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Research Article
Omega-3 Rich Oils Attenuate ADHD-Like Behaviour Induced by Dietary Monosodium Glutamate in Rats

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Abstract

Background and Objective: Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder characterized by inattention, hyperactivity and cognitive dysfunction. The present study was designed to examine the possible modulatory effect of Fish, Walnuts or Fenugreek Oils against Attention Deficit Hyperactivity Disorder (ADHD)-like Behavior induced by Monosodium Glutamate (MSG) in Rats. **Materials and Methods:** Fifty weaning rats were divided into five groups, (each group contain 10 rats) as follows: Group 1: Normal control rats were fed on a balanced diet. Groups from 2-5 rats were fed on a balanced diet+MSG (0.4 g kg^{-1} diet), Group 2 served as a positive control group whereas group 3, 4 and 5 treated with Fish, Walnuts and Fenugreek oil, respectively, (200 mg kg^{-1} b.wt.) by intra-gastric tube. Biochemical and behavioural parameters were tested as well as microscopic examination of brain tissue was done. **Results:** MSG ingestion caused marked disruption in locomotors activity, memory function and brain tissue structure along with significant abnormalities in some bio-markers and reduction in the gene expression level of Bcl-2 in brain tissue. However, treatment with the tested oils showed remarkable effect by reversing the condition. **Conclusion:** Dietary supplementation with walnut; fenugreek or fish oils at the tested dose could modulate the condition of ADHD in rats.

Key words: ADHD, MSG, fish oil, walnut oil, fenugreek oil, hippocampus

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Attention Deficit Hyperactivity Disorder (ADHD) is a neurodevelopmental disorder that makes the affected persons unable to concentrate and pay attention[1].ADHD can harm finances, career opportunities, income, retirement funds (due to impulsive spending), friendships groups, families and co-workers[2].

There are three types of ADHD: inattentive type, hyperactive-impulsive type and combination type[3]. In addition, poorly affected cognitive impulsiveness is a general trait in children with ADHD, including deficiencies in planning, forgetfulness, inadequate time usage and impetuous conduct[4].

The aetiology of the disease is still unknown; as shown by family and twin research, ADHD may come into view as a strong genetic component[5]. Around 80% of the prevalence of the disease depends on the genetic factor; also, catecholamine dysregulation plays a crucial role in the pathophysiology of ADHD[6]. In addition, environmental risk factors are etiologically associated with ADHD, such as biological and psychosocial adversity[7].

Monosodium glutamate (MSG) is commonly used as a food additive and taste enhancer; however, intake of a large dose of MSG causes oxidative stress[8], Renal and hepatotoxicity. Also, some sex hormones were adversely disrupted: testosterone, estrogen and progesterone[9].

The confirmation and potential risk of neurotoxicity is a long-standing concern with dietary glutamate intake, particularly MSG[10]. It also causes Alzheimer's disease, Parkinson's disease, depression, multiple sclerosis, addiction, schizophrenia, ADHD and autism[11].

Essential oils, particularly in the frontal lobe and limbic region of the brain, may have a direct chemical effect on cephalic function, stimulating or balancing hormonal/ dopaminergic activity, positively affecting memory, mental alertness, clarity and focus, balance, reaction time, mood, emotion and behaviour[12]. Omega-3 and omega-6 polyunsaturated fatty acids (PUFA) are essential oils for health. Omega-3 fatty acids (n3-FAs) are critical for the growth, physiology and perfusion of the human brain as well. In addition, it benefits learning and academic achievement[13].

A relative lack of omega-3 may contribute to extra psychiatric and neurodevelopmental disorders, so omega-3 fatty acids may play a role in the treatment of ADHD and related developmental disorders[14]. Eicosapentaenoic acid (EPA) and Docosahexaenoic acid (DHA) are two highly concentrated fatty acids in the brain that reveal anti-oxidative and anti-inflammatory properties that contribute to the protection of neurons[15].

There are many sources, including fish, walnut and fenugreek oils, that are rich in omega-3 fatty acids. Fish oil supplementation decreased oxidative stress and raised EPA and DHA levels, which were correlated with a substantial improvement in free glutamate levels[16].

Many studies reported the beneficial effects of walnuts as an antioxidant on the motor, cognitive functions, learning and memory[17-20].A positive impact on neurological wellbeing is also exercised by Fenugreek and its constituents. Few studies have identified the beneficial function of fenugreek in the pathological symptoms of Alzheimer's disease. Likewise, other studies have demonstrated the neuroprotective, anti-depressant, anti-anxiety and modulatory effects of fenugreek on cognitive functions and Parkinson's disease[21].

The objective of this study is to investigate the ameliorative effects of walnuts, fenugreek oils in comparison with fish oil on ADHD-like behaviour induced by MSG in weaning rats.

MATERIALS AND METHODS

Study site: The study was carried out at Biochemistry and Nutrition Department, Animal House of faculty of women for arts, science and education at Ain Shams University, Egypt, from March, 2019 to May, 2019.

Extraction and analysis of oils: Walnut oil was extracted by using a cold-press machine according to Çakaloğlu *et al.*[22]. Fenugreek oil was extracted by using cold hexane according to Gu *et al.*[23]. Fish oil was purchased from Pharco-pharmaceutical, DownTown, Cairo, Egypt. Analysis of free fatty acids in the tested oils by using the Gas Chromatography technique as described by Shirasawa *et al.*[24]. Antioxidant capacity in the tested oils was analyzed by using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) as described by Najafabad and Jamei[25].

Diets: The diets used in the study were the balanced diet according to Reeves *et al.*[26] and MSG supplemented diet; contained 0.4 g MSG kg^{-1} diet according to Onaolapo *et al.*[27]. MSG powder was purchased from Sigma Company of pharmaceutical industries, Egypt.

Animal trial: The experimental animals used throughout the work were 50 healthy weaning male albino rats Sprague-Dawley strain weighing 25±5 g supplied by the Nile Pharmaceuticals Company, Cairo, Egypt. All rats were adapted for 7 days. Then the animals were divided into 5 groups (10 rats/group); the first and second groups served as control

groups fed on a balanced diet[26] and MSG diet[27] respectively for 8 weeks. The other three groups were fed on MSG diet plus (200 mg kg^{-1} b.wt.) of tested oils given by intra-gastric tube daily for 8 weeks in the following manner; Group 3: Fish oil[28], Group 4: Walnut oil[29] and Group 5: Fenugreek oil[30].

Behavioural tests: At the last 10 days of the experiment, all rats were transferred to the Faculty of Pharmacy "girls", Al-Azhar University to do behavioural tests. All rats were adapted for 7 days then the open field task and Y-maze task were done as described by Beppe *et al.*[31].

Biological evaluation, collection of brain tissue samples and histological studies: At the end of the experiment biological evaluation was determined. The animals were then sacrificed by decapitation. Brain tissues were collected, washed by sterile saline solution (9 g L^{-1}), weighted and then frozen at -80°C. Parts of the brain were carefully removed and fixed in 10% neutral formalin for routine histological examination by using (H and E) stain.

Gene expression measurement of Bcl-2 in brain tissue by RT-qPCR: Measurement of mRNA levels of Bcl-2 and the housekeeping gene (β-actin) in brain tissue was done using the real-time quantitative polymerase chain reaction (RT-qPCR) by using Applied Biosystems step one plus equipment[32]. Total RNA was isolated using a Qiagen tissue extraction kit (Qiagen, USA) according to the instructions of the manufacturer. Reverse transcription of the extracted mRNA was done by using a sense fast cDNA synthesis kit (CAT No. BIO-65053)[33]. Data analyses were performed using an Applied Biosystem with software version 3.1 (StepOne™, USA). Primer sequences (Table 1) were designed as described by Briton-Jones *et al.*[34] for Bcl-2 and by Glare *et al.*[32] for β-actin.

Biochemical measurements in brain tissue: Glial Fibrillary Acidic Protein (GFAP) and Apoptosis-Inducing Factor (AIF) were determined by the Western Blot technique as described by Bargagna-Mohan *et al.*[35]. Glutamate and Brain-Derived Neurotrophic Factor (BDNF) were determined using an ELISA kit (CAT. No. MBS 756400) and (Catalog: ELR-BDNF)[36], respectively. Epinephrine, Norepinephrine and Dopamine were determined by using the ELISA kits (CAT. No. MBS031232), (CAT. No. MBS269993)[37] and (CAT. No. MBS026032) respectively. Calcium was determined as described by Valrance *et al.*[37]. Tumour necrosis factor-alpha (TNF-α) was determined using ELISA kit (CAT. No. MBS175904)[38]. MDA and GSH were determined calorimetrically[39-41].

Table 1: Forward and reverse sequence of the primer used for Bcl_2 and beta-actin genes

	Gene primer
Bcl-2	Forward primer: 5-CTACGAGTGGGATGCTGGAG-3'
	Reverse primer: 5-TTCTTCACGATGGTGAGCG-3'
Beta-actin	Forward primer: 5-GGTCGGTGTGAACGGATTTGG-3'
	Reverse primer: 5-ATGTAGGCCATGAGGTCCACC-3'

Statistical analyses: Data were statistically analyzed by Statistical Package for Social Science (SPSS) version 16.0 Microsoft Windows, SPSS Inc. Values were expressed as mean±standard deviation (SD). Statistical differences between groups were done by using one-way analysis of variance ANOVA; the mean difference was significant at the ($p<0.05$) level according to Levesque[42].

RESULTS

Some free fatty acids content and antioxidant capacity in fish, walnut and fenugreek oils: The free fatty acid content of walnuts, fish and fenugreek oils are illustrated in (Fig. 1a-c) respectively. Moreover, the results presented in (Table 2) showed the 1, 1-diphenyl-2-picryl hydrazyl DPPH free radical scavenging activity of tested oils. Every 1 mL of each of the tested oils scavenged 5.0 mL of DPPH radicals by 0.892±0.013 mg TE, 0.645±0.008 mg TE and 0.967±0.007 mg TE for fish oil, walnut oil and fenugreek oil, respectively.

Biological evaluation (food intake, feed efficiency ratio, gain body weight and relative brain weight): Results are presented in (Table 3), showed that there was a statistically significant increase in food intake in MSG fed rats group when compared with the healthy control group ($p<0.05$). However, oral ingestion of fish oil with MSG showed a significant decrease in food intake in comparison with MSG fed rats group ($p<0.05$). on the other hand, no significant change in food intake was observed in rats fed on MSG diet plus oral doses of either walnut or fenugreek oils versus MSG fed rats group ($p<0.05$).

Concerning feed efficiency ratio and gain in body weight, there was a statistically significant decrement in MSG fed rat groups when compared to the healthy control group. Oral ingestion with tested oils with MSG showed no significant change in FER and gain in body weight as compared to MSG fed rat group.

Table 2: Total antioxidant content of oils

Sample	DPPH (mg TE mL^{-1} oil)
Fish oil	0.892±0.013
Walnut oil	0.645±0.008
Fenugreek oil	0.967±0.007

Values are means of three replicates. Values are Mean±SD

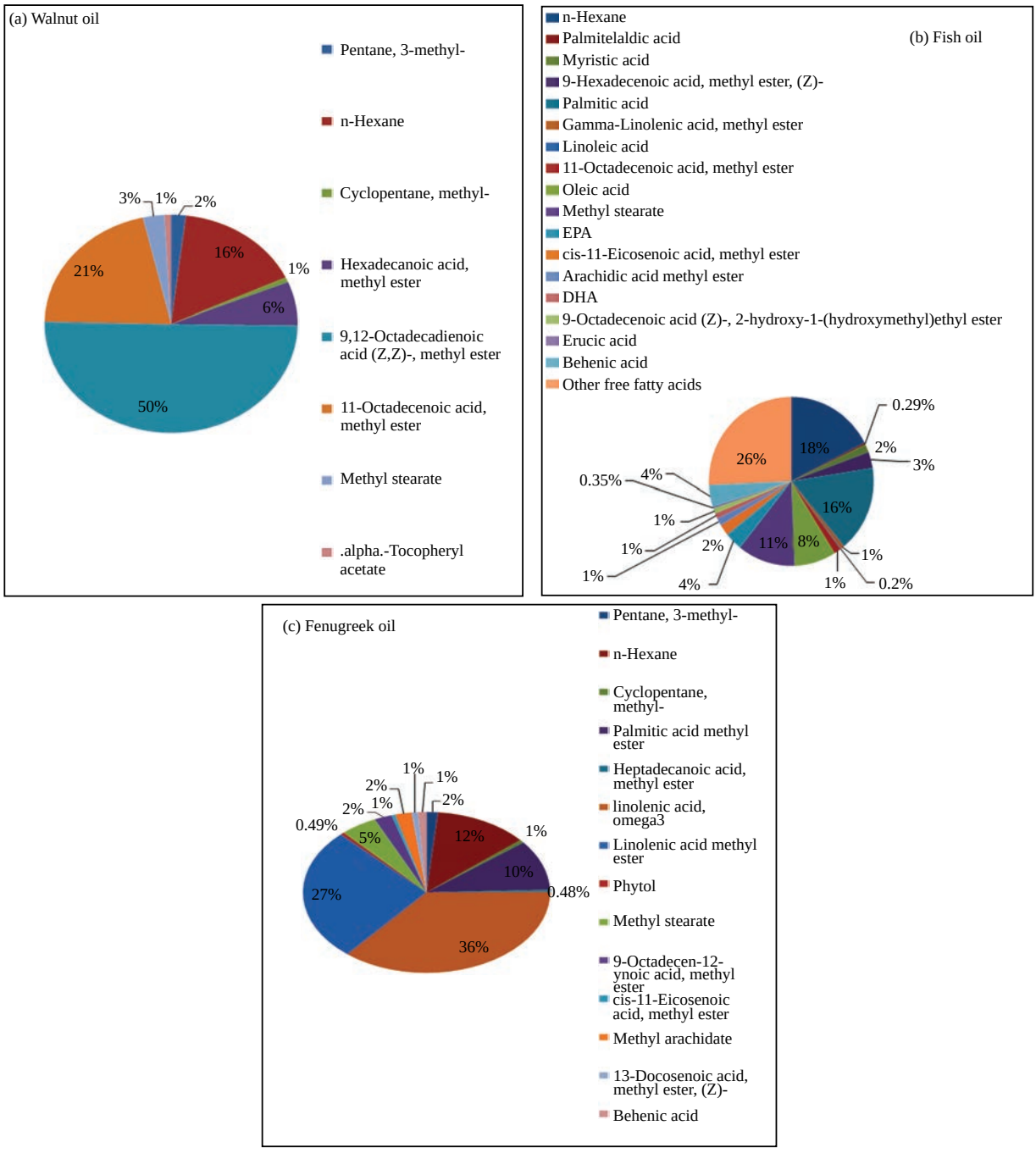

Fig. 1(a-c): Percentage of free fatty acids content in tested oils by using Gas chromatography
(a) Walnut oil, (b) Fish oil and (c) Fenugreek oil

Table 3: Effect of fish, walnut or fenugreek oil on biological evaluation (food intake, feed efficiency ratio, gain body weight and relative brain weight) in MSG fed rats

Parameter/group	Food intake	Feed efficiency ratio (FER)	Gain body weight	Relative brain weight (RBW)
Healthy control group	608.75±35.97^{b}	0.07±0.04^{a}	41.20±19.60^{a}	1.84±0.42^{c}
MSG fed rats group	664.40±33.98^{a}	0.01±0.01^{b}	8.09±5.51^{b}	2.67±0.36^{ab}
MSG fed rats plus fish oil group	587.80±21.10^{b}	0.03±0.03^{b}	18.76±15.95^{b}	2.35±0.47^{b}
MSG fed rats plus walnut oil group	645.50±28.33^{a}	0.02±0.02^{b}	12.19±9.79^{b}	2.86±0.57^{a}
MSG fed rats plus fenugreek oil group	645.89±27.24^{a}	0.02±0.02^{b}	12.27±7.69^{b}	2.36±0.36^{b}
LSD (p<0.05)	26.95	0.03	11.56	0.4

Values are represented as mean±SD (n = 10). There was no significant difference between means have the same letters in the same column (p<0.05)

Table 4: Effect of fish, walnut or fenugreek oil on assessed behavioral parameters (open-field test) in MSG fed rats

Behavioural test/group	Latency (sec)	Ambulation frequency (no. of squares/3-)	Rearing frequency (no. of rearing/3-)	Grooming (sec/3-)
Healthy control group	1.33±0.52^{c}	30.00±2.61^{a}	13.00±1.41^{a}	6.17±1.17^{a}
MSG fed rats group	6.00±1.79^{a}	11.83±2.32^{c}	4.50±1.87^{d}	3.83±1.94^{b}
MSG fed rats plus fish oil group	2.17±1.17^{bc}	24.17±2.84^{b}	10.50±1.87^{bc}	6.83±1.17^{a}
MSG fed rats plus walnut oil group	3.00±0.90^{b}	22.50±1.87^{b}	9.33±1.97^{c}	6.33±1.21^{a}
MSG fed rats plus fenugreek oil group	2.17±1.17^{bc}	24.00±2.61^{b}	12.00±1.41^{ab}	6.50±1.87^{a}
LSD (p<0.05)	1.41	2.85	2.05	1.80

Values are represented as mean±SD (n = 6). There was no significant difference between means have the same letters in the same column (p<0.05)

Table 5: Effect of fish, walnut or fenugreek oil on assessed behavioral parameters (Y-maze test) in MSG fed rats

Groups	Spontaneous alteration (%)
Healthy control group	97.00±1.41^{a}
MSG fed rats group	48.00±2.37^{d}
MSG fed rats plus fish oil group	88.17±2.32^{bc}
MSG fed rats plus walnut oil group	85.67±4.63^{c}
MSG fed rats plus fenugreek oil group	89.50±1.87^{b}
LSD (p<0.05)	3.28

Values are represented as mean±SD (n = 6). There was no significant difference between means have the same letters in the same column (p<0.05)

Considering the relative weight of the brain, there was a statistically significant increase in the relative weight of the brain in the MSG fed rats group when compared with a healthy control group (p<0.05). Whereas, the relative weight of the brain showed no significant change in the tested oils treated groups.

Behavioural parameters (open-field and Y-maze tests)

Effect on open-field test: The results are presented in (Table 4) showed that there were statistically significant increases in the mean latency in MSG fed rats group when compared with a healthy control group (p<0.05). On the contrary, a significant decrease in the mean latency in MSG fed rats plus oral fish oil, walnut oil, or fenugreek oil (p<0.05).

MSG fed rats group demonstrated a significant reduction in the mean ambulation frequency when compared with a healthy control group (p<0.05), While significant increases in the mean ambulation frequency in MSG fed rats plus oral fish oil, walnut oil and fenugreek oil from the value of MSG fed rats group (p<0.05).

It was observed that there was a significant decrease in the mean rearing frequency in MSG fed rats group when compared with a healthy control group (p<0.05). While significant increases in the mean rearing frequency in MSG fed rats plus oral fish oil, walnut oil and fenugreek oil from the value of MSG fed rats group (p<0.05).

Finally, there was a significant decrease in the mean grooming in MSG fed rats group when compared with a healthy control group (p<0.05), While oral ingestion with fish, walnuts or fenugreek oil showed a significant increase in these values as compared to MSG fed rats group.

Effect on Y-maze test: The results are summarized in (Table 5) revealed that there was a statistically significant decrease in the mean (%) spontaneous alteration in MSG fed rats group when compared with a healthy control group (p<0.05). On the other hand, significant increases in the mean (%) spontaneous alteration in MSG fed rats plus oral fish oil, walnut oil and fenugreek oil from the value of MSG fed rats group (p<0.05).

Some apoptotic factors: Results are presented in (Table 6) and illustrated in (Fig. 2) revealed a significant down regulation in Bcl-2 gene expression level in MSG fed rats group as compared to the healthy control group (p<0.05). Whereas AIF and GFAP protein expression levels, showed a significant increase in MSG fed rats as compared to the control group. On the other hand, oral administration of fish, walnut or fenugreek oils reversed these values to the normal levels.

Glutamate, calcium, malondialdehyde and reduced glutathione levels: From the results of the current study that are presented in (Table 7), it is clear that there was a significant increase in brain glutamate level in MSG-fed rats as compared to the healthy control group (p<0.05). On the other hand, dietary supplementation with fish, walnuts, or fenugreek oil significantly reduced glutamate level in brain tissue. The reduction in glutamate levels in fish, walnuts and fenugreek supplemented group.

From the results of the current study, it is clear that there was a significant decrease in brain calcium level in MSG fed rats as compared to a healthy control group (p<0.05). On the other hand, dietary supplementation with fish, walnuts, or fenugreek oil induced a significant increase in calcium levels in brain tissue. The induction in calcium levels was in fish, walnuts and fenugreek supplemented groups, respectively.

MSG loading induced oxidative stress as marked by a significant increase in MDA level which was accompanied by a significant depletion in GSH level in MSG fed rat group as compared in the healthy control group. While dietary supplementation with fish, walnut or fenugreek oils attenuated the oxidative stress. This reflected in a significant decrease in MDA level with a significant increase in GSH level.

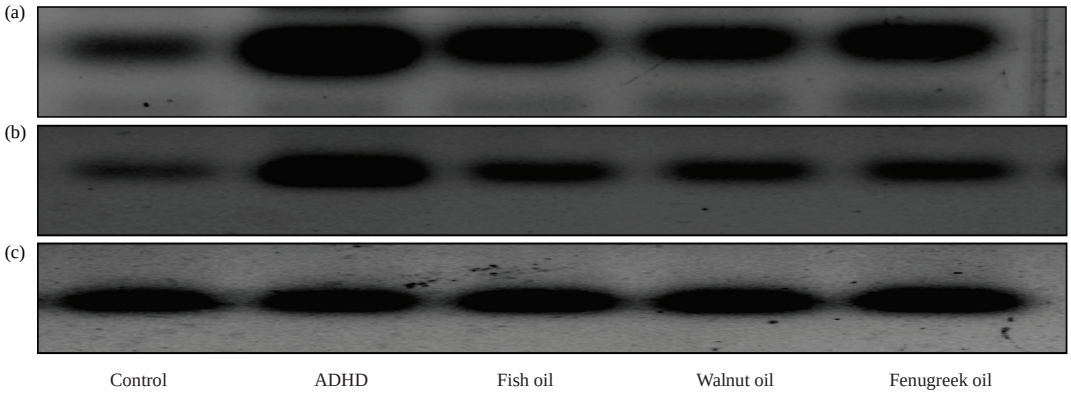

Fig. 2(a-c): Western blot analysis of protein expression of AIF, GFAP and β-actin in brain tissue of all tested groups
(a) AIF protein expression, (b) GFAP protein expression and (c) β-actin protein expression

Table 6: Effect of fish, walnut or fenugreek oil on some apoptotic factors in MSG fed rats

Parameter/group	Relative gene expression of (Bcl-2/β-actin)	Apoptosis-inducing factor (AIF) (relative protein expression)	Glial fibrillary acidic proteins GFAP (relative protein expression)
Healthy control group	1.06±0.03^{a}	1.08±0.07^{d}	1.12±0.09^{e}
MSG fed rats group	0.17±0.03^{d}	6.71±0.16^{a}	7.88±0.09^{a}
MSG fed rats plus fish oil group	0.67±0.09^{c}	2.95±0.06^{b}	2.08±0.04^{d}
MSG fed rats plus walnut oil group	0.68±0.03^{c}	2.86±0.04^{b}	3.55±0.08^{b}
MSG fed rats plus fenugreek oil group	0.80±0.03^{b}	2.06±0.11^{c}	3.34±0.05^{c}
LSD (p<0.05)	0.04	0.09	0.06

Resulting data were presented as mean±SD relative expression levels of three replicates. There was no significant difference between means have the same letters in the same column (p<0.05)

Table 7: Effect of fish, walnut or fenugreek oil on glutamate, calcium, malondialdehyde and reduced glutathione levels in MSG fed rats

Parameter/group	Glutamate (ng mg^{-1})	Calcium (mg %$^{-1}$)	MDA (nmol mg^{-1})	Reduced GSH (nmol mg^{-1})
Healthy control group	3.37±0.03^{c}	9.69±0.06^{a}	19.41±0.17^{e}	85.65±1.17^{a}
MSG fed rats group	5.93±0.08^{a}	8.17±0.09^{c}	67.35±0.75^{a}	31.88±0.76^{e}
MSG fed rats plus fish oil group	3.04±0.11^{e}	9.33±0.14^{b}	31.01±0.45^{c}	68.14±1.06^{c}
MSG fed rats plus walnut oil group	3.18±0.08^{d}	9.33±0.15^{b}	36.01±0.60^{b}	61.24±0.86^{d}
MSG fed rats plus fenugreek oil group	3.81±0.06^{b}	9.70±0.12^{a}	28.82±0.68^{d}	71.61±1.49^{b}
LSD (p<0.05)	0.07	0.11	0.51	0.99

Resulting data were presented as mean±SD (n = 10). There was no significant difference between means have the same letters in the same column (p<0.05)

Table 8: Effect of fish, walnut or fenugreek oil on dopaminergic substances (epinephrine, norepinephrine and dopamine) levels in MSG fed rats

Parameter/group	Adrenaline (ng mg^{-1})	Noradrenaline (pg mg^{-1})	Dopamine (ng mg^{-1})
Healthy control group	9.89±0.11^{a}	32.67±0.68^{a}	58.67±0.76^{a}
MSG fed rats group	3.58±0.13^{e}	11.06±0.33^{e}	29.25±0.59^{e}
MSG fed rats plus fish oil group	5.06±0.03^{d}	25.74±0.18^{b}	42.13±0.86^{d}
MSG fed rats plus walnut oil group	6.23±0. 30^{c}	16.24±0.32^{d}	46.50±0.51^{c}
MSG fed rats plus fenugreek oil group	7.39±0.11^{b}	23.44±0.23^{c}	48.08±0.69^{b}
LSD (p<0.05)	0.15	0.35	0.62

Resulting data were presented as mean±SD relative expression levels of three replicates (n = 10). There was no significant difference between means have the same letters in the same column (p<0.05)

Dopaminergic substances (epinephrine-norepinephrine-dopamine): The results are presented in (Table 8) reported that there was a significant decrease in epinephrine, norepinephrine and dopamine levels in MSG fed rats as compared to the healthy control group (p<0.05). On the other hand, dietary supplementation with fish, walnuts, or fenugreek oils significantly induced epinephrine, norepinephrine and dopamine secretions.

Brain-derived neurotrophic factor and tumour necrosis factor Alfa (TNF-α) levels: BDNF and TNF-α level in brain tissue of different rat groups are summarized in (Table 9). From the results, it is clear that there was a significant increase in brain TNF-α level with a significant decrease in BDNF in MSG-fed rat groups as compared to the healthy control group (p<0.05). While, dietary supplementation with fish, walnuts, or fenugreek oil significantly reversed these parameters to their normal levels.

Table 9: Effect of fish, walnut or fenugreek oil on brain-derived neurotrophic factor (BDNF) and tumour necrosis factor-alfa (TNF-α) levels in MSG fed rats

Parameter/group	Brain-derived neurotrophic factor (BDNF) (pg mg^{-1})	Tumour necrosis factor-alpha (TNF-α) (pg mg^{-1})
Healthy control group	132.14±1.27[a]	38.90±0.66[e]
MSG fed rats group	68.58±0.84[e]	119.55±1.60[a]
MSG fed rats plus fish oil group	104.61±1.07[d]	58.78±0.75[d]
MSG fed rats plus walnut oil group	117.64±1.23[c]	77.24±1.06[b]
MSG fed rats plus fenugreek oil group	123.59±1.03[b]	75.24±0.97[c]
LSD (p<0.05)	0.99	0.96

Resulting data were presented as mean±SD relative expression levels of three replicates (n = 10). There was no significant difference between means that have the same letters in the same column (p<0.05)

Microscopic examinations of brain tissues: Starting with (Fig. 3a-b, respectively), it shows no histopathological alteration in the regions of the brain as well as normal histological structure of the neurons in healthy control rats group consuming the balanced diet. When the MSG-fed rat's group showed nuclear pyknosis and degeneration in most of the neurons diffuse gliosis was also detected was recorded as shown in (Fig. 3c-d).

Oral supplementation with fish oil plus MSG fed rats group showed there was no histopathological alteration in the subiculum. Few neurons showed nuclear pyknosis and degeneration in fascia dentate and hilus. Nuclear pyknosis and degeneration were detected also in some neurons with diffuse gliosis in between in striatum; it was recorded in (Fig. 3e-f).

Also, supplementation of oral walnut and fenugreek oils plus MSG fed rats group showed there was atrophy associated with nuclear pyknosis and degeneration in the neurons in the subiculum. Nuclear pyknosis and degeneration were observed in the neurons in fascia dentate and hilus. The neurons were intact associated with diffuse gliosis in between in striatum, as recorded in the cerebellum was recorded in (Fig. 3g-j).

DISCUSSION

The current investigation was aimed to evaluate the ameliorative effects of walnuts and fenugreek oils in comparison with fish oil on ADHD like behaviour, induced by dietary consumption of MSG in male albino rats.

The present results indicated high content of omega-3 and many essential free fatty acids present in fish oil followed by fenugreek and walnut oils. The results were supported by the studies of Brotas *et al.*[43], Verardo *et al.*[44] and Sulieman *et al.*[45]. Also, fish oil was found to have high reactivity with DPPH[46]. The important factor underlying the increase in the antioxidant capacity of walnut oil was the increase in the content of natural antioxidants such as tocopherols and polyphenols[47]. However, Fenugreek extracts' antioxidant effects could be attributed to their hydrogen-donating activities, which convert the stable violet DPPH radical to the yellow DPPH-H[48].

In the present study, it was observed that MSG fed rat group showed a significant increase in food intake, with a significant decrease in body weight. These are in agreement with the studies of Hijazi *et al.*[49] and Wen *et al.*[50]. Moreover, Rolls[51] reported that by stimulating the orosensory receptors by enhancing the palatability of meals, MSG delivers its flavouring function. MSG consumption for an extended period causes stomach injury, which results in weight loss[52]. Also, the brain weight was found to be decreased significantly with a decrease in rats' body weight following MSG contained diet intake, thus, free glutamic acid from food sources can get into the hypothalamus, injuring and frequently killing its neurons[53]. However, diets rich in omega (3, 6 and 9) reduce food intake[54]. Omega-3 PUFA supplementation improved the bodyweight loss induced by caloric restriction[55].

MSG administration led to only temporary increases in locomotor behaviour (hyperactivity), which was more pronounced during the first few postnatal weeks, followed by a subtle hypoactivity at 2 months of age. Through the open field test, line crossing, rearing, grooming and defecation number parameters of rats can be assessed[56]. Eweka *et al.*[57] revealed that consumption of MSG affects the locomotor activities in the Adult Wistar rats. MSG can cause a significant increase in latency time (decision making), which might be due to the impact on dopaminergic and other neurotransmitter systems in the same brain region[58]. MSG can cause a reduction in spatial learning and memory in rats[59]. However, the behavioural benefits of fish oil were correlated with the restoration of the neurochemical homeostasis[60]. Our results showed that walnut oil treatment ameliorated the memory-impaired mice in the Morris water maze test as recorded by Liao *et al.*[61]. In the same trend, acute treatment by fenugreek oil has an excellent antidepressant effect in animal models of depression via serotonergic pathways[62].

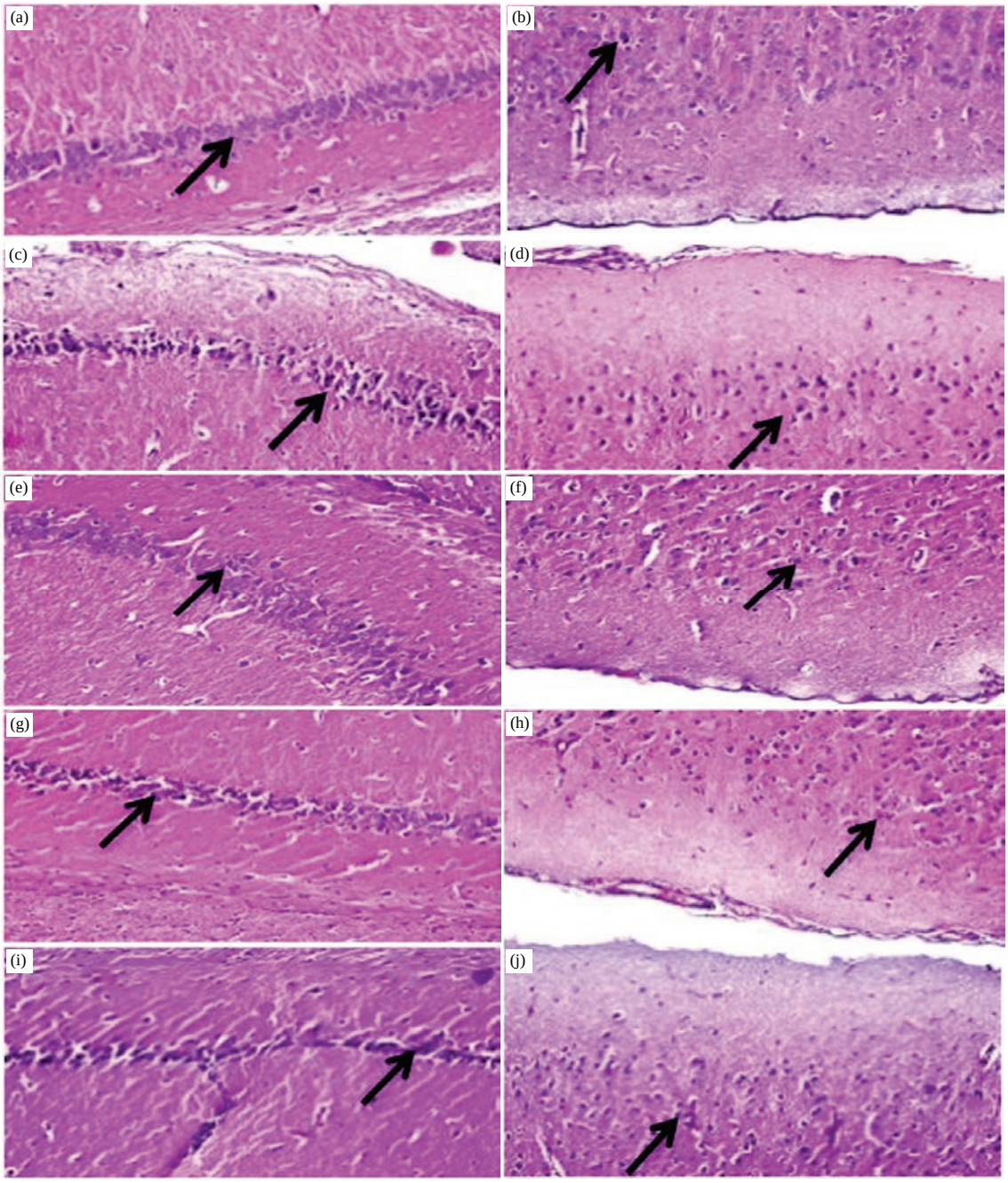

Fig. 3(a-j): Rats brain tissue photograph

(a) Brain tissue section from healthy control group showing Normal histological structure of the cerebral cortex. (b) Brain tissue section from healthy control group showing Normal histological structure of the subiculum of the hippocampus. (c) Brain tissue section ADHD rats fed on MSG contained diet group showing nuclear pyknosis and degeneration were observed in most of the neurons of the cerebral cortex. (d) Brain tissue section ADHD rats fed on MSG contained diet group showing most of the neurons showed nuclear pyknosis and degeneration of the subiculum of the hippocampus. (e) Brain tissue section from fish oil plus MSG contained diet group showing the neurons showed nuclear pyknosis and degeneration of the cerebral cortex. (f) Brain tissue section from fish oil plus MSG contained diet group showing there was no histopathological alteration of the subiculum of the hippocampus. (g) Brain tissue section from walnut oil plus MSG contained diet group showing nuclear pyknosis and degeneration were detected in most of the neurons of the cerebral cortex. (h) Brain tissue section from walnut oil plus MSG contained diet group showing there was atrophy associated with nuclear pyknosis and degeneration in the neurons of the subiculum of the hippocampus. (i) Brain tissue section from fenugreek oil plus MSG contained diet group showing The neurons showed nuclear pyknosis and degeneration of the cerebral cortex. (j) Brain tissue section from fenugreek oil plus MSG contained diet group showing there was atrophy with nuclear pyknosis and degeneration in the neurons of the subiculum of the hippocampus. Using by (H and E ×40) stain

The level of Bcl-2 gene expression was decreased in the MSG treated group as compared to a healthy control group[63]. MSG overfeeding can induce free radical flow obstruction and enhances degenerative diseases. Also, AIF probably plays a role in neuronal apoptosis induced by MSG, although other regulators may also be decisive in mediating neuronal death[64]. However, the level of GFAP in brain tissue was higher in MSG fed rat group than in a healthy control group[8]. Astrocytes respond through the overexpression of GFAP, an intermediate astrocyte-specific cytoskeletal filament protein, in cases of neuronal harm; as its expression is up-regulated in response to CNS injury. Mayurasakorn *et al.*[65] showed that DHA up-regulates the Bcl-2 family of anti-apoptotic proteins including the members Bcl-2, but down-regulates AIF apoptotic proteins expression in DHA cultured neurons. Fish oil, by up-regulating Bcl-2 gene expression, rescues neurons. In animal models, the omega-3 PUFAs found in fish oil modulate neuroinflammation and reduce the amount of GFAP in astrocytes[66]. The effect of fenugreek oil on the GFAP level in the brain might be due to its omega-3 content and antioxidant activity[67].

Excitotoxicity induced by treatment with neonatal MSG has an important pathophysiological effect on adulthood. It can cause a significant increase in brain glutamate level[68]. On the other hand, calcium level was decreased, it might be due to a decrease of catecholamine's levels; as neurotransmitter release is dependent on calcium influx, a disturbance in the concentration gradient of calcium across the cell membrane may decrease the influx of calcium ions into the cell and impaired neurotransmitter release[69]. High concentrations of MSG can also induce oxidative stress in different body organs especially in the brain. Consumption of a high MSG diet by weaning mice for eight weeks resulted in a significant increase in MDA level in brain tissue[8,70]. On the other hand, Owoeye and Salami[71] found that MSG administration in Wister rats induces a significant decrease in GSH level as compared with a control group. However, Omega-3 fatty acids could be influencing the overall glutamate uptake activity. It can reduce large variations in the flow of Ca^{2+} (i.e., no flow versus large flow), which can be at the root of the excitotoxicity of Ca^{2+}[72,73]. Arnal *et al.*[74] reported that the omega6/omega3 ratio could be a marker of antioxidant defence capability. Fish oil administration could increase the level of GSH by gavage in the brain when compared control group[75]. Also, oral administration of walnut oil to rats caused a significant decrease in MDA level in brain tissue[76]. Walnut extracts are rich in polyphenols significantly improved the adverse effects of MSG[77]. The fenugreek possesses a great antioxidant property that has a beneficial effect on the brain because of its high phenolic content and flavonoid components these findings agreed with the results recorded by Dixit *et al.*[67].

Administration of neonatal MSG causes dopaminergic and cholinergic neurons to degenerate, projecting from the arcuate nucleus to median eminence[78]. Grosso *et al.*[79] explained that given the effects of dietary fish oil on the lipid composition of several regions of the brain, it was important to link these changes to the neurochemical and behavioural parameters associated with those regions. Finally, Lei *et al.*[80] illustrated that fenugreek oil had a role in neuro development, neurocognition and neurodegenerative disorders.

The level of BDNF was found to be decreased in neonatal male and female Wistar rats who received MSG diet[81]. In this sense, a reduction of BDNF signalling has been implicated in the pathophysiology of both bipolar and anxiety disorders[82]. Also, administration of MSG in rats for 4 successive weeks showed a significant increase in TNF-α level[83] which might be explained by that high glutamate level could cause severe central nervous system inflammation responses characterized by excess TNF-α production. However, fish oil improves social interaction in a strain of mice displaying a reduction of BDNF levels in various brain regions[84] and decreased TNF-α level in rats exposed to chronic stress as compared with the control group[85]. It might be due to the high DHA content in fish oil. Walnut diets increased hippocampal neurogenesis and restored the decreased hippocampal BDNF expression caused by D-galactose[86]. Walnut oil showed both anti-inflammatory and pro-inflammatory properties[87]. Pretreatment with fenugreek for 7 days reversed lipopolysaccharide mediated behavioural and memory deficits, Also, reversed BDNF levels which depleted by lipopolysaccharide administration to their normal level, these effects due to omega-3 content[88]. The fenugreek oil that contains omega-3 is considered to be an imperative anti-inflammatory factor[89].

The histopathological examination of the brain of MSG fed rats showed a marked change in the tissue. Pyknotic neurons and destruction of the normal layers of the neurons demonstrated MSG toxicity to the pyramidal neurons[71] when rats received omega-3 in fish oil orally via a gastric tube, nerve cells of dentate gyrus with overall picture less severe[90]. Scopolamine-induced histological changes in neurons in the hippocampal CA1 and CA3 regions were prevented by walnut oil[61].

Finally, the current study confirms that supplementation with fish, walnut or fenugreek oils attenuate the pathogenic effects induced by consumption of MSG. The tested oils can also help improve ADHD symptoms. They contain omega-3

fatty acids and also high antioxidant content. Supplements with them may improve mental skills and cognition functions and have positive effects on brain functions. Fenugreek oil is the highest content of antioxidant also had a high content of free fatty acids.

CONCLUSION

Oral administration of MSG in diet can induce ADHD like behaviour in weaning rats. MSG altered behavioural activities, biochemical tests and histopathological examination of the brain. However, supplementation with fish, walnut, or fenugreek oils attenuate the pathogenic effects induced by MSG, these oils can improve ADHD symptoms due to their high content of omega-3 fatty acids and antioxidants. Also, may improve mental skills, cognition functions and have positive effects on other brain functions. The improvement was more pronounced in the fenugreek oil supplemented group than other remaining groups, due to the high content of antioxidants and free fatty acids.

SIGNIFICANCE STATEMENT

This study discovers the beneficial impact of fish, walnut as well as fenugreek oils supplementation against ADHD and its related behavioural, biochemical and brain tissue abnormalities, which might have a significant impact as protective means against the development of ADHD by intake of dietary MSG. This study will help the researchers to uncover the critical areas of hyperactivity disorder that many researchers were not able to explore. As a consequence; this study will open a modern approach for the researchers to discover a safer and more efficient treatment for ADHD.

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