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Pumpkin Seed Oil Exerted Antidepressant Effects through Ameliorating the Oxidative Stress and Neuroinflammation in the Hippocampus of Depressed Rats

Marwa H.A. EL-Azma[1], Nadia M. EL-Beih[2], Karima A. EL-Shamy[1,†], Khaled M.M. Koriem[1], Mahitab I. EL-Kassaby[1], Wael M. EL-Sayed[2,*]

[1]*Medical Physiology Department, Institute of Medical Research and Clinical Studies, National Research Centre, Cairo, Egypt. 12622*

[2]*Department of Zoology, Faculty of Science, Ain Shams University, Cairo, Egypt.11566*

Abstract

We hypothesized that pumpkin seed oil (PSO) exhibits antidepressant activity by modulating oxidative and neuroinflammatory pathways. This would be the first study to report the antidepressant effect of PSO in the hippocampus. Sixty adult male rats were divided into six groups. The first group served as the control. Groups two and three received venlafaxine (20 mg/kg) or PSO (40 mg/kg), respectively. Groups 4-6 were subjected to chronic mild stress (CMS); group four remained untreated, while groups five and six received venlafaxine or PSO, respectively. CMS induced a redox imbalance evidenced by elevated malondialdehyde and nitric oxide levels, and reduced activity of antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase) in the hippocampus. CMS increased neuroinflammation, decreased neurotransmitter levels and downregulated the expression of histamine-N-methyl transferase and tyrosine hydroxylase. These changes were associated with behavioral alterations, including reduced sucrose preference, body weight, and coat state score, alongside increased immobility time in the tail suspension test and freezing time in the open field test, and decreased rearing activity. Administration of PSO reversed all neurochemical and behavioral changes induced by CMS by attenuating oxidative stress and neuroinflammation induced by depression. PSO demonstrated efficacy comparable to or better than venlafaxine in mitigating these effects.

***Keywords*:** Chronic mild stress; Depression; Na^+/K^+-ATPase; Neurotransmitters; Tyrosine hydroxylase

1. Introduction

Approximately 350 million people worldwide suffer from depression, experiencing symptoms such as anhedonia, sadness, lack of interest, feelings of worthlessness, indifference, anxiety, appetite disturbances, and suicidal thoughts [1]. Both human and animal studies have highlighted the hippocampus as one of the brain regions most affected by depression [2]. Depression induced by chronic mild stress (CMS) serves as an environmental model that mimics depression induction in humans following exposure to stressful life events. This model successfully mirrors depression symptoms and pathophysiology, making it useful for testing new potential antidepressants [3]. Stressors in this model trigger hypercortisolemia, leading to oxidative stress and neuroinflammation depending on their duration, nature, and intensity [4].

Current antidepressants operate by altering neurotransmitter concentrations in the synapse according to the monoamine theory of depression. However, these antidepressants have a high relapse rate and are effective in treating only one-third of diagnosed depressed patients [5,6]. Conventional antidepressants have side effects such as weight gain, sexual dysfunction, dry mouth, and dizziness. They also carry risks of withdrawal symptoms and interactions with other medications [7].

Nutraceuticals and dietary supplements have demonstrated therapeutic potential in depression by modulating mood and providing essential components for normal bodily function [8]. Evidence regarding the efficacy of nutraceuticals in treating depression is growing but less robust compared to conventional antidepressants. Some nutraceuticals such as St. John's Wort have shown efficacy comparable to antidepressants in mild to moderate depression. Others, like S-adenosylmethionine, have shown promising results but require further research [8,9].

Pumpkin, an edible vegetable, is considered a nutraceutical due to its anti-inflammatory, antioxidative, and hypolipidemic properties. Pumpkin seed oil (PSO) is rich in tocopherols, phytosterols, and vitamins, which possess anti-inflammatory and antioxidant properties [11,12]. In this study, we investigated the potential of PSO to alleviate depressive symptoms by ameliorating oxidative stress and neuroinflammation in the hippocampus of a chronic mild stress-induced depression rat model.

*Corresponding author e-mail: waelelhalawany@hotmail.com,; (Wael M. EL-Sayed).
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2. Materials and Methods

2.1. Animals and diet

Eighty adult male rats weighing 160-180 g were obtained from the animal house of VACSERA (Helwan, Egypt). The current study was approved by the Ethics Committee of the National Research Centre (Approval No. 16004) and conducted in accordance with the guidelines of the National Institutes of Health (NIH) for the care and use of laboratory animals. Animal food was procured from El Hayani Co. (Al-Tal Al-Kabir, Ismailia, Egypt).

2.2. Materials

Venlafaxine (IDIXOR® 75 mg) was purchased from IDAPI (Cairo, Egypt). PSO (Pepon capsules 300 mg) was supplied by the ACPM Plants (Cairo, Egypt).

2.3. Chronic mild stress paradigm

The experimental rats were exposed daily to one or two stressors according to a specific schedule repeated weekly for six weeks in the stressed group. The schedule was as follows: on the 1st day, forced swimming at 45°C for 5 minutes; on the 2nd day, cage tilting and wet bedding; on the 3rd day, shaking for 10 minutes; on the 4th day, tail pinching for one minute; on the 5th day, forced swimming at 4°C for 5 minutes; on the 6th day, food deprivation for 24 hours; and on the 7th day, water deprivation for 24 hours with overnight illumination

2.4. Experimental design

The rats were allowed one week for acclimatization before being randomly assigned to eight groups, with 10 rats per group. The non-stressed groups (Groups 1 to 3) remained under normal, stress-free conditions throughout the experiment. The stressed groups (Groups 4 to 6) were subjected to the previously described stress paradigm. Group 1 (Control): Rats remained on a standard diet for six weeks. Group 2: Rats received oral administration of 20 mg/kg venlafaxine daily for four weeks [13]. Group 3: Rats were orally administered 40 mg/kg of pumpkin seed oil (PSO) daily for four weeks [14]. Group 4: Rats were exposed to the chronic mild stress (CMS) paradigm for six weeks. Groups 5 and 6: Rats were exposed to the CMS paradigm for six weeks and received venlafaxine or PSO orally from the 3rd to the 6th week. Throughout the study, the animals' body weights were measured weekly to monitor any changes.

2.5. Behavior evaluation

2.5.1. Sucrose preference

In the adaptation period, the animals were habituated to uptake sucrose. We put two bottles of sucrose solution (1%, w/v) in every cage for 72 h. Then, one bottle was replaced by a bottle filled with tape water. The consumed amount of both sucrose and water was assessed to calculate the sucrose preference [13].

2.5.2. Coat state score

Each rat was visually inspected for coat condition in specific regions: head, neck, dorsal coat, ventral coat, tail, forepaws, hind legs, and genital region. A kempt coat received a score of (1) in each region, while an unkempt coat received a score of (0). The coat state score for each animal was calculated by summing the scores across these eight regions. Subsequently, the average score for the entire group was compared to the average score of the control group [14].

2.5.3. Tail suspension test (TST)

The animals were hanged separately from their tails on 58 cm height from the floor for 5 minutes and the immobility time was recorded after the struggling phase [15].

2.5.4. Open field test

A special cage of 75 cm × 75 cm × 40 cm dimensions was divided in to 25 squares, the animals were tested individually by putting each one in the central square and the animal was allowed to explore the environment for five minutes and the number of crossings, rearings, central square entries and freezing time were calculated in each session [13].

2.6. Sampling

The animals were decapitated, and the brains were dissected rapidly on ice to collect hippocampus. It was homogenized in 1ml phosphate buffer (pH 7.4) and centrifuged at 3,000 rpm/min and 4 °C for 10 min, finally the tissue homogenate was stored at -80 °C for further analyses.

2.7. Biochemical assessments

The oxidative stress parameters (nitric oxide and malondialdehyde) and the antioxidant enzymes activities (glutathione peroxidase (GPx), superoxide dismutase (SOD), and catalase) were determined spectrophotometrically using commercial kits purchased from Biodiagnostic Co. (Giza, Egypt) as described elsewhere [8-12]. The activity of Na^+/K^+-ATPase activity was assessed spectrophotometrically as previously described [13]. The proinflammatory markers, interleukin-1β (IL-1β) and tumor necrosis factor-a (TNF-α), were estimated using ELISA kits supplied by SinoGeneclon Co. (Hangzhou, China) following the instructions of the manufacturer. Neurotransmitters (dopamine, norepinephrine, serotonin, and gamma-

aminobutyric acid (GABA)) were measured using ELISA kits supplied by SinoGeneclon Co. (Hangzhou, China) as described elsewhere [20,21].

2.8. Quantitative real time PCR (qPCR)

The RNA was extracted from hippocampus using PureLink® RNA Mini Kit manufactured by Invitrogen™, Thermo Fisher Scientific (Waltham, Massachusetts, USA) according to the instructions of the manufacturer. Then, RNA isolated was transformed to cDNA by a Kit supplied from Applied Biosystems, (Massachusetts, USA). qPCR was performed using Fast SYBR™ Green Master Mix (Applied Biosystems, USA) on 96 Real-Time PCR System (Bio-rad, USA). The primers for tyrosine hydroxylase (cat no. QT00185024), histamine-N-methyl transferase (cat no. QT00183750), and glyceraldehyde 3-phosphate dehydrogenase (cat no. QT00199633) were purchased from QuantiTect ® Primer Assay (Qiagen, Germany). The fold change for tyrosine hydroxylase $2^{-\Delta\Delta CT\ (Th)}$ and histamine-N-methyl transferase $2^{-\Delta\Delta CT\ (Hnmt)}$ was calculated by relative method and normalized against glyceraldehyde 3-phosphate dehydrogenase.

2.9. Statistical analyses

The sample size was calculated using the G Power software (statistical power at 80% and α at 0.05). The data distribution was tested by the Kolmogorov-Smirnov test. The data were analyzed using one-way analysis of variance (ANOVA), then post hoc least significant test (LSD) using Statistical Package of Social Sciences (IBM statistics SPSS version 25.0, US). The data are expressed as means ± SEM and were considered significant at p <0.05.

3. Results

3.1. Body weight measurements

Chronic mild stress paradigm significantly diminished the body weight in the stressed animals (p <0.05) by 7.9%, 11.4%, 13.1%, 15.1%, 20.1%, and 24.2%, respectively in comparison to control group from the 1st week to the 6th week **(Fig. 1A).** Administration of venlafaxine or PSO to the stressed rats significantly mitigated body weight from the 3rd week till the 6th week. Venlafaxine mitigated the decrease in body weight by 2.5%, 6.3%, 13.6%, and 19.6% over the four weeks, respectively. In comparison, PSO provided a better amelioration, alleviating the reduction in body weight by 7.0%, 12.1%, 17.8%, and 23.7% during the same period **(Fig. 1A)**.

3.2. Behavior evaluation assessments

3.2.1. Sucrose preference test

The sucrose preference was significantly (p <0.05) diminished by 19.8%, 41%, 42%, 42.4%,43.7%, and 44.6% respectively in comparison to control from the 1st week to the 6th week **(Fig. 1B).** The uptake of venlafaxine or pumpkin seed oil significantly attenuated the sucrose preference and hedonic behavior from 3rd week to 6th week in comparison to the CMS group. Venlafaxine mitigated the decrease in sucrose preference by 13.9%, 37.1%, 52%, and 62.3% over the four weeks, respectively. In comparison, PSO was as equivalent as venlafaxine in ameliorating the reduction in sucrose preference by 13.8%, 39.3%, 51.8%, and 62% during the same period **(Fig. 1B)**.

3.2.2. Coat state score

The coat state score was significantly (p <0.05) reduced in the CMS group by 19.7%, 32.7%, 34.9%, 45.9%, 61.6%, and 85.2%, respectively compared to the control group from the 1st week to the 6th week **(Fig. 1C).** Administration of venlafaxine or PSO to the stressed rats significantly ameliorated the coat state score from the 4th to the 6th week compared to the CMS group. Venlafaxine mitigated the reduction in coat state score by 18.2%, 95.7%, and 466% over the four weeks, respectively. In comparison, PSO provided a better amelioration, mitigating the reduction in coat state score by 24.2%, 130.4%, and 523% during the same period **(Fig. 1C)**.

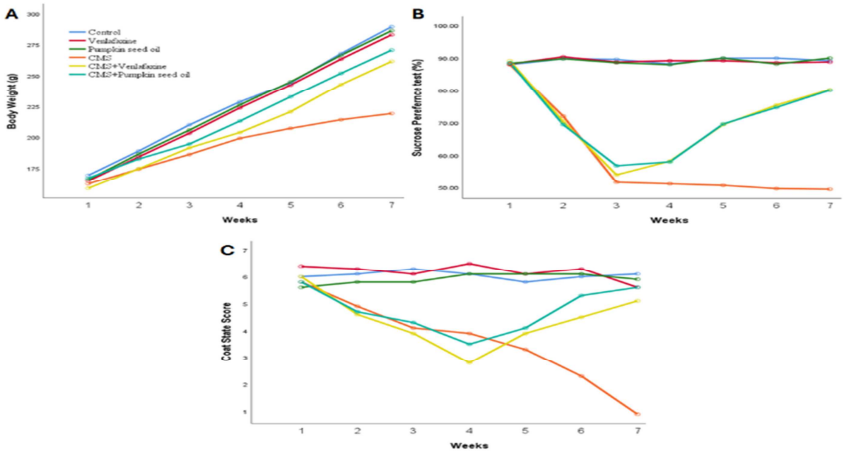

Figure 1: Effect of pumpkin seed oil (PSO) and venlafaxine (Venla) on (A): the body weight; (B): the sucrose preference test; (C): on the physical score test, in chronic mild stress (CMS)-induced depression rat model.

3.2.3. Tail suspension test (TST)

The immobility time significantly increased in the CMS group in comparison to the control group by 347%. Treatment of the stressed groups with venlafaxine and PSO resulted in statistically significant declines in the immobility time as they reduced the immobility time by 74% and 76%, respectively compared to the CMS group (Fig. 2).

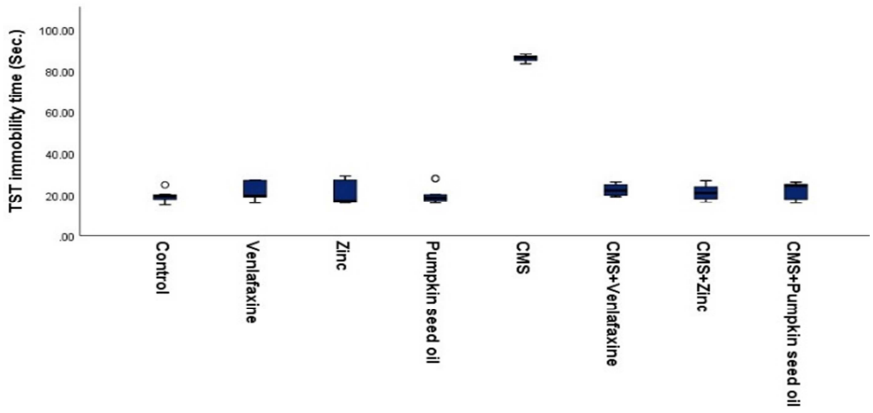

Figure 2: Effect of pumpkin seed oil (PSO) and venlafaxine (Venla) on the immobility time in the tail suspension test (TST) of chronic mild stress (CMS)- induced depression rat model. [a]Significant compared to control, [b]significant compared to CMS group at (p <0.05).

3.2.4. Open Field test

Significant reductions in the crossed lines, rearing, and numbers of central square entries were reported in the stressed group compared to the control animals by 90%, 91%, and 94% respectively. The stressed animals also showed a significant elevation in the freezing time by 427% when compared to the control animals. Treating the depressed rats with venlafaxine and PSO opposed the previous effects and restored normal behavior. Venlafaxine and PSO elevated the percentage of crossed lines by 845% and 863%, respectively compared to the CMS group. Venlafaxine and PSO also elevated the percentage of rearing and number of central squares by 835% and 935% (rearing), and 1540% and 1500% (number of central squares) respectively compared to the CMS group. Treatment with venlafaxine or PSO diminished the freezing time by 75% (**Table 1**).

Table 1: Effect of pumpkin seed oil (PSO) and venlafaxine (Venla) on the open field test in chronic mild stress (CMS)-induced depression rat model

	Control	**Venla**	**PSO**	**CMS**	**CMS+Venla**	**CMS+PSO**
No. of crossed Lines	40.1 ± 1.3	39.5 ± 1.6	39.3 ± 1.6	4.0 ± 0.7 [a]	37.8 ± 1.1[b]	38.5 ± 1.2[b]
No. of rearing	25.5 ± 1.9	29.1 ± 1.5	26.9 ± 1.7	2.3 ± 0.3 [a]	21.5 ± 1.7 [b]	23.8 ± 1.6 [b]
Freezing time (S)	32.8 ± 3.0	32.3 ± 2.9	29.5 ± 2.7	173.0 ± 6.4[a]	43 ± 1.9[b]	44.2 ± 1.9[b]
Central square entries	8.6 ± 0.4	8.7 ± 0.7	8.5 ± 0.5	0.5 ± 0.17[a]	8.2 ± 0.5 [b]	8.7 ± 0.5 [b]

Data are expressed as Means ±SEM. n= 10. The data were analyzed using one-way analysis of variance (ANOVA) followed by *post hoc* least significant test

3.3. Biochemical analyses measurements

In the hippocampus of the CMS rats, the oxidative stress indices (nitric oxide (NO) and malondialdehyde (MDA)) were significantly (p <0.05) elevated by 103% and 99.4%, respectively and the antioxidant enzymes activities (GPx, SOD, and catalase) were significantly (p <0.05) diminished by 65%, 55.7%, and 75%, respectively. A significant reduction in the Na^+/ K^+-ATPase activity by 26% compared to the control group was also reported in the CMS group (**Table 2**). Venlafaxine elevated the activities of GPx, SOD, catalase, and Na^+/ K^+-ATPase by 174%, 115%, 240%, and 32%, respectively compared to the CMS group. PSO was as equipotent as venlafaxine and resulted in significant attenuations of all the oxidative stress parameters investigated. PSO elevated the activities of GPx, SOD, catalase, and Na^+/ K^+-ATPase by 177%, 116%, 260%, and 33%, respectively compared to the CMS group. Venlafaxine reduced the NO and MDA levels by 45% and 44%, respectively compared to the CMS group. PSO reduced the NO and MDA levels by 47% and 47%, respectively compared to the CMS group **(Table 2).**

Table 2: Effect of pumpkin seed oil (PSO) and venlafaxine (Venla) on oxidative stress parameters levels in hippocampus tissue of chronic mild stress (CMS)-induced depression rat model

	Control	Venla	PSO	CMS	CMS+Venla	CMS+PSO
GPx (U/g)	781 ± 10	765 ± 18	779 ± 14	273 ± 23[a]	748 ± 19[b]	756 ± 15[b]
SOD (U/g)	2370 ± 150	2300 ± 170	2390 ± 150	1050 ± 41[a]	2260 ± 180[b]	2270 ± 160[b]
Catalase (µmol H_2O_2/min/mg)	0.21 ± 0.02	0.20 ± 0.02	0.19 ± 0.02	0.05 ± 0.01[a]	0.17 ± 0.01[b]	0.18 ± 0.01[b]
NO (µmol/g)	0.53 ± 0.02	0.56 ± 0.02	0.57 ± 0.01	1.08 ± 0.05[a]	0.59 ± 0.01[b]	0.56 ± 0.01[b]
MDA (µmol/g)	19.6 ± 0.9	19.0 ± 0.8	18.8 ± 1.2	39.1 ± 2.1[a]	22.0 ± 0.9[b]	20.8 ± 0.8[b]
Na^+/K^+-ATPase (nmol pi/min/ mg)	1300 ± 18	1270 ± 25	1280 ± 24	960 ± 26[a]	1270 ± 11[b]	1280 ± 13[b]

Data are expressed as Means ±SEM. n= 10. The data were analyzed using one-way analysis of variance (ANOVA) followed by *post hoc* least significant test (LSD). [a]Significant compared to control, [b]significant compared to the stressed group at (p <0.05). Abbreviations; GPx: glutathione peroxidase, SOD: superoxide dismutase, NO: nitric oxide, MDA: malondialdehyde.

The levels of the neurotransmitters measured (serotonin, norepinephrine, dopamine, and GABA) in the hippocampus of the CMS rats significantly (p <0.05) decreased by 62.6%, 75.6%, 35.3%, and 65.5%, respectively **(Table 3).** Venlafaxine elevated these neurotransmitters (serotonin, norepinephrine, dopamine, and GABA) significantly compared to the CMS group by 164%, 302%, 52%, and 189%, respectively. PSO also elevated these neurotransmitters (serotonin, norepinephrine, dopamine, and GABA) significantly compared to the CMS group by 162%, 305%, 53%, and 222%, respectively.

The proinflammatory markers (IL-1β and TNF-α) were elevated in the hippocampus of the stressed rats by 185% and 148.2%, respectively compared to the control group**).** These detrimental effects of the CMS on the neurotransmitters and proinflammatory markers measured were ameliorated by the treatment with venlafaxine or PSO. Venlafaxine reduced IL-1β and TNF-α by 58% and 50%, respectively. While PSO reduced IL-1β and TNF-α by 60% and 55%, respectively (**Table 3**).

Table 3: Effect of pumpkin seed oil (PSO) and venlafaxine (Venla) on the levels of cytokines and neurotransmitters in the hippocampus of chronic mild stress (CMS)-induced depression rat model

	Control	Venla	PSO	CMS	CMS+Venla	CMS+PSO
TNF-α (ng/g)	17.6 ± 1.3	17.2 ± 1.4	16.1 ± 1.5	43.7 ± 1.8[a]	21.7 ± 1.7[b]	19.6 ± 1.5[b]
IL-1β (ng/g)	0.70 ± 0.04	0.71 ± 0.03	0.67 ± 0.03	2.00 ± 0.07[a]	0.85 ± 0.03[b]	0.81 ± 0.04[b]
Serotonin (ng/g)	701 ± 14	702 ± 14	705 ± 12	262 ± 13[a]	691 ± 14[b]	687 ± 15[b]
NE (ng/g)	756 ± 14	757 ± 14	760 ± 12	184 ± 7.3[a]	739 ± 15[b]	746 ± 14[b]
DA (ng/g)	1070 ± 13	1070 ± 14	1080 ± 16	692 ± 20[a]	1050 ± 15[b]	1060 ± 14[b]
GABA (µmol/g)	0.29 ± 0.02	0.28 ± 0.02	0.31 ± 0.02	0.09 ± 0.01[a]	0.26 ± 0.03[b]	0.29 ± 0.02[b]

Data are expressed as Means ±SEM. n= 10. The data were analyzed using one-way analysis of variance (ANOVA) followed by *post hoc* least significant test (LSD). [a]Significant compared to control, [b]significant compared to the stressed group at p <0.05. Abbreviations; TNF-α: tumor necrosis factor-α, IL-1β: interleukin-1β, NE: norepinephrine, DA: dopamine, GABA: gamma-aminobutyric acid.

3.4. Quantitative real time PCR (qPCR) results

Chronic mild stress paradigm reduced the expression of *Th* and *Hnmt* significantly (p <0.05) by 62.6% and 30%, respectively. The administration of PSO or venlafaxine almost normalized the mRNA expression levels for both genes. Venlafaxine attenuated the reduction caused by CMS in *Th* and *Hnmt* by 139% and 28%, respectively. PSO attenuated the reduction caused by CMS in *Th* and *Hnmt* by 184% and 23%, respectively. **(Fig. 3A and 3B).**

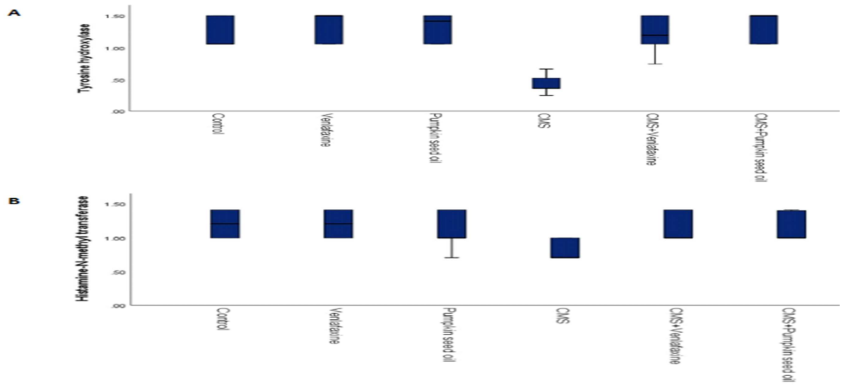

Figure 3: Effect of pumpkin seed oil (PSO) and venlafaxine (Venla) on fold change of (A) Tyrosine hydroxlase (*Th*) (B) Histamine-N-methyl transferase (*Hnmt*) in hippocampus tissue of chronic mild stress- induced depression rat model. [a]Significant compared to control, [b]significant compared to the stressed group at (p <0.05).

4. Discussion

The hypothesis of this study—that pumpkin seed oil (PSO) alleviates depressive symptoms by reducing oxidative stress and neuroinflammation in the hippocampus of a chronic mild stress-induced depression rat model—was confirmed by biochemical, molecular, and behavioral results. The chronic mild stress (CMS) model mimics depression induced by stressful life events in humans [3]. In this study, the CMS group exhibited significantly reduced sucrose intake, indicative of anhedonia, as well as decreased body weight and coat condition scores, mirroring the lack of self-interest and appetite disturbances seen in depressed individuals. Depressive-like behavior was further evidenced by a significant decrease in immobility time in the tail suspension test, consistent with prior research [25-27].

Anxiety-like behavior was indicated by reduced crossings, rearings, and entries into the central square, along with increased freezing time in the open field test, aligning with previous findings [28]. These behavioral changes correlated with disturbances in oxidative stress and resulting neuroinflammation [29]. Elevated levels of pro-inflammatory cytokines (TNF-α and IL-1β) and markers of oxidative stress (MDA and $^{\bullet}$NO) reflected neuroinflammation and oxidative stress, respectively, alongside reduced activity of antioxidant enzymes (catalase, superoxide dismutase, and glutathione peroxidase), consistent with prior studies [30]. The dysfunctional Na+/K+-ATPase activity resulting from oxidative stress also disrupted neurotransmitter signaling and transport [30-33], contributing to reduced levels of serotonin, norepinephrine, dopamine, and GABA observed in our study.

Administration of venlafaxine successfully attenuated hedonic behavior, normalized body weight, and improved performance in behavioral tests such as the TST and open field test, consistent with previous reports [36, 37]. These improvements were linked to reduced kynurenine/tryptophan ratios and increased levels of serotonin, norepinephrine, GABA, and dopamine [27, 28]. Venlafaxine also upregulated tyrosine hydroxylase *(Th)* mRNA expression and mitigated oxidative stress and neuroinflammation while enhancing Na^+/K^+-ATPase activity [27, 33, 38].

Despite its efficacy, venlafaxine can cause serotonin toxicity and various side effects such as hypertension, hypercholesterolemia, diabetes, nausea, and narrow-angle glaucoma [33]. Furthermore, one-third of depressed individuals do not respond to current antidepressants, and relapse rates are high [32]. Consequently, research is exploring alternative therapies, supplements, and nutraceuticals as potential treatments. Nutraceuticals offer promise in alleviating depressive symptoms by providing essential components that positively impact mental health [10].

Pumpkin seed oil (PSO) is rich in bioactive compounds including carotenoids, tocopherols (vitamins E and C), phytosterols, minerals, and unsaturated fatty acids [18]. In the current study, PSO administration reduced oxidative stress by lowering MDA and $^{\bullet}$NO levels and increasing antioxidant enzyme activities (catalase, SOD, and GPx), consistent with previous findings [27, 42]. This antioxidative effect likely contributed to the elevation of Na^+/K^+-ATPase activity [43], while PSO also attenuated neuroinflammation, possibly through modulation of toll-like receptor 4, nuclear factor kappa B pathways, and down-regulation of cyclooxygenase-2 [42, 44]. PSO-treated groups showed increased neurotransmitter levels and upregulated the expression of *Th* and *Hmnt*, correlating with improved hedonic behavior, body weight, coat condition, and reduced immobility time in the TST, and normalized parameters in the open field test.

Several studies have demonstrated the effectiveness of PSO components in modulating brain function and neurotransmitter levels via antioxidative and anti-inflammatory pathways. For instance, stigmasterol regulates monoamine oxidase, acetylcholine, and GABA [45], while β-sitosterol down-regulates ERK/p38 and NF-κB pathways [39], and tocopherols suppress cyclooxygenase-2 and neuroinflammation [46]. Thus, PSO's antidepressant activity may stem from the cumulative effects of these compounds.

5. Conclusion

While conventional antidepressants remain essential for treating moderate to severe depression due to their proven efficacy, nutraceuticals such as pumpkin seed oil (PSO) offer a promising complementary approach with potentially fewer

side effects. Further research is necessary to fully understand PSO's efficacy, safety, and optimal use in managing depression, including potential interactions with other drugs. This study demonstrated that PSO effectively alleviated chronic mild stress-induced depression in rats by mitigating oxidative insult, normalizing neurotransmitter levels, and reducing neuroinflammation in the hippocampus. These findings were supported by significant improvements in the animals' behavior. Future studies should investigate PSO's effects on specific molecular pathways, such as the NF-κB pathway.

Conflict of interest

Authors have no conflict of interest to report

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