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Effect of cannabinoids in global cerebral Ischemia in vivo

Omar M. E. Abdel-Salam[1], Rania Elgohary[1], Marawan Abd El Baset Mohamed Sayed[2], , Eman R. Youness[3], Amany A. Sleem[2], Nermeen Shaffie[4]

[1] *Narcotics, Ergogenics and Poisons Department, National Research Centre, El- Buhouth St., Dokki, Cairo 12622, Egypt*

[2] *Pharmacology Department, National Research Centre, El- Buhouth St., Dokki, Cairo 12622, Egypt*

[3] *Medical Biochemistry Department, National Research Centre, El- Buhouth St., Dokki, Cairo 12622, Egypt*

[4] *Clinical Pathology Department, National Research Centre, El- Buhouth St., Dokki, Cairo 12622, Egypt*

Abstract

Cannabinoid receptors have been suggested as potential therapeutic targets in cerebral ischemia. In this study, cannabinoid receptor (CBR) agonists and antagonists were tested for their ability to protect against the effects of global cerebral ischemia. For this purpose, rats were intraperitoneally (i.p.) treated with N-arachidonoyl dopamine (NADA; CBR1 agonist), Rimonabant (CBR1 antagonist), GW 405833 (CBR2 agonist), AM630 (CBR2 antagonist) at a dose of 1 mg/kg and subjected later to transient of their bilateral common carotid arteries (CCA) for 30 min. The control group received the vehicle dimethyl sulfoxide (DMSO). The brain content of the oxidative stress biomarkers malondialdehyde (MDA), reduced glutathione (GSH), nitric oxide (NO) was determined. In addition, paraoxonase-1 (PON-1) activity, acetylcholinesterase (AChE), and nuclear factor-□B (NF-□B) brain's content were measured, and histopathological study of brain tissue was done. In the ischemic group, brain tissue concentrations of malondialdehyde and nitric oxide were higher, and reduced glutathione was lower than the control animals. In addition, brain ischemia resulted in a significant decrease in PON-1 activity and increased NF-κB.

On the other hand, I/R had no significance on the AchE brain's content. Brain ischemia resulted in degeneration in the cerebral cortex, hippocampus, and substantia nigra. In the ischemic brain, either NADA or AM630 produced a significant decrease in lipid peroxidation. Cannabinoids inhibited nitric oxide and increased reduced glutathione contents in the ischemic brain. The level of NF-κB was decreased by either NADA or AM630 but AChE decreased significantly by cannabinoids. Moreover, NADA, GW 405833, or AM630 caused a significant decrease in PON-1 activity. AM630 markedly improved the ischemia-induced histopathological changes. In addition, protection of substantia nigra neurons was observed after treatment with NADA or GW 405833.

Keywords: AchE, Ischemia, Cannabinoid, Receptors, Brain, PON-1, Substantia nigra

1. Introduction

Ischemic stroke is the third highest cause of death in people after myocardial infarction and cancer. It is also the primary cause of disability [1]. A stroke affects 16 % of people at some point in their lives, with more than 15 million cases reported each year [2]. While a stroke can be divided into hemorrhage and ischemia depending on the mechanism of injury [3]. ischemic stroke can be further subdivided into global and focal ischemia[4]. global cerebral ischemia (GCI) may occur in many clinical settings, including severe hypotension, cardiac arrest, shock, severe cerebrovascular accident, and craniocerebral trauma [5]. In GCI, There is no cerebral blood flow to any part of the brain, causing neurons in some regions of the brain to be damaged [6] thus leading to neurological consequences including short- or long-term motor/cognitive dysfunction [7]. Acetylcholine (ACh) is a neurotransmitter that plays a critical role in the brain's physiological function. Specific components of mental processes, learning, and memory are all influenced by ACh signaling. ACh signaling changes play a role in the pathogenesis of various brain illnesses. The concentration of ACh in the brain falls after an ischemic injury. Inhibition of acetylcholinesterase (AChE) reduces the volume of cerebral infarction in rats after an experimental stroke[8].

*Corresponding author e-mail: Rony2003rania@gmail.com (Rania Elgohary)

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Clinically, vascular recanalization was always employed to restore blood supply; nevertheless, this reperfusion could cause additional brain damage, referred to as ischemia/reperfusion (I/R) injury [9]. Multiple insults to the cerebral microvasculatures occurred due to I/R, including the formation of oxygen free radicals, mast cell degranulation, and endothelial cell damage [10]. ROS that mediates oxidative insults during cerebral I/R injury may harm the vulnerable regions of the brain[11]. Specific brain regions, including the hippocampus and the cortex, are more susceptible to ischemia than other regions. Severe damage to the hippocampus results in difficulties in forming new memories[12]. It has been established that inflammatory response plays an essential role in the pathogenesis of cerebral I/R injury[13]. Therefore, one of the most promising strategies for treating stroke was the reduction of inflammatory damage. During I/R, the transcription factor nuclear factor-kappa β (NF-k β), which is implicated in inflammatory processes, was activated, resulting in the expression of several pro-inflammatory molecules such as interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α), as well as neuron death. [13].

Currently, the neuroprotective approach has become a novel direction in the treatment of stroke and has been studied in animal experiments, but the efficacy on patients remains limited [14]. As a result, new therapeutic options are needed to enhance the prognosis of ischemia-induced brain injury. Numerous studies suggested that anti-inflammatory and/or antioxidant medicines could reduce brain damage and improve neurological outcomes in stroke models because neuroinflammation and oxidative stress play essential roles in the pathophysiology of cerebral ischemia [15].

The consequences of ischemic injury in the heart, liver, and brain can be ameliorated by cannabinoids [16]. Most of the therapeutic effects of cannabinoids are mediated by the G-protein-coupled receptors cannabinoid receptor 1 (CB1) and cannabinoid receptor 2 (CB2)[17]. Cannabinoids have been proposed as a potential neuroprotective drug for stroke treatment [18]. This possibility has primarily been addressed in laboratory animals using experimental models. Clinically relevant models are critical for cerebral ischemia research. These models should be clinically relevant and repeatable to aid in the study of the pathophysiology of ischemic stroke and serve as a platform for developing novel stroke treatment techniques.

The present study was undertaken to evaluate the neuroprotective potential of cannabinoid CB1R and CB2R agonists and antagonists in bilateral common carotid artery (BCA) occlusion induced global cerebral ischemia model in rats.

2. Material and methods

Animals

Male Sprague-Dawley rats weighing 220-230 g obtained from the Animal House Colony of the National Research Centre, Cairo, were used in the study. Rats were kept under temperature- and light-controlled conditions and given standard laboratory rodent chow and water ad libitum. The experimental procedures followed the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 85-23, revised 1985) and the recommendations of the institutional Ethics Committee of the National Research Centre.

Drugs and chemicals

Dimethyl sulfoxide (DMSO) was used to dissolve the agonists and antagonists. CB1-receptor agonist NADA (N-arachidonoyl dopamine), CB2-receptor agonist GW 405833(1,2,3-Dichlorobenzoyl)-5-methoxy-2-methyl-3-[2- (4-morpholinyl)ethyl]-1H-indole), CB1-receptor antagonist Rimonabant (SR141716A), and CB2-receptor antagonist AM630 (6-Iodopravadoline) were all from Sigma-Aldrich Chemical, USA.

Methods

Experimental groups

Rats were randomly allocated into the following groups (6 rats each):

Group 1: Non-operated rats treated with the vehicle.

Group 2: Rats received the vehicle and underwent cerebral ischemia-reperfusion injury (I/R).

Group 3: Rats treated with NADA at 1 mg/kg[19], i.p., 30 min before ischemia.

Group 4: Rats treated with GW 405833 at 1 mg/kg, i.p., 30 min before ischemia.

Group 5: Rats treated with Rimonabant at 1 mg/kg, i.p., 30 min before ischemia.

Group 6: Rats treated with AM630 at 1 mg/kg, i.p., 30 min before ischemia.

Global cerebral ischemia

Global cerebral ischemia lasting 30 min, followed by reperfusion, was induced by carotid artery occlusion in anesthetized rats using thiopental (20 mg/kg; i.p.) [20].The common carotid arteries (CCAs) were exposed and ligated for 30 min with microvascular clips, and perfusion was then restored for two hours. Body temperature was maintained at 36.5 ± 0.5°C from the onset of the procedure using a heating pad, and respiration pattern was monitored.

Biochemical assessment

At the end of the experiment, the animals were sacrificed by decapitation under light anesthesia. The brain was dissected, then washed with saline, and placed in ice-cold phosphate buffer (pH 7.4) to prepare

20% homogenate using a tissue homogenizer (MPW-120, BitLab Medical instruments, Poland). Homogenized tissues were centrifuged at 4000 rpm/min for 10 min using a cooling centrifuge (Laboratory Centrifuge, 2 K15, Sigma Co., Germany)[21]. The supernatant was collected and stored at -80°C and then used for estimation of brain contents of reduced glutathione (GSH), malondialdehyde (MDA), nitric oxide (NO), paraoxonase-1 (PON-1) activity, acetylcholinesterase (AChE), and nuclear factor-κB (NF-κB) using Glory Science ELISA kits, China.

Determination of GSH

Reduced glutathione was determined in tissue homogenates using the procedure of Ellman[22]. The assay is based on the reduction of Ellman's reagent [DTNB; 5,5′-dithiobis (2-nitrobenzoic acid)] by the free sulfhydryl group on GSH to form yellow colored 5-thio-2-nitrobenzoic acid, which can be determined using a spectrophotometer at 412 nm.

Determination of lipid peroxides

Malondialdehyde was determined by measuring thiobarbituric reactive species using the method of Ruiz-Larrea et al. 1994. The thiobarbituric acid reactive substances react with thiobarbituric acid to produce a red-colored complex having peak absorbance at 532 nm [23].

Determination of NO

Nitric oxide was determined in serum according to the method of Miranda [24]. The total nitrite/nitrate level in serum samples was calculated using the standard curve constructed with the prepared serial dilutions of sodium nitrite.

Determination of PON-1 activity

Arylesterase activity of paraoxonase was measured spectrophotometrically using phenylacetate as a substrate [25]. In this assay, arylesterase/paraoxonase catalyzes phenylacetate's cleavage, resulting in phenol formation. The rate of formation of phenol is measured by monitoring the increase in absorbance at 270 nm at 25 °C. The working reagent consisted of 20 mM Tris/HCl buffer, pH 8.0, containing 1 mM $CaCl_2$ and 4 mM phenylacetate as the substrate. Samples diluted at 1:3 in the buffer are added, and the change in absorbance is recorded following a 20 s lag time. Absorbance at 270 nm was taken every 15 s for 120 s. One unit of arylesterase activity is equal to 1 μM of phenol formed per minute. The activity is expressed in kU/L, based on the extinction coefficient of phenol of 1 310 m/cm at 270 nm, pH 8.0 and 25 °C. Blank samples containing water are used to correct for the spontaneous hydrolysis of phenylacetate

Determination of AChE activity

The procedure used was a modification of the method of Ellman et al. [26] as described by Gorun et al. [27]. The principle of the method is the measurement of the thiocholine produced as acetylthiocholine is hydrolyzed. The color was read immediately at 412 nm.

Quantification of NF-κB

According to the manufacturer's manufacturer, the active form of NF-κB was measured in the supernatants using a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Glory Science, Del Rio, TX, USA) instructions. The kit uses a double-antibody sandwich ELISA assay to determine the level of active NF-κB.

Histological assessment studies

Brain samples of all animals were dissected immediately after death. The specimens were then fixed in 10 % neutral-buffered formalin saline for 72 hours at least. All the specimens were washed in tap water for half an hour and then dehydrated in ascending grades of alcohol, cleared in xylene, and embedded in paraffin. Serial sections of 6μm thick were cut and stained with hematoxylin and eosin for histopathological investigation[28].

Statistical analysis

The mean and standard error (SE) of the data was calculated. GraphPad Prism 5 Software version 5 (San Diego, CA, USA) was used for statistical analysis, using one-way analysis of variance test (ANOVA) followed by Tukey's multiple comparisons. The significance criteria were set at a 0.05 level of probability.

3. Results

Effect of cannabinoid agonists and antagonists on oxidative stress biomarkers after induction of cerebral ischemia in rats

Cerebral I/R resulted in a significant decrease in GSH brain content by 33% compared with the vehicle group. While, Pretreatment with NADA (CB1R agonist), Rimonabant (CB2R antagonist), GW 405833 (CB2R agonist), and AM630 (CB2R antagonist) significantly increased GSH brain content by 43%, 23%, 16%, and 29%, respectively, as compared to the I/R treated group (Fig. 1A). Cerebral I/R treated rats showed decreased levels of PON-1 in their brain homogenates by 39% compared with the vehicle group. Moreover, Rats were given NADA, GW 405833, and AM630 had a significantly decrease in brain PON-1 levels by 45%, 31%, and 46% respectively, as compared to theI/R treated group (Fig. 1B). Cerebral I/R induced lipid peroxidation as indicated by a significant increase in brain MDA levels by 83% compared to the vehicle group. Pretreatment with NADA and AM630 significantly decreased the

extent of lipid peroxidation by 21% and 38%, respectively, compared with the I/R group (Fig. 1C). The brain NO level of rats subjected to I/R was significantly increased by 62% compared to the vehicle group. On the other hand, pretreatment with NADA, Rimonabant, GW 40583, and AM630 had a significant decrease in NO brain levels by 44.4%, 28%, 23%, and 43%, respectively, compared with the I/R treated group (Fig. 1D). Relative to the I/R group in brain tissues. According to these results, we believed that the beneficial effects of cannabinoids administration under cerebral I/R are associated with anti-oxidative function.

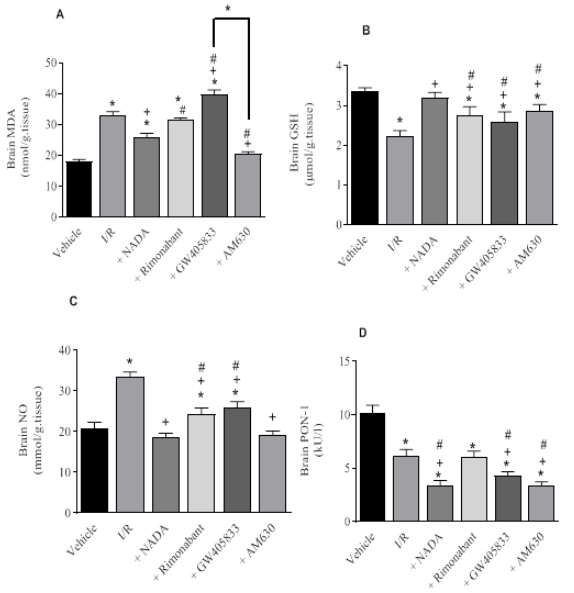

Figure 1: effect of cannabinoid agonists and antagonists on (A) MDA (B) GSH, (C) NO and (D) PON-1 activity, after induction of cerebral ischemia in rats

Data are provided as the mean ± S.E. of (n=6) for each group. The statistical analysis was carried out by one-way analysis of variance then followed by Tukey's multiple comparisons test. *Statistically significant from vehicle and between different groups. + Statistically significant from I/R group. #statistically significant from NADA group at P <0.05.

Effect of cannabinoid agonists and antagonists on brain NF-κB after induction of cerebral ischemia in rats

Rats subjected to 30 min of cerebral I/R followed by 2 h of reperfusion showed marked elevation in the brain content of NF-κB by 720% compared to the vehicle group. While, Pre-treatment with NADA and AM630 significantly decreased the brain content of NF-κB by 34% and 32%, respectively, as compared to the I/R group (Fig. 2).

Effect of cannabinoid agonists and antagonists on brain AChE after induction of cerebral ischemia in rats

The activity of AChE had not changed in I/R treated group as compared to the vehicle group. While rats treated with NADA, Rimonabant, GW 405833, and AM630 significantly decreased brain AChE activity by 41%, 58%, 44.3%, and 54%, respectively, compared to the I/R treated group (Fig. 3).

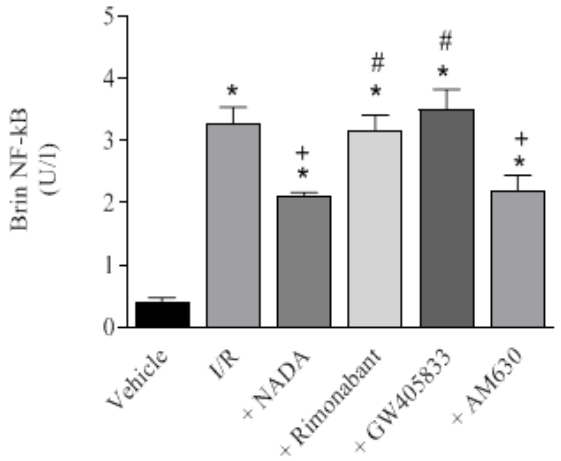

Figure 2: Effect of cannabinoid agonists and antagonists on brain NF-κB after induction of cerebral ischemia in rats

Data are presented as the mean ± S.E. of (n=6) for each group. Statistical analysis was conducted by one-way analysis of variance followed by Tukey's multiple comparisons test. *Statistically significant from vehicle group. + Statistically significant from I/R group. #statistically significant from NADA group at P <0.05.

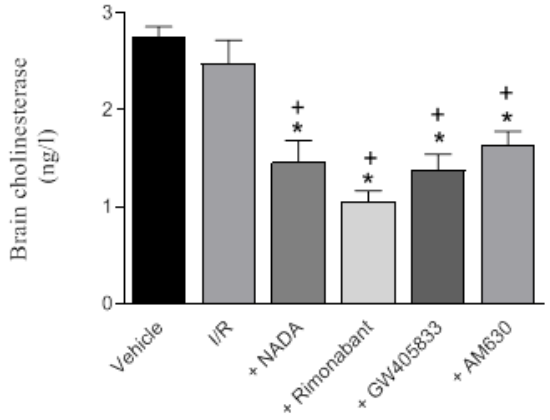

Figure 3: Effect of cannabinoid agonists and antagonists on brain AChE after induction of cerebral ischemia in rats

Data are available as mean ± S.E. of (n=6) for each group. Statistical analysis was done by one-way analysis of variance followed by Tukey's multiple comparisons test. *Statistically significant from

vehicle group. + Statistically significant from I/R group. #statistically significant from NADA group at P <0.05.

Histopathological study

Representative light microphotographs from the cerebral cortex of rats

Sections from the cerebral cortex tissue of the control -ve rat show the normal neurons with large vesicular nuclei (4A). Ischemic brain rat shows many neurons with deeply stained nuclei (4B). Ischemic brain rat treated with NADA shows some neurons with deeply stained nuclei are still observed (4C). Ischemic brain rat treated with GW 405833 shows many neurons with deeply stained nuclei (4D). Ischemic brain rat treated with AM 630 shows many cells with regeneration signs, although some cells with apoptotic nuclei (arrow) are observed (4E). Ischemic brain rats treated with Rimonabant shows neurons with nuclei slightly darker than normal (arrow) and some shrunken cells (arrowhead) (4F).

Representative light microphotographs of the hippocampus

Sections from the hippocampus area of the control -ve rat show the normal structure of this area (5A). Ischemic brain rat shows most neurons with deeply stained nuclei (5B). Ischemic brain rat treated with NAD shows deformation of the arrangement of the neurons; most of them are deeply stained (5C). Ischemic brain rat treated with GW 405833 shows many deeply stained cells but no deformation in arrangement (5D). Ischemic brain rat treated with AM 630 shows most of the cells appear normal. Only a few deeply stained cells are observed (5E). Ischemic brain rats treated with Rimonabant show many deeply stained neurons with mild deformation in arrangement (5F)**.**

Representative light microphotographs of the substantia nigra

Sections from the substantia nigra of the control -ve rat show the normal size and shape of pigmented neurons (6A). Ischemic brain rat shows marked decrease in size and number of pigmented (6B). Ischemic brain rat treated with NADA shows normalization of pigmented neurons concerning size and number (6C). Ischemic brain rat treated with GW 405833 shows a marked increase in size and number of pigmented neurons (6D). Ischemic brain rat treated with AM 630 shows some pigmented neurons regain their normal size, although their number is still below normal (6E). Ischemic brain rat treated with Rimonabant shows a decrease in size, and the number of pigmented neurons is still noticed (6F)**.**

4. Discussion

In the present study, a well-established animal model of I/R was used, the results showed that CB1R and CB2R agonist and antagonist, given i.p., 30 min before ischemia, protected the animals from the deleterious effects of the ischaemic insult as it improves antioxidant enzymes levels, reduces the brain lipid peroxidation, and alters the histopathological status in animals after bilateral cerebral artery occlusion. Previous studies showed that CB1R had been found to play an essential role in normal neuronal development; it is, therefore, possible that chronic deletion of the CB1R causes neurons to be much more susceptible to injury. In contrast, the selective CB1R antagonist SR141716 (Rimonabant) administration was neuroprotective following ischemia-reperfusion injury. Interpretation of these results is complicated because SR141716 may interact with other non-CB1Rs, including 5-HT1A and Vanilloid VR1 receptors [29]. Moreover, The CB2R is present on numerous cells involved in inflammatory responses following stroke [30]. The CB2R contributes to the downregulation of proinflammatory responses by these cells. Numerous studies have provided evidence that activation of the CB2R on endothelial cells decreases the expression of adhesion molecules. These actions decrease the inflammatory cell invasion of the brain and reduce their contribution to secondary injury following stroke [31].

Moreover, the pharmacological properties of AM 630 are more complex. It has been revealed that AM 630 behaves as an inverse agonist at CB2 receptors as well as an inverse agonist at CB1 receptors [32]. Thus, we may propose that both agonist and antagonist of CB2 receptors used in our study may improve neuroprotective effect through CB1 as well as CB2 receptors. A previous study revealed that the higher doses of AM 630 (2.0 and 3.0 mg/kg) induced a statistically significant increase in brain tissue's antioxidant properties [33].

Oxidative stress plays a vital role in the pathophysiology of cerebral ischemia, and the overproduction of reactive oxygen species (ROS) occurs during brain ischemia and reperfusion [34]. Additionally, the resultant oxidative stress and overproduction of ROS during the I/R event have a significant role in the degree of brain damage [35]. As potential oxidative stress biomarkers, various enzymatic antioxidant molecules such as SOD, GPx, and CAT, nonenzymatic antioxidants such as GSH, and LPO products such as MDA are commonly employed. GSH is transformed to glutathione disulfide and depleted during oxidative stress, resulting in LPO. As a result, GSH's role as a valuable marker for assessing oxidative stress is critical [36]. Results of the current study illustrated an increase in the level of MDA accompanied by depleted GSH level in I/R rat brain and pretreatment with NADA and AM630 attenuate the elevated level of MDA and a depleted level of GSH, which is concomitant with the previous

observations where antioxidants were used as a remedy in experimental stroke models [37]. Cannabinoids possess antioxidant and antiapoptotic properties.

Fernández-López et al. 2012 studied the effect of TAK-937. This cannabinoid receptor agonist protected the brain in t MCAO rats when given at the time of hypothermia by reducing the volume of infarction [38]. Abdel-salam et al. 2019 showed that anandamide CB1R ligand possesses a neuroprotective effect by decreasing MDA and increasing GSH contents in the brain of PTZ-treated rats [39]. Inducible nitric oxide (INOS) has been suggested during ischemia and reperfusion as a critical modulator of inflammatory reactions. Induction of iNOS results in delayed neuronal cell death and exacerbates glutamate toxicity [40].

Furthermore, NO produced by iNOS and its oxidative byproduct peroxynitrite are hypothesized to play a role in neuronal death during the late stages of cerebral ischemia [41]. In this study, global ischemia increased NO levels. However, cannabinoids treatment decreased NO level as compared to the I/R model. Exogenous cannabinoid agonists are neuroprotective in different paradigms of brain injury; they inhibit intracellular calcium influx, reduce glutamate and decrease stimulated iNOS expression, and exert antioxidant actions [42]. Our results support a role for these cannabinoids in preventing ischemia-induced damage in the rat brain.

Inflammation in the brain and oxidative stress are linked and cannot be regarded as distinct phenomena. Crosstalk between inflammatory systems is enhanced by oxidative stress [43]. The pathophysiology of ischemic stroke includes some key events such as activating glial cells to release nitric oxide, activation of microglia, increased levels of inflammatory cytokines in the brain, and adhesion and migration of peripheral leukocytes as a result of damage to the blood-brain barrier [44] . Inflammation plays a significant role in ischemic and reperfusion injury. Our study has shown that bilateral common carotid arteries (CCA) occlusion was accompanied by an increase in the level of transcripts of redox-sensitive factor NF-κB, controlling the development of the inflammatory reaction. Shortly after ischemia, phosphorylation and activation of IκB beta kinase (IKKß), the most critical regulator of NF-κB, lead to phosphorylation and enhance the expression of proinflammatory cytokines such as interleukin-1 beta (IL-1β) and tumor necrosis factor-alpha (TNF-α) [45]. At an early stage of cerebral ischemia/reperfusion, This cascade happens in glial cells and neurons, creating an overwhelming inflammatory response in the brain and resulting in severe damage, such as the disruption of the blood-brain barrier and brain edema, which is the leading cause of early death in stroke patients [46]. CB1 receptor activation triggers several protective signals involving phosphorylation of mitogen-activated protein kinase (MAPK) and nuclear factor-kappa B (NF-κB) [47]. These intracellular pathways might increase neuronal survival. It was observed that pretreatment with NADA and AM630 reduced brain damages by decreasing NF-κB brain content. Our data support a differential protective reaction of (CB) agonist WIN 55,212-2 (WIN) which reduced the widespread and notorious increase in inflammatory markers tumor necrosis factor (TNF)-α, interleukin (IL)-1β and IL-6 induced by Hypoxia–Ischemia [43].

On the other hand, the most beneficial effects of cannabinoids are associated with the activation CB2 receptor, which is classically described to inhibit proinflammatory immune functions. Suggest a potential direct regulation of CB2 in cerebral post-stroke inflammation and inflammatory brain cells [39]. The lack of efficacy for GW405833 in our experiment could be explained; it may have been that the dose used in our experiments may have been insufficient to suppress the inflammatory response or other pathological processes relevant to CB2 activation [48]. Possibly, these considerations could explain why our results contrast with those that show the efficacy of CB2 agonists in the treatment of models of stroke. In addition, there are essential differences between rodent and human immune systems, responses to cerebral ischemia, and in the CB2 receptor itself [49]. In line with our study, Rivers- Auty et al. 2014 [50] showed that administration of GW405833 doesn't ameliorate the ischemic injury.

Acetylcholine (ACh), the principal neurotransmitter of the cholinergic neurons, is also known to have a pivotal role in neuroinflammation after stroke [51]. A great link between inflammatory pathways and cholinergic signaling. Specifically, the so-called cholinergic anti-inflammatory pathway inhibits cytokine synthesis and release through activation of the ACh receptor. In cortical cells and hippocampal slice cultures, such activation prevents cell damage and cell death during experimental ischemia. Correspondingly, acetylcholinesterase (AChE) inhibition attenuates cerebral infarction volume in rats during the experimental stroke [52]. In this study, the administration of cannabinoids significantly decreased ACHE brain content. In line with our results, (tetrahydrocannabinol)THC has the capacity to inhibit AChE (acetylcholinesterase, the enzyme that catalyzes the breakdown of acetylcholine), thus increasing ACh levels [51].

The underlying mechanisms involved in I/R insult are various and include oxidative stress and inflammation. CB1R and CB2R agonists and antagonists demonstrated a potential for ischemic control through

attenuation of oxidative stress and inflammation, therefore adding to the significance of cannabinoids as neuroprotective agents. Further studies are needed to investigate the mechanisms underlying the anti-ischemic effects of cannabinoids and their potential application in the clinical setting.

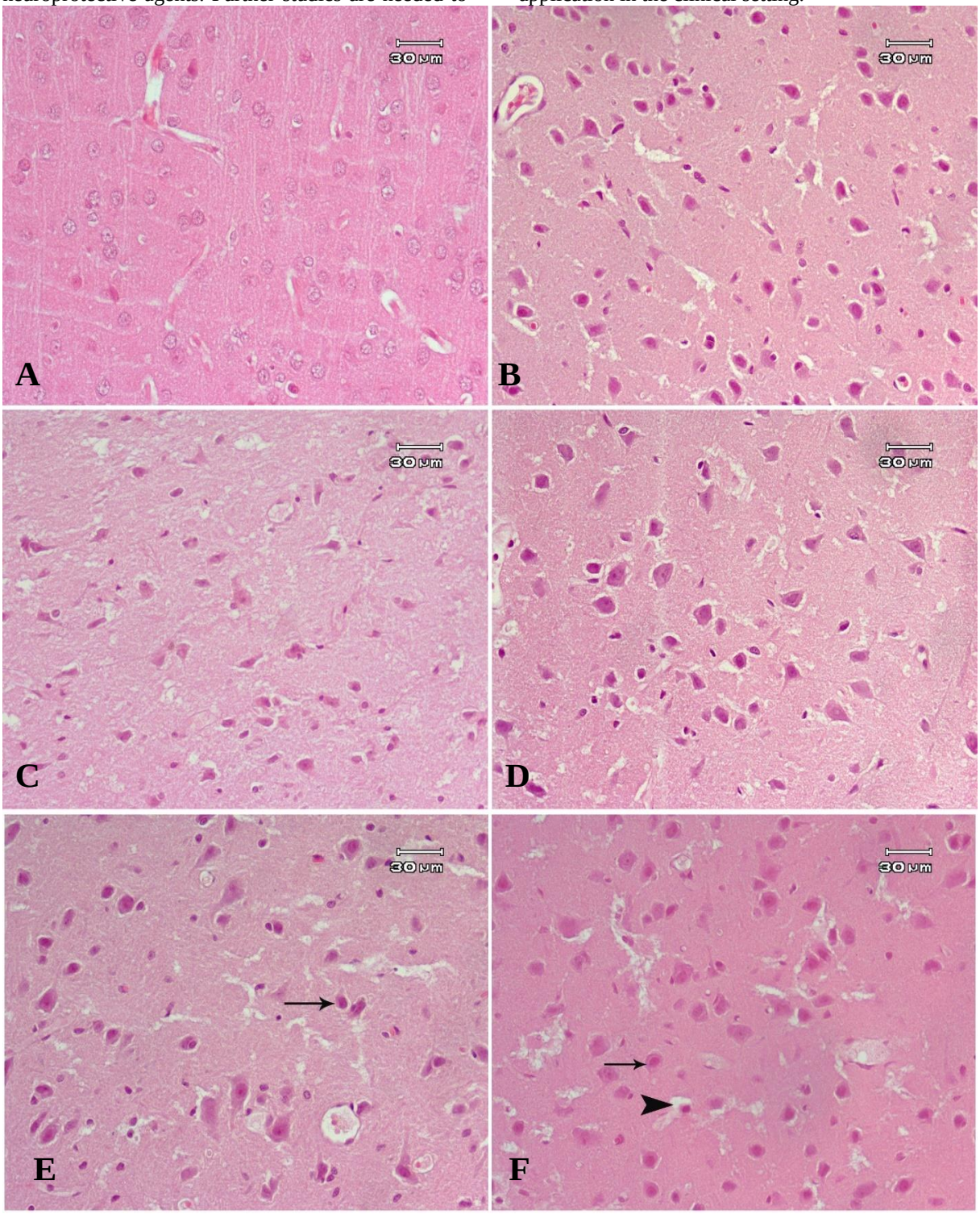

Figure 4: a photomicrograph of cerebral cortex tissue of (A) Control -ve rat shows the normal neurons with large vesicular nuclei. (B) Ischemic brain rat shows many neurons with deeply stained nuclei. (C) Ischemic brain rat treated with NADA shows some neurons with deeply stained nuclei are still observed. (D) Ischemic brain rat treated with GW 405833shows many neurons with deeply stained nuclei. (E) Ischemic brain rat treated with AM 630 shows many cells with regeneration signs, although some cells with apoptotic nuclei (arrow) are observed. (F)

Ischemic brain rat treated with rimonabant shows neurons with nuclei slightly darker than normal (arrow) and some shrunken cells (arrowhead) (H&E X 200).

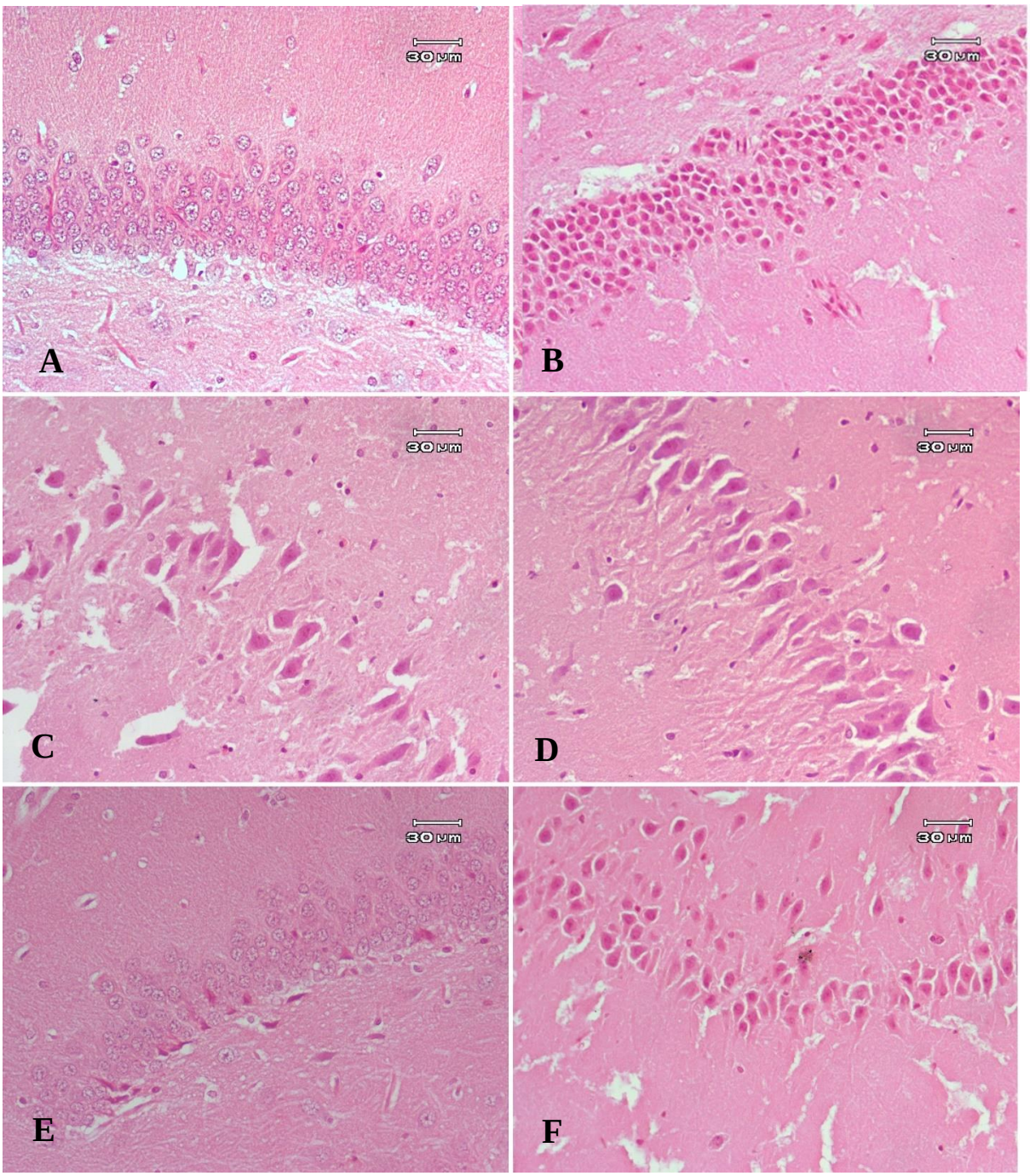

Figure 5*:* a photomicrograph of hippocampus area of (A) Control -ve rat shows the normal structure of this area. (B)Ischemic brain rat shows most of the neurons with deeply stained nuclei. (C)Ischemic brain rat treated with NAD shows deformation of the arrangement of the neurons, most of them are deeply stained. (D)Ischemic brain rat treated with GW 405833 shows many deeply stained cells but with no deformation in arrangement.(E) Ischemic brain rat treated with AM 630 shows most of cells appear normal. Only a few deeply stained cells are observed. (F) Ischemic brain rat treated with rimonabant shows many deeply stained neurons with mild deformation in arrangement (H&E X 200).

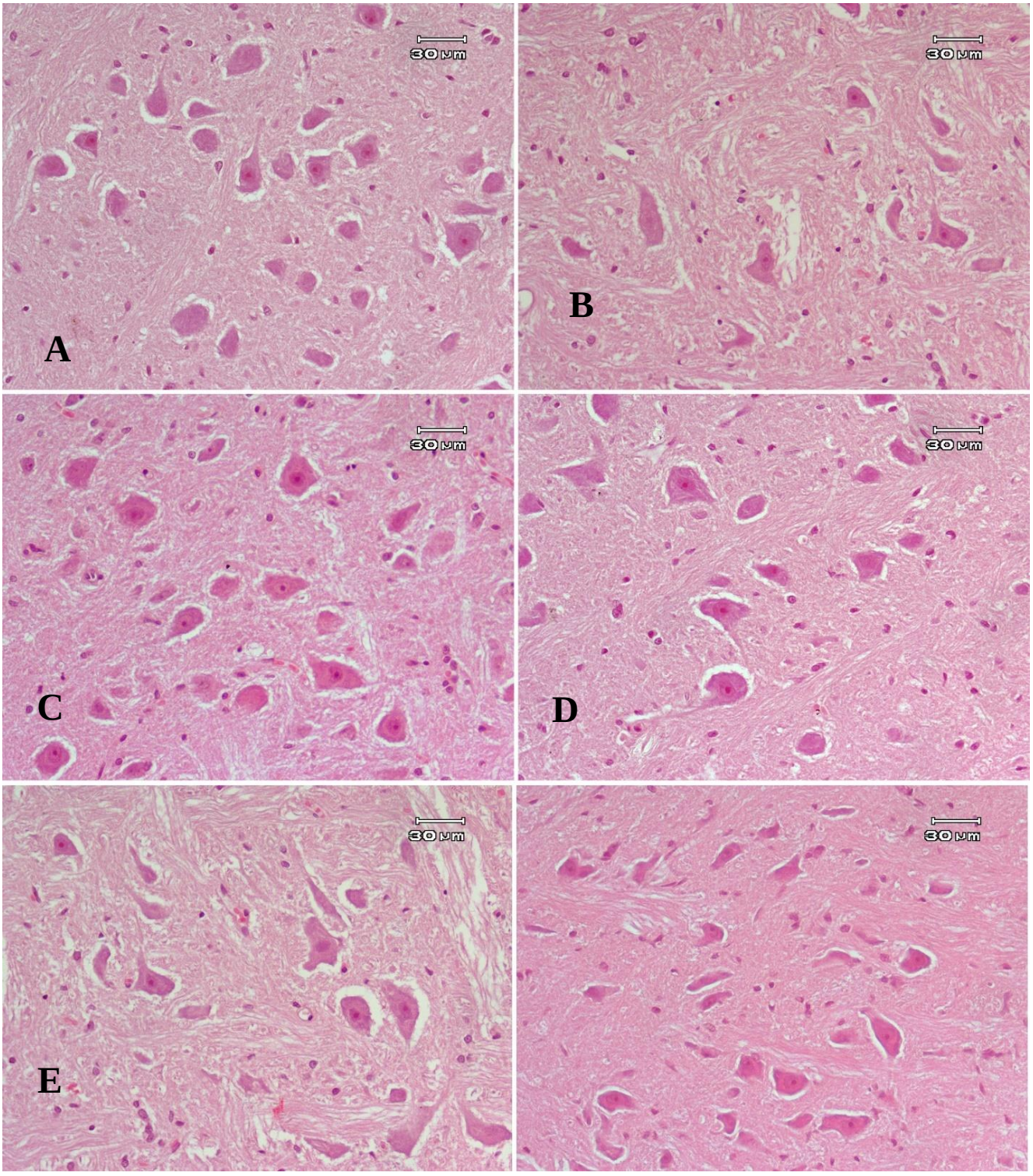

Figure 6*:* a photomicrograph of substantia nigra of (A) Control -ve rat shows the normal size and shape of pigmented neurons. (B) Ischemic brain rat shows marked decrease in size and number ofpigmented. (C) Ischemic brain rat treated with NADA shows normalization of pigmented neurons concerning size and number. (D) Ischemic brain rat treated with GW 405833 shows marked increase in size and number of pigmented neurons.(E) Ischemic brain rat treated with AM 630 shows some pigmented neurons regain their normal size, although their number is

still below normal. (F) Ischemic brain rat treated with rimonabant shows decrease in size and number of pigmented neurons is still noticed (H&E X 200).

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