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TIME-COURSE EFFECTS OF *Cannabis sativa* ON BRAIN ACETYLCHOLINESTERASE (AChE) ACTIVITY AND EXPRESSION OF DOPA DECARBOXYLASE GENE (*DDC*)

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Abstract

Cannabis sativa affects almost every system in the body of animals and humans resulting in impairments, especially of attention, memory, and ability to process complex information which may be a result of inhibition or activation of activities and expressions of neurotransmitters involved in these biological functions. The effects of doses of *Cannabis sativa* extract on brain acetylcholine esterase (AChE) activity and the expression of dopa decarboxylase gene (*DDC*) an enzyme involved in the biosynthetic pathway of Serotonin (a neurotransmitter) was investigated to correlate the duration of use with associated cholinergic neurotoxicity and behavioral implications. Male Wistar rats weighing between 90g ± 10 g were treated with graded doses of petroleum ether extract of C. *sativa* (12.5, 25, and 50 mg/kg body weight) orally for 4, 8, and 12 weeks. AChE activity was measured in the brain of the treated animals using the colorimeter method while the expression of *DDC* was obtained using reverse transcriptase-polymerase chain reaction (RT-PCR) method. There was a significant ($p<0.05$) increase (about 16.6 %) in the brain AChE activity in rats treated with the different doses of C. *sativa* at 4 weeks. A significant ($p<0.05$) decrease in the enzyme activity by 29.9 % and 24.5 % was however observed after 8 weeks' administration of 25 and 50 mg/kg doses of C. *sativa* respectively. Administration of all measured doses of C. *sativa* for 12 weeks resulted in significant($p<0.05$) increase in brain AChE activity by 17.3 % and 28.7 % and 39.3 % respectively, Upregulation of about 35.9 % and 30.6 % was recorded in the expression of *DDC* with the administration of only 50 mg/kg after 4 and 8 weeks, no significant($p>0.05$) difference was however observed after 12 weeks exposure to all doses of the extract. The important implication of our study is that prolonged cannabis exposure increases AChE activity in the brain and ultimately could decrease brain level of acetylcholine thereby affecting cognition and neurotransmission. Alteration of the expression of *DDC* in the brain after exposure to different doses for varying duration could also account for different behavioral tendencies observed in users.

Keywords: *C. sativa, acetylcholine esterase, serotonin, neurotransmission, cognition, gene expression.*

Introduction

Cannabis sativa is a popular recreational drug around the world, like alcohol, caffeine, and tobacco with tetrahydrocannabinol, THC as the principal psychoactive constituent responsible for the feeling of "high", mild euphoria and state of relaxation [1]. Exposure to *Cannabis sativa* usually starts in school, at the teenage years [2] with effects in almost every system of the body. It combines many of the properties of alcohol, tranquilizers, opiates, and hallucinogens [3], and could produce anxiolytic, sedative, analgesic, dysphoric reactions, including severe anxiety, panic, paranoia, and psychosis [4]. These impairments, especially of attention, memory, and ability to process complex information, maybe a result of inhibition or activation of different neurotransmitters and can last for many weeks, months, or even years after cessation of use [5]. Neuroanatomic alterations in brain regions rich in cannabinoid receptors, such as the hippocampus, prefrontal cortex, amygdala, and cerebellum are often linked to exposure[6].

Acetylcholinesterase (AChE) enzyme is present in high concentration in all types of conducting tissue, nerve and muscle, central and peripheral tissues, motor and sensory fibers. It is released by motor neurons to activate muscles [7] by rapid hydrolysis of acetylcholine (ACh). Acetylcholine functions in the peripheral nervous system (PNS) and central nervous system (CNS). It activates muscles in the PNS, while in the CNS, cholinergic projections from the basal forebrain to the cerebral cortex and hippocampus support the cognitive functions of those target areas [8]. It also has different effects on plasticity, arousal, and reward and in the enhancement of alertness, to maintain attention, learning, and memory [9].

Serotonin (5-HT) is a monoamine neurotransmitter that plays a central role in the modulation of anger, aggression, body temperature, mood, sleep, sexuality, and appetite as well as stimulation of vomiting reflex. It is mainly found in the gastrointestinal (GI) tract, the platelets, and the central nervous system of animals and it contributes to a sense of well-being and happiness *[10]*. Several classes of pharmacological antidepressants act by modulating serotonin at synapses [11].

The serotoninergic system is in a strategic position to regulate complex sensory and motor patterns during diverse behavioral states. 5-HT has been implicated in the etiology of numerous disease states, such as depression, anxiety, social phobia, schizophrenia, obsessive-compulsive, panic disorders, migraine, hypertension, pulmonary hypertension, eating disorders, vomiting, and irritable bowel syndrome [12].

Dopa decarboxylase (DDC), catalyzes the conversion of L-aromatic amino acids into amines. It is central in the synthesis of dopamine and serotonin and therefore a pharmacotherapeutic target. It metabolizes the serotonin precursor, 5-hydroxytryptophan to the transmitter serotonin. It is also known as Aromatic L-amino acid decarboxylase (AAAD), tryptophan decarboxylase and 5-hydroxytryptophan decarboxylase, [13]). The gene encoding the enzyme is referred to as *DDC* and located on chromosome 7 in humans [14]. It has been identified in monoaminergic neurons and neurons that do not contain other monoamine synthetic enzymes, as well as in kidney and blood vessels [15].

Methods

Animals

Juvenile Wistar rats, with average weights of 90 ± 10g were used. The Wistar rats were purchased from the College of Veterinary Medicine, Federal University of Agriculture, Abeokuta. The rats were maintained in polypropylene cages with steel wire tops at a temperature of 23 ± 2 ° C under a 12h light-dark cycle, food and water were provided *ad libitum.* The experimental protocol was approved by the ethical committee of the Biochemistry Department, which is in conformation with the NIH guidelines as outlined in NIH publication 80-23 (NRC, 1985).

Drugs and Chemicals

Cannabis sativa was obtained from the National Drug Law Enforcement Agency (Nigeria). Other chemicals and reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA).

Preparation of Cannabis resin extract

Cannabis extract was prepared from the whole plant, it was air-dried at room temperature and pulverized using a clean, dry electric blender. 250 g of milled marijuana was soaked in 1000 ml of petroleum ether in a round bottom flask for 24 hours, decanted. The filtrate was concentrated using a rotary evaporator and the concentrated extract was dissolved in olive oil.

Study Design

The animals were randomly divided into twelve groups of six animals each. Group A1 animals served as the control for groups A2, A3, and A4 that were given 12.5, 25, and 50 mg/kg oral doses by gavage of the concentrated extract daily for 4 weeks respectively. Similarly, group B1 served as the control for groups B2, B3 and B4 given 12.5, 25, and 50 mg/kg oral doses of the extract respectively for 8 weeks while animals in C1 were the control for groups C2, C3, and C4 exposed to 12.5, 25, and 50 mg/kg oral doses of the extract respectively for 12 weeks. Each group was housed in separate cages and allowed to acclimatize for two weeks before the commencement of marijuana exposure. At the end of each group treatment, rats were euthanized by exsanguinations by cardiac puncture. The brain was excised from the animals and 0.1 g was homogenized in 0.9 ml of 0.1 M Tris buffer (pH 7.4) [16]

Determination of Acetylcholinesterase Activity

Brain AChE activity was determined using the colorimetric method of Ellman *et al* (1961) [17] as described by Whittaker (1984) [18]. Briefly, 0.10 ml of Buffered Ellman's reagent, 5, 5'-dithiol-bis-2-nitrobenzoic acid (DTNB) (DTNB, 10 mmol/l, $NaHCO_3$, 17.85 mmol/l in phosphate buffer 100 mmol/l, pH 7.0) and 0.02 ml of acetylthiocholine solution (acetylthiocholine iodide, 75 mmol/l), were added to 3 ml phosphate buffer (pH 8.0) in a cuvette and incubated at 25°C for 10 minutes. 0.1 ml of the homogenate was then added and absorbance was monitored at 30 seconds interval for 3 minutes at 410 nm. A Jenway 6405 UV/Visible spectrophotometer (Jenway Ltd., Felsted, Dunmow, Essex, U.K.) was used for all measurements. Enzyme activity was expressed as u/g tissue.

Calculation (u/g tissue):

$$\frac{\Delta A \times V \times \mathbf{1000}}{\varepsilon \times \delta \times \Delta t \times v}$$

ΔA – Change in absorbance

V – Volume of assay

Δt – Change in time = 3 mins

v – Volume of the sample used in the assay

£ =1.361 x $mmol^{-1}$ x mm^{-1}

ɗ - Cuvette =10 mm

RNA extraction

RNA was extracted from RNAlater®–stabilized the brain using the Aidlab spin column RNA extraction kit according to the instructions of the manufacturer. Concentration and purity of extracted RNA were determined at 260 nm and 280 nm using a NanoDrop® 2000 spectrophotometer (Thermo Scientific). RNA samples were kept at –80 °C until gene expression analysis.

Expression of Dopa decarboxylase (DDC)

The levels of expression of Dopa decarboxylase were assessed in the brain using semi-quantitative reverse transcriptase-polymerase chain reaction (RT-PCR). The RT-PCR was carried out with a 500 ng RNA template using the TranGen EasyScript one-step RT-PCR kit according to the manufacturer's instructions. The RNA samples were subjected to an initial 30 min incubation at 45 °C for cDNA synthesis after which PCR amplification was carried out, using gene-specific primers (GSP) (Table 1), at 94 °C for 5 min followed by 40 cycles of 94 °C for 30 s, 5 min at the annealing temperature of GSP, and 1 min at 72 °C. All amplifications were carried out in C1000 Touch™ Thermal Cycler (Bio-Rad Laboratories, Hercules, CA). After PCR, amplicons were visualized on 1.2 % agarose gel in 1X Tris Borate EDTA buffer using UVP BioDoc-It-™ Imaging system (Upland, CA, USA). The intensity of the bands was analyzed using Image J software [19]. Results were presented as relative expression (ratio of the intensity of each gene to that of β-actin, Actb) of the gene in comparison with housekeeping (β-actin, Actb) gene.

Data analysis

The results obtained are expressed as the mean ± standard error of mean (SEM). One-way analysis of variance (ANOVA) followed by Tukey's multiple range test was used to analyze the results. Values with $p<0.05$ were regarded as being significant,

using the Statistical Package for Social Sciences (SPSS) version 20.0.

Results

At 4 weeks, there was a significant ($p < 0.05$) increase in the brain AChE activity in rats treated with all the different doses (12.5, 25, and 50 mg/kg body weight) of C. *sativa* compared to the olive oil control group (Figure 1). A significant inhibition in the enzyme activity by 29.9% and 24.6% was however observed after 8 weeks' administration of 25 and 50 mg/kg doses of the C. *sativa* (Figure 2). Prolonged administration (12 weeks) of C. *sativa* was accompanied with significant ($p<0.05$) increase in brain AChE activity by 17.3%, 28.7%, and 39.3% respectively compared to the control group (Figure 3).

At 4 weeks' exposure to marijuana, there was an upregulation in the expression of *DDC* only in the group administered 50 mg/kg body weight dose while there was no significant difference ($p > 0.05$) at 12.5 mg/kg and 25 mg/kg doses when compared to the control. At 8 weeks' exposure, there was a significant increase ($p < 0.05$) at 12.5 mg/kg and 50 mg/kg doses while 25 mg/kg dose produced no significant effect when compared to the control group. However, at 12 weeks' exposure to graded doses of marijuana, there was no significant difference ($p > 0.05$) in the expression of the gene at all the doses (Figure 4).

Discussion

This research work revealed an increase in the activity of brain acetylcholinesterase (AChE) following the administration of different doses of cannabis extract over a total period of 12 weeks. Communication between two regions of the brain that control memory and learning, the basal forebrain and the hippocampus is enabled when the receptors are activated. ACh's role in the brain enhances both neuron signaling intensity and quality by increasing theta waves. In particular, acetylcholine enhances the encoding of memories in the perirhinal and entorhinal cortex [8]. Also, ACh initiates synaptogenesis, the normal development of synapses throughout the brain [8]. This additionally improves memory encoding and attention during learning. AChE is a major enzyme that terminates the action of acetylcholine at post-synaptic junctions and its catalytic activity has been tuned to the highest possible level causing rapid hydrolysis of thousands of acetylcholine molecules in micro-seconds. This termination ensures that the nervous system is not unnecessarily stimulated [20]. A further increase, as observed in some of the doses employed over a certain period in this study, will consequently reduce the time of interaction of acetylcholine with receptors on the postsynaptic membrane.

Damage to the cholinergic (acetylcholine-producing) system in the brain is associated with memory deficits as seen in Alzheimer's disease and Attention Deficit Hyperactivity Disorder (ADHD) [21].

Some findings reported a decreased AChE activity after exposure to different toxicants [22-24] diseases [25] and drugs. Our findings revealed an increase at 4 and 12 weeks' exposure to all the doses of marijuana extract. There was a decrease at 8 weeks' exposure to 25 and 50 mg/kg doses, which was swiftly reversed, this corroborates the work of Abdel- Salam *et al.* [26] and Acquas *et al.* [27] who reported increased cortical and hippocampal acetylcholine release following intravenous administration of low doses (10-150 ug/kg) of delta 9-THC to freely moving rats and elevated AChE activity after 6 weeks of daily subcutaneous administration of cannabis resin respectively.

Although the effect of marijuana on the expression of *DDC* was not significant after 12 weeks of exposure to all the doses, there was a decrease in the expression when compared to 4 weeks and 8 weeks. This could be as a result of reduced transcription and/or tolerance developed by the body system to the cannabis. Reduced dopa decarboxylase activity has been suggested to have a role in schizophrenia, postural hypotension, oculogyric crisis, hallucination, and cognitive deficit [28; 29]. On the other hand, dopa decarboxylase inhibitors (DDCIs) such as carbidopa and benserazide, are of clinical importance in the therapeutic administration of levodopa for the treatment of Parkinson's disease [30]. This is to prevent the premature conversion of dihydroxyl phenylalanine (DOPA) to dopamine. The decrease observed in the expression of *DDC* after 12 weeks

exposure, might be the reason for the submission of Cherek *et al*. [31] that prolonged exposure to high doses of cannabis could result in decreased synthesis of serotonin and lead to "amotivational syndrome" i.e a state of decreased motivation, reduced ability to concentrate, loss of effectiveness, decreased capacity to carry out complex plans or prepare realistically for the future. Gruber *et al*. [32] also revealed that chronic exposure to high doses of cannabis worsens depression and other psychiatric conditions like psychosis. An association was also established between the intake of cannabis and increased prevalence of depressive disorders anxiety and mood disorders [33] [34]. Moreover, among patients with bipolar disorder, cannabis users exhibited less compliance and a higher level of overall illness severity compared with non-users [35]. The result from this study also corroborates the findings of Bambico *et al*. [36] that low doses of cannabis increased the production of serotonin through interaction with the CB1 receptors and responsible for its use as an antidepressant. However, exposure to higher doses completely reversed its effect.

The findings of this study suggest that the effects produced by cannabis use are linked to an alteration of the serotonergic system. Also, an important implication of our study is that marijuana exposure might be associated with activation of acetylcholinesterase activity which could cause a decrease in the brain levels of acetylcholine, due to rapid hydrolysis and a reduction in the time of interaction with the postsynaptic receptors which is required for it to elicit its numerous functions. This reveals to some extent an effect on neurotransmission and could explain at least in part, the cognitive and memory deficits observed in some users.

References

1. *Wayne, H. and Rosalie, L. 2003.* Cannabis Use and Dependence: Public Health and Public Policy. *Cambridge University Press p 38.*
2. Miller, P. M. C. and Plant, M. 1996. *Drinking, smoking and illicit drug use among 15 and 16 year olds in the United Kingdom. British Medical Journal*313: 394 -397.
3. Kathmann, M., Flau, K., Redmer, A., Tränkle, C. and Schlicker, E. 2006. Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. *Naunyn-Schmiedeberg's Archives of Pharmacology*372 (5): 354–361.
4. Hall, W., Degenhardt, L., and Teesson, M. 2004. Cannabis use and psychotic disorders: an update. *Drug Alcohol Review* 23:433–443.
5. Eubanks, L.M., Rogers, C.J., Beuscher, A., Koob, G.F., Olson, A.J., Dickerson, T.J. and Janda, K.D. 2006. A molecular link between the active component of marijuana and alzheimer's disease pathology. *Molecular Pharmaceutics* 3 (6): 773–777.
6. Paton, W.D.M. and Pertwee, R.G. 1973. *The actions of cannabis in man. In: Mechoulam,* R. (ed) Marijuana: Chemistry, Pharmacology, Metabolism and Clinical Effects. *pp* 288 -*334.*
7. Ridley, R.M., Bowes, P.M., Baker, H.F. and Crow, T.J. 1984. An involvement of acetylcholine in object discrimination learning and memory in the marmoset. *Neuropsychologia* 22 (3): 253–263.
8. *Whittaker, V.P. 1990. The Contribution of Drugs and Toxins to Understanding of Cholinergic Function.Trends in Physiological Sciences11 (1): 8–13.*
9. *Himmelheber, A.M., Sarter, M. and Bruno, J.P. 2000. Increases in cortical acetylcholine release during sustained attention performance in rats. Brain research cognitive brain research 9 (3): 313–325.*
10. Young, S.N. 2007. How to increase serotonin in the human brain without drugs. *Journal of Psychiatry and Neuroscience* 32 (6): 394–399.
11. Goodman, L.S., Brunton, L.L., Chabner, B. and Knollmann, B.C. 2001. Goodman and Gilman's pharmacological basis of therapeutics. New York: McGraw-Hill. ISBN 0-07-162442-2. pp 459–461
12. White, K.J., Walline, C.C. and Barker, E.L. 2005. Serotonin transporters: implications for antidepressant drug development. *American Association of Pharmaceutical Scientists Journal* 7:421-433.
13. Logan, C.M. and Rice, M.K. 1987. Logan's Medical and Scientific Abbreviations. J.B. Lippincott Company. Philadelphia. 3pp.

14. Scherer, L.J., McPherson, J.D., Wasmuth, J.J. and Marsh, J.L. 1992. Human dopa decarboxylase: localization to human chromosome and characterization of hepatic cDNAs. *Genomics* 13 (2): 469–471.

15. Brun, L., Ngu, L.H., Keng, W.T., Ch'ng, G.S., Choy, Y.S., Hwu, W.L., Lee, W.T., Willemsen, M. A., Verbeek, M. M., Wassenberg, T., Regal, L. and Orcesi, S. 2010. Clinical and biochemical features of aromatic L-amino acid decarboxylase deficiency. *Neurology* 75: 64-71.

16. Dosumu, O.A., Ugbaja, R. N., Popoola, A. R., Rotimi, S.O., Owolabi, O.P. 2017. Administration of Cannabis extracts causes alteration in brain and plasma nitric oxide concentration in rats. Biokemistri 29 (1), 2017.

17. Ellman, G.L., Courtney, K.D. and Andre,V.(Jr.) 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical* 7(2) 88-90

18. Whittaker. 1984. Segregation and developmental autonomy in expression of functional muscles acetylcholineserase mRNA in the Ascidian embryo. *Developmental Biology. 105: 479 – 487.*

19. Abràmoff, M.D., Magalhães, P.J. and Ram, S.J. 2004. Image Processing with ImageJ. *Biophotonics international* 11(7): 36-42.

20. Ademuyiwa , O., Ugbaja, R.N., Rotimi, S.O., Abam, E., Okediran, B.S., Dosumu, O.A. and Onunkwor, B.O. 2007. Erythrocyte acetylecholinesterase activity as a surrogate indicator of lead-induced neurotoxicity in occupational lead exposure in Abeokuta,Nigeria. *Environmental Toxicology and Pharmacology* 24 (2):183-188.

21. Francis, P.T., Palmer, A.M., Snape, M., Wilcock, G.K. 1999. The cholinergic hypothesis of Alzheimer's disease: a review of progress.*Journal of Neurology Neurosurgery and Psychiatry.66 (2): 137–147.*

22. Ugbaja, R.N., Olaniyan, T.O., Afolabi, O.K., Onunkwor, B.O., Dosumu, O.A., Akinhanmi, T.F., Tiamiyu, A.Y. and Aliyu-Banjo, N.O. 2017.Ionoregulatory Disruption and Acetylcholinesterase Activity in Aluminium Toxicity: Effects of Vitamins C and E. *Nigerian Journal of Physiological Sciences* 32: 207-212.

23. Madhavan, N., Jessy, J., Nitesh K, Jayesh, M., Gopalan, K.N., and Mallikarjuna, R.C. 2015. Modulatory Role of Simvastatin against Aluminium Chloride-Induced Behavioural and Biochemical Changes in Rats. *Behavioral Neuroscience* 210169: 9

24. Kumar, A., Dogra, S. and Prakash, A. 2009. Protective effect of curcumin (*Curcuma longa*), against aluminium toxicity: Possible behavioral and biochemical alterations in rats. *Behavioural Brain Research* 205 (2):384-390.

25. Habila, N., Inuwa, H.M.,Aimola, I.A.,Lasisi, O.I., Chechet, D.G. and Okafor, O.I. 2012. Correlation of acetylcholinesterase activity in the brain and blood of Wistar rats acutely infected with *Trypanosoma congolense. Journal of Acute Diseases 1 (1): 26-30.*

26. Acetylcholinesterase, butyrylcholinesterase and paraoxonase 1 activities in rats treated with cannabis, tramadol or both. *Asian Pacific Journal of Tropical Medicine* 9(11): 1089-1094.

27. Acquas, E; Cadoni, C; Pisanu, A; Solinas, M and Chiara, Gatano. 2001. Behavioural sensitization after repeated exposure to tetrahydrocannabinol and cross-sensitization with morphine. *Springer.* 158(3): 259-266.

28. Brebion, G., David, A.S., Bressan, R.A., Ohlsen, R.I. and Pilowsky, L.S. 2009. Hallucinations and two types of free-recall intrusion in schizophrenia. *Psychological Medicine* 39(6):917-926.

29. Kellendonk, C., Simpson, E., Polan, H., Malleret, G., Vronskaya, S., Winiger, V. 2006. Transient and selective overexpression of dopamine D2 receptors in the striatum causes persistent abnormalties in prefrontal cortex functioning. *Neuron* 49 (4):603-615.

30. *Gnegy, M.E. and Siegel G.J. 2012. Catecholamines. Basic Neurochemistry (8th edition) Principles of molecular, cellular and medical neurobiology. pp 283-299.*

31. Cherek, D.R., Lane, S.D. and Dougherty, D.M. 2002. Possible amotivational effects following marijuana smoking under laboratory conditions. *Experimental and Clinical Psychopharmacology* 10: 26–38.

32. Gruber, A.J., Pope, H.G. and Brown, M.E. 1996. Patient's use of marijuana as an antidepressant. *National Institute of Health* 4 (2):77- 80.

33. Bovasso, G.B. 2001 Cannabis abuse as a risk factor for depressive symptoms. *American Journal of Psychiatry* 158:2033-2037.

34. Cheung, J.T., Mann, R.E., Ialomiteanu, A., Stoduto, G., Chan, V., Ala-Leppilampi, K., and Rehm,

J. 2010. Anxiety and mood disorders and cannabis use. *American Journal of Drug & Alcohol Abuse* 36:118-122.
35. Van-Rossum, I., Boomsma, M., Tenback, D., Reed, C. and Van Os, J. 2009. EMBLEM Advisory Board. Does cannabis use affect treatment outcome in bipolar disorder? A longitudinal analysis. *Journal of Nervous and Mental Disease* 197:35-40.
36. Bambico, F., Katz, N. and Debonnel, G. 2007. Cannabis: Potent antidepressant in low doses, worsens depression at high doses. ScienceDaily, Retrieved September 14, 2018 from www.sciencedaily.com/releases/2007/10/071023183937.htm

Table 1: Sequences of gene-specific primer

Gene Specific Primers	Sequence (5`-3`)	Template
DDC	Forward: TTGCAGAGCTGGACTGAGTG	NM_012545.4
	Reverse: GCATAGCTGGGTAGGAGCTG	
β-Actin	Forward: GTCAGGTCATCACTATCGGCAAT	NM_031144.3
	Reverse: AGAGGTCTTTACGGATGTCAACGT	

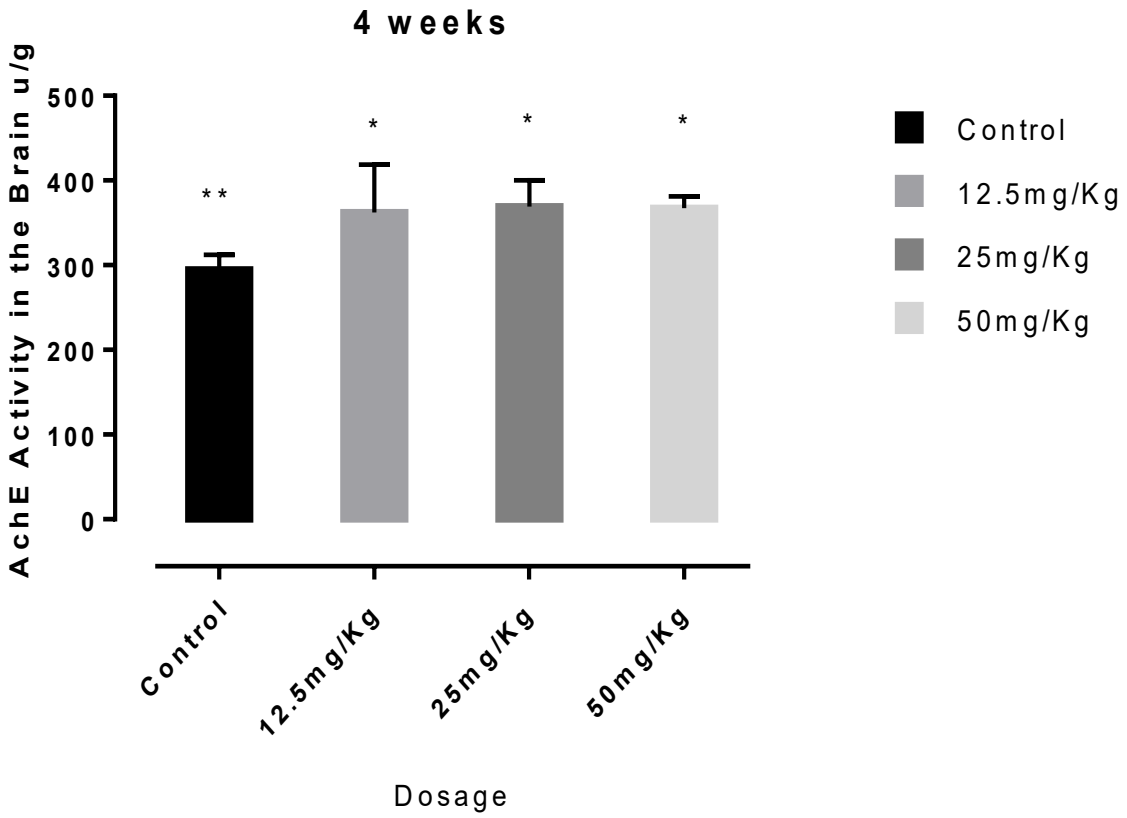

Figure 1: Effects of C. *sativa* on Brain AChE activity after 4 weeks of exposure. Data represent mean ± S.E.M (n=6). Bars with different no of * are significantly different from one another ($p<0.05$).

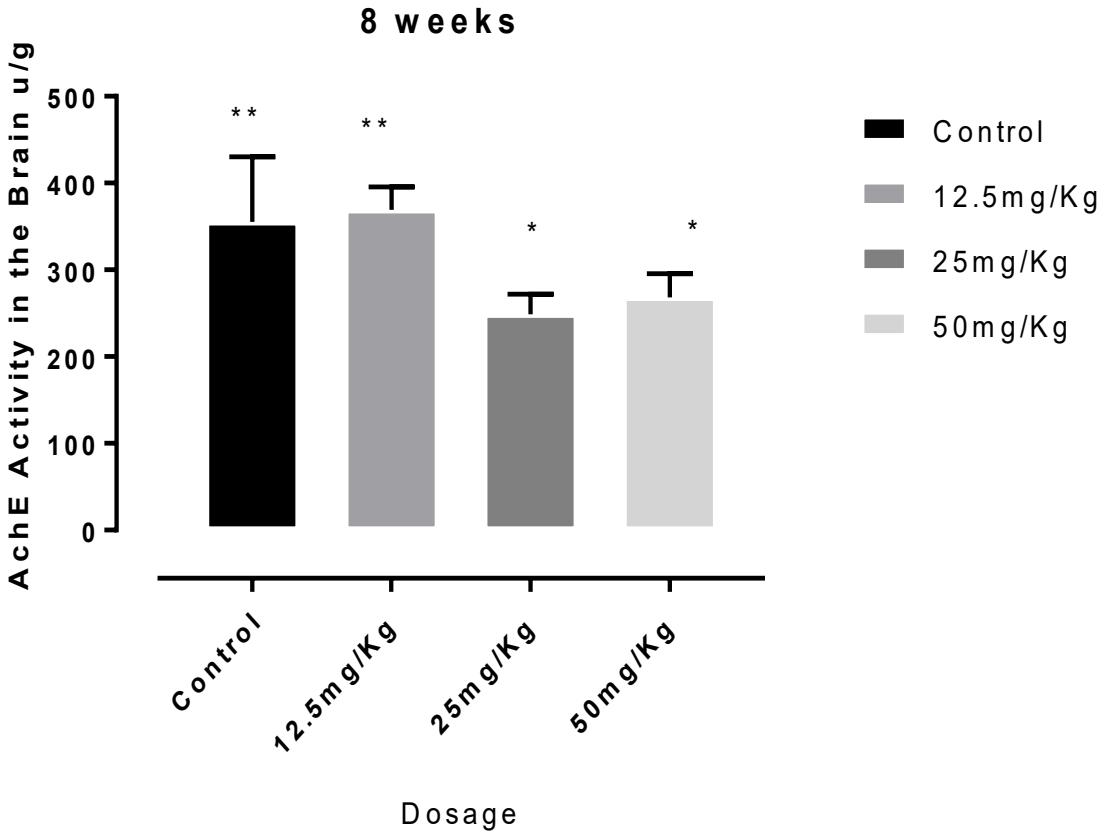

Figure 2: Effects of C. *sativa* on Brain AChE activity after 8 weeks of exposure. Data represent mean ± S.E.M (n=6). Bars with different no of * are significantly different from one another ($p<0.05$).

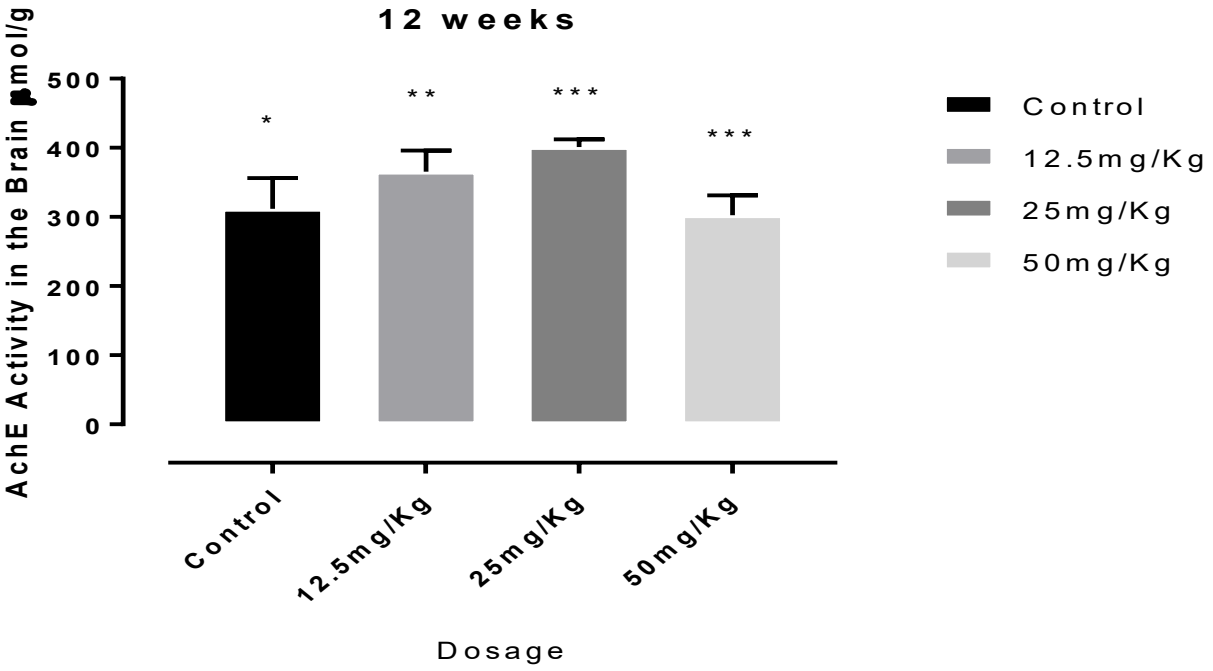

Figure 3: Effects of C. *sativa* on Brain AChE activity after 12 weeks of exposure. Data represent mean ± S.E.M (n=6). Bars with different no of * are significantly different from one another ($p<0.05$).

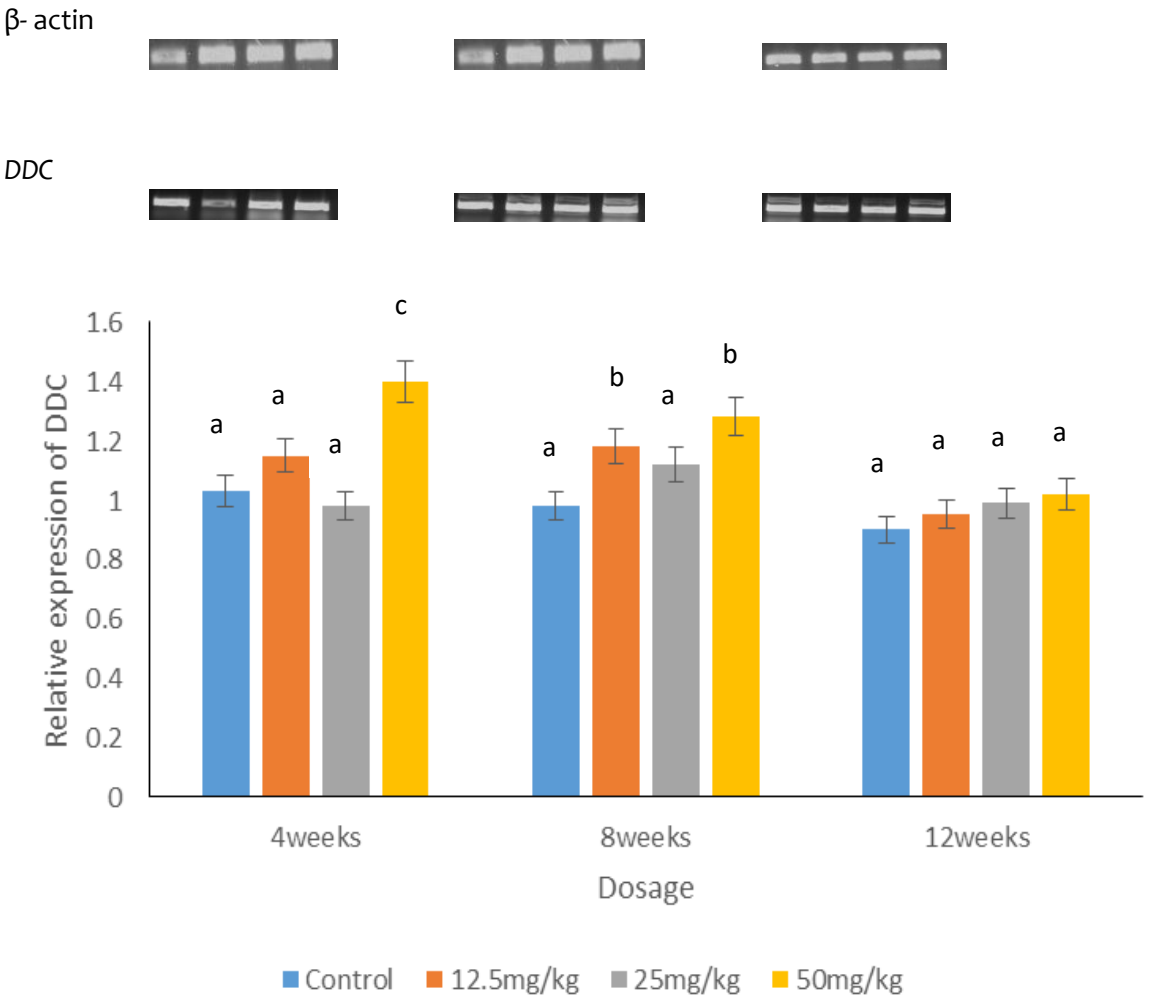

Figure 4: Effects of C. *sativa* on relative expressions of dopa decarboxylase gene (*DDC*) in the brain after 4, 8 and 12 weeks of exposure. Data represent mean ± S.E.M (n=6). Bars with different alphabets are significantly different from one another ($p<0.05$).