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# Trichostatin A Ameliorates Spatial Memory Deficits and Suppresses Hippocampal Pro- Inflammatory Cytokines in A Rat Model of Prenatal Stress

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## Abstract

**Introduction:** Maternal stress during pregnancy leads to neuroinflammation of the fetus and cognitive disorders in children. On the other side, Trichostatin A (TSA) as a histone deacetylase inhibitor has anti-inflammatory and neuroprotective effects. The aim of the present study was an evaluation of TSA effect on amelioration of spatial memory deficit and modification of pro-inflammatory cytokines in rat's hippocampus of prenatal footshock stress model.

**Methods:** In the present study, 24 pregnant Wistar rats were divided into 4 groups including control, PFS+Saline, PFS+TSA5, and PFS+TSA10. In order to induce prenatal footshock stress (PFS), the pregnant rats were subjected to electrical shock at 1 mA, 50 Hz for 2 seconds with 5 repetitions at 3-minute intervals from 12 to 18 days of pregnancy. Two hours before applying the shock intraperitoneal administration of TSA was performed daily. When the offsprings were one-month old, their spatial memory was assessed by the Morris water maze. Finally, the levels of pro-inflammatory cytokines (TNF-α, IL-1β, and IL-6) were measured by ELISA method.

**Results:** a reduction in spatial memory and an increase in the level of TNF-α, IL-1β, and IL-6 were observed in PFS+Saline group compared to the control. Besides, spatial learning showed a significant increase in TSA-treated groups compared to PFS+Saline.

**Conclusion:** TSA with anti-inflammatory effects led to the reduction of hippocampal inflammatory cytokines and amelioration of spatial memory in offsprings who were exposed to stress in the prenatal period.

**Keywords:** Prenatal Stress, Inflammatory Cytokine, Hippocampus, Rat

## Introduction

Maternal stress by increasing the level of glucocorticoid hormones and noradrenaline in blood circulation results in a delay in fetal development and cognitive-behavioral disorders in newborns (1). Besides, pregnancy stress affects glucocorticoid receptors of the hippocampus by changing the activity of the embryo's hypothalamus- pituitary- adrenal axis (HPA) (2). However, the interaction between the HPA axis and the hippocampus plays an important role in memory and learning process (3). Exposure of embryos to high levels of cortisol during pregnancy can delay the growth and development of the brain cells, resulting in a decreased size of the hippocampus and pathological changes in the hippocampal neurons, which leads to hippocampus-dependent learning disorder (4). Moreover, in the final weeks of pregnancy, stress causes the reduction of neurogenesis in the hippocampus' embryo (5). The hippocampus as a part of the prefrontal cortex in the mammalian brain is the main structure in spatial learning and memory consolidation and has several vital functions in the memory process, including storage, consolidation, and retrieval of information (6). Damage to the hippocampus causes severe deficit in spatial memory (7). Although the causes of pregnancy stress-induced cognitive and neurodevelopmental disorders are not well

known, recently pro-inflammatory cytokines have been addressed as a major risk factor (8). In the brain of the fetus, pregnancy stress results in significant changes in microglial density (9). Increasing microglias by activating the mother's immune system during pregnancy increases the risk of cognitive and behavioral disorders in newborns (10). Increased levels of pro-inflammatory mediators such as IL-6 and TNF-α have been observed in the hippocampus of infants exposed to prenatal stress (11). Histone deacetylase inhibitor (HDACi) is described as a category of medicines that are used to improve the formation of long-term memory (12). Generally, HDAC is modify and adjust genomic transcription (13). Hydroxamic acids such as TSA have also been effective in improving aging-caused cognitive disorders and the treatment of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease and Huntington's disease (14). Therefore, the aim of the present study was evaluating the effect of repetitive stress during pregnancy on pro-inflammatory cytokine levels (IL-1β, IL-6, and TNF-α) in the hippocampus and evaluation of spatial memory, effects of neuroprotective and anti-inflammatory of TSA on 30-day rat offsprings.

## Methods

In the present experimental study, the offsprings of 24 pregnant female Wistar rats with an average weight of 180±10 gr were used. The animals were purchased from the center for animal labratory of Shiraz University of Medical Sciences. After transferring them to laboratory, they were kept 8 days in standard laboratory condition to adapt to new conditions. At all stages of operation, the animals were placed under the standard temperature (25±2$^{\circ}$C) humidity (50±10%), and the 12-hour lighting cycle (6 am to 6 pm). Observance of ethical principles was in accordance with international laws and regulations of the faculty of the animal faculty ethics committee. After an adaptation period, each virgin female rat was caged with a mature male rat to induce pregnancy. Before mating, vaginal smears were prepared from rats and the animals that were in the estrus cycle were selected for mating. After 8 hours, the vaginal sample was taken from a female rat to determine the presence of spermatozoa and zero-day of pregnancy. Then, the pregnant rats were weighted and randomly divided into 4 groups (n=6) including the healthy control group, PFS+Saline (which received shock and Saline), PFS+TSA5 (which received a shock and 5mg/kg of TSA), and PFS+TSA10 (which received a shock and 10mg/kg of TSA). From 12th to 18th day of pregnancy, pregnant rats were placed in a shock induction chamber with steel bars on the floor and were subjected to electrical shock at 1 mA, 50 Hz for 2 seconds with 5 repetitions at 3-minute intervals (15). In TSA groups, 2 hours before subjecting to shock, the pregnant rats intraperitoneally received different doses of normal saline from 12 to 18 days of pregnancy. After birth, newborns were kept in the standard condition. Then, 20 male 30-day newborns were selected from each group to study the spatial memory. Morris water maze test was used to assess spatial memory. The maze, a circular black color pond with a diameter of 136 cm and a height of 60 cm was filled with 25 cm water (24±1$^{\circ}$C). A hidden platform with 10 cm diameter was in one cm below the water in the center of one of the pool's quadrants. In each test block, each rat was tested four times (four trials). The position of the platform and its coordinates were the same in each block and for all experimental groups. But the starting point at each test time was randomly determined by tracker software. An indicator of latency in platform finding (EL) and speed of swimming was recorded in 60 seconds. To maintain learning, 4 blocks were performed consecutively and then the probing test was conducted. In this test, by removing the hidden platform, the animal was abandoned for 60 seconds at the pool, and the duration of swimming in the quadrant was considered as a

probe indicator (16). After completing the Morris Water Maze test, the animals were deeply anesthetized by inhalation of the chloroform gas in the desiccator and animal's brain was immediately separated and put on ice. Then, the hippocampus was carefully removed from other parts under a stereoscope (Olympus, Japan). At least 10 hippocampal samples were used from each group. After washing the sample, the hippocampus was homogenized with saline solution and Tris buffer (Sigma, Germany) for 5 minutes by Homogenizer (IKA, Germany) at 5000 rpm and then centrifuged with a refrigerated centrifuge machine (Hermle, Germany). Besides, 0.5 mM of phenyl methyl sulfonyl fluoride (Sigma-Aldrich, Germany) was used as an inhibitor of proteases (17). After centrifugation, the supernatant was taken by the sampler, and then the tissue level of the TNF-α, IL-1β and IL-6 were measured by ELISA method and ELISA reader Model 2100 (Stat Fax, USA) and kits of Fine Test Co. (Fine Test, China). Statistical analysis was performed among different groups using SPSS V.22 software. One-way ANOVA and Tukey's post hoc test were used to determine the significant difference between the groups. In the learning process of the Morris Water Maze Test, Repeated Measure ANOVA was employed. Statistically, significant value was considered to be P <0.05.

## Results

The results of Repeated Measure ANOVA on 4 blocks showed the progressive decrease in the time to reach the platform in all studied groups (Figure 1). In fact, the time to reach the hidden platform in the PFS+Saline group has a significant increase compared to the control group in the second to fourth blocks ($p$ <0.001). Also, in TSA groups, the average EL time in the fourth learning block showed a significant reduction compared to PFS + Saline group ($p$ <0.01). There was no significant difference between PFS+TSA5 and PFS+TSA10 groups in none of the studied blocks. There was no significant difference in swimming speed between PFS + Saline and control and between PFS+TSA5, PFS+TSA10 groups, and PFS+Saline group ($p$ > 0.05). This result suggests that the PFS model does not induce motor impairment (Figure 2). The mean time spent on the objective quadrant of Morris water maze showed that PFS+Saline group had a significant decrease compared to the control (Figure 3. $p$ <0.001). On the other hand, in swimming time, PFS + TSA5 and PFS + TSA10 groups showed a significant increase in the objective quadrant compared to the PFS + Saline group (Figure 3. $p$ <0.001). Moreover, there was a significant difference between PFS+TSA5 and PFS+TSA10 groups ($p$ <0.05). The results of a one-way ANOVA test showed that the expression level of pro-inflammatory cytokines in the hippocampus of rats exposed to embryonic stress was significantly increased in comparison with the control group. In fact, there was a significant increase in the level of TNF-α, IL-1β, and IL-6 in the hippocampus of the PFS + Saline group compared to the control group ($p$ < 0.05). On the other hand, in treated groups with different dosage of TSA, the level of hippocampal pro-inflammatory cytokines was significantly decreased compared to PFS+Saline group (Table 1, $p$ <0.05). Based on Tukey's post hoc test, there was a significant difference between PFS+Saline group with PFS+TSA5 and PFS+TSA10 groups, while there was no significant difference between PFS+TSA5 and PFS+TSA10 groups.

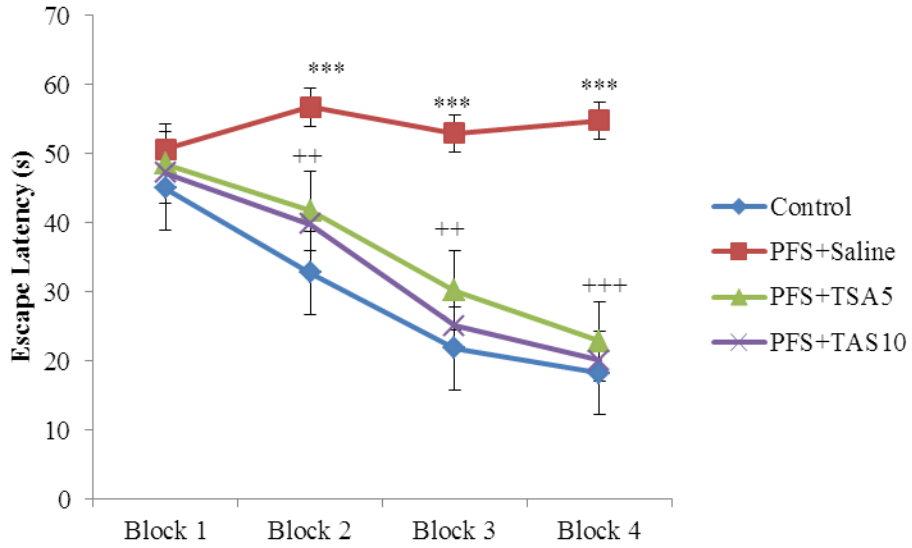


**Figure 1.** Mean ± standard deviation of escape latency (EL) in four trial blocks of Morris water maze groups. The results indicate that there is significant different between PFS+Saline group and control group ($^{***}p < 0.001$) and treated TSA groups compared with PFS+Saline ($^{++}p < 0.01$, $^{+++}p < 0.001$).

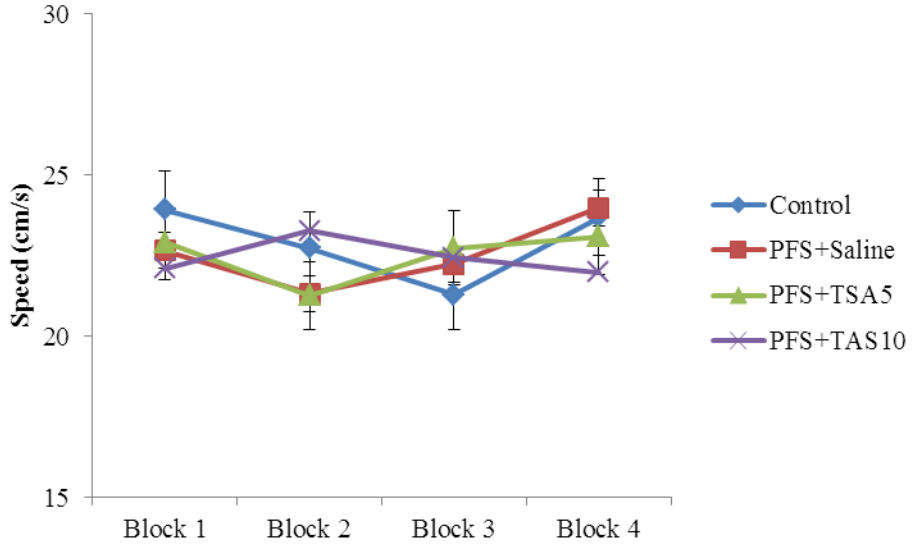


**Figure 2.** Mean ± standard deviation of swimming speed in four trial blocks (learning blocks) of Morris water maze groups. The results indicate no significant difference between these groups.

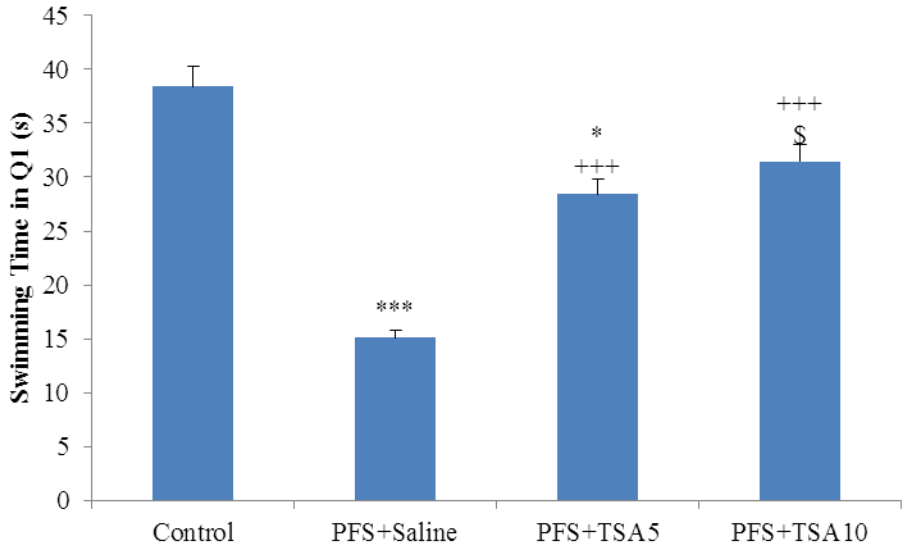


**Figure 3.** Mean ± standard deviation of average time the subject spends in in target quadrant (Q1) in trial stage (probe). There is a significant difference between control and PFS+Saline groups ($^{***}p < 0.001$, $^{*}p < 0.05$). There is a significant difference between TSA-treated and PFS+Saline groups ($^{+++}p < 0.001$) and between PFS+TSA5 compared with PFS+TSA10 ($^{\$}p < 0.05$).

**Table 1.** Average forebrain level of inflammatory cytokines per group

| Group / Parameter (n-10) | TNF-α (pg/ml) | IL-1β (pg/ml) | IL-6 (pg/ml) |
|---|---|---|---|
| Control | 103.27±11.87 | 55.43±3.17 | 78.19±5.29 |
| PFS+Saline | 186.74±9.43 † | 123.11±5.98 † | 176.41±10.86 † |
| PFS+TSA5 | 127.45±10.27 * | 89.34±4.63 * | 93.39±8.08 * |
| PFS+TSA10 | 118.97±9.96 * | 92.18±5.09 * | 88.54±9.47 * |
| The significance level (ANOVA) | 0.003 | 0.017 | 0.001 |

Data are shown in mean±SEM and analysed by one way ANOVA with Tukey post hoc:
†: $p < 0.05$, control vs. PFS+Saline
*: $p < 0.05$, PFS+Saline vs. PFS+TSA5,10

## Discussion

The present study has evaluated the effect of TSA on the amelioration of spatial memory and modification of prenatal stress-induced neuroinflammation in 30- day old newborn rats. The results showed that maternal stress causes cognitive and spatial memory deficits, which are along with neuroinflammation of the hippocampus and increase the level of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 in the hippocampus of newborns. Also, TSA could reduce the spatial learning deficits and improve spatial memory by significant reduction of pro-inflammatory cytokines level in the hippocampus. In the last trimester of pregnancy, chronic stresses will cause severe learning disabilities, including a disorder in short-term, long-term, spatial, and non-spatial memory at the beginning of the child's life (18). In pregnant rats, by increasing the footshock stress, the functional activity of limbic and paralimbic areas was increased

(19). However, the blood flow to the posterior region of the hippocampus, which is associated with cognitive functions, was decreased (20). Besides, the reduction in the prefrontal cortex, premotor cortex, temporal lobe, and large neuronal death in the CA3 region of the hippocampus was observed in newborns exposed to prenatal stress (21). The induction of neuroinflammation following pregnancy stress and microglial activity in different regions of the fetal brain is one of the proposed mechanisms to justify cognitive-behavioral disorders following pregnancy stress (22). Inflammation results in secretion of the high amount of pro-inflammatory cytokines such as TNF-α, IL-1β, and IL-6 (23), which is along with the reduction of neurogenesis and inhibition of neurotrophic system and neuronal death (24). Memory and cognitive functions deficits have also been seen after activation of astrocytes and the release of pro-inflammatory cytokines in the brain (25). Bittle and Stevens showed that injection of cortisol hormone and IL-1β leads to a change in function of the microglial system in fetal brain and induction of psychopathological conditions in newborns (26). On the other hand, Zhang *et al*. indicated that the incidence of anxiety-like behaviors and depression associated with pregnancy stress depend on the increase in the hippocampus inflammatory mediators and administration of anti-depressant drug such as duloxetine results in a decrease level of IL-6 (27). Moreover, pregnancy stress increases mRNA level of IL-1β and the number of microglial cells in the hippocampus of female offsprings (28). Trichostatin A (TSA) as a histone deacetylase inhibitor ameliorates cognitive disorder in various types of cognitive lesions including Alzheimer's disease model in rodents (29). Treatment with TSA reduces plasma corticosterone levels and acetylation of histone H3 in the pre-limbic cortex and hippocampus in animals under chronic restraint stress and ameliorates memory (30). In this study, the amelioration of spatial memory, which was measured by Morris water maze, was seen in PFS groups treated with TSA compared to untreated group. On the other hand, various studies showed that histone deacetylase inhibitor such as TSA suppresses inflammatory responses in various autoimmune and inflammatory diseases (31). In fact, treatment with TSA has reduced serum levels of TNF-α, INF-ϒ, IL-10, and IL-18 in chronic liver failure compared to untreated patients (32). Similar to the present study, TSA significantly reduced tissue levels of TNF-α, IL-1β, and IL-6 in pulmonary lesions, and thus exhibited its anti-inflammatory effects (33). The results of this study showed that TSA with anti-inflammatory and neuroprotective effects can reduce cognitive disorder and chronic pregnancy stress-induced nervous inflammation in one-month-old newborns. Therefore, it can be used as a therapeutic approach to treating cognitive-behavioral diseases caused by pregnancy stress.

## Conclusion

According to results of this study it concluded that TSA with anti-inflammatory effects led to the reduction of hippocampal inflammatory cytokines and amelioration of spatial memory in offsprings who were exposed to stress in the prenatal period.

## Ethical issues

Not applicable.

## Authors' contributions

All authors equally contributed to the writing and revision of this paper.

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