amino acid synaptic transmission in developing mouse cerebellar cortex. Brain Res 2005; 160:19-27.
13. Todorov T. Paraquat neurotoxicity. Revista Médica Sinergia 2018; 3:3-6.
14. Niveditha S, Ramesh S, Shivanandappa T. Paraquat-induced movement disorder in relation to oxidative stress-mediated neurodegeneration in the brain of Drosophila melanogaster. Neurochem Res 2017; 42:3310-3320.
15. Brouwer M, Huss A, van der Mark M, Nijsse PC, Mulleners WM, Sas AM, et al. Environmental exposure to pesticides and the risk of Parkinson’s disease in the Netherlands. Environ Int 2017; 107:100-110.
16. Richardson JR, Quan Y, Sherer TB, Greenamyre JT, Miller GW. Paraquat neurotoxicity is distinct from that of MPTP and rotenone. Toxicol Sci 2005; 88:193-201.
17. Mitra S, Chakraborti N, Bhattacharyya A. Differential regional expression patterns of α-synuclein, TNF-α, and IL-1β; and variable
status of dopaminergic neurotoxicity in mouse brain after Paraquat treatment. J Neuroinflammation 2011; 8:1-22.
18. Liu H, Tsai M, Chen C, Jeng J, Chang Y, Chen S, et al. Environmental risk factors and Parkinson’s disease: a case-control study in Taiwan. Neurology 1997; 48:1583-1588.
19. McCormack AJ, Johnston L, C., Andersen JK, Vu S, Di Monte DA. Role of oxidative stress in paraquat-induced dopaminergic cell degeneration. J Neurochem 2005; 93:1030-1037.
20. Bartlett RM, Holden JE, Nickles RJ, Murali D, Barbee DL, Barnhart TE, et al. Paraquat is excluded by the blood brain barrier in rhesus macaque. An in vivo pet study. Brain Res 2009; 1259:74-79.
21. Yasaka T, Okudaira K, Fujito H, Matsumoto J, Ohya I, Miyamoto Y. Further studies of lipid peroxidation in human paraquat poisoning. Arch Intern Med 1986; 146:681-685.
22. Gawarammana IB, Buckley NA. Medical management of paraquat ingestion. Br J Clin Pharmacol 2011; 72:745-757.
23. 6 Proinias K, Giedy M, Gryko D. Vitamin B 12: chemical modifications. Chem Soc Rev 2013; 42:6605-6619.
24. Birch CS, Brash NE, McCaddon A, Williams JH. A novel role for vitamin B12: cobalamins are intracellular antioxidants in vitro. Free Radic Biol Med 2009; 47:184-188.
25. McCaddon A. Vitamin B12 in neurology and ageing: clinical and genetic aspects. Biochimie 2013; 95:1066-1076.
26. Lalonde R, Barraud H, Ravey J, Guéant J-L,Bronowicki J-P, Strazielle C. Effects of a B-vitamin-deficient diet on exploratory activity, motor coordination, and spatial learning in young adult Balb/c mice. Brain Res 2008; 1188:122-131.
27. Al-Daghri NM, Rahman S, Sabico S, Yakout S, Wani K, Al-Attas OS, et al. Association of vitamin B12 with pro-inflammatory cytokines and biochemical markers related to cardiometabolic risk in Saudi subjects. Nutrients 2016; 8:460.
28. World Health Organization FaAOotUN. Vitamin and mineral requirements in human nutrition: vitamin A. 2nd ed. Geneva: World Health Organization 2004:279-300.
29. Madenci G, Bilin S, Arli B, Saka M, Ak F. Serum iron, vitamin B12 and folic acid levels in Parkinson’s disease. Neurochem Res 2012; 37:1436-1441.
30. Shen L. Associations between B vitamins and Parkinson’s disease. Nutrients 2015; 7:197-204.
31. Hosseinzadeh H, Moallem S, Moshiri M, Sarnavazi M, Etemad L. Anti-nociceptive and anti-inflammatory effects of cyanocobalamin (vitamin B12) against acute and chronic pain and inflammation in mice. Arzneimittelforschung 2012: 62:324-329.
32. Scalabrino G, Corsi M, Veber D, Buccellato F, Pravettoni G, Manfridi A, et al. Cobalam in (vitamin B12) positively regulates interleukin-6 levels in rat cerebrospinal fluid. J Neuroimmunol 2002; 127:37-43.
33. Lee Y-J, Wang M-Y, Lin M-C, Lin P-T. Associations between vitamin B12 status and oxidative stress and inflammation in diabetic vegetarians and omnivores. Nutrients 2016; 8:118.
34. Roy S, Sable R, Khaire A, Randhir K, Kale A, Joshi S. Effect of maternal micronutrients (folic acid and vitamin B12) and omega-3 fatty acids on brain function. J Biomed Sci 2016; 23:1-7.
35. Gupta J, Sana QS. Potential benefits of methylcobalamin: A review. Austin J Pharmacol Ther 2015; 3:1076.
36. Attia HN, Maklad YA. Neuroprotective effects of coenzyme Q10 on paraquat-induced Parkinson’s disease in experimental animals. Behav Pharmacol 2018; 29:79-86.
37. Richter F, Gabby L, McDowell KA, Mulligan CK, De La Rosa K, Sioshansi PC, et al. Effects of decreased dopamine transporter levels in nigrostriatal neurons and paraquat/maniae neurotoxicity in mice. Neurobiol Aging 2017; 51:54-66.
38. Majumdar S, Maiti A, Kamarak S, Sekhar D, Mukherjee S, Das D, et al. Antiapoptotic efficacy of folic acid and vitamin B12 against arsenic-induced toxicity. Environ Toxicol 2012; 27:351-363.
39. Li X, Matsumoto K, Murakami Y, Tetzuka Y, Wu Y, Kadota S. Neuroprotective effects of Polygonum multiflorum on nigrostriatal dopaminergic degeneration induced by paraquat and maneb in mice. Pharmacol Biochem Behav 2005; 82:345-352.
40. Pitteljohn D, Mangano EN, Hayley S. Cylooxygenase-2 deficiency modifies the neurochemical effects, motor impairment and colloidmorbidity anxiety provoked by paraquat administration in mice. Eur J Neurosci 2008; 28:707-716.
41. Ping C, Zhen C, An L, Xiao-Chu L, Xiao-Kang W, Chun-Jun Z, et al. Catalytic metalloporphyrin protects against paraquat neurotoxicity in vivo. Biomed Environ Sci 2008; 21:233-238.
42. Fernagut P-O, Hutson C, Fleming S, Tetreaut N, Salcedo J, Masliha E, et al. Behavioral and histopathological consequences of paraquat intoxication in mice: Effects of dexamethasone overfl expression. Synapse 2007; 61:991-1001.
43. Brook A, Chadwick C, Gelbard H, Cory-Slechta D, Fedoroff H. Paraquat elicited neurobehavioral syndrome caused by dopaminergic neuron loss. Brain Res 1999; 823:1-10.
44. Fernandes L, Santos A, Sampaio T, Sborgi S, Prediger R, Ferro M, et al. Exposure to paraquat associated with periodontal disease causes motor damage and neurochemical changes in rats. Hum Exp Toxicol 2011; 40:81-89.
45. Rudyk C, Dwyer Z, McNeill J, Salmaso N, Farmer K, Prowse N, et al. Chronic unpredictable stress influences the behavioral but not the neurodegenerative impact of paraquat. Neurobiol Stress 2019; 11:1001.79.
46. Ishola IO, Akatoabi OE, Alade AA, Adeyemi OO. Gilimpireide prevents paraquat-induced Parkinsonism in mice: involvement of oxidative stress and neuroinflammation. Fundam Clin Pharmacol 2019; 33:277-285.
47. Pitteljohn D, Mangano E, Shukla N, Hayley S. Interferon-γ deficiency modifies the motor and co-morbidity behavioral pathology and neurochemical changes provoked by the pesticide paraquat. Neurosci 2009; 164:1894-1906.
48. Chanyachkul T, Yovathavorn K, Thongsaard W, Chongthammakun S, Navasumrit P, Satayavivad J. Attenuation of paraquat-induced motor behavior and neurochemical disturbances by L-valine in vivo. Toxicol Lett 2004; 150:259-269.
Cyanocobalamin on paraquat-induced Brain injury

Jafari Fakhrabad et al.

58. Ait-Bali Y, Ba-Mhamed S, Bennis M. Prenatal Paraquat exposure induces neurobehavioral and cognitive changes in mice offspring. Environ Toxicol Pharmacol 2016; 48:53-62.
59. Pellegrino IJ, Altman J. Effects of differential interference with postnatal cerebellar neurogenesis on motor performance, activity level, and maze learning of rats: a developmental study. J Comp Physiol Psychol 1979; 93:1.
60. Hamm RJ, Pike BR, O’DELL DM, Lyeth BG, Jenkins LW. The rotorod test: an evaluation of its effectiveness in assessing motor deficits following traumatic brain injury. J Neurotrauma 1994; 11:187-196.
61. Reynolds E. Vitamin B12, folic acid, and the nervous system. Lancet Neurol 2006; 5:949-960.
62. Akaiae A, Tamura Y, Sato Y, Yokota T. Protective effects of a vitamin B12 analog, methylcobalamin, against glutamate cytotoxicity in cultured cortical neurons. Eur J Pharmacol 1993; 241:1-6.
63. Hung K-L, Wang C-C, Huang C-Y, Wang S-J. Cyanocobalamin, vitamin B12, depresses glutamate release through inhibition of voltage-dependent Ca2+ influx in rat cerebrocortical nerve terminals (synapsesomes). Eur J Pharmacol 2009; 602:230-237.
64. Tamaddondar E, Farshid A, Samadi F, Eghdami K. Effect of vitamin B12 on functional recovery and histopathological changes of tibial nerve-crushed rats. Drug Res (Stuttg) 2014; 64:470-475.
65. Taherian N, Vaezi G, Neamati A, Etemad L, Hojjati V, Gorji-Valokola M. Vitamin B12 and estradiol benzene improve memory retrieval through activation of the hippocampal AKT, BDNF, and CREB proteins in a rat model of multiple sclerosis. Iran J Basic Med Sci 2021; 24:256.
66. Arora K, Sequeira JM, Hernández AI, Alarcon JM, Quadros EV. Behavioral alterations are associated with vitamin B12 deficiency in the transcobalamin receptor/CD320 KO mouse. PloS one 2017; 12:e0177156.
67. Awuah EB. Effects Of Shmt1 Heterozygosity On Motor Coordination And Peripheral Neuropathy. 2019.
68. Jadavji NM, Emmerson JT, MacFarlane AJ, Willmore WG, Smith PD. B-vitamin and choline supplementation increases neuromplasticity and recovery after stroke. Neurobiol Dis 2017; 103:89-100.
69. Bendich A, Machlin L, Scandurra O, Burton G, Wayner D. The antioxidant role of vitamin C. Adv. in Free Radical Biology & Medicine 1986; 2:419-444.
70. Sauberlich HE. Pharmacology of vitamin C. Annu Rev Nutr 1994; 14:371-391.
71. Agus DB, Gambhir SS, Partridge WM, Spielholz C, Baselga J, Vera JC, et al. Comparative cytoprotective activity of vitamin C, E and beta-carotene against chromium induced oxidative stress in murine macrophages. Biomol Pharmacother 2006; 60:71-76.
72. Huang J, Agus DB, Winfree CJ, Kusi S, Mack WJ, McCaggart RA, et al. Dehydroascorbic acid, a blood-brain barrier transportable form of vitamin C, mediates potent cerebroprotection in experimental stroke. Proc Natl Acad Sci U S A 2001; 98:11720-11724.
73. Zhang N, Zhao W, Hu Z-J, Ge S-M, Huo Y, Liu L-X, et al. Protective effects and mechanisms of high-dose vitamin C on sepsis-associated cognitive impairment in rats. Sci Rep 2021; 11:1-10.
74. Ward MS, Lamb J, May JM, Harrison FE. Behavioral and monoamine changes following severe vitamin C deficiency. J Neurochem 2013; 124:363-375.
75. Collé D, Farina M. Oxidative stress in paraquat-induced damage to nervous tissues. Toxicology; Elsevier; 2021. p. 69-78.
76. Sun Y, Zheng J, Xu Y, Zhang X. Paraquat-induced inflammatory response of microglia through HSP60/TLR4 signaling. Hum Exp Toxicol 2018; 37:1161-1168.
77. Dinis-Oliveira R, Remiao F, Carmo H, Duarte J, Navarro AS, Bastos M, et al. Paraquat exposure as an etiological factor of Parkinson's disease. Neurotoxicology 2006; 27:1110-1122.
78. Peng J, Stevenson FF, Oo ML, Andersen JK. Iron-enhanced paraquat-mediated dopaminergic cell death due to increased oxidative stress as a consequence of microglial activation. Free Radic Biol Med 2009; 46:312-320.
79. Purisa MG, McCormack AL, Cumine S, Li J, Isla MZ, Di Monte DA. Microglial activation as a priming event leading to paraquat-induced dopaminergic cell degeneration. Neurobiol Dis 2007; 25:392-400.
80. Hou L, Sun F, Sun W, Zhang L, Wang Q. Lesion of the locus coeruleus damages learning and memory performance in paraquat and maneb-induced mouse Parkinson’s disease model. Neuroscience 2019; 419:129-140.
81. Kang MJ, Gil SJ, Koh HC. Paraquat induces alternation of the dopamine catechol pathways and glutathione levels in the substantia nigra of mice. Toxicol Lett 2009; 188:148-152.
82. Zhao X, Wang R, Xiong J, Yan D, Li A, Wang S, et al. JWA antagonizes paraquat-induced neurotoxicity via activation of Nrf2. Toxicol Lett 2017; 277:32-40.
83. Keeney PM, Xie J, Capraldi RA, Bennett JP. Parkinson's disease brain mitochondrial complex I has oxidatively damaged subunits and is functionally impaired and misassembled. J Neurosci 2006; 26:5256-5264.
84. Gazewood JD, Richards DR, Clebak K. Parkinson disease: an update. Am Fam Physician 2013; 87:267-273.
85. Nita M, Graybowksi A. The role of the reactive oxygen species and oxidative stress in the pathomechanism of the age-related ocular diseases and other pathologies of the anterior and posterior eye segments in adults. Oxid Med Cell Longev 2016; 2016.
86. Suarez-Moreira E, Yun J, Birch CS, Williams JH, McCaddon A, Brash NE. Vitamin B12 and redox homeostasis: cob (II) alamin reacts with superoxide at rates approaching superoxide dismutase (SOD), sepis, severe sepis, and septic or traumatic shock. Med Hypotheses 2006; 67:124-142.
87. Wheatley C. A scarlet pinpricker for the resolution of inflammation? The role of supra-therapeutic doses of cobalamin, in the treatment of systemic inflammatory response syndrome (SIRS), sepsis, severe sepis, and septic or traumatic shock. Med Hypotheses 2006; 67:124-142.
88. Moshir M, Hosseiniany SM, Moallem SA, Hadizadeh F, Jafari AH, Ghadiri A, et al. The effects of vitamin B12 on the brain damages caused by methamphetamine in mice. Iran J Basic Med Sci 2018; 21:434.
89. Khastar H, Garmabi M, Mehrjerdi Z, Rahimi MT, Shamsaei N, Ali A-H, et al. Cyanocobalamin improves memory impairment via inhibition of necrosis and apoptosis of hippocampal cell death after transient global ischemia/reperfusion. Iran J Basic Med Sci 2021; 24:160.
90. Vandana S, Ram S, Ilavazhagan M, Kumar GD, Banerjee PK. Comparative cytoprotective activity of vitamin C, E and beta-carotene against chromium induced oxidative stress in murine macrophages. Biomol Pharmacother 2006; 60:71-76.
91. Dinis-Oliveira R, Duarte J, Sanchez-Navarro A, Remiao F, Bastos M, Carvalho F. Paraquat poisonings: mechanisms of lung toxicity, clinical features, and treatment. Crit Rev Toxicol 2008; 38:13-71.
92. Nagatsu T, Ichinose H, Togari A. Cytokines in toxicology, clinical features, and treatment. Crit Rev Toxicol 2008; 38:13-71.
93. Ding W, Lin H, Hong X, Ji D, Wu F. Poloxamer 188-mediated antioxidant role of vitamin C. Adv. in Free Radical Biology & Medicine 2018; 38:13-71.
94. Tian T, Zhang B, Wang K, Zhang B, Huang M. Protective effects of taurine on neurons and microglia in Parkinson’s disease-like mouse model induced by paraquat. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi 2020; 38:801-808.
95. Li Q, Xiao H, Shao Y, Zhang X, Zhang Y, Zhou Z. Paraquat increases Interleukin-1β in hippocampal dentate gyrus to impair hippocampal neurogenesis in adult mice. Ecotoxicol Environ Saf 2020; 200:110733.
96. Ishola IO, Akinyede A, Adeluwa T, Michal C. Novel action of vinpocetine in the prevention of paraquat-induced parkinsonism in mice: involvement of oxidative stress and neuroinflammation.
Cyanocobalamin on paraquat-induced Brain injury

107. Kim S-M, Lim S-M, Yoo J-A, Woo M-J, Cho K-H. Consumption of high-dose vitamin C (1250 mg per day) enhances functional and structural properties of serum lipoprotein to improve anti-oxidant, anti-atherosclerotic, and anti-aging effects via regulation of anti-inflammatory microRNA. Food Funct 2015; 6:3604-3612.

108. Ellul MS, Rahmat A, Patimah I, Khaza‘ai H, Abed Y. Effect of vitamin C on inflammation and metabolic markers in hypertensive and/or diabetic obese adults: a randomized controlled trial. Drug Des Devel Ther 2015; 9:3405-3412.

109. Ranjbar A, Asl SS, Firozian F, Dartoti HH, Seyedabadi S, Azandariani MT, et al. Role of cerium oxide nanoparticles in a paraquat-induced model of oxidative stress: emergence of neuroprotective results in the brain. J Mol Neurosci 2018; 66:420-427.

110. Li H-f, Zhao S-x, Xing B-p, Sun M-l. Ulinastatin suppresses endoplasmic reticulum stress and apoptosis in the hippocampus of rats with acute paraquat poisoning. Neural Regen Res 2015; 10:467.

111. Li K, Cheng X, Jiang J, Wang J, Xie J, Hu X, et al. The toxic influence of paraquat on hippocampal neurogenesis in adult mice. Food Chem Toxicol 2017; 106:356-366.

112. Kumar A, Ganini D, Mason RP. Role of cytochrome c in alpha-synuclein radical formation: implications of alpha-synuclein in neuronal death in Maneb- and paraquat-induced model of Parkinson's disease. Mol Neurodegener 2016; 11:70.

113. de Queiroz KB, Cavalcante-Silva V, Lopes FL, Rocha GA, D’Almeida V, Coimbra RS. Vitamin B12 is neuroprotective in experimental pneumococcal meningitis through modulation of hippocampal DNA methylation. J Neuroinflammation 2020; 17:1-12.

114. Lv FH, Gao JZ, Teng QL, Zhang JY. Effect of folic acid and vitamin B12 on the expression of PPARγ, caspase-3 and caspase-8 mRNA in the abdominal aortas of rats with hyperlipidemia. Exp Ther Med 2013; 6:184-188.

115. Naseer MI, Ullah I, Ullah N, Lee HY, Cheon EW, Chung J, et al. Neuroprotective effect of vitamin C against PTZ induced apoptotic neurodegeneration in adult rat brain. Pak J Pharm Sci 2011; 24:263-268.