Azhar Int J Pharm Med Sci 2024; Vol 4 (1):40-51.

Open Access

Print ISSN: 2735-4598
Online ISSN: 2735-4601

(Research Article.)

Sesamol protects against monosodium glutamate-induced attention-deficit/hyperactivity disorder (ADHD) in rats' Offsprings focused on regulating the GSK-3β/Nrf2/NF-kβ/Bax/Bcl-2 signaling pathways

Rahma S. Zedan[1], Magdy M. Awny[1*], Karema Abu-Elfotuh[2] and Azza A. Ali[2]

[1] *Department of Pharmacology and Toxicology, Faculty of Pharmacy, October 6 University, Cairo, Egypt*
[2] *Department of Pharmacology and Toxicology, Faculty of Pharmacy (Girls), Al-Azhar University, Cairo, Egypt*
* Correspondence: magdyawny@o6u.edu.eg (M. M. Awny); tel: +2 01144716406

Article history: **Received** 17-05- 2023 **Revised** 23-06-2023 **Accepted** 20-07-2023.

Abstract: Background: Monosodium glutamate (MSG), a sodium salt of l-glutamic acid, is used in many different types of cuisine to improve flavor and palatability. Food containing MSG was associated with excitotoxicity which induced oxidative stress and neuroinflammation; the most common mechanism that linked with neurological disorders. Attention-deficit/ hyperactivity disorder (ADHD) is a neurodevelopmental disorder that affects children most frequently around the globe. Sesamol (SML) is one of the promising bioactive flavonoids with multiple pharmacological activities. **Objective:** we aim to examine the potential neuroprotective effects of sesamol against MSG by modulating the GSK-3β/Nrf2/NF-kβ /Bcl-2 pathways. **Materials and Methods:** thirty-six male young rats (4-6 weeks old) were randomly distributed over three groups, (12/group). Control group (1): rats received 2% DMSO in saline. ADHD group (2): rats received 400mg/kg MSG daily by intra-gastric tube for 60 consecutive days. ADHD + SML group (3): rats treated like group 2, in addition to receiving SML (20mg/kg i.p.) in concurrent with MSG. behavioral tests (y-maze and open field test) were conducted over the final two days of the experimental period and after the experiment's conclusion, rats were sacrificed and Biochemical parameters were measured in brain tissue (assessment of brain monoamines, GSK-3 β, Nrf2, inflammatory, and apoptotic biomarkers by ELISA technique along with colorimetric assay of Calcium, MDA, SOD and TAC levels). **Conclusion:** According to our results, SML may be able to protect the brain from neurological disorders resembling ADHD by targeting the GSK-3 β /Nrf2/NF-kB/Bax/Bcl-2 signaling pathways.

Keywords: ADHD; Sesamol; monosodium glutamate; GSK-3β; Nrf2; NF-kβ; Bcl-2..

1. INTRODUCTION

Attention Deficit Hyperactivity Disorder (ADHD), a diverse neurodevelopmental ailment with high incident, affects 8-12% of children worldwide and can also affect adults (4%)[1-2], males are more prevalent than females (5.2% vs. 2.7%, respectively)[3]. This disorder's heterogeneity is caused by a confluence of various elements, collectively can prevent healthy brain growth and alter how neural networks function [4]. 76-80% of ADHD is related to genetic factors which increase its heritability and comorbidity [5]. Several other environmental factors strongly participate in ADHD development [6-7].

ADHD is highly associated with catecholamine dysregulation, which affects prefrontal cortex (PFC) development and results in executive dysfunction[8-9]. As a result, amphetamine, atomoxetine, and methylphenidate—the most often prescribed ADHD medication which restore catecholamine level, hence control ADHD symptoms [10]. The exact pathophysiology of ADHD is still unknown. However, several studies strongly reported oxidative stress and neuroinflammation incidence in ADHD [11-12].

Cite this article Zedan, R., Awny, M., Abu-Elfotuh, K., Ali, A. Sesamol protects against monosodium glutamate-induced attention-deficit/hyperactivity disorder (ADHD) in rats' Offsprings focused on regulating the GSK3-β/Nrf2/NF-kβ/Bax/Bcl-2 signaling pathways. Azhar International Journal of Pharmaceutical and Medical Sciences, 2024; 4 (1): 40-51. doi: 10.21608/AIJPMS.2023.211678.1212
DOI : 10.21608/AIJPMS.2023.211678.1212

Monosodium glutamate (MSG), a prominently used flavor enhancer, is a white crystalline powder of L-glutamic acid sodium salt[13-14]. Overconsumption of MSG developed renal and hepatic toxicity [15-16], and some studies reported gonadal dysfunction[17]. Glutamate is the main excitatory neurotransmitter in CNS that plays a vital role in normal brain development, regulates learning and memory function[18-19]. However, exposure to high level of MSG, disrupt blood osmolality and increase glutamate influx in the brain, induce excitotoxicity[20-21,22,23]. Excitotoxicity is one of the main hallmarks recognized in ADHD cases, associated with increased intracellular calcium level that disrupt mitochondrial function, increase ROS and RNS release leading to apoptosis or necrosis[24-25]. Administration of MSG was associated with certain damage in the PFC and implicated in impaired attention and locomotor activity in animal model[26-27]. Nuclear factor erythroid 2-related factor 2(Nrf2) is a crucial anti-oxidant and anti-inflammatory agent[28-29]. Excess O&Ns over the antioxidant capacity, induce Nrf2 nuclear translocation that binds to antioxidant response element (ARE) sequentially upregulates expression of several antioxidant genes. Nrf2-dependent antioxidants increase the production of anti-inflammatory compounds, inhibit nuclear factor kappa-B (NF-KB) signaling and NLRP3 inflammasome activation, result in decrease the expression of pro-inflammatory cytokines and limit neuronal apoptosis [30]. These findings were further supported by Suryakant K. Niture, et al., who described the vital role that Nrf2 played in the up-regulation of Bcl-2, which preserved cellular viability.

On the other hand, glycogen synthase kinase-3B (GSK-3β) is a kinase enzyme that maintains in an inactive state under normal conditions. Prolonged exposure to oxidative stress, activates GSK-3β that implicated in psychiatric and neurodegenerative disorders[31-32]. Activated GSK-3β phosphorylates Nrf2 and induces its proteosomal degradation[33-34,35]. In addition, Inhibition of GSK-3β was linked to a reduction in NF-κβ transcriptional activity and a marked reduction in the level of pro-inflammatory cytokines, which support stabilization of the brain blood barrier[36]. Therefore, targeting GSK-3β and Nrf2 is considered as therapeutic strategies in ADHD.

Sesamol (SML) is a white crystalline powder with peculiar odor, extracted from roasted sesame seeds (Sesamum indicum L.), and their pressed oil[37]. SML is the most bioactive methylene dioxyphenyl sesame lignin with biphasic solubility [38]. Regarding its chemical structure, SML is the best antioxidant and free radical scavenger among the bioactive phenolic compounds since it functions as a primary and secondary antioxidant [39]. Primary antioxidants are substances that donate electrons or hydrogen to free radicals, causing them to immediately interact and change into inactive metabolites such as phenolic compounds. Meanwhile, secondary metabolites indirectly slow down oxidation by chelating pro-oxidant metals, supplying primary antioxidants with hydrogen, absorbing UV rays, or acting as singlet oxygen buffers[57]. By investigating numerous studies, SML demonstrated variety of pharmacological actions such as anti-oxidant, anti-inflammatory[40], anti-apoptotic, antihyperlipidemic [41], neuroprotective [42], cardioprotective [43], and anti-cancer activities [44]. In this study we seek to assess the potential neuroprotective property of SML against MSG induced ADHD like symptoms by ameliorating oxidation, inflammation and apoptosis regulated by GSK-3β /Nrf2/NF-kβ /Bax/Bcl-2 pathways.

2. METHODS

2.1. Drug and Chemicals

MSG, SML and other utilized agents were of analytical quality, and sourced from Sigma-Aldrich Chemical Co. in St. Louis, Missouri.

2.2. Animals

Thirty-six healthy male western albino rat offsprings, at age of 4-6 weeks old (25-40 g), were obtained from the Nile Co. for Pharmaceuticals and Chemical Industries, Cairo, Egypt. Rat pups were sheltered in standard housing conditions, 25°C, 55% humidity, 12-hour light and dark cycle with full access to normal feed and water ad libitum. Our study received approval from Al-Azhar University's Ethics Committee for the Faculty of Pharmacy (Girls) (No. 323), which adheres to NIH guidelines regarding animal studies.

2.3. Experimental design

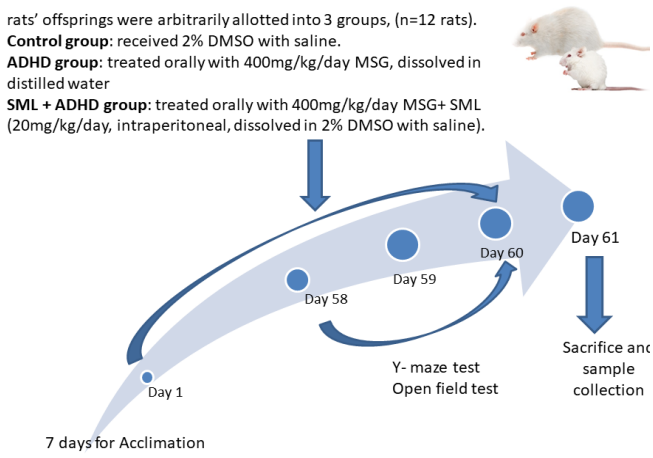

Figure 1. Schematic diagram illustrates schedule of the experimental design [55-56, 26].

2.4. Behavioral experiments:

Behavioral tests were performed during the last 2 days of the experiment.

2.4.1. Y-maze test for assessing of attention behavior.

Animals' ability to pay attention during exploring the Y-maze was assessed by % spontaneous alternation behavior (SAB). As mentioned by Kim HJ, et al [45], Y-maze used in the study has 3 arms, each arm (l×w×h= 50×10×20) with equal angles (120°) in-between and an equilateral triangular central area. The three arms were labeled as A, B and C, arm A was used as the start arm. Rats were individually sited in the first arm, permitted to explore the three arms for 8 minutes where their movements were tracked and evaluated with specialist software (Any-maze™, Stoelting Co., Chicago, USA). Between each trial, the apparatus was disinfected with 70% ethyl alcohol and allowed to dry [46]. The rat was thought to have inputted the arm when its four paws were entirely inside of it. The description of SAB was enrollment into all three arms on sequential decisions. The following formula was used to calculate the percentage of SAB, it equals (number of alternations) / (total arm entries −2) ×100.

2.4.2. Open-field test for assessing locomotor alterations.

Open field apparatus used in this study was a black rectangular box (l×w×h =72×72×36) with a floor divided into 16 square (18 x 18 cm for each square) [47]. Rats were set individually in the middle of the instrument and given ten minutes to explore it. When their movements were tracked and evaluated with specialist software (Any-maze™, Stoelting Co., Chicago, USA). Between each trial, the apparatus was disinfected with 70% ethyl alcohol and allowed to dry. Four parameters were measured throughout this test: the ambulation frequency (the total number of lines that the animal crossed with all 4 limbs), time mobile (the total time that animal spent moving), mean speed (the total distance travelled by rat divided by the duration of mobility) and rearing frequency (number of times the animal rose on his hind paws).

2.5. Samples collection

After accomplishing the experiment, dilute ether was used to anesthetize the animals to be sacrificed and then the brains were removed and soaked with cold saline (0.9% w/v). The experiment complied with NIH Publications No. 85-23, amended 2011—guidelines for the proper handling of laboratory animals.

2.6. Biochemical evaluations:

2.6.1. Enzyme-linked immunosorbent assay (ELISA)

ELISA kits obtained from MyBioSource (Southern California, San Diego, USA) were used for assessing several brain monoamines, including glutamate (Cat. #: MBS756400), dopamine (Cat. #: MBS262606) and norepinephrine (Cat. #: MBS269993). In addition to tumor necrosis factor alpha (TNF-α) (Cat. #: MBS175904), and NF-kβ (Cat. #: MBS453975)**.** Nrf2 (Cat. #: SL0985Ra)**,** GSK-3β (Cat. #: SL0319Ra), IL-1b (Cat. #: SL0402Ra) and apoptotic biomarkers B-cell leukemia/lymphoma 2 protein (Bcl-2) (Cat. #: SL0108Ra) and Bcl-2-associated X protein (Bax) (Cat. #: SL0109Ra) kits were assessed by SUNLONG BIOTECH CO., LTD., CHINA**.**
The procedures were followed exactly as directed by the manufacturer, and then the plate's content was examined spectrophotometrically.

2.6.2. Colorimetric assessment of malondialdehyde (MDA), superoxide dismutase (SOD), total antioxidant capacity (TAC) and calcium ion concentrations

Colorimetric analysis was performed using available kits provided by Bio diagnostic, Inc., Giza, Egypt, using 10% of brain supernatant.
Calcium level was measured as the key factor for glutamate excitotoxic effect. Thiobarbituric acid was used to measure brain lipid peroxidation revealed by the MDA level. The SOD enzyme's activity was examined according to enzyme's capacity to prevent the phenazine methosulphate-mediated reduction of the nitro blue tetrazolium dye. Finally, TAC was evaluated using the antioxidant reaction with exogenously supplied H_2O_2.

2.7. Statistical analysis:

The data were analyzed using GraphPad Prism® statistical analysis tools version 9.5.0 (730) for Windows (GraphPad Software, San Diego, CA, USA), and Tukey's multiple comparison test was performed to compare the results between the various groups. All data were expressed as mean ± standard deviation (SD), and the difference between groups deemed statistically relevant if P-value < 0.05.

3. RESULTS

3.1. Effect of Sesamol on MSG-induced changes in attention and locomotor activity in rats' offsprings assessed by Y-maze and open-field test.

3.1.1. Y-maze test

Attentive rats can enter the three arms of y-maze consecutively, therefore, the more attentive rats, the higher spontaneous alternation behavior percentage (% SAB). As verified in table (1), MSG induced a significant decrease in % SAB in ADHD rat model to 79%, compared to normal control number. However, concurrent administration of SML (20mg/kg) with MSG improved attention behavior and significantly increased % SAB to 116.35%, compared to MSG value.

Table 1. Sesamol improves attention in ADHD rat model induced by MSG.

Groups	% SAB
Control	72.68 ± 1.69
ADHD	57.47 ± 3.69$
ADHD + SML	66.87 ± 4.88 $#

Data expressed as mean ± SD (n = 12). $, #: values are significant when compared to the control and ADHD group respectively. One way ANOVA followed by Tukey's as post-hoc test presented P-value < 0.05. ADHD: attention-deficit hyperactivity disorder; SML: sesamol; %SAB: percentage of spontaneous alternation behavior.

3.1.2. Open-field test

As summarized in table (2), rats receiving MSG orally showed significant decrease in the ambulation frequency, time mobile, mean speed and rearing frequency by 55.7%, 57%, 67% and 35.1% respectively, contrasted to normal control readings. Contrarily, administration of 20 mg/kg of SML with MSG increased the ambulation frequency, time mobile, means speed and rearing frequency by 44.8%, 65%, 90% and 34% respectively, compared to ADHD group.

Table 2. Sesamol modifies locomotor alterations induced by MSG in ADHD rat model.

	Open field test			
Group	Ambulation frequency	Time mobile	Mean speed	Number of Rearing
Control	73 ± 3.22	211.6 ± 63.83	0.0298 ± 0.006	25.67 ± 1.21
ADHD	32.33 ± 5.35 $	91.45 ± 14.54 $	0.0099 ± 0.003 $	16.67 ± 1.03 $
ADHD + SML	46.83 ± 1.602$#	150.9 ± 5.53 $#	0.0189 ± 0.003 $#	22.33 ± 2.06 $#

Results expressed as mean ± SD (n = 12). $, #: values are significant when compared to the control and ADHD group respectively. One way ANOVA followed by Tukey's as post-hoc test presented, P-value < 0.05. ADHD: attention-deficit hyperactivity disorder; SML: sesamol.

3.2. Sesamol protects against brain catecholamine dysfunction and elevated calcium level induced by MSG in ADHD rat model.

As depicted in figures (2), MSG damaging impact induced significant decrease in dopamine and Nor-epinephrine levels in brain tissue by 2.5-folds and 3.9-folds correspondingly, in contrast to significant increase in glutamate and calcium levels by 3.98-folds and 3.5-folds correspondingly, contrasted with normal control. However, administration of SML 20mg/kg reverted MSG effect and significantly increased dopamine and Nor-epinephrine by 1.56-folds and 2.8-folds respectively, and significantly decreased glutamate and calcium level by 2.57-folds and 1.5-folds respectively, compared to ADHD group.

3.3. Sesamol repairs brain oxidative stress induced by MSG in ADHD animal model.

Figures (3) (A, B, C and D) demonstrated that MSG -mediated oxidative stress in rats' brain, presented by significant elevation in MDA level by 1717% and significant decrease in levels/activity of NRF2, TAC and SOD by 57.2%, 81% and 95.4% respectively, regarding normal control animals. As expected, SML decreased MDA level by 40.5%, and increased the level/activity of NRF2, TAC and SOD by 169.7%, 112.7% and 789.5% individually, contrasted with ADHD group.

3.4. Sesamol re-establishes inflammatory biomarkers induced by MSG in ADHD rat model.

Figure (4) displayed that MSG significantly increased the brain inflammatory biomarkers NF-kβ, TNF-α and IL-1b by 6 folds, 5.48-folds and 1.7-fold respectively, in comparison to normal control. By contrast, concurrent administration of SML with MSG decreased NF-kβ, TNF-α and IL-1B by 1.68-fold, 2.4-folds and 1.6-fold respectively, compared to ADHD group.

3.5. Sesamol protects against MSG- induced apoptosis in ADHD rat model.

Figures 5 (A, B and C) depicted that MSG treated animals revealed significant increase in brain level of Bax to 147.5% along with significant decrease in anti-apoptotic biomarker Bcl-2 to 52%. Therefore, Bax/ Bcl2 ratio increased by 2.7-folds, compared to value of normal control. On the other hand, administration of SML decreased Bax level by 45% and increased Bcl-2 by 44%. Hence, Bax/Bcl2 ratio decreased by 2.7-folds, contrasted with ADHD group.

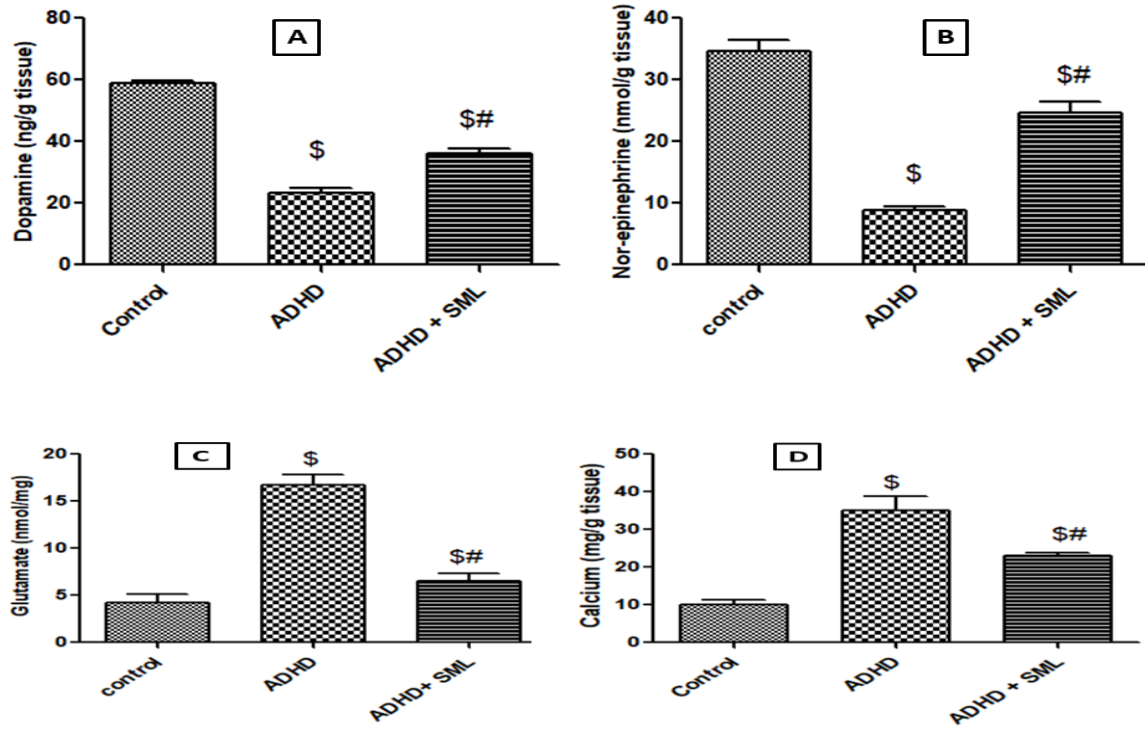

Figure 2. Sesamol modulates catecholamines' dysregulation and elevated calcium level induced by MSG. Data presented as mean ± SD (n=12) $, #: values are significant when compared to the control and ADHD groups respectively. One way ANOVA followed by Tukey's as post-hoc test presented P-value < 0.05. Quantification of; A) dopamine; B) nor-epinephrine; C) glutamate; D) calcium. ADHD: attention-deficit hyperactivity disorder; SML: Sesamol.

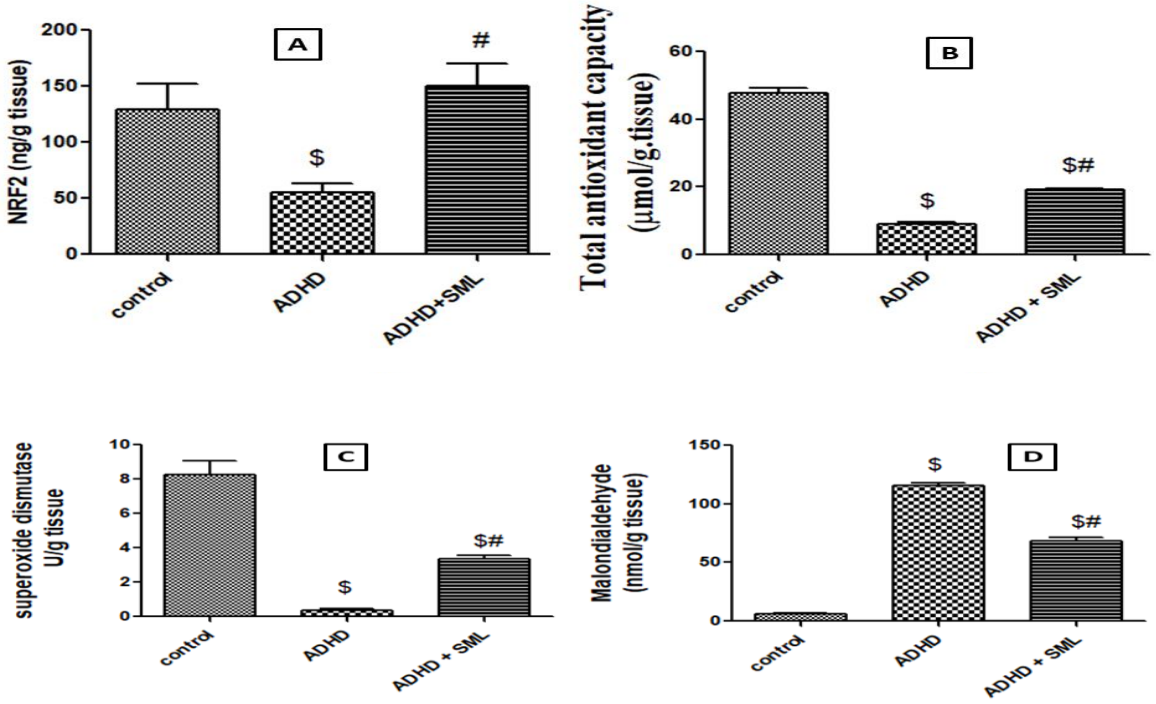

Figure 3. Sesamol inhibits oxidative stress induced by MSG.

Data presented as mean ± SD (n=12). $, #: values are significant when compared to the control and ADHD groups, correspondingly. One way ANOVA followed by Tukey's as post-hoc test presented P-value < 0.05. Quantification of; A) NRF2; B) TAC; C) SOD; D) MDA. ADHD: attention-deficit hyperactivity disorder; SML: sesamol; NRF2: Nuclear factor erythroid 2-related factor 2; SOD: superoxide dismutase; TAC: total antioxidant capacity; MDA: malondialdehyde.

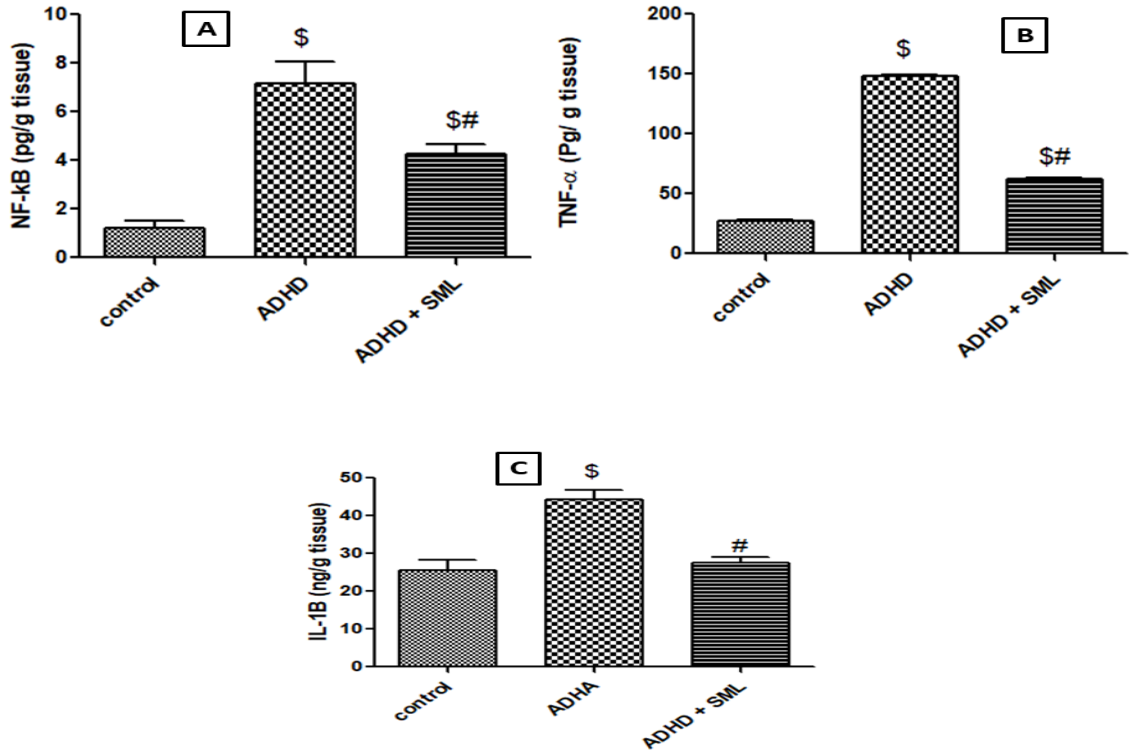

Figure 4. Sesamol decreased inflammatory biomarkers induced by MSG in ADHD rat model.

Data presented as mean ± SD (n=12). $, #: values considered significant when compared to the control and ADHD groups respectively. One way ANOVA followed by Tukey's as post-hoc test presented P-value < 0.05. Quantification of; A) NF-kβ; B) TNF-α; C) IL -1B. ADHD: attention-deficit hyperactivity disorder; SML: Sesamol; NF-kβ: nuclear factor kappa-B; TNF-α: tumor necrosis factor alpha; IL-1B: interleukin – 1 Beta.

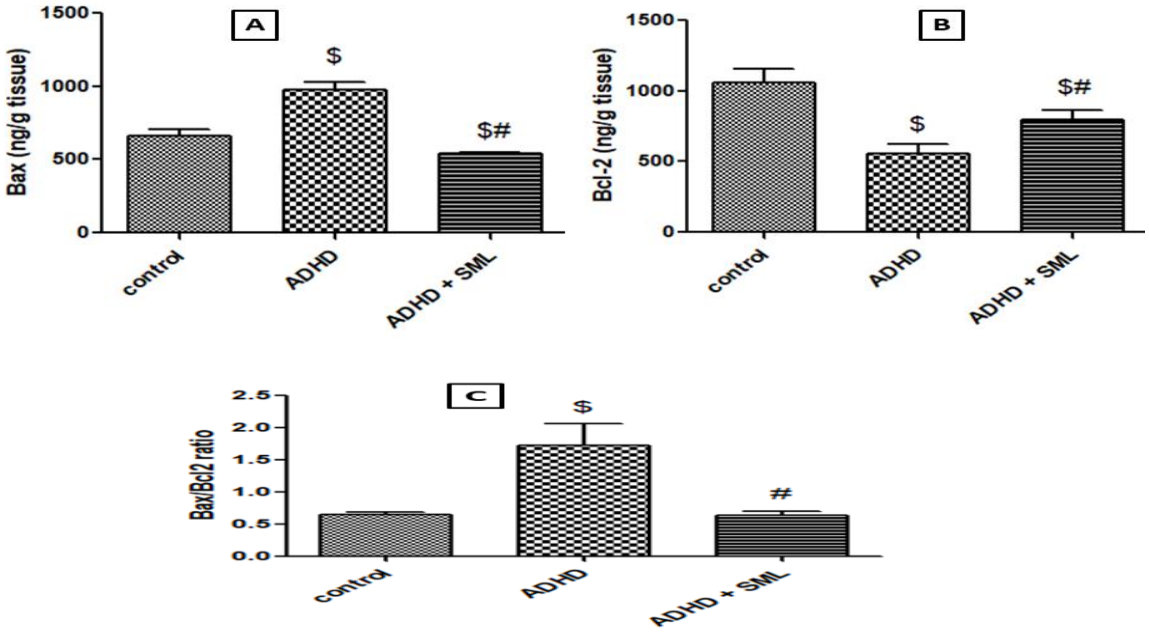

Figure 5. Sesamol improves apoptotic/anti-apoptotic markers induced by MSG.

Data presented as mean ± SD (n=12). $, #: values are significant when compared to the control and ADHD groups respectively. One way ANOVA followed by Tukey's as post-hoc test presented P-value < 0.05. Quantification of; A) Bax; B) Bcl-2; C) Bax/Bcl2 ratio. ADHD: attention-deficit hyperactivity disorder; SML: sesamol; Bax: Bcl-2-associated X protein; Bcl-2: B-cell leukemia/lymphoma 2 protein.

3.6. Sesamol suppresses MSG-induced glycogen synthase kinase-3B in ADHD rat model.

In figure (6), MSG significantly increased GSK-3β level in rat pups by 1.54-folds, regarding normal control value. However, concurrent administration of 20 mg/kg SML with MSG decreased GSK-3B content by 1.7-fold, compared to ADHD group.

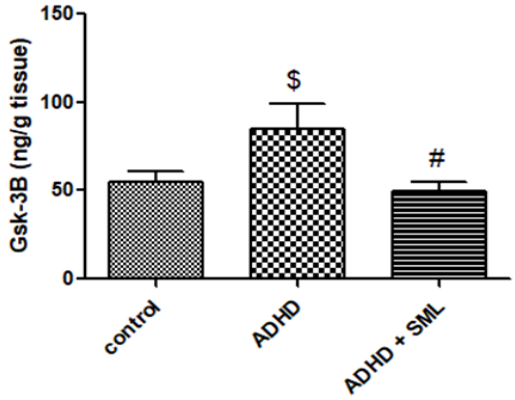

Figure 6. Sesamol attenuates GSK-3β induced by MSG in ADHD rat model.

Data expressed as mean ± SD (n=12). $, #: values are significant when compared to the control and ADHD groups respectively. One way ANOVA followed by Tukey's as post-hoc test presented P-value < 0.05. ADHD: attention-deficit hyperactivity disorder; SML: Sesamol; GSK-3β: glycogen synthase kinase-3B.

4- DISCUSSION

As ADHD prevalence rises over time, it increases the necessity to investigate potential factors that could be contributing to ADHD pathophysiology. The umami taste of MSG promotes its wide use in many different types of cuisine to improve flavor and palatability. MSG consumption either by oral gavage or mixed with food achieves higher brain glutamate level, associated with attention deficit and locomotor alterations in young animals [48-27].

Although glutamate is an essential neurotransmitter for normal brain function, increase in brain glutamate level induces excitotoxicity, oxidative stress and neuroinflammation which impair normal brain development in rat pups. Sesamol (SML) is a bioactive compound obtained from roasted sesame seeds (Sesamum indicum L.) and their pressed oil, which demonstrated multiple-pharmacological activities.

In our study, small rat pups treated orally with 400 mg/kg of MSG for two months, developed significantly high level of brain glutamate and calcium than normal group. The increase in calcium level was correlated with a considerable elevation of MDA, a biomarker of lipid peroxidation, and a decrease in Nrf2 and its antioxidant downstream, SOD and TAC activity/level, that promote oxidative stress. These findings were analogous to earlier studies [26-27].

High levels of extracellular glutamate activate the glutamate ionotropic receptor viz; NMDA, which induced Calcium influx, as well as the metabotropic receptor viz; mGluR1s and mGluR5s, which cause more Calcium to be released from the endoplasmic reticulum [20].

The elevation of cellular calcium is considered detrimental. High intracellular calcium activates various mechanisms associated with neuronal death. Besides calpains, other destructive enzymes and death associated protein kinase 1 activation, high intracellular calcium induces neuronal nitric oxide synthase (nNOS), increase NO level that would mediates lipid peroxidation and impair mitochondrial function result in oxidative stress [48].

As a results of oxidative stress, the cellular level of ROS elevated and induced Nrf2 nuclear translocation, which binds to antioxidant response element and consequently upregulates the antioxidant enzymes mRNA and elevated total antioxidant capacity (TAC) to detoxify cellular free radicals and inhibited neuroinflammation, as has been showed by Ngo V, et al [28]. However, chronic exposure to significant high level of oxidative stress, as shown in our investigation through the high level of MDA, was associated with significantly low brain level of Nrf2 and its antioxidants [27].

Moreover, rats received MSG expressed significantly high level of GSK-3β, NF-Kβ and its downstream inflammatory markers, TNFα and IL-1B. In addition, a significant high level of Bax and lower level of Bcl-2 apoptotic biomarkers was reported.

Elevated ROS associated with reduction in Nrf2 transcription factor level may be a function of GSK-3β. As shown in Misrani A, et al study[49], GSK-3β kinetic activity was induced in response to excessive O &Ns, induced neurodegenerative disease. Moreover, activation of GSK-3β, phosphorylates Nrf2 transcription factor even after nuclear translocation, induce ubiquitination and proteosomal degradation[56-57]. Accordingly, our investigations revealed that MSG-induced oxidative stress was associated with high level of GSK-3β and significant decrease in Nrf2 level.

GSK-3β and Nrf2 both modulate neuroinflammation in different ways. Toll-like receptors (TLRs) stimulation, followed by GSK-3β activation was associated with NF-kβ nuclear translocation which increased the release of numerous cytokines, IL-6, IL-1B, and TNFα and decreased anti-inflammatory cytokines. Typically, inhibitors of GSK-3β reduced TLR expression and consequently decreased the release of inflammatory cytokines by inhibiting the transcriptional activation of NF-κB[36].

Furthermore, activated Nrf2 can induce HO-1 expression which exhibits anti-inflammatory activity. Numerous studies have shown that HO-1 and its metabolites significantly inhibit NF-kβ signaling pathway and consequently reduce inflammation. Additionally, Nrf2 prevents NLRP3 inflammasome activation, which results in a reduction in IL-1B release and promotes cell survival[30]. Therefore, GSK-3β and Nrf2 are considered as a target for suppressing neuroinflammation.

Oxidative perturbations and pro-inflammatory cytokines induced by MSG increased Bax level and decrease Bcl2, which resulted in significant increase in Bax/Bcl-2 ratio. Oxidative stress disturbs mitochondrial function was associated with increase cytochrome C release in the cytoplasm that ultimately induces cellular apoptosis[52].
On the other hand, rats that received MSG showed significant reduction in brain monoamines viz; Dopamine and Nor-epinephrine as have been reported by Abu-Elfotuh K, et al[26] and Salem HA, et al[27]. Dopamine and Nor-epinephrine play essential roles in executive function, and they interact together in PFC, regulating attention, cognition and locomotion [58]. Consequently, their deficiency impaired attention and spatial working memory which suppressed the animals' ability to enter the three arms of Y-maze consecutively. In addition to locomotor alterations demonstrated by significant decrease in moving time and speed in open field test which may related to NE induced spontaneous locomotor hyperactivity[59].

MSG decreased brain NTs may be related to high extracellular glutamate level which disturbs calcium homeostasis and impairs the calcium-mediated neurotransmitter release[26-27]. In addition, increased Bax/Bcl-2 ratio induced neuronal death that consequently result in overwhelming NTs levels.
Interestingly, concurrent administration of SML with MSG significantly decreased glutamate excitotoxicity as revealed by low glutamate and calcium levels, and due to its highly potent antioxidant activity, SML significantly increased Nrf2, SOD and TAC levels as well as decreased MDA levels.

Furthermore, SML impact extended beyond its antioxidant capacity to involve suppression of neuroinflammation and apoptosis, these effects were verified by significant decrease in the brain levels of GSK-3β, NF-kβ, IL-1B, TNF-α and Bax, and increased Bcl-2 level, hence it reduced neuroinflammation and maintained neuronal survival, these results come in harmony with those of Mahendra Kumar C, et al [38], Liou XWC, et al [40] , Abou-zeid SM, et al [42] and John J, et al [53].

Moreover, the lower glutamate and calcium levels in SML group may be attributed to its antioxidant and anti-inflammatory activity, as the decrease in oxidative stress and inflammatory cytokine help to maintain glial function and consequently increase the uptake of extracellular glutamate by glial cells [54], hence maintains neuronal calcium level, which decrease the risk for excitotoxicity and maintain neurotransmission. Therefore, SML preserved the level of dopamine and Nor-epinephrine which modulated locomotor activity, increased rearing frequency, and improved rats' attention and spatial memory function in Y-maze test.

5. CONCLUSION

In summary, our work focused on the GSK-3β /Nrf2/NF-kB/Bax/Bcl-2 pathways to examine the potential neuroprotective mechanism of sesamol against MSG-induced ADHD in rats' offsprings. Our findings from studying the brain tissue of ADHD rats showed elevated levels of oxidative stress, apoptotic, and inflammatory indicators along with a large increase in GSK-3β, which sesamol greatly decreased. In the Y-maze and open-field tests, sesamol's antioxidant, antiapoptottic, and anti-inflammatory effects eliminated monoamines shortage, markedly enhanced attention, and restored locomotor behavior

Funding: There is no funding for this research.

Conflicts of Interest: The authors assert they have no competing interests.

Acknowledgement: we would particularly like to thank our co-worker A. Elfouly, teaching assistant at department of pharmacology, Faculty of pharmacy, October 6TH University, for his constructive practical advice and tireless help to get this work done.

Ethical Statement: The study was carried out in accordance with the ethical principles and procedures that were established by the Ethics Committee of the Faculty of Pharmacy, (Girls), Al-Azhar University (No.323), that are acceptable to the NIH standards for lab animals.

Author Contribution: RZ performed the experiment, collected the data, performed the graphical and statistical analysis, and wrote the manuscript. MA supervised the data analysis, writing, and revised the manuscript. KA participated in the creation of the research concept and oversaw the data analysis. AA participated in the research idea, supervised the data analysis, writing and made edits to the paper. All authors have read and agreed to the published version of the manuscript.

List of Abbreviations: ADHD: Attention-deficit/ hyperactivity disorder; MSG: monosodium glutamate; SML: sesamol; ROS: reactive oxygen species; Nrf2: Nuclear factor erythroid 2-related factor 2; GSK-3β: glycogen synthase kinase -3 Beta; nNOS: neuronal nitric oxide synthase; NF-kβ: nuclear factor kappa B; IL-1B: interleukine-1 beta; TNF-α: tumor necrosis factor alpha; Bcl-2: *B-cell leukemia/lymphoma 2*; Bax: Bcl-2 associated-x-protein; NMDA: *N-methyl-D-aspartate receptor*; NTs: neurotransmitters; TAC: total antioxidant capacity; MDA: malondialdehyde; HO-1: heme oxygenase 1; NLRP3: Nod-like receptor protein 3; SOD: superoxide dismutase.

REFERENCES

1. Amin SA, Bmmki A, Reddy A. The Prevalence of Vitamin D Deficiency in ADHD-Affected Children and Adolescents in the Appalachian Region of Virginia. :5–7.

2. Daugherty AM, Bender AR, Raz N, Ofen N. Childhood to Late Adulthood. Hippocampus. 2016;26(2):220–8.

3. Akinbami LJ, Liu X, Pastor PN, Reuben CA. Attention deficit hyperactivity disorder among children aged 5-17 years in the United States, 1998-2009. NCHS Data Brief. 2011;(70):1–8.

4. Luo Y, Weibman D, Halperin JM, Li X. A review of heterogeneity in attention deficit/hyperactivity disorder (ADHD). Front Hum Neurosci. 2019;13(February):1–12.

5. Faraone S V., Mick E. Molecular genetics of attention deficit hyperactivity disorder. Psychiatr Clin North Am [Internet]. 2010;33(1):159–80. Available from: http://dx.doi.org/10.1016/j.psc.2009.12.004

6. Brookes KJ, Mill J, Guindalini C, Curran S, Xu X, Knight J, et al. A common haplotype of the dopamine transporter gene associated with attention-deficit/hyperactivity disorder and interacting with maternal use of alcohol during pregnancy. Arch Gen Psychiatry. 2006;63(1):74–81.

7. Banerjee T Das, Middleton F, Faraone S V. Environmental risk factors for attention-deficit hyperactivity disorder. Acta Paediatr Int J Paediatr. 2007;96(9):1269–74.

8. Kirley A, Hawi Z, Daly G, McCarron M, Mullins C, Millar N, et al. Dopaminergic system genes in ADHD: Toward a biological hypothesis. Neuropsychopharmacology. 2002;27(4):607–19.

9. Caylak E. Biochemical and genetic analyses of childhood attention deficit/hyperactivity disorder. Am J Med Genet Part B Neuropsychiatr Genet. 2012;159 B(6):613–27.

10. Brown KA, Samuel S, Patel DR. Pharmacologic management of attention deficit hyperactivity disorder in children and adolescents: A review for practitioners. Transl Pediatr. 2018;7(1):36–47.

11. Corona JC. Role of oxidative stress and neuroinflammation in attention-deficit/hyperactivity disorder. Antioxidants. 2020;9(11):1–17.

12. Dunn GA, Nigg JT, Sullivan EL. Neuroinflammation as a risk factor for attention deficit hyperactivity disorder. Pharmacol Biochem Behav. 2019;182(July 2018):22–34.

13. Walker R, Lupien JR. The Safety Evaluation of Monosodium Glutamate. J Nutr. 2000;130(4S):1049S-52S.

14. Chakraborty SP. Patho-physiological and toxicological aspects of monosodium glutamate. Toxicol Mech Methods [Internet]. 2019;29(6):389–96. Available from: http://dx.doi.org/10.1080/15376516.2018.1528649

15. Kassab RB, Theyab A, Al-Ghamdy AO, Algahtani M, Mufti AH, Alsharif KF, et al. Protocatechuic acid abrogates oxidative insults, inflammation, and apoptosis in liver and kidney

associated with monosodium glutamate intoxication in rats. Environ Sci Pollut Res. 2022;29(8):12208–21.

16. Hassan HA, EL-Kholy WM, EL-Sawi MRF, Galal NA, Ramadan MF. Myrtle (Myrtus communis) leaf extract suppresses hepatotoxicity induced by monosodium glutamate and acrylamide through obstructing apoptosis, DNA fragmentation, and cell cycle arrest. Environ Sci Pollut Res. 2020;27(18):23188–98.

17. Hamza RZ, Diab AEAA. Testicular protective and antioxidant effects of selenium nanoparticles on Monosodium glutamate-induced testicular structure alterations in male mice. Toxicol Reports [Internet]. 2020;7(January):254–60. Available from: https://doi.org/10.1016/j.toxrep.2020.01.012

18. Featherstone DE. Intercellular glutamate signaling in the nervous system and beyond. ACS Chem Neurosci. 2010;1(1):4–12.

19. Skerry TM, Genever PG. Glutamate signalling in non-neuronal tissues. Trends Pharmacol Sci. 2001;22(4):174–81.

20. Balkhi HM, Gul T, Banday Z, Haq E. " Glutamate Excitotoxicity: An Insight into the Mechanism ". Int J Adv Res Journalwww.journalijar.com Int J Adv Res. 2014;2(7):361–73.

21. Datta A, Hossain A, Roy S. An overview on monosodium glutamate: Its direct and indirect effects. Res J Pharm Technol. 2019;12(12):6187–92.

22. Farhat F. Monosodium Glutamate Safety, Neurotoxicity and Some Recent Studies. Al-Azhar J Pharm Sci. 2021;64(2):224–45.

23. Škultétyová I, Tokarev D, Ježová D. Stress-induced increase in blood-brain barrier permeability in control and monosodium glutamate-treated rats. Brain Res Bull. 1998;45(2):175–8.

24. Glaser PEA, Batten SR, Gerhardt GA. The Role of Glutamate Dysregulation in the Etiology of ADHD. Glutamate Neuropsychiatr Disord. 2022;467–92.

25. Elia J, Izaki Y, Ambrosini A, Hakonarson H. Glutamatergic Neurotransmission in ADHD: Neurodevelopment and Pharmacological Implications. J Pediatr Neonatol. 2020;2:1006.

26. Abu-Elfotuh K, Abdel-Sattar SA, Abbas AN, Mahran YF, Alshanwani AR, Hamdan AME, et al. The protective effect of thymoquinone or/and thymol against monosodium glutamate-induced attention-deficit/hyperactivity disorder (ADHD)-like behavior in rats: Modulation of Nrf2/HO-1, TLR4/NF-κB/NLRP3/caspase-1 and Wnt/β-Catenin signaling pathways in rat. Biomed Pharmacother [Internet]. 2022;155:113799. Available from: https://doi.org/10.1016/j.biopha.2022.113799

27. Salem HA, Elsherbiny N, Alzahrani S, Alshareef HM, Abd Elmageed ZY, Ajwah SM, et al. Neuroprotective Effect of Morin Hydrate against Attention-Deficit/Hyperactivity Disorder (ADHD) Induced by MSG and/or Protein Malnutrition in Rat Pups: Effect on Oxidative/Monoamines/Inflammatory Balance and Apoptosis. Pharmaceuticals. 2022;15(8):1–24.

28. Ngo V, Duennwald ML. Nrf2 and Oxidative Stress: A General Overview of Mechanisms and Implications in Human Disease. Antioxidants. 2022;11(12).

29. Satta S, Mahmoud AM, Wilkinson FL, Yvonne Alexander M, White SJ. The Role of Nrf2 in Cardiovascular Function and Disease. Oxid Med Cell Longev. 2017;2017.

30. Ahmed SMU, Luo L, Namani A, Wang XJ, Tang X. Nrf2 signaling pathway: Pivotal roles in inflammation. Biochim Biophys Acta - Mol Basis Dis [Internet]. 2017;1863(2):585–97. Available from: http://dx.doi.org/10.1016/j.bbadis.2016.11.005

31. Matsuda S, Ikeda Y, Murakami M, Nakagawa Y, Tsuji A, Kitagishi Y. Roles of PI3K/AKT/GSK3 Pathway Involved in Psychiatric Illnesses. Diseases. 2019;7(1):22.

32. Hooper C, Killick R, Lovestone S. The GSK3 hypothesis of Alzheimer's disease. J Neurochem. 2008;104(6):1433–9.

33. Gameiro I, Michalska P, Tenti G, Cores Á, Buendia I, Rojo AI, et al. Discovery of the first dual GSK3β inhibitor/Nrf2 inducer. A new multitarget therapeutic strategy for Alzheimer's

disease. Sci Rep. 2017;7(March):1–15.

34. Zang H, Mathew RO, Cui T. The Dark Side of Nrf2 in the Heart. Front Physiol. 2020;11(July):1–8.

35. Rojo AI, Sagarra MR De, Cuadrado A. GSK-3β down-regulates the transcription factor Nrf2 after oxidant damage: Relevance to exposure of neuronal cells to oxidative stress. J Neurochem. 2008;105(1):192–202.

36. Cortés-Vieyra R, Silva-García O, Gómez-García A, Gutiérrez-Castellanos S, Álvarez-Aguilar C, Baizabal-Aguirre VM. Glycogen Synthase Kinase 3β Modulates the Inflammatory Response Activated by Bacteria, Viruses, and Parasites. Front Immunol. 2021;12(May):1–13.

37. Singh N, Kushwaha P, Gupta A, Prakash O, Swarup S, Usmani S. Phytochemical and Pharmacological Insight on Sesamol: An Updated Review. Curr Bioact Compd. 2020;17(2):112–9.

38. Mahendra Kumar C, Singh SA. Bioactive lignans from sesame (Sesamum indicum L.): evaluation of their antioxidant and antibacterial effects for food applications. J Food Sci Technol. 2015;52(5):2934–41.

39. Zhou S, Zou H, Huang G, Chen G. Bioorganic & Medicinal Chemistry Letters Preparations and antioxidant activities of sesamol and it ' s derivatives. Bioorg Med Chem Lett [Internet]. 2021;31(September 2020):127716. Available from: https://doi.org/10.1016/j.bmcl.2020.127716

40. Liou XWC, Lai ZLX. Sesamol suppresses the inflammatory response by inhibiting NF- j B / MAPK activation and upregulating AMP kinase signaling in RAW 264 . 7 macrophages. Inflamm Res. 2015;

41. Biswas A, Dhar P, Ghosh S. Antihyperlipidemic Effect of Sesame (Sesamum indicum L .) Protein Isolate in Rats Fed a Normal and High Cholesterol Diet. 2010;75(9):274–9.

42. Abou-zeid SM, Elkhadrawey BA, Anis A, Abubakr HO, El-bialy BE. Neuroprotective effect of sesamol against aluminum nanoparticle-induced toxicity in rats. 2021;

43. Jayaraj P, Narasimhulu CA, Rajagopalan S, Parthasarathy S, Desikan R. Function from sesame oil for cardioprotection. 2020;(Ldl):1198–210.

44. Siriwarin B, Weerapreeyakul N. Sesamol induced apoptotic effect in lung adenocarcinoma cells through both intrinsic and extrinsic pathways. Chem Biol Interact [Internet]. 2016;254:109–16. Available from: http://dx.doi.org/10.1016/j.cbi.2016.06.001

45. Choi I, Kim P, Joo SH, Kim MK, Park JH, Kim HJ, et al. Effects of preconceptional ethanol consumption on ADHD-like symptoms in sprague-dawley rat offsprings. Biomol Ther. 2012;20(2):226–33.

46. Kraeuter A, Guest PC. Chapter 10. 2019;1916:105–11.

47. Arika WM, Kibiti CM, Njagi JM, Ngugi MP. Effects of DCM Leaf Extract of Gnidia glauca (Fresen) on Locomotor Activity, Anxiety, and Exploration-Like Behaviors in High-Fat Diet-Induced Obese Rats. Behav Neurol. 2019;2019.

48. Qin YWZ. Molecular and cellular mechanisms of excitotoxic neuronal death. 2010;1382–402.

49. Misrani A, Tabassum S, Yang L. Mitochondrial Dysfunction and Oxidative Stress in Alzheimer ' s Disease. 2021;13(February):1–20.

50. Zhang J, Wang X, Vikash V, Ye Q, Wu D, Liu Y, et al. ROS and ROS-Mediated Cellular Signaling. 2016;2016(Figure 1).

51. Ciotti S, Iuliano L, Cefalù S, Comelli M, Mavelli I, Giorgio E Di, et al. GSK3 β is a key regulator of the ROS-dependent necrotic death induced by the quinone DMNQ. Cell Death Dis [Internet]. 2020; Available from: http://dx.doi.org/10.1038/s41419-019-2202-0

52. Al H, Elmorsy E, Al-ghafari A, Ghulam J. Saudi Journal of Biological Sciences Roles of oxidative stress , apoptosis , and inflammation in metal-induced dysfunction of beta pancreatic cells isolated from CD1 mice. Saudi J Biol Sci [Internet]. 2021;28(1):651–63. Available from: https://doi.org/10.1016/j.sjbs.2020.10.056

53. John J, Nampoothiri M, Kumar N, Mudgal J, Nampurath GK, Chamallamudi MR. Sesamol, a lipid lowering agent, ameliorates aluminium

chloride induced behavioral and biochemical alterations in rats. Pharmacogn Mag. 2015;11(42):327–36.

54. Liao SL, Chen CJ. Differential effects of cytokines and redox potential on glutamate uptake in rat cortical glial cultures. Neurosci Lett. 2001;299(1–2):113–6.

55. Zhang P, Wang Y, Wang H, Cao J. Sesamol alleviates chronic intermittent hypoxia-induced cognitive deficits via inhibiting oxidative stress and inflammation in rats. Neuroreport. 2021;105–11.

56. Kumar A, Choudhary S, Adhikari JS. Sesamol Ameliorates Radiation Induced DNA Damage in Hematopoietic System of Whole Body c -Irradiated Mice. 2018;90(June 2017).

57. Gutiérrez-Del-río I, López-Ibáñez S, Magadán-Corpas P, Fernández-Calleja L, Pérez-Valero Á, Tuñón-Granda M, et al. Terpenoids and polyphenols as natural antioxidant agents in food preservation. Antioxidants. 2021;10(8).

58. Viggiano D, Ruocco LA, Arcieri S, Sadile AG. Involvement of norepinephrine in the control of activity and attentive processes in animal models of attention deficit hyperactivity disorder. Neural Plast. 2004;11(1–2):133–49.

59. Jones MD, Hess EJ. Norepinephrine regulates locomotor hyperactivity in the mouse mutant coloboma. Pharmacol Biochem Behav. 2003;75(1):209–16.

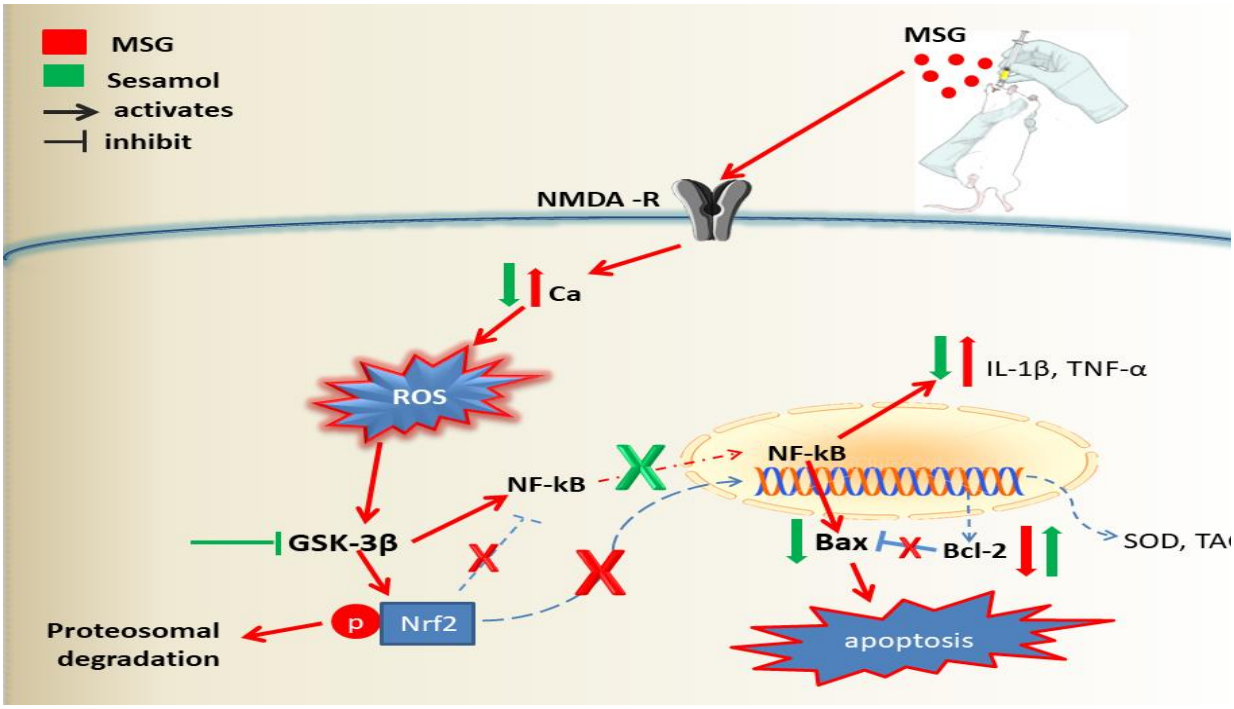

Figure 7. Graphical summary illustrates the prospective modulatory effect of Sesamol on biochemical alterations triggered by MSG in ADHD rats' model.

MSG: monosodium glutamate; NMDA-R: N- methyl D-aspartate receptor; ROS reactive oxygen species; GSK-3β: glycogen synthase kinase 3- beta; Nrf2: Nuclear factor erythroid 2-related factor 2; NF-Kb: nuclear factor kappa beta; Bax: Bcl-2-associated X protein; Bcl-2: B-cell leukemia/lymphoma 2 protein; SOD: superoxide dismutase; TAC: total antioxidant capacity; IL-1β: interleukin 1-beta; TNF-α: tumor necrosis factor alpha. **Red color** represents MSG effect, **green color** represents Sesamol protective effect.