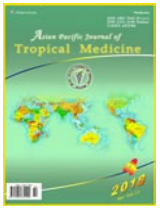

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Preventive effects of cannabis on neurotoxic and hepatotoxic activities of malathion in rat

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ABSTRACT

Objective: To investigate the effect of *Cannabis sativa* extract on the development of neuro- and hepato-toxicity caused by malathion injection in rats. **Methods:** The extract of *Cannabis sativa* was obtained from the plant resin by chloroform treatment. Δ^9-Tetrahydrocannabinol content of the extract (20%) was quantified using gas chromatography–mass spectrometry. The doses of cannabis extract were expressed as Δ^9-tetrahydrocannabinol content of 10 or 20 mg/kg. Malathion (150 mg/kg) was intraperitoneally administered followed after 30 min by the cannabis extract (10 or 20 mg/kg, subcutaneously). Rats were euthanized 4 h later. Malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide and paraoxonase-1 (PON-1) activity were determined in brain and liver. Brain 5-lipoxygenase and butyrylcholinesterase (BChE) activity were measured as well. Histopathological examination of brain and liver tissue was also performed. **Results:** Compared to controls, malathion resulted in increased oxidative stress in brain and liver. MDA and nitric oxide concentrations were significantly increased (P<0.05) and GSH significantly decreased with respect to control levels (P<0.05). Malathion also significantly inhibited PON-1 and BChE activities but had no effect on brain 5-lipoxygenase. Brain MDA concentrations were not altered by cannabis treatment. Cannabis at 20 mg/kg, however, caused significant increase in nitric oxide and restored the GSH and PON-1 activity. Brain BChE activity significantly decreased by 26.1% (P<0.05) after treatment with 10 mg/kg cannabis. Cannabis showed no effect on brain 5-lipoxygenase. On the other hand, rats treated with cannabis exhibited significantly higher levels of liver MDA, nitric oxide and PON-1 activity compared with the malathion control group. Rats treated with only malathion exhibited spongiform changes, neuronal damage in the cerebral cortex and degeneration of some Purkinje cells in the cerebellum. There were also hepatic vacuolar degeneration and dilated and congested portal vein. These histopthological changes induced by malathion in brain and liver were reduced to great extent by cannabis administration at 20 mg/kg. **Conclusions:** Our data suggest that acute treatment with cannabis alleviates the malathion-induced brain and hepatic injury in rats possibly by maintaining the levels of GSH and PON-1 activity.

1. Introduction

Organophosphorous compounds are widely used as insecticides in agriculture, gardens, veterinary and in the household. The use of these agents is associated with the risk of causing human toxicity[1]. These compounds irreversibly inhibit the enzyme acetylcholinesterase (AChE) which functions to hydrolyze acetylcholine into acetate and choline and thus terminates its action at the neuronal synapse, membrane, autonomic ganglia, myoneural junction as well as at post-ganglionic parasympathetic nerve endings[2]. This causes the accumulation of acetylcholine which results in excessive central and peripheral cholinergic

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activity[2,3]. Symptoms can be mild and include diarrhoea, urination, excessive salivation and lacrimation, headache, dizziness, and muscle twitches. However, convulsions, respiratory muscle paralysis and respiratory failure can occur as well as coma and death[4]. Subjects exposed to organophosphates for prolonged period might experience neuropsychiatric and mood changes, cognitive and memory deficits, polyneuropathy, and extrapyramidal symptoms[5-7]. Recent studies also implicate these agents in the development of neurodegenerative disorders like Parkinson's disease or dementia[8,9]. The neurotoxic effects of organophosphates might not result only from cholinesterase inhibition and mechanisms such as oxidative tissue damage and neuro-inflammation also contribute to the neurotoxicity[10,11]. Oxidative/nitrosative stress arises when the cell's antioxidant mechanisms can not cope with an increasingly produced reactive oxygen and nitrogen metabolites, resulting in damage to cell membrane, enzymes, and DNA. These reactive species are produced within the cell during normal metabolism, *e.g.*, from the mitochondrial respiratory chain, cellular enzymes such as monooxygenases, xanthine oxidase or from astrocytes and microglia during inflammation and toxic states[12,13]. Organophosphates, *e.g.*, malathion, causes the activation of glia cells[14], impairment of mitochondrial functions[15,16] and increased generation of reactive oxygen metabolites[15].

Cannabis preparations from the plant *Cannabis sativa* L (family Cannabidaceae) are the most widely used illicit substances worldwide. These include herbal cannabis or marijuana, cannabis resin or hashish and sinsemilla[17]. Cannabis are well known for their psychotropic properties such as the sense of euphoria or being "high", distortion in time perception and relaxation[18]. Cannabis also exerts a wide spectrum of effects on the gastrointestinal tract, liver and the endocrine and immune systems[19,20]. Cannabis is characterized by their content of cannabinoids, a group of C21 terpenophenolic compounds peculiar to the plant[21] and among them Δ-9-tetrahydrocannabinol (Δ^9-THC) was shown to be the principal psychoactive constituent[22]. Other important cannabinoids are cannabidiol, cannabigerol, Δ^9-tetrahydrocannabivarin, and cannabidivarin[23]. Some of these cannabinoids have distinctive pharmacological actions from that of Δ^9-THC and might prove of value as an anti-inflammatory agents in such conditions[24]. Whole plant extracts are also in use for medicinal purposes, *e.g.*, spasticity in multiple sclerosis[25], diabetic neuropathy[26], chronic pain from arthritis or fibromyalgia[27], and inflammatory bowel diseases[28]. The actions of cannabinoids are mediated by G-protein coupled receptors. The cannabinoid receptor type 1 is expressed primarily in the brain but is also found in peripheral tissues (vasculature and immune tissues). On the other hand, the cannabinoid receptor type 2 is expressed mainly on immune cells in the peripheral tissues and to a small extent in the brain[29]. Studies reported neurotoxic actions for cannabis[30-32]. Neuroprotective effects have also been described in models of excitotoxicity and attributed to an antioxidant and radical scavenging actions[33,34]. Cannabis has been shown to accelerate liver fibrosis and steatosis in hepatitis C virus patients[35,36] and enhance acute hepatic damage caused by CCl_4, acetaminophen or thioacetamide in rats[37,38]. Cannabis modulates colinesterase activity and increases paraoxonase-1 (PON-1) activity[39]. PON-1 is important in the detoxification of organophosphorus compounds by hydrolyzing their active metabolites[40] and increases enzyme activity exerting therapeutic action against acute toxicity by organophosphates[41]. The present study thus aimed to investigate the possible modulation by *Cannabis sativa* of the neurotoxic and hepatotoxic effects of acute malathion exposure.

2. Materials and methods

2.1. Animals

In this study we used male Sprague–Dawley rats weighing 180–200 g, which were obtained from the National Research Centre (Egypt) and housed in light/dark cycle for 12-h with free access to standard laboratory water and food. The study followed the recommendations of the Institutional Ethical Committee and the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

2.2. Drugs and chemicals

Malathion (Commercial grade, 57%) from El-Naser Chemical Co., Cairo was used. *Cannabis sativa* L. resin (Hashish) was kindly provided from the Ministry of Justice-Egypt. Other chemicals and reagents were purchased from Sigma (St Louis, MO, USA).

2.3. Cannabis extract

The dry cannabis extract was prepared according to Turner and Mahlberg method[42] with some modifications. In brief, 10 g of hashish cut into small pieces subjected to heat (in a glass baker in boiling water at 100 ℃) for a period of 2 h with the aim to decarboxylate the acidic cannabinoids founded[42]. Cannabis was then kept in chloroform overnight and extracted 3 times with chloroform. Fractions were combined and filtered. Fractions were then collected in a 100 mL volumetric flask. The filtrate was then evaporated under a gentle stream of nitrogen. The extract was kept at 4 ℃ and protected from light. The extract was re-dissolved in 2 mL of 96% ethanol and 100 mL distilled water when needed. The extract content of Δ^9-THC was found to be about 20% as determined by gas chromatography–mass spectrometry. The doses of cannabis extract used in the study were equivalent to Δ^9-THC content of 10 or 20 mg/kg, respectively.

2.4. Study design

Animals were divided into four groups, six rats each. Group 1 (normal control) was injected with saline intraperitoneally (i.p.)

(0.2 mL/rat). Group 2 received malathion (150 mg/kg, i.p.) followed by saline injection subcutaneously after 30 min. Groups 3 & 4 received malathion (150 mg/kg, i.p.) after 30 min followed by the cannabis extract at doses of 10 or 20 mg/kg, subcutaneously. Four hours after malathion injection rats were euthanized by decapitation. Brains and livers were then removed, washed with ice-cold saline solution (0.9% NaCl), weighed and stored at 80 ℃ for the investigations. Tissue homogenization was done using 0.1 mol/L phosphate buffer saline (pH 7.4) (20% w/v).

2.5. Biochemical analyses

2.5.1. Determination of oxidative stress parameters

Lipid peroxidation was determined by measuring thiobarbituric reactive species (TBARS)[43]. Reduced glutathione (GSH) was determined using Ellman´s reagent[44]. The concentration of nitric oxide was determined using Griess reagent[45].

2.5.2. Determination of PON-1 activity

PON-1 arylesterase activity was determined using phenylacetate as a substrate and enzyme activity is expressed in kilo International Unit/Liter (kU/L)[46].

2.5.3. Determination of DNA fragmentation

Quantitation of DNA fragmentation in the liver was done according to the method according to Gercel-Taylor[47].

2.5.4. 5-Lipoxygenase

5-Lipoxygenase was determined using a double-antibody sandwich enzyme-linked immunosorbent assay (Rat (5-LO/LOX) ELISA kit) from Shanghai Biovision Co., Ltd, Jufengyuan Road, Baoshan District, Shanghai.

2.5.5. Butyrylcholinesterase (BChE) activity

The activity of BChE (EC 3.1.1.8) was measured using commercially available kit from Ben Biochemical Enterprise (Milan, Italy).

2.6. Histopathology

Brain and liver of different groups were dissected out and fixed in 10% formol saline. Sections of 5 μm thickness were cut, stained with haematoxlin and eosin and investigated by light microscope.

2.7. Statistical analysis

Data are presented as mean±SE. The data are analyzed using way analysis of variance, followed by Duncan's multiple range test for post hoc comparison of group means. Effects with a probability of $P<0.05$ are considered statistically significant.

3. Results

3.1. Oxidative stress

3.1.1. Lipid peroxidation

Exposure to malathion caused a significant increase in brain and liver malondialdehyde by 105.3% ($P<0.05$) and 56.0% ($P<0.05$), respectively as compared to the saline control group. Treatment with cannabis extract had no significant effect on brain malondialdehyde. In the liver, however, 78.1% ($P<0.05$) and 26.8% ($P<0.05$) increments in malondialdehyde concentrations were noticed following treatment with 10 and 20 mg/kg of cannabis extract, respectively, compared to the malathion control group (Table 1).

3.1.2. Nitric oxide

Malathion resulted in markedly raised levels of nitric oxide in brain and liver by 69.0% ($P<0.05$) and 78.2% ($P<0.05$), respectively. Nitric oxide increased further by 29.7% ($P<0.05$) and 23.9% ($P<0.05$) in both the brain and liver tissue by treatment with 20 mg/kg of cannabis extract, compared to the malathion control group (Table 1).

3.1.3. GSH

In malathion intoxicated rats, the brain and liver tissue levels of GSH fell by 56.4% ($P<0.05$) and 24.0% ($P<0.05$) of their corresponding saline control values, respectively. The depletion of GSH by malathion in brain tissue was restored to its saline control value by cannabis given at 20 mg/kg. Cannabis, however, had no significant effect on liver GSH in malathion intoxicated rats (Table 1).

Table 1

Effect of malathion or malathion + cannabis on malondialdehyde (MDA), nitric oxide, reduced glutathione (GSH) and paraoxonase-1 (PON-1) activity in rat brain and liver.

Group	Brain				Liver			
	MDA (nmol/g•tissue)	Nitric oxide (μmol/g•tissue)	GSH (μmol/g•tissue)	PON-1 activity (kU/L)	MDA (nmol/g•tissue)	Nitric oxide (μmol/g•tissue)	GSH (μmol/g•tissue)	PON-1 activity (kU/L)
Saline	23.84±1.28	26.00±1.30	4.36±0.15	15.30±1.62	44.55±2.77	33.00±2.11	7.37±0.31	37.00±2.62
Malathion	48.94±2.90*	43.93±2.81*	1.90±0.07*	4.90±0.33*	69.50±2.10*	58.81±1.41*	5.60±0.40*	19.10±1.54*
Malathion + cannabis 10 mg/kg	48.00±3.50*	46.00±1.70*	1.81±0.23*	6.64±0.41*#	123.78±8.50*#	52.49±2.51*	5.34±0.39*	28.14±2.19*#
Malathion + cannabis 20 mg/kg	47.97±1.00*	57.00±1.63*#	4.57±0.41#	13.67±0.47#	88.14±6.00*#	72.88±4.3*#	5.10±0.70*	33.38±2.45#

Results are means±SEM. Statistical analysis was done using one-way ANOVA and Duncan multiple range test. *$P<0.05$ *vs.* saline treated group. #$P<0.05$ *vs.* malathion control group.

3.1.4. PON-1 activity

In rats treated with malathion, PON-1 activity in the brain and liver was inhibited by 68.0% (P<0.05) and 48.4% (P<0.05), respectively as compared to the corresponding saline control values. Cannabis given at 10 and 20 mg/kg caused significant increase in PON-1 activity in brain by 35.5% (P<0.05) and 179.0% (P<0.05), respectively, compared to the malathion control group. There was also a marked and significant increase in PON-1 activity in the liver by 37.3% (P<0.05) and 74.8% (P<0.05) following treatment with cannabis at doses of 10 and 20 mg/kg, respectively (Table 1).

3.2. Liver DNA fragmentation

DNA fragmentation in liver significantly increased by malathion administration (28.50±2.16 *vs.* 2.10±0.34). Cannabis administration at 20 mg/kg resulted in 20.3% decrease in DNA fragmentation. Values for DNA fragmentation in rats treated with malathion along with cannabis at 10 and 20 mg/kg were 27.80±3.00 and 22.70±1.60, respectively.

3.3. 5-Lipoxygenase

Malathion had no significant effect on brain 5-lipoxygenase compared to the saline control group. There was 16.1% increase in 5-lipoxygenase in the brain of rats treated with both malathion and 10 mg/kg of cannabis extract, compared to the malathion control group. In contrast, cannabis given at 20 mg/kg showed no significant effect on 5-lipoxygenase (Table 2).

3.4. BChE activity

A 73.9% decrease in brain BChE activity was observed in malathion-treated rats compared to its saline control value. Cannabis given at 10 mg/kg caused further decrease in BChE activity by 26.1% (P<0.05). The higher dose of the extract, however, resulted in non-significant increase in BChE activity by 13.2%, compared to the malathion only group (Table 2).

Table 2

5-Lipoxygenase and butyrylcholinesterase (BChE) activity in brain of rats treated with malathione alone or malathion and cannabis.

Group	5-Lipoxygenase (ng/mL)	BChE activity (U/L)
Saline	70.00±3.16	174.10±9.50
Malathion	68.52±4.93	45.42±3.49*
Malathion + cannabis 10 mg/kg	79.53±7.72$^{\#}$	34.10±1.17$^{*\#}$
Malathion + cannabis 20 mg/kg	66.73±2.18	51.43±2.63

Results are means±SEM. Statistical analysis was done using one-way ANOVA and Duncan multiple range test. $^{*}P$<0.05 *vs.* saline treated group. $^{\#}P$<0.05 *vs.* malathion control group.

3.4. Histopathological results

3.4.1. Brain tissue

The cerebral cortex and cerebellum from the saline control group showed a normal appearance (Figures 1A & 2A). Rats treated with malathion only exhibited spongiform changes consisting of relatively small delicate vacuoles in the cerebral cortex, and thrombotic vessels (vessels with membrane bound vacuoles). There were clear sings of neuronal degeneration in the form of some neuronal nuclei that stained poorly, with an indistinct membrane, with the nucleoli being shifted towards membrane or with pyknotic nucleoli (Figure 1B). Degeneration of some Purkinje cells in the cerebellum was observed (Figure 2B). Sections from the cerebral cortex of rats treated with malathion along with cannabis at 10 mg/kg showed normal granular layer and neurons but dilated and congested cerebral blood vessels were observed (Figures 1C & 1D). There was degeneration of some Purkinje cells in the cerebellum (Figure 2C). Rats treated with malathion and cannabis at 20 mg/kg showed normal appearance of the cortex and cerebellum (Figures 1E & 2D).

3.4.2. Liver tissue

Microscopic examination of sections of liver from the saline control group showed a normal appearance the normal architecture and cells with granulated cytoplasm and small uniform nuclei, the hepatocytes appeared as cord (Figure 3A). Malathion caused dilated and congested portal vessel, sings of degeneration in the form of karyolysis, karyorrhexis, pyknosis and vacuolar degeneration. Red blood cells in dilated blood sinusoids and foci of necrosis were seen

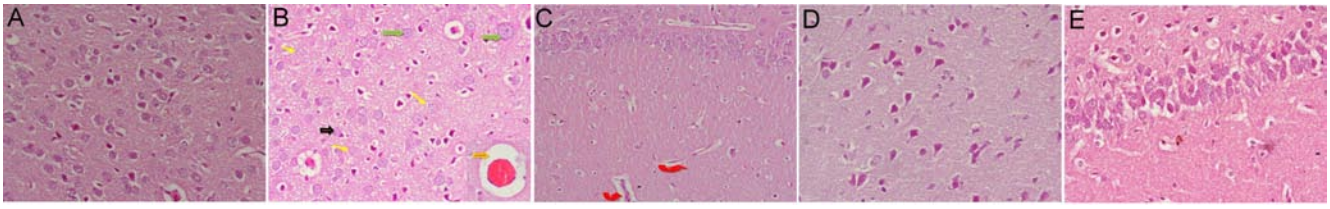

Figure 1. Representative light microphotographs from the cerebral cortex of rats.

(A) Saline control group: The larger cells are neurons. The pink substance between cells is the neuropil. A few capillaries are also seen (H&E, ×200). (B) Malathion control group showing spongiform changes consisting of relatively small delicate vacuoles in the cortex, thrombotic vessels: vessels with membrane bound vacuoles (orange arrow). Signs of damage are found in neurons in the form of some neuronal nuclei that stain poorly (yellow arrow), with an indistinct membrane, with the nucleoli being shifted towards periphery of nuclear membrane (black arrow) or pyknotic nucleoli (light green arrow) (H&E, ×400). (C) Malathion + cannabis 10 mg/kg: The granular layer appears normal but dilated and congested cerebral blood vessel was observed (arrow) (H&E, ×400). (D) Malathion + cannabis 10 mg/kg: Normal neurons seen (H&E, ×200). (E) Malathion + cannabis 20 mg/kg: normal structure of cerebral cortex (H&E, ×400).

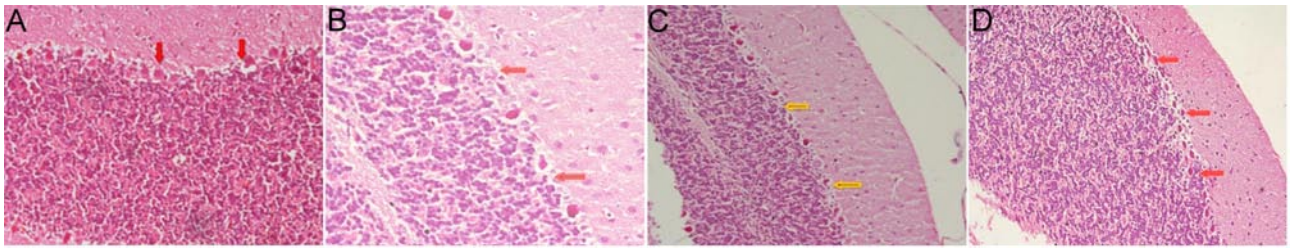

Figure 2. Representative light microphotographs from the cerebellum of rats.

(A) Saline control group: normal Purkinje cells. (B) Malathion control group: degeneration of some Purkinje cells (arrow) (H&E, ×400). (C) Malathion + cannabis 10 mg/kg: degeneration of some Purkinje cells (arrow) (H&E, ×100). (D) Malathion + cannabis 20 mg/kg: normal appearance of Purkinje cells (arrow) (H&E, ×100).

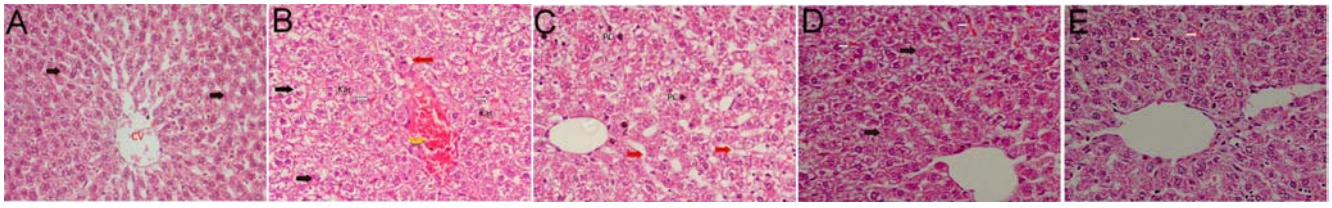

Figure 3. Representative light microphotographs from the liver of rats.

(A) Saline control group: normal histological structure of hepatic lobules and central vein (CV). Cells with granulated cytoplasm and small uniform nuclei (arrow) (H&E, ×200). (B) Malathion control group: dilated and congested portal vein (orange arrow). Sings of degeneration are found in the form karyolysis (Kar), karyorrhexis (black arrow), and vacuolar degeneration (red arrow) (H&E, ×400). (C) Malathion control group: another filed showing pyknotic cells (PC) and dilated blood sinusoids (red arrow) (H&E, ×200). (D) Malathion + cannabis 10 mg/kg: Most hepatocyte appeared normal but with some cells with karyolysis (black arrow) and red blood cells in blood sinusoids (white arrow) (H&E, ×400). (E) Malathion + cannabis 20 mg/kg: Most cells appeared normal. Few blood cells in blood sinusoids (arrow) (H&E, ×400).

(Figures 3B & 3C). Liver sections of rats treated with malathion and cannabis at 10 mg/kg showed that most hepatocyte appeared normal, although few cells with karyolysis and red blood cells in blood sinusoids were seen (Figure 3D). In liver sections of rats treated with malathion and cannabis at 20 mg/kg most hepotocytes appeared normal but few red blood cells in blood sinusoids were still present (Figure 3E).

4. Discussion

In this study malathion treatment resulted in neuronal cell injury and liver damage. Oxidative stress was increased in the brain and liver as shown by the increased lipid peroxidation (malondialdehyde) and the decrease in GSH, suggesting increased generation of free radicals as a likely mechanism of the malathion-induced tissue injury. These observations are consistent with other studies that showed increased lipid peroxidation in brain, liver and erythrocytes[48-52] and decreased antioxidant enzyme activities, *e.g.*, glutathione peroxidase and glutathione reductase activities and total antioxidant capacity in brain[51,53] of rats treated with malathion. *In vitro*, human erythrocytes exposed to malathion showed an increase in malondialdehyde and a decrease in glutathione peroxidase, catalase, and superoxide dismutase enzyme activities[54]. There were also increased lipid peroxidation, and oxidized glutathione in mice cerebellar granule neurons by chlorpyrifos and diazinon caused[55]. The role of oxidative stress in mediating the malathion toxicity is also supported by the observations that vitamin C and vitamin E were able to reduce the biochemical changes caused by malathion in erythrocytes[48,54]. Our results also showed markedly increased nitric oxide concentrations in brain and liver following malathion exposure which is in agreement with previously published data[14,51]. The increased expression of the inducible form of nitric oxide synthase by malathion was reported in the rat brain and liver[51]. Moreover, the injurious effects of malathion on the brain and liver and the DNA damage of peripheral blood lymphocytes in the rat could be alleviated with nitric oxide synthase inhibitors[52]. These observations provided evidence for nitric oxide in contributing to the malathion-induced tissue injury.

Our results also shows that malathion caused significant and marked decrease in PON-1 activity in the brain and liver which is in agreement with previous studies[14,51,52]. The enzyme act to hydrolyse the active metabolites, *i.e.*, "oxons" of some organophosphates such as parathion, diazinon, chlorpyrifos and nerve agents[40] and a decrease in its activity due to genetic variation in enzyme activity increases the likelihood of developing Parkinson's disease in those exposed to organophosphates[8]. Variation in the maternal activity level of PON-1 also determines the adverse effects of exposure to organophosphates on the fetal brain[56]. In mice, deficiency of the enzyme increases the susceptibility to toxicity by chlorpyrifos[57]. Conversely, it was found the systemic injection of PON-1 that was purified from rabbit serum into rats resulted in increased plasma PON-1 activity and decreased acute toxicity of paraoxon, chlorpyrifos oxon and dichlorvos as indicated by the degree of AChE inhibition. This suggested a role for PON-1 in the treatment of acute poisoning due to organophosphates and increasing the ability of PON-1 to hydrolyze organophosphates might be a promising therapeutic option[58].

As shown in this study, malathion caused a significant and marked inhibition of brain BChE activity by 73.9% (P<0.05). Organophosphate insecticides inhibit cholinestersaes and this accounts for their neurotoxic manifestations due to the excessive stimulation of the cholinergic receptors by the raised acetylcholine levels[2,3]. In the management of acute toxicity by these agents, cholinergic blockade with muscarinic anticholinergic agent atropine and cholinesterase reactivators, *e.g.*, pralidoxime, are used[4]. Organophosphates inhibit both types of cholinesterase, *i.e.*, AChE and BChE. The latter is present in brain, plasma, smooth muscles, and heart[59]. Like AChE, it also hydrolyzes acetylcholine and in addition ester-containing drugs[59,60]. Purified human BChE was reported to protect against organophosphate toxicity in animals, thereby, suggesting a possible role for the enzyme in treating poisoning due to organophosphates[61,62].

The present study examined the effect of *Cannabis sativa* extract on the malathion-induced neuronal degeneration and liver injury in the rat. Our findings show that cannabis was able to protect against neurodegeneration and liver injury caused by acute malathion intoxication. The spongiform changes and signs of neurodegeneration in the cerebral cortex, the degeneration of Purkinje cells in the cerebellum and the vacuolar degeneration and foci of necrosis in the liver were all ameliorated by cannabis. These findings were unexpected in view of other studies indicating increased extent of the liver damage caused by acetaminophen or carbon tetracholoride by cannabis[37]. Cannabis also increased liver tissue damage and neuronal degeneration due to thioacetamide[38]. Histological evidence of liver injury was seen after both short- and long-term administration of *Cannabis sativa* in rats (in the form of mild vacuolar degeneration, cellular infiltration, increased number of Kupffer cells, increased DNA fragmentation, dilatation of the portal vein and fibrosis). There were also increased caspase-3 immunoreactivity in brain and liver of rats given cannabis[32,37,38]. Other researchers found elevated serum alanine aminotransferase, γ glutamyltransferase activities, and increased serum total bile acids, and bilirubin in marijuana smokers with no previous history of liver disorders[63]. Subjects with chronic hepatitis C virus infection and who smoke cannabis appear to have increased severity of steatosis and more rapid rate of fibrosis compared to non-smokers[35,36]. Cannabis given alone also resulted in the appearance of dark neurons, and cellular infiltration in the brain of rats[32] and in models of neuronal damage due to rotenone and/or lipopolysaccharide, cannabis failed to prevent neuronal damage despite the improved redox status of the cell as indicated by the decrease in lipid peroxidation and increased catalase activity and GSH[64,65]. Other *in vitro* studies showed that cannabis exerted neurotoxic effects and increased the generation of free radicals[30,31]. Neuroprotective effects, however, have been reported for cannabis in models of excitotoxicity and attributed to an antioxidant and radical scavenging actions[33,34].

Clearly, the protective effect of cannabis observed in the current study against tissue injury caused by malathion was not due to a decrease in oxidative stress or nitric oxide levels. The action of cannabis on brain 5-lipoxygenase also seems unlikely to contribute to the cannabis effect because of increased brain 5-lipoxygenase by the lower dose of the extract that resulted in improved brain histology. In rats, BChE has been shown to be inhibited in serum by cannabis extract[39]. AChE activity in serum was also inhibited by *Cannabis sativa* extract (Unpublished observations). Other researchers reported competitive inhibition of AChE by Δ^9-THC[66]. These data suggest that cholinesterases are amenable to modulation by cannabis or Δ^9-THC. In this study, cannabis given at 10 mg/kg caused further decrease in brain BChE activity by 26.1% (P<0.05) while a mild non-significant increase was observed after treatment with the higher dose of the extract. This finding suggests that cannabis also modulates brain BChE activity. Whether an inhibitory effect for cannabis on BChE or AChE activity underlies the protection by cannabis observed here is not clear, but in the same time still an intriguing possibility. Cannabis might competitively inhibit AChE and BChE and in this case will act to prevent the irreversible binding of the organophosphate metabolites onto the enzyme BChE and AChE. Cannabis, however, resulted in the restoration of the depleted GSH and PON-1 activity in both the brain and liver of malathion intoxicated rats. In other studies, cannabis was able to counteract the inhibition of PON-1 or GSH by rotenone in rat brain[65]. Cannabis given in normal rats was shown to increase serum PON-1 activity by 25.7%[39]. Studies indicated depressed plasma PON-1 activity in patients with chronic liver disease[67] and also after heptotoxicity in experimental animals[38]. The protective effect of cannabis can thus be explained by maintaining PON-1 activity and GSH levels which lend support to the notion that theses antioxidants play an important role in protecting against organophosphate toxicity.

In summary, we have shown administering a cannabis extract rich in Δ^9-THC was able to alleviate neurotoxicity and hepatotoxicity caused by acute malathion exposure in rats. Cannabis might mediate these effects through maintenance of PON-1 activity and restoration of the depleted GSH.

Conflicts of interest statement

The authors declare that they have no conflicts of interest.

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