

Monosodium glutamate Induces cortical oxidative, apoptotic, and inflammatory challenges in rats: The potential neuroprotective role of apigenin

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Research Article

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Abstract

Monosodium glutamate (MSG) is used as a flavor, and a taste enhancer was reported to evoke marked neuronal impairments. This study investigated the neuroprotective ability of flavonoid apigenin against neural damage in MSG-administered rats. Adult male rats were allocated into four groups; control, apigenin (20 mg/kg, orally), MSG (4 g/kg, orally), and apigenin + MSG at the aforementioned doses for 30 days. Regarding the levels of neurotransmitters, our results revealed that apigenin augmented the activity of acetylcholinesterase (AChE) markedly, and levels of brain monoamines (dopamine, norepinephrine, and serotonin) accompanied by lessening the activity of monoamine oxidase (MAO) as compared to MSG treatment. Moreover, apigenin counteracted the MSG-mediated oxidative stress by decreasing the malondialdehyde (MDA) levels together with elevating the glutathione (GSH) levels. In addition, pre-treatment with apigenin induced notable increases in the activities of cortical superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR). Further, apigenin attenuated the cortical inflammatory stress as indicated by lower levels of pro-inflammatory mediators as interleukin-1 β (IL-1 β), tumor necrosis factor- α (TNF- α), and nitric oxide (NO) as well as downregulated inducible nitric oxide synthase (iNOS) expression levels. Histopathological screening validated the abovementioned results and revealed that apigenin restored the distorted cytoarchitecture of the brain cortex. Thus, the present findings collectively suggest that apigenin exerted significant protection against MSG-induced neurotoxicity by enhancing the cellular antioxidant response and attenuating inflammatory machineries in the rat brain cortex.

Introduction

The distinguished flavor and taste enhancing abilities of monosodium glutamate (MSG) have provided it a global concern as one of the most widely used food additives (Gaffen &Liu 2004). It is encoded as E621 in a variety of foods products such as soups, sauces, mixed condiments, and puddings (Ren &Torres 2009). The ingested glutamate is metabolized, and most of its carbon atoms are converted into CO₂ or used for amino acids synthesis (Bickel 1993). However, when excess glutamate is ingested, the glutamic acid accumulates and rises in the blood (Ren &Torres 2009). A vast of studies have shed light on the resulted deleterious effects of a high daily intake MSG on many organs such as the brain, liver, and kidney (Albrahim &Binobead 2018, Gaffen &Liu 2004, Ren &Torres 2009).

Glutamic acid can be considered as a “double-edged weapon” because it serves as a neurotransmitter and neurotoxin (El Okle et al. 2018). It acts as a vital excitatory neurotransmitter in the central nervous system (CNS), supplies the energy for other tissues, and contributes to glutathione synthesis (Ren &Torres 2009). MSG exerts its excitotoxic effect through over-excitation of the nerve cells that may result in damage or even apoptotic death in many brain areas, including the hippocampus (Mekkawy et al. 2020). Further, the binding of glutamate with its receptors encourages the calcium influx with subsequent induction of neural apoptosis (Khafaga et al. 2021). Besides, excess activation of glutamate receptors induces neuronal necrosis due to persistent neuronal depolarization (El Okle et al. 2018). Former studies unveiled that glutamate remarkably decreased the levels of norepinephrine (NE) and dopamine (DA) and

suppressed the activity of acetylcholine esterase in different brain areas (Liu et al., Odenwald & Turner 2017, Pravda 2005). Actually, a high level of circulating glutamate has been linked with the formation of toxic reactive oxygen (ROS) and nitrogen species (RNS) (Bickel 1993, Gaffen & Liu 2004). Most tissue injuries caused by MSG in experimental models resulted from oxidative stress and activation of inflammatory pathways (Reifen et al. 2004, Seiva et al. 2012).

Using naturally occurring molecules as neuroprotective agents represents a promising therapeutic strategy that minimizes the toxic insults to the brain. Among these, apigenin is a plant-derived flavonoid that is found at significant levels in various vegetables and fruits, such as parsley, celery, and oranges (Al Olayan et al. 2020). Outstandingly, apigenin has the ability to cross the blood-brain barrier without having any neurotoxic effect (Alvi et al. 2021). Due to the notable neuroprotective activities of apigenin reported previously in both in vitro and in vivo models [15–18], it attracts much research attention, particularly those related to nervous system disorders. Apigenin alleviated markedly cognitive dysfunction and neuronal damage induced by scopolamine in mice (Al Olayan et al. 2020). Further, apigenin exerted noteworthy anti-inflammatory activities against chronic neuroinflammation in a mouse model (Taiwe et al. 2021). It has also been reported that apigenin reduced the infarct volume and inflammatory response associated with hypoxic-ischemic brain injury in neonatal rats (Taskiran & Ergul 2021). Dourado et al. (Alvi et al. 2021) observed that neurons and glial cell cultures treated with apigenin counteracted markedly the microglia activation and neuroinflammation induced by lipopolysaccharide.

These promising neuroprotective effects may endorse apigenin's antioxidant activities and anti-inflammatory activities. The marked decline was detected in the oxido-inflammatory status of the brain tissue exposed to acetonitrile and co-treated with apigenin (Singh et al. 2020). Apigenin increased the antioxidant activities of SOD, CAT, and GST and decreased the lipid peroxidation levels in the parkinsonian rat model (Lim et al. 2018). In addition, apigenin subsided the neuroinflammation in three different models induced by lipopolysaccharide, IL-1b or Ab oligomers (Alvi et al. 2021). Apigenin also effectively attenuated lipopolysaccharide-mediated production of pro-inflammatory cytokines (IL-1 β and TNF- α) in the prefrontal cortex of treated mice (Gao et al. 2020). The bioactivity of apigenin is linked to the hydroxyl in its chemical structure that can combine with unstable, highly reactive free radicals (Singh et al. 2020).

Despite the therapeutic effect of apigenin on neuronal function in various models that have been studied, the protective role of apigenin against MSD-induced neurotoxicity, to our knowledge, has not yet been investigated. Hence, the existing study was aimed to explore the potential therapeutic activity of apigenin on brain dysfunctions associated with MSG exposure in rats. The alterations in oxidant/antioxidant status, neuroinflammation, and neurotransmitters in cortical tissues of treated rats were elucidated.

Materials And Methods

Animals and ethical considerations

This study was carried out on adult male Wister albino rats weighed 150-200 g. They were purchased from VACSERA (Helwan, Cairo, Egypt). They were reared under controlled conditions of temperature (22-24 °C), humidity (50-60 %) and 12 h light/dark cycle. Throughout the experimental duration, standard laboratory animal chow and water were offered to rats *ad libitum*.

All experimental procedures were performed according to the principles of the Ethics Committee of Taif University (Approval No. TU 33-062) (Taif, Saudi Arabia).

Experimental protocol

Rats (N= 40) were assigned into 4 equal groups (10 rats/ group): control, apigenin, MSG, and MSG + apigenin groups. The first group (control): rats were orally received normal saline (0.9% NaCl). The second group (apigenin); rats were administered orally with 20 mg/ kg of apigenin as reported by Asma et al. (Bao et al. 2018). The third group (MSG) received 4 mg/Kg of MSG following the method of Eid et al. (Eid et al. 2019). Finally, the fourth group (apigenin+MSG) was received 20 mg/kg of apigenin and 4 mg/Kg of MSG.

Both of MSG and apigenin were orally administered per day for 30 days and apigenin was given to rats 2 hours before MSG. After 24 hours from the last treatment, animals were euthanized with ketamine (90–100 mg/kg) and xylazine (10 mg/kg) intraperitoneally. The brain of each animal was immediately dissected and divided into three portions. The first part was mixed with ice-cold 50 mM Tris-HCl buffer (pH 7.4) followed by centrifuging at $3000 \times g$ for 10 min at 4 °C for preparation of tissue homogenates (10% w/v). The resultant supernatant was stored at -20°C for biochemical analysis. The second portion was kept at -80 °C for analysis of gene expression and the third portion was preserved in 10% of neutral-buffered formalin for histopathological examination.

Neurochemical analysis

The activity of acetylcholinesterase (AChE) in brain samples was assessed according to the procedure described by Elman et al. (Ellman et al. 1961). AChE activity was determined based on the yellow color developed following the addition of thionitrobenzoic acid, measured at 412 nm. HPLC reports and chromatograms were obtained using the data acquisition program (ChemStation). Hippocampal samples were subjected to a solid-phase extraction using a CHROMABOND column (Cat. No. 730031) to remove trace elements and lipids, and the NH_2 phase was retrieved. The NH_2 phase was then injected into an AQUA column (150 mm; 5 μm ; C18, Phenomenex, USA). After 12 minutes, dopamine (DA), norepinephrine (NE), and serotonin (5-HT) were separated. Each monoamine position and concentration in the sample was identified by comparison of the resulting chromatogram with that of the corresponding standard (Sigma Chemical Co., St. Louis, MO, USA). According to Pagel et al. (Pagel et al. 2000), the concentration of each monoamine was quantified relative to total brain tissue ($\mu\text{g/g}$).

MAO activity

The activity of MAO in the brain tissue was determined using ELISA kits purchased from Elabscience (catalogue numbers: E-EL-R2563).

Oxidant/antioxidant status

Cortical levels of glutathione (GSH), the non-enzymatic antioxidant marker, were measured colorimetrically utilizing Elaman's reagent according to the method of by Ellman (Ellman 1959). Lipid peroxidation was assessed in terms of malondialdehyde (MDA) according the method mentioned by Ohkawa et al. (Ohkawa et al. 1979).

Evaluation of the enzymatic activities of superoxide dismutase (SOD) was done based on the ability of SOD to inhibit the reduction of nitroblue tetrazolium dye as described by Sun et al. (Sun et al. 1988). Moreover, catalase (CAT) activities was measured based on the breakdown rate of H₂O₂ according to the method of Aebi (Aebi 1984). GSH peroxidase (GPx), and GSH reductase (GR) were measured via determination of the oxidation rate of NADPH at 340 nm and reduction rate of NADPH in the presence of glutathione as stated by Paglia and Valentine (Paglia & Valentine 1967), and Factor et al. (Factor et al. 1998), correspondingly.

Inflammatory marker determination

ELISA kits obtained from Thermo Fisher Scientific, USA were employed for assessment of pro-inflammatory markers, i.e.; tumor necrosis factor- α (TNF- α ; Cat. no. BMS607-3), and interleukin-1 β (IL-1 β ; Cat. no. BMS6002) according to the manufacturer's instructions. Moreover, nitric oxide (NO) level was measured in the brain cortex through dye formation after adding the Griess reagent at 540 nm, as previously described (Green et al. 1982).

RNA extractions, reverse transcriptions and qRT-PCR

Isolation of total RNA from the brain cortical samples was employed by TRIzol reagent (Qiagen, Germantown, MD, USA) based on the manufacturer instructions. After that, the cDNA synthesis was done by a Super ScriptVILO cDNA Synthesis Kit (Life Technologies, Thermo Fisher Scientific, Waltham, MA, USA). Quantification of target mRNA levels were performed by qRT-PCR using SYBR Green MasterMix (Life Technologies, CA, USA) with the Applied Biosystems 7500 Instrument. Primer of the *Nos2* gene (Table 1) were prepared by Jena Bioscience GmbH (Jena, Germany). *B-actin* was used as the housekeeping gene.. The altered gene expression was assessed in comparison with the control based on the standard $2^{-\Delta\Delta C_t}$ method (Pfaffl 2001).

Histopathological examination

Brain specimens were fixed by neutral buffered formalin (10%), dehydrated, embedded in paraffin wax and cut into sections (5 μ m in thickness). Next, tissue sections were deparaffinized, and stained with hematoxylin and eosin. They were examined under a Nikon Eclipse E200-LED microscope (Nikon Corporation, Tokyo, Japan) for histopathological alterations.

Statistical analysis

The differences between different treatment groups was assessed by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test. P value less than 0.05 was considered to be statistically different. Analyzed data were presented as the mean $\pm$ SD.

Results

Apigenin increased the MSG-mediated declines in cortical neurotransmitters levels in rats

The enzymatic activity of AChE was measured to evaluate the impact of MSG on cholinergic functions of the neurons. Our results revealed noteworthy decreases ($P < 0.05$) in AChE in MSG-treated group when compared to control. Further, we observed that MSG toxicity caused notable increases ($P < 0.05$) in MAO activity that catalyzes the oxidative deamination of biogenic amines. As a result, noteworthy reductions ($P < 0.05$) were observed in DA, 5-HT and NE levels in the brain cortex of MSG-treated group in respect to the control rats. Marked rise was observed in the level of 5-HT only in the group received apigenin in relation with the control. In contrast, apigenin pre-treatment counteracted MSG-mediated changes in neurotransmitters as indicated by notable elevations ($P < 0.05$) in the levels of monoamines and AChE besides marked reductions in MAO in comparison with the sole treatment with MSG. These results revealed that apigenin could improve the neurotransmitters disturbance elicited by MSG in rat cortical tissue (Fig.1).

Apigenin enhanced cortical antioxidant enzymes in rats received MSG

Regarding the enzymatic activities upon MSG treatment, we found marked depletions ($P < 0.05$) in SOD, CAT, GPx, and GR enzymatic activities in brain cortex related to the control rats. In contrast, the pre-administration of apigenin (20 mg/kg) boosted significantly ($P < 0.05$) the activities of SOD, CAT, GR, and GPx in rats' brain in respect to those treated with MSG only (Fig. 2).

Apigenin modulated MSG-related cortical oxidative markers in rats

In order to further investigate the antioxidant effect of apigenin administration on MSG-intoxicated rats, the levels of non-enzymatic oxidative stress biomarkers were assessed. Marked elevations ($P < 0.05$) were observed in the levels of MDA in MSG-exposed group compared to untreated control group. Moreover, MSG treatment caused noteworthy depletion ($P < 0.05$) in GSH levels of the brain cortex relative to the control. Remarkably, apigenin pre-administration significantly attenuated the MSG-evoked cortical oxidative injury in comparison with the sole treatment of MSG as indicated by elevations ($P < 0.05$) in GSH levels along with declines ($P < 0.05$) in MDA levels. Compared to the control, noteworthy increases ($P < 0.05$) were detected in the GSH level in apigenin-treated group in respect to the control (Fig. 3).

Apigenin mitigated the inflammatory stress in the brain induced by MSG

Next, we illustrated the anti-inflammatory activity of apigenin on brain cortex by ELISA analysis. As shown in Fig. 4, levels of TNF- α , and IL-1 β were notably increased ($P < 0.05$) in the MSG group related to the sham group. Moreover, pre-treatment with apigenin declined notably the levels of inflammatory cytokines after MSG insult which suggests apigenin's anti-inflammatory potential against MSG-mediated inflammation in rats' brain.

Since the activated iNOS results in production of toxic levels of NO which in turn aggravates the neuroinflammatory process, the levels of NO and the expression levels of iNOS were detected in rat cortical tissue. We found marked upregulation ($P < 0.05$) in iNOS mRNA expression with noteworthy elevations ($P < 0.05$) in the NO levels in MSG-treated rats as compared with the control group. Adversely, pretreatment with apigenin noticeably lessened MSG-induced iNOS expression level and elevated NO levels in brain cortex in respect to MSG administration. The above findings suggest that apigenin could decrease the NO level in rat brain through inhibition of the iNOS expression induced by MSG (Fig. 4).

Apigenin alleviated the histopathological alterations in brain of MSG-treated rats

Histopathological screening of cortical slices of the control (Fig. 5A) and apigenin-treated (Fig. 5B) groups revealed normal neurons and normal glial cells without vacuolation. However, brain tissue from MSG-treated group showed large area of hemorrhage and necrosis in addition to degeneration in some glial cells and marked pericellular edema (Fig. 5C). Remarkably, apigenin pretreatment did marked recovery of the affected areas as showed by moderate neuronal degeneration and slight pericellular edema with marked restoration of the neuron architecture (Fig. 5D).

Discussion

MSG is a commonly used food additive worldwide especially in Chinese and Asian dishes (El Okle et al. 2018). Ingestion of meals with high MSG content was associated with some symptoms as asthma, urticaria, and neuropathy which is known as Chinese restaurant syndrome (Gaffen & Liu 2004). However, glutamate is a vital excitatory neurotransmitter in the CNS, its accumulation in the brain provokes prolonged neurotoxicity and neurodegeneration (Kwon et al. 2010). MSG is dissociated in water into sodium ion and L-glutamate which extremely augments the plasma levels of glutamate than the normal basal levels (El Okle et al. 2018, Gaffen & Liu 2004). Glutamate stimulates the glutamate receptors in CNS; this could aggravate reactive oxygen species (ROS) generation, peroxidation of lipids and neuroinflammation (Mirzakhani et al. 2020).

Catecholamines (NE, DA and 5-HT), a group of neuroactive substances, are of core importance in the building of memory functions and learning skills (Shah et al. 2021). Dopamine is a pivotal catecholamine that is abundant in the CNS and involved in controlling of movement, motivation and learning (Pravda 2005). Moreover, alterations of 5-HT levels had been linked with defect the learning and cognitive activities (Rebai et al. 2017). In line with former studies (Odenwald & Turner 2017, Pravda 2005), significant declines were detected in the levels of the monoamines (NE, DA and 5-HT) in the brain cortex of MSG group. Further, Hamza et al. (Odenwald & Turner 2017) reported that the levels DA, 5-HT,

epinephrine, and NE were markedly diminished with the increase in the dosage of MSG. These findings indicated that the MSG exerts its neurotoxic effect via the depletion of the neurotransmitters in the brain cortex. In addition, we observed notable increase in the level of MAO which is responsible for oxidation of monoamines in the brain and this is in harmony with a former study (Liu et al.). Such increase in MAO activity may indirectly responsible for monoamines depletion in our study. The elevated MAO level has been reported in neurodegenerative disorders as Alzheimer's disease and dementia (Shah et al. 2021).

Corroborating with our study, the inhibitory action of apigenin on MAO activity has been formerly assessed (Guo & Li 2017, Liu et al. 2020). It is known that MAO inhibitors augment the levels of brain monoamines (Gong et al. 2019). Yi et al. (Hwang et al. 2014) found that apigenin administration to rats with chronic mild stress resulted in increases in 5-HT levels in some brain areas as prefrontal cortex, hippocampus, hypothalamus and nucleus accumbens. Further, apigenin exhibited notable monoamine uptake activity in Chinese hamster ovary cells and the uptake for DA was greater than that for NE and 5-HT. In our study, apigenin did a significant modulating effect on the disturbed monoamines that suggested the neuroprotective ability of apigenin might be arbitrated by action on monoaminergic system and MAO in rats exposed to MSG.

Acetylcholinesterase (AChE) is abundant in the neuromuscular junctions and synaptic cleft and is used for estimating the cholinergic functions of the neurons. In agreement with a former study (Odenwald & Turner 2017), our results revealed marked decline in AChE activity in MSG-treated group when compared to control. This suppressed activity of AChE suggested increased ACh concentration which may evoke over stimulation of cholinergic, muscarinic and nicotinic receptors (Chen et al. 2018). Thus, over activation of these receptors results in excess neuronal excitation and paralysis of cholinergic transmission (Fang et al. 2016). Lower AChE activity was linked with learning, memory and visuospatial and motor impairments (Chen et al. 2018). However, these changes were reversed in rats pretreated with apigenin. AChE stimulation lessens the accumulation of acetylcholine at nerve synapses, which subsequently mitigates neurotoxicity (Yuan et al. 2020). Thus, the modulation of AChE activity is incorporated in the ability of apigenin to mitigate MSG-induced neurotoxicity in rats.

Oxidative injury is a key mechanism of neuronal injury and is incorporated in the progress of neurodegenerative diseases [47]. It is a consequence of uncontrolled production of ROS that results in antioxidant depletion and imbalance between pro-oxidant and antioxidant status [48]. The vulnerability to brain tissue to free radical effect may refer to high oxygen consumption rate, low levels of antioxidant enzymes and the high lipid content (polyunsaturated fatty acids) [40]. Supporting former studies (Bickel 1993, El Okle et al. 2018, Mekkawy et al. 2020), we observed notable decreases in SOD and CAT activities in the group received MSG. SOD converts the superoxide anion into oxygen and hydrogen peroxide (H_2O_2). H_2O_2 is dissociated into water and oxygen by the action of CAT (Kim et al. 2012). The pro-oxidant effect of MSG is mediated by over-generation of ROS that is a possible cause of the excitotoxicity of MSG. In addition, MSG induced decreases in the levels of antioxidants (GSH, GR and GPx) with concomitant increase in MDA levels. Similar findings were formerly reported (Abdel-Rahman et al. 2013, Odenwald & Turner 2017, Wang et al. 2018). GSH plays pivotal roles in the antioxidant defense system

and the maintaining the redox homeostasis in neurons (Gautam et al. 2021). It can suppress the production or directly reacting of free radicals by promoting the action of GPx. Besides its action as a radical scavenger, GSH maintains the membrane structure by removing the acyl peroxides that resulted from lipid peroxidation (Wang et al. 2018). Accordingly, MSG-mediated peroxidation of lipid may endorse for tissue depletion of GSH and this indicates the incapacity of primary antioxidant system to counteract the over-production of free radicals. The oxidative damage in brain cortex is also confirmed by altered histopathological features.

Similar to other flavonoids, apigenin showed noteworthy antioxidant activities and potent free radical scavenging ability in *in vitro* and *in vivo* experimental models (Aghaie et al. 2021, Singh et al. 2020). In this study, apigenin pretreatment significantly restored enzymatic (SOD, CAT, GR and GPx) and non-enzymatic (GSH) antioxidants compared with MSG treatment. Free radical scavenging property of apigenin is attributed for the presence of hydroxyl and keto groups in its chemical structure. The main contributor in this function is the 4-hydroxyl group in its B ring by donating its hydrogen atom and electron to the hydroxyl, peroxy, peroxy nitrite radicals to form stable flavonoid radicals (Abd Allah et al. 2021). In addition, apigenin possess another free radical-scavenging site; *m*-5,7-dihydroxy arrangements in A ring and 4-oxo group in C ring (Kazmi et al. 2020). Further, scavenging excess free radicals may rely on the existence of C2–C3 double bond and 4 keto group in C ring that are involved in electron transfer from B ring (Miyazaki et al. 2020). Moreover, our results showed that apigenin boosted the antioxidant capacity of the neural cells exposed to MSG via enhancing the antioxidant enzymes. These findings corroborate with previous authors (Al Olayan et al. 2020, Lim et al. 2018, Mishra &Goel 2013, Miyazaki et al. 2020). In former studies, apigenin was reported to upregulate the expression of nuclear factor E2-related factor 2 (Nrf2)/heme oxygenase (HO-1) signaling pathways (Mishra &Goel 2013, Taskiran &Ergul 2021). Nrf-2 is a transcriptional factor that is involved in cell protection against oxidative damage via enhancement of the genes encoding for phase II detoxification enzymes (Giorgi et al. 2004). Huang et al. (Mori et al. 1987) found that apigenin upregulates the glutamate cysteine ligase (GCL) gene transcription in in rat primary hepatocytes which further enhances GSH synthesis. GCL triggers the rate limiting step in GSH synthesis. Therefore, these results suggest that apigenin protect against MSG-induced neurotoxicity via boosting the cellular antioxidant system.

Excitotoxicity, oxidative injury, and neuro-inflammation are intimately intertwined and explain the pathogenicity of neurodegenerative brain disorders (Yan et al. 1994). The activation of microglia and astrocytes evoke the release of inflammatory cytokines, thus excitotoxicity can cause or be caused by inflammation (Koriem &Soliman 2014). Oxidative stress triggers the expression of pro-inflammatory genes which resulted in elevated levels of pro-inflammatory cytokines (TNF- α , and IL-1 β) (Al-Megrin et al. 2020). TNF- α is a neurotoxic agent that causes neuronal cell death in direct and indirect manners via induction of NO and free radicals in neuronal cells. In the existing study, MSG triggered the inflammatory responses by elevating the levels IL-1b and TNF- α in cortical tissues of exposed rats that is in harmony with previously reported results (Abdel-Rahman et al. 2013, Li et al. 2021, Reifen et al. 2004, Yan et al. 1994). Furthermore, the exposure to high glutamate was reported to increase the levels of TNF- α in cerebral and intestinal tissues (Xu et al. 2005). The neuroinflammation was suggested to disrupt blood

brain barrier which permit the entry of more MSG with subsequent more damage and inflammation (Li et al. 2021).

In contrast, apigenin administration brought the levels of IL-1 β and TNF- α back near to normal in brain cortex of rat exposed to MSG. These results were in harmony with those reported in rats exposed to acrylonitrile and co-treated with apigenin (Singh et al. 2020). Remarkable anti-inflammatory actions of apigenin were also reported in experimental models of Parkinson's disease, hypoxic-ischemic brain damage and lipopolysaccharide (LPS)-induced depression (Gao et al. 2020, Lim et al. 2018, Taskiran & Ergul 2021). Li et al. (Gao et al. 2020) reported that pretreatment with apigenin reduced NF- κ B activation induced by LPS-induced in prefrontal cortex in depressive mice. The activation of NF- κ B signaling is needed for the expression of inflammatory cytokines (Othman et al. 2021a). Therefore, the decline in pro-inflammatory cytokine expression by apigenin may refer to the modulation of NF- κ B signaling pathway. In addition to the action on NF- κ B, apigenin was reported to suppress the inflammasome activation that resulted from chronic stress and inflammation (Li et al. 2016). The NLRP3 inflammasome is expressed in microglia, and regulates the expressions of IL-1 β and IL-18 (Nader et al. 2018). Thus, the inhibition of the NLRP3 inflammasome which reduce IL-1 β release may be an additional mechanism of apigenin's anti-inflammatory effects.

Further, marked increases in NO levels (measured as nitrite) were detected in MSG administered group which agree with previous findings (Abdel-Rahman et al. 2013, Bickel 1993, Lokman et al. 2022, Ren & Torres 2009). NO is an important inflammatory mediator, and its release is controlled by the level of NOS expression in activated macrophages (Bickel 1993). It displays pro-inflammatory and destructive effects in neuronal systems and strongly implicated in neurotoxicity (Othman et al. 2021b). Our study also revealed marked upregulation in iNOS gene expression after MSG administration into rats. MSG administration was reported to induce the mRNA and protein expressions of NOS isoforms, especially nNOS and iNOS in rat cortex (Abdel-Rahman et al. 2013, Wang et al. 2014). NO could convert the superoxide anion to form harmful peroxynitrite radicals. Both nitrogen and oxygen radicals contribute to neural cell-death by detrimental post-translational modification of proteins (Kassab et al. 2020).

Recent studies revealed that modulation of the NO pathway is a new therapeutic approach to treat various neural disorders (Kassab et al. 2020). Supporting former studies (Lim et al. 2018, Singh et al. 2021), apigenin pretreatment attenuated levels of NO and gene expression of iNOS in cortical tissues of MSG-exposed rats. The suppressive effect of apigenin on iNOS activity has been reported in a diabetes-induced cognitive decline in a rat model (Lee et al. 2020). These results suggested that apigenin was able to protect neurons from cytotoxic levels of NO via decreasing iNOS activation. Hence, it is possible that iNOS signaling is related to neuroprotection of apigenin against MSG-induced neural stress.

Conclusion

It may be concluded from the current results that oral administration of MSG significantly impaired neural functions in exposed rats via induction of oxido-inflammatory stress as well as disturbance in the

neurotransmitters. This study also illustrated the effects of apigenin pre-administration in mitigating the neurotoxic effects of MSG as witnessed by restoring the neurotransmitters levels, boosting the cellular antioxidant defense and lessening the inflammatory machineries. Therefore, apigenin could be considered as a suitable candidate to protect the brain cortical tissue against the neurotoxicity of MSG which is being ascribed to its distinguished antioxidant and anti-inflammatory activities.

Declarations

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Ethics approval

All experimental protocols, including the use of animals, were approved by the Committee of Research Ethics for Laboratory Animal Care, Taif University (approval no. MMI-21-T-709).

Data Availability

The collected data in this study are available from the corresponding author on reasonable request.

Consent to participate

Not applicable.

Consent for publication

Consented.

Conflict of interest

The author declares no competing interest.

Author's contribution: A. Albrakati has performed all the research items.

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Table

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Figures

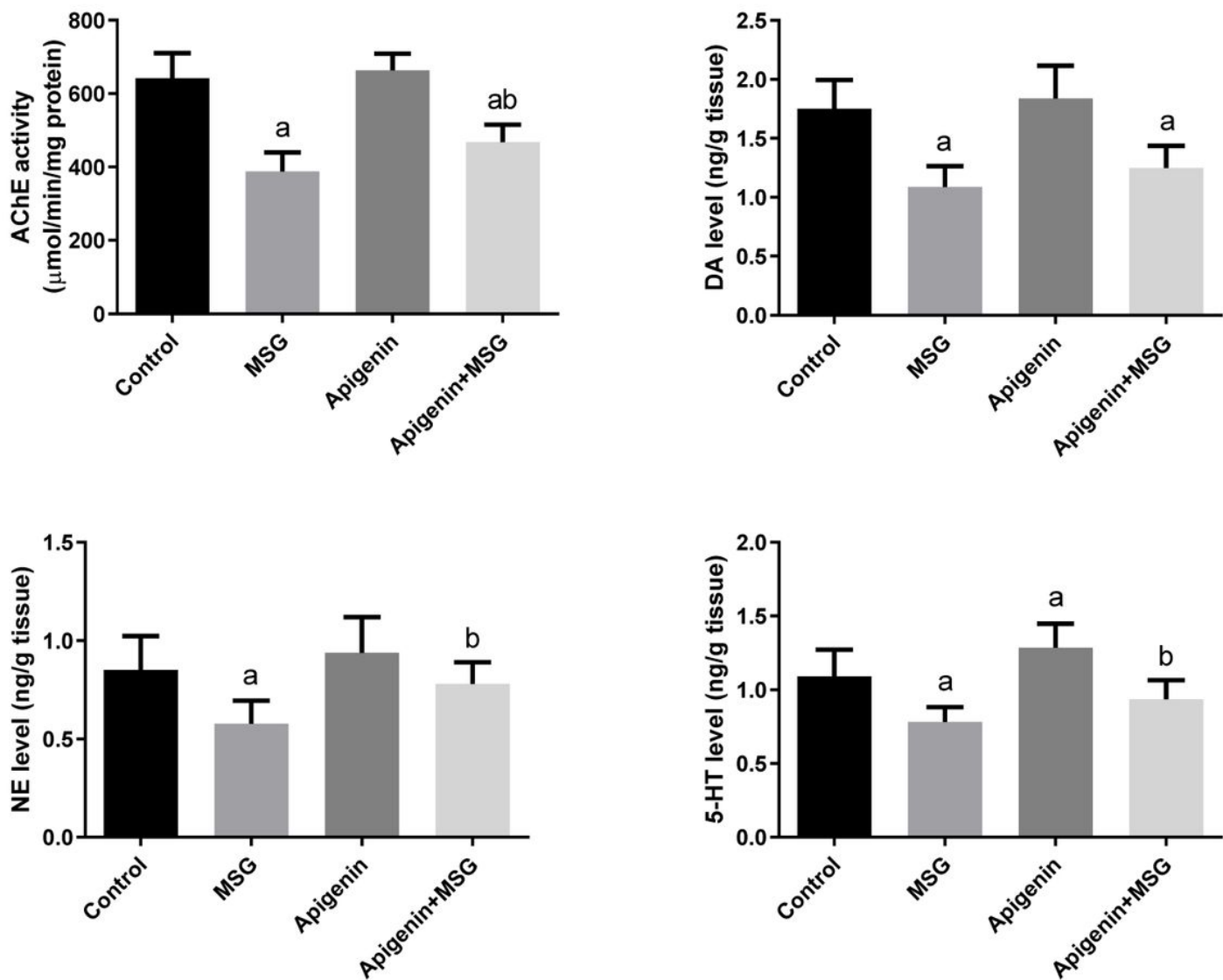


Figure 1

The effect of apigenin on monosodium glutamate (MSG)-induced alterations in neurotransmitters levels in the brain of male rats. Each value represents mean $\pm$ SD ($n = 7$). ^a $P < 0.05$ versus the control rats, ^b $P < 0.05$ versus the MSG-treated rats.

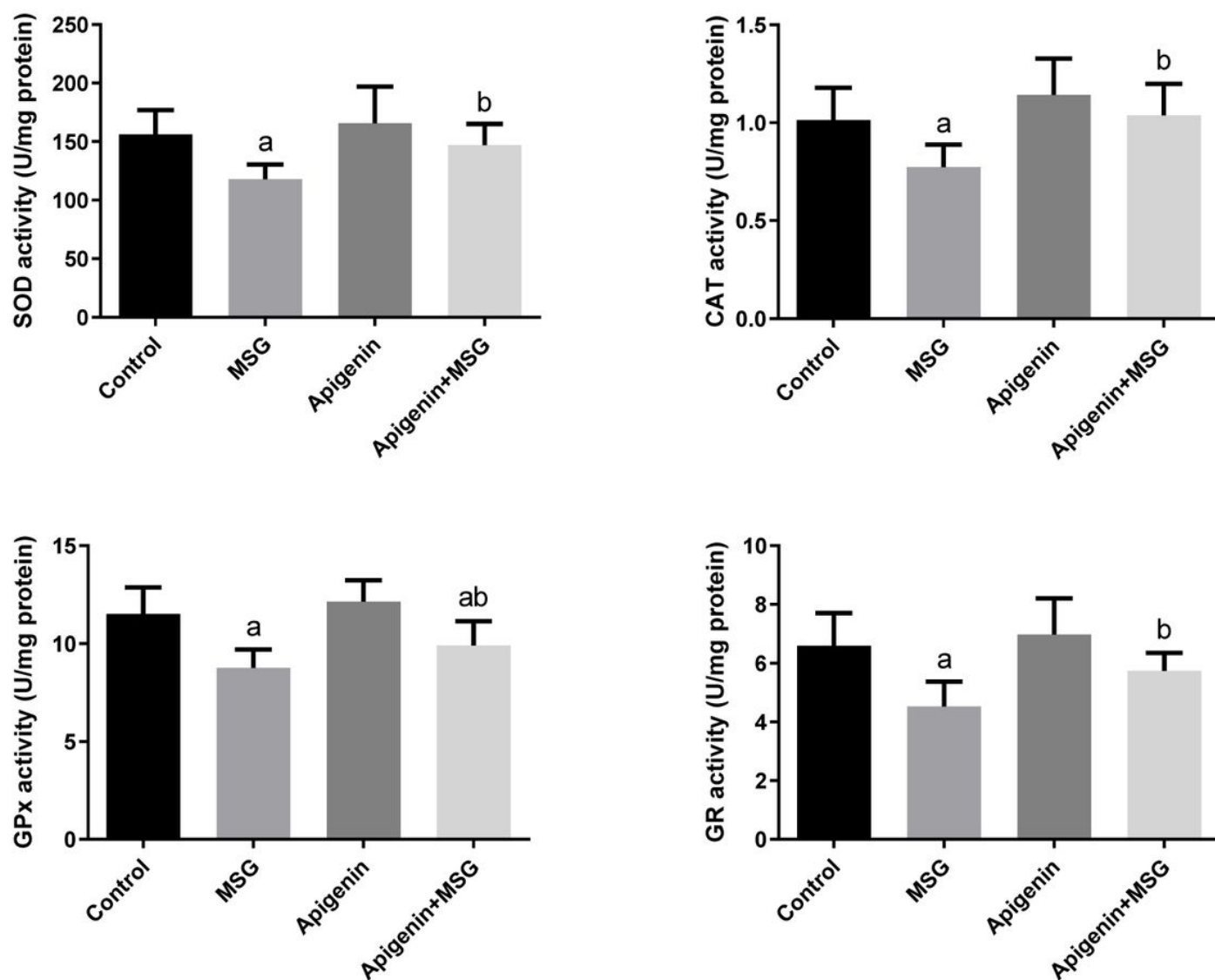


Figure 2

The effect of apigenin on monosodium glutamate (MSG)-induced oxidative stress in the cortex of male rats. Each value represents mean $\pm$ SD ($n = 7$). ^a $P < 0.05$ versus the control rats, ^b $P < 0.05$ versus the MSG-treated rats.

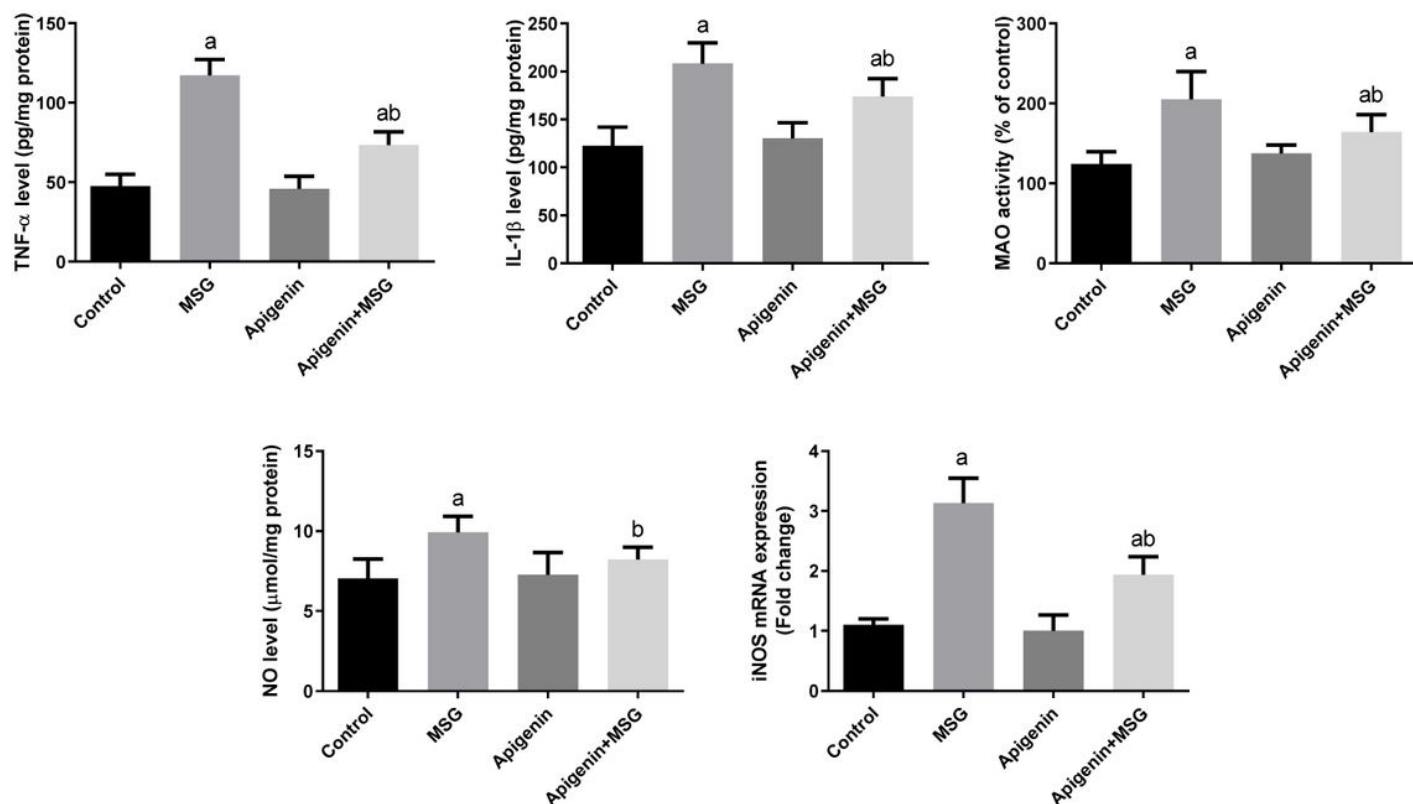


Figure 3

The effect of apigenin on antioxidant enzyme activities in brain cortex of monosodium glutamate (MSG)-treated rats. The enzyme activity values are presented as mean $\pm$ SD ($n = 7$), ^a $P < 0.05$ versus the control rats, ^b $P < 0.05$ versus the MSG-treated rats.

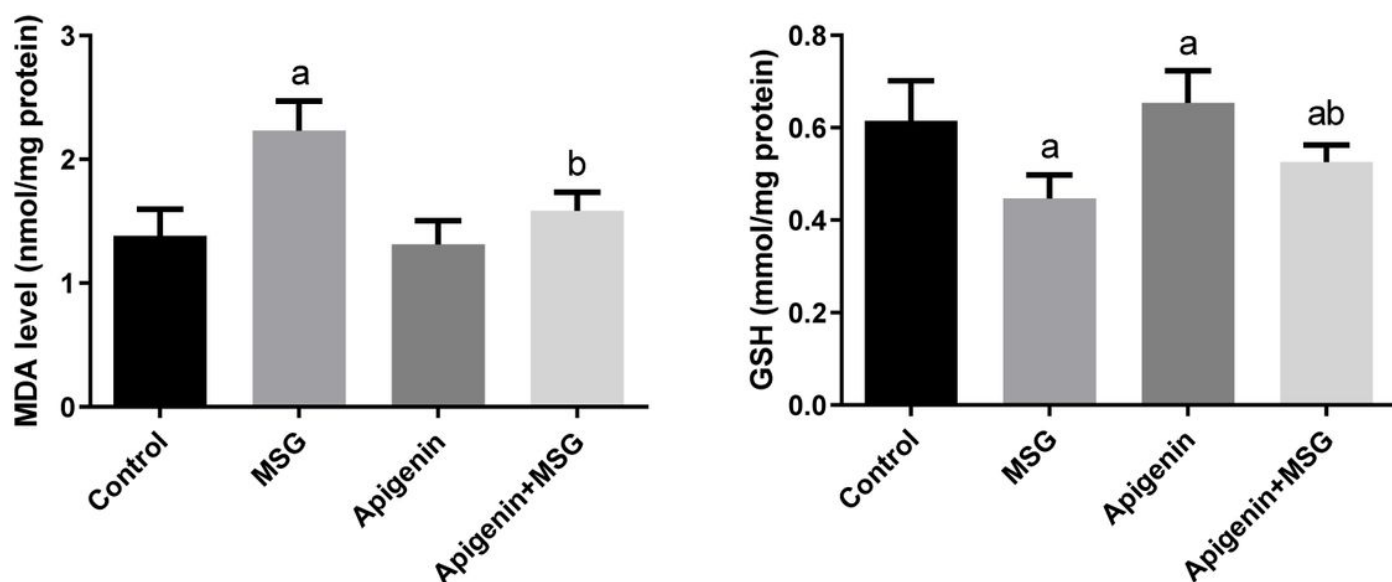


Figure 4

The effect of apigenin on levels of IL-1b, TNF- α , and NO as well as the iNOS expression levels in the brain cortex of monosodium glutamate (MSG)-treated rats. Each value represents mean $\pm$ SD (n=7). Results of gene expression are expressed as mean $\pm$ SD of triplicates that were normalized to the housekeeping *B-actin* gene and represented as fold-change. ^a $P < 0.05$ versus the control rats, ^b $P < 0.05$ versus the MSG-treated rats.

Figure 5

H&E stained micrographs of brain cortex tissues from (A) control, (B) apigenin, (C) MSG, and (D) apigenin + MSG-treated male rats. X= 400.