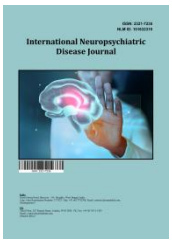

International Neuropsychiatric Disease Journal

Volume 21, Issue 5, Page 32-44, 2024; Article no.INDJ.120086
ISSN: 2321-7235, NLM ID: 101632319

Caffeine, Nicotine and Mdma Effects on the Brain Hippocampal Formation of Juvenile Experiential Models

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Authors' contributions

This work was carried out in collaboration among all authors. Author LAA wrote and prepared the original draft. Authors OSF and OEO supervised the study. Author IK did the project administration of the manuscript. Author JOO designed the study, reviewed and edited the manuscript. . All authors read and approved the final manuscript.

Article Information

DOI: https://doi.org/10.9734/indj/2024/v21i5447

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/120086

Original Research Article

Received: 06/06/2024
Accepted: 03/08/2024
Published: 09/08/2024

ABSTRACT

Introduction: Chronic exposure of MDMA in humans has been shown to produce negative neuroplastic alterations to the brain's white matter and microvasculature, as well as significant neurodegeneration in the striatal, hippocampal, prefrontal, and occipital serotonergic axon terminals. Adolescent exposure to nicotine damages hippocampus cells, and as a result, damages

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Cite as: *Adelakin, Lola Adeola, Oluseyi Sunday Fabiyi, Olubunmi Esther Ogunbiyi, Isioma Konyeme, and Joshua Oladele Owolabi. 2024. "Caffeine, Nicotine and Mdma Effects on the Brain Hippocampal Formation of Juvenile Experiential Models". International Neuropsychiatric Disease Journal 21 (5):32-44. https://doi.org/10.9734/indj/2024/v21i5447.*

memory retention. Caffeine suppresses the actions of adenosine which is crucial for energy transfer and sleep promotion as long as it enters the brain, as it crosses the crosses the blood-brain barrier. The hippocampus is critical for the formation of new autobiographical and fact memories, hence, severe damage to the hippocampi in both hemispheres result in profound difficulties in forming new memories. This also affects the memory formed before the damage, resulting in anterograde and retrograde amnesia, respectively. This study compared the effect of Nicotine, MDMA and Caffeine on the hippocampus and memory of juvenile male Wister rats.

Materials and Methods: Fifty (n=50) juvenile male Wistar rats (120g) were randomly distributed into 7 groups labeled A-G. Group A served as Control, Group B was administered 30mg/kg Caffeine, Group C was administered 50mg/kg Caffeine, Group D was administered 10mg/kg Nicotine, Group E was administered 20mg/kg Nicotine, Group F was administered 30mg/kg MDMA and Group G was administered 40mg/kg MDMA, for a period of 30 days. Rats were sacrificed after the experiment and their brains were harvested. Their hippocampi were excised and processed for histological, immunohistochemical and biochemical observations. Neurobehavioral studies were done before sacrifice. Analysis was done using Graph Pad Prism 8.0. P-value of ≤0.05 was regarded as significant, and data was expressed as mean ± SEM.

Results: MDMA and caffeine caused neuron degeneration at low and high dose. There was no tissue disruption attributable to nicotine. Myelination was preserved generally across the treated groups, except groups F and G. There was general disruption in the dopamine and acetylcholine neurotransmitters levels, except group c, and a significant increase in serotonin neurotransmitters especially, in groups D-G.

Conclusion: Caffeine, nicotine and MDMA induced neuronal disruptions of varying degrees in the hippocampus of the brain, and as such caused deleterious effects in the long/short-term memories, as evidenced in the behavioral analyses. The damage was dose dependent.

Keywords: Caffeine; nicotine; MDMA; hippocampus; neurodegeneration.

1. INTRODUCTION

Nicotine, Caffeine and MDMA (ecstasy) are stimulants commonly consumed by the world population non-medically, primarily for quality-of-life purpose and casually for wakefulness or performance enhancement. The effects of the psycho-stimulants have been notably intertwined.

The impact of nicotine on the mind is differential among age brackets, adolescent and adults [1]. It's been reported that nicotine ameliorated the neurotoxic effect of β-amyloid protein in hippocampal cultures, through nicotinic receptors, in Alzheimer's disease [2]. Nicotine exposure at adolescence elicits hippocampal cell damage, leading to the dysfunction of synaptic receptors and resultantly, behavior abnormalities. Nicotine can exist in both charged and uncharged forms since it is a tertiary amine. Nicotine can enter the brain in its uncharged form and cross the blood-brain barrier, to bind to receptors where it changes into its charged form [3]. Thus, nicotine acts on nicotinic acetylcholine receptors, (nAChRs) to indirectly affect intracellular processes; but, when nicotine enters the cytoplasm, it may also directly affect these processes [4]. Recent studies have additionally indicated that activation of nicotine receptors through low dose nicotine ended in apoptotic cell death in primary hippocampal progenitor cells [5].

Caffeine is rapidly and completely absorbed in humans, with 99 percent being absorbed within 45mins of ingestion and distributed throughout the body. Maximum concentration within the blood stream is reached about 1 to 2 hours after intake [6]. Caffeine crosses the blood-mind-barrier, and blocks the activities of adenosine [7, 8], which is critical for energy transfer; sleep promotion, learning and memory [9]. The rationale for the caffeine dosage in this study was premised on previous studies, especially owing to the fact that, the established lethal dose (LD_{50}) for rats is 367/mg/kg [10].

MDMA (ecstasy) on the other hand, is a psychostimulant whose elicited experience is dependent on dose, location, and the user [11,12]. Long-term exposure to MDMA in humans has been shown to cause significant neurodegeneration in striatal, hippocampal, prefrontal, and occipital serotonergic axon terminals. Adverse neuroplastic changes to brain microvasculature and white matter have also been seen in humans taking moderate doses of MDMA [13]. According to [14], MDMA treatment to experimental rats resulted in enhanced

anxiety-like behavior in the open field test and avoidant behavior in the light-dark box test at 10 mg/kg. Furthermore, 10 mg/kg MDMA raised 5-hydroxytryptamine (5-HT) and 5-hydroxy indole acetic acid (5-HIAA) in the amygdala, and did not alter levels in the hippocampus, but lowered 5-HT in the dorsal raphe, implying that, there was no significant disruptions in the hippocampus at 10 mg/kg. [15] also supported that standard dosages of MDMA that induce persistent 5-HT depletions in rats (10–20 mg/kg) do not consistently elevate indicators of neurotoxic harm, such as reactive gliosis or cell death; while [16] claimed that MDMA caused differential reaction based on brain locations, at high doses (5–20 mg/kg) in adults and adolescents. This suggest that the need to assess the chronic effect in rats, even at a higher dose. MDMA usage has also been linked to increased impulsivity and depression. Serotonin depletion resulting from MDMA use may cause depression [16]. According to research, recurrent recreational ecstasy users had higher rates of sadness and anxiety even after they stopped using the substance [17].

The hippocampus is significantly, an intricate structure that is often associated with memory consolidation and decision-making. It is a convex elevation of grey matter tissue located within the parahippocampal gyrus of inferior temporal horn of the lateral ventricle. The subfields are named using the abbreviation of Cornu Ammonis (CA). It is divided into CA1, CA2, CA3 and the subiculum [18]. Research has shown that the hippocampus is a part of the larger temporal lobe memory system responsible for general declarative memory [19]. Severe damage to the hippocampi in both hemispheres result in profound difficulties in forming new memories and also affects the memory formed before the damage that is anterograde and retrograde amnesia respectively [20].

2. MATERIALS AND METHODS

2.1 Procurement of Reagents

The psychoactive substances (Nicotine, Caffeine and MDMA) were acquired in crystalline form, from Sigma Aldrich, USA, and diluted in water for oral administration, with the use of cannula.

2.2 Animal Procurement and Tissue Processing

Fifty (n=50) juvenile male Wistar rats with an average body weight of 120g were procured for the research from the institutional animal protective facility and randomly divided into 7 groups, labeled A-G. Group A served as Control, Group B was administered 30mg/kg Caffeine (10), Group C was administered 50mg/kg Caffeine (10), Group D was administered 10mg/kg Nicotine (3), Group E was administered 20mg/kg Nicotine (3), Group F was administered 30mg/kg MDMA (13-15) and Group G was administered 40mg/kg MDMA [13-15], for a period of 30 days.

2.3 Neurobehavioral Tests

Elevated Plus Maze: The elevated plus maze was used as a tool for studying anxiety as a consequence of the treatments administered and to define brain regions and mechanisms underlying anxiety-related behavior [21].

Barnes Maze: The Barnes maze was used to assess spatial learning and memory [22].

2.4 Neurotransmitter Assay

After sacrificing the animals, the tissues needed were excised and stored in phosphate buffer saline (PBS), homogenized and centrifuged. Supernatants were taken for neurotransmitter assay. Neurotransmitters assayed for are: dopamine, according to [23], acetycholine, according to [24] and serotonin, according to [25].

2.5 Enzyme Assay

The following were assayed to assess enzyme activities and levels of oxidative analysis in the treated rats: Succinate Dehydrogenase [26] and Lactate dehydrogenase [27].

2.6 Staining Techniques

The H&E staining technique was finished following the methods of [28], Luxol Fast Blue technique was achieved following the techniques of [29], while Cresyl Fast Violet was completed following the methods of [30].

2.7 Immunohistchemistry

Glial Fibrillary Acidic Protein (GFAP): Immunohistochemical Staining was done using IHC standard procedure credited to [31].

3. RESULTS

3.1 Histology and Immunohistochemistry (Figs. 1-4)

The histoarchitecture of the hippocampi showing the different CA regions and the dentate gyri were generally preserved, clearly defined and demonstrated at low magnification, across all the treated groups. The layers of the hippocampus (polymorphic, pyramidal and molecular) were also clearly demonstrated across the groups at high magnification. However, localized neuron degeneration and vacuolation was observed in groups F and G (low and high dose MDMA). Group D and E (administered nicotine) showed no disruption in hippocampi tissues, a contrast to previous findings.

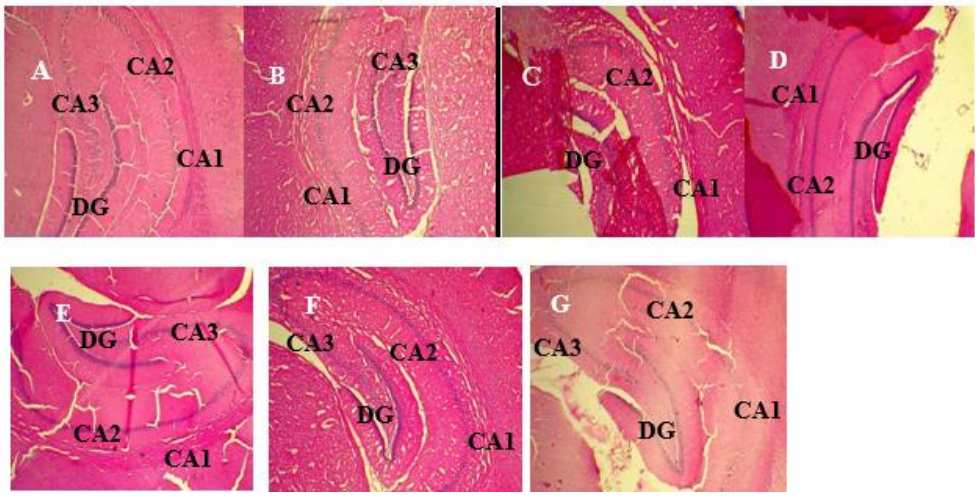

Fig. 1a. Photomicrographs of the hippocampi of rats showing the histoarchitecture and cell morphology of the different cornu ammonis regions and dentate gyrus in Groups A-G, stained with haematoxylin and eosin [H&E A-G, X40], as well preserved, clearly defined and demonstrated

Legend: Dentate gyrus (DG), Cornu ammonis (CA))

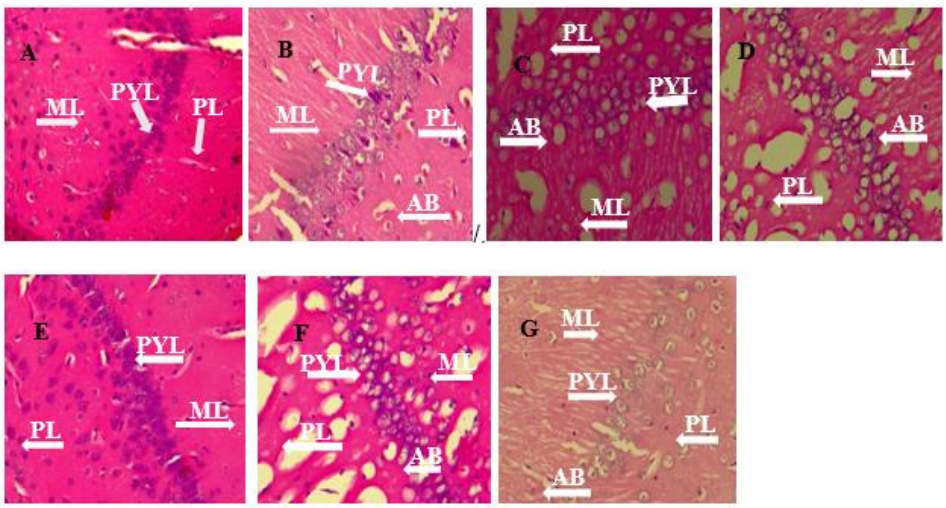

Fig. 1b. Photomicrographs of the hippocampi of rats showing the histoarchitecture and cell morphology of CA1 in Groups A-G, stained with the haematoxylin and eosin [H&E A-G, X400]. Localized neuron degeneration and vacuolation was observed across the treated groups, especially in the groups F and G (low and high dose MDMA). This observation is applicable to Figs. 1c and d.

Legend: Polymorphic Layer (PL), Pyramidal layer (PYL), Molecular layer (ML), Aberration (AB).

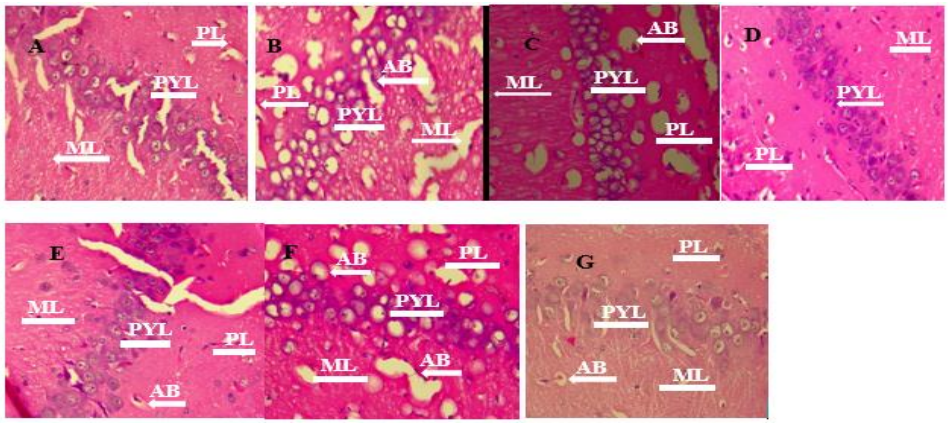

Fig. 1c. Photomicrographs of the hippocampi of rats showing the histoarchitecture and cell morphology of CA3 in Groups A-G, stained with the haematoxylin and eosin [H&E A-G, X400].

Legend: Polymorphic Layer (PL), Pyramidal layer (PYL), Molecular layer (ML), Aberration (AB).

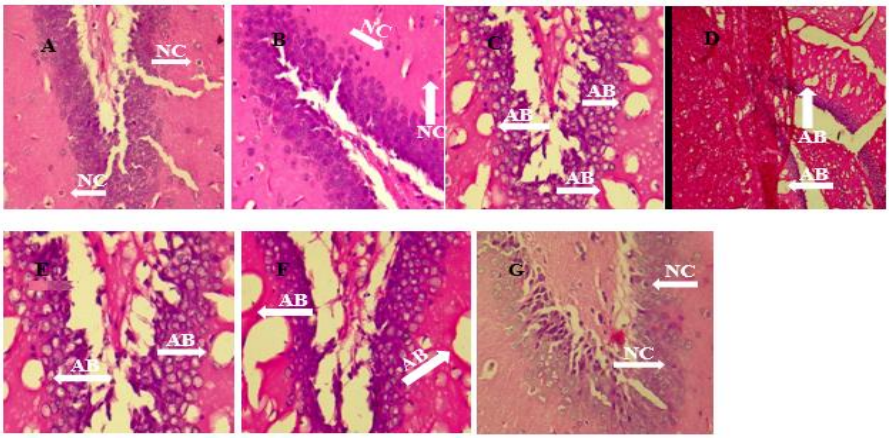

Fig. 1d. Photomicrographs of the hippocampi of rats showing the histoarchitecture and cell morphology of the dentate gyri in Groups A-G, stained with the haematoxylin and eosin [H&E A-G, X400]

Legend: Normal cell (NC), Aberration (AB).

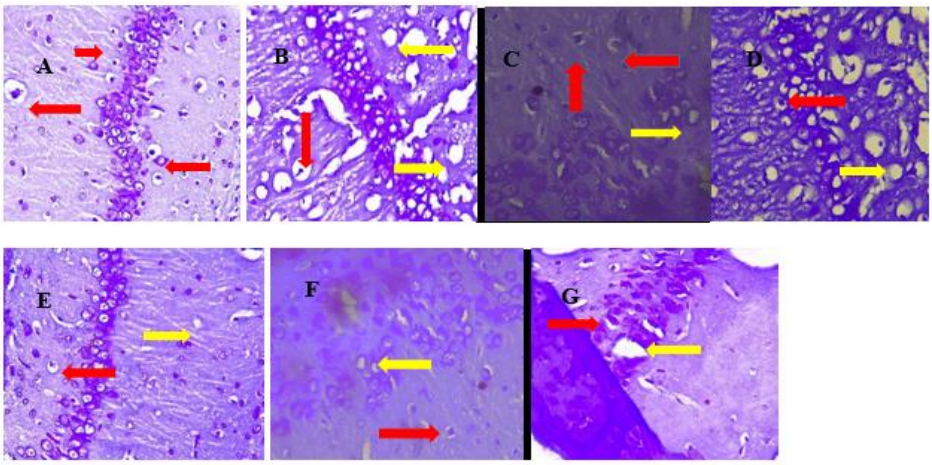

Fig. 2a. Photomicrographs of the hippocampi of rats showing the CA1 regions in Groups A-G, stained with Cresyl fast violet [CFV A-G, X400]. There was a general reduction of nissl substance observed across all the treated groups

Legend: Red arrows: Nissl substance, Yellow arrows: loss of Nissl substance

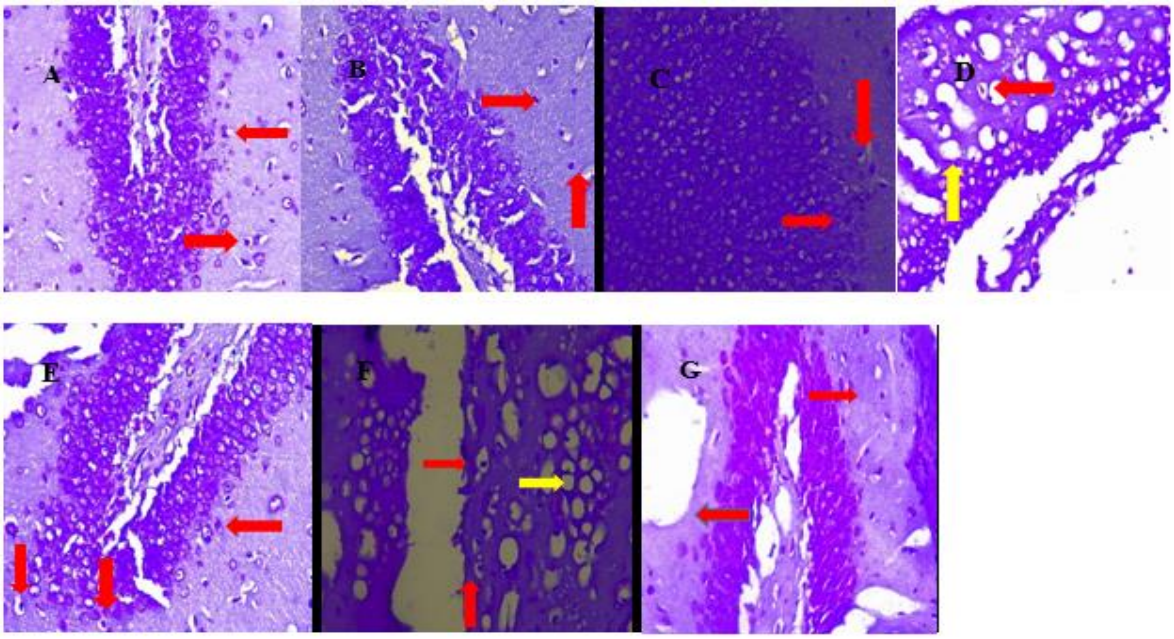

Fig. 2b. Photomicrographs of the hippocampi of rats showing the dentate gyri in Groups A-G, stained with Cresyl fast violet [CFV A-G, X400].

Legend: Red arrows: Nissl substance, Yellow arrows: loss of Nissl substance.

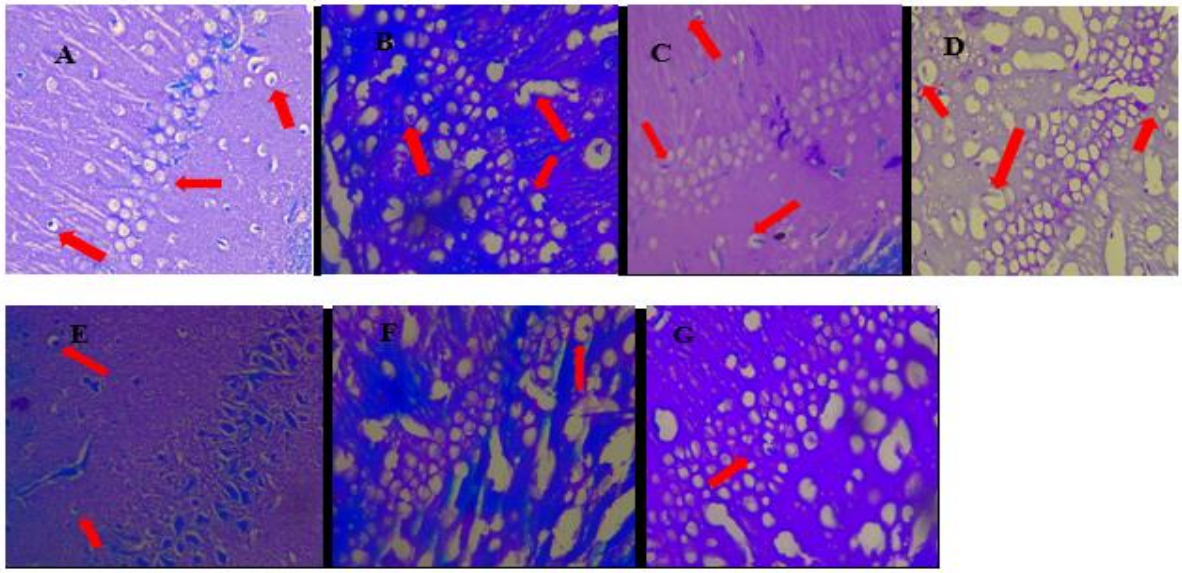

Fig. 3a. Photomicrographs of the hippocampi of rats showing the cytological conditions and myelin sheath integrity of the CA1 regions in Groups A-G, stained with Luxol fast blue [LFB A-G, X400]. Myelination was observed in the groups B, C, D and E (low and high dose caffeine; low and high dose nicotine), while a high degree of demyelination, vacuolation and degeneration was observed in groups F and G (low and high dose MDMA)

Legend: Red arrows: Myelination

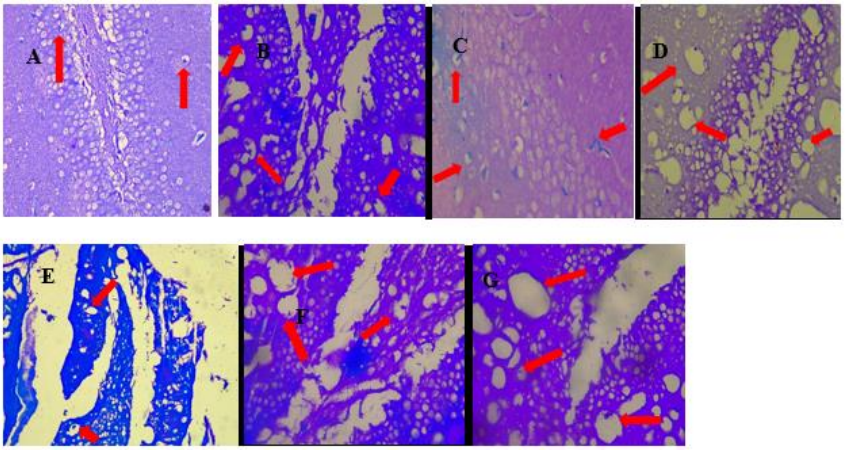

Fig. 3b. Photomicrographs of the hippocampi of rats showing the cytological conditions and myelin sheath integrity of the dentate gyri in Groups A-G, stained with Luxol fast blue [LFB A-G, X400]. Myelination was relatively preserved across the experimental groups

Legend: Red arrows: Myelination

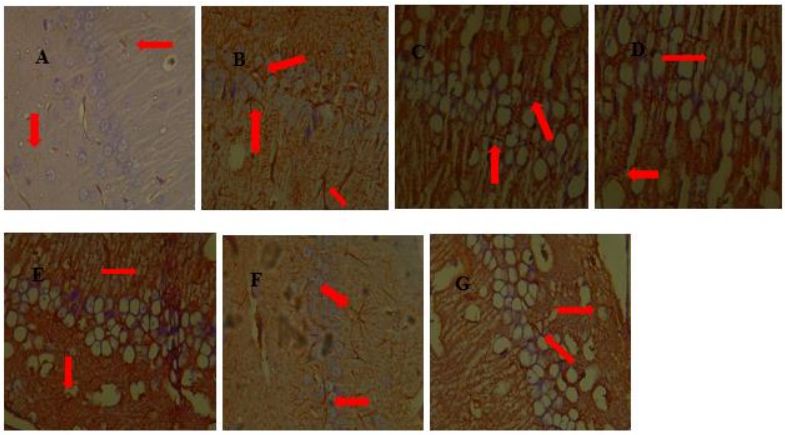

Fig. 4a. Photomicrographs of the hippocampi of rats showing astrocytic reactions in response to treatments administered in the CA1 regions of Groups A-G, demonstrated with GFAP [GFAP A-G, X400].

Legend: Red arrows: Astrocytes

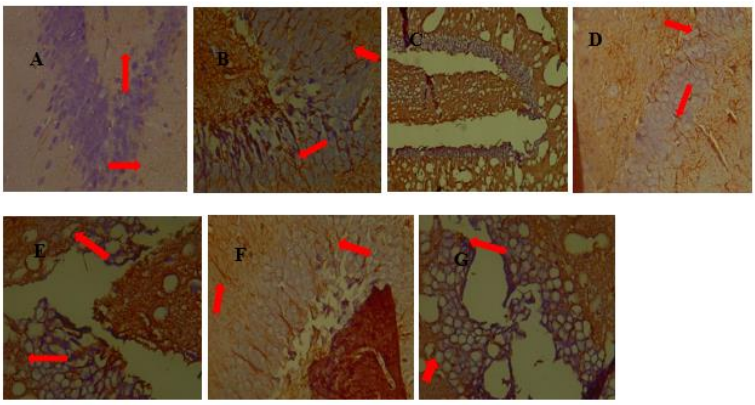

Fig. 4b. Photomicrographs of the hippocampi of rats showing astrocytic reactions in response to treatments administered in the dentate gyri of Groups A-G, demonstrated with GFAP [GFAP A-G, X400]

Legend: Red arrows: Astrocytes

Cytological conditions and integrity of the myelin sheath/nissl bodies were demonstrated using the Luxol fast blue stain and Cresyl fast blue stain. Myelination was observed in groups B, C, D and E (low and high dose caffeine; low and high dose nicotine) while a high degree of demyelination, vacuolation and degeneration was observed in groups F and G (low and high dose MDMA). General reduction in nissil bodies level was observed across all the treated groups.

Glia fibrilary acidic protein (GFAP) was employed to express the integrity of astrocytes. Increased astrocytic activity was observed in all the treated groups, relative to the control.

3.2 Neurochemical Changes (Figs. 5 - 6)

For neurotransmitters, there was significant increase of dopamine and acetylcholine across the treated groups relative to the control, except group C (high dose caffeine); and significant disruption in serotonin neurotransmitter levels, among all experimental groups. Groups D, E, F and G (high and low dose caffeine; high and low dose MDMA) showed higher levels of serotonin neurotransmitter activity.

Succinate dehydrogenase significantly increased in groups C, E, F and G (high dose caffeine and nicotine; low and high dose MDMA) relative to the control group. There was significant increase in lactate dehydrogenase enzyme level among all experimental groups except the group C (high dose caffeine). These results suggest that the psychoactive stimulants induced tissue damage.

3.3 Neurobehavioral changes (Figs. 7 - 8)

Anxiety was used as a measure of balance and motor activity, while latency was used as a marker of memory quality.

Neurotransmitters

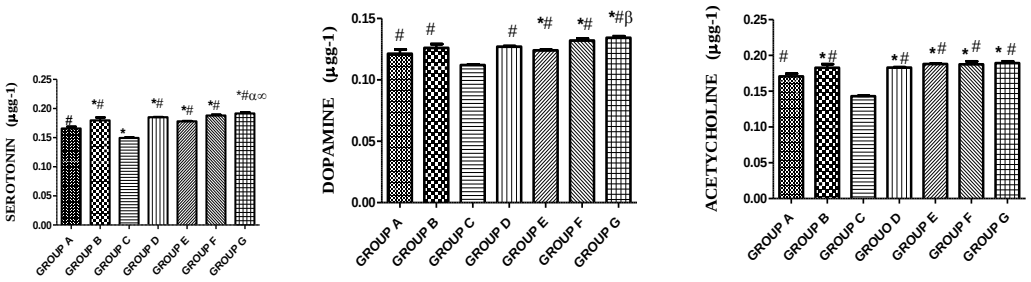

Fig. 5. Bar chart showing the neurotransmitter levels of the rat brains across the control and treated groups, at the end of treatment. There was general disruption in the dopamine and acetylcholine neurotransmitters levels across all the treated groups, except the group C and a significant disruption in serotonin neurotransmitter levels, among all the treated groups while groups D, E, F and G showed higher levels of serotonin neurotransmitter activity

Enzymes Activities

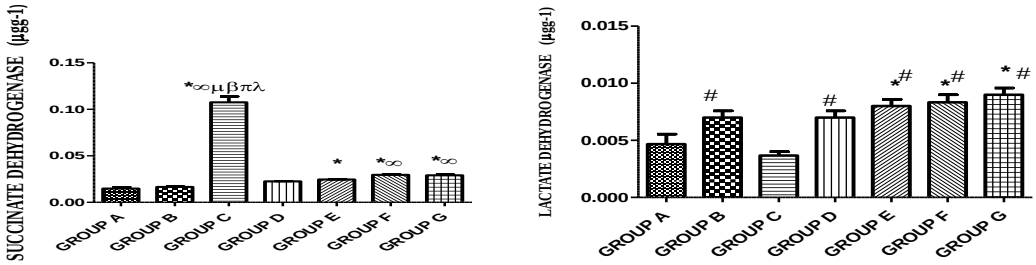

Fig. 6. Bar chart showing enzyme activities in the rat brains across the control and treated groups, at the end of administration. Succinate dehydrogenase enzyme showed statistically significant increase in the groups C, E, F and G, while Lactate dehydrogenase enzyme significantly increased among all the treated groups except the group C

The elevated plus maze results showed slight increase in the time spent in the open arm across all the treated groups, except the group treated with nicotine, which showed no significant difference, relative to the control. The group administered MDMA, spent a longer time in the open arm than the closed arm, indicating the extinction of fear associations attributable to MDMA, as established in previous works.

Results of the total latency obtained from the probe for Barnes maze test, showed no statistical significant difference among all the treated groups, though they were all slightly higher than the control group especially groups E, F and G (high dose nicotine; low and high dose MDMA respectively). This contrasts previous findings which claims nicotine enhances learning and memory by activating receptors for the neurotransmitter acetylcholine.

3.4 Neurobehavioral Study

Elevated Plus Maze was used to assess anxiety as a measure of balance and motor activity, while Barne's Maze assessed latency as a marker of memory quality.

4. DISCUSSION

Caffeine, Nicotine and MDMA (ecstasy) are well known psychoactive stimulants consumed casually and non-medically for wakefulness or overall performance enhancement during the last centuries [17]. Prolonged ingestion of these psycho-stimulants has been studied to show inhibition of hippocampal neurogenesis, neurotoxicity and memory impairment respectively, hence, this study compared their effect on the hippocampus using juvenile male Wistar rats [19].

4.1 Nicotine is Not Implicated in Hippocampal Tissue Disruption

The histological results from this study revealed the histoarchitecture of the cornu ammonis regions and the dentate gyri were preserved, clearly defined and demonstrated. The layers of the hippocampus (polymorphic, pyramidal and molecular) were

Elevated Plus Maze

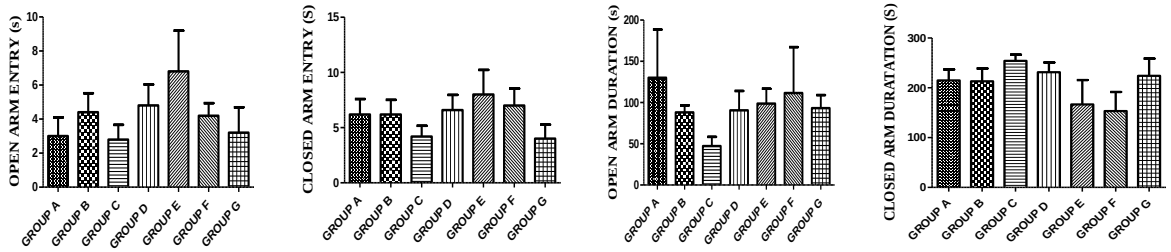

Fig. 7. Bar chart, showing balance and motor activity as a measure for anxiety. There was a slight increase in the time spent in the open arm across all the treated groups, except groups D and E, when compared to the control. Groups F and G spent a longer time in the open arms than the closed arms, indicating the extinction of fear associations.

Barnes Maze

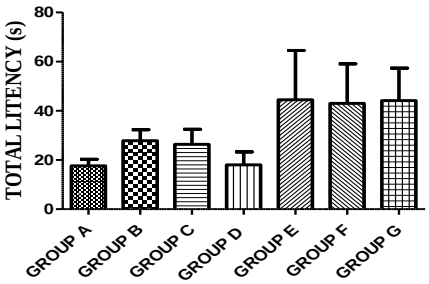

Fig. 8. Bar chart, showing latency as a measure of memory quality. There was no statistically significant difference observed among all the treated groups, when compared to the control, though they were all slightly higher than the control group, especially groups E, F and G

also demonstrated in all the treated groups. However, localized neuron degeneration and vacuolation was observed, especially in groups F and G (low and high dose MDMA) which is similar to previous work by [32] that claimed MDMA diminishes neurogenesis. Groups B and C (low and high dose caffeine) also showed mild degeneration which confirms previous claims that caffeine suppresses neurogenesis. There was no disruption observed in the tissues of the hippocampus in groups D and E on the other hand, which contrasts previous claims that nicotine decreased the number of neurons [12].

4.2 Psychoactive Substances Caused Deleterious Effects/Loss of Cellular Integrity

A general reduction of nissl bodies with concomitant reduction in cell functionality was observed across the treated groups. Myelination was observed in groups B, C, D and E (low and high dose caffeine; low and high dose nicotine) while a high degree of demyelination, vacuolation and degeneration was observed in groups F and G (low and high dose MDMA). This is similar to the findings that MDMA induces myelin disruption in developing adolescent brain [33].

Increased astrocytic activity was seen in all the experimental groups, relative to the control, which is suggestive of the deleterious outcomes of the psychoactive agents administered. The improved astrocytic activities shows the presence of toxicants within the brain.

4.3 Neurotransmitters Activities

Analysis on dopamine and acetylcholine neurotransmitters confirmed remarkable increase (P=0.05) in all the experimental groups relative to the control, except Group C (high dose caffeine). This validates the claims that nicotine stimulates nAChRs, resulting in the release of some neurotransmitters [34,35] and that, MDMA blocks the reuptake of neurotransmitters [36], resulting in abundant neurotransmitters level inside the synaptic cleft and consequentially, excito-toxicity; but contrasts the findings that caffeine induces dopamine and acetylcholine release inside the brain [37].

4.4 Serotonin Neurotransmitter Release not Attributable to Caffeine

Results gotten from serotonin neurotransmitter level analysis showed significant difference (P=0.05) among all the treated groups, relative to the control. Groups D, E, F and G (high and low dose nicotine; high and low dose MDMA) showed higher levels of serotonin neurotransmitter activity, which is similar to findings that, nicotine elevates the serotonin level [38] and that MDMA enhances the release of neurotransmitters, but contrasts the findings that claim, high dose caffeine can increase serotonin levels [39].

4.5 Enzymes Activities

A significant increase (* P=0.05) in succinate dehydrogenase enzyme was seen in groups C, E, F and G (high dose caffeine, high dose nicotine, low and high dose MDMA) relative to the control, a strong indication that the psychostimulants induced oxidative pressure [40]. Lactate dehydrogenase enzyme revealed significant increase (*P=0.05) across all the experimental groups, except the group C (high dose nicotine). This indicates that the psychoactive stimulants precipitated tissue damage [32] and nicotine is not implicated in neuron degeneration/tissue damage, as supported by [41].

4.6 Neurobehavioral Analysis

Anxiety was used as a measure of stability and motor activity, while latency was used as a marker of memory quality in determining neurobehavioral change. Elevated plus maze results showed no remarkable difference in all arms and this contrasts preceding researches claiming prolonged caffeine consumption causes anxiety [42], because the time spent by the caffeine-treated groups in the open arm was lower than the control.

Research has confirmed that anxiety is the foremost reason humans resort to smoking, and nicotine having anxiolytic uses has been used to lessen tension in human and experimental rats [43]. Although the result from this study shows no significant difference in the time spent in the open arm between the groups administered nicotine and the control group, there was a slight increase in the time spent in the open arm compared to the other treated groups. Likewise, the group that was administered MDMA, spent a longer time in the open arm than the closed arm which is similar previous researches made [44].

Results of the total latency obtained from the probe showed no statistical significant difference among all groups, but were all slightly higher

than the control group especially groups E, F and G (high dose nicotine, low and high dose MDMA respectively), which is similar to the findings that, long term ingestion of MDMA has been seen to cause impairments in multiple aspects of cognition, including attention, learning and memory [37]; and contrasts previous findings that nicotine complements studying and memory by activating receptors for the neurotransmitter acetylcholine [45-46].

5. CONCLUSION AND RECOMMENDATION

According to the results obtained from this study, it can be deduced that, caffeine, nicotine and MDMA induced neuronal disruptions of varying degrees in the hippocampus of the brain. The damage was dose dependent, and prominently revealed in the MDMA treated groups, as shown in the enzyme analysis, behavioral and histological test results. Although MDMA and nicotine did not increase anxiety levels according to this research, it only demonstrated the limitation of the study with histoarchitectural, immunohistochemical and biochemical parameters. It is therefore recommended that the long-term effects of nicotine and MDMA be further investigated at the mitochondrial (molecular) level.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL

Standard protocols for animal use and handling were observed consistently with Babcock University's Health Research Ethical Committee (BUHREC), Babcock University, Ilishan –Remo, Ogun state, with BUHREC No: 651/17**.**

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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