

## Ameliorative Effect of *Mentha spicata* on Dichlorvos-Induced Oxidative Stress in the Rat Brain

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### Original Research Article

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**Abstract:** The present study was carried out to evaluate the protective role of aqueous extract of *Mentha spicata* (MSE) leaf on dichlorvos (DDVP) induced oxidative stress in rat brain. Male rats were divided into 4 groups, the first group served as control (C). Group (E1) received 5.33mg/Kg BW of DDVP and group (E2) received 5.33mg/Kg BW of DDVP with MSE (100.0 mg/kg) and the group (E3) received MSE (100.0 mg/kg). Rats under all the groups were treated with their respective constituents for 30 days. The results suggested that rats under E1 suffered from significant ( $p < 0.01$ ) decline in the activity of superoxide dismutase, catalase, glutathione peroxidase with a significant elevation in the levels of malondialdehyde of their brain. Histological changes were also found to be prominent in the brain of E1 group as compared to others. Rats under E2 showed recuperation tendencies which were evidenced by partial restoration of antioxidant enzyme activity and the lesser degree of damage in histological aspect as compared to E1. No significant changes were observed in either biochemical constituents or histological aspects of rats under E3. The current study suggests that DDVP can cause oxidative damage and brain injury in male rats and co-administration of MSE with the selected dose partially attenuates the toxic effect caused by DDVP.

**Keywords:** *Mentha spicata*, Brain, Dichlorvos, Oxidative stress and Xenobiotics.

### INTRODUCTION

Chemical contamination as a result of pesticide exposure in agriculture sector has been postulated as one of the major factors for the deterioration of natural fauna [1, 2].

Organophosphate pesticides (OPs) are the neurotoxin they inhibit acetylcholinesterase and pseudocholinesterase leading to the accumulation of acetylcholine and subsequent activation of cholinergic muscarinic and nicotinic receptors [3, 4]. Interference of these OPs in the human physiological system through consumption of contaminated foods, breathing air near pesticide applications and drinking pesticide contaminated water would cause negative effects in many organs, including the liver [5], heart [6], neuron [7] and respiratory dysfunction [8]. Dichlorvos [O, O-dimethyl-2, 2-dichlorovinyl phosphate (DDVP)] is one among the organophosphates, is extensively used to kill agricultural and veterinary pests [11]. DDVP has shown potential antagonist on the acetylcholinesterase (AChE) and cholinesterase (ChE) the enzyme that degrades the neurotransmitter ACh in cholinergic synapses, and disrupts nerve function activities, thereby loss of efficient neuron function in the nervous system [7, 9, 10]. DDVP is poisonous if inhaled, swallowed or absorbed through skin and eyes [11]. Exposure to high doses of DDVP leads to severe health effect; it includes headache, blurred vision, muscle tremors, breathing problems, coma and even death [12]. According to the

literature studies brain is very sensitive to DDVP [13, 14], it causes excessive generation of reactive oxygen species (ROS) and subsequently increased oxidative stress as well as neural cell death [14-17].

Currently, there is an increased demand for neuroprotective medicinal to overcome the xenobiotics induced neurotoxicity [18]. Many plants have medicinal property, which are enriched by antioxidants, which can play an important role in neutralizing free radicals and protect the key biomolecules such as DNA, proteins, and lipids by scavenging ROS [19]. *Mentha spp.* are a rich source of polyphenols and essential oil like carvone, limonene, dihydrocarvone [20-22], vitamin C, vitamin E and carotenoids, these acts as a strong antioxidant by neutralizing free radicals [23, 24]. A previous report shows *Mentha spp.* are well known folk remedy for treatment of several disorders [25]. According to recent pharmacological studies, *Mentha spp.* shows various biological activities, such as antiallergenic, antimicrobial, anti-inflammatory, antioxidant, antiviral, gastrointestinal protective, hepatoprotective, chemopreventive, anticancer and radioprotective activities [26, 61].

However, only few data about the neuroprotective effects of *M. spicata* against pesticide toxicity. Hence, the present study was designed to assess the ameliorative effect of aqueous extract of *M. spicata* leaf against DDVP induced neurotoxicity in male albino rats.

## MATERIALS AND METHODS

### Chemicals

Glacial acetic acid, Trichloroacetic acid (TCA), Ethylenediaminetetraacetic acid (EDTA), Thiobarbituric acid (TBA), Glutathione, Hydrogen peroxide ( $H_2O_2$ ), Phenazine methosulphate, NADH, NADPH, Sodium pyrophosphate, Sodium hydroxide, Hydroxylamine hydrochloride, Ferric chloride, all chemicals used were of analytical grade and obtained from HiMedia.

### Treatment of animals

Sixty days old clinically healthy adult male Wistar Albino rats, weighing  $250 \pm 25$  g. which were housed in polypropylene cages, at a room temperature of  $22 \pm 1^\circ C$ . The animals were given a basic diet and tap water *ad libitum*. The experimental group of Wistar rat were divided into four different groups, of five in each ( $n=5$ ). Rats were treated by intragastric administration. Rats in group C received corn oil and served as control. Group E1 received 5.33 mg/kg b.w of DDVP ( $1/9^{th}$  of  $LD_{50}$ ) [59], group E2 receives 5.33 mg/kg b.w of DDVP and MSE (100 mg/kg of b.w) and group E3 received only MSE (100 mg/kg b.w) for 30 days. At the end of treatment period, animals were euthanized under mild ether anesthesia and brain was harvested, rinsed in bouin's fluid for histology and in PBS for biochemical analysis.

### Preparation of *Mentha spicata* leaf aqueous extracts (MSE)

Fresh leaves of *Mentha spicata* plants were collected, washed, dried in shade, powdered and extracted with distilled water (DW) by refluxing for 36 h at  $80^\circ C$  and was given to each rat by intragastric administration with a dose 100mg/kg of b.wt./day [27].

### Superoxide dismutase activity (SOD)

SOD activity was estimated by the method of Kakkar *et al.*, [28]. Reaction mixture consists of 0.2 ml of phenazine methosulphate (0.186 mmol), 2.4 ml of sodium pyrophosphate buffer (0.052 mmol; pH 7.0) and 0.6 ml of the supernatant of tissue homogenate after centrifugation at 1500 rpm for 10 min, was added to the reaction mixture. Enzyme reaction was initiated by adding 0.2 ml of NADH (0.780 mmol) and stopped after 1 min by adding 1 ml of glacial acetic acid. Reaction mixture measured at 560 nm. The results are expressed in units/mg protein.

### Catalase activity

CAT activity was assayed by the method of Luck [29], wherein the breakdown of  $H_2O_2$  being measured at 240 nm. Briefly, the assay mixture consisted of 3 ml of  $H_2O_2$  (2mmol), phosphate buffer 0.067 M (pH 7.0) and 10% of 0.05 ml of supernatant of whole brain homogenate and the changes in absorbance were recorded at 240 nm using spectrophotometer. Enzyme activity was calculated using the mM extinction coefficient of  $H_2O_2$ . The results were expressed as  $\mu mol H_2O_2$  decomposed/min/mg protein.

### Glutathione peroxidase (GSH-Px)

The GSH-Px activity was determined by Paglia and Valentine [30]. The reaction medium was composed of potassium phosphate buffer (171 mmol), sodium azide (4.28 mmol), EDTA (2.14 mmol), reduced glutathione (6 mmol), NADPH (0.9 mmol), and glutathione reductase ( $2 \mu mol^{-1}$ ). The reaction took place at  $22^\circ C \pm 1$ , starting with the addition of  $H_2O_2$  0.72 mmol. The absorbance of the samples was measured at 340 nm using a spectrophotometer. The enzymatic activity expressed in enzymatic units per mL of sample  $U.g^{-1}$  of protein.

### Malondialdehyde (MDA)

Lipid peroxidation levels were assessed according to the method described by Buege and Aust [31] and was estimated by thiobarbutyric acid reactive substance (TBARS) assay performed by optically measuring malondialdehyde (MDA) in reaction with thiobarbituric acid. Optical density was measured by spectrophotometer at 530 nm. TBARS levels were expressed as nmol MDA/mg protein

### Histopathology

Histopathology was done by according to the method of Humason [32]. After the exposure period, rats were killed under the ether anaesthesia to excise brain and fixed in 10% neutral formalin, dehydrated in ascending grades of alcohol, and imbedded in paraffin wax. Paraffin sections ( $5 \mu m$  thick) were stained for routine histological study using haematoxylin and eosin (H&E).

### Ethical statement

All the experiments were conducted in accordance with IAEC, and the Committee for the Purpose of Control and Supervision of Experiments on Animals (animal house registration number 639/02/a/CPCSEA).

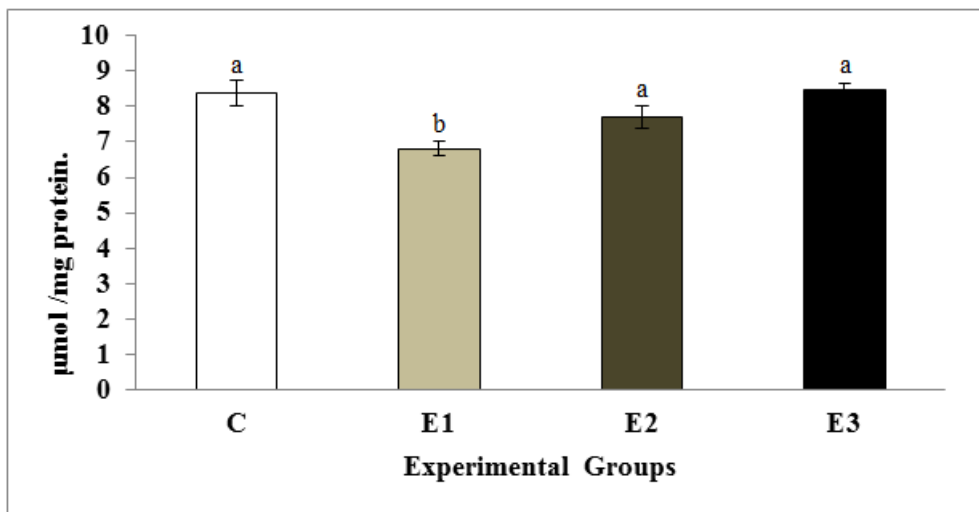
### Statistical Analysis

Statistical analysis was done using SPSS 21 program. All values expressed were as mean  $\pm$  SEM. Post-hoc analysis was done by turkey test. The statistical significance was assessed by one way ANOVA. P values  $<0.01$  were considered to be significant.

**RESULTS****Evaluation of enzymatic antioxidant activities in rat brain**

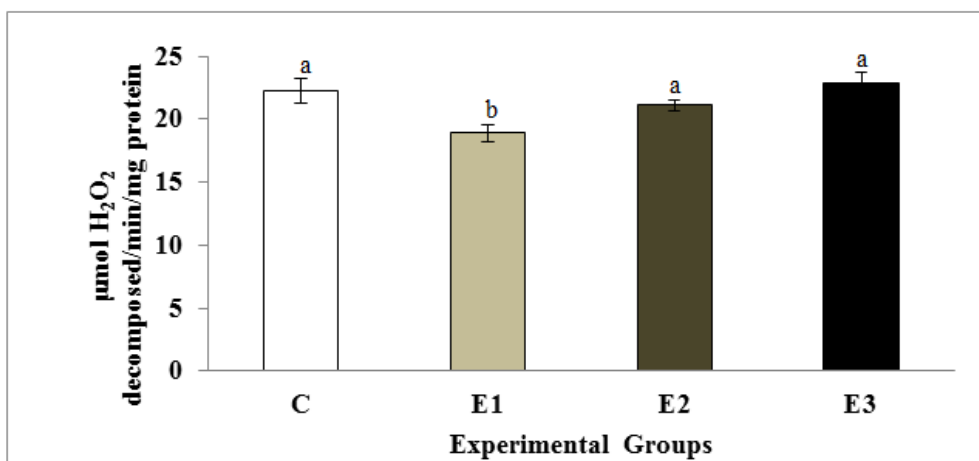
Compared to the control group the significant ( $p < 0.01$ ) decrease in the activity of superoxide dismutase (Fig-1) catalase (Fig-2), and glutathione peroxidase (Fig-3) in E1 group to tunes of -18.68, -

15.23 and -18.68%, respectively. Co-treatment of MSE to the rats intoxicated with DDVP exhibits significant ( $p < 0.01$ ) protection of the activities by -8.18, -5.45 and -2.11% in the E2 group respectively. Nevertheless, these enzymatic antioxidants were found to be insignificant in rats receiving MSE (100.0 mg/kg of b.wt) only (E3) as compared to control at 30 days of exposure tenure.



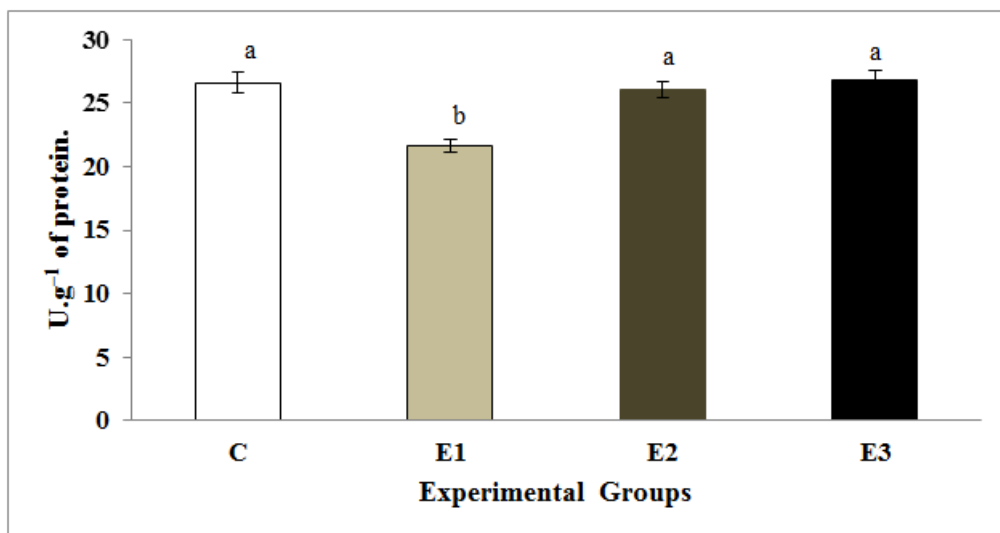
**Fig-1: Effect of *Mentha spicata* leaf aqueous extracts (MSE) on dichlorvos (DDVP)-induced alterations in superoxide dismutase (SOD) activity in brain tissue of control and treated rats.**

Each value is a mean of 5 rats  $\pm$  SE; Means having the same letters are insignificant from each other, at  $p < 0.01$ .



**Fig-2: Effect of *Mentha spicata* leaf aqueous extracts (MSE) on dichlorvos (DDVP)-induced alterations in catalase (CAT) activity in brain tissue of control and treated rats.**

Each value is a mean of 5 rats  $\pm$  SE; Means having the same letters are insignificant from each other, at  $p < 0.01$ .



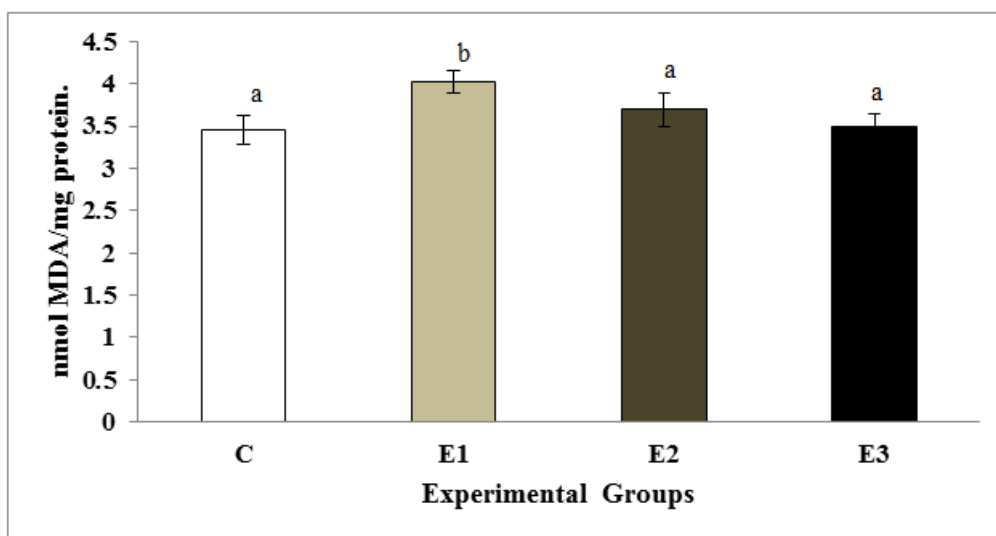
**Fig-3: Effect of *Mentha spicata* leaf aqueous extracts (MSE) on dichlorvos (DDVP)-induced alterations in glutathione peroxidase (GSH-Px) activity in brain tissue of control and treated rats.**

Each value is a mean of 5 rats  $\pm$  SE; Means having the same letters are insignificant from each other, at  $p < 0.01$ .

#### Malondialdehyde level (MDA)

The results shown in figure 4 indicated a significant ( $P < 0.01$ ) increased in the level of MDA in the brain tissue of rat at 30 days, by 16.55% in E1 group as compared to the control group. However, co-

administered with MSE resulted +7.18% of significant recovery in the E2 group. Moreover, the MDA levels were found to be 1.1% increased in the rat brain receiving only MSE (E3) as compared to control group.



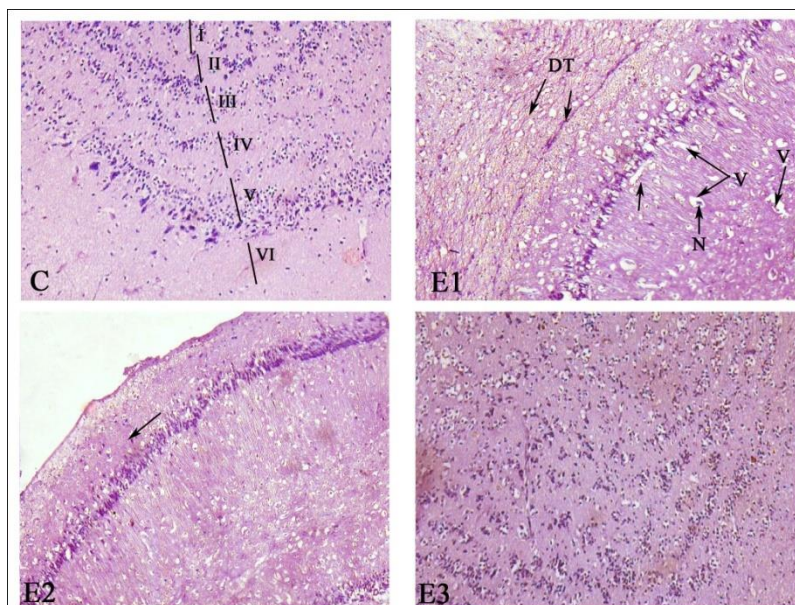
**Fig-4: Effect of *Mentha spicata* leaf aqueous extracts (MSE) on dichlorvos (DDVP)-induced alterations in Melondialdehyde (MDA) level in brain tissue of control and treated rats.**

Each value is a mean of 5 rats  $\pm$  SE; Means having the same letters are insignificant from each other, at  $p < 0.01$ .

### Histological results

As shown in Fig-5, Control rat brain displayed normal structure of six distinguished layers with no sharp boundaries in molecular layer, outer granular layer, outer pyramidal layer, inner granular layer, inner pyramidal layer and polymorphic layer (Fig-5C). Exposure to DDVP at given dose causes the severe

damage which includes increased vacuole size, necrosis and tissue degeneration in the brain (Fig-5E1) as compared to control. Co-administration of MSE could partially recover the damage in the brain by; diminution in vacuoles size, necrosis and tissue degeneration against DDVP induced histopathological alteration at 30 days of exposure. Nevertheless only MSE administration shows normal histological structure in rat brain as compared to control (Fig-5E3).



**Figure 5: Paraffin sections of brain stained by haematoxylin and eosin (viewed in 40X objectives) for histopathological changes. Control group (C) showing the normal structure of six distinguished layers with no sharp boundaries in molecular layer (I), outer granular layer (II), outer pyramidal layer (III), inner granular layer (IV), inner pyramidal layer (V) and polymorphic layer (VI). Group E1 showing, necrosis (N), vacuole (V), degenerative tissue (DT). Group E2 showing, vacuole. Group E3 showing normal histoarchitecture of brain.**

### DISCUSSION

Several studies have demonstrated that lipophilic nature of OPs interacts with cell membrane and causes lipid peroxidation there by increased generation of hydrogen peroxidase and other reactive oxygen species [39, 40]. However these produced hydrogen peroxidase interacts with  $Fe^{2+}$  and  $Cu^{+}$  generates hydroxyl radicals (OH) which are highly damaging factors to mtDNA [41]. Furthermore, production of toxic 4-hydroxynonenal aldehyde by hydroxyl radicals reacting with membrane lipids induces mitigation of proteins, lipids and DNA in brain tissues that leads neurodegenerative disorders [42-44]. The enzymatic antioxidants such as SOD, CAT and GSH-Px are the most important defenses against toxic effects of ROS. SOD is one of the key enzymes that provide the first line of defense against pro-oxidants and catalyses conversion of superoxide radicals to hydrogen peroxide, whereas CAT and GSH-Px helps in the removal of the  $H_2O_2$  formed during the reaction catalyzed by SOD [45-49]. Maintaining the balance between ROS and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and

glutathione peroxidase (GSG-Px), is therefore, crucial and could be an important mechanism for preventing oxidative damage [50]. Our results revealed that DDVP caused a statistically significant decrease in the activity of SOD, CAT, and GSH-Px in brain of rats. Therefore, any impairment in this pathway will affect the activities of antioxidant enzymes in the cascade [51]. However, reduction in the activity of SOD will result in an increased level of  $O_2^{\cdot -}$ , while a decrease in the activity of CAT will lead to accumulation of  $H_2O_2$  in the cell, which leads to peroxidation of membrane lipids [49, 53].

MDA is one of the most widely used indicators of the cellular redox state and final metabolites of peroxidized polyunsaturated fatty acids are considered as a late biomarker of oxidative stress [52]. In the present study, we observed significant ( $P < 0.01$ ) increase in the levels of MDA in the brain of DDVP treated rat. The possible explanation for this effect could be that the increase in the formation of brain lipid peroxidation in DDVP-intoxicated animals acted as a signal to maintain lower levels -of antioxidant enzymes

in order to enhance the detoxification process for the DDVP. So, the decrease in the activity of SOD, CAT, and GSH-Px in DDVP-intoxicated animals indicates insufficient detoxification of DDVP in rats. The decline of antioxidant enzymes activity in our study is supported by earlier findings [53, 54]. Further, exposure of animals to organophosphates decreased antioxidant enzymes activity following increment in lipid peroxidation, as assessed by the elevated levels of MDA, has been well documented in previous reports [55-58].

Furthermore, histological impairments in the DDVP intoxicated rat brain are more pronounced, with symptoms of vacuoles, necrosis and tissue degeneration. This histological damage is due to increased levels of TBARS, and H<sub>2</sub>O<sub>2</sub>, which certainly play a vital role in the pathogenesis of brain injury in DDVP treated rat [59]. Moreover, various studies reported that exposure to DDVP leads to the oxidative stress and neural tissue damage [9, 17]. Brain of rats treated with MSE (E3) showed no obvious lesions on histopathological examination which is similar to that of the control. This is evidence of an ameliorative and possibly neuroprotective effect of the MSE

Nowadays, natural antioxidants are of take greater interest due to their safety and beneficiary effects against toxicant induced oxidative stress. In present investigation, we demonstrated that long term oral intake of leaf aqueous extract of *Mentha spicata* (100mg/kg) protected the antioxidant status of brain against the DDVP-induced oxidative stress, with significant increase in SOD, CAT, and GSH-Px activity and decrease in the levels of MDA. According to the previous reports administration of *Mentha spp.* extract reduces the lipid peroxidation and up-regulation of the CAT and SOD, in the brain of gamma radiated rat brain [60]. Further, this study coincides with Ojo *et al.*, [54] who demonstrated *Alstonia boonei* showed a significant protective effect by decreasing the level of lipid peroxidation and elevating the activities of CAT, SOD and GSH-Px enzymes in the brain. The current neuroprotective activity of MSE may be assigned to its different functional constituent like antioxidants flavonoids, caffeic acid, eugenol,  $\alpha$ -tocopherol [21, 61]. Polyphenols and Vit C and Vit E, and carotenoids these are able to convert the reactive free radicals to inactive products [20, 60, 57]. Polyphenols regulate mRNA levels of CAT, GSH-Px, SOD, thus counteract oxidative stress induced by DDVP [59, 58].

This study provides evidence that antioxidant rich plant extract offers safety against DDVP induced oxidative stress. The antioxidant products present in the *Mentha spicata* are potential neuroprotective and need further experimental investigation for their use as therapeutic agents for treating neurodegenerative disorders involving oxidative stress.

## CONCLUSION

In the present study, dichlorvos causes the oxidative damage and brain injury in the rat brain by altering the enzymatic and non-enzymatic antioxidants. Further, administration of *Mentha spicata* leaf aqueous extract (MSE) could partially ameliorate oxidative damage in the rat brain. But, the exact mechanism of prevention of brain tissues damage remains to be ascertained. However, MSE appeared to be a promising agent for protection against dichlorvos-induced oxidative damage in the brain. Therefore, results suggest that MSE can be used as pharmacological compounds to protect humans exposed to OPs against their neurodegenerative diseases.

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