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Research Article
Evaluation of Antidepressant Activity of Triptolide in Lipopolysaccharide Induced Depressive like Behavior in Experimental Mice

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Abstract

Background and Objective: Depression is a psychiatric disorder and inflammation facilitates the depression. Complexity of symptoms related to depression among different individuals and different therapeutic responses exhibited by the use of available antidepressants show that a need to identify novel antidepressants is there. Thus present study evaluates the effect of Triptolide (TPL) on depressive like behavior induced by lipopolysaccharide (LPS) in mice. **Materials and Methods:** All the mice were challenged with LPS (0.83 mg kg^{-1}, i.p.) 30 min after the administration of 5 mg kg^{-1} of TPL. Several behavioral parameters were studied including open field test, forced swim test, tail suspension test and sucrose preference test. The level of pro-inflammatory cytokines and oxidative stress parameters were estimated in the brain tissues of LPS challenged mice. Moreover, level of brain derived neurotrophic factor (BDNF) in the brain tissues and plasma concentration of corticosteroid was estimated in LPS challenged mice. **Results:** Data given in the study suggested that treatment with TPL attenuates the behavioral parameters affected by LPS in mice. Level of pro-inflammatory cytokines and oxidative stress parameters were significantly decreases in the prefrontal cortex and hippocampus of TPL treated group compared to negative control group. Plasma concentration of corticosteroid significantly decreases and level of BDNF in brain tissue significantly increases in TPL treated group than negative control group of mice. **Conclusion:** Present study concludes that treatment with TPL attenuates the depressive behavior by decreasing the level of cytokines and oxidative stress parameter in the brain tissues of LPS challenged mice.

Key words: Lipopolysaccharide, depression, oxido-nitrosative stress, pro-inflammatory cytokines, BDNF levels, hippocampus, prefrontal cortex

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

INTRODUCTION

Depression is an important psychiatric disorder that is facilitated by inflammation[1]. A high level of pro-inflammatory cytokines like TNF-α and IL-1β is reported in blood and cerebrospinal fluid of patients suffering from depression[2]. More than 350 million people suffer from this disorder worldwide and 1.5-19% people suffer from it throughout their life[3]. Lipopolysaccharide (LPS) and cytokines are reported to produce depressive like behavior in animals[4]. The production of anti-inflammatory cytokines have been reported to increase by antidepressants, however, the level of pro-inflammatory cytokines have been decreased by their use[5]. Most of the antidepressants used are slow to respond, that is, they take about 6-8 weeks for producing any prominent response[6]. Besides slow onset of response, side effects and drug-drug interactions also pose a problem for proper activity of antidepressants[7]. Complexity of symptoms related to depression among different individuals and different therapeutic responses exhibited by the use of available antidepressants show that a need to identify novel antidepressants is there, moreover, the prevention of inflammatory can also be a possible therapeutic target for curing such psychiatric disorders.

A major component of Chinese herb *Tripterygium wilfordii* Hook F. is Triptolide that has been used traditionally for the treatment of inflammatory diseases in China. Studies have shown that it possesses anti-inflammatory, antitumor and immunosuppressive features[8]. Triptolide is demonstrated to have low molecular size and lipophilic characteristics that give it a potential to treat disorders of central nervous system and it is being investigated worldwide[9]. *Triptergiumis* used in Chinese allopathic medicine since 1960s and diterpenoid tripoxide (Triptolide) which it contains is thought to have therapeutic potential against disorders like cancer, hepatitis and chronic nephritis[10,11]. Thus present study evaluated the antidepressant activity of Triptolide.

MATERIALS AND METHODS

Chemicals: Triptolide was purchased having 98% purity from National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China). Lipopolysaccharide was purchased from Sigma-Aldrich, St. Louis, MO, USA obtained from *E. coli* (serotype 0127: B8) along with all other chemicals. Immunoassay kits were purchased from Invitrogen Co., Carlsbad, CA, USA for this study.

Animals: Adult Kun-Ming mice (male) were used for carrying out the experiments weighing 22-25 g and were obtained from Shanghai Medical College, China. All the animals were kept in a 12 h light/dark cycle at 20±2°C. An unlimited excess to food and water was provided to animals in a humidity controlled environment. All the experiments were performed according to the ethical guidelines of Hubei Cancer Hospital, China (HCH/IAEC/2016/32). The study was performed in the lab of Hubei Cancer Hospital, China during the duration of February-June, 2016.

Experimental: Normal saline was used to dissolve LPS and it was administered at 0.83 mg kg^{-1} intraperitoneally. This dose was used in accordance with the previous studies as depressive like behavior is induced in adult mice by it O'Connor *et al.*[12]. Administration of Triptolide was carried out at 5 mg kg^{-1}/day according to the previously described studies regarding the effect of Triptolide on inflammatory pathway after spinal cord injury in rats[13]. Animals were distributed in three different groups for the study where (n = 10). Control was treated with PBS (vehicle of Triptolide), 30 min before administering the saline. Negative control group was treated with PBS 30 min prior to intraperitoneal administration of LPS as 0.83 mg kg^{-1} that served as control group for LPS. In TPL treated group treated with 5 mg kg^{-1} Triptolide 30 min before administration of LPS (0.83 mg kg^{-1}) intraperitoneally.

Behavioral parameters were assessed that included open field test, forced swim test and sucrose preference tests after 24 h of administering LPS. Different biochemical parameters were also assessed and level of corticosterone (plasma) was investigated at time interval of 4 h after lipopolysaccharide challenge.

Assessment of behavior

Open field test: For the determination of behavioral changes in rodents under different environments, open field test is performed[14]. For this purpose, a specially designed cage, divided into nine quadrants was used that as devoid of bedding or litter. During a 5 min period the number of line crossings and rearing were counted and locomotor activity was measured.

Forced swimming test: According to previously described protocols, forced swimming test (FST) was performed[15]. For this purpose, water was poured into a cylinder that had (diameter 15 cm, height 25 cm) dimensions 15 cm of cylinder

was filed with water at 25±1°C. For experiment mice were placed in cylinder for 6 min. For atleast 4 min video data and immobility time were recorded. In case any animal remained motionless while floating and did not struggle except for necessary movement required to keep head above water level, such animals were considered motionless.

Tail suspension test: According to previous studies tail suspension test was performed[16]. At 50 cm height above the floor each experimental animal was suspended from edge of the table. It was done by placing adhesive tap at 1 cm distance from the tale tip. The process was carried out in dark room. Immobility time was video recorded for 6 min and time utilized for 4 min was reported.

Sucrose preference test: For evaluating anhedonia sucrose preference test was used as reported previously[17]. About 2% sucrose solution was used along with water for 5 days to acclimatize all experimental animals. Sucrose preference at a baseline level was determined for each mouse after administering LPS. Sucrose solution was placed in mice cages after filling it in drinking water bottles having stopper valve. Relative position was also changed on each day. For performing experiment, food and water was not given to mice at least 2 h before the test. Then sucrose preference was analyzed by 48 h post LPS fluid content analysis.

Preparation of tissue homogenate: Cervical dislocation procedure was used to kill mice at time interval of 24 h after administering LPS. Dissection of hippocampus and Prefrontal cortex was performed after an immediate removal of brains. 10% w/v 0.1 M phosphate buffer having pH 7.4 was used to homogenize both dissected parts for performing biochemical analysis.

Estimation of oxidative stress parameters: Estimation of reduced glutathione levels and lipid peroxidation was performed to measure oxidative stress level in prefrontal cortex and hippocampus. The level of thiobarbituric acid (TBARS) was estimated as done previously[18]. Spectrophotometer was used to measure the absorbance of TBARS level. The absorbance at 532 nm was recorded and reported in the form of mmol of malondialdehyde (MDA)/g wet tissue. To estimate changes in glutathione level the method described by Beutler *et al.*[19] was used.

Estimation of level of cytokines, corticosterone and BDNF: Estimation of IL-6, TNF-α and IL-1β was performed using ELISA. Kits were purchased from Invitrogen Co., Carlsbad, CA, USA and manufacturer's protocol was followed for analysis. To determine quantitative BDNF level in hippocampus, ELISA kits (Promega, Madison, WI, USA) were used. These kits had intra-assay coefficient variation of <10% and a minimum detection limit 15.6 pg mL^{-1}. The BDNF concentration was determined and reported as ng mg^{-1} of protein. For measuring corticosterone level, ELISA kit was purchased from Abnova Corporation, Taiwan to determine circulating corticosterone level (CORT). The level was expressed as ng mL^{-1} and manufacturer's instructions were followed for performing the test.

Statistical analysis: Statistical analysis and results reported in the form of Mean±SEM. One-way analysis of variance (ANOVA) was performed for the comparison of results and $p<0.05$ was significant value. Graph Pad Prism version 5.0 for windows (San Diego, CA, USA) was used to analyze the results.

RESULTS

Effect of Triptolide on behavioral parameters: Effect of TPL was observed on the behavioral parameter in LPS challenged mice as shown in Fig. 1. For open field test, it was observed that there wasn't significant difference between control, LPS and Triptolide groups in rearing numbers. Little decrease was observed in mice that were treated with lipopolysaccharide but this difference wasn't significant as shown in Fig. 1a. In force swim test, there was significant increase in the immobility in LPS treated rat as compared to control group. However, treatment with TPL significantly ($p<0.01$) decreases the immobility in force swim test as compared to negative control group as shown in Fig. 1b. It was observed that immobility time in tail suspension test was significantly increases in negative control group than control group. There was significant decrease in the immobility time in TPL treated group than negative control group as shown in Fig. 1c. A decreased preference for sucrose was observed in LPS treated group as analyzed by the sucrose preference index, where $p<0.05$ and sucrose preference increased significantly in group of mice treated with Triptolide (Fig. 1d).

Effect of Triptolide on oxidative stress parameters: Effect of Triptolide on different stress parameters in LPS challenged

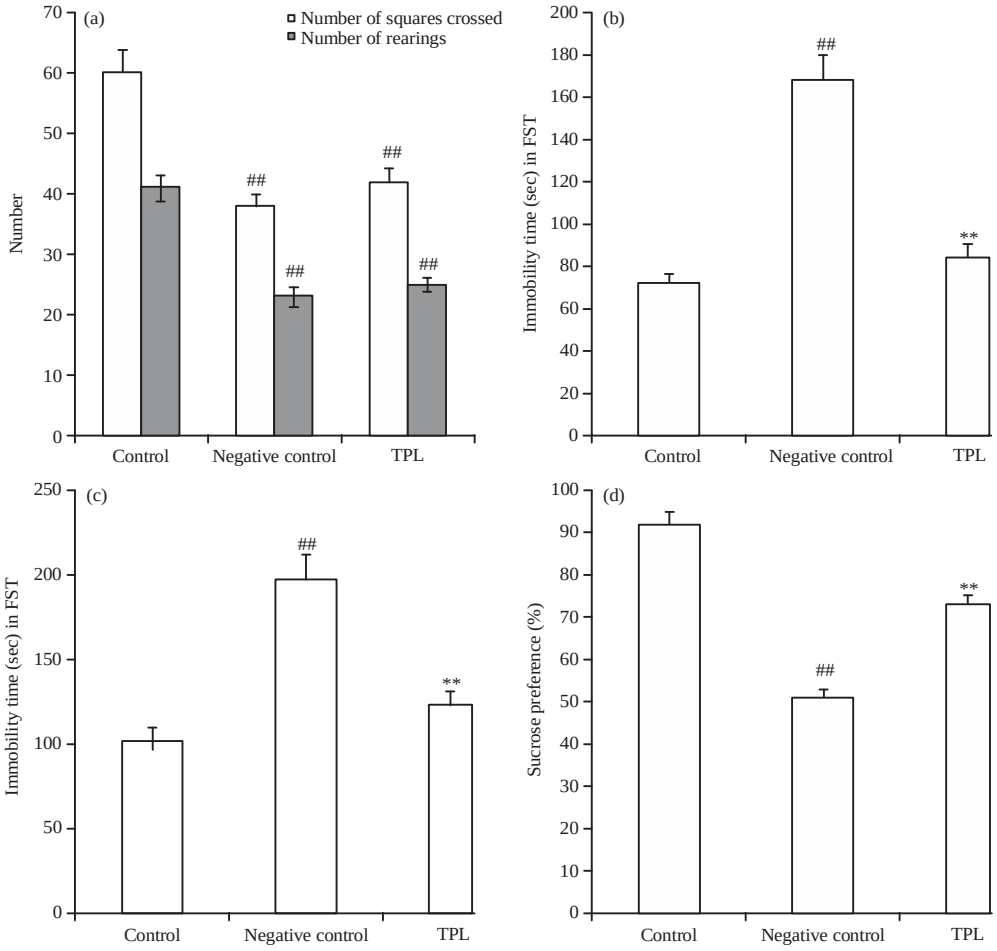

Fig. 1(a-d): Effect of Triptolide on behavioral parameters in LPS challenged mice, (a) Open field test, (b) Forced swim test, (c) Tail suspension test and (d) Sucrose preference test

Mean±SD (n = 10), ##p<0.01 compared to control, **p<0.01 compared to Negative control

mice was shown in Fig. 2. There was significant ($p<0.01$) increase in the level of MDA and decrease in the level of GSH was found in the brain tissue homogenate of negative control group than control group of mice. However, treatment with TPL was significantly ($p<0.01$) decreases the level of MDA and increases the level of GSH in the brain tissue homogenate of LPS challenged mice than negative control group.

Effect of Triptolide on the level of cytokines: Effect of Triptolide pretreatment on level of cytokines in the brain tissue homogenate of LPS challenged mice was shown in Fig. 3. It was observed that due to LPS administration level of IL-6, TNF-α and IL-1β significantly ($p<0.01$) increases in brain tissue homogenate of negative control group compared to control group of mice. However, treatment with TPL significantly ($p<0.01$) decreases the level of cytokines in the brain tissue homogenate of LPS challenged mice than negative control group.

Effect of Triptolide on the level of corticosteroids and BDNF: Effect of Triptolide pretreatment on the plasma concentration of corticosteroid and the level of BDNF in the hippocampus tissue of LPS challenged mice was shown in Fig. 4. There was significant ($p<0.01$) increase in the plasma concentration of corticosteroid and decrease in the level of BDNF in the brain tissues of negative control group compared to control group of mice. However, treatment with TPL significantly ($p<0.01$) decrease the concentration of corticosteroid in the plasma and increase in the level of BDNF in the hippocampus tissues of LPS challenged mice compared to negative control group.

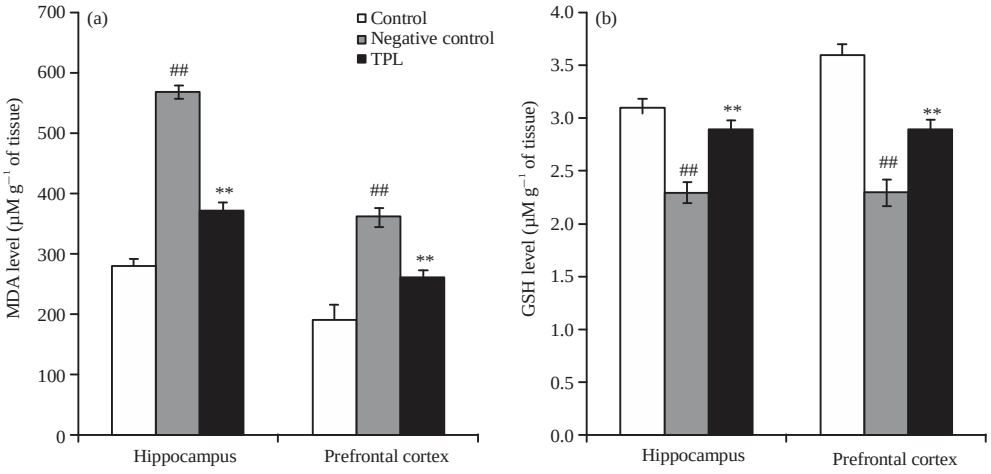

Fig. 2(a-b): Effect of Triptolide pretreatment on the oxidative stress parameters in the brain tissue homogenate of LPS challenged mice

Mean±SD (n = 10), ##p<0.01 compared to control, **p<0.01 compared to negative control

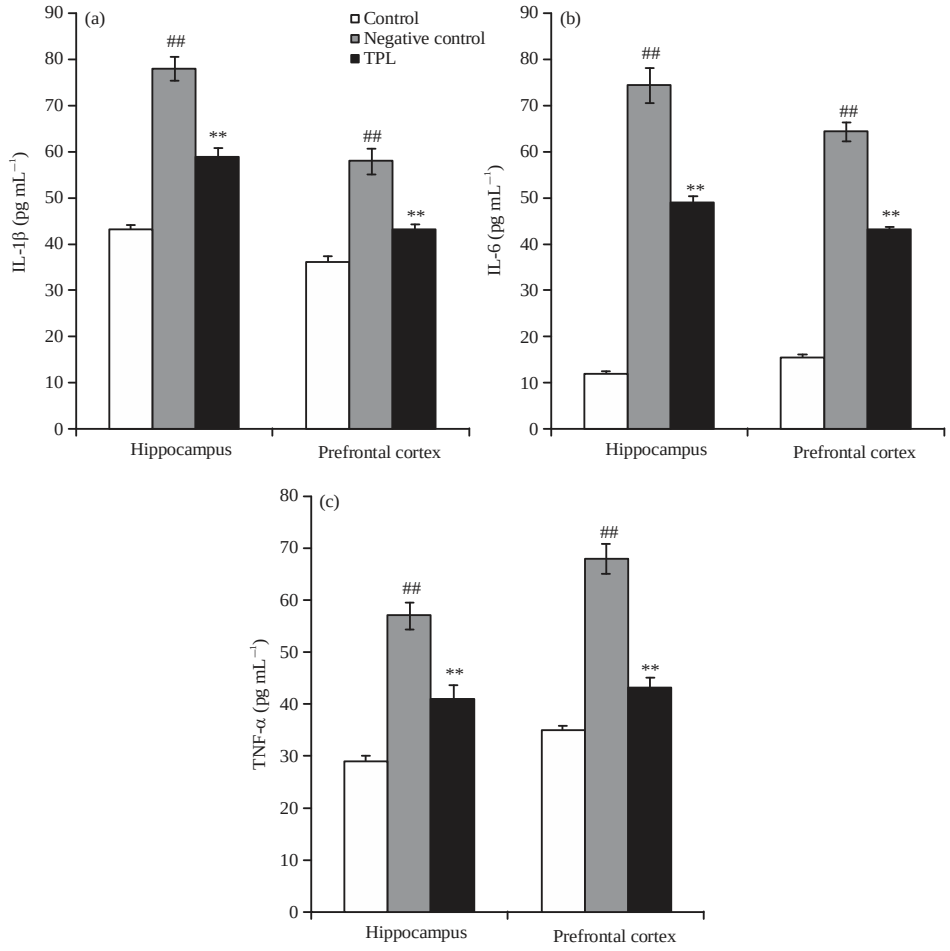

Fig. 3(a-c): Effect of Triptolide pretreatment on level of cytokines in the brain tissue homogenate of LPS challenged mice

Mean±SD (n=10), ##p<0.01 compared to control, **p<0.01 compared to negative control

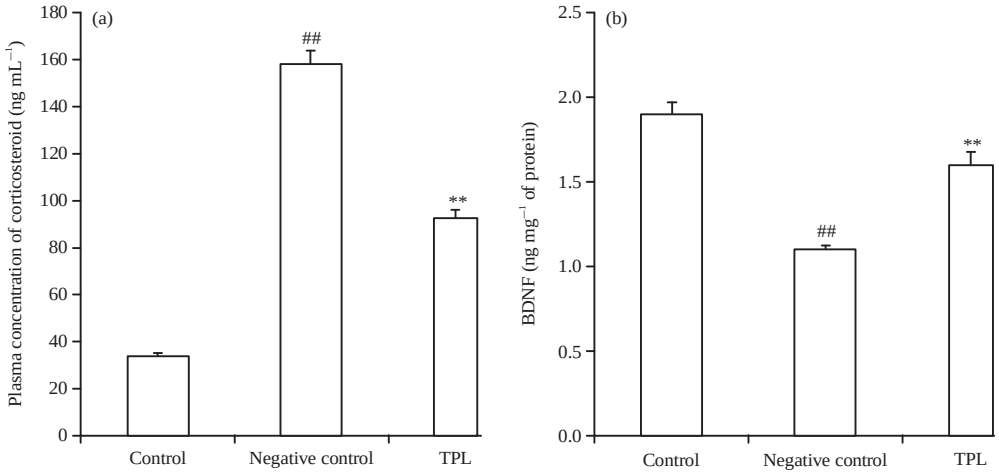

Fig. 4(a-b): Effect of Triptolide pretreatment on plasma concentration of corticosteroid and level of BDNF in hippocampus of LPS challenged mice

Mean±SD (n=10), ##p<0.01 compared to control, **p<0.01 compared to negative control

DISCUSSION

The results of this study indicate that antidepressant activity is induced by Triptolide in case of pretreatment of LPS challenged mice. A reduction in oxidonirosative stress parameters and proinflammatory cytokines level indicate a positive activity of Triptolide.

Previous studies have shown that different depressive like behaviors can be generated in experimental mice after systemic administration of LPS that include a reduced social interaction, a reduced tendency for exploration, increased anhedonia[5,20]. Different parameters like tail suspension test and open field tests are used for analyzing the positive effect of pretreatment in mice treated with Triptolide. Proinflammatory cytokines level was measured as it is a good indicator of depression[21]. This study has shown that the level of proinflammatory cytokines decreases by the pre-treatment of mice with Triptolide and therefore, it will be helpful in reducing depressive like behavior. A reduction in oxido-nitrosative stress values and hippocample BDNF levels is an indicator of the positive effect of Triptolide in LPS challenged mice that possess a depressive like behavior.

Triptolide has been used for treating different ailments traditionally in Chinese medicine which include arthritis and inflammation[22]. A close relation exists between inflammation and stress like behavior according to previous studies and serotinine transporters play a major role in this regard[23].

It has been reported that in case of tail suspension test and forced swimming test the level of proinflammatory cytokines favored the immobile behavior under the action of indoleamine 2,3 dioxygenase enzyme (IDO)[24]. In this study, it was observed that due to treatment of LPS an increased immobility is observed in mice which are exhibited by the forced swim test and tail suspension test. This finding is in accordance with previous studies[4]. Data of the present study suggests that Triptolide has a potential treat the depression and it could be used clinically for the management of depression. However, one can also study its effect on the gene level for the better understanding of it anti depression mechanism.

CONCLUSION

Present study concludes that treatment with TPL attenuates the depressive behavior by decreasing the level of cytokines and oxidative stress parameter in the brain tissues of LPS challenged mice.

SIGNIFICANT STATEMENT

This study discovers the antidepressant activity of Triptolide in lipopolysaccharide induced depressive like behavior in experimental mice. This investigation provides the alternative treatment for the management of depressive behavior as Triptolide attenuates the altered level of inflammatory mediators and oxidative stress.

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