Future Natural Products
doi: 10.34172/fnp.195 2024;10(2):67-75 https://fnp.skums.ac.ir

Original Article

Effects of *Tanacetum polycephalum* Essential Oil on Depression: Enhancement of Hippocampal Antioxidant Capacity

Najmeh Asgharzadeh[1], Khadijeh Reisi[1], Hossein Amini-Khoei[1], Zahra Lorigooini[1], Marzieh Mardani[1], Elham Bijad[1], Diana Shahrani Korrani[1], Mohamad Shahrani Korrani[2], Mehrdad Shahrani Korrani[1]*

[1]Medical Plants Research Center, Basic Health Sciences Institute, Shahrekord University of Medical Sciences, Shahrekord, Iran
[2]Neurosciences Research Center, Isfahan University of Medical Sciences, Isfahan, Iran

Abstract

Background and aims: Depression is a complex, persistent, and debilitating condition that affects individuals worldwide. This study aimed to investigate the antidepressant properties of essential oil derived from *Tanacetum polycephalum*.

Methods: Fifty-six mice were randomly assigned to seven groups: (1) the control group, which received normal saline (10 mL/kg); (2) the depressed model group, which received reserpine (5 mg/kg); (3 to 6) groups that received reserpine in combination with *T. polycephalum* essential oil at doses of 25, 50, 75, and 100 mg/kg, respectively; and (7) the positive control group, which was administered Fluoxetine (20 mg/kg) alongside reserpine. All injections were administered via the intraperitoneal route. Depression was assessed using the forced swimming test (FST), Splash test, and open field test (OFT). Levels of malondialdehyde (MDA) and total antioxidant capacity (TAC) were measured in hippocampal and serum samples.

Results: The administration of reserpine significantly prolonged the duration of immobility in the FST ($P<0.001$), whereas *T. polycephalum* essential oil significantly reduced this duration across all four doses tested ($P<0.05$, $P<0.001$). In the Splash test, both the duration and frequency of licking were significantly reduced in the reserpine group ($P<0.001$), while all doses of *T. polycephalum* essential oil significantly increased both the duration and frequency of licking ($P<0.001$). The reserpine group exhibited a significant reduction in the number of line crossings, rearing, and grooming behaviors ($P<0.01$). Notably, *T. polycephalum* essential oil at doses of 50, 75, and 100 mg/kg significantly enhanced these behaviors ($P<0.001$). Furthermore, reserpine treatment resulted in a significant decrease in TAC and an increase in MDA levels in both the hippocampus and serum. In contrast, *T. polycephalum* essential oil at various doses significantly increased TAC and decreased MDA levels ($P<0.001$).

Conclusion: *Tanacetum polycephalum* essential oil exhibited significant efficacy in alleviating depression-like behaviors induced by reserpine. This effect is likely associated with its capacity to reduce oxidative stress parameters. The findings underscore its potential as a natural antidepressant agent. Further research is warranted to validate its therapeutic applications.

Keywords: Depression, Essential oil, Malondialdehyde, Grooming, Rearing, Total antioxidant capacity

***Corresponding Author:**
Mehrdad Shahrani Korrani,
Email: mehrdadeshahrani2000@gmail.com

Received: January 4, 2025
Revised: April 27, 2025
Accepted: May 5, 2025
ePublished: October 18, 2025

Cite this article as: Asgharzadeh N, Reisi K, Amini-Khoei H, Lorigooini Z, Mardani M, Bijad E. Effects of *Tanacetum polycephalum* essential oil on depression: Enhancement of hippocampal antioxidant capacity. Future Nat Prod. 2024;10(2):67–75. doi: 10.34172/fnp.195

Introduction

Depression is a prevalent, debilitating, and hazardous condition that significantly impacts individuals' lives and behaviors, affecting millions globally. Currently, it is estimated that approximately 5% of adults worldwide experience depression, making it a leading cause of disability (1). Depression ranks as the fourth most significant health issue today and is anticipated to become the second leading cause of global disability by 2030. Research conducted in Iran indicates that nearly 7 million individuals struggle with depression, with 15%-25% of the population experiencing mild to severe forms of the condition (2-4).

The implications of depression extend beyond personal suffering; it adversely affects performance in various domains, including education, employment, and interpersonal relationships, and is associated with elevated rates of crime and substance misuse. Furthermore, individuals with depression face a heightened risk of suicide compared to those with other mood disorders.

Pharmacological treatments for depression often entail numerous side effects and potential interactions with other medications and foods. Approximately 30% of patients exhibit an inadequate response to existing treatments, while the remaining 70% do not achieve full recovery. Therefore, it is essential to identify effective therapies that present fewer adverse effects. Recent studies have increasingly emphasized the exploration of herbal remedies as complementary or alternative options for managing depression, owing to their affordability and potential for safe interaction with prescribed medications (5). The species *Tanacetum polycephalum* is an aromatic, perennial plant. Laboratory studies have demonstrated the antioxidant, antibacterial, and anticancer properties of the plant's essential oil (6,7). Phytochemical analysis conducted via gas chromatography-mass spectrometry (GC-MS) has identified the presence of various terpene compounds in the essential oil, including camphor, 1,8-cineole, cys-togen, trans-togen, cis-chrysanthenol, and borneol.

Research has reported sedative and anxiolytic effects associated with 1,8-cineole, as well as antidepressant effects linked to borneol. Additionally, borneol has been noted for its anti-ischemic and antioxidative stress properties, while camphor has also been recognized for its antioxidative stress effects. In this study, reserpine was utilized to induce a depressive state in mice (4,8,9). Fluoxetine, a widely prescribed initial treatment for depression and one of the most commonly used antidepressant medications, served as the positive control in the investigation of the antidepressant effects of *Tanacetum polycephalum* essential oil (10).

Materials and Methods

Plant preparation, essential oil extraction, and identification of phytochemical compounds of Tanacetum polycephalum

The *T. polycephalum* plant was collected during its flowering stage from the mountainous regions and natural habitats of Ab Nahr in the Kohgiluyeh and Boyer Ahmad provinces, located at an altitude of 3220 m (longitude 51°39', latitude 30°40'). The samples were authenticated at the Botanical Department of the Shahrekord Research Center and subsequently documented in the Herbarium of the Medicinal Plants Research Center at Shahrekord University of Medical Sciences. Essential oil extraction was performed using a Clevenger apparatus through water distillation. Specifically, 50 g of powdered plant material and 500 mL of water were placed in a distillation flask and heated to achieve a distillation rate of 2–3 mL per minute. After a duration of four hours, the essential oil was collected and dried with anhydrous sodium sulfate for 24 hours (11). The phytochemical components of the essential oil were analyzed using a Thermo Finnigan Trace GC-MS instrument at Shahid Beheshti University of Tehran.

Experimental animals

Fifty-six mice were maintained under standard laboratory conditions and randomly divided into seven groups for the study. The groups were as follows:

1. Control group: Administered normal saline at a dosage of 10 ml/kg.
2. Depression model group: Received reserpine at a dose of 5 mg/kg to induce a depressive state.
3. Treatment groups: Groups 3 through 6 were administered *Tanacetum polycephalum* essential oil at doses of 25, 50, 75, and 100 mg/kg, respectively, 18 hours after the reserpine injection.
4. Positive control group: Received reserpine followed by Fluoxetine at a dose of 20 mg/kg, administered 18 hours later.

All injections were conducted intraperitoneally (10).

Behavioral assessments were performed thirty minutes following the administration of normal saline and essential oil. The tests included open field test (OFT), Splash test, and forced swimming test (FST).

Subsequently, the mice were fully anesthetized using ketamine and xylazine at doses of 100 mg/kg and 10 mg/kg, respectively. Blood samples were collected for further analysis (12). Under complete anesthesia, the heads of the mice were severed, and the brain tissue was stored at -80 °C until the necessary experiments were conducted (Figure 1).

Plant preparation, essential oil extraction, and identification of phytochemical compounds

The leaves and stems of *T. polycephalum* were collected in June 2017 during the flowering stage from the mountainous regions and natural habitats of Ab Nahr in the Kohgiluyeh and Boyer Ahmad provinces. The collection site is located at an altitude of 3220 m, with coordinates of 51°39' E longitude and 30°40' N latitude. The identification of the samples was conducted in the Botany Department of the Research Center in Chaharmahal and Bakhtiari province. The essential oil was extracted using water distillation in a Clevenger apparatus. Fifty grams of plant powder and 500 mL of water were placed in a distillation flask and heated until the distillation rate reached 2 to 3 mL/min. Using this method, the essential oil was collected after four hours and subsequently dried over anhydrous sodium sulfate for 24 hours. The phytochemical components of the essential oil were analyzed using a gas chromatography-mass spectrometer (GC/MS), specifically the TRACE MS model manufactured by Thermo Quest-Finnigan, located at Shahid Beheshti University in Tehran. The column used was a DB5 type, with a length of 30 m and an internal diameter of 0.25 mL. The thermal programming of the column ranged from 60 °C to 250 °C, with a temperature increase rate of 5 °C per minute. Helium, with a purity level of 99.999%, served as the carrier gas. The injection method was split, with an injection volume of 0.2 µL and a gas flow rate of 1.1 mL/min. The identification of compounds was performed using relative inhibition

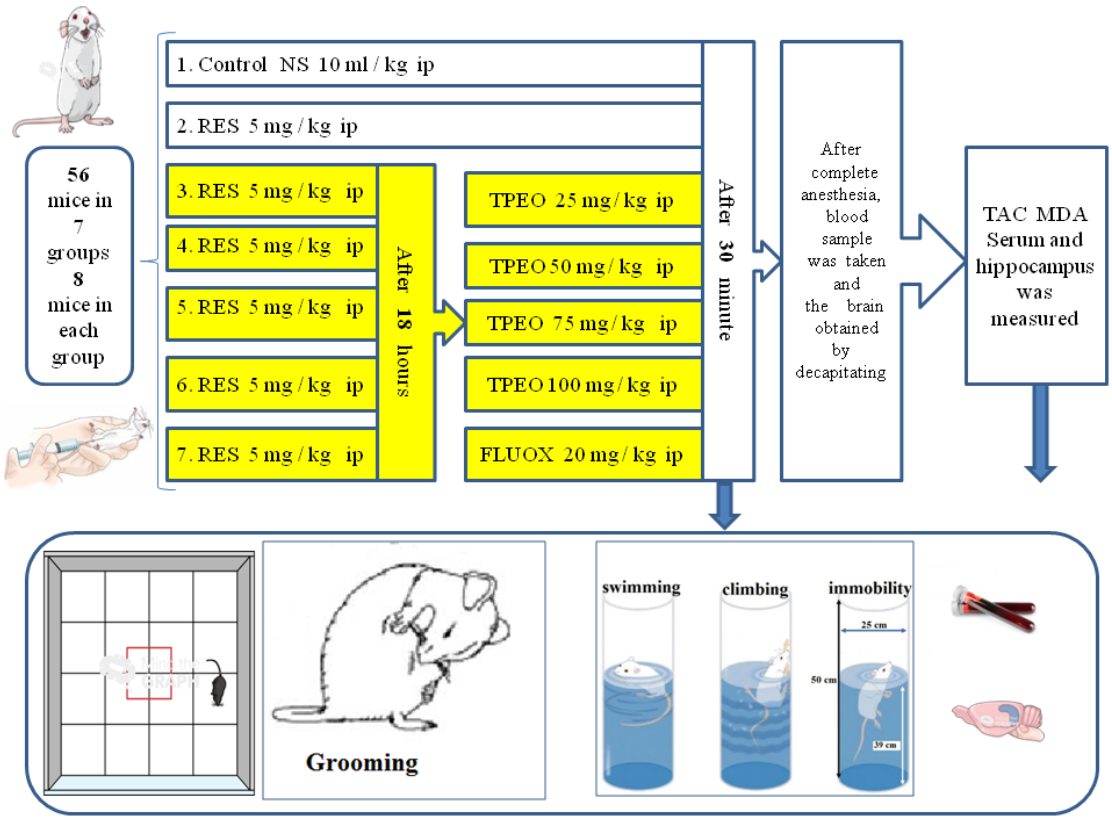

Figure 1. Schematic diagram of the study design. NS: Normal saline, RES: Reserpine, TPEO: *Tanacetum polycephalum* essential oil, FLUO: Fluoxetine, ip: Intraperitoneal, TAC: Total antioxidant capacity, MDA: Malondialdehyde

indices, based on comparisons of their retention times and mass spectra with those obtained from the original sample and the Wiley library.

Animal model of depression

A depression model in animals was induced using reserpine, which was administered intraperitoneally at a dose of 5 mg/kg. This method is widely utilized in preclinical studies to mimic depression-like symptoms. Reserpine induces a depletion of monoamines, resulting in oxidative stress and subsequent behavioral changes. This approach is considered a reliable means of evaluating antidepressant therapies (13).

Behavioral tests

Three behavioral tests were conducted: OFT, Splash test, and FST.

OFT

The OFT is primarily employed to assess anxiety, depression, and exploratory behavior in rodents. The testing apparatus consists of a Plexiglas box measuring 40×50×60 cm, divided into 16 equal squares, and is located in a quiet room. To ensure accurate results, each animal was placed in the testing room at least one hour prior to the test. Additionally, on the day before the test, each mouse was acclimatized to the box for a duration of 10 minutes.

On the test day, the animal was placed in the box, and its behavior was recorded for 5 minutes. The number of times the animal crossed the lines, stood on its hind legs (rearing), and engaged in self-grooming (licking and cleaning) were recorded as indicators of depressive behavior. Mice exhibiting depressive symptoms demonstrated significantly less rearing and grooming behavior compared to normal controls (14).

Splash test

The Splash test is a validated method for assessing depression in rodents, specifically evaluating aversive behavior induced by depression. In this test, depressed mice typically exhibit a reduced preference for sucrose and spend less time licking the sweet liquid. To conduct the test, a 10% sucrose solution is sprayed onto the animal's back, and the duration and frequency of licking the sweet liquid (referred to as grooming activity time) are recorded over a 5-minute period (15).

In behavioral tests such as the Splash test and the OFT, specific behaviors are measured to assess the animals' emotional states:

- Rearing: This behavior refers to the mouse standing on its hind legs, often indicative of exploratory behavior. In the OFT, frequent rearing signals curiosity and reduced anxiety. While it may be less prominent in the Splash test, it can still reflect overall activity levels.

- Grooming: This behavior encompasses self-cleaning actions, such as licking or scratching. In the Splash test, grooming (particularly licking) is a crucial measure of self-care and motivation, commonly used to assess depression-like states. In the OFT, grooming can indicate stress or adaptation to the environment.

FST

FST is a validated method for assessing depression in rodents. In this test, a glass container measuring 25 cm in length, 12 cm in width, and 8 cm in height is filled with water maintained at a temperature of 25 °C. The animal is gently lowered into the water from a height of 20 cm. Typically, the cessation of limb movements is interpreted as immobility.

The total duration of the test is 6 minutes, with the first 2 minutes designated for the animal to acclimate to the environment. During the subsequent 4 minutes, the time during which the animal exhibits no movement or reaction is recorded as immobility time. Depressed mice generally demonstrate a longer immobility time compared to normal mice, indicating a lack of escape behavior and reflecting depressive-like symptoms (16).

Serum MDA levels

To initiate the procedure, 0.5 g of thiobarbituric acid (TBA) was mixed with 80 mL of 20% acetic acid, and the pH of the solution was adjusted to 3.5 using sodium hydroxide (NaOH). The final volume was then brought up to 100 mL with additional acetic acid. A 100 µL serum sample was combined with 2.5 mL of the prepared TBA solution and 100 µL of a 1% sodium dodecyl sulfate (SDS) solution, which was prepared by dissolving 1 g of SDS in 100 mL of distilled water. The mixture was subsequently heated in a boiling water bath for one hour. After heating, the mixture was allowed to cool and then centrifuged at 4000 rpm for 10 minutes. The optical absorbance of the resulting supernatant was measured at a wavelength of 523 nm, and the concentration of MDA was determined using a standard curve (17).

Hippocampus MDA

Lipid peroxidation levels in hippocampus homogenates were evaluated using a colorimetric technique that relies on the production of TBA-reactive substances. The procedure began by dissolving 0.8 g of TBA in 19 mL of 20% acetic acid. A 100 µL sample of the hippocampus homogenate (prepared at a concentration of 10% w/v in 50 mM phosphate buffer, pH 7.4) was mixed with 1.5 mL of the prepared TBA solution, 200 µL of an SDS solution (prepared by dissolving 8.05 g of SDS in 50 mL of distilled water), and 10 µL of butylated hydroxytoluene (BHT) solution (prepared by dissolving 2.2 g of BHT powder in 20 mL of ethanol). The samples were then heated in a boiling water bath for one hour, allowed to cool, and subsequently treated with 3 mL of butanol. Afterward, the samples were centrifuged at 2000 rpm for 10 minutes. The optical absorbance of the resulting supernatant was measured at a wavelength of 523 nm, and the concentration of MDA was calculated using a standard curve (18).

Serum and hippocampus antioxidant capacity

To assess serum and tissue antioxidant capacity via the ferric reducing antioxidant power (FRAP) method, three solutions were prepared: Buffer: 1.55 mL of sodium acetate and 8 mL of concentrated acetic acid, diluted with distilled water to a total volume of 500 mL. Ferric chloride solution: 270 mg of ($6H_2O$) $FeCl_3$, diluted to 50 mL with distilled water. Triazine solution: 47 mg of triazine dissolved in 40 mL of 40 mM HCl. The working solution was created by combining 10 mL of solution 1, 1 mL of solution 2, and 1 mL of solution 3. A 25 µL sample of serum or liver and kidney tissue homogenate was added to 1.5 mL of the working solution, which was incubated at 37°C for 10 minutes. The optical absorbance was measured at a wavelength of 593 nm (19).

Data analysis method

In this research, statistical analysis was carried out using Prism (Version 8) software. The Kolmogorov–Smirnov test was applied to evaluate normality, and the Brown-Forsythe test was used to verify homogeneity of variance. One-way ANOVA was conducted, followed by Tukey's post hoc test for group comparisons. Statistical significance was set at a P value below 0.05, with the findings presented as mean ± standard deviation.

Results

Composition of the essential oil of Tanacetum polycephalum

Table 1 shows the phytochemical compounds identified for the essential oil of *Tanacetum polycephalum* by GC/MS. According to the table's results, the major compound identified for the plant's essential oil was beta-thujone, which constituted 84.4% of the essential oil. Other major compounds included borneol (3%), 1.8-cineole (2.5%), and camphor (1.3%).

The effect of Tanacetum polycephalum essential oil on immobility time in the FST test

In the FST, reserpine-treated mice exhibited significantly prolonged immobility compared to the control group ($P<0.001$). However, administering *Tanacetum polycephalum* essential oil at doses of 25, 50, 75, and 100 mg/kg significantly shortened immobility duration compared to the reserpine group ($P<0.05$, $P<0.001$). Likewise, treatment with Fluoxetine notably reduced immobility time in reserpine-treated mice ($P<0.001$) (Figure 2).

The effect of Tanacetum polycephalum essential oil on the duration of self-licking and the number of grooming behaviors in the Splash test

In the Splash test, reserpine-treated mice exhibited significantly reduced licking duration and frequency

Table 1. Phytochemical compositions of the essential oil of the *Tanacetum polycephalum* by GC/MS

Compound name	Inhibition index	GC/MS Percentage
α-Pinene	933	0.3
Camphene	948	0.36
Sabinene	973	0.18
β-Pinene	978	0.22
α-Phellandrene	977	0.4
p-Cymene	1025	0.6
1,8-Cineole	1031	2.5
cis-Sabinene hydrate	1069	0.2
linalool	1105	0.6
α-Thujone	1108	1.1
β-Thujone	1125	84.4
Chrysanthenone	1129	0.76
trans-Pinocarveol	1144	0.14
Camphor	1148	1.3
cis-Chrysanthenol	1167	0.6
Borneol	1171	3
Terpinen-4-ol	1181	0.3
α-Terpineol	1196	0.13
Myrtenal	1200	0.16
Myrtenol	1203	0.07
cis-Crysanthenyl acetate	1262	0.3
Bornyl acetate	1286	1.12
p-Cymen-7-ol	1300	0.07
Germacrene D	1483	0.1
Spathulenol	1582	0.07
Caryophyllene oxide	1586	0.2
γ-Eudesmol	1636	0.12
Methyl jasmonate	1642	0.07
β-Eudesmol	1654	0.07
Selin-11-en-4-α-ol	1660	0.56
	Total: 100	

compared to the control group ($P<0.001$). However, administration of *Tanacetum polycephalum* essential oil at doses of 25, 50, 75, and 100 mg/kg, as well as Fluoxetine, significantly increased the licking frequency compared to the reserpine group ($P<0.001$) (Figure 3a) The number of grooming actions was markedly lower in reserpine-treated mice compared to the control group ($P<0.01$). Nonetheless, *Tanacetum polycephalum* essential oil treatment at doses of 75 and 100 mg/kg significantly enhanced grooming frequency relative to the reserpine group ($P<0.05$, $P<0.01$). Similarly, Fluoxetine treatment significantly elevated grooming activity compared to the reserpine group ($P<0.01$) (Figure 3b).

The effect of Tanacetum polycephalum essential oil on the number of rearing and line crossings in the OFT

The frequency of rearing observed in the open-field test was significantly lower in reserpine-treated mice

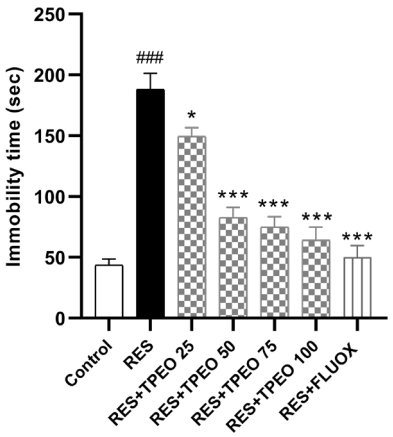

Figure 2. Comparison of immobility time in the FST. Values are shown as mean ± standard error. ### $P<0.001$ compared to the control group, * $P<0.05$, *** $P<0.001$ compared to the RES group. RES = Reserpine, TPEO = *Tanacetum polycephalum* essential oil, FLUOX = Fluoxetine

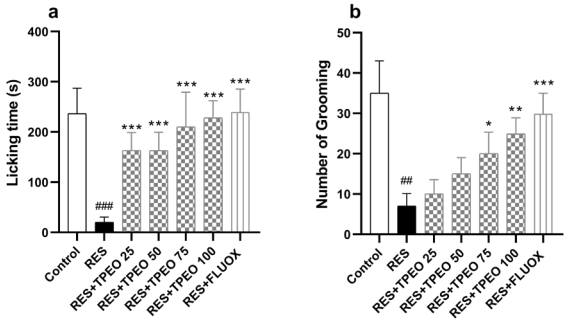

Figure 3. Comparison of body licking time (a) and grooming number (b) in the Splash test. Values are shown as mean ± standard error. ### $P<0.001$, ## $P<0.01$ compared to the control group, * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to the RES group RES = Reserpine, TPEO = *Tanacetum polycephalum* essential oil, FLUOX = Fluoxetine

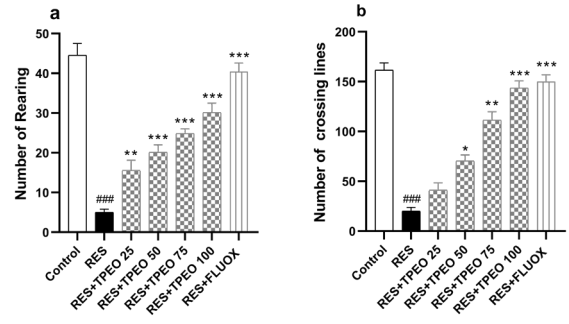

Figure 4. Comparison of rearing (a) and crossing lines (b) in the OFT. RES = Reserpine, TPEO = *Tanacetum polycephalum* essential oil, FLUOX = Fluoxetine, ### $P<0.001$ compared to the control group, * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to the RES group

compared to the control group ($P<0.001$). Administration of *T. polycephalum* essential oil at 25, 50, 75, and 100 mg/kg significantly enhanced rearing activity compared to the reserpine group ($P<0.01$, $P<0.001$). Similarly, treatment with Fluoxetine notably increased rearing behavior compared to the reserpine group ($P<0.001$) (Figure 4a). The findings showed that the number of line crossings

in the open-field test was substantially reduced in mice treated with reserpine compared to the control group ($P<0.001$). However, administration of *T. polycephalum* essential oil at doses of 50, 75, and 100 mg/kg, as well as Fluoxetine, significantly elevated the frequency of line crossings in comparison to the reserpine group ($P<0.05$, $P<0.01$, $P<0.001$) (Figure 4b).

The effect of Tanacetum polycephalum essential oil on TAC and MDA in hippocampus tissue

The TAC of the hippocampus was significantly diminished in reserpine-treated mice compared to the control group ($P<0.001$). However, administration of *T. polycephalum* essential oil at doses of 25, 50, 75, and 100 mg/kg, as well as Fluoxetine, significantly enhanced hippocampus TAC compared to the reserpine group ($P<0.05$, $P<0.001$) (Figure 5a). MDA levels in the hippocampus were considerably higher in mice treated with reserpine than in the control group ($P<0.001$). Nonetheless, treatment with *T. polycephalum* essential oil at doses of 25, 50, 75, and 100 mg/kg, along with Fluoxetine, significantly decreased hippocampus MDA levels relative to the reserpine group ($P<0.05$, $P<0.001$) (Figure 5b).

The effect of Tanacetum polycephalum essential oil on TAC and MDA in serum

The TAC of serum was significantly reduced in mice treated with reserpine compared to the control group ($P<0.001$). However, administration of *T. polycephalum* essential oil at doses of 25, 50, 75, and 100 mg/kg, as well as Fluoxetine, substantially increased serum TAC relative to the reserpine group ($P<0.05$, $P<0.01$, $P<0.001$) (Figure 6a). Serum MDA levels were notably elevated in reserpine-treated mice compared to the control group ($P<0.001$). Treatment with *T. polycephalum* essential oil at doses of 25, 50, 75, and 100 mg/kg, along with Fluoxetine, significantly lowered serum MDA levels compared to the reserpine group ($P<0.05$, $P<0.01$, $P<0.001$) (Figure 6b).

Discussion

Depression is a widespread mental health disorder affecting approximately 5% of adults globally, with women being more commonly affected than men. It is a leading cause of disability, impacting over 280 million people worldwide. Medicinal plants like St. John's Wort, saffron, and turmeric have shown promise in alleviating depressive symptoms by modulating neurotransmitters and reducing oxidative stress. These natural remedies offer potential as complementary treatments, though further research is needed to standardize their use (20-22). Over the years, various animal models have been created to investigate the underlying pathophysiology of depression and evaluate new antidepressant treatments. These models include chronic mild stress and early-life maternal separation stress, among many others (23). An alternative method for inducing depression involves the use of specific pharmaceutical compounds that alter brain neurotransmitter levels. reserpine, a drug used to lower blood pressure and treat psychosis, has been primarily employed to induce depression in animal models (24,25). Reserpine irreversibly obstructs vesicular monoamine transporters, namely vesicular monoamine transporter-1 and vesicular monoamine transporter-2. By inhibiting vesicular monoamine transporter-2, reserpine hinders the uptake. It reduces the storage of monoamines—including norepinephrine, dopamine, serotonin, and histamine—within the synaptic vesicles of neurons (26-28). In this research, the injection of reserpine in mice induced depression-like behaviors, as demonstrated by a rise in immobility during the FST. The FST is broadly acknowledged as one of the most reliable approaches for assessing rodent depression.

According to Martin Seligman's learned helplessness theory, continuous exposure to inescapable stress causes an animal to lose hope and cease mobility and activity gradually. This experiment models a stage of hopelessness in depression, with depressed mice exhibiting more immobility due to diminished hope. In this research, the open-field test was employed to assess exploratory activity, depression, and anxiety in mice. Reserpine administration resulted in a notable reduction in line crossings, rearing,

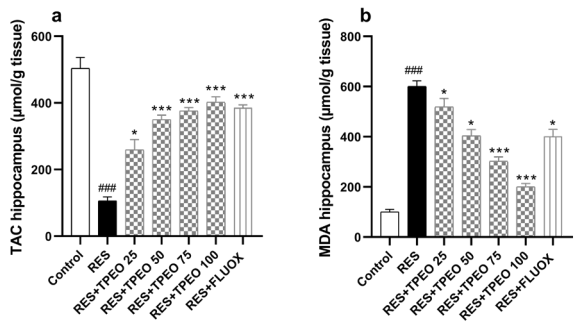

Figure 5. Comparison of brain TAC (a) Brain MDA (b) in the study groups. RES = Reserpine, TPEO = *Tanacetum polycephalum* essential oil, FLUOX = Fluoxetine, TAC = total antioxidant capacity, MDA = malondialdehyde, µmol/g tissue = micromoles per gram of tissue, ### $P<0.001$ compared to the control group, * $P<0.05$, *** $P<0.001$ compared to the RES group

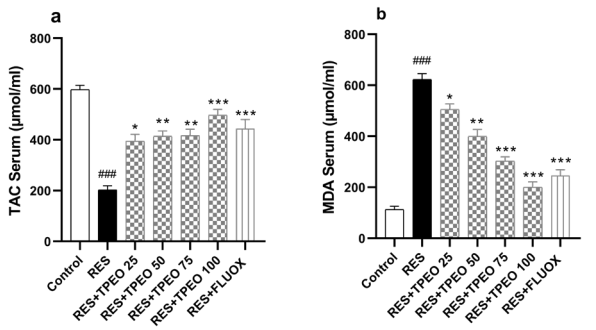

Figure 6. Comparison of serum TAC (a) and Serum MDA (b) in the study groups. RES = Reserpine, TPEO = *Tanacetum polycephalum* essential oil, FLUOX = Fluoxetine, TAC = total antioxidant capacity, MDA = malondialdehyde, µmol/g tissue = micromoles per gram of tissue, ### $P<0.001$ compared to the control group, * $P<0.05$, ** $P<0.01$, *** $P<0.001$ compared to the RES group

and grooming behaviors compared to the control group, indicating diminished exploratory behavior alongside heightened depression and anxiety. The open-field test evaluates motor activity, anxiety, depression, and the exploratory drive in animals (29,30). This test evaluates line crossings, rearing, grooming, time spent in center and edge squares, defecation, and urination. Line crossings are used to assess motor activity, while rearing and grooming behaviors indicate anxiety and depression. Researchers use these indicators to measure the animal's behavior and psychological state (31). The Splash test in this study was used to assess animal depression, and reserpine injection led to a decrease in the duration and frequency of licking, indicating depressive behavior. The lack of pleasure behavior caused by depression was evaluated through sucrose preference, with depressed mice showing lower sucrose consumption. In this research, reserpine-treated mice displayed depression-like behaviors, increased MDA levels, and diminished antioxidant capacity in both serum and brain, signifying oxidative stress. It is widely accepted among researchers that oxidative stress plays a crucial role in the onset of depression in humans and animal models alike (32).

In this research, administering *T. polycephalum* essential oil at doses of 25, 50, 75, and 100 mg/kg significantly alleviated depressive symptoms in reserpine-treated mice, as indicated by reduced immobility in the FST and enhanced licking behavior in the Splash test. *T. polycephalum* essential oil also increased rearing and grooming behaviors. This study is the first to demonstrate the antidepressant, sedative, and anti-anxiety effects of *T. polycephalum* essential oil. Major compounds identified in *T. polycephalum* essential oil included beta-pathogen (84.4%), borneol (3%), 1,8-cineole (2.5%), and camphor (1.3%). The relationship between beta-pathogen and depression is not well-documented in the latest scientific literature.

However, beta-adrenergic receptors, which may be related, play a significant role in the neurobiology of depression. Studies suggest that norepinephrine, acting on these receptors, influences stress resilience and antidepressant efficacy. Additionally, beta-blockers, which interact with these receptors, have been studied for their potential link to depression. However, findings indicate that the association may not be causal. Further research is needed to clarify these mechanisms and their implications for treatment (33).

According to the study results, the protective effects of *T. polycephalum* essential oil against reserpine-induced depression appear to be linked to the reduction of oxidative stress parameters in the brains of mice. Reducing oxidative stress helps prevent nerve cell damage, crucial in mitigating depression symptoms. Oxidative stress is known to play a significant role in the pathophysiology of depression, as it leads to the production of free radicals that can damage neurons. *T. polycephalum* essential oil, rich in antioxidant compounds, is pivotal in combating oxidative stress, a major contributor to neuronal damage and neurodegenerative diseases. Neutralizing free radicals helps preserve the structural and functional integrity of nerve cells. This protective mechanism not only supports cognitive functions but also mitigates inflammation, a key factor in the development of depression and anxiety.

Furthermore, studies have highlighted its ability to enhance neurotransmitter balance, promoting mental well-being. Regularly using this essential oil may also improve resilience against stress-induced neurological disorders. These findings underscore its potential as a natural therapeutic agent for maintaining brain health and preventing the progression of depression.

Conclusion

Reserpine injection induced depressive-like behaviors and oxidative stress in mice, as evidenced by reduced antioxidant capacity and increased lipid peroxidation. Treatment with *T. polycephalum* essential oil alleviated oxidative stress and prevented depression, potentially by modulating brain antioxidant levels. Upcoming research should investigate the influence of antioxidant capacity on the antidepressant properties of *T. polycephalum* essential oil.

Authors' Contribution

Conceptualization: Hossein Amini-Khoei, Mehrdad Shahrani Korrani.
Data curation: Najmeh Asgharzadeh, Khadijeh Reisi.
Formal analysis: Hossein Amini-Khoei, Zahra Lorigooini.
Funding acquisition: Khadijeh Reisi.
Investigation: Elham Bijad.
Methodology: Hossein Amini-Khoei, Marzieh Mardani.
Project administration: Zahra Lorigooini.
Resources: Mehrdad Shahrani Korrani.
Software: Najmeh Asgharzadeh, Diana Shahrani Korrani, Mohamad Shahrani Korrani.
Supervision: Mehrdad Shahrani Korrani.
Validation: Najmeh Asgharzadeh.
Visualization: Najmeh Asgharzadeh, Hossein Amini-Khoei.
Writing–original draft: Hossein Amini-Khoei, Diana Shahrani Korrani.
Writing–review & editing: Najmeh Asgharzadeh, Khadijeh Reisi, Hossein Amini-Khoei, Zahra Lorigooini, Marzieh Mardani, Elham Bijad, Diana Shahrani Korrani, Mohamad Shahrani Korrani, Mehrdad Shahrani Korrani.

Competing Interests

Although one of the authors in this article is the journal's editor-in-chief, the whole process of reviewing and publishing the article is like that of other articles in the journal, and there is no difference in its review from other ones.

Ethical Approval

This project was designed and implemented based on the license number IR.SKUMS.REC.1397.164 of the Ethics Committee of Shahrekord University of Medical Sciences (Shahrekord, Iran). The ARRIVE guidelines were utilized for reporting experiments involving live animals, ensuring the promotion of ethical research practices.

Funding

The Medicinal Plants Research Center of Shahrekord University

of Medical Sciences, Shahrekord, Iran, financially supported this project (Grant number: 3770-45).

References

1. Chen-Li DCJ, Mansur RB, Di Vincenzo JD, Chisamore N, Kaczmarek E, McIntyre RS, et al. Effect of intravenous ketamine on suicidality in adults with treatment-resistant depression: a real-world effectiveness study. Psychiatry Res. 2025;343:116282. doi: 10.1016/j.psychres.2024.116282.
2. Taylor WD, Butters MA, Elson D, Szymkowicz SM, Jennette K, Baker K, et al. Reconsidering remission in recurrent late-life depression: clinical presentation and phenotypic predictors of relapse following successful antidepressant treatment. Psychol Med. 2025:1-12. doi: 10.1017/s0033291724003246.
3. Cheng YY, Yao Q, Miao Y, Guan W. Metformin as a potential antidepressant: mechanisms and therapeutic insights in depression. Biochem Pharmacol. 2025;233:116773. doi: 10.1016/j.bcp.2025.116773.
4. De Filippis S, Martinotti G, Nicoletti F, Mastrostefano A, Trovini G, Pugliese A, et al. Major depression in comorbidity with substance use disorders: patients' features and clinical-neurobiological rationale of antidepressant treatments. Curr Neuropharmacol. 2025;23(3):256-75. doi: 10.2174/1570159x22666240827165327.
5. Farzan M, Farzan M, Amini-Khoei H, Shahrani M, Bijad E, Anjomshoa M, et al. Protective effects of vanillic acid on autistic-like behaviors in a rat model of maternal separation stress: Behavioral, electrophysiological, molecular and histopathological alterations. Int Immunopharmacol. 2023;118:110112. doi: 10.1016/j.intimp.2023.110112.
6. Soleiman-Dehkordi E, Reisi-Vanani V, Hosseini S, Lorigooini Z, Azimian Zvareh V, Farzan M, et al. Multilayer PVA/gelatin nanofibrous scaffolds incorporated with *Tanacetum polycephalum* essential oil and amoxicillin for skin tissue engineering application. Int J Biol Macromol. 2024;262(Pt 1):129931. doi: 10.1016/j.ijbiomac.2024.129931.
7. Gaur R, Chauhan A, Kanta C. A critical review of antioxidant potential and pharmacological applications of important *Ficus* species. J Herbmed Pharmacol. 2024;13(4):537-49. doi: 10.34172/jhp.2024.52557.
8. Muhammad RN, Albahairy MA, Abd El Fattah MA, Ibrahim WW. Empagliflozin-activated AMPK elicits neuroprotective properties in reserpine-induced depression via regulating dynamics of hippocampal autophagy/inflammation and PKCζ-mediated neurogenesis. Psychopharmacology (Berl). 2024;241(12):2565-84. doi: 10.1007/s00213-024-06663-0.
9. Deyama S, Kaneda K, Minami M. Resolution of depression: antidepressant actions of resolvins. Neurosci Res. 2025;211:85-92. doi: 10.1016/j.neures.2022.10.006.
10. Zhu BL, Tang JY, Chen WJ, Qian JJ, Zhang F, Zhang XL, et al. Fluoxetine treatment reverses chronic stress-induced promotion on Fk506-binding protein 5 expression and multiple effects on glucocorticoid receptor phosphorylation in the paraventricular nucleus of mice. Pharmacol Biochem Behav. 2025;246:173916. doi: 10.1016/j.pbb.2024.173916.
11. El-Assri EM, El-Assri Y, El Brahimi R, El Fadili M, Baghouz A, Abuelizz HA, et al. Molecular characterization, chemical profile and biological properties of essential oils from *Chamaemelum nobile* (L.) flowers of Morocco: in vitro and in silico studies. Front Chem. 2025;13:1539872. doi: 10.3389/fchem.2025.1539872.
12. Abdulhamid Kadhim R, Erfanparast A, Tamaddonfard E, Amirkashani D, Imani M. Effects of linalool on acute hyperglycemia and hypoinsilinemia induced by ketamine-xylazine in rats: the role of alpha 2 adrenergic receptors. Iran J Physiol Pharmacol. 2025;8:183-94.
13. Kang DW, Choi SR, Shin H, Lee H, Park J, Lee M, et al. Modulation of brain-derived neurotrophic factor expression by physical exercise in reserpine-induced pain-depression dyad in mice. Exp Neurobiol. 2024;33(4):165-79. doi: 10.5607/en24014.
14. Amini-Khoei H, Mohammadi-Asl A, Amiri S, Hosseini MJ, Momeny M, Hassanipour M, et al. Oxytocin mitigated the depressive-like behaviors of maternal separation stress through modulating mitochondrial function and neuroinflammation. Prog Neuropsychopharmacol Biol Psychiatry. 2017;76:169-78. doi: 10.1016/j.pnpbp.2017.02.022.
15. Stoyanova T, Nocheva H, Nenchovska Z, Krushovlieva D, Ivanova P, Tchekalarova J. Prenatal constant light exposure induces behavioral deficits in male and female rat offspring: effects of prenatal melatonin treatment. Int J Mol Sci. 2025;26(3):1036. doi: 10.3390/ijms26031036.
16. Clark EA, Wang L, Hanania T, Kretschmannova K, Bianchi M, Jagger E, et al. 5-HT(1B) receptor activation produces rapid antidepressant-like effects in rodents. Pharmacol Biochem Behav. 2025;247:173917. doi: 10.1016/j.pbb.2024.173917.
17. Alcon E, Hidalgo FJ, Zamora R. Alkylresorcinols trap malondialdehyde in whole grain crackers. Food Chem. 2025;463(Pt 2):141128. doi: 10.1016/j.foodchem.2024.141128.
18. Park HJ, Nam MH, Park JH, Lee JM, Hong HS, Kim TW, et al. Comparison of malondialdehyde, acetylcholinesterase, and apoptosis-related markers in the cortex and hippocampus of cognitively dysfunctional mice induced by scopolamine. Biomedicines. 2024;12(11):2475. doi: 10.3390/biomedicines12112475.
19. Admasu FT, Demissie B, Yitbarek GY, Geto Z, Tesfaw A, Zewde EA, et al. Evaluation of total oxidative stress and antioxidant capacity of brain tumour patients attending referral hospitals in Addis Ababa, 2020: a comparative cross-sectional study. Ecancermedicalscience. 2022;16:1391. doi: 10.3332/ecancer.2022.1391.
20. Condominas E, Sanchez-Niubo A, Domènech-Abella J, Haro JM, Bailon R, Giné-Vázquez I, et al. Exploring the dynamic relationships between nocturnal heart rate, sleep disruptions, anxiety levels, and depression severity over time in recurrent major depressive disorder. J Affect Disord. 2025;376:139-48. doi: 10.1016/j.jad.2025.02.010.
21. Rekha S, Kumar A, Kafeel M, Mathew SR, Surya Harshitha S, Muthukumaran T, et al. Phyto-pharmacological effects of medicinal plants for the treatment of depression. J Adv Zool. 2024;45(2):780-7. doi: 10.53555/jaz.v45i2.4005.
22. Nurzyńska-Wierdak R. Plants with potential importance in supporting the treatment of depression: current trends, and research. Pharmaceuticals (Basel). 2024;17(11):1489. doi: 10.3390/ph17111489.
23. Liu S, Xiao Q, Tang J, Li Y, Zhu P, Liang X, et al. Running exercise decreases microglial activation in the medial prefrontal cortex in an animal model of depression. J Affect Disord. 2025;368:674-85. doi: 10.1016/j.jad.2024.09.124.
24. Wang M, Li H, Zhang W, Zhang L, Wang S, Jia M, et al. Saikosaponin A alleviates depressive-like behavior induced by reserpine in mice by regulating gut microflora and inflammatory responses. PLoS One. 2025;20(2):e0311207. doi: 10.1371/journal.pone.0311207.
25. Kamaly NA, Kamel AS, Sadik NA, Shahin NN. Milnacipran and vanillin alleviate fibromyalgia-associated depression in reserpine-induced rat model: role of Wnt/β-catenin signaling. Mol Neurobiol. 2025. doi: 10.1007/s12035-025-04723-w.
26. Tod P, Varga A, Román V, Lendvai B, Pálkovács R, Sperlágh B, et al. Tetrabenazine, a vesicular monoamine transporter 2 inhibitor, inhibits vesicular storage capacity and release of monoamine transmitters in mouse brain tissue. Br J Pharmacol. 2024;181(24):5094-109. doi: 10.1111/bph.17348.
27. Shen R, Wang J, Zhao Y, Dang Z, Zhang K, Li M, et al. Polysaccharides from *Scrophularia ningpoensis* Hemsl.

improve reserpine-induced depression-like behavior by inhibiting HTR2A/HTR2C mediated AKT/GSK3β/β-catenin/CBP/BDNF signalling. Int J Biol Macromol. 2025;301:140445. doi: 10.1016/j.ijbiomac.2025.140445.

28. Brum ES, Landini L, Souza Monteiro de Araújo D, Marini M, Geppetti P, Nassini R, et al. Characterisation of periorbital mechanical allodynia in the reserpine-induced fibromyalgia model in mice: the role of the Schwann cell TRPA1/NOX1 signalling pathway. Free Radic Biol Med. 2025;229:289-99. doi: 10.1016/j.freeradbiomed.2025.01.040.
29. Sohail A, Shams F, Nawaz A, Ain QU, Ijaz B. Antifibrotic potential of reserpine (alkaloid) targeting Keap1/Nrf2; oxidative stress pathway in CCl4-induced liver fibrosis. Chem Biol Interact. 2025;407:111384. doi: 10.1016/j.cbi.2025.111384.
30. Cheung M, Parmar M. Reserpine (archived). In: StatPearls [Internet]. Treasure Island, FL: StatPearls Publishing; 2025.
31. Birmann PT, Sinott A, Zugno GP, Rodrigues RR, Conceição FR, Sousa FS, et al. The antidepressant effect of *Komagataella pastoris* KM 71 H in maternal separation mice model mediated by the microbiota-gut-brain axis. Behav Brain Res. 2025;476:115287. doi: 10.1016/j.bbr.2024.115287.
32. Wu X, Cai H, Liao R, Tedesco AC, Li Z, Wang F, et al. Bio-inspired carbon dots as malondialdehyde indicator for real-time visualization of lipid peroxidation in depression. Small. 2024;20(46):e2400671. doi: 10.1002/smll.202400671.
33. Zhang H, Cui M, Cao JL, Han MH. The role of beta-adrenergic receptors in depression and resilience. Biomedicines. 2022;10(10):2378. doi: 10.3390/biomedicines10102378.