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Research Article

Evaluation of Anti-Inflammatory Effect of Combination Therapy of Silymarin Nanomicelles plus Berberine Nanomicelles in LPS-induced Depressive-like Behavior in Mice

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Abstract

Depression is one of the most common mental illnesses globally, and unfortunately, current medications as a treatment, in this case, have a wide range of side effects. Therefore, finding new drugs is a valuable step in the treatment of depressive disorders. Berberine and Silymarin have many biological therapeutic properties like antioxidant, anti-inflammatory, and anti-cancer, while their bioavailability is not strong.

In order to increase the effectiveness and improve the bioavailability of drugs, new tools like nano micelles are suggested. Therefore, we aimed to investigate the effect of Berberine and Silymarin nanomicelles (NB+ NS) on the model of depression induced by Lipopolysaccharides (LPS) in mice.

LPS (1 mg/kg, i.p.) was injected to induce depression-like behavior, then, 24 h later, the treatment began, and behavioral tests like forced swimming test (FST), open field test (OFT), and tail suspension test (TST) *were carried out. Also, the brain level of Tumor Necrosis Factor- α (TNF-α) and Interleukin-1 Beta (*IL-1ß) were measured as well*. In this study, the mice were divided into different groups including, control, LPS, Nanomicelle, Fluoxetine, Berberine, Silymarin, and NB 5+ NS 25.*

All data were analyzed by Graph pad prism (version 5), and SPSS ($P < 0.05$ was considered significant).

This study showed that FST immobility time, TST immobilization time, brain levels of TNF-α and IL-1ß significantly decreased in NS 25+ NB 5 and fluoxetine groups compared to LPS and control groups ($P < 0.05$).

Keywords: Nanomicelles; Silymarin; Berberin; LPS; Depression

Introduction

Depression is one of the most common mental illnesses that has involved a large number of people in the world [1,2]. This disease is characterized by persistent sad, anxious or "empty" mood, feelings of hopelessness or pessimism, irritability, feelings of guilt, worthlessness or helplessness, loss of interest or pleasure in hob-

bies and activities, decreased energy or fatigue, moving or talking more slowly, feeling restless, difficult concentrating or making decisions, difficult sleeping, early-morning awakening or oversleeping, appetite and/or weight changes, thoughts of death, and suicide or suicide attempts [3].

Causes of depression are usually due to neurochemical changes that can occur on account of physical and psychological trauma, social factors, unpleasant events, genetic factors, and the use of certain medications. Antidepressants, like fluoxetine, are one of the main methods in treating depression [2,4]. However, about one-third of patients do not respond to existing medications or experience multiple side effects; Therefore, finding new drugs that do not have the problems of existing drugs is a valuable step in the treatment of depressive disorders [1,2,5].

Changes in the interactions of neurotransmitters in the serotonergic, dopaminergic, and adrenergic nerve pathways by decreased secretion of serotonin and catecholamines at synapses, lead to impairing the uptake and reabsorption of presynaptic and postsynaptic signals in the neurons system. These complicated changes clinically manifest themselves in the form of depressive symptoms. Antidepressants with various mechanisms are prescribed to balance the levels of neurotransmitters in synaptic spaces and compensate for the lack of brain amines, especially norepinephrine (NE) and serotonin (5 HT). However, in more than half of the cases, the lack of proper response and the occurrence of annoying side effects will cause the patient to stop taking the drugs [4,6,7]. Despite extensive research, the neurobiological processes that lead to depression are still poorly understood, and recent treatments for depression have not provided complete recovery and have made unwanted side effects [8].

The bacterial toxins and lipopolysaccharide (LPS) cause depression by activating microglia and producing pro-inflammatory factors such as nitric oxide, eicosanoids, and other inflammatory cytokines in the brain. Following depression, inflammatory pathways get activated, and some factors such as TNFα and IL1β would increase [4,7]. Berberine is an alkaloid plant that is abundant in the roots and bark of the barberry stem and is found in many plants such as *Hydrastis canadensis, Coptidis Rhizoma, Berberis aquifolium, Berberis aristata* and *Berberis vulgaris* [9]. Berberine has shown several effects, for instance, anti-anxiety, analgesic, anti-inflammatory, antidepressant, anti-amnesia effects, anti-viral, anti-cancer, and antioxidant effects [10-12]. Although Berberine has many biological therapeutic properties and its bioavailability and productivity are limited because of poor aqueous solubility. Many studies using nanotechnology to increase the effectiveness and improve the bioavailability of Berberine have been shown. The nano-drug preparation methods are used to increase the solubility and bioavailability of water-soluble drugs [13-15]. Today, nanoscience researchers are looking for drug delivery tools with suitable properties for the drugs to enter the body. One of these applicable and new tools for drug delivery is micelles [15,16]. Micelles create systems that physically trap the drug inside the nucleus, optimizing the accommodation between the drug and the dissolution medium. It can mainly improve drug loading, drug barriers, and consequently the chemical stability of the compound [13,14,16].

Micelles affect its stability during drug storage and subsequent in-body performance. However, some studies indicate that various drugs have loaded successfully into micelles with different techniques [13-16].

Silymarin is a flavonolignan derived from the seeds of the sage (*Silybum marianum*) [17,18]. Silymarin is a plant extract that consists of approximately 70-80% of Silymarins flavonolignan-like (Silybin**,** Isosilybin, Silydianin, Silychristin), and the rest parts of it include polyphenolic and polymeric oxidized compounds [17,18]. Properties such as antioxidant, anti-inflammatory, anti-fibrotic, multiple protection, anti-viral and anti-cancer are found in the use of the Silymarin [18-20].

The extraordinary effectiveness of sage seed extract on improving liver function has been confirmed in various sources [21-23]. According to studies, inflammation of nerve cells is an essential factor in aggravating brain cell damage [17,18]. Silymarin effectively prevents brain damage by inhibiting inflammation of brain cells [17,18,24]. Silymarin's aqueous solubility limits its medical applications; therefore, to improve the biosorption and bioavailability of Silymarin, many methods have been studied to change its physical and chemical nature [25,26]. Nanotechnology is a powerful tool to be used in the development of a new drug delivery system; moreover, this technology is useful for compounds with weak aqueous solubility, as this system can provide an increase in the level of available area and increase the dissolution process [25,26]. Be-

cause of the low solubility of Silymarin compounds in water and its low bioavailability, researchers are trying to synthesize improved formulations [27,28].

Considering the anti-inflammatory effects of these compounds, in this study, we aimed to investigate the effect of Silymarin nanomicelles with Berberine nanomicelles on the model of depression in mice induced by LPS [29].

Results

Results of FST immobility time in control, LPS, nanomicelles, fluoxetine, Berberine NB 5 and NB 10 groups

The effects of NB 5, NB 10, Berberine, and fluoxetine on depression induced by LPS injection in the FST test are shown in figure 1A. The graph showed that immobility time significantly increased in the LPS group compared to the control group that indicates the induction of depression ($P < 0.05$). As it shows, immobility time has no difference between LPS and nano micelles groups. Based on the results, inactivity duration significantly reduced in NB 5, NB 10, Berberine, and fluoxetine groups compared to the LPS group ($P < 0.05$). The difference between NB 5 and fluoxetine was significant ($P < 0.05$). Fluoxetine and Berberine both reduced immobility more than different doses of NB ($P < 0.05$).

Results of OFT crossings number in control, LPS, nanomicelles, fluoxetine, Berberine NB 5 and NB 10 groups

According to figure 1B, the LPS group showed a significantly lower number of crossing than the control group ($P < 0.05$). Also, in the fluoxetine group, the number of crossings significantly increased rather to the LPS group ($P < 0.05$). However, none of the NB 5, NB 10, and Berberine doses significantly increased or decreased the number of crossings compared to the control group.

Results of TST immobility time in control, LPS, nanomicelles, fluoxetine, Berberine NB 5 and NB 10 groups

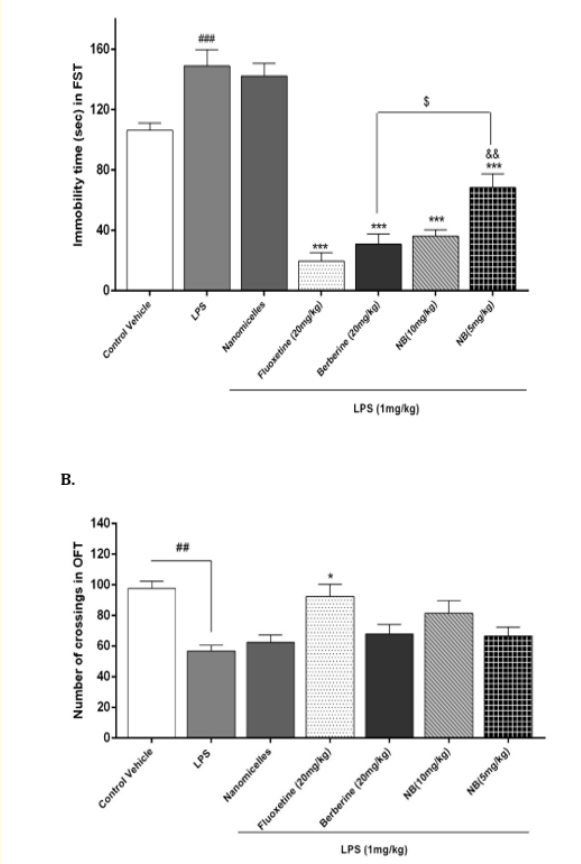
According to figure 1C, the duration of immobility in the tail suspension test increased significantly in the LPS group compared to the control group, which indicates the induction of depression ($P < 0.05$). Also, NB 5, NB 10, Berberine, and fluoxetine significantly reduced the duration of immobility in the tail suspension test compared to the LPS group ($P < 0.05$).

Results of brain TNF-α level in control, LPS, nanomicelles, fluoxetine, Berberine NB 5 and NB 10 groups

As shown in figure 1D, the level of TNF-α in the brain was increased significantly in the LPS group compared to the control group ($P < 0.05$). Brain TNF-α levels significantly decreased in NB 5, NB 10, Berberine, and fluoxetine groups compared to the LPS group ($P < 0.05$). The Fluoxetine group showed a significant reduction compared to NB 5, NB 10, Berberine groups ($P < 0.05$).

Results of brain IL-1ß level in control, LPS, nanomicelles, fluoxetine, Berberine NB 5 and NB 10 groups

As it was shown in figure 1E, brain IL-1ß levels in the LPS group increased significantly compared to the control group ($P < 0.05$). Also, the level of IL-1ß in the groups of NB 10, Berberine, and fluoxetine significantly decreased compared to the LPS group ($P < 0.05$). In NB 10 and fluoxetine groups, the amount of brain IL-1ß decreased significantly compared to the NB 5group ($P < 0.05$).



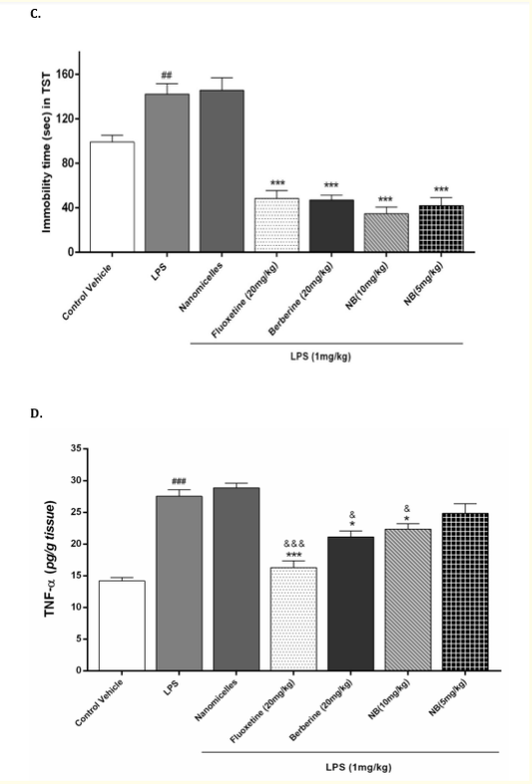


Figure 1: Comparison results in control, LPS, nanomicelles, fluoxetine, berberine NB 5 and NB 10 groups.

A. Immobility time in FST for 6 minutes.

B. Number of crossing in OFT

C. Immobility time in TST

D. Brain TNF-α levels

E. Brain IL-1ß levels

Comparison between control and LPS, * Comparison between fluoxetine and berberine and NB with LPS, & Comparison between NB and fluoxetine, $ Comparison between berberine and NB

Results of FST immobility time in control, LPS, nanomicelles, fluoxetine, Silymarin, NS 25 and NS 50 groups

The results showed that the duration of immobility in LPS nanomicelle groups increased significantly compared to the control group ($P < 0.05$). Also, the duration of immobilization Silymarin, fluoxetine, NS 25, and NS 50groups compared to the control group ($P < 0.05$). Also, the time of immobility in the fluoxetine group was significantly reduced compared to the NS 25 and NS 50 groups (Figure 2A).

Results of OFT crossing number in control, LPS, nanomicelles, fluoxetine, Silymarin, NS 25 and NS 50 groups

The results showed that there was no significant difference in the number of movements in different groups with control and LPS groups (Figure 2B).

Results of TST immobility time in control, LPS, nanomicelles, fluoxetine, Silymarin, NS 25 and NS 50 groups

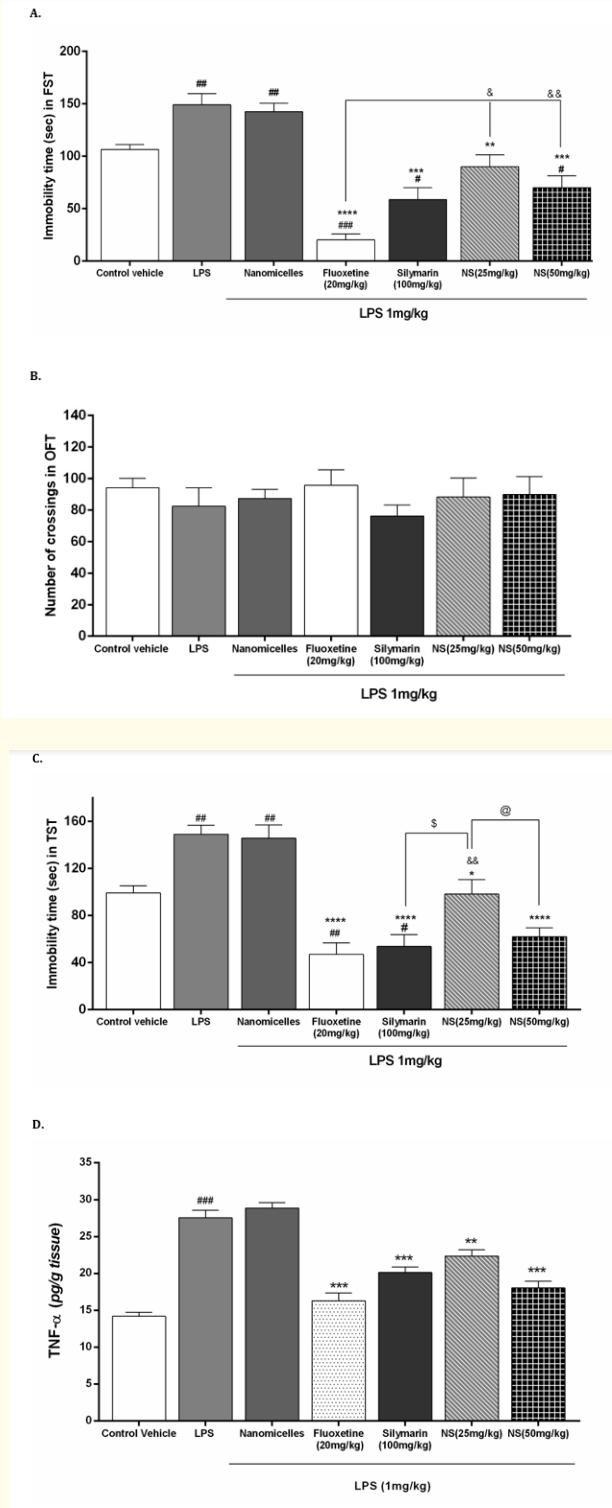
The results showed that the duration of immobility in LPS and nanomicelle groups increased significantly compared to the control group ($P < 0.05$). Also, the duration of immobilization in Silymarin and fluoxetine groups was significantly reduced compared to the control group ($P < 0.05$). Duration of immobilization showed a significant decrease in Silymarin NS 25, NS 50, and fluoxetine groups in comparison to the LPS group ($P < 0.05$). Also, the time of immobility in the Silymarin group was significantly reduced compared to NS 25 group (Figure 2C). On the other hand, the duration of immobility in the group treated with NS 50 showed a significant decrease compared to NS 25 group ($P < 0.05$).

Results of brain TNF-α level in control, LPS, nanomicelles, fluoxetine, Silymarin, NS 25 and NS 50 groups

According to the graph (Figure 2D), the amount of TNF-α in the brain tissue in the LPS group showed a large increase compared to the control group, indicating the induction of depression ($P < 0.05$). The amount of TNF-α in the fluoxetine group was significantly lower than that in NS 50 and Silymarin groups ($P < 0.05$). This inflammatory factor showed a more significant decrease in fluoxetine, Silymarin, NS 25, and NS 50 groups than the nanomicelle group ($P < 0.05$).

Results of brain IL-1ß level in control, LPS, nanomicelles, fluoxetine, Silymarin, NS 25 and NS 50 groups

According to figure 2E, the level of IL-1ß in brain tissue increased in the LPS group compared to the control group, which indicates the induction of inflammation ($P < 0.05$). The brain IL-1ß level was significantly lower in fluoxetine, Silymarin, NS 25, and NS 50 groups in comparison to the LPS group ($P < 0.05$).



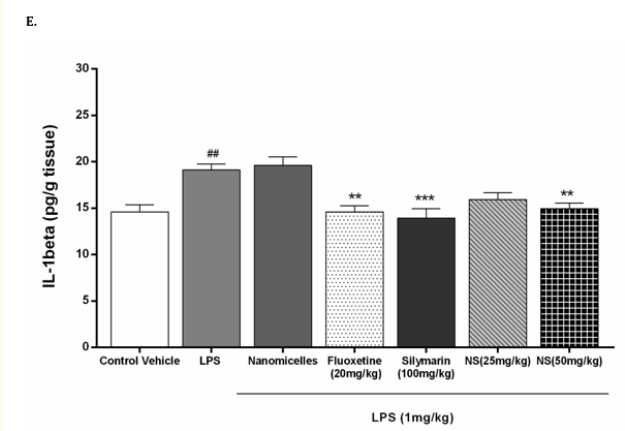


Figure 2: Comparison results in control, LPS, nanomicelles, fluoxetine, silymarin, NS 25 and NS 50 groups.

A. Immobility time in FST for 6 minutes.

B. Number of crossing in OFT

C. Immobility time in TST

D. Brain TNF-α levels

E. Brain IL-1ß levels

Comparison between different groups and control

* Comparison between different groups and LPS

& Comparison between NS 25 and fluoxetine

$ Comparison between silymarin and NS 25

@ Comparison between NS 25 and NS 50

The meaning of a star is * P < 0.05, ** p < 0.01, *** p < 0.001 and **** p < 0.0001.

Results of FST immobility time in control, LPS, fluoxetine, and NS 25+ NB 5 groups

The results of this study showed that NS 25+ NB 5 and fluoxetine groups significantly decreased immobility compared with LPS and control groups (P < 0.05). The duration of immobility in fluoxetine showed a significant decrease compared to NS 25+ NB 5 group (P < 0.05) (Figure 3A).

Results of TST immobilization time in control, LPS, fluoxetine, and NS 25+ NB 5 groups

As shown in figure 3B, immobilization time in fluoxetine and NS 25+ NB 5 groups significantly reduced in comparison to the LPS

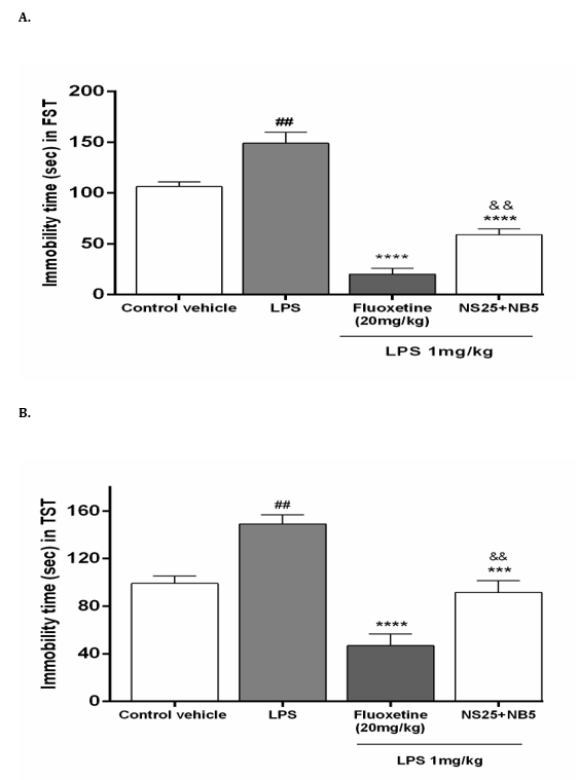
group (P < 0.05). Also, the fluoxetine group showed a significant reduction in immobility time in TST compared to NS 25+ NB 5 group (P < 0.05).

Results of brain TNF-α level in control, LPS, fluoxetine and NS 25+ NB 5 groups

According to figure 3C, the brain level of TNF-α factor in the LPS group increased compared to the control group, which indicates inflammation in the brain tissue (P < 0.05). Both fluoxetine and NS 25+ NB 5 groups significantly reduced TNF-α in brain tissue compared to the LPS group (P < 0.05).

Results of brain IL-1ß level in control, LPS, fluoxetine, and NS 25+ NB 5 groups

Based on figure 3D, the level of IL-1ß in the LPS group increased compared to the control group (P < 0.05). Both fluoxetine and NS 25+ NB 5 groups reduced IL-1ß in brain tissue compared LPS group (P < 0.05).



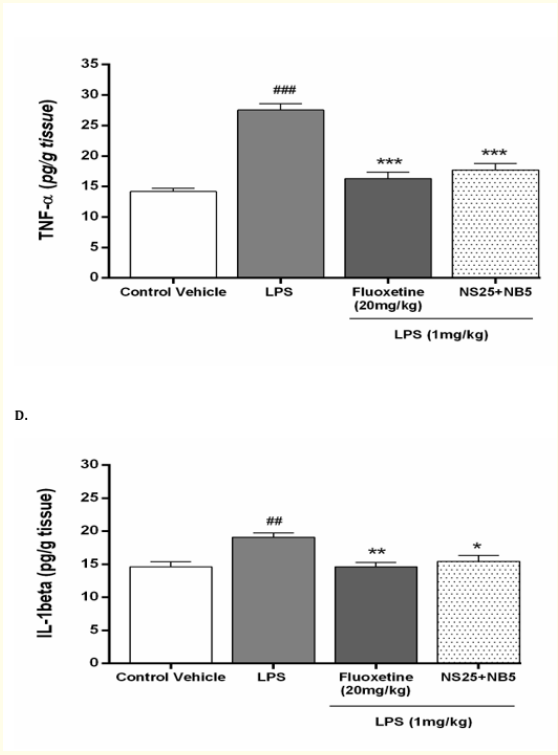


Figure 3: Comparison results in control, LPS, fluoxetine and NS 25+ NB 5 groups.

A. Immobility time in FST for 6 minutes.

B. Immobility time in TST

C. Brain TNF-α levels

D. Brain IL-1ß levels

Comparison between LPS and control group

* Comparison between different groups and LPS and

Comparison between NS 25+ NB 5 and fluoxetine.

Discussion

This study aimed to investigate the effects of Silymarin nanomicelles with Berberine nanomicelles on the model of depression induced by LPS in mice.

One of the behavioral methods in psychopharmacology is to use the forced swimming test (FST), OFT, and TST tests to measure the effect of drugs on depression. The Swimming test is one of the

most valid and common animal tests for depression. According to the theory of helplessness by Mr. Martin Seligman, if the animal is exposed to constant stress and has no way out of it, they gradually lose hope of escaping from this condition and stop their movement and activity, and become completely motionless and helpless. As already mentioned, the open field test (OFT) has been used as a model to study and evaluate depression. The OFT makes it possible to measure the movement, exploration, and anxiety of the animals simultaneously. In this method, the number of lines that the animal crosses are considered as an indicator of exploratory and emergency behavioral activities. In other words, the high frequency of such behaviors indicates high activity and exploratory behaviors.

As it was shown, LPS was significantly increased immobility time in FST, duration of immobility in TST, brain TNF-α and IL-1ß levels, moreover, decreased the number of crossing in OFT in comparison to the control group that indicated the induction of depression by LPS.

In line with our data, a strong relationship between depression and increased level of brain TNF-α in animal, human, and *in vitro* studies [30,31].

Also, it is reported that fluoxetine reduces TNF-α in depressed patients and inhibits its production [32].

As mentioned earlier, Silymarin has an anti-inflammatory effect. It is demonstrated in previous studies that Silymarin decreases TNF-α levels in the serum considerably [33].

In this context, it is showing that Silymarin enhances depressive-related behavior and reduces TNF-α levels in the hippocampus and cerebral cortex of rats as well [19].

Also, it is reported that Silymarin is effective to reduce the immobility time and reduce symptoms that are related to depression in FST and TST tests [34,35].

On the other hand, it is documenting that Berberine has an antidepressant-like effect as it inhibits MAO action [36]. It is reported that monoamine oxidase (MAO) inhibitors have antidepressant effects by increasing brain norepinephrine, serotonin (5-HT), and dopamine concentration [37]. Also, Kulkarni et al. conducted a study that showed Berberine modulates brain biogenic amines and is involved in the nitric oxide pathway that leads to antidepressant effects [10].

Our findings in this study suggested that Berberine, Silymarin, and fluoxetine attenuated the IL-1 and TNF-α in the brain.

Recently, some methods such as nano-based approaches have been used to enhance the bioavailability of drugs. Based on these studies, nanostructured biomaterials showed various advantages over conventional biomaterials. Nanostructured biomaterials are applicable and lead to cellular interaction and bioavailability improvement [27,38,39].

Simoes *et al.* reported that micelles improve the water solubility of hydrophobic drugs, increased absorption of free drugs, and release the drug in the target site of the gastrointestinal tract together with a high concentration gradient near the epithelium [40].

Azadi *et al.* conducted a study to investigate the efficacy and effects of Berberine micelle on inflammatory cytokines secretion in cerebral ischemia in an animal model. Their study showed that Berberine micelle decreased inflammatory factors level in cerebral ischemia [41].

In line with past studies, fluoxetine improves depression behavior, and in this context, fluoxetine showed better antidepressant activity than Berberine and Silymarin. On the other hand, it illustrated that fluoxetine has multiple side effects like tremors and hyperactivity and exhibits a delayed onset of action [42].

As Berberine and Silymarin have an herbal origin, they are possibly safe. As mentioned earlier, nanomicelle can increase its effectiveness and improve bioavailability. Hence it can improve drug loading and barriers, and consequently, the chemical stability of the compound [13-16].

Methods

Chemicals

LPS from *Escherichia coli*, serotype 0127:B8 was purchased from Sigma-Aldrich, St. Louis, MO, USA. Also, Berberine hydrochloride, Fluoxetine, and Silymarin were purchased from Sigma-Aldrich (USA). Berberine nano micelle has been developed in the Nanotechnology Research Center of Mashhad University of Medical Sciences, Mashhad, Iran, that contained 5% Berberine. Berberine

nanomicelles have an average diameter of 2.7 nm. Berberine (BBR) hydrochloride and Silymarin (Sigma, St. Louis, MO, USA) were diluted freshly in saline (0.9%) and Carboxymethyl cellulose (CMC, 03%) before using in this project. Silymarin nanomicelle was prepared from Exir Nano Sina Co., Tehran, Iran.

Animals and experimental groups

In this study, 88 male NMRI mice weighing 25 ± 5 g (Tehran University of Medical Sciences, Tehran, Iran) were used all over the study. Animals were kept at standard temperatures and environmental conditions and had free access to normal food and water; 12 hours of light - 12 hours of darkness; the temperature was 23 ± 1°C throughout the study. They were kept in the laboratory for about a week before the experiment to adapt to environmental conditions, then all the experiments were conducted between 9:00 and 12:00 A.M each day. The minimum number of samples was considered and tested according to the protocol of working with laboratory animals of Tehran University of Medical Sciences based on animal ethics. Also, animals were handled according to the criteria proposed by the Guide for the Care and Use of Laboratory Animals (NIH US publication, no. 23-86, revised 1985).

Drug-treated animals with Berberine nanomicelles (5 and 10 mg/kg), Berberine hydrochloride (20 mg/kg), Silymarin nanomicelles (25 and 50 mg/kg), Silymarin (100 mg/kg), and fluoxetine (20 mg/kg) after 14 days drugs administration as pretreatment in a volume of 10 ml/kg of the mice's body weight (b.w.), to induce depression-like behavior, LPS (1 mg/kg) soluble in isotonic saline was injected intraperitoneally (i.p) 22.5 hours before the last dose of the studied drugs (on 13th day). Then, on the 14th day, 1 hour after the last dose of drugs administrations, the depression tests, including the Open-field test (OFT), Forced swimming test (FST), and Tail suspension test (TST), were implemented on them [43]. Also, the brain level of TNF-α and IL-1ß was measured after finishing the behavior tests on the supernatant of whole brain tissue of mice in all groups [43].

The mice were divided into 11 groups including:

- Control group
- LPS(1mg/kg) group
- Nanomicelle with LPS (Nano micelle)
- Fluoxetine (20mg/kg) with LPS (Fluoxetine)

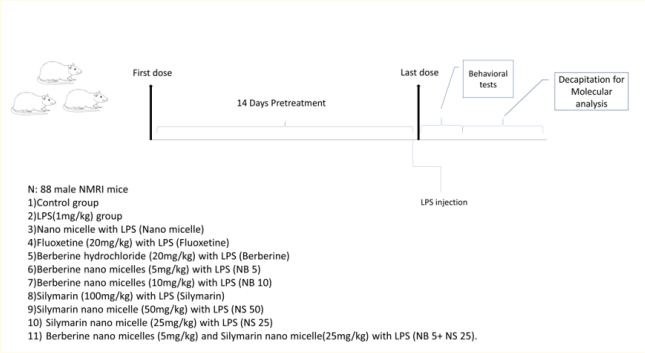


Figure a

- Berberine hydrochloride (20mg/kg) with LPS (Berberine)
- Berberine nanomicelle (5mg/kg) with LPS (NB 5)
- Berberine nanomicelle (10mg/kg) with LPS (NB 10)
- Silymarin (100mg/kg) with LPS (Silymarin)
- Silymarin nanomicelle (50mg/kg) with LPS (NS 50)
- Silymarin nanomicelle (25mg/kg) with LPS (NS 25)
- Berberine nanomicelle (5mg/kg) and Silymarin nanomicelle (25mg/kg) with LPS (NB 5+ NS 25).

Behavioral tests

In the force swimming test, we use a 25 cm high glass cylinder containing 19 cm of water according to the room temperature. Then we put the mouse in it for 6 minutes, and to adapt to the conditions after 2 minutes, the movement and immobility time of the mouse was recorded in the last 4 minutes.

In the open field test, the number of lines that the animal crosses during 10 minutes were recorded then counted, and it was compared in each group to measure and show the differences. Between each mouse test, we used 95% Ethanol to wipe the chamber prior to use and before subsequent tests to remove any scent clues left by the previous subject mouse.

To perform the TST, we hung the mice from the end of the tail using glue at the height of 50 cm above the ground and recorded a time of inactivity of 6 minutes.

Tissue preparation for biochemical assays

To evaluate the levels of TNF-α and IL-1ß, animals were anesthetized (complete analgesia) with ketamine 10% at a dose of 50 mg/kg and xylazine 2% at a dose of 10 mg/kg by intraperitoneal injection [44]. They underwent surgery and used buprenorphine at a dose of 0.05 mg/kg subcutaneously (SC) to reduce pain. Also, to collect brain tissue, animals under complete anesthesia, the spinal cord was amputated, and after death, the animal's head was cut off with scissors, followed by a craniotomy and the whole brain excision. Finally, the collected brain samples were homogenized, and the supernatant was used to measure the levels of TNF-α and IL-1ß to evaluate the anti-inflammatory effects by enzyme-linked immunoassay (ELISA) and its protocol. It was performed according to the instructions of the ELISA kit (Sigma-Aldrich, St. Louis, MO, USA).

Statistical analysis

All data were analyzed with a two-way analysis of variance for the drug interactions followed by Tukey's posttest (Graph pad prism, version 5), except for the main effect of Berberine and Silymarin nanomicelles which was performed with One-Way ANOVA. The value of $P < 0.05$ was considered significant.

Conclusion

Our clinical data indicated that the combination of berberine and silymarin nanomicelles showed significant reduction in brain TNF-α and IL-1ß levels, immobility time in FST, duration of immobility in TST which indicate decreased depression- related symptoms in mice.

Also, the results of the study suggest that using nanomicelle can increase the effectiveness and improve the bioavailability and consequently the chemical stability of the compound.

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Nanotechnology research center, Pharmaceutical technology institute, Mashhad University of Medical Sciences, Mashhad, Iran.

Bibliography

1. Nestler EJ., *et al*. "Neurobiology of Depression". *Neuron* 34.1 (2002): 13-25.
2. Millan MJ. "Multi-target strategies for the improved treatment of depressive states: Conceptual foundations and neuronal substrates, drug discovery and therapeutic application". *Pharmacology and Therapeutics* 110.2 (2006): 135-370.
3. Malhi GS and Mann JJ. "Depression". *The Lancet* 392.10161 (2018): 2299-2312.
4. Widner B., *et al*. "Neopterin production, tryptophan degradation, and mental depression—What is the link?" *Brain, Behavior, and Immunity* 16.5 (2002): 590-595.
5. Cipriani A., *et al*. "Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis". *The Lancet* 391.10128 (2018): 1357-1366.
6. Takikawa O., *et al*. "Interferon-Gamma-Dependent/Independent Expression of Indoleamine 2,3-Dioxygenase". In: Huether G, Kochen W, Simat TJ, Steinhart H, editors. Tryptophan, Serotonin, and Melatonin: Basic Aspects and Applications. Boston, MA: Springer US; (1999): 553-557.
7. Fujigaki H., *et al*. "The signal transducer and activator of transcription 1alpha and interferon regulatory factor 1 are not essential for the induction of indoleamine 2,3-dioxygenase by lipopolysaccharide: involvement of p38 mitogen-activated protein kinase and nuclear factor-kappaB pathways, and synergistic effect of several proinflammatory cytokines". *Journal of Biochemistry* 139.4 (2006): 655-662.
8. Baghai TC., *et al*. "Drug treatment of depression in the 2000s: an overview of achievements in the last 10 years and future possibilities". *The World Journal of Biological Psychiatry* 7.4 (2006): 198-222.
9. Kumar A., *et al*. "Current knowledge and pharmacological profile of berberine: An update". *European Journal of Pharmacology* 761 (2015): 288-297.
10. Kulkarni SK and Dhir A. "On the mechanism of antidepressant-like action of berberine chloride". *European Journal of Pharmacology* 589.1 (2008): 163-172.
11. Chatuphonprasert W., *et al*. "Suppression of beta-naphthoflavone induced CYP1A expression and lipid-peroxidation by berberine". *Fitoterapia* 82.6 (2011): 889-895.

12. Cai Z., *et al*. "Role of berberine in Alzheimer's disease". *Neuropsychiatric Disease and Treatment* 12 (2016): 2509-2520.

13. Zhang Z., *et al*. "Solid dispersion of berberine–phospholipid complex/TPGS 1000/SiO2: preparation, characterization and in vivo studies". *International Journal of Pharmaceutics* 465.1 (2014): 306-316.

14. Wen-zhuan Ma., *et al*. "Preparation characterization and antitumor activity in vitro of berberine hydrochloride polymeric micelles". *China Journal of Chinese Materia Medica* 40 (2015): 1001-5302.

15. Zahmatkeshan M., *et al*. "Improved drug delivery and therapeutic efficacy of PEgylated liposomal doxorubicin by targeting anti-HER2 peptide in murine breast tumor model". *European Journal of Pharmaceutical Sciences* 86 (2016): 125-135.

16. Wang T., *et al*. "Preparation of an anhydrous reverse micelle delivery system to enhance oral bioavailability and anti-diabetic efficacy of berberine". *European Journal of Pharmaceutical Sciences* 44.1 (2011): 127-135.

17. Wang M-J., *et al*. "Silymarin protects dopaminergic neurons against lipopolysaccharide-induced neurotoxicity by inhibiting microglia activation". *European Journal of Neuroscience* 16.11 (2002): 2103-2112.

18. Dixit N., *et al*. "Silymarin: A review of pharmacological aspects and bioavailability enhancement approaches". *Indian Journal of Pharmacology* 39.4 (2007): 172-179.

19. Thakare VN., *et al*. "Therapeutic potential of silymarin in chronic unpredictable mild stress induced depressive-like behavior in mice". *Journal of Psychopharmacology* 32.2 (2017): 223-235.

20. Neha Jaggi AS and Singh N. "Silymarin and Its Role in Chronic Diseases". In: Gupta SC, Prasad S, Aggarwal BB, editors. Drug Discovery from Mother Nature. Cham: Springer International Publishing (2016): 25-44.

21. Priya LB., *et al*. "Chapter 21 - Phytonanoconjugates in oral medicine". In: Andronescu E, Grumezescu AM, editors. Nanostructures for Oral Medicine: Elsevier (2017): 639-668.

22. Cai CX., *et al*. "Chapter 29 - Nutritional and Dietary Interventions for Nonalcoholic Fatty Liver Disease". In: Watson RR, Preedy VR, editors. Dietary Interventions in Liver Disease: Academic Press (2019): 357-72.

23. Bijak M. "Flavonolignans - compounds not only for liver treatment". *Polskiego Towarzystwa Lekarskiego* 42 (2017): 34-37.

24. El-Elimat T., *et al*. "Silymarin Prevents Memory Impairments, Anxiety, and Depressive-Like Symptoms in a Rat Model of Post-Traumatic Stress Disorder". *Planta Medica* 85.1 (2019): 32-40.

25. Junghanns JU and Müller RH. "Nanocrystal technology, drug delivery and clinical applications". *International Journal of Nanomedicine* 3.3 (2008): 295-309.

26. Kakran M. "Fabrication of Nanoparticles of Silymarin, Hesperetin and Glibenclamide by Evaporative Precipitation of Nanosuspension for Fast Dissolution". *Pharmaceutica Analytica Acta* 06 (2014).

27. Ahmad U., *et al*. "Silymarin nanoemulsion against human hepatocellular carcinoma: development and optimization". *Artificial Cells, Nanomedicine, and Biotechnology* 46.2 (2018): 231-241.

28. Yousefdoost S., *et al*. "Evaluation of Nano and Microcapsules of Silymarin in Simulated Gastrointestinal Conditions for Animal Target Delivery". *Iranian Journal of Applied Animal Science* 9.2 (2019): 247-255.

29. Kosari-Nasab M., *et al*. "Anxiolytic- and antidepressant-like effects of Silymarin compared to diazepam and fluoxetine in a mouse model of mild traumatic brain injury". *Toxicology and Applied Pharmacology* 338 (2018): 159-173.

30. Del Grande da Silva G., *et al*. "Pro-inflammatory cytokines and psychotherapy in depression: Results from a randomized clinical trial". *Journal of Psychiatric Research* 75 (2016): 57-64.

31. Miller AH and Raison CL. "The role of inflammation in depression: from evolutionary imperative to modern treatment target". *Nature Reviews Immunology* 16.1 (2016): 22-34.

32. Amitai M., *et al*. "The Relationship Between Plasma Cytokine Levels and Response to Selective Serotonin Reuptake Inhibitor Treatment in Children and Adolescents with Depression and/or Anxiety Disorders". *Journal of Child and Adolescent Psychopharmacology* 26.8 (2016): 727-732.

33. Al-Drees A and Khalil MS. "Histological and immunohistochemical effects of L-arginine and silymarin on TNBS-induced inflammatory bowel disease in rats". *Histology and Histopathology* 31.11 (2016): 1699-5848.

34. Khoshnoodi M., *et al*. "Possible involvement of nitric oxide in antidepressant-like effect of silymarin in male mice". *Pharmaceutical Biology* 53.5 (2015): 739-745.

35. Thakare VN., *et al*. "Potential antidepressant-like activity of silymarin in the acute restraint stress in mice: Modulation of corticosterone and oxidative stress response in cerebral cortex and hippocampus". *Pharmacological Reports* 68.5 (2016): 1020-1027.

36. Peng W-H., *et al*. "Berberine produces antidepressant-like effects in the forced swim test and in the tail suspension test in mice". *Life Sciences* 81.11 (2007): 933-938.

37. Bryant Sg., *et al*. "Current concepts in clinical therapeutics: major affective disorders, Part 1". (1986): 0278-2677.

38. Javed S., *et al*. "Reassessing bioavailability of silymarin". *Alternative Medicine Review* (2011): 1089-5159.

39. Shi J., *et al*. "Nanotechnology in Drug Delivery and Tissue Engineering: From Discovery to Applications". *Nano Letters* 10.9 (2010): 3223-3230.

40. Simões SMN., *et al*. "Polymeric micelles for oral drug administration enabling locoregional and systemic treatments". *Expert Opinion on Drug Delivery* 12.2 (2015): 297-318.

41. Azadi R., *et al*. "Anti-inflammatory Efficacy of Berberine Nanomicelle for Improvement of Cerebral Ischemia: Formulation, Characterization and Evaluation in Bilateral Common Carotid Artery Occlusion Rat Model". *BMC Pharmacology and Toxicology* (2020).

42. Posternak MA., *et al*. "Is there a delay in the antidepressant effect? A meta-analysis". *The Journal of Clinical Psychiatry* (2005): 0160-6689.

43. Mousavi SE., *et al*. "Licofelone Attenuates LPS-induced Depressive-like Behavior in Mice: A Possible Role for Nitric Oxide". *Journal of Pharmacy and Pharmaceutical Sciences* 21.1 (2018): 184-194.

44. Pishva SP., *et al*. "The effect of berberine nanomicelles on hepatic cirrhosis in bile duct-ligated rats". *Nanomedicine Journal* 5.4 (2018): 199-209.

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