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Neuroprotective Effect of Apigenin in induced Cerebral Ischemia-Reperfusion Injury in Male Mice

[1] Widad Abd AL-Jabbar, [2,3] Yasmeen Ali Hussein, [4] Saad Mashkoor Waleed,Maytham [5] Ahmed Abdul Aemah, [6] Najah R. Hadi

[1,5] Department of Pharmacology, College of Pharmacy, Al Mustaqbal University ,Babylon, Iraq
[2] ARCPMS, University of Alkafeel, Najaf, Iraq
[3] Department of Pharmacology, College of Pharmacy, University of Al Kafeel, Najaf, Iraq.
[4] Department of Physiology, College of Pharmacy, University of Al Kafeel, Najaf, Iraq
[6] Department of Pharmacology and Therapeutics, College of Medicine, University of Kufa, Iraq
[2] Yasmeen.alamri@alkafeel.edu.iq

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Abstract

Background: The main causes of death is ischemic strokes in addition to cerebral ischemia. We have two types of ischemia that might happen: localized and global, that they all influence the central nervous system and can be extremely leading to patient's disability. In this research, flavonoids are utilized which are consider as a naturally occurring bioactive substance that is present in large amounts in fruits, vegetables, and traditional herbs. Method: An A total of 32 experimental model Swiss Albino male mice at 10 weeks, weighing 28–36 g used in our study were divided into four groups at random method .Sham group, I\R group, I\R+(dimethyl formamide (DMF), act as a vehicle) , I\R with intraperitoneal Apigenin 40mg/kg 1hour before induction of Bilateral Common Carotid Artery Occlusion (BCCAO). The level of All markers utilized in our study (IL-1β, Bax, Bcl, and F2-isoprostanewere) measured in brain tissue for every research groups. Results; (40mg/kg) Apigenin was significantly diminish the cerebral tissue level of IL-1β, Bax, and F2-isoprostane and significantly increased Bcl levels when compared to I\R and I\R with vehicle groups. Conclusions; Treatment with flavonoids such as Apigenin has a neuroprotective effect due to its anti-inflammatory, anti-oxidant, and anti-apoptotic properties against injury caused by ischemia.

Keywords; *Interleukin 1β(IL-1β), Ischemia reperfusion injury(I\R), Bilateral common carotid artery occlusion, Apigenin, Bax, Bcl-2,F2-isoprostane.*

1. Introduction

One of the main causes of disability and death is the ischemic stroke, now a day, the clinical treatment techniques for individuals with acute ischemic stroke are intravenous thrombolysis and intra-arterial thrombectomy[1]. Brain ischemia is typically a neurological condition caused by either a partial or total lack of blood supply in the brain [2]. cerebral ischemia/reperfusion injury (CI/RI) is becoming a more serious problem for stroke patients. [3]. The primary mechanisms of CI/RI were oxidative stress complement activation, leukocyte infiltration, platelet activation and aggregation, mitochondrial malfunction, calcium overload(inflammatory response), and the activation of apoptotic and autophagic pathways. [4].If drainage of blood to cerebral tissue is disrupted, due to blood clots generated by atrial fibrillation or as a result of thrombus formed on fatty deposits known as atherosclerotic plaque, an ischemic stroke develops very quickly within minutes [5]. Ischemic Core (ischemic penumbra) an area of cells surrounding the ischemia core and frequently the focus of therapy [6]. ischemia stroke is characterized by many of alterations inside the affected ischemia core and the surrounding penumbra as other neurodegenerative disorders. [7]. Excitotoxicity, oxidative stress, autophagy, apoptosis, and

neuroinflammation, these are the general five macro and microscopic changes [8]. Oxidative stress and decreased energy metabolism result from disruptions in blood flow to the brain, reperfusion after blood flow obstruction has been identified as a contributing factor to both oxidative stress-induced injury and autophagy [9]. The oxidant–antioxidant mechanism is upset by oxidative stress, a main cause of ischemic stroke, especially in brain cells with high level of polyunsaturated fatty acids. [10]. The main component of IS is neuroinflammation, which include several immune cells including innate and adaptive immune cells [11]. Necrosis and apoptosis developed by insult of brain that follows an ischemic stroke trigger an inflammatory response which is regulated by the release of chemokines, cytokines, and ROS[13]. The blood–brain barrier (BBB) is disrupted due to sever neuroinflammation in acute phase of ischemic stroke in addition to damage to neurons, and a poor prognosis[12]. Apoptosis involve a sequence of internal and/or external processes that make neurons to condense in cytoplasm then shrinkage [14]. Now a day the treatment for ischemic stroke include: thrombolytics agents which is initially found to treat cardiac thrombolysis, the intravenous thrombolytic (IVT) treatment finally, discovered there importance in Ischemic Stroke [15]. Different treatment options available to conserve the ischemic penumbra, like cytoprotectants, cellular therapy and antithrombotic drugs, these strategies make brain remodeling and repair then increase the rate and volume of reperfusion, and halt the progression of ischemic penumbra. [16]. Aspirin, ticagrelor, and clopidogrel are the antiplatelet medicines that are given to stroke patients in the early stages [17]. Cellular therapies have a role on patients' recovery, they should be taken into account when managing ischemic stroke[18]. Flavonoids which is a phytochemical molecules found in many plants like fruits, vegetables, and leaves have a medical roles as anticancer, antioxidant, anti-inflammatory, antiviral, and antiapoptotic agents additionally, they have cardio- and neuroprotective properties. [19]. Apigenin (4′, 5, 7,-trihydroxyflavone) is a glycoside that belongs to a class of flavonoids, naturally occurring in various forms in plants and vegetables such grapefruits, parsley, onions, oranges, tea, chamomile, wheat, sprouts, oregano, artichokes, and some seasonings[20]. Since it is less toxic, non-mutagenic, and an anticancer drug, so it considers as antioxidant, neuroprotective, and anti-inflammatory agents, apigenin has been formulated in a variety of ways with other polymers to improve its biological function. [21]. Aim of current study is to assess the protective effect of Apigenin in brain ischemia reperfusion injury.

2. Materials and Methods

2.1. Animals

The study protocol was approved by the Ethics committee of the Alkafeel University Health Science Center. The experiments were carried out following international laboratory animal use and care guidelines (approval date: 15/11/2022, decision number: 2022-13).Thirty two male Swiss Albino mice weighing between 28 and 36 grams, ten weeks of age were employed in our investigation. The experimental groups that the mice were randomly assigned to are: sham, IR, [dimethyl formamide (DMF) vehicle], and Apigenin treatment group. Unrestricted access of food and water were given to animals, 25°C ambient temperature, and a 65% relative humidity, the animal kept in a controlled environment with a 12-hour light/dark cycle, observations were made once a day before surgery.

2.2. Surgery

An intraperitoneal(IP) injection of diazepam and ketaminr used to induce anesthesia to animals. To kept the temperature at 37 ± 0.5°C, a heating pad was employed . Toe pinching was used to examine the degree of anesthesia prior to beginning surgery, if there be no reaction, the surgery would begin.

2.3. Animal grouping and drug administration

Randomly distributed the mice in to four groups: (Sham group): in this group the animals had surgery and anesthesia but did not receive bilateral common carotid artery occlusion (BCCAO). (Control group): the animals were anesthetized then the BCCAO was done for thirty min. followed by one hour of reperfusion period. (Vehicle group): The animals in this group had an DMF intraperitoneal injection one hour before the BCCAO which is done for thirty minutes followed by one hour of reperfusion. (Treated group): 40 mg/kg Apigenin was received by the

animals in this group intraperitoneally one hour before to BCCAO for 30 minutes, followed by an hour of reperfusion period. Drug preparation: from Med Chem Express Co. USA apigenin (4′,5,7-trihydroxyflavone) was purchased . Before using the medication, the dose was made by dissolving each 40 mg of the medication in 1 ml of DMF. [22] 40mg\kg of Apigenin administered to the animal intraperitoneally (i.p) [23].The limbs of animal fixed by plasters and the animal put in a supine posture position [24]. In the neck a slight median incision was produced, then isolation for two carotid arteries was done from the vagal nerves, vascular clamps used to make the obstruction for about thirty minute, the period of reperfusion start when the clamps removed which is continue for 1 hour [25]. Then scarification done to all animals and tissue of brain was isolated for ELISA and histopathological technique.

By using ELISA technique we measured IL-1, F2-isoprostane(8-isoprostaglandin), Bax and Bcl-2, then homogenization by using ultrasonic liquid processer was made for all particles of brain tissue, the solution of homogenization mixed with triton-x100 and 0.25% cocktail protease inhibitor these samples were centrifugation at 2000 RPM at 4°c for 30min. IL-1β, F2-isoprostane Bax and Bcl-2 concentration was measured by using the supernatant according to the information protocols of these kits. The histopathoclogical tests was done according to the instructions protocol.

2.4. Statistical analysis

The data were analyzed by a statistical package of Social Sciences (SPSS) version 24. Data were expressed as mean ± standard error of mean (SEM). Analyzed statistically using student t-test and in order to evaluate multiple comparisons between groups we using ANOVA post Hoc tukey test. Statistical significance is considered if the value of $p \geq 0.05$

3. Results

Brain ischemia reperfusion responsible for a significant increase in mediator of inflammation like IL-1 and oxidative stress marker like F2-isoprostane level that induced damage of the cell. The concentration of IL-1 inflammatory mediator significant increased($P \geq 0.05$) for IR Group when compared to Sham Group, and there is insignificant difference in the concentration of IL-1 between IR and vehicle groups. When we treated the animal with (40mg/kg) of Apigenin leading to a significant decrease ($p \geq 0.05$) of IL-1 concentration in brain tissue compared to the Vehicle Group. Figure (1) illustrate the variation in IL-1 concentrations among the research groups.

Concentration of oxidative stress marker [F2- isoprostane (8-isoprostaglandin f2α)] in the brain significant increased ($p \geq 0.05$) for IR Group compared to Sham Group. According to F2-isoP concentration, there was no significant difference between IR & Vehicle Groups. Significant decrease in F2-isoP concentration for treated group with Apigenin when compared with the vehicle group. Figure (2) illustrate the variation in F2-isoP concentrations among the research groups.

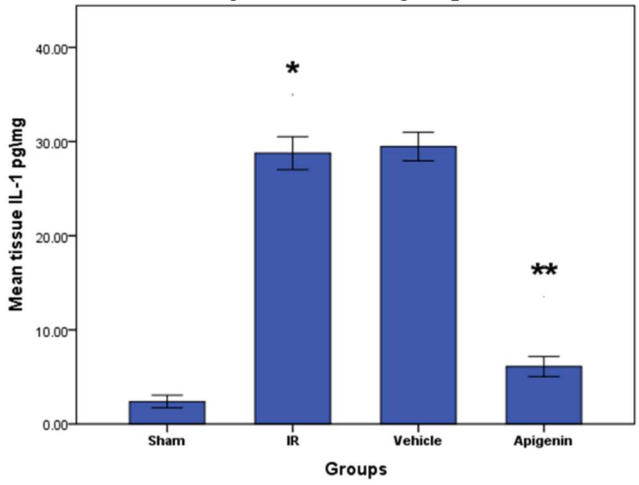


Figure 1: The mean ±SEM of IL-1 concentration in the brain for each of the four research groups

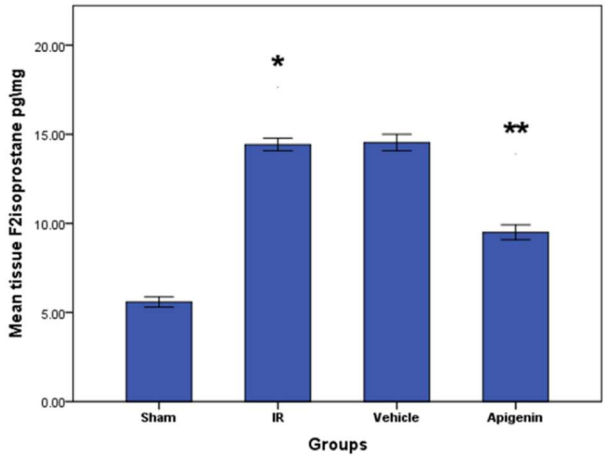


Figure 2: The mean ±SEM of F2isoProstane concentration in the brain for each of the four research groups

The concentration of the marker of apoptosis Bax (Bcl-2 associated X protein) in brain tissue was significantly elevated ($p \geq 0.05$) in the IR Group compared to Sham Group. No Significant Difference for Bax concentration among IR and vehicle groups. Significant decrease in Bax concentration for treated group with Apigenin when compared with the vehicle group, that's mean Apigenin ameliorated apoptosis, Figure (3) illustrate the variation in Bax concentrations among the research groups.

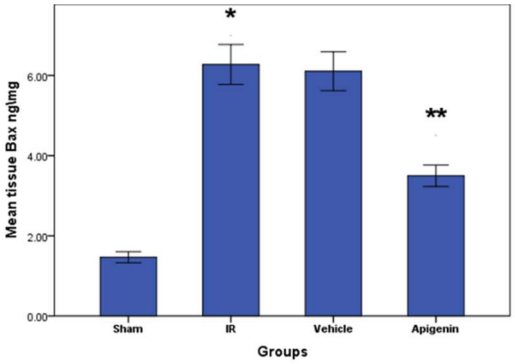


Figure 3: The mean ±SEM of Bax concentration in the brain for each of the four research groups

There was a significantly decreased in Bcl-2 (anti-apoptotic protein) concentration in IR group when compared with sham group ($p < 0.05$), and insignificant difference between Vehicle and IR, group treated with Apigenin has a significantly increased in Bcl-2 concentration when compared with Vehicle group ($p < 0.05$). Figure (4) illustrate the variation in Bcl-2 concentrations among the research groups

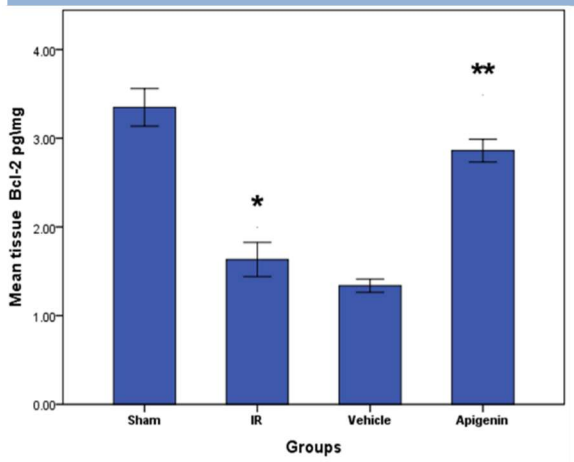
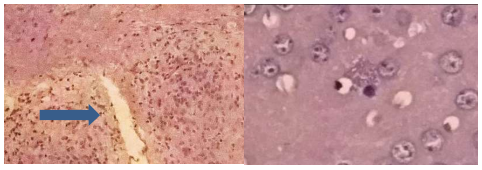


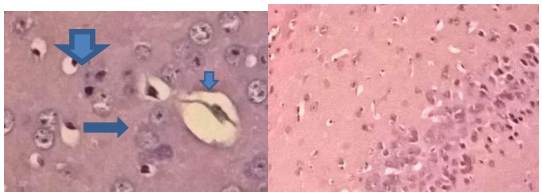
Figure 4: The mean ±SEM of Bcl-2 concentration in the brain for each of the four research groups

Histological findings

A normal tissue appearance was showed in a cross section of sham mouse brain, between IR group and sham group there was statistically significant difference ($P < 0.05$), severe cerebral injury (70 %) was the score of IR group and it (30%) showed moderate injury , between IR group and Vehicle Group there was no statistically significant difference, and the score of the Vehicle group showed (30%) moderate injury and (70%) severe cerebral injury, improved cerebral injury score significantly ($P < 0.05$) when treated the mice with Apigeninas compared with Vehicle group and (40%) was the total score of this group had normal histological appearance, and (50%) of the group had slight cerebral injury and (10 %) had moderate injury, these alterations displayed in Figure 5



(A) **(B)**



(C) **(D)**

Figure(5) Photograph for mice brain stained with H&E. A: represent Sham group which show normal brain tissue. B: IR group show edema (blue arrows) . C:Vehicle group also show edema

(blue arrows) . D: represent Apigenin treated groupshows moderate to little edema

4. Discussion

Oxidative stress, apoptosis and inflammation are major factors of I\R injury[26]. The most essential regions of our research is discovering compounds that reduce oxidative stress, inflammation, apoptosis and boost the antioxidant defense system mechanism [27]. Apigenin in our study showed a neuroprotective effects in cerebral I/R conditions since it has antioxidant , anti-apoptosis and anti-inflammatory properties . In our research, high oxidative stress in the ischemic region was resulted after the induction of cerebral I\R which is consider one of the damaging pathways

that plays an important role in reperfusion injuries after ischemia due to production of high level of oxygen-free radicals (Fesharaki-Zadeh, A. (2022))[28]. The study results indicate that, The brain's antioxidant defense system was reduced and there was an increase in F2 isop. after cerebral ischemia in mice due to damaging of neurons membrane (Kashyap P et al., 2022)[29]. F2 isop. was significantly reduced and recover the brain motor activities after the administration of apigenin, (Kashyap, D., et al 2018)[30] supports the previous results. When nitrogen-free radicals combined with oxygen free radicals creates a very dangerous compound in the affected area of ischemic region (peroxynitrite) leading to necrosis and stimulation of apoptosis (Mai, H., et al 2023)[31]. IL-1β pro-inflammatory cytokine level are higher during ischemic stroke, which also help with stroke associated with brain damage [32]. These mechanisms responsible for death of neuron cells because of stimulation to cascade of inflammation which leads to accumulation of fluid in cerebral area due to loss of blood-brain barrier integrity [33]. About the present study, BCCO exhibited an elevation in IL-1β level in affected area, our results agreed with other studies, which stated that increased IL-1β levels could play a significant role in reperfusion damage after brain ischemia. [34]. Because of its anti-inflammatory properties, Apigenin significant reduced the expression of proinflammatory cytokines.

Proteins belonging to the Bcl-2 family have the ability to permanently damage cells and regulate important apoptotic signal transduction pathways, the regulation of apoptotic cell death is done by Bcl-2/Bax ratio [35]. According to our study, the Bax level increased & decreased in Bcl-2 level in ischemic tissue with BCCO, Apigenin restore the level of apoptotic agents to normal ratio so prevent the process of apoptosis, Previous research has suggested that apigenin possesses anti-apoptotic action (Lotfi MS, et al 2024 [36]). The histopathological findings in our study exhibit swelling of the endoplasmic reticulum with sever edema which seems to be as a clefts at neurons periphery after BCCO, this was in agreement with (Dettmeyer RB., 2011)[37].

5. Conclusion

The neuroprotective effect on cerebral tissue in contact with BCCO-reperfusion and ischemic damage in mice is suggested by Apigenin in the present study which is mainly due to the mitigation of inflammation and apoptosis responses, inhibition of neurological deficiencies and recovery of antioxidants. Considering the findings of this study, Apigenin might be a good option for the clinical management of a number of oxidative stress-related brain ischemia disorders, inflammation and apoptotic effect.

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