doi.org/10.36721/PJPS.2022.35.2.SP.671-678.1

Thymol mitigates cadmium-induced behavioral and cognitive deficits by up-regulating hippocampal BDNF levels in rats

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Abstract: Cadmium is a potent neurotoxin and induces adverse impact on brain function. Protective effects of monoterpenes on the CNS have been reported previously. The present study was designed to investigate the beneficial effect of thymol on cadmium-induced neurotoxicity. Rats were initially divided into 2 groups, vehicle control and thymol. Thymol (40mg/kg) was given orally for 14 days. Each group was subdivided into two groups (Vehicle control and Cadmium, Thymol and Thymol+Cadmium). Cadmium Chloride (5mg/kg) was given for last 3 days only to the groups assigned as Cadmium and Thymol+Cadmium. Behavioral parameters were assessed after 24h of last dose of cadmium. Brain sample were collected and BDNF was measured in hippocampus. The present study suggests that pre-administration of thymol provides a protective therapy against cadmium-induced intoxication by enhancing the brain BDNF levels and plasticity. Results further suggest that thymol not only ameliorates cadmium-induced learning and memory impairment but also reduced anxiety, motor incoordination and depression assessed by various behavioral tests. The study may provide a better apprehension of the neuroprotective role of thymol and highlighting its significance in the diet for human health particularly in cadmium intoxication.

Keywords: Thymol, cadmium, BDNF, hippocampus, memory.

INTRODUCTION

Cadmium is one of the most commonly occurring toxic industrial and environmental pollutants. Toxicity of cadmium has been reported to produce damaging effects on various vital organs including the CNS (Levin-Schwartz *et al*., 2022). Studies have shown that cadmium is a neurotoxin has the ability to bypass the blood-brain barrier and therefore accumulate in the brain (Branca *et al*., 2018). Cadmium is capable of causing alteration in the synthesis of various important neurotransmitters after entering in brain (Vijaya *et al*., 2020). Neurobehavioral disturbances induced by cadmium are supposed to be associated with Alzheimer's disease, developmental delays, neurological defects, learning disabilities and parkinsonian like symptoms (Lamtai *et al*., 2020; Rahimzadeh *et al*., 2017). Exposure to cadmium chiefly occurs by the consumption of contaminated water, food and most commonly through cigarette smoke and inhalation of cadmium fumes or dust in workers associated with battery manufacturing, soldering, and pigment production industries (Rahimzadeh *et al*., 2017; Genchi *et al*., 2020). It has been reported that an individual's dietary pattern may modify vulnerability to metal toxicity (Fernstrom and Fernstrom, 2007). In recent times, due to fewer side effects nutraceuticals have attracted the attention of researchers as the potential therapy for the treatment of heavy metals poisoning (Mehrandish *et al*., 2019). They improve health by delaying the aging process, increase life span, prevent chronic diseases, or support the function and structure of the body. Thymol (2-isopropyl-5-methylphenol) has been identified as one of the active constituent in thyme species with various pharmacological properties including free radical scavenging, antioxidant, anti-inflammatory, antispasmodic, analgesic, antibacterial, antiseptic antifungal and antitumor activities (Kuete, 2017; Meeran *et al*., 2017; Escobar *et al*., 2020; Gholami-Ahangaran *et al*., 2020). Overall thymol plays an important role in improving general health and immunity and reduces the problems of many animal and human disorders (Alagawany *et al*., 2021).

The potential of thymol to mitigate cadmium-induced neurobehavioral changes in rat and the role of hippocampal BDNF levels have not been investigated yet. Hence the aim of this study was to assess the effect of thymol administration on cadmium-induced neuro-chemical and behavioural impairments in rats and its association to the hippocampal BDNF levels.

MATERIALS AND METHODS

Thirty two male Albino-Wistar rats weighing 180-200 gm were purchased from animal house of Dow University of Health Sciences (DUHS), OJHA campus Karachi, Pakistan. To avoid effect of social interaction, animals were housed individually with ad libitum access to cubes of standard rodent diet (Bocarsly *et al*., 2012) and tap water under a 12:12 h light/dark cycle (lights on at 7:00

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am) at controlled room temperature (22 ± 2°C). In order to reduce the stress of novelty and handling animals were subjected to 3 days of acclimatization period and to various handling trials before experiments. All experiments were carried out in a balanced design (8 animals/group) to avoid influence of order and time. Thymol and cadmium were purchased from Merck (Germany).

Ethical approval

The experimental procedures were approved by the institutional Advanced Studies and Research Board (ASRB/03202/Sc.) and performed in strict accordance with National Institute of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985).

Experimental protocol

Animals were divided into two groups, vehicle control (VC) and thymol (THY). An oral dose of thymol (40 mg/kg/ml in slightly warm water) was administered daily for 14 days to the THY group while the VC group was given saline and water orally. On the 12th day each group was subdivided into 2 groups (VC and Cd, THY and THY+Cd). Cadmium ($CdCl_2$) was given orally at a dose of 5 mg/kg for last three days (i.e. 12^{th}, 13^{th}, 14^{th} days) to cadmium assigned groups only. Doses of thymol and cadmium were selected on the basis of previously reported studies (Saravanan and Pari, 2016; Renugadevi and Prabu, 2010). After 24 h of last dose of cadmium administration different behavioral tests were performed. Memory function was assessed using the EPM and NOR. The depressogenic effect was monitored in TST. Anxiogenic behaviors were assessed by LDT and MBT. Motor coordination and locomotor activities were done by using IST and OFT. Thymol administration was continuous till the end of the behavioral investigation. After behavioral analysis rats were decapitated and brain samples were collected. The hippocampal region was separated for the estimation of BDNF levels which then stored at -20°C.

Behavioral analysis

Activities of all groups were monitored in a balance design in order to avoid the order effect.

Tests to access memory and cognition

Elevated Plus Maze

Elevated plus maze test is employed to access spatial memory and learning in animals. Elevation and open arms of the maze serve as aversive stimuli for rodents as they are nocturnal animals and tend to prefer to move to closed arm for safety. The method is same as described by Mutlu *et al*. (2011). The test comprised of two sessions (acquisition and retention). In the acquisition session each rat was positioned at the end of open arm facing away from the central platform and the transfer latency to come into one of the closed arm with all four paws was monitored. After 24h retention session was performed and transfer latency was again monitored.

Novel object recognition test

The test is used to estimate cognitive function principally recognition memory in rats. It is based on the natural tendency of rats to pass more time exploring a new object than an old one. The apparatus was an open box (40 x 40 x 40 cm) made up of wood painted with grey colour. The floor was covered with saw dust. The method was fundamentally the same as defined previously (Batool *et al*., 2016).

Test to access depression-like symptoms

Tail suspension test

The test was established by Steru *et al*. (1985) and is most commonly employed to estimate despair like symptoms in rats. Exposure of rats to inescapable, short term stress of being hanged by their tail will develop an immovable posture is the bases of this test (Naqvi *et al*., 2012). The time spend by rat in an immobile posture is recorded.

Tests to access anxiety-like symptoms

Light/dark transition test

The light/dark transition test is most commonly employed to evaluate anxiety-like symptoms in rats. The natural aversion of rats to brightened areas is the bases of the test. The apparatus consists of two cubicles joined with a door. First the rat was placed in the light cubicle and the extent of anxiety was determined by monitoring the time spent in the light box (Khaliq *et al*., 2012).

Marble burying test

The test is broadly employed to explore repetitive, obsessive-compulsive like behaviors and anxiety in rats (Brouwer *et al*., 2018; Dixit *et al*., 2020). The rats will bury both harmless and harmful objects in their bedding considering them as noxious stimuli (Kedia and Chatteiji, 2014). In this test 20 glass marbles in a regular grid pattern were placed in a cage containing sawdust and then allowed to explore the cage for 20 minutes. The number of marble covered in sawdust by each rat is counted.

Test to access ambulatory activity and motor-coordination

Inverted screen test

This test is widely employed to assess motor coordination/ strength in rats. The test was established by Kondziela in 1964. The rats were placed separately on top of a square screen made up of wire. The screen was then rotated 180o so that rat were on the base of the square screen. The time taken by rat to fall off was noted for 1 min.

Open field test

Open field test is used for the evaluation of ambulatory performance and examination in a new atmosphere. It is

simple assessment of behaviors that require no animal training. The locomotor activity of animals was monitored in the open field apparatus that consisted of a square area (76 × 76 cm) having 42 cm high walls. The floor of the arena was equally divided by the help of lines into 25 equal squares. To determine locomotor activity, the animal was placed in the central square of the open field arena and allowed to explore the area. The number of squares crossed by animal with its four paws was counted for 5 min as reported earlier (Perveen *et al*., 2009).

Estimation of hippocampal BDNF levels

The levels of brain-derived neurotropic factor in hippocampal region were analyzed through ELISA assay method using commercially available kit (Rat BDNF Elisa Kit, Picokine. Cat. No. EK0308, Boster Biological Technology, CA, USA). Homogenization along with centrifugation of hippocampal region in PBS buffer (pH =7.4) was done as described earlier with slight modification (Afzal *et al*., 2021). Supernatant was collected which was then used for the estimation of BDNF levels by using the manufacturer's protocol. The BDNF levels were expressed as pg/g of hippocampal tissue. Fig.8(a) showing the standard curve used to calculate the hippocampal BDNF levels.

STATISTICAL ANALYSIS

The statistical evaluation was executed by ANOVA (two-way) and while post-hoc analysis was done by Tukey's test via SPSS version 20. Results are indicated as the mean ± SD; $p<0.05$ was observed significant.

RESULTS

Effect of Thymol on Cd-induced memory impairments

Fig. 1(a) shows the effect of pre-administration of thymol on STM and LTM assessed by EPM. The test was employed to measure retention of unpleasant memory in rats. The ANOVA (two-way) showed a significant effect of thymol on STM ($F_{3,28}$=12.998, $p<0.01$) and LTM ($F_{3,28}$=16.157, $p<0.01$), a significant effect of administration of cadmium for last three days on STM ($F_{3,28}$=15.937, $p<0.01$) and LTM ($F_{3,28}$=21.487, $p<0.01$), and non-significant interaction between the two factors for STM ($F_{3,28}$=1.055) and LTM ($F_{3,28}$=1.832). Tukey's test showed that prior administration of thymol in THY+Cd group for two weeks significantly ($p<0.01$) improved the retention of memory in rats as evident by decrease in transfer latency in STM and LTM as compared to the Cd treated group only. The significant ($p<0.01$) improvement in memory retention was also observed in THY group compared to VC controls in LTM only. In addition to this, cadmium treated rats showed impaired learning abilities as the rats exhibited increase transfer latency in both types of memory forms (STM and LTM) as compared to their respective controls.

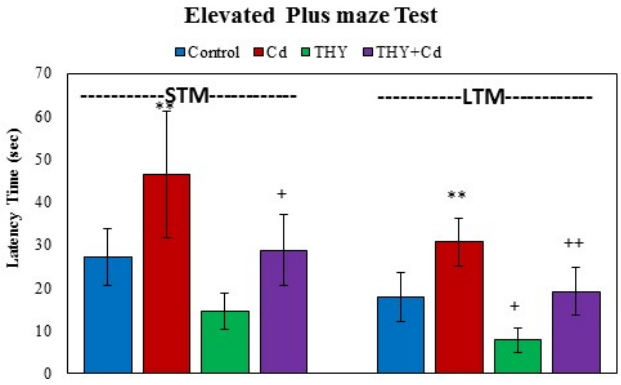

Fig. 1(a): Effect of pre-treatment of Thymol on Cd-induced memory impairments in EPM. Data represented as mean ± SD; (n=8) rats per group. Significance difference was done by Tukey's test. **p<0.01; +p<0.05; ++p<0.01 verses respective controls.

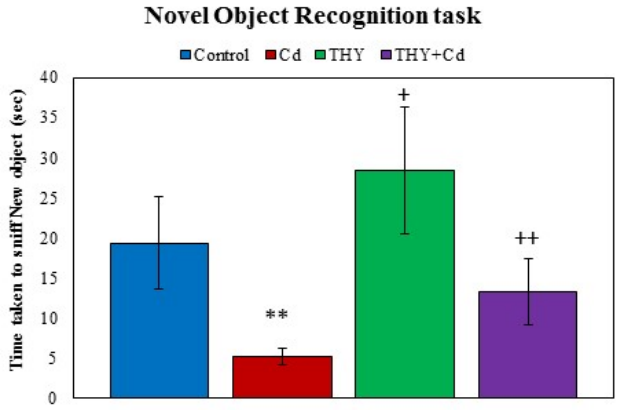

Fig. 1(b): Effect of pre-treatment of Thymol on Cd-induced memory impairments in NOR. Data represented as mean ± SD; (n=8) rats per group. Significance difference was done by Tukey's test. **p<0.01; +p<0.05; ++p<0.01 verses respective controls.

The cognitive ability of rats was evaluated by NOR (Fig. 1b). The ANOVA (two-way) showed a significant effect of pre-administration of thymol ($F_{3,28}$=10.696, $p<0.01$), a significant effect of administration of cadmium for last three days ($F_{3,28}$=38.372, $p<0.01$) on cognitive function. Following administration of thymol Tukey' test showed a significant ($p<0.01$) improvement in cognitive function as compared to the VC group. The significant ($p<0.01$) improvement was also observed in a THY+Cd group as compared to the Cd treated rats only. In Cd treated group impaired cognitive ability ($p<0.01$) was found. In essence a greater consolidation of memory was found in rats that were pre-administered with thymol.

Effect of Thymol on Cd-induced Depressogenic behavior

The depression-like behavior was assessed by TST with a measure of immobility time. The ANOVA (two-way) showed a significant effect of pre-administration of

thymol ($F_{3,28}$=36.536, p<0.01) on immobility time. Cadmium administration for last three days significantly affected immobility time ($F_{3,28}$=20.442, p<0.01) in TST (Fig. 2). Tukey' test showed administration of cadmium elicited depressogenic like effects, as increased (p<0.01) immobility time was observed in Cd-induced rats as compare to the respective control rats. Moreover Cd-induced depressogenic behaviors were significantly mitigated by pre-treatment with thymol. The THY and THY+Cd groups exhibited significantly decreased immobility time (p<0.01) compared to the rats treated with VC and only cadmium respectively.

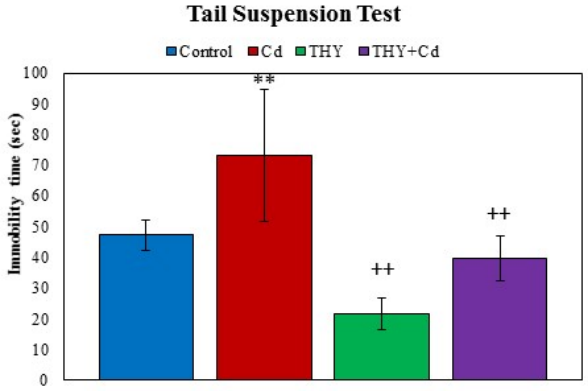

Fig. 2: Effect of pre-treatment of Thymol on Cd-induced Depressogenic behavior in TST. Data represented as mean ± SD; (n=8) rats per group. Significance difference was done by Tukey's test. **p<0.01; +p<0.05; ++p<0.01 verses respective controls.

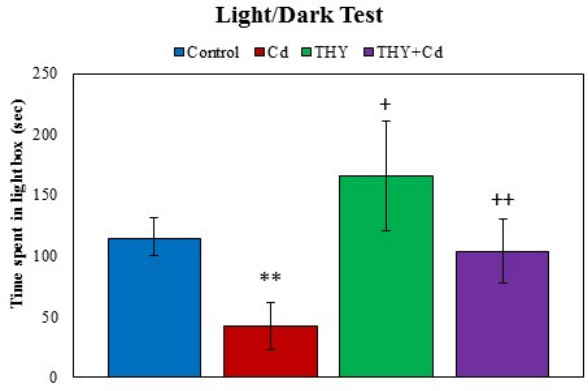

Fig. 4(a): Effect of pre-treatment of Thymol on Cd-induced motor dysfunction in IST. Data represented as mean ± SD; (n=8) rats per group. Significance difference was done by Tukey's test. **p<0.01; +p<0.05 verses respective controls.

Effect of Thymol on Cd-induced Anxiogenic behavior

Effects of pre-administration of thymol and cadmium administration for last three days were also assessed in the LDT (Fig. 3a). Statistical analysis by two way-ANOVA showed that time spent in the bright compartment was significantly changed by the treatment of thymol ($F_{3,28}$=23.581, p<0.01) and cadmium ($F_{3,28}$=33.741, p<0.01). Tukey' test indicated that pre-treatment of thymol for two weeks significantly increased time spent in bright compartment (p<0.05) compared to VC group. Cadmium administration for the last three days exerted anxiogenic effect indicated by decreased in time spent in the bright compartment (p<0.01) when compared with VC rats. The THY+Cd group also showed a significant (p<0.01) increased in time spent in bright compartment compared to Cd-treated rats only, reflecting that pre-administration of thymol attenuates the Cd-induced anxiety.

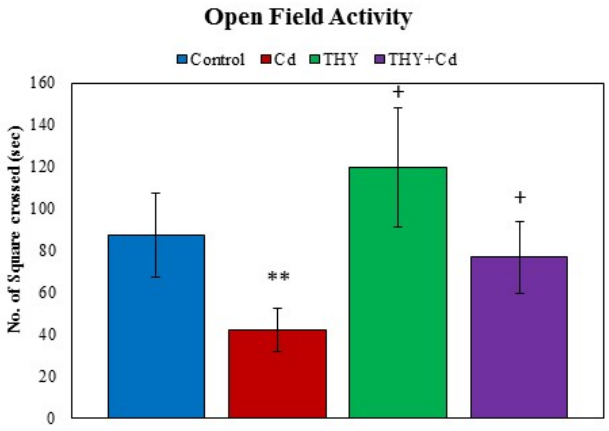

Fig. 4(b): Effect of pre-treatment of Thymol on Cd-induced reduced locomotion in OFT. Data represented as mean ± SD; (n=8) rats per group. Significance difference was done by Tukey's test. **p<0.01; +p<0.05 verses respective controls.

Anxiogenic behavior was also assessed in marble burying test (Fig. 3b). Statistical analysis by two-way ANOVA showed a significant effect of pre-administration of thymol ($F_{3,28}$=17.102, p<0.01) and cadmium ($F_{3,28}$=33.106, p<0.01) on % burying behavior. Tukey' test indicated that pre-treatment of thymol significantly decreased (p<0.01) in % marble burying in THY+Cd group as compared to Cd treated rats only, while rats treated with THY showed a non-significant increase in % marble burying compared to VC group. In addition to this cadmium treatment for the last three days exerted anxiety like behavior as shown by significant (p<0.01) increased in burying behavior compared with respective controls.

Effect of Thymol on Cd-induced motor dysfunction and reduced locomotion

The effect of pre-administration of thymol on motor co-ordination was assessed by IST (Fig. 4a). Data analysis by two-way ANOVA revealed a significant ($F_{3,28}$=14.346, p<0.01) effect of thymol and cadmium ($F_{3,28}$=20.690, p<0.01) on motor co-ordination. Tukey' test determined that cadmium treatment for the last three days significantly (p<0.01) increased the latency to fall compared to the respective control. On other hand pre-treatment of thymol significantly decreased (p<0.05) the latency to fall in THY+Cd group as compared to

cadmium treated rats only. THY treated group also showed a decreased in latency to fall in IST as compared to VC group but the result was non-significant.

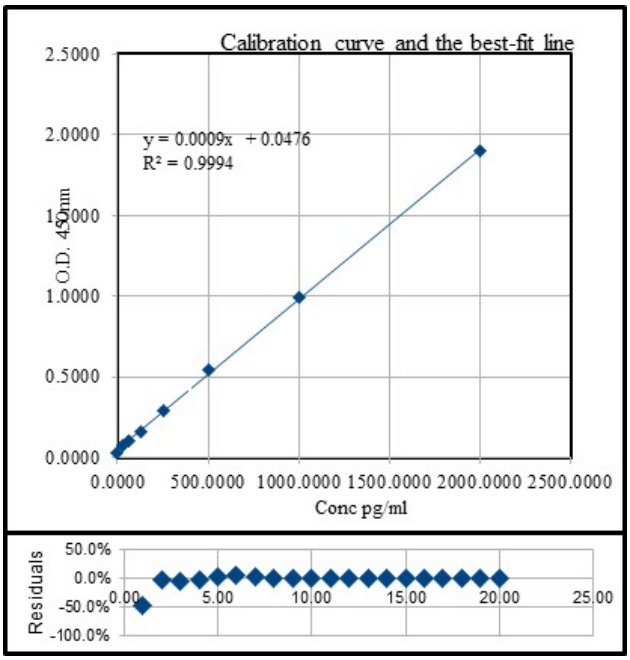

Fig. 5(a): Standard curve for the estimation of Hippocampal BDNF levels

The effect of pre-administration of thymol on locomotor activity was assessed by OFT (Fig. 4b). Data analysis by two-way ANOVA revealed a significant ($F_{3,28}$=20.520, p<0.01) effect of thymol and cadmium ($F_{3,28}$=30.257, p<0.01) on locomotor activity. Tukey' test determined that cadmium treatment for the last three days significantly (p<0.01) decreased the number of square crossed with compared to the respective control. In addition to this, pre-treatment of thymol significantly increased the number of square crossed in THY (p<0.05) and THY+Cd (p<0.01) group as compared to VC group and cadmium treated rats respectively.

Effect of Thymol on Cd-induced Decreased Hippocampal BDNF levels

Fig. 5(a) shows the standard curve for the estimation of Hippocampal BDNF levels. In ANOVA (two-way) it was observed that there was also a significant effect of pre-treatment of thymol ($F_{3,28}$=770.50, p<0.01), cadmium ($F_{3,28}$=411.6, p<0.01) and interaction between the two factors ($F_{3,28}$=97.163, p<0.01) on hippocampal BDNF levels (Fig. 5b). Tukey' test indicated that pre-treatment of thymol in THY and THY+Cd groups exhibited significantly increased levels (p<0.01) of BDNF as compared to that of VC controls and cadmium treated group respectively. Cadmium treated rats showed significantly (p<0.01) reduced levels of BDNF with compared to the respective control rats. Increased levels of BDNF in both thymol administered groups compared to cadmium treated and VC groups showed improvement in hippocampal functions by thymol treatment.

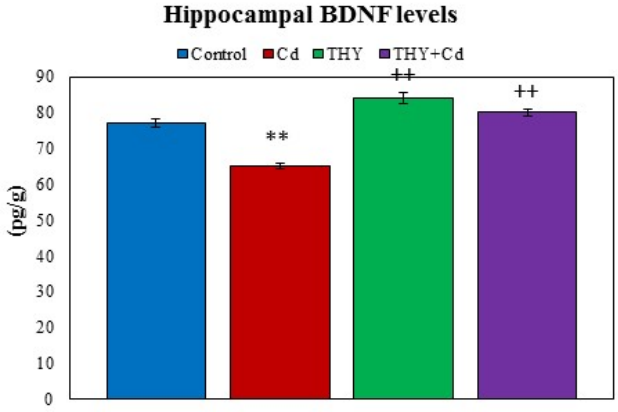

Fig. 5(b): Effect of pre-treatment of Thymol on Cd-induced reduced Hippocampal BDNF levels. Data represented as mean ± SD; (n=8) rats per group. Significance difference was done by Tukey's test. **p<0.01; ++p<0.05 verses respective controls.

DISCUSSION

The present study was designed to investigate the effect of thymol on cadmium induced toxicity by assessing the different behavioral parameters to understand the brain function. The study indicated that pre-administration of thymol significantly attenuated the cadmium induced toxicity. In the present study acute administration of cadmium significantly impaired memory while pre-administration of thymol for 14 days significantly attenuated the memory decline as shown by decreased transfer latency in EPM and increased time to sniff new object in NOR. Previously it has been reported that cadmium intoxication resulted in oxidative burden (Agha *et al*., 2020). Exposure to heavy metals have shown to induced production of free-radicals and resulted in denaturation of proteins that may lead numerous diseases (Fu and Xi, 2020). Cadmium exposure has shown to induce increased lipid peroxidation and produced an inhibitory effects on antioxidant enzymes which are involved in removal of reactive radicals and resulted in the reduction of oxidative stress (Renugadevi and Prabu, 2010; Branca *et al*., 2018). The high reactivity of free radicals leads to an increase propagation of oxidative stress which can additionally weaken the antioxidant mechanism leading to impaired neuronal activity, apoptosis and ultimately memory dysfunction (Ferdinando Franzoni *et al*., 2021; Lobo *et al*., 2010). Earlier studies demonstrate that cadmium is a neurotoxin and its administration modifies memory and learning functions (Lukawski *et al*., 2005). Exposure to cadmium has previously shown to inhibit acetyl choline esterase activity (Alshammari *et al*. 2021). This inhibition of enzyme activity not only decreases the breakdown of acetylcholine but also the reuptake of its precursor

molecule choline (Haider *et al*., 2015). This decrease in acetylcholine may be related to decreased memory function exerted by cadmium administration. Consumption of dietary source of exogenous antioxidants is essential in curbing damages exerted by free-radicals, particularly in the CNS to extenuate heavy metal-induced oxidative stress (Agha *et al*., 2020). The results of present study are in agreement with the previously reported studies (Abdolkarim Hosseini *et al*., 2021; Luminita Capatina *et al*., 2020; Kristina Bacova *et al*., 2020) as the pre-treatment of thymol helped in the attenuation of cadmium induced memory impairment evident by decreased transfer latency and increased time to sniff new object in both behavioral assessment tests.

In the present study, acute cadmium administration produced a significant reduction in locomotor activity in OFT and impaired motor coordination in IST. Impaired activities in different behavioral assessment tests following cadmium exposure has also been reported earlier (Gagnaire *et al*., 2011). Oxidative stress alters the neurotransmission and neuronal function (Schieber and Chandel, 2014). Data also showed that parental exposure to cadmium reduces serotonin, dopamine and acetylcholine levels in brain of first generation offspring (Tian *et al*., 2021). Thymol treatment in rat model of Parkinson disease significantly mitigated loss in the dopaminergic neurons, inflammation and oxidative stress (Javed *et al*., 2019). The current study also strengthened the previous finding and suggesting the neuroprotective effects of thymol against cadmium induced motor incoordination.

In the present study acute cadmium administration produced a significant decrease in time spent in the central square in OFT and the light compartment in LDT box. Similarly increases in burying behavior in MBT and immobility time in TST have also been observed in the present study. Significant alterations in different behavioral paradigms in the present study in rats following acute cadmium administration may be explainable as an index of anxiogenic and depressogenic effect. Hypo-locomotor activity has been associated with depression-like symptoms in rats (Chkhartishvili *et al*., 2011). Studies have shown that cadmium treated rats exhibited increase in anxiety in a dose related manner and cadmium produces anxiogenic effect by alteration in biochemical activity of the brain (Levin-Schwartz *et al*., 2022; Lamtai *et al*., 2018). The cadmium induced depressogenic effects have been associated to the oxidative deterioration and concomitant modified serotonin levels (Romero *et al*., 2011). Moreover in the present study pre-administration of thymol significantly attenuates cadmium induced behavioral alterations evident by increase time spent in bright compartment, decrease in the burying behavior and immobility indicating anxiolytic and antidepressant effect respectively. Previous data suggested that thymol retrieved depression like symptoms and increases the BDNF levels in hippocampus in corticosterone induced depression model (Capibaribe *et al*., 2019). Up-regulation of neurotransmitters and inhibition of pro-inflammatory cytokines by the administration of thymol have also been reported in chronic unpredictable mild stress mice (Deng *et al*., 2015). In the present study acute administration of cadmium declines the hippocampal BDNF levels as compared to control rats. Reduced neurogenesis particularly impaired neuronal differentiation and axonogenesis following cadmium induced neurotoxicity resulted to neuronal death. The brain-derived neurotrophic factor has neuroprotective effects in various brain disorders (Flores *et al*., 2020). It is a main protein implicated in neuroplastic changes associated with memory and learning process. Changes in the BDNF expression in hippocampus and parahippocampal areas that particularly involve in memory process have also been reported (Mourao *et al*., 2019). Pre-administration of thymol in the present study increases the BDNF levels compared to the cadmium administered rats. Our findings clearly indicate that thymol helps to regulate hippocampal functions by increasing BDNF levels and improve brain plasticity. Hence this increase in BDNF levels is not only responsible for decrement in cadmium induced depression like symptoms as reported previously but also responsible for improved motor coordination, learning and memory.

CONCLUSION

In conclusion BDNF is responsible for the increased plasticity and hence improved brain function. Increased BDNF levels in brain in thymol administered rats played a crucial role in reversing cadmium induced toxicity in brain and represent a significant contribution to understand the pathogenesis of cadmium toxicology

REFERENCES

Agha F, Batool Z, Batool TS, Nisar R, Naqvi F, Saleem S, Sajid I and Haider S (2020). Tree nuts supplementation instigates the oxidative status and improves brain performance in male rats. *Pak. J. Pharm. Sci*., **33**(Ssuppl.): 2785-2791.

Alagawany M, Farag MR, Abdelnour SA and Elnes SS (2021). A review on the beneficial effect of thymol on health and production of fish. *Reviews in Aquaculture*, **13**(1): 632-641.

Alshammari GM, Al-Qahtani WH, Alshuniaber MA, Yagoub AE, Al-Khalifah AS, Al-Harbi LN, Alhussain MH, AlSedairy SA and Yahya MA (2021). Quercetin improves the impairment in memory function and attenuates hippocampal damage in cadmium chloride-intoxicated male rats by suppressing acetylcholinesterase and concomitant activation of SIRT1 signaling. *J. Funct. Foods*., **86**: 104675.

Bacova K, Zitterl-Eglseer K, Chrastinova L, Laukova A, Madarova M, Gancarcikova S, Sopkova D, Andrejcakova Z and Placha I (2020). Effect of thymol addition and withdrawal on some blood parameters, antioxidative defence system and fatty acid profile in rabbit muscle. *Animals*, **10**(8): 1248.

Batool Z, Sadir S, Liaquat L, Tabassum S, Madiha S, Rafiq S, Tariq S, Batool TS, Saleem S, Naqvi F and Perveen T (2016). Repeated administration of almonds increases brain acetylcholine levels and enhances memory function in healthy rats while attenuates memory deficits in animal model of amnesia. *Brain. Res. Bull*., **120**: 63-74.

Bocarsly ME, Barson JR, Hauca JM, Hoebel BG, Leibowitz SF and Avena NM (2012). Effects of perinatal exposure to palatable diets on body weight and sensitivity to drugs of abuse in rats. *Physiol. Behav*., **107**(4): 568-575.

Branca JJ, Morucci G and Pacini A (2018). Cadmium-induced neurotoxicity: Still much ado. *Neural. Regen. Res*., **13**(11): 1879.

Capatina L, Todirascu-Ciornea E, Napoli EM, Ruberto G, Hritcu L and Dumitru G (2020). *Thymus vulgaris* essential oil protects zebrafish against cognitive dysfunction by regulating cholinergic and antioxidants systems. *Antioxidants*, **9**(11): 1083.

Capibaribe VC, Vasconcelos Mallmann AS, Lopes IS, Oliveira IC, de Oliveira NF, Chaves RD, Fernandes ML, de Araujo MA, da Silva DM, Valentim JT and Maia Chaves Filho AJ (2019). Thymol reverses depression-like behaviour and upregulates hippocampal BDNF levels in chronic corticosterone-induced depression model in female mice. *J. Pharm. Pharmacol*., **71**(12): 1774-1783.

Chkhartishvili E, Maglakelidze N, Babilodze M, Chijavadze E and Nachkebia N (2011). Changes of open field behavior in animal model of depression. *Georgian Med. News*., **11**(200): 107-112.

Deng XY, Li HY, Chen JJ, Li RP, Qu R, Fu Q and Ma SP (2015). Thymol produces an antidepressant-like effect in a chronic unpredictable mild stress model of depression in mice. *Behav. Brain. Res*., **291**: 12-19.

Dixit PV, Sahu R and Mishra DK (2020). Marble-burying behavior test as a murine model of compulsive-like behavior. *J. Pharmacol. Toxicol. Methods*., **102**: 106676.

EscobarA, Perez M, Romanelli G and Blustein G (2020). Thymol bioactivity: A review focusing on practical applications. *Arab. J. Chem*., **13**(12): 9243-9269.

Fernstrom JD and Fernstrom MH (2007). Tyrosine, phenylalanine, and catecholamine synthesis and function in the brain. *J. Nutr.*, **137**(6): 1539S-1547S.

Flores KR, Viccaro F, Aquilini M, Scarpino S, Ronchetti F, Mancini R, Di Napoli A, Scozzi D and Ricci A (2020). Protective role of brain derived neurotrophic factor (BDNF) in obstructive sleep apnea syndrome (OSAS) patients. *PloS One*, **15**(1): e0227834.

Franzoni F, Scarfò G, Guidotti S, Fusi J, Asomov M and Pruneti C (2021). Oxidative Stress and Cognitive Decline: The Neuroprotective Role of Natural Antioxidants. *Front. Neurosci.*, **13**: 1294.

Fu Z and Xi S (2020). The effects of heavy metals on human metabolism. *Toxicol. Mech. Methods*, **30**(3): 167-176.

Gagnaire B, Adam-Guillermin C, Bouron A and Lestaevel P (2011). The effects of radionuclides on animal behavior. *Rev. Environ. Contam. Toxicol*., **210**: 35-58.

Genchi G, Sinicropi MS, Lauria G, Carocci A and Catalano A (2020). The effects of cadmium toxicity. *Int. J. Environ. Res.*, **17**(11): 3782.

Gholami-Ahangaran M, Ahmadi-Dastgerdi A and Karimi-Dehkordi M (2020). Thymol and carvacrol; as antibiotic alternative in green healthy poultry production. *Plant. Biotechnol. Persa.*, **2**(1): 22-25.

Haider S, Anis L, Batool Z, Sajid I, Naqvi F, Khaliq S and Ahmed S (2015). Short term cadmium administration dose dependently elicits immediate biochemical, neurochemical and neurobehavioral dysfunction in male rats. *Metab. Brain. Dis.*, **30**(1): 83-92.

Haider SA, Shameem SA, Ahmed SP, Perveen TA and Haleem DJ (2005). Repeated administration of lead decreases brain 5-HT metabolism and produces memory deficits in rats. *Cell. Mol. Biol.*, **10**(4): 669.

Hosseini A, Azizi V and Allahyari F (2021). Effects of Thymus vulgaris on passive avoidance learning and oxidative stress in pentylenetetrazole-induced model of memory impairment in the male Wistar rats. *J. Cell. Neurosci. Oxidative Stress.*, **13**(1): 985-993.

Javed H, Azimullah S, Meeran FMN, Ansari SA and Ojha S (2019). Neuroprotective effects of thymol, a dietary monoterpene against dopaminergic neurodegeneration in rotenone-induced rat model of Parkinson's disease. *Int. J. Mol. Sci*., **20**(7): 1538.

Kedia S and Chattarji S (2014). Marble burying as a test of the delayed anxiogenic effects of acute immobilisation stress in mice. *J. Neurosci. Methods.*, **233**: 150-154.

Khaliq S, Haider S, Naqvi F, Perveen T, Saleem S and Haleem DJ (2012). Altered brain serotonergic neurotransmission following caffeine withdrawal produces behavioral deficits in rats. *Pak. J. Pharm. Sci*., **25**(1): 21-25.

Kondziela W (1964). A new method for the measurement of muscle relaxation in white mice. *Arch. Int. Pharmacodyn. Ther*., **152**: 277-284.

Kuete V (2017). *Thymus vulgaris*. Medicinal spices and vegetables from Africa (first ed.)., **1**: 599-609.

Lamtai M, Azirar S, Zghari O, Ouakki S, El Hessni A, Mesfioui A and Ouichou A (2021). Melatonin ameliorates cadmium-induced affective and cognitive impairments and hippocampal oxidative stress in rat. *Biol. Trace. Elem. Res.*, **199**(4): 1445-55.

Lamtai M, Chaibat J, Ouakki S, Berkiks I, Rifi EH, El Hessni A, Mesfioui A, Hbibi AT, Ahyayauch H, Essamri A and Ouichou A (2018). Effect of chronic administration of cadmium on anxiety-like, depression-like and memory deficits in male and female rats: Possible involvement of oxidative stress mechanism. *J. Behav. Brain. Sci.*, **8**(5): 240-68.

Levin-Schwartz Y, Cowell W, Hsu HH, Enlow MB, Amarasiriwardena C, Andra SS, Wright RJ and Wright RO (2022). Metal mixtures are associated with increased anxiety during pregnancy. *Environ. Res.*, **204** (Part C): 112276.

Lobo V, Patil A, Phatak A and Chandra N (2010). Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn. Rev.*, **4**(8): 118.

Lukawski K, Nieradko B and Sieklucka-Dziuba M (2005). Effects of cadmium on memory processes in mice exposed to transient cerebral oligemia. *Neurotoxicol. Teratol.*, **27**(4): 575-584.

Mehrandish R, Rahimian A and Shahriary A (2019). Heavy metals detoxification: A review of herbal compounds for chelation therapy in heavy metals toxicity. *J. Herbmed. Pharmacol.*, **8**(2): 69-77.

Mourao AM, Vicente LC, Abreu MN, Sant'Anna RV, Vieira EL, de Souza LC, de Miranda AS, Rachid MA and Teixeira AL (2019). Plasma levels of brain-derived neurotrophic factor are associated with prognosis in the acute phase of ischemic stroke. *J. Stroke. Cerebrovas. Dis.*, **28**(3): 735-740.

Mutlu O, Ulak G, Celikyurt IK, Akar FY and Erden F (2011). Effects of citalopram on cognitive performance in passive avoidance, elevated plus-maze and three-panel runway tasks in naïve rats. *Chin. J. Physio*., **54**(1): 36-46.

Nagoor Meeran MF, Javed H, Al Taee H, Azimullah S and Ojha SK (2017). Pharmacological properties and molecular mechanisms of thymol: Prospects for its therapeutic potential and pharmaceutical development. *Front. Pharmacol.*, **8**: 380.

Naqvi F, Haider S, Batool Z, Perveen T and Haleem DJ (2012). Sub-chronic exposure to noise affects locomotor activity and produces anxiogenic and depressive like behavior in rats. *Pharmacol. Rep.*, **64**(1): 64-69.

Perveen T, Haider S, Kanwal S and Haleem DJ (2009). Repeated administration of Nigella sativa decreases 5-HT turnover and produces anxiolytic effects in rats. *Pak. J. Pharm. Sci*., **22**(2): 139-144.

Rahimzadeh RM, Rafati Rahimzadeh M, Kazemi S and Moghadamnia AA (2017). Cadmium toxicity and treatment: An update. *Caspian. J. Intern. Med*., **8**(3): 135-145.

Renugadevi J and Prabu SM (2010). Cadmium-induced hepatotoxicity in rats and the protective effect of naringenin. *Exp. Toxicol. Pathol.*, **62**(2): 171-181.

Romero A, Caride A, Pereiro N and Lafuente A (2011). Modulatory effects of melatoninon cadmium-induced changes in biogenic amines in rat hypothalamus. *Neurotox. Res*., **20**: 240-249.

Saravanan S and Pari L (2016). Protective effect of thymol on high fat diet induced diabetic nephropathy in C57BL/6J mice. *Chem. Biol. Interact.*, **245**: 1-11.

Schieber M and Chandel NS (2014). ROS function in redox signaling and oxidative stress. *Curr. Biol.*, **24**(10): R453-462.

Steru L, Chermat R, Thierry B and Simon P (1985). The tail suspension test: a new method for screening antidepressants in mice. *Psychopharmacology*, **85**(3): 367-370.

Tian J, Hu J, Liu D, Yin J, Chen M, Zhou L and Yin H (2021). Cadmium chloride-induced transgenerational neurotoxicity in zebrafish development. *Environ. Toxicol. Pharmacol.*, **81**: 103545.

Vijaya P, Kaur H, Garg N and Sharma S (2020). Protective and therapeutic effects of garlic and tomato on cadmium-induced neuropathology in mice. *J. Basic. Appl. Zool.*, **81**(1): 1-11.