

Neuroprotective Effect of Cannabis Sativa on Stress Induced Biochemical and Histological Changes in the Brain of Wistar Rats

Olalekan Oladimeji, Uduak Umana, Abel Agbon, Serene Daodu, Aisha Usman.

Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University (A.B.U), Zaria- Nigeria

*Correspondence: O Oladimeji.. Department of Human Anatomy, Faculty of Basic Medical Sciences, College of Medical Sciences, Ahmadu Bello University (A.B.U), Zaria- Nigeria. Email: Oladimejerry21@gmail.com

Submitted: 20 September 2022. Published: 12 March 2023

ABSTRACT

Background: Stress continues to be a rising global health concern that can trigger neurodegenerative diseases. Available treatment options focus more on the alleviation of specific symptoms without effort to control/delay the progression of neurodegenerative diseases triggered by stress. Cannabis sativa L. (C. sativa) at moderate doses has potent psychoactive and antioxidative properties. **Objectives:** This study assessed the neuroprotective effect of a hydroethanolic extract of C. sativa (HCS) on stress induced biochemical changes in the brains of Wistar rats. **Methods:** Twenty-five (25) Wistar rats were used in the present study and were divided into 5 groups (n=5). Group I (H₂O, 1 ml/kg); group II (2 hours of restraint stress); group III (diazepam, (5 mg/kg) + stress; group IV (250 mg/kg C. sativa. + stress); and group V (750 mg/kg of C. sativa + stress). Treatment was via oral route for a period of 21 days. The neuroprotective effect of HCS was evaluated using, biochemical and histological analysis of oxidative stress biomarkers (MDA, SOD, CAT, and GPx) and endogenous enzyme (AChE) activity of the brain of Wistar rats and the CA1 and CA3 regions of the hippocampus of Wistar rats using brain H&E stains, respectively. **Results:** Biochemical results revealed alterations in the concentration levels of oxidative stress parameters in the hippocampus in stress exposed groups. HCS significantly (p<0.05) ameliorated stress-induced alterations of biochemical parameters. Histoarchitecture alterations were observed in the hippocampus of the stress exposed group, which were mitigated in HCS group in a dose-dependent manner. **Conclusion:** Findings from this study suggest the efficacy of low dose C. sativa. in ameliorating stress induced biochemical and histological alterations in the brain and could be a potential candidate for application in the management and treatment of stress induced neurodegeneration

Keywords: Antioxidant, Neurodegeneration, Neuroprotective, Oxidative stress

INTRODUCTION

Stress, a state of disturbed homeostasis in biological system, is consequent to endogenous or exogenous stimuli referred to as

stressors. Stress is a physiologic response that enables an organism to handle a wide variety of detrimental conditions encountered on daily bases, aiding survival in a dynamic environment^[1, 2].

Access to the article



Website: www.jmbsr.com.ng
eISSN: 2971-7833
pISSN: 2971-7108



How to cite this article

O Oladimeji, U Umana, A Agbon, S Daodu, A Usman. Neuroprotective Effect of Cannabis Sativa on Stress Induced Biochemical and Histological Changes in the Brain of Wistar Rats. *J Med & Bas Sci Res.* 2023;4(1-2):71-84. DOI: 10.5281/zenodo.7743964

Systems including the autonomic nervous system and the release of certain biochemicals like catecholamines, (norepinephrine and epinephrine), corticotropin-releasing hormone, adrenocorticotropin hormone, cortisol and acetylcholine play critical roles in response to stress and cognitive functions like attention, learning and memory^[3, 4]. Exposure to long-term stressing conditions has been associated with deleterious impacts on the physiology of bodily systems and structures including the brain, which results to inflammation, accelerated aging and accompanying body-weight loss. These negative manifestations have been attributed to biochemical, neurochemical, and structural alterations in the body systems and several brain regions like the hippocampus, prefrontal cortex and amygdala^[2, 5].

The hippocampus plays important roles in the regulation of stress and, under chronic stress conditions, changes including impaired synaptic plasticity, decreased hippocampal volume and inhibited hippocampal neurogenesis have been reported^[6, 7, 8]. Hippocampal damage is thought to play an important role in the etiology of stress-related psychiatric disorders and, changes in hippocampal volume have been associated with the development of several psychological conditions in humans, such as working memory impairment, anxiety and depressive-like behaviours^[9, 10].

Cannabis sativa L. (*C. sativa*) is a dioecious plant that belongs to the *Cannabaceae* family^[11, 12]. It is widely distributed in several countries including Central and Eastern Asia, Morocco, South Africa, America, Nigeria, India, and parts of Europe^[13]. *C. sativa* is the most widely used illicit substance globally, with upwards of ~180 million users^[14]. Folkloric uses of *C. sativa* across different ethnic groups around the world ranges from smoking to concoction preparations to treat diseases and other cultural purposes^[15]. Cannabis is composed of chemical constituents including cannabinoids, nitrogenous compounds, flavonoids, glycosides,

steroids, terpenes, hydrocarbons, non-cannabinoid phenols, vitamins, amino acids, proteins, sugars and other related compounds^[16]. *C. sativa* at moderate exposure is postulated to be relatively safe and has been reported to be of medical benefits in epilepsy^[17], pain^[18], cancer^[19], antipsychotic and anxiolytic diseases^[20]. Hence, the need to explore the certain health beneficial potentials of *C. sativa* products especially, in the management of stress in animal model.

This study assessed the effects of hydro-ethanol leaf extract of *C. sativa* in ameliorating biochemical alterations and hippocampal changes following stress exposure in Wistar rats.

MATERIALS AND METHODS

Plant Material

600 g dried leaves of *C. sativa* L. was obtained by permission from the National Drug Law Enforcement Agency (NDLEA), Kaduna State Command and authenticated in the Herbarium Unit of the Department of Botany, Faculty of Life Sciences, Ahmadu Bello University (ABU), Zaria. The voucher number: 02438 was provided.

Plant Extraction and Phytochemical Screening

The maceration method of extraction as described by Sofowora *et al.*^[21] was adopted for this study. This method is convenient and very suitable for thermolabile plant molecules. Briefly, the extraction protocol was as follows:

Dried leaves of *Cannabis sativa* L. were pulverized into a fine powder using laboratory mortar and pestle. 600 g of the fine powder was weighed and soaked in 2 liters of 50% hydro-ethanol (v/v; 1:1 water to ethanol) in a conical flask. After 24 hours, the solution (mixture of the fine powder and hydro-ethanol) was filtered using filter rag and funnel. The filtrate was allowed to settle for a while, followed by decantation of the supernatant. The supernatant was gently evaporated to dryness in an oven at 50°C. Residue obtained was kept in a sealed container until when used. A yield of 4.07% (24.4 g) was obtained.

Extracted plant material (hydro-ethanol leaf extract

of *Cannabis sativa*, HCS) was subjected to qualitative phytochemical screening for secondary metabolites adopting the method described by Trease and Evans ^[22]. Extraction and phytochemical analysis was conducted in the Department of Pharmacognosy and Drug Development, Faculty of Pharmaceutical Sciences, ABU, Zaria.

Experimental Animals

Twenty-five (25) adult male Wistar rats were obtained from the Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, ABU, Zaria, Nigeria and transferred to the Animal House of the Department of Human Anatomy, ABU, Zaria and housed in new cages to acclimatize for two weeks prior to the commencement of experimentation. Rats were housed under standard laboratory conditions, light and dark cycles of 12 h was provided and, fed with rat chow and clean water *ad libitum*.

Drugs and Chemicals

Diazepam: Diazepam tablet (5 mg) was obtained from the Pharmacy Unit, ABU, Teaching Hospital, Shika, Zaria and used as a reference anti-stress drug. The product is manufactured by Swipha Ltd,

Lagos, Nigeria.

Ketamine: Ketamine Hydrochloride injection USP, 50 mg/ml was used as anesthetic agent. The product is manufactured by Swiss Parenterals PVT Ltd, Gujarat, India.

Stress Induction

Stress induction in rats was achieved by adopting the Modified Repetitive Restraint Stress (MRRS) model reported by Smith *et al.* ^[23] using a restraint cage. The restraint cage, made up of a Perspex material, was designed with five compartments, each with the following dimensions: 5 cm in height, 4 cm in width and 15 cm in length. Compartments were perforated at both ends to provide for sufficient ventilation.

Briefly, the protocol for stress induction is as follows:

The rats were kept immobilized in restraint cage for a duration of 120 minutes daily with no access to food or water. The compartment allows the rat to turn around once or twice (invariably getting stuck halfway through the turn), and then stop trying to move. The cage was sanitized with 70% alcohol after each session. Stress induction was for a period of 21 days (See Figure 1).



Figure 1: Restrain stress model

Experimental Design

25 rats were categorized into 5 groups (groups I – V; n= 5). Group I served as the control and was administered distilled water (1 ml/kg); group II were subjected to restraint stress only; group III was administered diazepam (5 mg/kg; *Suleiman et al.* [24]) and subjected to stress concurrently; groups IV and V were administered HCS (250 mg/kg and 750 mg/kg, respectively; 5% and 15% LD_{50} respectively, *Richard et al.* [25]) and subjected to stress concurrently. Administration was *via* oral route, once daily for 21 days (Table 1). Ethical approval with number of ABUCAUC/2021/010 was obtained from the research and ethics committee on Animal Use and Care of Ahmadu Bello University Zaria before the commencement of this research work.

Table 1: Experimental protocol

Group	Treatment
I	Distilled water (1 ml/kg)
II	Stress (MRRS)
III	Diazepam (5 mg/kg) + Stress
IV	HCS (250 mg/kg) + Stress
V	HCS (750 mg/kg) + Stress

Table 1:

n= 5; administration was *via* oral route for 21 days; MRRS= modified repetitive restraint stress; hydro-ethanol leaf extract *C. sativa*. (HCS).

Physical Observation and Animal Sacrifice

During the period of experimentation, rats' physical behavioral patterns including agility, aggressiveness and feeding were observed. The rats were weighed before and after the study, and absolute body weight change was computed as described by Amber et al. [26].

At the end of the experiment, rats were euthanized using ketamine (75 mg/kg i.p, Kurdi et al. [27]) anesthesia. The brain was dissected out of the cranial cavity by a median incision through the cranium. Harvested whole brain was weighed and sectioned sagittally into two halves; one part homogenized in M1 phosphate buffer (pH 7.4) for biochemical assessments and the other half was immediately fixed in Bouin's fluid (fixative).

Biochemical Analysis

Collected brain homogenate were centrifuged and aliquots of supernatant obtained for biochemical assay: oxidative stress bio-makers (malondialdehyde, MDA; superoxide dismutase, SOD; catalase, CAT and glutathione peroxidase, GPx) and endogenous neurotransmitter enzyme (acetylcholinesterase, AChE) using Assay Kits (manufactured by Sigma-Aldrich Pty Ltd, Darmstadt, Germany Suite 1, Level 1 Building B 11 Talavera Road) according to the manufacturer's instruction.

Histological Studies

The fixed brains were processed for light microscopic examination using histological techniques stained with Hematoxylin and Eosin (H&E) stains. Sections of the hippocampus were obtained as reported by Agbon et al. (2021) [28] and general cyto-architectural features of *Cornu ammonis* (CA) of the hippocampal regions (CA1 and CA3) assessed.

Data Analysis

Data obtained were expressed as mean \pm standard error of mean (SEM); paired sample t-test was employed for the comparison of means within groups and one way analysis of variance (ANOVA) with least significant difference (LSD) *post hoc test* for presence of significant difference among means of the groups. Confidence interval was set at $p < 0.05$, for values to be considered as statistically significant. The statistical softwares, Statistical Package for the Social Sciences (IBM SPSS version 25.0 SPSS IBM., Armonk, NY) and Microsoft Office Excel 2013 (for charts) were employed for data analysis.

RESULTS

Phytochemical Analysis

Qualitative phytochemical screening of HCS produced positive reaction for secondary metabolites like, flavonoids, saponins and tannins (Table 2).

Table 2: Phytochemical Constituents of the hydro-ethanol leaf extract of *Cannabis sativa*

Constituents	HCS
Flavonoids	+
Saponins	+
Tannins	+
Cardiac glycoside	+
Carbohydrates	+
Alkaloid	-
Steroid	+
Unsaturated Tripenone	+
Antraquinones	-

Key: + = Positive (present); - = Negative (absent); **HCS**= hydro-ethanol leaf extract of *Cannabis sativa*.

Physical Observation

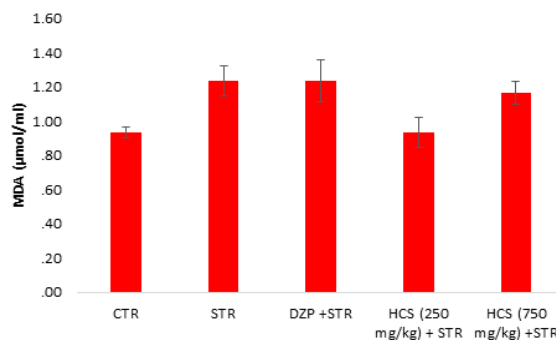
During the experimental period, changes in physical activity and behavioral patterns were observed in the rats. Reduced agility including sluggishness and weakness were observed in the stress group, whereas cannabis-treated groups exhibited some aggressive like-behaviors however, not quantified.

Biochemical Studies

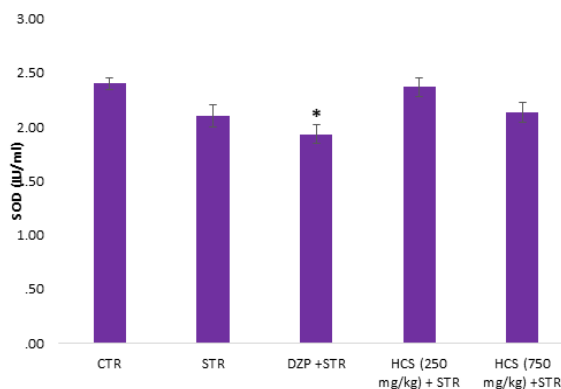
Analysis of oxidative stress biomarkers in tissue (brain) revealed, remarkably ($p < 0.05$) increased MDA levels in Stress-exposed groups, except in HCS-treated (250 and 750 mg/kg) groups when compared with the control (Figure 2). Relative to the control, SOD levels decreased in the Stress-exposed groups, especially ($p < 0.05$) in Diazepam + Stress- treated group (Figure 3).

CAT levels decreased significantly ($p < 0.05$) in Diazepam + Stress- and HCS (750 mg/kg) + Stress-treated groups when compared with the control (Figure 4). GPx levels showed no difference in all treated groups when compared with the control (Figure 5).

Tissue (brain) endogenous neurotransmitter enzyme (AChE) levels revealed decrease in Stress-exposed groups, especially ($p < 0.05$) in HCS (250 mg/kg) + Stress-treated group when compared with the control (Figure 6).

**Figure 2: Effect of hydro-ethanol leaf extract of *Cannabis sativa* on tissue (brain) MDA levels in Wistar rats**

n= 5; mean ± SEM, One-way ANOVA, * = $p < 0.05$ significant difference when compared to control. **MDA**= Malondialdehyde; **CTR**= Control (distilled H₂O 1 ml/kg); **HCS**=hydro-ethanol leaf extract of *Cannabis sativa*; **DZP**= Diazepam (5 mg/kg); **STR**= Stress.

**Figure 3: Effect of hydro-ethanol leaf extract of *Cannabis sativa* on tissue (brain) SOD activity in Wistar rats**

n=5; mean± SEM, One-way ANOVA, * = $p < 0.05$ significant difference when compared to control. **SOD**= Superoxide dismutase; **CTR**= Control (distilled H₂O 1 ml/kg); **HCS**=hydro-ethanol leaf extract of *Cannabis sativa*; **DZP**= Diazepam (5 mg/kg); **STR**= Stress.

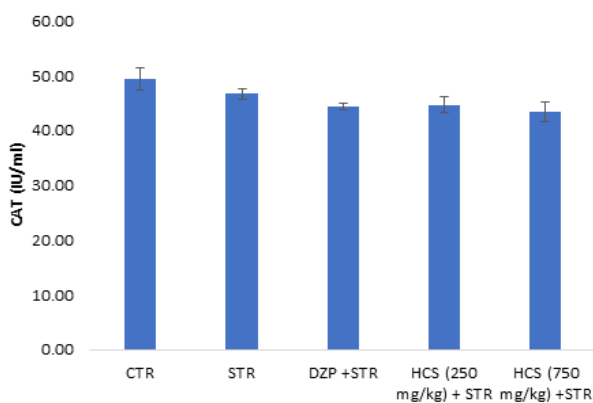


Figure 4: Effect of hydro-ethanol leaf extract of *Cannabis sativa* L. on tissue (brain) CAT activity in Wistar rats

n=5; mean± SEM, One-way ANOVA, $*=p<0.05$ significant difference when compared to control. CAT= Catalase; CTR= Control (distilled H₂O 1 ml/kg); HCS=hydro-ethanol leaf extract of *Cannabis sativa*; DZP= Diazepam (5 mg/kg); STR= Stress.

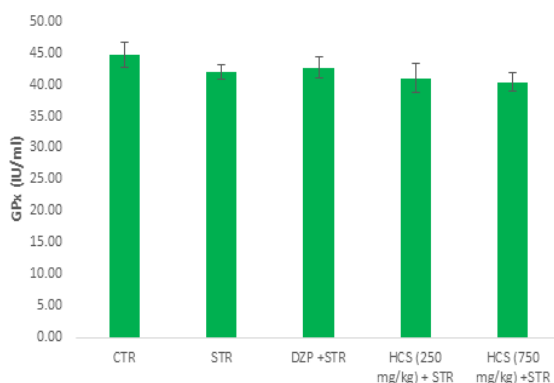
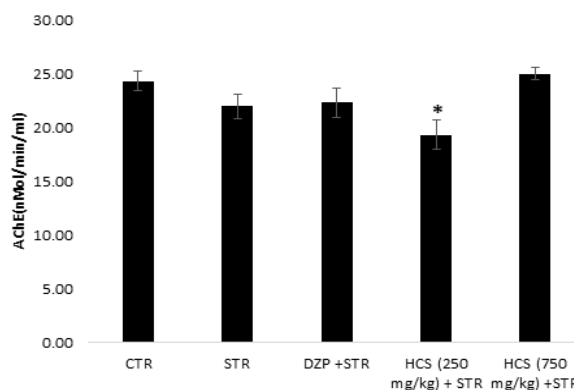


Figure 5: Effect of hydro-ethanol extract of *Cannabis sativa* on tissue (brain) GPx activity in Wistar rats

n=5; mean± SEM, One-way ANOVA. GPx= Glutathione peroxidase; CTR= Control (distilled H₂O 1 ml/kg); HCS=hydro-ethanol leaf extract of *Cannabis sativa*; DZP= Diazepam (5 mg/kg); STR= Stress.



Histological Studies

Histological examination of sections of rats' hippocampal regions (CA1 and CA3) revealed normal histoarchitecture in the control with the characteristic pattern having three layers; molecular, pyramidal and multiform layers. The pyramidal layer consists of an array of neurons (pyramidal and granules cells) with cell bodies closely packed together. The pyramidal cells were larger and obliquely oriented in the CA3 region relative to CA1 region (Plates 1A and 2A).

Relative to the control, histological sections of the Stress-exposed groups demonstrated neuropathologic changes as marked histoarchitectural distortion of the CA1 and CA3 regions including karyolysed cells, necrosed cells with pyknotic nuclei and depolarization or irregular arrangement of CA1 neurons (Plates 1B and 2B). Sections of the Diazepam + Stress-treated group revealed neuronal shrinkage, perineuronal vacuolation and karyorrhexis of the CA1 and CA3 regions (Plates 1C and 2C).

However, sections of HCS + Stress-treated groups revealed preserved histoarchitecture of the CA1 and CA3 regions, especially in HCS (250 mg/kg)-treated group (Plates 1D and 2D). Histoarchitectural distortion of the hippocampal regions were observed in HCS (750 mg/kg)-treated group (Plates 1E and 2E).

Figure 6: Effect of hydro-ethanol leaf extract of *Cannabis sativa* on tissue (brain) AChE activity in Wistar rats

n=5; mean± SEM, paired *t*-test, $*=p<0.05$ significance difference when compared to control. AChE= Acetylcholinesterase; CTR= Control (distilled H₂O 1 ml/kg); HCS=hydro-ethanol leaf extract of *Cannabis sativa*; DZP= Diazepam (5 mg/kg); STR= Stress.

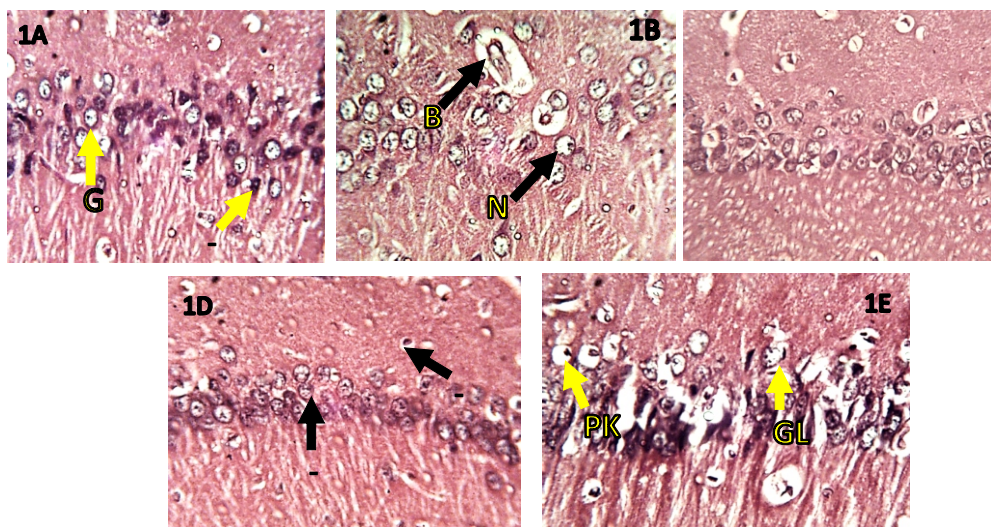


Plate 1: Section of the hippocampus (CA1 region) of Wistar rat treated with hydro-ethanol leaf extract of *Cannabis sativa* following exposure to stress. H&E (Mag x 250)

Pyramidal Cells (P); Granule cells (G); Oligodendrocytes (O); Blood Vessel (B) Neurodegeneration (N); Perineuronal vacuolation (PV); Pyknosis (PK); Gliosis (GL)

A=control, B=stress, C=diazepam 5mg/kg +stress D= HCS 250 mg/kg +stress,E= HCS 750 mg/kg +stress

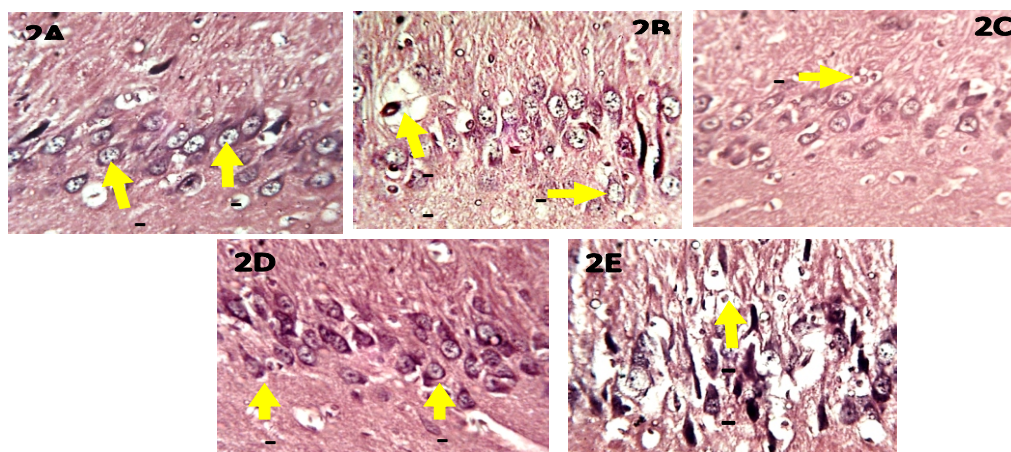


Plate 2: Section of the hippocampus (CA3 region) of Wistar rat treated with hydro-ethanol leaf extract of *Cannabis sativa* following exposure to stress. H&E (Mag x 250)

Pyramidal Cells (P); Granule cells (G); Oligodendrocytes (OG); Blood Vessel (BV) Neurodegeneration (N); Perineuronal vacuolation (PV); Chromatolysis Condensation (CC)

A=control, B=stress, C=diazepam 5mg/kg +stress D= HCS 250 mg/kg +stress, E= HCS 750 mg/kg +stress

DISCUSSION

In this study, qualitative phytochemical screening of HCS was conducted and, the neuroprotective property assessed following stress-triggered changes in oxidative stress bio-markers and histoarchitecture of CA1 and CA3 hippocampal

regions of Wistar rats.

Phytochemical analysis of HCS revealed the presence of health beneficial secondary metabolites including flavonoids, saponins, and tannins which have been reported to possess

antioxidant and neuroprotective properties in models of neurological disorders^[29]. Findings agree with reported phytochemicals present in leaf extracts of *Cannabis sativa*^[30].

Alternations in the homeostasis of biochemical and physiological state and processes have been associated with exposure to stressing conditions at cellular and subcellular levels^[31]. Stress increases generation of oxygen free radicals which are unstable and readily reacts with components including lipids, proteins, and DNA in the body, impairing the antioxidant defense system and consequently, triggering apoptotic changes and neuronal damage in neurological conditions like neurodegenerative diseases^[32, 33]. Oxidative stress biomarkers are established indicators of the state (healthy or pathologic) of biological systems depicting lipid peroxidation (LPO) levels and alterations in the activity of endogenous antioxidant enzymes^[34].

Observed elevated MDA levels following stress exposure in this study is indicative of changes in lipid metabolism during stress resulting to elevated LPO levels in the system. Findings are in line with the reports of El- sokkary (2008)^[35] and Eger et al., (2016)^[36] who associated stress exposure with altered enzymatic activity in the rats. Remarkably elevated MDA levels observed in diazepam + Stress-treated group is suggestive that, metabolism of diazepam in stressing condition could enhance the production of reactive oxygen species (ROS) leading to peroxidation of membrane lipids which the antioxidant defenses were unable to scavenge effectively^[37]. ROS generated by the bimodal effect of stress and diazepam metabolism could probably lead to cell injury including neuronal apoptotic and degenerative changes^[38,39]. Treatment with *C. sativa* played a pivotal role in maintaining MDA levels relative to the control. This finding could be attributed to the potential ability of Cannabinoid (CBD) beneficial phytochemicals to boost the antioxidant activity of terpenes and/or polyphenols through its synergistic effect and free radical

scavenging properties^[40,41].

Endogenous antioxidant enzymes including SOD, CAT and GPx play critical roles in the breaking down and scavenging of ROS within the system^[42]. A decline or down-regulation of these enzymes have been associated with accumulation of free radicals within cells and subsequently, cytotoxicity or cell injury^[43]. In this study, SOD and CAT activity were observed to be reduced in diazepam + Stress-treated group relative to the control. This finding corroborates the possibility of oxidative stress generation in response to the bimodal effect of stress and diazepam's active metabolite (desmethyldiazepam) to inhibit the ROS-scavenging action of the antioxidant enzymes. Altered homeostasis of CAT activity has been associated with neurological disease conditions including Alzheimer's and Parkinson's diseases, bipolar disorder and schizophrenia^[42]. Findings are in consistence with the reports of Eger *et al.*^[36].

Administration of *C. sativa*, especially at dose 250 mg/kg, maintained SOD and CAT activity relative to the control. This finding is suggestive of the possible modulatory role of *C. sativa* metabolites in enhancing endogenous enzymatic antioxidant activity and consequently, reducing oxidative stress^[43]. Reducing oxidative stress could probably be by acting at transient receptor potential cation channel sub-family V member 1 (TRPV₁), endogenous cannabinoid receptors Type 2 (CB₂), and G protein coupled receptor (GPR₅₅) sites; these interactions lead to down-regulation of enzymes involved in the production of prostaglandins, cytokines and ROS^[44]. Relative to the control, GPx activity revealed no remarkable changes in the stress exposed groups. This may imply slight or no reactivity or response in the treatment-enzyme biochemical process. Thus, finding is suggestive of the beneficial property of *C. sativa*, especially at dose 250 mg/kg, in the amelioration of oxidative stress following exogenous stress exposure.

AChE activity revealed no remarkable changes, except in HSC (250 mg/kg)-treated group, relative to the control. This is in agreement with the work of

Malathi et al.^[45] who proposed the influence of stress on AChE levels to be dependent on the kind and duration of stressors. Thus, it is possible that two hours of daily restrain stress was unable to alter AChE levels in rats. Significantly decreased AChE levels observed with HSC (250 mg/kg)-treated group could be attributed to cholinesterase enzyme inhibitor activity of CBD rich phytochemicals associated with improvement of cognitive functions. This finding is in line with the work of Osaretin et al.^[46] who reported reduction in brain AChE activity in rats following acute exposure to a diet supplemented with *C. sativa*.

Cascades of event leading to LPO in the brain has been associated with major alternations in the mechanism of cells and in some instances, cell injury and neuronal death^[32,4]. Hippocampal histoarchitectural changes including perineuronal vacuolation and pyknosis observed in Stress-exposed group is suggestive of treatment-triggered pathologies. Saravana et al.^[48] and McEwen et al.^[49] have reported that, glucocorticoids released during stressful events accelerate morphological deformation and functional retardation in the hippocampal pyramidal neurons. Finding is in accordance with stress-related reports in animals and humans that, stressful events accelerate morphological deformation and functional retardation in the hippocampal circuitry and pyramidal cells^[50,51,52].

Similarly, Diazepam + Stress-treated group revealed histoarchitectural changes in the hippocampal region, which was rather mild compared the Stress-treated group. Findings corroborates the possibility of neuronal pathologic changes subsequent to oxidative stress generation in response to the bimodal effect of stress and desmethyldiazepam on the hippocampus. Furthermore, findings could attributed to the inhibitory ability of diazepam in modulation on GABA receptor to exert inhibitory neurotransmission in the central nervous system, consequently reducing the excitability of neurons and subsequent histopathological changes^[53,54]. El-

sokkary^[55] reported that long-term use of diazepam terminates in a complex liver damage marked by an increase in systemic lipid peroxides activity plus a decrease in GPx concentration and SOD activity in diazepam-treated rats.

Interestingly, HCS (250 mg/kg) treatment preserved hippocampal histoarchitecture following stress exposure, which is suggestive of the neuroprotective properties *C. sativa*. This finding is in line with the reported health benefits of reduced doses of *C. sativa* as a potentials neuroprotective agent against exogenous neurotoxins^[56, 57, 58, 59]. *C. sativa* contains phenolics (linalool, α -pinene, β -caryophyllene, flavonoids and anthocyanin) established for their antioxidative nature and radical scavenging properties; thus, preventing cellular damage^[59]. Conversely, histopathologic changes observed with HCS (750 mg/kg) treatment could be related to the reports of Solanke et al.^[60] and Imam et al.^[61] who observed neurodegenerative in rats treated with 20 mg/kg of aqueous extract of *C. sativa* for 7 days.

Possibly, higher concentrations of HCS could contain high amount of Tetrahydrocannabinol (THC), a psychoactive component of *C. sativa*, which binds to cannabinoid receptors and may interfere with the well maintained inhibitory signaling regulated by endogenous cannabinoids [60]. Some studies have reported that, cannabinoids at high doses induce excessive secretion of glutamate resulting to neurotoxicity. This finding thus, support the assumption that exposure to *C. sativa* at high doses may contribute to increasing incidence of brain damage in animal models and humans^[61,62,63].

Neuroprotective therapies often target oxidative stress and excitotoxicity-both of which are highly associated with central nervous system disorders. Relative to the reference drug (Diazepam), HCS especially at dose 250, proved more efficacious as it confers better neuroprotective property following stress exposure. Findings support the

health benefits and potentials of *C. sativa* products in the management of stress-induced neurodegenerative changes in animal model. Neuroprotective property could be attributed to constituent pharmacologically active substance(s), phytochemicals including phenolic compounds like flavonoids, which possess neuroprotective activity, efficacious as an agent for the treatment and management of neurological conditions in traditional practice of medicine^[63,28].

CONCLUSION

Findings suggests that hydro-ethanol leaf extract of *C. sativa* at low dose may prove efficacious in ameliorating biochemical alterations and hippocampal changes following stress exposure in Wistar rats. Thus, hydro-ethanol extract of *C. sativa* is a potential candidate for application in the management and treatment of stress-related neurodegenerative disease conditions including dementia and Alzheimer's disease. Hence its formulation into pharmacological therapies for the management of stress and related conditions in modern day health care. However, in-depth study including ultra-microscopy and immunohistochemistry and, molecular assessments with respect to alternate routes of administration (intramuscular, intraperitoneal or inhalation) are required to fully elucidate the findings of this study.

REFERENCES

1. Archana A, Vidya H. M , and Ravindran R [2018]. Effect of Eclipta Alba Administration On Chronic Stress Induced Behavioral Changes In Wistar Albino Rats. *European Journal of Biomedical and Pharmaceutical sciences*, 5[2]: 633-639.
2. Maria Morena, Sachin Patel, Jaideep S Bains and Matthew N Hill [2016]. Neurobiological Interactions between Stress and the Endocannabinoid System. *Neuropsychopharmacology reviews*, 41: 80–102.
3. Branka Markovi T, Nevena V. RadonjiT, Milan AksiT, Branislav FilipoviT, and Nataša Petronijevit T [2014]. Long-Term Effects of Maternal Deprivation on Cholinergic System in Rat Brain. *BioMedical Research International*, 2014: 1-7.
4. Chaves, T.; Fazekas, C.L.; Horváth, K.; Correia, P.; Szabó, A.; Török, B.; Bánrévi, K.; Zelena, D. [2021]. Stress Adaptation and the Brainstem with Focus on Corticotropin-Releasing Hormone. *International Journal of Molecular Science*, 22, 9090.
5. Weishun Tian, Md Rashedunnabi Akanda, Anowarul Islam, Hae-Dong Yang, Sang-Cheon Lee, Jeong-Ho Lee, Sang-Ki Kim, Yu-Jin Choi, So-Yeon Im and Byung-Yong Park, [2018]. The Anti-Stress Effect of Mentha arvensis in Immobilized Rats. *International Journal of Molecular Sciences*, 2[19]: 355.
6. Vidita A. [2000]. Stress, depression and hippocampal damage. *Journal of Bioscience*, 21[2]: 123-124.
7. Kim, J. I., Lee, J. W., Lee, Y. A., Lee, D. H., Han, N. S and Choi, Y. K., [2013]. Sexual activity counteracts the suppressive effects of chronic stress on adult hippocampal neurogenesis and recognition memory. *Brain Research*, 1538: 26–40.
8. Grigoryan, G., Biella, G., Albani, D., Forloni, G., and Segal, M. [2014]. Stress impairs synaptic plasticity in triple-transgenic Alzheimer's disease mice: rescue by ryanodine. *Neurodegenerative. Diseases*. 13: 135–138.
9. Patki, G., Solanki, N., Atrooz, F., Allam, F., Salim, S. [2013]. Depression, anxiety-like behavior and memory impairment are associated with increased oxidative stress and inflammation in a rat model of social stress. *Brain Research*, 1539: 73–86.
10. Xu, B., Lang, L., Li, S.-Z., Guo, J.-R., Wang, J.-F., Wang, D and Lian, S. [2019]. Cortisol Excess-Mediated Mitochondrial Damage Induced Hippocampal Neuronal Apoptosis in Mice Following Cold Exposure. *Cells*, 8[6]: 612.
11. LaVigne, J.E., Hecksel, R., Keresztes, A. John

- M. Streicher [2021]. *Cannabis sativa* terpenes are cannabimimetic and selectively enhance cannabinoid activity. *Science Report* 11, 8232. <https://doi.org/10.1038/s41598-021-87740-8>
12. Flemming, R., Muntendam, T., Steup, T., Kayser, O. [2007]. Chemistry and biological activity of tetrahydrocannabinol and its derivatives. *Topics in Heterocyclic Chemistry*, 10: 1-42.
 13. Thafeni, M., Sayed, Y., Motadi, L. [2012]. *Euphorbia mauritanica* and *Kedrostis hirtella* extracts induces cell death in lung cancer cells. *Journal of Molecular Biology*, 39[12]: 10785–10794
 14. Valentina L., Yann C., Pedro., Nadia S., and Carl A. [2019]. Does regular cannabis use affect neuroanatomy? An updated systematic review and meta-analysis of structural neuroimaging studies. *European Archives of Psychiatry and Clinical Neuroscience*, 10[2]: 10-23.
 15. Li-Weber, M. [2013]. Targeting apoptosis pathways in cancer by Chinese medicine. *Cancer Letters*, 332: 304–312.
 16. Thafeni, M., Sayed, Y., Motadi, L. [2012]. *Euphorbia mauritanica* and *Kedrostis hirtella* extracts induces cell death in lung cancer cells. *Journal of Molecular Biology*, 39[12]: 10785–10794.
 17. Tzadok M., Uliel-Siboni S., Linder I., Kramer U., Epstein O., Menascu S., Nissenkorn A., Yosef O.B., Hyman E., Granot D., Dor M., Lerman-Sargie T., Ben-Zeev B. [2016]. CBD-enriched medical cannabis for intractable pediatric epilepsy: the current Israeli experience. *Seizure*, 35: 41-44.
 18. Lynch M.E. [2016]. Cannabinoids in the management of chronic pain: a front line clinical perspective. *Journal of Basic Clinical Physiology and Pharmacology*, doi: 10.1515/jbcpp2015-0059.
 19. Velasco G., Hernandez-Tiedra S., Davila D., Lorente M. [2016]. The use of cannabinoids as anticancer agents. *Progress Neuro-Psychopharmacology, Biology and Psychiatry*, 64: 259-266.
 20. Bergamaschi, M.M., Queiroz, R.H., Chagas, M.H., et al. [2014]. Rimonabant effects on anxiety induced by simulated public speaking in healthy humans: a preliminary report. *Human Psychopharmacology*, 29: 94–99.
 21. Soforowa, E. A. [1982]. Medicinal plants and traditional medicine in Africa. *John Wiley and sons, New York*, 256
 22. Trease GE, Evans WC. *Phytochemicals. Pharmacognosy*. 15th eds. Saunders Publishers, London; 2002. 42-393
 23. Smith, C. [2012]. Using Rodent Models to Simulate Stress of Physiologically Relevant Severity: When, Why and How. *Glucocorticoids - New Recognition of Our Familiar Friend*
 24. Suleiman J. Bagi, Eze E. Daniel, Karimah M. Rabi, Iliya Ezekiel, Sheu O. Sulaiman (2017). Effects of oral administration of some selected sweeteners on anxiety in Wistar rat models. *Research in Neuroscience*, 6(2): 28-34.
 25. Zachariah, R., Adebisi, S. S., Uduak, E. U., Sadeeq, A. A., & Adamu, P. P. (2020). Effect of n-butanol extract of *Cannabis sativa* L. extract on the cerebral cortex of adult Wistar rats. *African Journal of Cellular Pathology*, 12(2), 6-15.
 26. Amber WS, Musa SA, Sambo SJ, Agbon AN. [2020]. Nephroprotective effect of *Citrus sinensis* L. on mercury exposed Wistar rats. *Annals of Tropical Pathology*, 11:157-65.
 27. Kurdi SM, Theerth KA, Derva RS. Ketamin: Current applications in anesthesia, pain and critical care. *Anesthesia Essays Research*, 2014;8:283-90.
 28. Agbon A.N., Kwanashie H.O., Hamman W.O.1, Ibegbu A.O., Henry R., Sule H., Yahaya M. H., Shuaib Y.M., Usman I.M., Ivang A.E., and Oladimeji O.J. [2021] Comparative Microscopic Assessments of the Effect of Aqueous and Ethanol Extracts of *Phoenix dactylifera* L. in a Rat Model of Mercury- Triggered Hippocampal Changes

- Nigerian journal of physiological sciences: official publication of the Physiological Society of Nigeria 36:91-100
29. Henry, R., Agbon, A.N., Yakubu, U., Simon, D., Shuaib, Y.M., Sule, H., Yahaya, M.H., Usman, I M., Ivang, A.E., Oladimeji, O., Bobbo, K.A. and Mahdi, O. [2021]. Neurobehavioral and Microscopic assessments of methanol fruit extract of Phoenix dactylifera on lead acetate-induced cerebellar changes in Wistar rats. *Journal of Anatomical Sciences*, 12[1]: 95-108
 30. Andre CM, Hausman J-F and Guerriero G [2016]. Cannabis sativa: The Plant of the Thousand and One Molecules. *Frontiers in Plant Science* 7:19.
 31. Cordaro M, D'Amico R, Morabito R, Fusco R, Siracusa R, Peritore AF, Impellizzeri D, Genovese T, Crupi R, Gugliandolo E, Marino A, Di Paola R, Cuzzocrea S. Physiological and Biochemical Changes in NRF2 Pathway in Aged Animals Subjected to Brain Injury. *Cell Physiology and Biochemistry*, 55[2]:160-179. doi: 10.33594/000000353. PMID: 33811485.
 32. Qairunnisa G. Gajalakshmi, G. Purushothaman, B.A. Madhuri and M. Chandrasekhar [2014]. A study to evaluate the effect of restraint stress on antioxidants status in rats. *International Journal of Pharmaceutical Science and Research*, 5[1]: 156-160.
 33. Wang, F., Pan, F., Tang, Y., & Huang, J. H. [2021]. Editorial: Stress Induced Neural Changes in Emotional Disorders. *Frontiers in psychiatry*, 12, 710691. <https://doi.org/10.3389/fpsy.2021.71069>
 34. Dhama Kuldeep, Latheef Shyma K., Dadar Maryam, Samad Hari Abdul, Munjal Ashok, Khandia Rekha, Karthik Kumaragurubaran, Tiwari Ruchi, Yattoo Mohd. Iqbal, Bhatt Prakash, Chakraborty Sandip, Singh Karam Pal, Iqbal Hafiz M. N., Chaicumpa Wanpen, Joshi Sunil Kumar [2019]. Biomarkers in Stress Related Diseases/Disorders: Diagnostic, Prognostic, and Therapeutic Values. *Frontiers in Molecular Biosciences*, 6-91
 35. El-Sokkary GH, Kamel ES, Reiter RJ [2003]. Prophylactic effect of melatonin in reducing lead-induced neurotoxicity in the rat. *Cell Molecular Biology Letter* ;8[2]:461-70. PMID: 12813579.
 36. Eger G. A., V. V. Ferreira, C. R. Batista et al., [2016]. "Acute administration of diazepam provokes redox homeostasis imbalance in the rat brain: prevention by simvastatin". *Journal of Biochemical and Molecular Toxicology*, 10: 506–512
 37. Emmanuel O Ogueji, Stanley C Iheanacho, Christopher D Nwani, Christian E Mbah, Ogochukwu C Okeke, and Ibrahim B Usman [2017]. Toxicity of diazepam on lipid peroxidation, biochemical and oxidative stress indicators on liver and gill tissues of African catfish *Clarias gariepinus* [Burchell, 1822]. *International Journal of Fisheries and Aquatic Studies*, 5[3]: 114-123
 38. Ogueji, E.O., Iheanacho S.C., Nwani, C.D., Mbah, C.E., Okeke, O.C., and Usman I.B. [2017]. Toxicity of diazepam on lipid peroxidation, biochemical and oxidative stress indicators on liver and gill tissues of African catfish *Clarias gariepinus* [Burchell, 1822]. *International Journal of Fisheries and Aquatic Studies*, 5[3]: 114-123
 39. Rafia, A., Ahmad Rafia, Shahrbanoo Oryan, Akram Eidi, and Hedayat Sahraei [2020]. Memantine, Stress, Learning and Memory. *Basic and Clinical Neuroscience*, 11[4]: 447-456
 40. David, B., Wolfender, J.L., Dias, D.A. [2015]. The pharmaceutical industry and natural products: historical status and new trends. *Phytochemistry Review*, 14:299-315.
 41. Hacke, A.C.M.; Lima, D.; de Costa, F.; Deshmukh, K.; Li, N.; Chow, A.M.; Marques, J.A.; Pereira, R.P.; Kerman, K [2019]. Probing the antioxidant activity of Δ^9 -tetrahydrocannabinol and cannabidiol in Cannabis sativa extracts. *Analyst*, 144, 4952–4961.

42. Umoren, E. B., Wopara, I., Adebayo, O. G., Ezike, U. A., & Obembe, A. O. [2020]. The Effect of Ethanolic Extract of Cannabis sativa Leaves from Nigeria on the Antioxidants Markers in Albino Wistar Rats. *International Journal of Biochemistry Research & Review*, 29[9]: 58-65.
43. Liu Zewen, Ren Zhangpin, Zhang Jun, Chuang Chia-Chen, Kandaswamy Eswar, Zhou Tingyang, Zuo Li [2018]. Role of ROS and Nutritional Antioxidants in Human Diseases *Frontiers in Physiology*, 9, 477-491
44. Federica Pellati, Vittoria Borghonetti, Virginia Brighenti, Marco Biagi, Stefania Benvenuti, and Lorenzo Corsi. [2018]. Cannabis sativa L. and Nonpsychoactive Cannabinoids: Their Chemistry and Role against Oxidative Stress, Inflammation, and Cancer. *BioMed Research International*, 2018: 1691428.
45. Malathi S [2016]. Restoration of memory and acetylcholinesterase activity by michelia champaca in chronically noise stressed Wistar albino rats, *Asian Journal of Pharmaceutical and Clinical Research*:9[6]
46. Osaretin Albert Taiwo Ebuehi, Nosarieme Omoregie Abey [2016]. Impact of Marijuana [*Cannabis sativa*] on Some Neurochemicals and Cognitive Function of Sprague-Dawley Rats, *Research in Neuroscience*, 5[1]1-9. doi: 10.5923/Journal Neuroscience.20160501.01.
47. Saikarthik Jayakumar, Gunapriya Raghunath, Saraswathi Ilango, J Vijayakumar and R Vijayaraghavan [2017]. Effect of Fluoxetine on the Hippocampus of Wistar Albino Rats in Cold Restraint Stress Model. *Journal of Clinical and Diagnostic Research*, 11[6]: 1-6
48. S.S.K., P.S.R, V. [2015]. *Effect of Bacopa Monniera on Cold Stress Induced Neurodegeneration in Hippocampus of Wistar Rats: A Histomorphometric Study*, *Journal of Clinical and Diagnostic Research*. 9[1]: 05-07.
49. McEwen, B. S., Nasca, C., & Gray, J. D. [2016]. Stress effects on neuronal structure: hippocampus, amygdala, and prefrontal cortex. *Neuropsychopharmacology*, 41[1]: 3-23.
50. Radley, J. J., Kabbaj, M., Jacobson, L., Heydendael, W., Yehuda, R., & Herman, J. P. [2011]. Stress risk factors and stress-related pathology: Neuroplasticity, epigenetics and endophenotypes. *Stress*, 14[5]:481-497. doi:10.3109/10253890.2011.604751
51. Bruce S. McEwen and Huda Akil [2020]. Revisiting the Stress Concept: Implications for Affective Disorders. *The Journal of Neuroscience*, 40[1]: 12-21
52. Rafia, A., Ahmad Rafia, Shahrbanoo Oryan, Akram Eidi, and Hedayat Sahraei [2020]. Memantine, Stress, Learning and Memory. *Basic and Clinical Neuroscience*, 11[4]: 447-456
53. Griffin, C.E., A. M. Kaye, F. R. Bueno, and A. D. Kaye [2013]. "Benzodiazepine pharmacology and central nervous system-mediated effects" *The Ochsner Journal*, 13[2]: 214-223.
54. Casasola-Castro, C., Weissmann-Sánchez, L., Calixto-González, E., Aguayo-Del Castillo, A., & Velázquez-Martínez, D. N. [2017]. Short-term and long-term effects of diazepam on the memory for discrimination and generalization of scopolamine. *Psychopharmacology*, 234[20]: 3083-3090. doi:10.1007/s00213-017-4692-8
55. El-Sokkary G [2008]. Melatonin and vitamin C administration ameliorate diazepam-induced oxidative stress and cell proliferation in the liver of rats. *Cell Proliferation*, 41[1]:168-176.
56. Ibeas Bih, C., Chen, T., Nunn, A.V.W. et al. Molecular Targets of Cannabidiol in Neurological Disorders. *Neurotherapeutics* 12, 699 – 730 [2015]. <https://doi.org/10.1007/s13311-015-0377-3>
57. Alline C. Campos, Manoela V. Fogaça, Andreza B. Sonogo and Francisco S. Guimarães [2016]. Cannabidiol, neuroprotection and neuropsychiatric disorders, *Pharmacological Research*, 112, 119-127.
58. Campos, A. C., Fogaça, M. V., Scarante, F. F.,

- Joca, S., Sales, A. J., Gomes, F. V., Sonego, A. B., Rodrigues, N. S., Galve-Roperh, I., & Guimarães, F. S. [2017]. Plastic and Neuroprotective Mechanisms Involved in the Therapeutic Effects of Cannabidiol in Psychiatric Disorders. *Frontiers in pharmacology*, 8, 269. <https://doi.org/10.3389/fphar.2017.00269>
59. Raja, A., Ahmadi, S., de Costa, F., Li, N., & Kerman, K. [2020]. Attenuation of Oxidative Stress by Cannabinoids and Cannabis Extracts in Differentiated Neuronal Cells. *Pharmaceuticals [Basel, Switzerland]*, 13 [1 1] : 3 2 8 . <https://doi.org/10.3390/ph13110328>
60. Solanke Adebayo Solomon, Ebuehi Osaretin Albert T., Adetona Mutiat Olutope [2016]. Toxicological Effect of Ethanolic Extract of *Cannabis sativa* on Brain Serotonin in Adult Wistar Rats, *American Journal of Biochemistry*, 6 [4] : 9 7 - 9 9 . doi:10.5923/j.ajb.20160604.02
61. Imam A., Ajao M. S., Akinola O. B., Ajibola M. I., Ibrahim A., Amin A., Abdulmajeed, Z W.I., Lawal A. and Ali-Oluwafuyi A. [2016]. Repeated Acute Oral Exposure to Cannabis sativa Impaired Neurocognitive Behaviours and Cortico-hippocampal Architectonics in Wistar Rats. *Nigeria Journal of Physiological Sciences*, 153-159
62. Weston-Green, K., Clunas, H., & Jimenez Naranjo, C. [2021]. A Review of the Potential Use of Pinene and Linalool as Terpene-Based Medicines for Brain Health: Discovering Novel Therapeutics in the Flavours and Fragrances of Cannabis. *Frontiers in psychiatry*, 12, 583211. <https://doi.org/10.3389/fpsyt.2021.583211>
63. Sarian MN, Ahmed QU, Mat So'ad SZ, Alhassan AM, Murugesu S, Perumal V, Syed Mohamad SNA, Khatib A, Latip J [2017]. Antioxidant and Antidiabetic Effects of Flavonoids: A Structure-Activity Relationship Based Study. *Biomedical Research International.*;2017:8386065. doi: 10.1155/2017/8386065. Epub 2017 Nov 28. PMID: 29318154; PMCID: PMC5727842.