Artículo Invitado | Invited Article
Preventive effects of Topiramate on methylphenidate induced behavioral disorders

[Efectos preventivos de topiramato en los trastornos del comportamiento inducidos por metilfenidato]

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Abstract: Neuronal cell damage is often caused by prolonged misuse of Methylphenidate (MPH). Topiramate (TPM) carries neuroprotective properties but its assumed mechanism remains unclear. The present study evaluates *in vivo* role of various doses of TPM and its mechanism against MPH-induced motor activity and related behavior disorder. Thus, we used domoic acid (DOM), bicuculline (BIC), Ketamine (KET), Yohimibine (YOH) and Haloperidole (HAL) as AMPA/kainite, GABAA, NMDA, α2 adrenergic and D2 of dopamine receptor antagonists respectively. Open Field Test (OFT), Elevated Plus Maze (EPM) and Forced Swim Test (FST) were used to study motor activity, anxiety and depression level. TPM (100 and 120 mg/kg) reduced MPH-induced rise and inhibited MPH-induced promotion in motor activity disturbance, anxiety and depression. Pretreatment of animals with KET, HAL, YOH and BIC inhibited TPM-improves anxiety and depression through the interacting with Dopaminergic, GABAA, NMDA and α2-adrenergic receptors.

Keywords: Methylphenidate; Topiramate; Neurobehavioral changes; Glutamatergic; GABAergic; Adrenergic; Dopaminergic

Resumen: El daño a las células neuronales a menudo es causado por el uso prolongado de metilfenidato (MPH). El topiramato (TPM) tiene propiedades neuroprotectoras, pero su mecanismo de acción no es claro. El presente estudio evalúa el papel *in vivo* de varias dosis de TPM y su mecanismo contra la actividad motora inducida por MPH y el trastorno de comportamiento relacionado. Utilizamos ácido domoico (DOM), bicuculina (BIC), ketamina (KET), yohimbina (YOH) y haloperidol (HAL), así como antagonistas AMPA/kainato, GABAA, NMDA, α2-adrenérgico y D2 dopaminérgicos, respectivamente. Se utilizaron las pruebas de campo abierto (OFT), elevación de laberinto (EPM) y natación forzada (FST) para estudiar la actividad motora, la ansiedad y el nivel de depresión. El TPM (100 y 120 mg/kg) redujo el aumento inducido por MPH e inhibió la promoción inducida por MPH en la alteración de la actividad motora, la ansiedad y la depresión. El tratamiento previo de animales con KET, HAL, YOH y BIC inhibió el TPM, mejora la ansiedad y la depresión a través de la interacción con los receptores dopaminérgicos, GABAA, NMDA y α2-adrenérgico.

Palabras clave: Metilfenidato; Topiramato; Cambios neuroconductuales; Glutamatérgico; Gabaérgico; Adrenérgico; Dopaminérgico

INTRODUCTION

MPH, an amphetamine-like psychostimulant, is generally used for the management of Attention-Deficit Hyperactivity Disorder (ADHD) (Challman & Lipsky, 2000; Motaghinejad *et al.*, 2016). Through inhibition of dopamine and norepinephrine reuptake into presynaptic terminals, MPH moderates the activity of these transporters (Patrick & Markowitz, 1997). Regarding MPH similarity to methamphetamine, structurally and functionally, it carries a high potential of abuse and dependency. However, it is less potent and has a longer duration of action as compared to methamphetamines (Huss & Lehmkuhl, 2001; Tagaya, 2010). Recent reports have indicated that recreational use of MPH has become frequent, especially among college students (Babcock & Byrne, 2000; Gibbs *et al.*, 2016). The neurochemical alterations underlying prolonged MPH misuse remain unknown and the effect of MPH abuse on brain and behavior is still under research (Barrett & Pihl, 2002; Williams *et al.*, 2004). Prior studies have established MPH-induced oxidative stress and apoptosis in brain cells (Réus *et al.*, 2014; Banihabib et al., 2016). Moreover, the neurotoxic effect of MPH is more noticeable in some brain areas like hippocampus (Martins *et al.*, 2006). Studies directed on experimental animals and humans have proposed that activation of α_2 adrenergic and D2 receptor of dopaminergic system contributes to the clinical effects of MPH (Russell *et al.*, 2000; Vles *et al.*, 2003, Wilens, 2008). Clinically-applicable dose of MPH has shown to alter NMDA receptor composition and synaptic plasticity in prefrontal cortex of brain (Husson *et al.*, 2004; Prieto-Gomez *et al.*, 2005).

Topiramate (TPM) as an anticonvulsant drug is used for managing alcohol, methamphetamine and cocaine addiction (Garnett, 2000, Arnone, 2005). The neuroprotective properties of TPM have been stated in several studies but the supposed mechanism of action remains unspecified (Lee *et al.*, 2000; Kudin *et al.*, 2004). TPM has shown to decrease lipid peroxidation and increase antioxidant vitamin levels in epileptic rats (Kutluhan *et al.*, 2009). TPM exerts a synergistic effect on superoxide dismutase and catalase activities in striatum and mid brain regions (Armağan *et al.*, 2008; Kutluhan *et al.*, 2009). Furthermore, TPM has anti-inflammatory, immunomodulatory and neuroprotective properties (Armağan *et al.*, 2008; Dudley *et al.*, 2011). Due to

antioxidant and antiapoptotic activity of TPM, it has been considered as an appropriate candidate for counteracting oxidative stress-indiced destruction and neurodegeneration (Vajda, 2004). Research have proposed that TPM prompts neuroprotection against some neurodegenerative malaises via inhibition of AMPA/kainate and NMDA receptor or via activation of GABA$_A$ receptors (Raffa *et al.*, 2010; Mao *et al.*, 2012). Additionally, some studies have revealed that TPM can act as an analgesic agent by interacting with α_2-adrenergic and D2 receptor of dopaminergic system and these two receptors, show critical role in TPM-induced neuroprotective properties (Bischofs *et al.*, 2004; Paranos *et al.*, 2013). Latest studies have also proposed that TPM can cause inhibition of Ca^{2+} ion entry and PKC activity (Shank *et al.*, 2000; Muriach *et al.*, 2010; Demirci *et al.*, 2013). Keeping in view the role of AMPA/kainate, NMDA, GABA$_A$, α_2-adrenergic and D2 of dopaminergic receptors in intermediating TPM-induced neuroprotection, we aimed this study to evaluate the stimulation of these receptors in conferring protective role of topiramate against MPH prompted motor activity and mood associated behavior disorder.
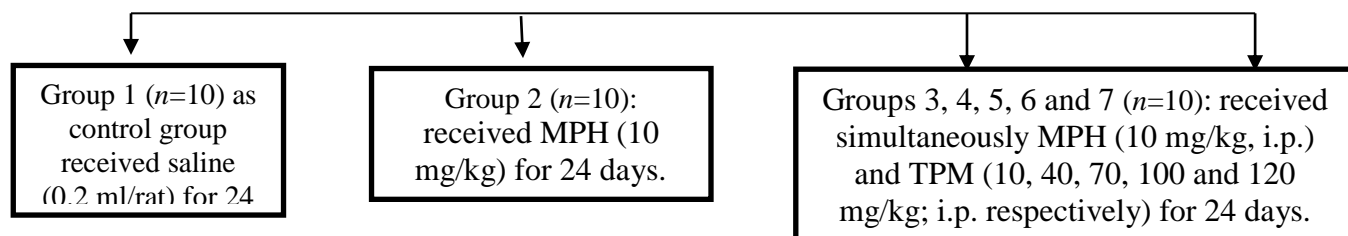
MATERIALS & METHODS

Drugs

TPM, MPH, domoic acid (DOM), bicuculline (BIC), Ketamine (KET), Yohimibine (YOH), and Haloperidole (HAL) were purchased from Sigma-Aldrich (USA) and dissolved in normal saline for injection (volume adjusted to 0.7 ml/rat per injection). The drug solutions were freshly prepared before use.

Experimental design
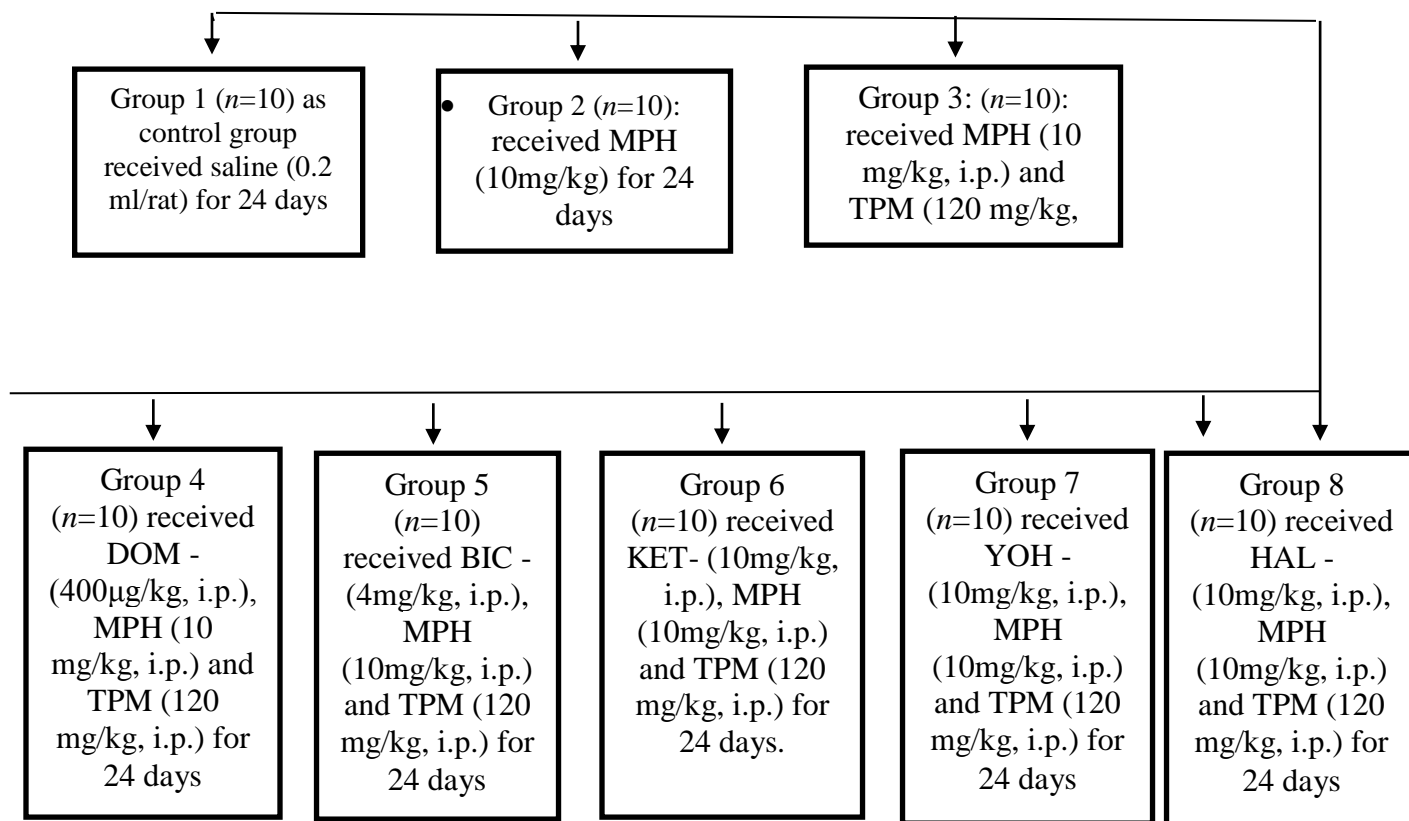
One hundred and filty male Wistar adult rats, with weighing 200±8.0 g were obtained from experimental research center, Iran university of medical science, were adapted to experimental conditions (12 hr light dark cycle, 24°C and free access to standard food and tap water) and randomly were divided for doing of two separate phases experiment as shown in the diagram. The present study was performed in accordance with the guidelines for the care and use of laboratory animals published by the US National Institutes of Health (NIH Publication No.85-23, revised 1996). And was approved by the Research Council of Iran University of Medical Sciences, Tehran, Iran (This research is supplementary data

from Ph.D thesis with code number: 14 and Research
Code: 6237).

Phase 1: Rats (n=70)

Group 1 (*n*=10) as control group received saline (0.2 ml/rat) for 24	Group 2 (*n*=10): received MPH (10 mg/kg) for 24 days.	Groups 3, 4, 5, 6 and 7 (*n*=10): received simultaneously MPH (10 mg/kg, i.p.) and TPM (10, 40, 70, 100 and 120 mg/kg; i.p. respectively) for 24 days.

Phase 2: Rats (n=80)

Group 1 (*n*=10) as control group received saline (0.2 ml/rat) for 24 days	• Group 2 (*n*=10): received MPH (10mg/kg) for 24 days	Group 3: (*n*=10): received MPH (10 mg/kg, i.p.) and TPM (120 mg/kg,

Group 4 (*n*=10) received DOM - (400μg/kg, i.p.), MPH (10 mg/kg, i.p.) and TPM (120 mg/kg, i.p.) for 24 days	Group 5 (*n*=10) received BIC - (4mg/kg, i.p.), MPH (10mg/kg, i.p.) and TPM (120 mg/kg, i.p.) for 24 days	Group 6 (*n*=10) received KET- (10mg/kg, i.p.), MPH (10mg/kg, i.p.) and TPM (120 mg/kg, i.p.) for 24 days.	Group 7 (*n*=10) received YOH - (10mg/kg, i.p.), MPH (10mg/kg, i.p.) and TPM (120 mg/kg, i.p.) for 24 days	Group 8 (*n*=10) received HAL - (10mg/kg, i.p.), MPH (10mg/kg, i.p.) and TPM (120 mg/kg, i.p.) for 24 days

After both phase on days 11, 17 and 24 some standard behavior tests such as Open Field Test (OFT), Elevated Plus Maze (EPM) and Forced Swim Test (FST) were used to examine motor activity, anxiety and depression level of animals under study.

Behavioral Tests
OFT protocol

This behavioral apparatus was applied to evaluate locomotor activity, anxiety and exploratory in experimental animal model. This equipment is a white table with walls, frequently marked in grid crossings, used to evaluate the conflict between the innate fears that animal have of the central area of a novel or brightly light open field against their desire to explore new environments. When anxious, the natural tendency of rodents is to prefer staying close to the walls. The animals were placed in the center of the field and their following activity is evaluated for 5 minutes 5 typical behaviors were assessed and scored.

1. Line crossing (ambulation) number: Frequency with which the rat crossed one of the grid lines with all four paws.
2. Line crossing (ambulation) distance: distance which the rat crossed the grid lines
3. Center Square Entries: Frequency with which the rat crossed one of the red lines with all four paws into the central square.
4. Center Square Duration: Duration of time the rat spent in the central square.
5. Rearing: Frequency with which the rat stood on their hind legs in the maze (Prut & Belzung, 2003; Gould *et al*., 2009).

FST protocol

The test was applied for valuation of depressive-like behaviors in animal models. Immobility and swimming activity was recorded in FST. The antidepressant properties of drugs reduce the duration of immobility and increase the duration of swimming. In this apparatus a transparent Plexiglas cylinder of 25 cm diameter and 60 cm height was used. Water level of 40 cm was maintained in the cylinder throughout the experiment. In order for the animals to adjust to this test, they were subjected individually to force swim for a period of 15 minutes, one day before the experiment. On the day of experiment animals were placed individually in water filled glass cylinder for a period of 5 minutes. The duration of immobility and swimming was recorded (Petit-Demouliere et al., 2005, Can et al., 2012).

EPM protocol

This model is used for assessment of anxiety pattern in experimental animal model. This apparatus includes two opposite arms 50 × 10 cm, connected with a central square (10 × 10 cm), giving the equipment a form of plus symbol. One arm with white color was kept open, while the other arm was closed with 40 cm high walls and was painted black. The maze was raised 50 cm above the floor. The experimental animals were located individually in the center of the maze facing an enclosed arm and the time spent on the open and closed arms were recorded during the next 5 minutes for each rat. An entrance was recorded when all four paws of the rat entered the arm. Lower level of stress and anxiety is indicated by animal entry and the length of stay in open arm (Carobrez & Bertoglio, 2005; Komada *et al*., 2008).

Statistical analysis

The data were analyzed by GraphPad PRISM v.6 software (2016) (Graph Pad Company, San Diego, USA). First, the normality of continuous variables (behavioral and molecular parameters) was measured using Kolmogorov–Smirnov test. Based on this test, all variables were normally distributed. All data were described as means ± standard error of the mean. The difference between treatment groups was evaluated by one-way ANOVA with Bonferroni's *post hoc*-test for group-by-group comparisons. Results were considered to be statistically significant at $P<0.001$ level.

RESULTS
OFT
Phase 1

As shown in Figure-1, the animals in control group had more ambulation distance, rearing number, frequency of central square entries and also spent more time in the central region of the OFT compared to groups receiving MPH with doses of 10 mg/kg in days11, 17 and 24 ($P<0.05$) (Figure No. 1A, 1B, 1C and 1D). Furthermore, TPM (100 and 120 mg/kg) treatment reduced the MPH-induced decrease in the frequency of central square entries, time spent in the central region, ambulation distance and frequency of rearing in the OFT, when compared to MPH (10 mg/kg) treated group in days 17 and 24 ($P<0.05$) (Figure No. 1A, 1B, 1C and 1D).

Phase 2

As shown in Figure-No. 2, the animals in control

group had more frequency of central square entries and also spent more time in the central region of the OFT compared to groups receiving MPH with doses 10 mg/kg in days 17 and 24 (*P*<0.001) (Figure-2A and 2B). It seems that the animals receiving doses of 10 mg/kg of MPH has less ambulation distance and rearing number in comparison to control group in days 17 and 24 (*P*<0.001) (Figure No. 2C and 2D). Furthermore, TPM (120 mg/kg) treatment reduced the MPH-induced decrease in the frequency of central square entries, time spent in the central region, ambulation distance and frequency of rearing in the OFT, when compared to MPH (10 mg/kg)

treated group in days 17 and 24 (*P*<0.001) (Figure No. 2A, 2B, 2C and 2D). Pretreatment of animals with KET, HAL, YOH and BIC (NMDA receptor antagonist, dopamine D_2 receptor antagonist, α_2 adrenergic receptor antagonist and GABA receptor antagonist respectively) abolished the protective effect of TPM against MPH-induced behavioral disturbances as observed by the decrease in frequency of central square entries, time spent in the central region, ambulation distance and rearing in the OFT in days 17 and 24 (*P*<0.001) (Figure No. 2A, 2B, 2C and 2D).
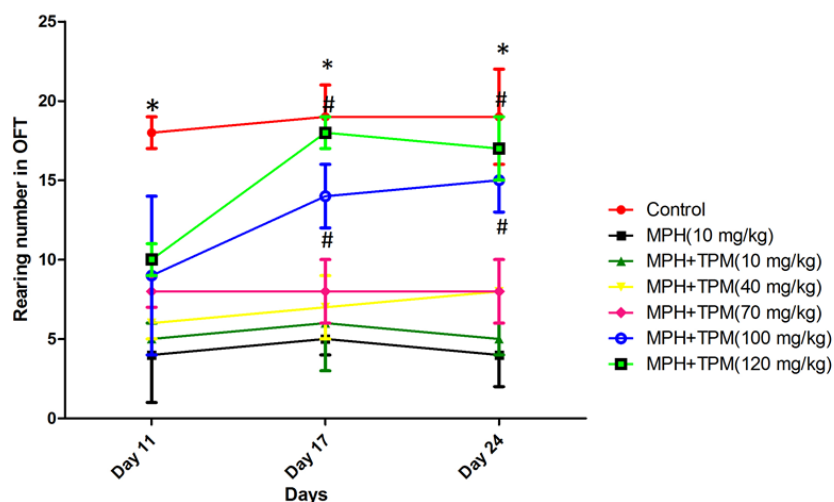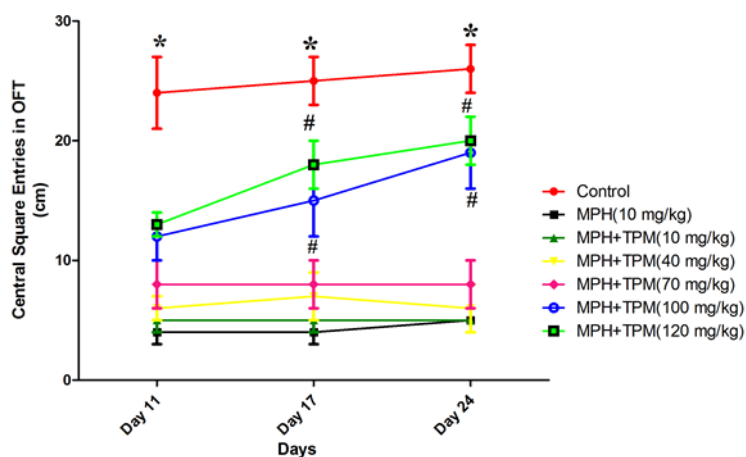

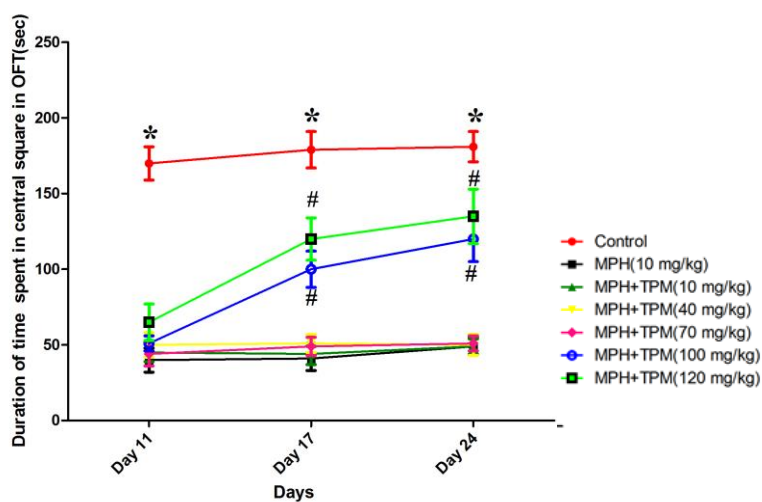**Figure No. 1A**

Figure No. 1B

Figure No. 1C

Figure No. 1D

Figure No. 1
Effect of various doses of TPM (10, 40, 70, 100 and 120 mg/kg) on MPH-induced alterations in OFT behavior such as ambulation distance(A), rearing number(B), central square entries(C), duration of time spent in central square(D) All data are presented as Mean ± SEM (N=8). * $P<0.05$ vs. control group, # $P<0.05$ vs. MPH treated group.
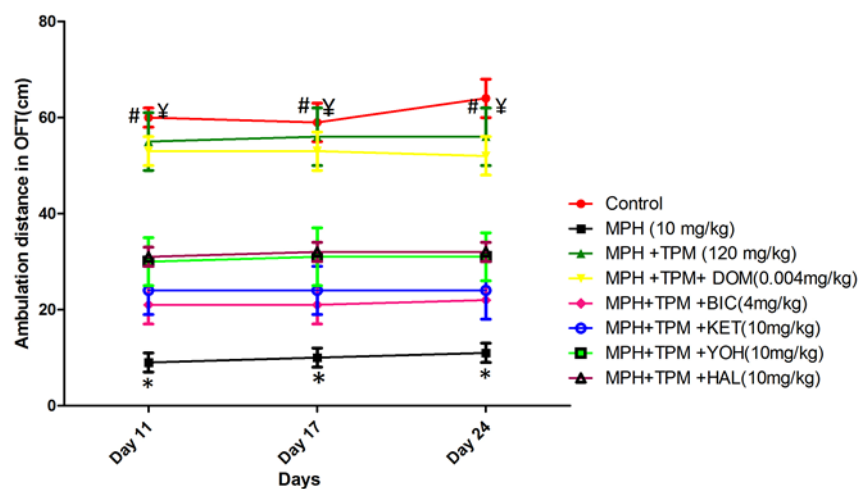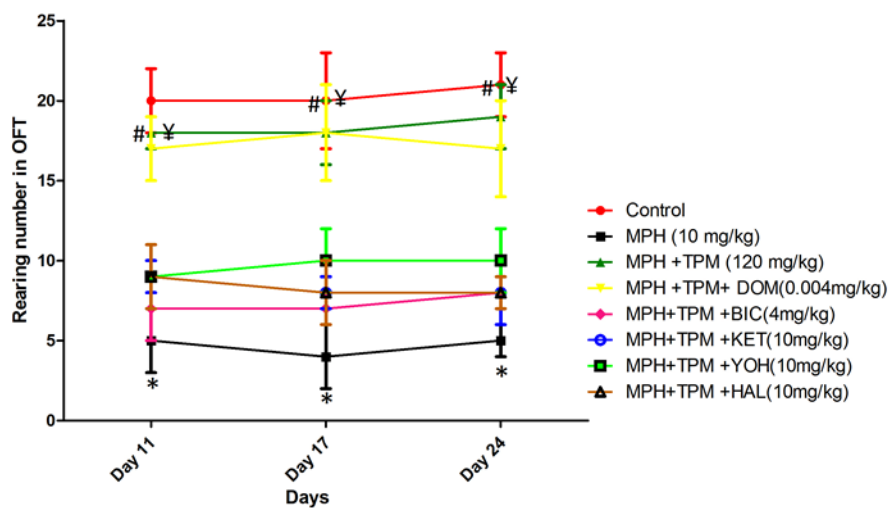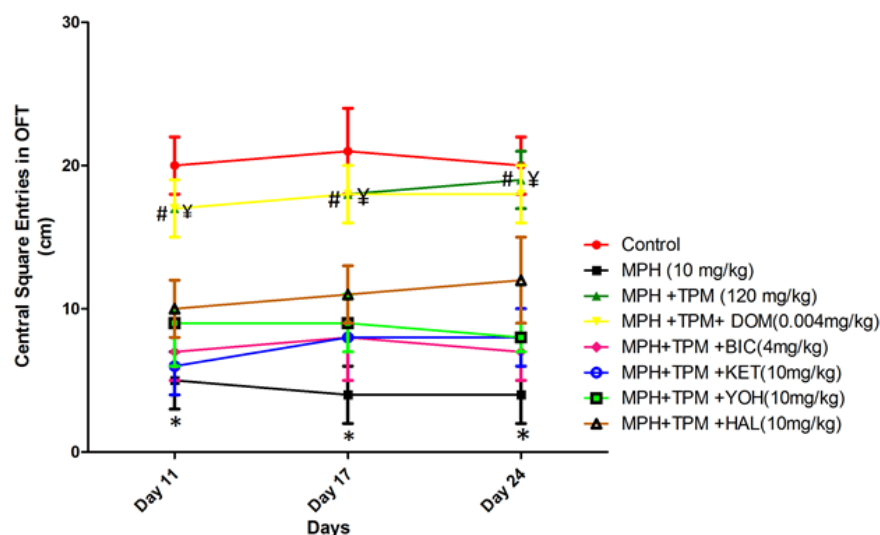
Figure No. 2A
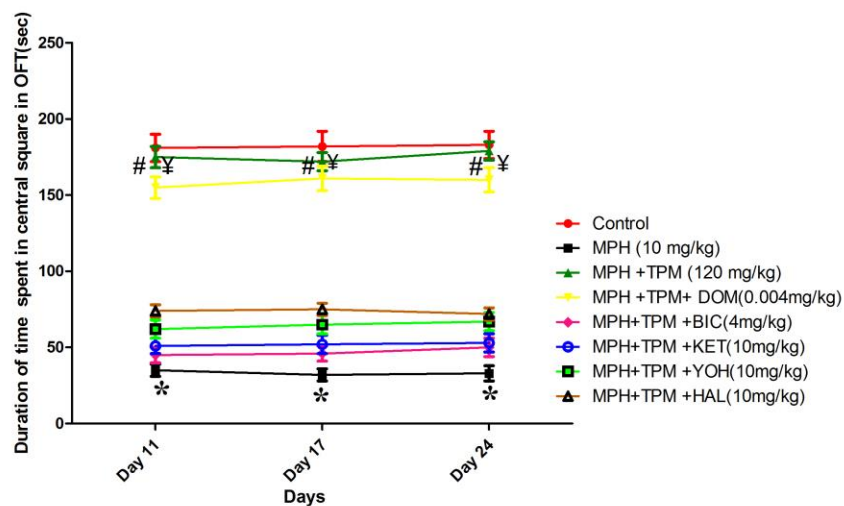

Figure No. 2B

Figure No. 2C

Figure No. 2D

Figure No. 2
Effect of DOM, BIC, KET, HAL and YOH on TPM-mediated effects in MPH-induced alterations in OFT behavior such as ambulation distance (A), rearing number (B), central square entries (C), duration of time spent in central square (D) All data are presented as Mean ± SEM (n=8). * *P*<0.05 vs. control group, # *P*<0.05 vs. MPH treated group, ¥ *P*<0.05 vs. MPH in combination with KET, HAL, YOH and BIC treated group.
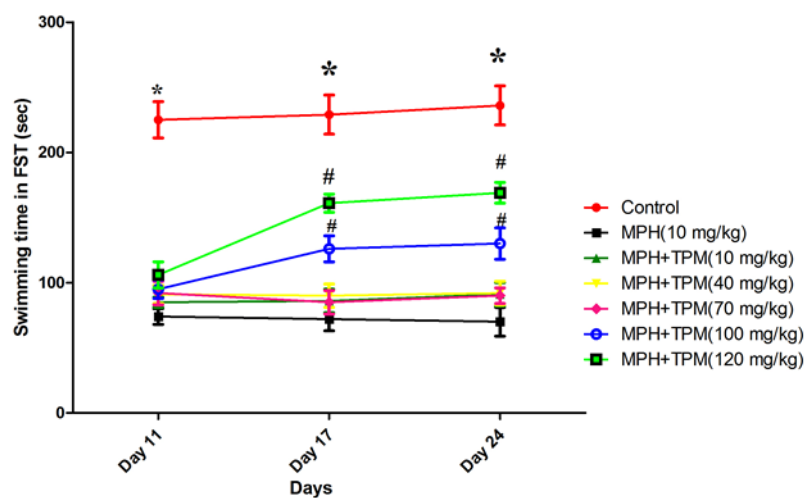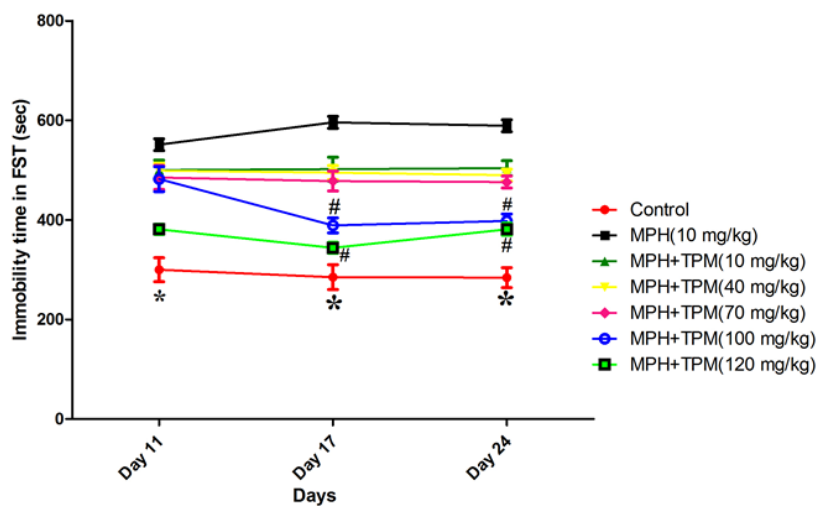
Figure No. 3A

Figure No. 3B

Figure No. 3
Effect of various doses of TPM (10, 40, 70, 100 and 120 mg/kg) on MPH-induced alterations in FST behavior such as Swimming time (A) and immobility time (B). All data are presented as Mean ± SEM (n=8). * $P<0.05$ vs. control group, # $P<0.05$ vs. MPH treated group

FST

Phase 1

As shown in figures 3, the animals in control group compared to MPH (10 mg/kg), spent more time swimming in the FST and spent less time immobility in days11, 17 and 24 in comparison to control group (*P*<0.05) (Figure No. 3A and 3B). Furthermore, TPM (100 and 120 mg/kg) treatment reduced the MPH-induced decrease in swimming time in the FST and MPH induced increase in immobility time in days 17 and 24 compared to MPH treated group (*P*<0.05) (Figure No. 3A and 3B).

Phase 2

As shown in Figures No. 4, the animals in control group compared to MPH at doses of 10 mg/kg, spent more time swimming in the FST and spent less time immobility in days 17 and 24 compared to control group (*P*<0.001) (Figure No. 4A and 4B). Furthermore, TPM (120 mg/kg) treatment reduced the MPH-induced decrease in swimming time and increased in immobility time in the FST through days 17 and 24 in comparison to MPH treated group (*P*<0.001) (Figure 4A and 4B). Pretreatment of animals with KET, HAL, YOH and BIC (NMDA receptor antagonist, dopamine D₂ receptor antagonist, α₂ adrenergic receptor antagonist and GABA receptor antagonist respectively) eliminated the MPH-induced behavioral disturbances as observed by the decrease in swimming time and increase in immobility time in FST, days 17 and 24, in comparison to TMP treated group (*P*<0.001) (Figure No. 4A and 4B).
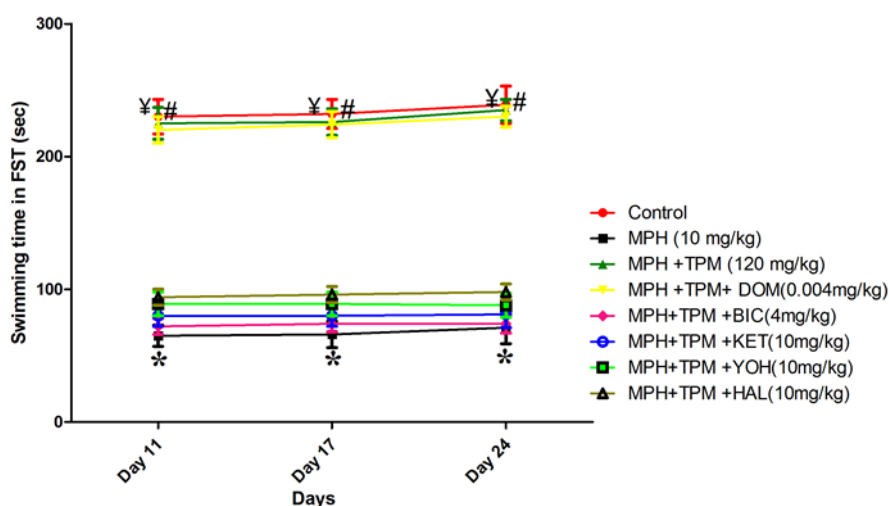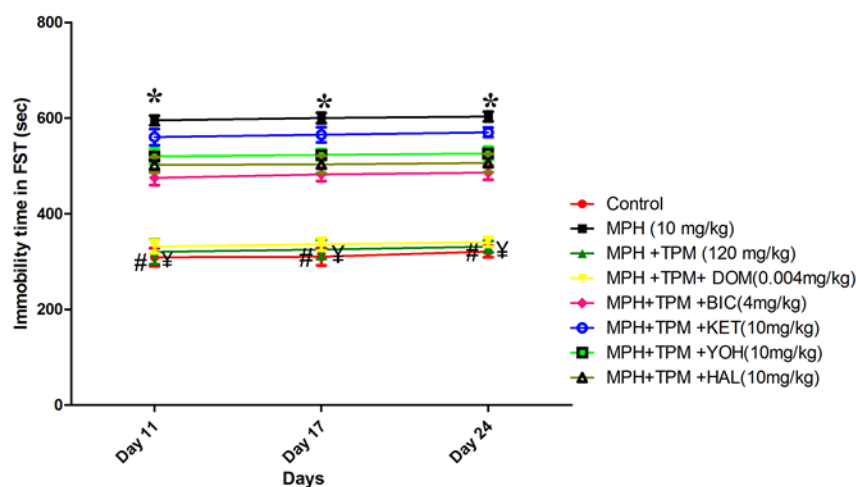

Figure No. 4A

Figure No. 4B

Figure No. 4
Effect of DOM, BIC, KET, HAL and YOH on TPM-mediated effects in MPH-induced alterations in FST behavior such as Swimming time (A) and immobility time (B). All data are presented as Mean ± SEM (n=8). * *P*<0.05 vs. control group, # *P*<0.05 vs. MPH treated group, ¥ *P*<0.05 vs. MPH in combination with KET, HAL, YOH and BIC treated group

EPM
Phase 1
As indicated in Figure No. 5 the animals treated by MPH (10 mg/kg), have spent shorter times in open arm and indicated reduction count of entrance to open arm, in EPM, in days 11, 17 and 24 in comparison to control group (*P*<0.05) (Figure No. 5A and 5B). MPH treated group, have spent longer times in closed arm and showed increase in count of entrance to closed arm, in EPM, in days 17 and 24 compared to control group (*P*<0.05) (Figure No. 5C and 5D). Furthermore, TPM (100 and 120 mg/kg) treatment reduced the MPH-induced decrease in time spent in open arm, count of entrance to open arm. Also reduced MPH prompted increase in time spent in open arm, count of entrance to open arm in days 17 and 24 in comparison to MPH treated group (*P*<0.05) (Figure No. 5A, 5B, 5C and 5D).

Phase 2
As indicated in figure-6, animals treated by MPH (10 mg/kg), have spent shorter times in open arm and showed decrease in count of entrance to open arm. Also this group spent longer times in closed arm and displayed increase in count of entrance to closed arm, in EPM, in days 17 and 24 in comparison to control group (*P*<0.001) (Figure No. 6A, 6B, 6C and 6D). Furthermore, TPM (120 mg/kg) treatment reduced all mentioned MPH-induced behavioral changes in EPM in days 17 and 24, compared to MPH treated group (*P*<0.001) (Figure No. 6A, 6B, 6C and 6D). Pretreatment of animals with KET, HAL, YOH and BIC (NMDA receptor antagonist, dopamine D_2 receptor antagonist, α_2 adrenergic receptor antagonist and GABA receptor antagonist respectively) ended the MPH-induced behavioral disorders as observed by the shorter times spent in open arm, reduced count

of entrance to open arm and also spent longer times in closed arm and increase in count of entrance to closed arm, in EPM, in days 17 and 24 in comparison to MPH treated group ($P<0.001$) (Figure No. 6A , 6B, 6C and 6D).
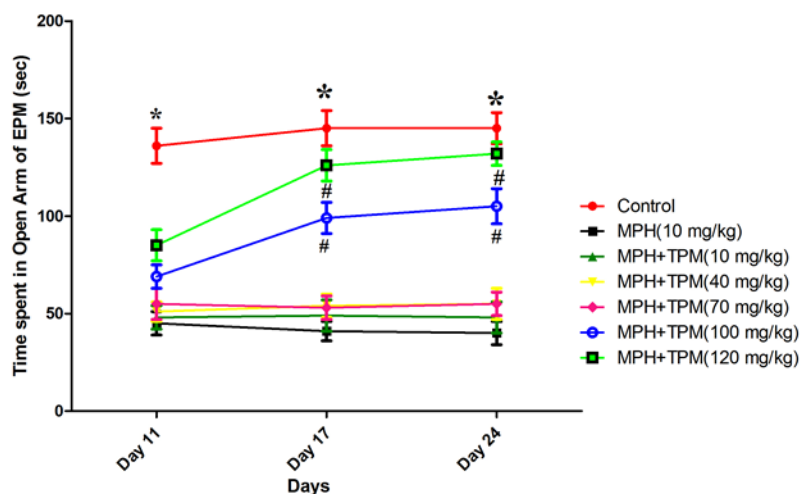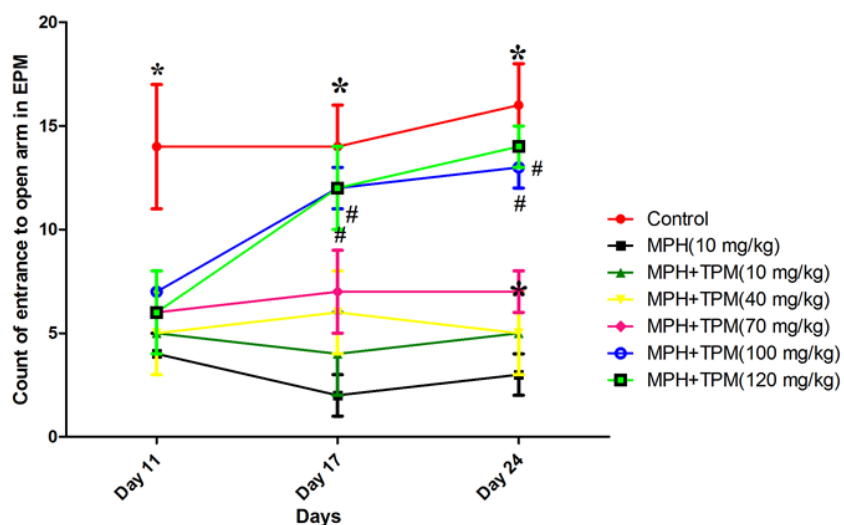

Figure No. 5A

Figure No. 5B

Figure No. 5C

Figure No. 5D

Figure No. 5
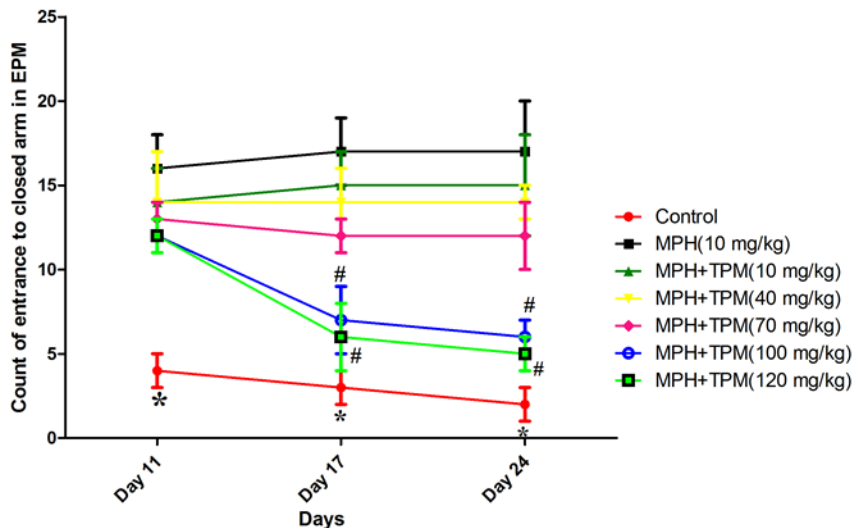**Effect of various doses of TPM (10, 40, 70, 100 and 120 mg/kg) on MPH-induced alterations in EPM behavior such as time spent in open arm (A), count of entrance to open arm (B), time spent in closed arm (C) and count of entrance to closed arm (D). All data are presented as Mean ± SEM (n=8). * $P<0.05$ vs. control group, # $P<0.05$ vs. MPH treated group**

Figure No. 6A

Figure No. 6B

Figure No. 6C

Figure No. 6D

Figure No. 6
Effect of DOM, BIC, KET, HAL and YOH on TPM-mediated effects in MPH-induced alterations in EPM behavior such as time spent in open arm (A), time spent in closed arm (B), count of entrance to open arm (C) and count of entrance to closed arm (D). All data are presented as Mean ± SEM (n=8). * $P<0.05$ vs. control group. # $P<0.05$ vs. MPH treated group, ¥ $P<0.05$ vs.
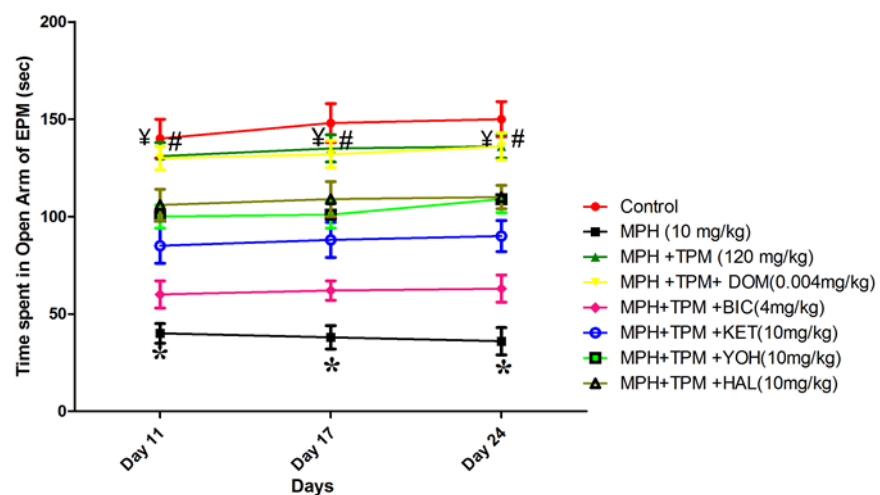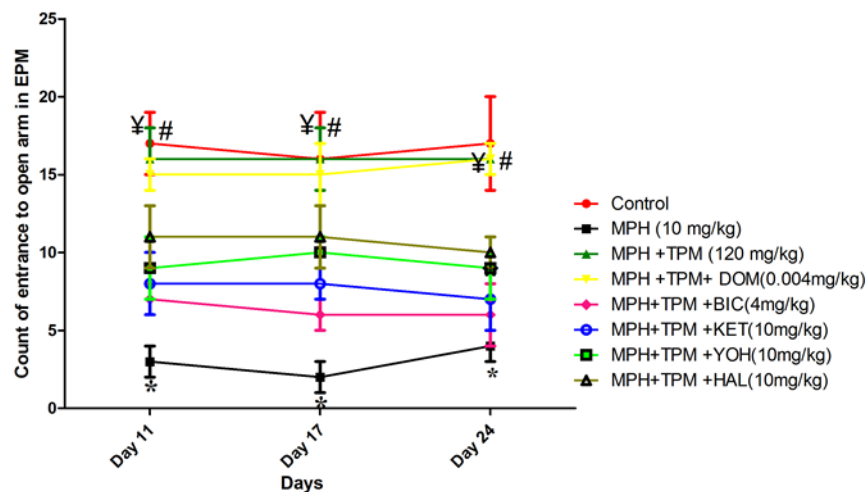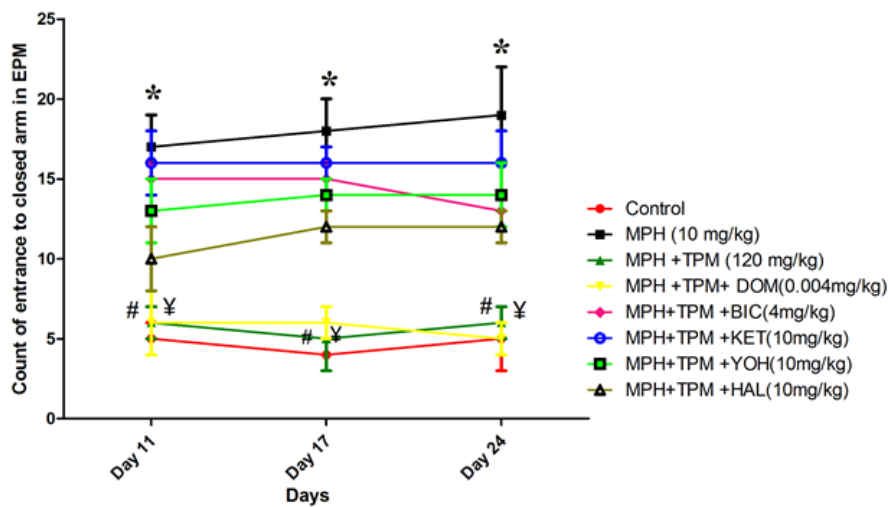
DISCUSSION

In first parts of study we found that prolonged administration of MPH at high doses reduces the frequency of rearing, ambulation distance, central square entries and time spent in Central Square in OFT in days 17 and 24. However, these changes were not significant on day 11, which is indicative of MPH-induced disturbances in motor activity and anxiety disorder in high doses and chronic administration. This finding is in agreement with the prior works which have confirmed MPH-induced anxiety-like behavior and disturbances in motor activity (Izenwasser *et al*., 1999; Motaghinejad *et al*., 2015b). According to this data we observed that administration of MPH (10 mg/kg) for 24 days causes depressive like behavior and increases mood disturbance. These findings have been confirmed by previous studies, showing MPH-induced depression in both human and animal subjects (Martins *et al.*, 2006). In addition, it has been shown that high doses of MPH reduce the mood of rodents in experimental procedure (Bolaños *et al*., 2008; Kaczmarczyk *et al*., 2013). Current study demonstrated that animals treated by MPH (10 mg/kg) have spent shorter times in open arm and indicated reduction count of entrance to open arm, in EPM, in days 17 and 24 but this changes was not significant on day 11. MPH treated group, have spent longer times in closed arm and indicated increase in count of entrance to closed arm, in EPM, in days 17 and 24 but this changes was not significant on day 11. According to previous studies and this current work, MPH increases the anxiety like behavior in rodent and human subjects (Bolaños *et al*., 2008). In contrast, we observed that TPM at doses of 100 and 120 mg/kg improved the motor activity and has anxiolytic effects in MPH treated rats in days 17 and 24, but these changes were not considerable on day 11. Several prior works have stated the anxiolytic properties of TPM and that it can be constructive against motor disturbances and anxiety prompted by the misuse of methamphetamine derived compounds (Chengappa *et al*., 2001; Motaghinejad *et al*., 2015a; Motaghinejad *et al*., 2015b; Motaghinejad *et al*., 2016).Animals treated by MPH (10 mg/kg), spent less time swimming in the FST and more time immobility in days 17 and 24. Furthermore, TPM (100 and 120 mg/kg) reduced the MPH-induced decrease in swimming time in the FST and MPH induced increase in immobility time in days 17 and 24 but these changes were not significant

on day 11. Moreover, TPM (100 and 120 mg/kg) treatment reduced the MPH-induced decrease in time spent in open arm, count of entrance to open arm and also in MPH induced increase in time spent in open arm, count of entrance to open arm in days 17 and 24. Previous studies have also demonstrated that anticonvulsants agents increase neurotrophic factors and thereby, mediate anxiolytic and antidepressant against mood related disorder (Cagetti *et al*., 2004). Anticonvulsant drugs like TPM have shown to confer antidepressant and anxiolytic by unknown mechanism (Garnett, 2000; Arnone, 2005; Bertges *et al*., 2011). This may suggest the role of TPM benefit effects in mood related disorder (Arnone, 2005). Previous studies have also demonstrated that anticonvulsants agents decreased anxiety and depression and thus, mediate neurobehavioral changes which are induced by MPH. In conclusion of first part, high doses and chronic administration of TMP has anxiolytic and antidepressant effects and also modulates motor activity disorder induced by MPH. It should be emphasized that the goal of this parts of study was to confirm our previous work about TPM neuroprotective role in neurobehavioral disorder and then usage of this data for second part of current study.

The second part is to clarify the role of some neurotransmitter receptors such as GABA, NMDA, AMPA/kainate, D_2 and α_2 in TPM anxiolytic and antidepressant and motor activity modulator neurobehavioral changes. Thus in order to gain some insights on the mechanism , we concurrently treated the animal with MPH, TPM, AMPA/kainate receptor agonist (Domoic acid), GABA$_A$ receptor antagonist (Bicuculline), NMDA receptor antagonist (Ketamine), α_2-adrenergic receptor antagonist (Yohimibine) and dopamine receptor D_2 antagonist (Haloperidole).

Briefly, in second parts of study we found that pretreatment of animals under treatment by TMP and MPH, with KET, HAL, BIC and YOH, can cause inhibition of protective effects of TPM in management of MPH induced motor activity disorder. Also pretreatment by mentioned agonist or antagonist causes increase of depressive and anxiety like behavior. Pretreatment of animals with KET, HAL, BIC and YOH eliminated the protective effects of TPM against MPH induced reduction in the ambulation distance, number of central square entries, rearing frequency and time spent in Central

Square in OFT. However, pretreatment with DOM attenuated the TMP effects but did not significantly affect the OFT behavior. According to recent studies, the neuroprotective, anxiolytic, and analgesic effects of TPM are mediated via inhibition of glutamatergic receptor or via activation of $GABA_A$, α_2-adrenergic and D_2 dopaminergic receptors (Bischofs *et al*., 2004; Paranos *et al*., 2013; Motaghinejad & Motevalian, 2016). In consistent with our data, some previous study demonstrated that attenuation of glutamate NMDA receptors is involved in motor activity and induction of anxiety like behavior (McEwen, 2005), Also some other study indicated that $GABA_A$, D_2 ,NMDA or α_2-adrenergic receptors activation can modulate motor activity and to somehow decreases anxiety, which the role of D_2 and α_2-adrenergic is less known and less likely (Brambilla *et al*., 2003; Brunello *et al*., 2003; McEwen, 2005; Nestler & Carlezon Jr, 2006).

According to this concept we conclude that in current study, chronic TPM by modulation of $GABA_A$, D_2, NMDA or α_2-adrenergic receptors can inhibit anxiety like behavior and motor activity disturbance in OFT during days 17 and 24 in MPH treated animal but these changes were not significant on day 11.Thus, it can be suggested that inhibition of NMDA, $GABA_A$, α_2-adrenegic and D_2 dopaminergic receptors can eliminate the protective effects of TPM against MPH-induced motor activity disturbance and anxiety. Many previous studies showed that NMDA receptor is involved in anxiety and depression and can modulate motor activity, also other studies showed that $GABA_A$, α_2-adrenergic and D_2 dopaminergic receptor is responsible for antidepressant and anxiolytic properties of several agents (Bergink *et al*., 2004; Kalueff & Nutt, 2007; Gowing *et al*., 2009; de la Mora *et al*., 2010).Several prior studies showed that TPM can activate $GABA_A$, D_2, NMDA or α_2-adrenergic receptors in multiple effects of TPM on seizure and pain perception methods (Garnett, 2000; Arnone, 2005). These concepts support the idea that NMDA, and $GABA_A$ receptors are possibly involved in protective effects of TPM against MPH induced behavioral changes. Present study demonstrated that pretreatment of animals with KET, HAL, BIC and YOH abolished the protective effects of TPM against MPH induced decrease in swimming time and increase in immobility time, in FST, through days 17 and 24 but these changes were not significant on day 11.

Additionally, TPM (120 mg/kg) reduced the MPH-induced decrease in swimming time in the FST and MPH induced increase in immobility time on days 17 and 24. We found that pretreatment with HAL, BIC, KET and YOH, reduced the protective effect of TPM against MPH-induced depression and led to a significant elevation in depressive like behavior. Many studies have presented that anticonvulsants like TPM can inhibit depression in brain by interacting with NMDA, D_2, $GABA_A$ and α_2 adrenergic receptors (Garnett, 2000; White *et al*., 2000; Arnone, 2005). Previous studies have expressed that single dose or repeated administration of TPM can antagonize depressant signaling pathway (Hargreaves & McGregor, 2007). On the other way prior data suggested that normal physiological patterns of NMDA, D_2, $GABA_A$ and α_2 adrenergic receptor activity can promote brain function against depression (Bergink *et al*., 2004; Kalueff & Nutt, 2007; Gowing *et al*., 2009; de la Mora *et al*., 2010), these data also demonstrated that activation of mentioned receptor can inhibit depression (Bergink *et al*., 2004; Kalueff & Nutt, 2007; Gowing *et al*., 2009; de la Mora *et al*., 2010,). Discussing the study concepts, we can conclude that probably MPH by inhibition of NMDA, D_2, $GABA_A$ and α_2 adrenergic receptor activity can trigger depressive like behavior in FST. On the other way TMP, by blockade of MPH action on mentioned receptor, can inhibit depressive like behavior in FST.

The current study suggests that the anti-depressive effect of TPM, in MPH treated rat, is mediated by activation of NMDA, $GABA_A$ and α_2-adrenergic receptors. In addition, pretreatment with DOM, BIC, KET and YOH abolished the beneficial effects of TPM and causes reduction of times spent in open arm and count of entrance to open arm, and also increased the times spent and count of entrance in closed arm in EPM, in days 17 and 24 but this changes were not significant on day 11. Many studies support our findings regarding the participation of these receptors in TPM induced anxiolytic effects (Bergink *et al*., 2004; Kalueff & Nutt, 2007; Gowing *et al*., 2009; de la Mora *et al*., 2010). previous data suggested that NMDA, D_2, $GABA_A$ and α_2 adrenergic receptor by multiple mechanisms can modulate anxiety pathway, in this manner there is some disagreements, but totally many previous data showed that activation of NMDA ,D_2,$GABA_A$ and α_2 adrenergic receptor antagonist ,in physiological

concentration, can cause inhibition of anxiety (Webster, 2001; Werner & Covenas, 2010). In our study we can discuss that MPH by inhibition of NMDA, D2, GABAA and α₂ can cause anxiety and TMP, by blockade of MPH effects on mentioned receptors, can protect against anxiety. Our results confirm that these receptors are involved in improving TPM neuroprotection in behavioral changes, induced by MPH.

CONCLUSION
The current study confirms the neuroprotective effects of chronic administration of TMP at high doses against MPH-induced motor activity disturbance, depression and anxiety. This can be applied for therapy of patients abusing MPH and suffering from its neurobehavioral disorder effects Furthermore, our findings suggest the role of NMDA, GABA$_A$, D$_2$ and α₂ adrenergic receptors play critical role in mediating the protective effects of TPM. Thus, TPM treatment can be considered as a potential therapy for MPH abusers. Therefore, we suggest that TPM has the potential of being helpful in managing problems associated with use of MPH, although further studies are required with human subjects.

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