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Oxidative stress, inflammation and apoptosis are the main mediators in AMB-FUBINACA induced brain injury in male Albino rats

Asmaa F. Esawy[1*], Nadia A. Eltablawy[2], Abeer I. Abd El-Fattah[1]and Wesam A. Abdelwahab[3]

[1]Department of Biochemistry and Molecular Biology, Faculty of Pharmacy (Girls), Al-Azhar University Cairo, Egypt.
[2] Department of Biochemistry, National Organization for Drug and Control Research (NODCAR, currently Egyptian drug authority, EDA) Cairo, Egypt.
[3]Department of Forensic Medicine and Clinical Toxicology, Faculty of Medicine (Girls), Al-Azhar University Cairo, Egypt.
* Corresponding Author: dr.asmaaesawy@gmail.com

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Abstract: Synthetic cannabinoids (SCs) abuse is a serious social problem worldwide. It can cause severe toxicity, including seizures and even death. This study aims to examine the role of sub-chronic administration of AMB-FUBINACA SC in the induction of brain injury. 32 Male adult rats divided into four groups (8 rats per cage) were used, **Control group:** Intraperitoneally (IP) injected with 0.5 ml of the vehicle, and the others each one of them IP injected with 0.5 ml of the three different doses of AMB-FUBINACA as following: **Group1:** 1mg/kg, **Group 2:** 3mg/kg and **Group 3:** 4mg/kg. The drug was injected once a day for six consecutive days. Tremors were observed 30 minutes following drug injection especially with 3 and 4 mg/kg examined doses followed by depressant effect. Then rats became hyperactive and aggressive. The drug administration induced a significant disturbance in the redox status of brain and significantly increased tumor necrosis factor-α (TNF-α), interlukin-6 (IL-6) and the soluble calcium binding protein B (S100B) serum levels. In addition, the mRNA expression levels of nuclear factor kappa B (NF-κB), mitogen activated protein kinase p38 (MAPK p38) and Caspase-3 were significantly up-regulated. Meanwhile, the mRNA expression levels of Cannabinoid receptors (CB1R, CB2R) and brain derived neurotrophic factor (BDNF) were significantly down-regulated. The obtained results demonstrated that, AMB-FUBINACA harmful effects increase with the dose, which was supported by the brain histopathological examination. We concluded that, AMB-FUBINACA has a functional and structural deleterious central nervous system (CNS) effects with sub chronic exposure in adult male rats. The induced brain injury and seizures that accompanied with the synthetic cannabinoids abuse might be mediated through the generation of oxidative stress, activating inflammatory cellular signaling mechanism and neural cell death via apoptosis.

Keywords: Oxidative stress, Inflammation, Caspase-3 and Synthetic Cannabinoids

1. INTRODUCTION

Synthetic Cannabinoids considered the biggest class of new psychoactive substances (NPS) reported by the European monitoring center for drugs and drug addiction (EMCDDA) recently.[1] Poisoning cases and ultimately death are relevant among the users of these substances. [2-5] SCs were firstly, synthesized for curative goals, but nowadays, some workrooms produce them as recreational substances, where it is treated with organic solvents and combined with dry plants to give the impress of being natural; or condensed in tablet for oral consumption. [6, 7]

Oxidative stress can be considered as an important underlying cause of cannabinoid toxicity.[8] Reactive oxygen species (ROS) accumulation leads to oxidative stress due to the pro-oxidant–antioxidant balance gets disturbed. The disturbance in this redox equilibrium causes severe irreversible interruption to the normal functions of cells, thereby affecting the entire system.[9, 10] ROS has a great effect in brain pathology in the context of neurology[11] such as stroke which is

a famous toxic effect of cannabinoid use, as shown in several studies.[12-14] ROS accumulation is also implicated in behavioral changes such as anxiety, depression and memory impairments.[15]

Free radicals also trigger neuron inflammation through the up-regulated production of pro-inflammatory factors as cytokines and chemokines. These factors, especially tumor necrosis factor-α (TNF-α) which is one of the main protein involved in an inflammatory and immune response that is associated with the up-regulation and expression of other cytokines, such as interlukin-6 (IL-6) in microglial cells, astrocytes, leukocytes and endothelial cells as part of a response to brain damage leading to the loss of synapses, neuronal death, and consequently cognitive dysfunction.[16, 17] Therefore neuron inflammation and the accumulation of these oxidative modified proteins in the brain enhance neuronal degeneration.[18, 19, 20]

It has been demonstrated that TNF-α stimulates brain pericytes to increase the synthesis of IL-6 by activating nuclear factor kappa B (NF-κB)[21], where NF-kB activation in the mitochondria leads to cytochrome c release, thus triggering caspase cascades and programed cell death.[19] Also the release of pro-inflammatory cytokines trigger mitogen activated protein kinase p38 (MAPK p38) pathway activation[22], which participate in the regulation of a variety of cellular processes, including cell proliferation, differentiation, and apoptosis.[23] Thus the enhancement of pro-inflammatory cytokines produces aggression and contributes to neuronal apoptosis and memory impairment through Caspase-3 pathway,[20] which is an enzymes crucial for initiation and execution of apoptosis within a cell and plays a key role in cleavage of cytoskeletal proteins that can further contribute to chronic axonal and microvascular damage.[24, 25]

Brain derived neurotrophic factor (BDNF) is a member of the neurotrophin family that has a wide array of functions within the brain. It is involved in plasticity, neuronal survival, formation of new synapses, and dendritic branching, as well as modulation of excitatory and inhibitory neurotransmitter profiles. Many brain pathologies cause reduction of BDNF protein levels both in the brain and serum of patients.[26] S100B, is a calcium sensor protein, appears to regulate a variety of activities, transferring signals from second messengers and interacting with different molecules in different cell types. Serum S100B level as well, is a marker of brain injury and damage and found to be increased in a variety of pathological conditions of the nervous system.[27]

An indazole-3-carboxamide SC called AMB-FUBINACA appeared 2014 in Sweden. It is one of the most widely abused NPS.[28] It is mainly marketed as a more potent substitutes of cannabis through the internet.[29] It has more cannabinoid receptors affinity and thus potency than trans-Δ9-tetrahydrocannabinol (Δ9-THC).[30-32] AMB-FUBINACA causes more intense psychotropic effects and increased severity of the cardiovascular (CVC) and neurological toxicities, in relation to Δ9-THC, even with the consumption of small amounts[33, 34] through the activation of cannabinoids receptors**.** A "zombie outbreak" – was reported as a hallmark of AMB-FUBINACA toxicity.[35, 2]Yet, the knowledge of toxicological mechanisms of AMB-FUBINACA remains unclear, therefore this work was performed to explore the impact of this drug in the induction of brain cellular injury, through the characterization of inflammation, oxidative stress and neural cell death in experimental animals.

2. METHODS

2.1 AMB-FUBINACA

AMB-FUBINACA was kindly obtained from the Forensic chemistry Laboratories, Forensic Medicine Authorization (Cairo, Egypt) after permission from the Ministry of Justice. AMB-FUBINACA was confirmed by GC-MS. It is dissolved in absolute ethanol, Cremophor EL, and saline solution (1:1:18). The vehicle was prepared as solution made of ethanol, Cremophor EL, and saline.[36]

2.2. Experimental design

Study was performed on 32 male Wister rats, weighing 120 - 140 g. Animals were allowed free access of food and water, maintained at about 22°C, pathogen free animal house. They were classified into four groups (8 rats per cage), **Control group:** IP injected with 0.5 ml of the vehicle, and the others each IP injected with 0.5 ml of the three different doses of freshly prepared AMB-FUBINACA in vehicle used as following: **Group1:** 1mg/kg, **Group 2:** 3mg/kg and **Group 3:** 4mg/kg. The doses were chosen according to **Canaza et al.** [37]. All groups were received the treatment once a day for six consecutive days and were observed in their cages daily throughout the study for seizures and aggressiveness.

2.3 Sampling

After AMB-FUBINACA last dose by 24 hours, all animals were lightly anaesthetized with diethyl ether inhalation. Blood was collected, left to clot and separated for measurement of S100B, TNF-α and

IL-6 levels. Animals were sacrificed by cervical dislocation while they were under anesthesia. Brains then removed and cut into three partitions. One fixed in formalin for the histopathological examination, and the other two halves were homogenized in different buffers for undergoing two different techniques of parameters estimation: **The first half** was reserved for malondialdehyde (MDA) and reduced glutathione (GSH) determination. **The second one** was reserved for the quantitative estimation of brain CB1R, CB2R, BDNF, MAPK, NF-κB, and Caspase-3 mRNA expression levels.

2.4 Biochemical analysis

2.4.1 Estimation of S100B, TNF-α and IL6 serum levels

Levels of S100B, TNF-α and IL-6 were measured by enzyme linked immunosorbent assay (ELISA) technique, using Mybiosource, USA (CAT. No. MBS2023945), Cusabio, China (CAT. No. CSB-E11987r) and Quantikine® R and D system, USA (CAT. No. R6000B) kits individually as directed by the manufacturers.

2.4.2 Assessment of MDA and GSH content in brain tissue

Lipid per-oxidative end product MDA and GSH content in brain tissue homogenate were assayed by colorimetric assay kits provided by Biodiagnostic, Egypt (CAT. No. MD 25 28) as directed by the manufacturers.

2.4.3 Quantitation of mRNA expression levels of CB1R, CB2R, BDNF, MAPK, NF-κB and Caspase-3 in brain tissue by quantitative real time PCR:

Qiagen tissue extraction kit was used for brain RNA isolation. Fermentas Reverse transcription kit was used for RNA reverse transcription into complementary DNA. Hasting Software was used for designing PCR primers using RNA sequences obtained from gene bank **(Table 1)**. QuanitativeRT-PCR was done by using 25 ml reaction volume containing SYBR Green. The relative CB1R, CB2R, BDNF, MAPK, NF-κB and Caspase-3 mRNA expression was calculated by comparative threshold cycle method (Ct). β-actin gene internal control was concluded,[38] and the results were reported as fold change.

Table 1. Sequence of the primers used for real-time PCR**.**

Gene	Primer sequence
CB1-R	Forward, 5′ CCATTTCAAGCAAGGAGCAC3′ Reverse, 5′ GTCATTCGAGCCCACGTAGA3′
CB2-R	Forward, 5′ GGGTCGACTCCAACGCTATC3′ Reverse, 5′ AGGTAGGCGGGTAACACAGA3′
BDNF	Forward 5′ ATAATGTCTGACCCCAGTGCC3′ Reverse 5′ CTGAGGGAACCCGGTCTCAT3′
p38 MAPK	Forward 5′ AGGGCGATGTGACGTTT3′ Reverse 5′ CTGGCAGGGTGAAGTTGG3′
NF-κB	Forward 5′ CATGAAGAGAAGACACTGACCATGGAAA3′ Reverse 5′ TGGATAGAGGCTAAGTGTAGACACG3′
Caspase-3	Forward 5′ GTGGAACTGACGATGATATGGC3′ Reverse 5′ CGCAAAGTGACTGGATGAACC3′
β-actin	Forward 5′ TCTGTGTGGATTGGTGGCTCTA3′ Reverse 5′ CTGCTTGCTGATCCACATCTG3′

2.5 Histological studies of brain tissue

Brains were kept in 10% neutral formalin. The brain samples were preserved in paraffin blocks, 4-micron thickness sections were prepared, collected on glass slides and deparaffinized followed by staining with haematoxylin and eosin for examination using light microscopy. [39]

2.6 Statistics

Mean ± standard error (S.E) was used for data expression. One way analysis of variance (ANOVA) was used to assess variance between groups.

Subsequent multiple comparison between the different groups were done by Duncan's comparisons test. Statistical package for social sciences16 (SPSS) software was used for Statistics. GraphPad Prism software was used for graph construction. Significances was considered where $p < 0.05$.

3. RESULTS

3.1 General observations

AMB-FUBINACA resulted in substantial depressant effects in all rats during the first 10 min, and maximal depression occurred between 10-40 min and continued up to 3 to 6 h with the highest tested dose (4 mg/kg). Meanwhile tremors

happened 30 minutes after drug injection especially with 3 and 4 mg/kg examined doses. Then rats became hyperactive and aggressive.

3.2 Effect of AMB-FUBINACA on S100B, TNF-α and IL-6 serum levels:

Table 2 reveals that, administration of three different doses of AMB-FUBINACA (1, 3 and 4mg/kg) for 6 consecutive days induced significant increases ($p< 0.5$) of S-100B, TNF-α and interlukine-6 (IL-6) in relation to the control group. Recorded data also reveal that the undue effect of AMB-FUBINACA on the examined serum parameters significantly increases with increasing the examined dose

3.3 AMB-FUBINACA disturbs the Redox status of the brain

The administration of AMB-FUBINACA in three different doses induced a significant increase in lipid per oxidative end product (MDA). This escalation in brain malondialdehyde level is in concomitant with a significant reduction in GSH content compared with the control group **(Table 3)**.The obtained data also declared that the disturbance in the redox status of the brain to a great extent depended on the dose of AMB-FUBINACA.

Table 2. Serum levels of S100B, TNF-α and IL-6 in normal rats and under the influence AMB-FUBINACA administration in three doses

Parameters Groups	S100 (ng/ml)	TNF-α (pg/ml)	IL-6 (pg/ml)
Control	1.82 ± 0.15^{a}	15.98 ± 1.16^{a}	35.37 ± 1.69^{a}
Group 1	4.43 ± 0.34^{b}	62.73 ± 4.36^{b}	$104 .0 \pm 2.65^{b}$
Group 2	7.93 ± 0.16^{c}	94.59 ± 2.40^{c}	121.33 ± 2.34^{c}
Group 3	13.37 ± 0.90^{d}	118.86 ± 3.97^{d}	147.37 ± 5.84^{d}

Data are expressed as mean values ± SE (n = 8 rats for each group). In the same column, values with different superscript letters are significantly different ($p < 0.05$).

Table 3. Effect of three different doses of AMB-FUBINACA on lipid per-oxidative index (MDA) and reduced GSH in brain of rats

Parameters Groups	MDA (nM/mg protein)	GSH (mM/mg protein)
Control	18.07 ± 1.66^{a}	97.97 ± 5.10^{d}
Group 1	63.69 ± 3.86^{b}	56.07 ± 1.76^{c}
Group 2	85.19 ± 4.03^{c}	36.47 ± 1.12^{b}
Group 3	121.31 ± 2.53^{d}	26.41 ± 1.34^{a}

Data are expressed as mean ± S.E. (n = 8 rats). In the same column, values with different superscript letters are significantly different ($P < 0.05$).

3.4 Effect of AMB-FUBINACA on mRNA expression levels of NFκB, MAPK, Caspase-3, CBR 1, CB2R and BDNF

The administration of AMB-FUBINACA in three different doses induced a significant increase the expression levels of mRNA expression of NF-κB (A), MAPK P38(B) and Caspase-3(C) in association with a significant decrease in the mRNA expression of CB1 R (A), CB2R (B) and BDNF (C) in the brain tissue comparing to the control group **(Fig. 1, 2).** The obtained data also demonstrate that, as the examined dose increases the obtained disturbance in the examined mRNA expression levels were augmented.

3.5 The histological changes in brain tissue under the influence of different doses of AMB-FUBINACA

The histological examination of control group shows normal cerebral cortex and hippocampus **(Fig. 3A, B)**, meanwhile the administration of 1 mg / kg body weight of AMB-FUBINACA (Group 1) caused mild pathological alteration where hippocampus and choroid plexus normally appeared, however focal inflammatory aggregates in cerebral cortex could be observed **(Fig. 4A, B and C)**,while the administration of 3 mg / kg body weight of AMB-FUBINACA (Group 2) showing focal area of pyknotic cells in hippocampus (**Fig. 5A)**,with sever hemorrhage (**Fig. 5B)**, and inflammatory cell aggregates in ventricle (**Fig. 5C)**, and the microscopical examination of group 3

revealed that, administration of 4mg / kg induced a decrease in cellularity and apoptotic cells in hippocampus (**Fig. 6A**) dilated congested blood vessel with perivascular inflammation and hemorrhage in ventricle were noticed (**Fig. 6B, C**)

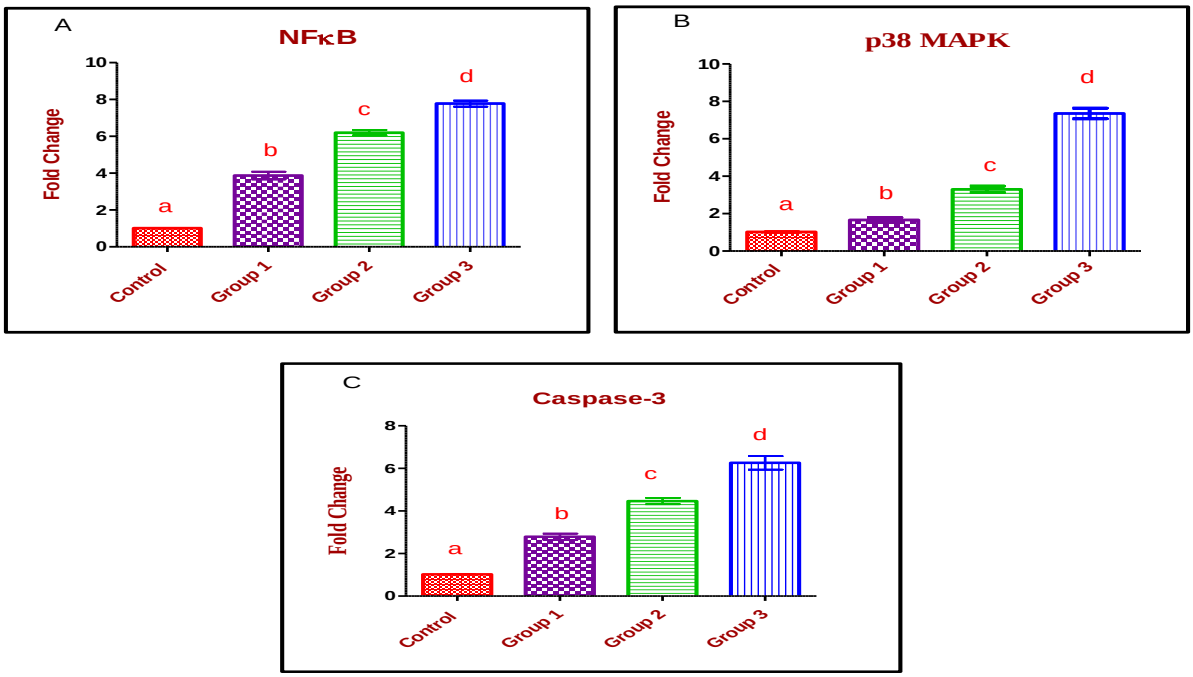

Figure 1. The up-regulation of mRNA expression levels of mRNA expression level of NF-Kb (A), MAPK (B)and Caspase-3 (C) in brain tissue under the influence of three doses of AMB-FUBINACA. The steady state levels of mRNA in the brain were analyzed by RT-PCR assay. β-actin was used as an invariant internal control for calculating mRNA fold changes. Data are expressed as mean values ± SE (n = 8 rats). Mean values with different superscript letters are significantly different ($p<0.05$)

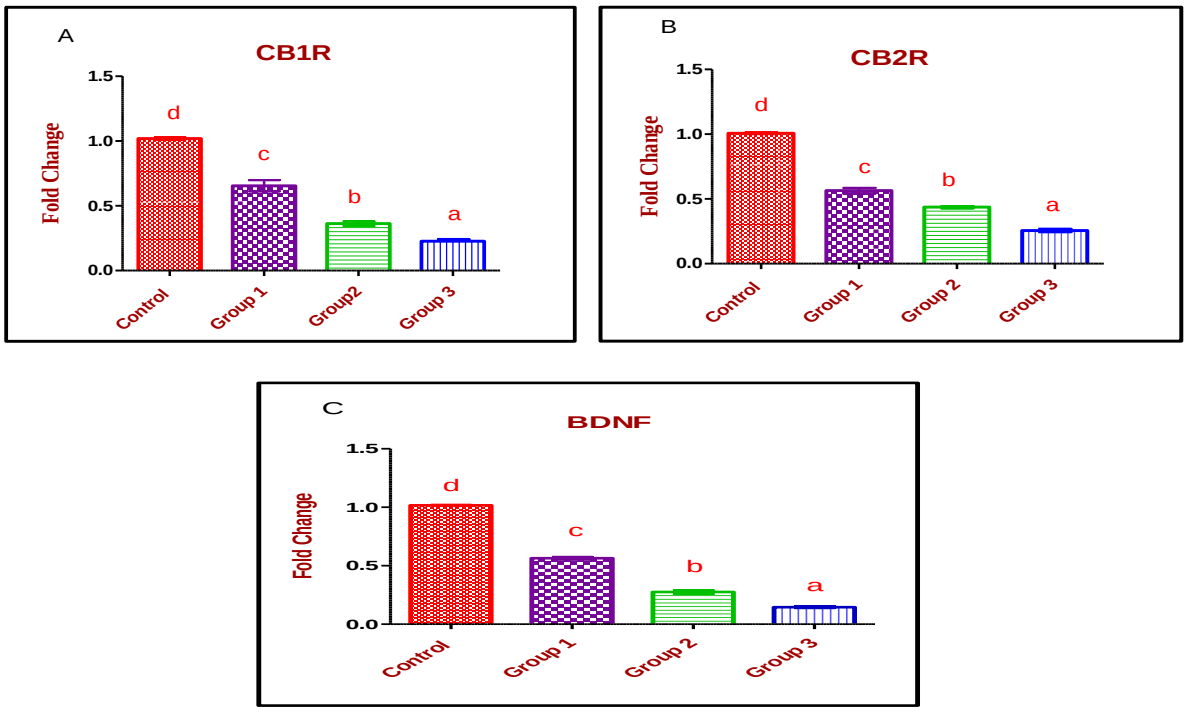

Figure 2. The down- regulation of mRNA expression levels of CB1R (A), CB2R (B)and BDNF (C) in brain tissue under the influence of three doses of AMB-FUBINACA. The steady state levels of mRNA in the brain were analyzed by RT-PCR assay. β-actin was used as an invariant internal control for calculating mRNA fold changes. Data are expressed as mean values ± SE (n = 8 rats). Mean values with different superscript letters are significantly different ($p<0.05$)

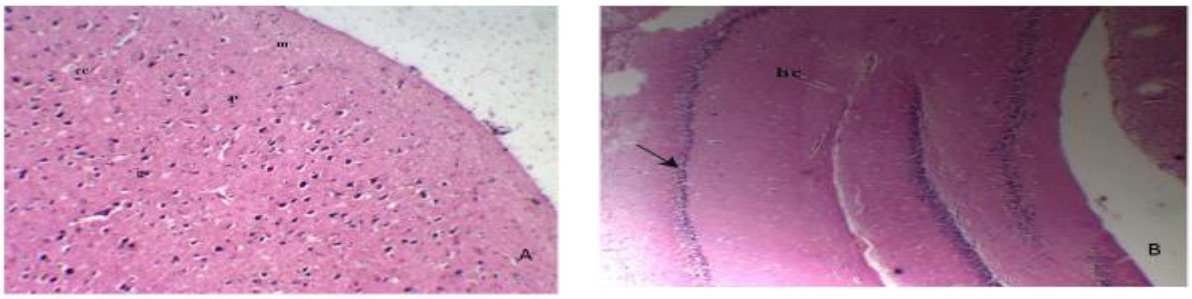

Figure 3. Photomicrograph of brain tissue from control group (A)showing normal cerebral cortex (cc), molecular layer (m), pyramidal layer (p), granular layer (g) (X : 40) and (B) showing normal hippocampus (hc), pyramidal layer (arrow) (X: 100)

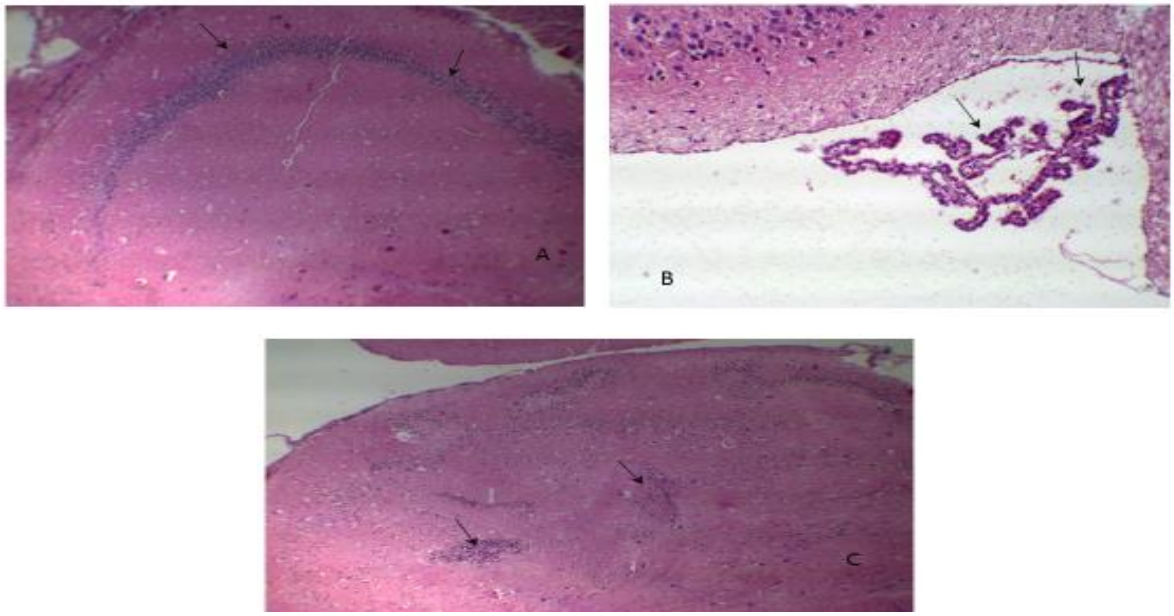

Figure 4. Photomicrograph of brain tissue from group 1: (A) showing normal hippocampus (arrow) (X: 100); (B) showing normal choridate plexus (arrow) (X: 200) and (c) focal inflammatory aggregates in cerebral cortex (arrow) (X: 100)

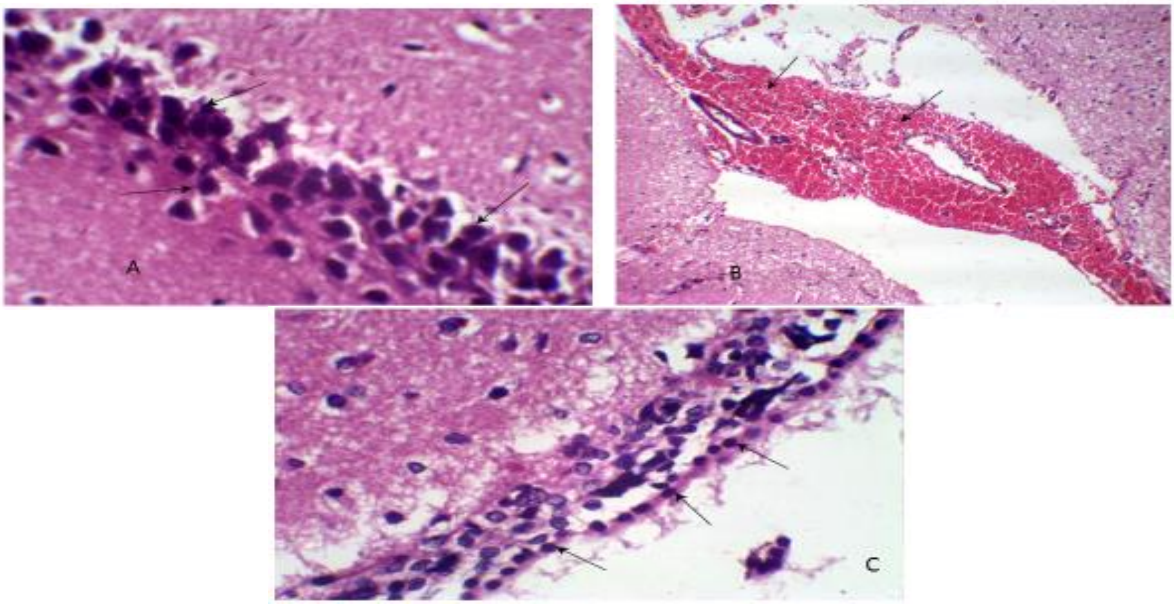

Figure 5. Photomicrograph of brain tissue from group (2) (A) showing hippocampus with pyknotic cells (arrow) (X: 400) (B) showing hemorrhage in ventricle (arrow) (X: 200) and (C) showing inflammatory cells in ventricle (arrow) (X: 400)

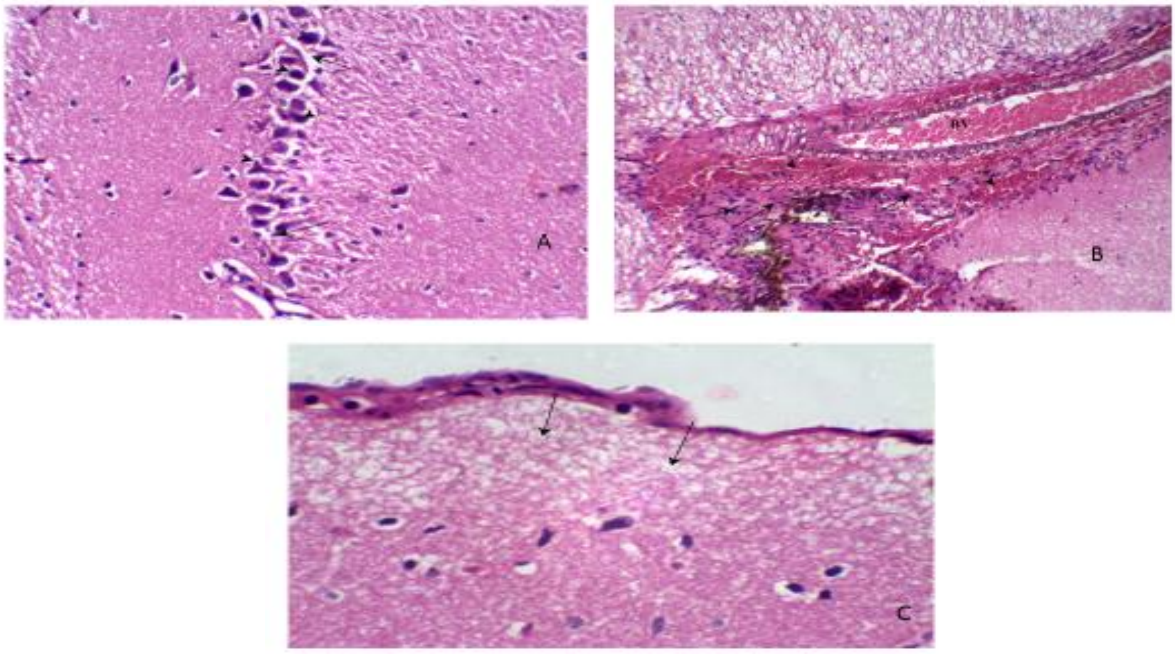

Figure 6. Photomicrograph of brain tissue from group (3) (A)showing hippocampus with less in cellularity (arrow), apoptotic cells (arrow head); (B)showing congested, dilated blood vessel (BV), perivascular inflammation (arrow), hemorrhage (arrow head) and (C) showing mild vacuolation in neuropil (arrow) (X: 400)

4. DISCUSSION

In the present study, AMB-FUBINACA injection with the three examined doses resulted in a significant elevations in lipid peroxidation process as manifested by the recorded increase in MDA levels in brain tissue. This increment is dose dependent and accompanied with a significant reduction in the brain content of GSH. This disturbance in the redox status of the brain proves the production of free radicals and the generation of oxidative stress which has been involved as a mechanism underlying the neuronal oxidative injury. **[10]** Moreover, MDA, is a neuronal toxin which could affect protin.**[40]** Therefore, ROS have indispensable role in the pathology of neurological disease.**[11]** This finding agrees with several studies that confirm the role of ROS in the induction of toxic effects among cannabinoid users,**[41,12,14]** in addition ROS production is a mechanism involved in stroke.**[42]**Additionally, free radicals activate neuron inflammation by up-regulating the production of cytokines and chemokines, which could cause loss of synapses, neuronal apoptosis, and so cognitive dysfunction.**[43, 44]** ROS are also substantially involved in numerous behavioral alteration, including depression, memory impairments and anxiety**.[15]**

Activation of microglial cells within CNS by harmful stimuli is the first step inducing neuron inflammation. [45] As activated microglia (MG) release cytotoxic factors such as interlukin-1 (IL-1), IL-6, TNF-α, adhesion molecules and ROS, [46-48] in the CNS. These factors could result in neuronal cell death and lead to more MG activation with more exacerbation of neuronal injury. [49] In the present study the IL-6 and TNF- α levels were increased with increasing the dose. The obtained data of the current study are in the same line with that obtained by Oztas et al.,[50] who reported the impact of the synthetic cannabinoid AKB48 in the induction of a dose dependent significant increment in TNF-α and IL-6, revealing the neurotoxicity of AKB48 which is one of the third-generation psychoactive synthetic cannabinoid (Apinca). The obtained data in the current study also confirmed the finding of Guler et al.[51] who recorded a significant elevation of IL-6, and TNF-α serum levels in synthetic cannabinoids users. So the present study proved the impact of inflammatory mediators among cannabinoid users.

Nuclear factor kappa B (NF-κB) has a pivotal function in expression of inflammatory mediators. It affects transcriptional activity via binding with certain sequences in genes that are responsible for immunological and inflammatory processes.[52]In the present study, the mRNA expression level of NF-κB was significantly increased in rats treated with the examined drug. This finding reinforces the role of AMB- FUBINACA in the induction of brain injury. Moreover, the obtained increase in the circulatory TNF-α and IL-6 is in concomitant with their

mediator NF-κB expression that occurred and propagated in the injured brain as the dose of the examined drug increased, which support the positive correlation between TNF-α, IL-6 and NF-κB. This finding proved the impact of SCs in the generation and development of brain injury.

It has been reported that, p38 MAPK and NF-κB pathways in brain participate in the production of cytokines and other harmful molecules.[53-55] In addition Roy et al.[56] found that p38 MAPK has a significant effect in the process of neuronal cell death after ischemia in animals with stroke. In the current study the I.P. administration of AMB-FUBINACA induced a dose dependent significant elevation in the mRNA expression levels of p83 MAPK. This finding clearly declares the contribution of the synthetic cannabinoids in the induction of neuronal damage and confirmed the findings of researchers who reported that,[53-55] in harmony with the obtained results Zhu et al. [57] reported that, MAPK and NF-κB signaling pathway mediates microglial inflammatory response in the induction of brain injury. Additionally, MAPK/NF-κB pathway activation also generates ROS which could also participate in neuronal damage as recorded by Zhang et al. [47]

In the current study the obtained increment in serum S100 is a dose dependent and is considered a biomarker of several brain diseases such as brain tumors, neuron inflammation, neuron degeneration and acute brain injury.[27] Liu et al., [58]reported neurotoxicity as a result of S100B overexpression. Also, studies have revealed that serum level of S100B could be a dependable biomarker in prediction and prognosis of cerebral infraction[59, 60].Therefore, S100B has a vital role as a biomarker for brain damage, which could result in psychiatric disorders, substance abuse as well as structural brain damage [61, 62]

Brain derived neurotrophic factor (BDNF) has a role in the pathology of neurodegenerative disorders.[63]In the current work administration of the examined drug at the three dosage levels caused a significant decline in BDNF mRNA expression. Oxidative stress may be increased in conditions with decreased BDNF. [64, 65]This fact is emphasized in the current study through the negative correlation between the increment in lipid per-oxidative index end products MDA and the obtained decline in BDNF mRNA expression levels. In addition the recorded results of this study confirmed the role of inflammatory mediators in the down- regulation of BDNF among synthetic cannabinoids users .[63, 66, 67]

It has been shown that, caspase-3 is a well-recognized hallmark of neuronal cell death in many CNS disorders.[68-70]Assessement of caspase-3 mRNA expression level in the current study was performed to ascertain apoptosis in brain. The recorded increase in caspase-3 mRNA expression provides evidence about the contribution of synthetic cannabinoid abuse in the activation of caspase-3 to promote cellular apoptosis in brain in a dose dependent manner. This finding is in harmony with that of Almada et al.,[71] the authors demonstrated the impact of JWH-018, JWH-122, UR-144 SCs and the THC in increasing the production of ROS with the induction of apoptotic pathways through the activation of caspases-9, 7 and 3.

It has been reported that SCs often act by modulating the action of endo-cannabinoids on CB1 [50, 72, 73] and CB2 receptors [74, 75] to exert their effects as a result of synthetic cannabinoids administration. Oztas et al.,[50] reported that cannabinoid B1receptor expression was well markedly increased under the influence of synthetic cannabinoid AKB48 administration in a dose dependent manner, meanwhile cannabinoid CB2 receptor was not expressed in SH-SY5Y cells. On the other hand our data recorded a significant decline in the mRNA expression of the both examined receptors under the influence of AMB-FUBINACA administration at the three examined doses. In addition the magnitude of this decrement increased as the dose of the examined drug increase. These contradictory findings recorded in the current study may be pointed out that the synthetic cannabinoid, AMB-FUBINACA used in the current study with doses 1, 3 and 4mg /kg for six days which mimic and exceed the sub-chronic (four days) administration that reported by Dalton et al.[76] The authors revealed a pronounced decline in CB1 receptor density in rat brain following acute, 4 days (sub chronic) and 14 days (chronic) administration of HU210, which is a potent SC, that totally agree with the obtained results of the current study. So in the present study the obtained down- regulation in the CB receptors may reflect the effect of dose concentration and the duration of the experiment in inducing receptors down-regulation through drug tolerance, since receptors down-regulation together with the retention of G-protein activity considered a mechanism for tolerance, whereas cannabinoids tolerance was recorded after chronic use.[77]

Several molecular mechanisms mediating the down regulation of CB1 receptors have been proposed. Sim-Selley et al., [78] reported that CB1 receptors down-regulation was achieved through the

receptor protein rather than gene expression. Moreover Villares[79] demonstrated that the CB1R cDNA expression in brain decline with long term cannabis abuse causing CB1R down-regulation, and thus decline in the binding capacity which might illustrate that the primary action of synthetic cannabinoid could be on the expression of CB1 receptor gene rather than the protein. Using HEK293 cells that contain neurons express CB1R by Martini et al.,[80] showed that treatment with cannabinoid agonist (WIN55, 212–2) resulted in CB1R endocytosis and degradation and that the GASP1 has a major role in this process that could explain a molecular mechanism for the decline of receptor density. Nguyen *et al.;* Schmid *et al.;* Walsh and Andersen[59, 81, 82] demonstrated the function of GRK in recruitments of both β-arrestin 1 and 2 to receptors that leads to desensitization and internalization of CB1R.

In the current study CB2 receptor mRNA was expressed in control rats confirmed its prevalence in the brain tissue[83, 84] and down regulated in AMB-FUBINACA challenged rats. This finding may declare that CB2 receptor plays a significant role in immunological function rather than the other neuronal function in neuronal processes. The increased NF-κB expression and the increased levels of serum IL-6 and TNF-α play a key role in inducement and continuation of inflammation in macrophage,[85] therefore, the down regulation of CB2 R in this study can be considered as important signal in the pathogenesis of neuronal disorders which can be assured by the increased expression of NF-κB that is mediated by successive increase of MAPK p38. Accordingly, we can speculate that CB1 and CB2 receptors work independently to regulate CNS physiological activities. The down regulation of CB1R and CB2R that obtained in the current study should be taken into consideration by future studies investigating ß-arrestin recruitment to synthetic cannabinoids receptors to avoid misplaced conclusions.

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5. Conclusion

In the light of all findings, the current study concluded that: AMB-FUBINACA was found to have a functional and structural deleterious CNS effects with sub chronic exposure in adult male albino rats. The induced brain injury and seizures that accompanied with the synthetic cannabinoids abuse might be mediated through the generation of oxidative stress, activating inflammatory cellular signaling mechanism and neural cell death via apoptosis.

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Ethical Statement: The protocol approval number given by Faculty of Pharmacy Al-Azhar University Research Ethics Committee is (185/2018).

Conflicts of Interest: No.

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Authors' Contribution: All authors participated in conducting the experiment, analysis of samples and writing the manuscript.

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