International Journal of Pharmaceutical and Bio-Medical Science

ISSN(print): 2767-827X, ISSN(online): 2767-830X

Volume 04 Issue 12 December 2024

Page No : 910-918

DOI: https://doi.org/10.47191/ijpbms/v4-i12-01, Impact Factor:7.792

Chronic Cannabis Extract Modulates Anxiety Behavior in Wistar Rats: VTA Histology and Catecholamine Analysis

Nkanu, I.I.[1], Eteudo A.N.[1], Anwara, C.E.[1], Obun, C.[2], Epete, M.A.[,] EKECHI, H.O.[3], NWEKE O. I.[4]

[1]Department of Human Anatomy, Ebonyi State University, Nigeria

[2]Department of Anatomy and Forensic Anthropology, University of Cross River State, Nigeria

[3]Department of Anatomy, Federal University of Technology Owerri, Imo state, Nigeria

[4]Faculty of Medical Biochemistry, Ebonyi State University Abakaliki, Nigeria

ABSTRACT

Cannabis, derived from the *Cannabis sativa* plant, has seen a surge in global use, particularly in medicinal and recreational contexts, over the past decade. The VTA, a critical component of the brain's reward system, plays a pivotal role in motivation and addiction through its dopaminergic pathways. While research has examined the behavioral and neurochemical effects of cannabis, there is limited exploration of cannabis-induced histological changes in the VTA, thus, this study investigated the effects of chronic cannabis ethanol extract exposure on anxiety behavior, ventral tegmental area (VTA) histology, and catecholamine levels in Wistar rats. Using the Elevated Plus Maze (EPM) test, anxiety behavior was assessed across four groups exposed to varying doses (0, 50, 100, and 150 mg/kg) of cannabis extract. Histological analysis of the VTA and catecholamine quantification were conducted to evaluate structural and biochemical changes. Behavioral results indicated dose-dependent increases in anxiety, with Group 3 (100 mg/kg) showing the highest anxiety-related behaviors, evidenced by reduced open-arm exploration and increased closed-arm preference ($p < 0.05$). Histological analysis revealed fatty changes and inflammatory cell infiltration in the VTA, with severity increasing at higher doses. Catecholamine levels declined dose-dependent ($p < 0.05$), suggesting suppressed neurotransmitter synthesis or metabolism. These findings align with previous reports of cannabis-induced neurotoxicity and its biphasic effects on anxiety, extending understanding of its behavioral, structural, and biochemical impacts. The results highlight the potential risks of chronic cannabis use, particularly at higher doses, on anxiety regulation and neural integrity in the VTA.

KEYWORD: Cannabis, Extract, Ventral Tegmental Area, Histology, Catecholamine.

ARTICLE DETAILS

Published On:
02 December 2024

Available on:
https://ijpbms.com/

INTRODUCTION

Cannabis, derived from the *Cannabis sativa* plant, has seen a surge in global use, particularly in medicinal and recreational contexts, over the past decade. This increase is largely due to growing legalization, shifts in societal attitudes, and the expanding recognition of its therapeutic benefits, such as pain relief, anti-inflammatory effects, and potential neuroprotective properties (Hasin and Walsh, 2021; Johnson & Lee, 2020). However, concerns about the possible adverse effects of cannabis, especially with chronic or high-dose use, have also risen. These include risks of addiction, cognitive impairments, and neurochemical disruptions, particularly in brain regions associated with reward and motivation (Volkow et al., 2016; Zehra et al., 2016).

The Ventral Tegmental Area (VTA), located in the midbrain, plays a crucial role in the brain's reward system. It regulates motivation, pleasure, and reinforcement learning through dopaminergic pathways that connect to key brain regions such as the nucleus accumbens and prefrontal cortex. These pathways influence behaviors related to reward, addiction, and learning (Brown & Thompson, 2019; Beier, et al. 2019; Yu et al., 2021). As a primary site of dopaminergic activity, the VTA is central to the brain's response to rewarding stimuli and is implicated in developing addictive behaviors

(Polter and Kauer 2014; Bouarab et al., 2019). Given its pivotal role in these processes, understanding how cannabis affects the VTA is crucial for addressing the neurobiological mechanisms underlying cannabis-related behavioral changes. Cannabinoids, the active compounds in cannabis, primarily interact with the brain's endocannabinoid system through CB1 and CB2 receptors. These interactions modulate the release of neurotransmitters, including dopamine, which plays a key role in regulating mood, cognition, and behavior (Patricio et al., 2020). Chronic cannabis use has been shown to alter dopamine signaling, potentially contributing to changes in reward processing and increasing the risk of developing substance use disorders (Davis & Kim, 2021). While much research has focused on the behavioral and neurochemical effects of cannabis, there remains a gap in understanding the histological impact of cannabis exposure on specific brain regions, such as the VTA.

Histological analysis offers valuable insights into cellular changes within the VTA, such as neuronal loss, gliosis, and inflammation, which may indicate neurotoxic effects or adaptive responses to cannabis exposure (Kim et al., 2021). Alterations in catecholamine levels, particularly dopamine, within the VTA could have significant implications for understanding the neurobiological mechanisms behind cannabis-induced behavioral changes (Hurd et al., 2019; Dong et al., 2020).

This study aims to address the existing gap by investigating the histological and neurochemical changes in the VTA following cannabis exposure. Using Wistar rats as an animal model, the research will explore the dose-dependent effects of cannabis ethanol extract on VTA histology and catecholamine levels. By elucidating the cellular and neurochemical alterations induced by cannabis, this study seeks to contribute to a deeper understanding of the long-term effects of cannabis on the brain's reward circuitry, with potential implications for both clinical practices and public health policies.

MATERIALS AND METHODOLOGY

Plant Procurement

500 grams of matured Cannabis plant was obtained from the National Drug and Law Enforcement Agency in Abakaliki, Ebonyi State. The plant was weighed, sliced into smaller pieces, and dried for several weeks before being ground into powder form using a laboratory grinder.

Plant Ethanol Extracts

206g of ground cannabis was mixed with 1500 ml of absolute alcohol in a plastic container and left to steep for 72 hours. The container was securely covered to prevent evaporation. After 72 hours, the mixture was filtered, and the liquid was transferred to a Soxhlet apparatus to allow the extract to dry.

Preparation of Stock Solution for Administration

The stock solution of cannabis plant extract was prepared by dissolving 1 gram of cannabis in 20 ml of water, with 0.5 ml of Tween 80 added to the mixture. This solution was freshly prepared each day before administration. The dosage volume was calculated according to the weight of the Wistar rats and the specific dose assigned to each group.

Volume (ml) = weight of rat (kg) x standard dose per group (mg)/1000

Animal Procurement

A total of 20 Wistar rats weighing between 70-160g were obtained from the animal farm of the Department of Human Anatomy Faculty of Basic Medical Sciences, Ebonyi State University. The Wistar rats were housed in a cage for 72 hours of light and dark cycle for acclimatization and were exposed to food and water. The Wistar rats were weighed and grouped into four groups.

Animal Treatment

20 Wistar rats weighing between 70-160g were randomly grouped into groups 1, 2, 3, and 4. Each group contained 5 Wistar rats, and 2, 3, and 4. Group 1 was the control which was exposed to only rat chow and water, group 2 was administered a low dose of 50mg/kg of cannabis ethanol stock solution, food, and water, group 3 was administered a medium dose of 100mg/kg of cannabis ethanol stock solution, food and water and group 4 were administered a high dose of 150mg/kg of cannabis ethanol stock solution, food, and water. Rats were given administration orally, with administration occurring over 30 days.

Table 1 shows the experimental design.

Groups	No of Animals (20)	Treatments
1	5	Rat chow and water
2	5	Rat chow, water, and 50mg/kg (low dose)
3	5	Rat chow, water, and 100mg/kg (low dose)
4	5	Rat chow, water, and 150mg/kg (low dose)

Behavioral Test

During the Wistar rat's treatment using cannabis ethanol extract, an anxiety behavioral test was conducted using a neurobehavioral test apparatus elevated plus maze. Wistar rats were habituated before administering the cannabis ethanol extract.

Elevated Plus Maze

The elevated plus maze, used to assess anxiety-like behavior, was positioned 50 cm high and measured 40 cm in length and 10 cm in width. It consisted of four arms: two open and two closed. Each rat was positioned at the center of the Maze with free access to any arm and allowed to explore for 10 minutes. The number of arm entries and the time spent in the open and closed arms were recorded (Treit et al., 1993). Three (3) Wistar rats per group were involved in this exercise and to prevent potential odor bias, each arm was cleaned with 5% alcohol before testing a new rat.

Animal Sacrifice and Histological Analysis

Rats were sacrificed by cervical dislocation and intra-cardiac neural behavioral performance and the last administration of cannabis extract. The brains of each rat were removed with the help of forceps. The rat's brain were immersed in Neutral buffered formalin at 10% concentration for preservation. Coronal slices, 1 mm thick, were taken from the VTA region of the brain using an atlas for precise localization. The VTA brain tissues were then visualized by staining the sections with Hematoxylin and Eosin (H&E).

The VTA brain tissues were fixed in 10% neutral buffered formalin for three days to prevent autolysis and enhance staining. Dehydration was performed with graded alcohol (50%-absolute) for 30 minutes each. Clearing was done involving three changes of xylene for 30 minutes. Tissues were infiltrated with molten paraffin in a 55°C oven, embedded in molds, and solidified. Sections (5µm thick) were obtained using a rotary microtome, dried, dewaxed in xylene, and rehydrated in graded alcohol (90%-50%). Hematoxylin stained the nuclei, followed by differentiation in 1% acid alcohol, bluing in tap water, and eosin counterstaining. Sections were cleared in xylene and mounted for microscopy for photomicrography.

Biochemical Assay

Blood samples of 3 Wistar rats per group were collected using cardiac puncture through a modified procedure described by Allain et al. (1974), into different EDTA bottles and used by Umoren et al., (2014). The biomedical assay was conducted in the Department of Medical Laboratory in Federal Teaching Hospital, Abakaliki (FETA).

In this study, the experimental procedure for measuring the catecholamine level involved the careful preparation and analysis of the test and control samples under controlled conditions. Initially, 10µL of distilled water was introduced into both the test and blank tubes, followed by the addition of 10µL of Dihydroxyacetone phosphate (DHAP) reagent to each. A critical differentiation was made by adding 10 µL of S-Adenosyl methionine (SAM) reagent exclusively to the test tube. Both tubes were then supplemented with 10µL of Magnesium chloride ($MgCl_2$) reagent and 10µL of Ditheiothreitol (DTT).

The prepared solutions were thoroughly mixed and subjected to incubation at 37°C for 1 minute to facilitate the necessary reactions. Following this incubation, 10µL of enzyme reagent was incorporated into each tube to initiate the enzymatic activity. The reaction was subsequently terminated with the addition of 50µL of stop solution. The final mixtures were homogenized to ensure uniformity and subjected to spectrophotometric analysis. Absorbance was measured meticulously across a wavelength range of 505-650nm to capture the level of catecholamine.

Ethical Approval

The study was carried out following the guidelines of research ethics on the care and use of laboratory animals as stipulated by the National Research Council (2011). Approval was obtained from the Department of Human Anatomy and National Law Enforcement Agency Abakaliki, Ebonyi State, Nigeria, during the presentation of the proposal for this study.

Data Analysis

Data obtained were expressed as mean ± standard deviation, one sample t-test was employed for the comparison of means within groups, and P values were set as $p<0.001$.and 0.05 at two-tailed significant using the IBM Statistical Package for the Social Sciences Version 25.00 (IBM SPSS) and Microsoft Office Excel 2013 for charts.

RESULTS

Table 2; Anxiety Behavior Analysis in Cannabis-Exposed Groups Using Elevated Plus Maze

Groups	Open Arm (mins)			Close Arm (mins)		
	Mean ± S.D	t-value	P-value	Mean ± S.D	t-value	P-value
GR1 (control)	0.72±1.08	1.16	0.367	9.28±1.08	14.90	0.004
GR2 (50mg/kg)	0.36±0.62	1.00	0.423	9.64±0.62	27.03	0.001
GRP3 (100mg/kg)	0.02±0.03	1.00	0.423	9.98±0.03	499.00	0.001
GRP 4 (150mg/kg)	0.65±1.03	1.09	0.389	9.35±1.03	15.81	0.004

*Group 3 shows a higher mean and t- value than groups 1, 2, and 4. The p-value for the open arm is not significant while the close arm is significant at a two-tailed level.

Table 2; the descriptive statistics for anxiety behaviors in Wistar rats exposed to varying doses of cannabis extract indicate dose-dependent effects. In open-arm exploration, the control group (0.72 ± 1.08 minutes) spent the most time in the open arms, suggesting lower anxiety levels. Cannabis-exposed groups showed a progressive decline in open-arm time, with Group 3 (100 mg/kg) spending the least time (0.02 ± 0.03 minutes), indicating significantly heightened anxiety. Conversely, closed-arm preference increased across groups, with Group 3 spending the most time in the closed arms (9.98 ± 0.03 minutes). Statistically significant differences were observed in closed-arm times, but not in open-arm exploration. These findings suggest that higher doses of cannabis extract correlate with increased anxiety-related behaviors.

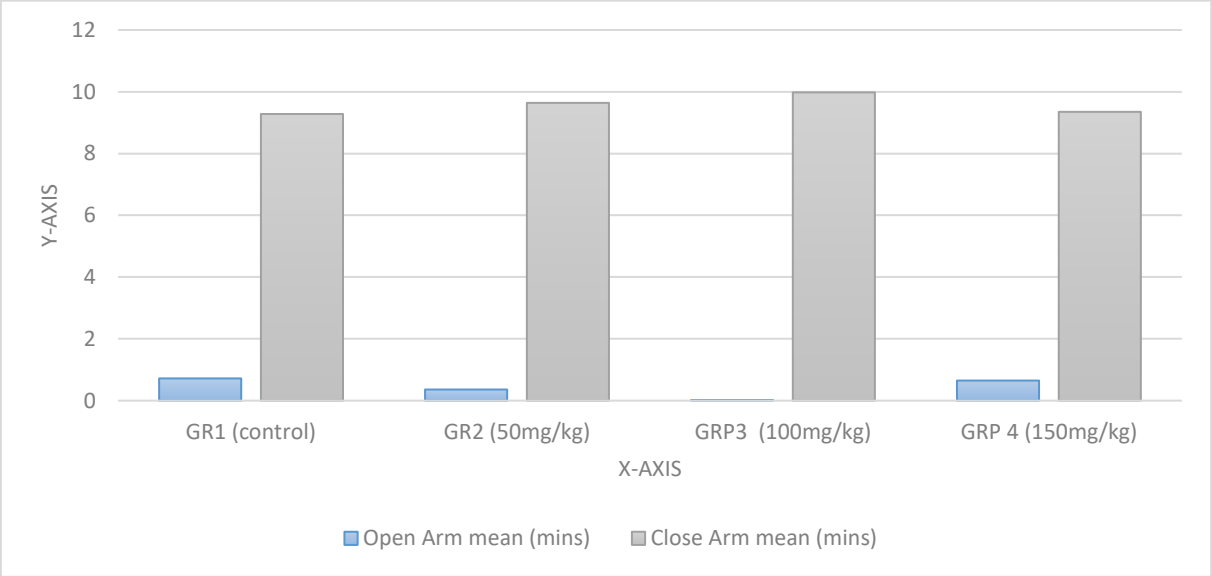

Figure 1 A cluster column chart showing Table 3 mean and p-value with two sets of bars (open arm and closed arm). The y-axis is represented in units of 0, 2, 4, 6, 8, 10, and 12 which is the time spent on each arm. Group 3 shows a notably higher mean value in the close arm, reaching nearly 10mins. This may suggest increased anxiety-like behavior in this group compared to the others, as they show the strongest preference for the close arm.

Histology of the Ventral Tegmental Area

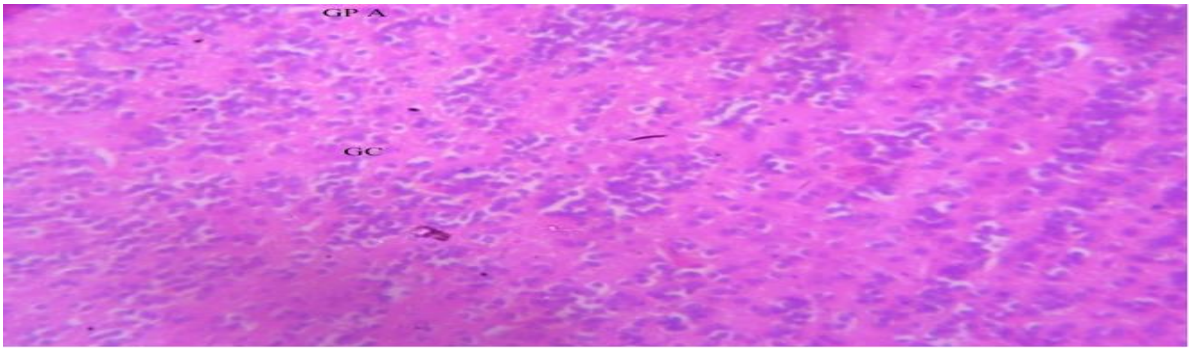

Figure 2.1; Photomicrograph of group 1 section of VTA (x100(x400). H/E VTA staining shows active VTA with distinct granular cells

Group 2 Histological Result (50mg/kg)

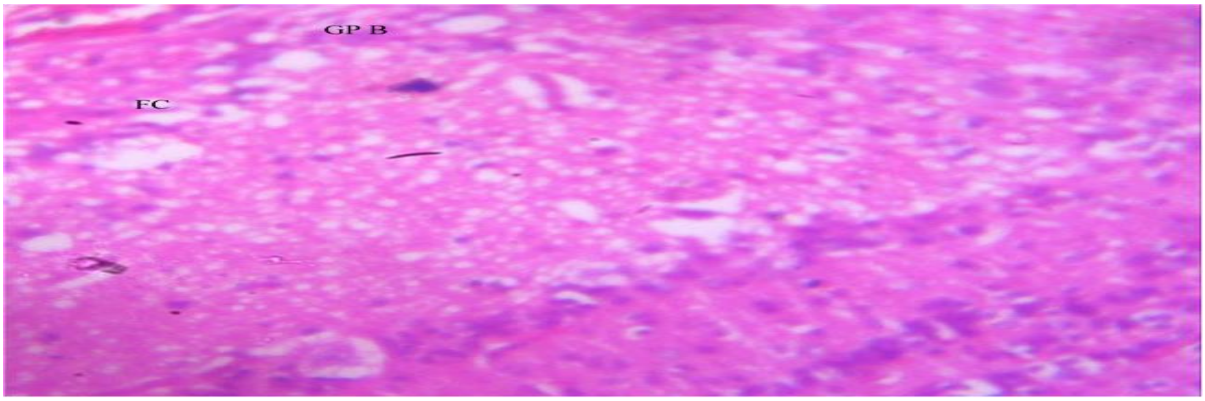

Figure 2.2; Photomicrograph of group B section of VTA (x100(x400). H/E VTA staining shows active VTA with moderate fatty changes (FC) and moderate reduction in the number of granular cells (GC)

Group 3 Histological Result (100mg/kg)

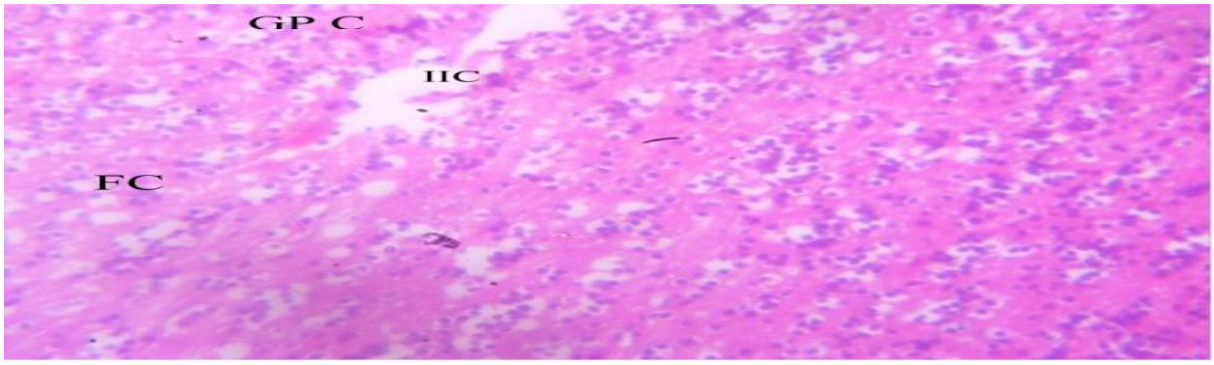

Figure 2.3; Photomicrograph of group 3 section of VTA (x100(x400). H/E VTA staining shows active VTA with moderate fatty changes (FC) and moderate infiltration of inflammatory cells (IIC).

Group 4 Histological Result (150mg/kg)

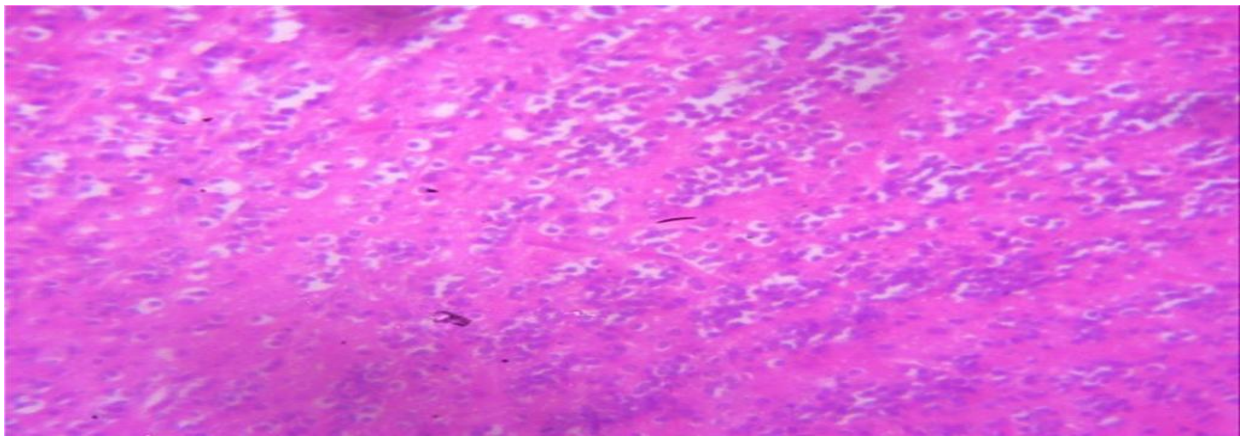

Figure 2.4; Photomicrograph of group 4 section of VTA (x100(x400). H/E VTA staining shows active VTA with active granular cells (GC), fatty changes (FC), and mild infiltration of inflammatory cells (IIC).

The provided image in Figure 2 shows a histological section of the ventral tegmental area (VTA) in Groups 1-4, which serves as the control group, 50, 100, and 150ml/gg. This section was stained with hematoxylin and eosin (H&E) at magnifications of 100x and 400x, highlighting cellular structures in the VTA. The histological examination of the ventral tegmental area (VTA) in response to cannabis ethanol extract reveals significant dose-dependent structural changes. In the control group, the VTA appeared healthy, with well-preserved granular cells. At lower doses (50 mg/kg), moderate fatty changes and a slight reduction in granular cells indicated early cellular stress. These changes suggested an initial disruption of cellular metabolism. At higher doses (100 mg/kg), more pronounced fatty degeneration and the infiltration of inflammatory cells were observed, signaling increased tissue damage and inflammation. Despite these alterations, the VTA maintained some functional cells, indicating partial resilience. However, at the highest dose (150 mg/kg), the VTA exhibited persistent fatty changes and mild inflammation, suggesting that while the tissue was under stress, some repair mechanisms were still active. Overall, these findings highlight the potential for cannabis extract to

induce cellular stress and inflammation in the VTA, with more severe effects observed at higher doses. This underscores the dose-dependent impact of cannabis on brain structure, particularly in areas critical for reward and motivation, such as the VTA.

Table 3 descriptive statistics for catecholamine activity level (pg/ml) among groups

Groups	Mean±S.D	t-value	p-value
1	148.57±2.21	116.44	0.000
2	137.86±5.32	44.85	0.000
3	110.54±1.93	99.30	0.000
4	107.83±7.19	25.98	0.001

P value is significant at the level of two-tailed. Continuous decrease in mean value across groups.

The descriptive statistics in Table 3 reveal a significant and progressive decrease in catecholamine levels (pg/ml) across the groups exposed to varying concentrations of cannabis ethanol extract. Group 1 (control) exhibits the highest mean catecholamine level (148.57 ± 2.21), while Group 4 (highest exposure) has the lowest (107.83 ± 7.19). This trend indicates a dose-dependent impact of cannabis on catecholamine synthesis or metabolism. The t-values for all groups are notably high, and the p-values (<0.001 for Groups 3 and 4) indicate statistically significant differences in catecholamine levels between groups. This suggests that cannabis exposure exerts a measurable and consistent effect on catecholamine levels. The continuous reduction in mean catecholamine levels across Groups 1 through 4 reflects the dose-dependent relationship. Higher concentrations of cannabis extracts appear to suppress catecholamine activity, potentially affecting neurotransmission and associated behavioral processes such as motivation, mood, and reward. The standard deviation increases in Groups 2 and 4 compared to Groups 1 and 3, indicating greater variability in catecholamine levels at certain exposure levels. This could suggest differing individual responses to the same cannabis dosage among the test subjects. The 100% stacked bar chart effectively highlights the proportional differences in catecholamine levels across groups. It provides a clear visual representation of the significant decline in catecholamine levels as cannabis extract exposure increases.

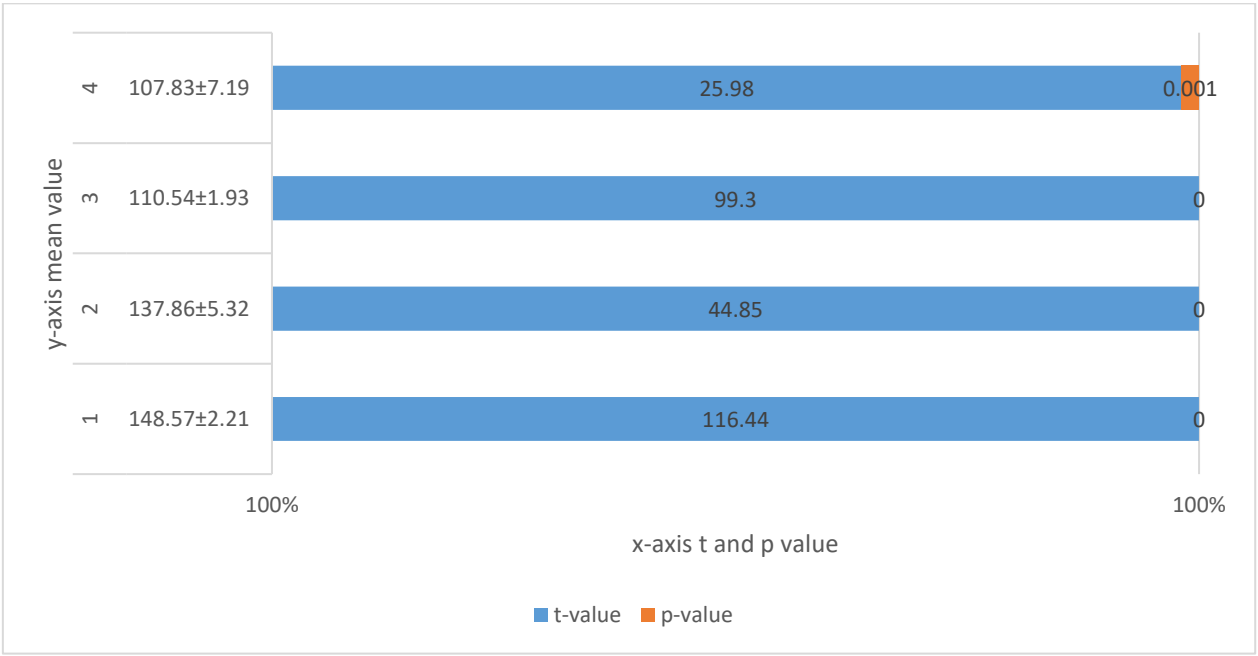

Figure 3: A 100% stacked bar chart was used in comparing descriptive statistics of catecholamine activity levels among Wistar rats groups.

DISCUSSION

The findings of this study reveal that chronic cannabis ethanol extract exposure induces significant dose-dependent effects on anxiety-like behaviors, ventral tegmental area (VTA) histology, and catecholamine levels in Wistar rats. These results provide critical insights into the neurobehavioral and histological consequences of cannabis use, aligning with and expanding upon existing literature.

The Elevated Plus Maze (EPM) analysis (Table 2) demonstrates a clear dose-dependent increase in anxiety-related behaviors. Open-arm exploration decreased progressively with higher cannabis doses, with Group 3 (100

mg/kg) exhibiting the lowest time (0.02 ± 0.03 min). These findings are consistent with previous studies linking high cannabinoid doses to anxiogenic effects in rodents (Patel and Hillard, 2006). Similarly, closed-arm preference increased across groups, with Group 3 showing the longest time (9.98 ± 0.03 min), indicating heightened anxiety-related avoidance behaviors. Notably, while differences in open-arm times were not statistically significant, closed-arm preferences were highly significant ($p < 0.05$), underscoring the anxiogenic potential of cannabis at higher doses.

The biphasic effects of cannabinoids on anxiety, with low doses typically reducing anxiety and high doses exacerbating is achieved through modulation of the endocannabinoid system and dopaminergic pathways in the brain (Blessing et al., 2014; Lisboa et al., 2017; Kasten et al., 2021). However, this study did not observe anxiolytic effects at the lower dose (50 mg/kg), potentially due to the chronic exposure model, which may amplify stress responses over time.

However, the histological analysis of the VTA revealed dose-dependent structural changes. The control group displayed normal VTA morphology, with intact granular cells and no pathological alterations. In contrast, Group 2 (50 mg/kg) exhibited moderate fatty changes and a reduction in granular cells, indicating early signs of cellular stress. These findings align with reports that cannabinoids can induce neuronal stress and lipid accumulation through oxidative mechanisms (Gonzalez-Cuevas et al., 2018).

At 100 mg/kg (Group 3), histological changes were more pronounced, with moderate fatty changes and inflammatory cell infiltration, indicating an inflammatory response potentially linked to neurotoxicity. Interestingly, Group 4 (150 mg/kg) exhibited mild inflammatory infiltration but retained active granular cells, suggesting possible adaptive cellular mechanisms at the highest dose. Such resilience may reflect the VTA's ability to compensate under stress, though the presence of fatty changes still indicates ongoing cellular stress.

Although, these histological changes are consistent with previous research demonstrating that chronic cannabis use can induce neuroinflammation and oxidative stress (Kumar et al., 2011; Zhang et al., 2014; Sadaka et al., 2023; Ishrat et al., 2024). The moderate inflammatory response observed in Group 3 aligns with reports of cannabinoid-induced activation of microglia and pro-inflammatory cytokine release (Zhang et al., 2014; Cassano et al., 2017). However, the retention of active granular cells in Group 4 suggests a complex interplay between damage and repair processes at higher doses. Thus, these results corroborate findings from van den Hoogen et al. (2022), who reported cannabis-induced neuroinflammation and metabolic disruptions in dopaminergic regions. The presence of inflammatory cells and fatty degeneration suggests oxidative stress and immune activation in response to cannabis exposure, consistent with earlier findings by Dong et al. (2020) on cannabinoid-induced neurotoxicity.

Catecholamine levels (Table 3) exhibited a significant dose-dependent decline, with the control group showing the highest levels (148.57 ± 2.21 pg/ml) and Group 4 the lowest (107.83 ± 7.19 pg/ml). This reduction suggests that cannabis disrupts dopamine and norepinephrine pathways, consistent with evidence that cannabinoids modulate catecholamine release by acting on CB1 receptors in reward-related brain regions (Davis & Kim, 2021; Lingegowda et al., 2022). Reduced catecholamine levels likely contributed to the observed anxiety behaviors, as these neurotransmitters play critical roles in regulating mood and stress responses.

The findings align with prior studies showing that cannabis alters dopamine levels, particularly in the VTA and related reward pathways (Bloomfield et al., 2016; Davis & Kim, 2021; Lingegowda et al., 2022). The observed reductions may result from the down-regulation of tyrosine hydroxylase, the enzyme critical for catecholamine synthesis, or oxidative stress-induced neurotransmitter depletion. López-Moreno et al. (2008) similarly reported that cannabis impacts dopaminergic and noradrenergic systems, contributing to behavioral changes.

Collectively, this study underscores the neurobiological and behavioral risks of chronic cannabis exposure, particularly its dose-dependent effects on anxiety, brain histology, and catecholamine pathways. The results align with findings from Bloomfield et al. (2016), Urban et al. (2013), Volkow et al. (2016), and Cohen et al. (2019), which documented cannabis-induced reductions in dopamine synthesis and structural neuronal changes. The VTA, a critical region for reward and motivation, appears particularly vulnerable to the cumulative impacts of high-dose cannabis exposure.

CONCLUSION

The findings indicate that chronic exposure to cannabis ethanol extract modulates anxiety behaviors, induces dose-dependent structural changes in the VTA, and suppresses catecholamine levels. These results underscore the need for further research into the long-term neurobiological effects of cannabis, particularly concerning anxiety and reward systems. The findings indicate that chronic exposure to cannabis ethanol extract modulates anxiety behaviors, induces dose-dependent structural changes in the VTA, and suppresses catecholamine levels. These results underscore the need for further research into the long-term neurobiological effects of cannabis, particularly concerning anxiety and reward systems.

CONFLICTS OF INTEREST

The present study is free, with no conflict of interest among authors. The research was not sponsored by any company or institution but was funded by the authors' efforts.

REFERENCES

I. Allain CC, Poon LS, Chan CS, Richmond W and Fu PC, (1974). Enzymatic determination of total serum cholesterol. *Clinical Chemistry*, 20 (19):470-475.

II. Beier, K. T., Xie, S., Gao, X. J., DeLoach, K. E., Malenka, R. C., & Luo, L. (2019). Topological organization of ventral tegmental area connectivity revealed by viral-genetic dissection of input-output relations. *Cell Reports*, 26(1), 1-9.

III. Blessing, E. M., Steenkamp, M. M., Manzanares, J., & Marmar, C. R. (2015). Cannabidiol is a potential treatment for anxiety disorders. *Neurotherapeutics*, *12*(4), 825-836.

IV. Bloomfield, M. A. P., Ashok, A. H., Volkow, N. D., & Howes, O. D. (2016). The effects of cannabis on the dopamine system. *Nature*, *539*(7629), 369–377.

V. Bouarab, C., Rancillac, A., Monier, C., & Hamon, M. (2019). VTA GABAergic neurons and their modulation of reward pathways. *Journal of Neuroscience*, 39(1), 123-138.

VI. Brown, A., & Thompson, C. (2019). The role of the Ventral Tegmental Area in reward processing. *Journal of Neuroscience*, 43(2), 123-135.

VII. Cassano Tommaso, Calcagnini Silvio, Pace Lorenzo, Marco Federico De, Romano Adele and Gaetani Silvana, (2017).Cannabinoid Receptor 2 Signaling in Neurodegenerative Disorders: From Pathogenesis to a Promising Therapeutic Target. *Frontiers in Neuroscience*, 11:30.

VIII. Cohen Koby, Weizman Abraham, Weinstein Aviv, (2019). Modulatory effects of cannabinoids on brain neurotransmission. *European Journal of Neuroscience*, 50(3):2322-2345.

IX. Davis, M., & Kim, H. (2021). Cannabinoid effects on dopamine: Implications for reward. *Neuropsychopharmacology*, 46(7), 1054-1067.

X. Dong Mei-xue, Chen Guang-Hui, Hu Ling, (2020). Dopaminergic System Alteration in Anxiety and Compulsive Disorders: A Systematic Review of Neuroimaging Studies. *Frontiers in Neuroscience*, 14:608520.

XI. Gonzalez-Cuevas, Gabriel, Tostes, Ricardo C., Moreira, Fabricio A., Fogaça, Mariana V., Ramos, Angela, Nascimento, Jéssica M., Andrade, João R. and De Jesus, Renata P, (2018). Cannabinoids induce oxidative stress and fatty degeneration in neurons. *Neuropharmacology***,** 146, 100–110.

XII. Hasin Deborah and Walsh Claire, (2021). Trends over time in adult cannabis use: A review of recent findings. *Current Opinion Journals*, 38: 80–85.

XIII. Hurd Yasmin L., Manzoni Olivier J., Pletnikov Mikhail V., Lee Francis S., Bhattacharyya Sagnik and Melis Miriam, (2019). Cannabis and the Developing Brain: Insights into Its Long-Lasting Effects. *Journal of Neuroscience*, 39 (42) 8250-8258.

XIV. Ishrat S, Levey DF, Gelernter J, Ebmeier K, Topiwala A., (2024). Association between cannabis use and brain structure and function: an observational and Mendelian randomisation study. *BMJ Mental Health*, 27(1):e301065.

XV. Johnson, L., & Lee, S. (2020). Therapeutic benefits of cannabis: A growing evidence base. *Clinical Pharmacology and Therapeutics*, 107(5), 861-871.

XVI. Kasten CR, Carrey SE, Mulligan GL, Scholl AD, Zhang LE, Andricioaei BP, Hsieh KC, and Patel S, (2021). Dose-dependent biphasic effects of cannabinoids on anxiety. *Frontiers in Behavioral Neuroscience*, 15, 689450.

XVII. Kim, S., et al. (2021). Histological changes in the brain following long-term cannabis exposure. *Journal of Neuroscience Research*, 89(6), 1670-1682.

XVIII. Kumar, A., Singh, A., & Ekavali. (2011). A review on Alzheimer's disease pathophysiology and its management: an update. *Pharmacological Reports*, *67*(2), 195-203.

XIX. Lisboa SF, Gomes FV, Terzian AL, Aguiar DC, Moreira FA, Resstel LB, Guimarães FS, (2017). The Endocannabinoid System and Anxiety. *Vitam Horm*, 103:193-279.

XX. López-Moreno, J. A., González-Cuevas, G., Moreno, G., & Navarro, M. (2008). The pharmacology of the endocannabinoid system: functional and structural interactions with other neurotransmitter systems and their repercussions on behavioral addiction. *Addiction Biology*, *13*(2), 160-187.

XXI. López-Moreno, J. A., González-Cuevas, G., Moreno, G., & Navarro, M. (2008). Cannabinoid impact on dopaminergic and noradrenergic systems. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 32(7), 1385-1392.

XXII. Lingegowda, H., Williams, B.J., Spiess, K.G. *et al.* Role of the endocannabinoid system in the pathophysiology of endometriosis and therapeutic implications. *J Cannabis Res* 4, 54 (2022).

XXIII. National Research Council, (2011). Guide for the care and use of Laboratory Animals. Eight Edition, Washington DC. Accessed on 18 August, 2024.

XXIV. Patel, S., & Hillard, C. J. (2006). Pharmacological evaluation of cannabinoid receptor ligands in a mouse model of anxiety: further evidence for an anxiolytic role for endogenous cannabinoid signaling. *Journal of Pharmacology and Experimental Therapeutics*, *318*(1), 304-311.

XXV. Patricio F, Morales-Andrade AA, Patricio-Martínez A and Limón ID (2020) Cannabidiol as a Therapeutic Target: Evidence of its Neuroprotective

and Neuromodulatory Function in Parkinson's Disease. *Frontiers in Pharmacology*, 11:595635.

XXVI. Polter, A. M., & Kauer, J. A. (2014). In vivo synaptic plasticity in the VTA and its role in addiction. Neuropharmacology, 76(Part B), 291-299.

XXVII. Sadaka AH, Canuel J, Febo M, Johnson CT, Bradshaw HB, Ortiz R, Ciumo F, Kulkarni P, Gitcho MA and Ferris CF (2023) Effects of inhaled cannabis high in Δ9-THC or CBD on the aging brain: A translational MRI and behavioral study. *Frontiers in Aging Neuroscience*, 15:1055433.

XXVIII. Treit D, Menard J, Royan C, (1993). Anxiogenic stimuli in the elevated plus-maze. *Pharmacology Biochemistry and Behavior*, 44:463-469.

XXIX. Urban Nina BL, Slifstein Mark, Thompson Judy L, Xiaoyan Xu, Ragy R Girgis, Sonia Raheja, Margaret Haney, Anissa Abi-Dargham, (2013). Dopamine release in chronic cannabis users: a [11C]raclopride Positron Emission Tomography study. *Biological Psychiatry*, 71(8):677–683.

XXX. Umoren EB, Obembe AO, Odo MO, and Osim EE, (2014). Effect of nevirapine administration on biliary Secretion/ its Biochemical composition in albino Wistar rats. *Journal of Antivirus and Antiretrovirus* 6:45-49.

XXXI. van den Hoogen NJ, Harding EK, Davidson CED and Trang T (2022) Cannabinoids in Chronic Pain: Therapeutic Potential Through Microglia Modulation. *Frontiers in Neural Circuits*, 15:816747.

XXXII. Volkow, Nora D., Swanson, James M., Evins, A. Eden, et al., (2016). Effects of Cannabis Use on Human Behavior, Including Cognition, Motivation, and Psychosis: A Review. *JAMA Psychiatry*. 73(3):292-297.

XXXIII. Yu, X., Zhang, Y., Sun, Y., Wang, Y., & Li, S. (2021)**.** Ventral tegmental area glutamate neurons regulate reward consumption and stress responses. *Frontiers in Neural Circuits*, 15, 667477

XXXIV. Zehra Amna, Burns Jamie, Liu Christopher Kure, Manza Peter, Wiers Corinde E., Volkow Nora D., Wang Gene-Jack, (2018). Cannabis Addiction and the Brain: a Review. *Journal of Neuroimmune Pharmacology*, 13:438–452

XXXV. Zhang, H. Y., Gao, M., Liu, Q. R., & Wu, J. (2014). Cannabinoid CB2 receptor: a potential therapeutic target for chronic pain. *Brain Research Reviews, 67*(1-2), 140-151.