Original Article

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The Preventive/Protective Effect of Testosterone on Haloperidol-Induced Extrapyramidal Disorders in Male Rats

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ABSTRACT

BACKGROUND AND OBJECTIVE: Many non-motor symptoms of Parkinson's disease such as depression are associated with testosterone deficiency. On the other hand, the effect of testosterone therapy on the motor symptoms of Parkinson's disease is unknown. Therefore, the present study was conducted to evaluate the effect of testosterone on haloperidol-induced extrapyramidal disorders.

METHODS: In this experimental study, 60 male Wistar rats weighing 180–220 g were randomly divided into 10 groups of 6. Rats were intraperitoneally pretreated with saline, polyethylene glycol, flutamide (10 mg/kg) or testosterone (1 mg/kg) for 1 or 7 consecutive days and before administration of haloperidol. The effects of testosterone and flutamide on catalepsy and motor disorder caused by haloperidol were measured by bar test and rotarod test, respectively.

FINDINGS: Haloperidol (1 mg/kg) significantly ($p<0.004$) resulted in catalepsy (170±17.25) and balance impairment (52±8). Pretreatment with testosterone for 7 days had a preventive effect on haloperidol-induced extrapyramidal disorders and improved catalepsy (55±8) ($p<0.001$) and balance impairment (178±8) ($p<0.05$) in mice, whereas co-administration of flutamide prevented the ameliorative effects of testosterone on extrapyramidal disorders.

CONCLUSION: The results of the study showed that pretreatment with testosterone has a significant effect on the improvement of catalepsy.

KEY WORDS: *Parkinson's disease, Testosterone, Flutamide, Haloperidol, Catalepsy, Bar test, Rotarod, Extrapyramidal disorders.*

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Introduction

Parkinson's disease is a progressive disorder of the nerves that occurs due to the destruction of dopaminergic neurons in the substantia nigra pars compacta and affects the pathways of the basal ganglia (1). Some of the common motor symptoms are tremor, bradykinesia, muscle stiffness, imbalance and catalepsy (2, 3). Catalepsy is the inability to control and change the muscles movement and stiffness caused by the disease (4). The prevalence of Parkinson's disease in men is 1.5 to 2 times higher than in women (1). Testosterone deficiency is common in 20 to 25% of men over 65 years of age. Recent studies have shown that testosterone deficiency occurs in 35% of patients with Parkinson's disease over 65 years of age (5).

The symptoms of extrapyramidal neuroleptic drugs are very similar to those of Parkinson's disease. For this, the catalepsy caused by neuroleptic drugs is used to examine Parkinson's disease in laboratory models (6–8). One of the standard methods for creating motor symptoms of Parkinson's disease is the administration of haloperidol neuroleptic drug (8). Haloperidol antipsychotics disrupts dopaminergic receptors on the substantia nigra pathway, resulting in extrapyramidal motor disorders (9–11).

Many of Parkinson's non-motor symptoms, such as fatigue, depression, and impotence, are associated with the lack of testosterone. Meanwhile, testosterone therapy has not yet been conclusively proven to treat Parkinson's motor side effects (5, 12, 13). This study was performed to investigate the effect of pre-treatment and acute injection of testosterone on haloperidol-induced extrapyramidal disorders (imbalance and catalepsy).

Methods

Animals: This experimental study was performed on 60 male Wistar rats from the Animals Laboratory of Hamedan University of Medical Sciences with a weight range of 180 – 220 g. All animals were kept in an environment with a temperature of 22±2, a 12-hour dark/light cycle, and adequate ventilation. Throughout the course of the study, animals had access to enough water and food.

Animals were transferred to the laboratory room 1–2 hours prior to the experiment, in order to get used to the laboratory atmosphere. All experiments were performed under the supervision of the Research Ethics Committee of Hamedan University of Medical Sciences with the code of ethics IR.UMSHA.REC.1395.536. Animals were randomly assigned to ten groups and each of the six animals was placed in a standard cage.

1).Normal group (healthy intact animals),

2).The sham group (normal saline solvent recipient),

3).The negative control group receiving the polyethylene glycol solvent,

4).The testosterone receiving group (receiving testosterone for 7 days intraperitoneally at a dose of 1 mg (4) per kg in healthy animals),

5).The flutamide receiving group (receiving flutamide for 7 days intraperitoneally at a dose of 10 mg (14) per kilogram in healthy animals),

6).The haloperidol group (receiving haloperidol at a dose of 1 mg (15) per kg by intraperitoneal injection)

7).Protective group (acute co-administration of testosterone at a dose of 1 mg / kg intraperitoneally and haloperidol)

8).Preventive group (receiving testosterone for 7 days intraperitoneally at a dose of 1 mg/kg and receiving haloperidol after the last injection of testosterone)

9).The group receiving flutamide (10 mg/kg) and testosterone (1 mg/kg) for 7 days simultaneously and then receiving haloperidol,

10).The group receiving flutamide (10 mg/kg) and testosterone (1 mg/kg) simultaneously and then co-administration of haloperidol.

Medications: Haloperidol, Flutamide, and Testosterone were purchased from Sigma Company. Haloperidol in 0.9% normal saline, testosterone and flutamide were dissolved in polyethylene glycol to achieve a desired concentration. All solutions were prepared on the day of injection. All injections were done intraperitoneally.

Induction of Extrapyramidal Disorder and its Measurement: Extrapyramidal disorder was induced by the administration of haloperidol (1 mg / kg, intraperitoneally) with a neuroleptic drug (haloperidol). To measure the catalepsy, the standard bar test (6, 16, 17) was used, and to measure the motor coordination, the standard rotarod test (3, 16, 18) was used.

In the bar test, the front legs of the animal were placed on a wood rod of 0.9 cm in diameter and 9 cm in height from the ground and measured for the duration of the animal's gripping in fixed position. The test is finished when the animal holds back one of its front legs from the bar or slips its head exploratively. The test was finished within 360 seconds. Experiments were performed at 15, 60, 120, and 180 minutes after the

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injection of drugs (6, 16, 17). In the rotarod method, the animal was placed on a rotating rod at a speed of 18 rpm. The time that the animal was able to stand and keep its balance on the rod was recorded in seconds (2,3,16).

Data analysis method: The data are expressed as Mean±SEM. To compare the difference between the mean data, ANOVA test was used and if there was a significant difference, Tukey post hoc test was used, and $p<0.05$ was considered significant.

Results

Effect of intraperitoneal injection of haloperidol on catalepsy: The results showed that the mean time of gripping the rod in the animals receiving the haloperidol neuroleptic drug (170±17.25) was significantly higher that the control group (20±4.45) and sham (22±3.5) ($p<0.001$). This was predominant at all times of 15, 60, 120, and 180 minutes after drug injection. Intraperitoneal injection of testosterone (18±5.2) or flutamide (22±5.15) alone for 7 days had no effect on the duration of time spent on the bar. Compared to normal and sham groups, there was no significant difference (Fig. 1a).

Preventive/Protective Effect of Testosterone on Haloperidol-induced Catalepsy: Comparing the bar test results of the haloperidol receptor group and the preventive group showed that the duration of gripping the bar in stasis mode in the group pre-treated with testosterone prior to administration of haloperidol in all periods of 15, 60, 120, and 180 minutes ((61±11), (55±8), (62±5) and (48±12), respectively) significantly decreased ($p<0.001$) compared to the group receiving haloperidol alone ((175±24), (158±25), (180±18) and (167±2), respectively).

Furthermore, in the protective group, acute simultaneous injection of testosterone with haloperidol significantly ($p<0.01$) decreased the duration of gripping the bar at 15 and 60 minutes ((68±24) (74±12), respectively), while at 120 and 180 minutes ((112±28) and (133±32), respectively), there was no significant difference in the duration of the animals' gripping compared to the haloperidol alone group, which is probably due to the low blood testosterone levels (Fig. 1b).

Effect of simultaneous injection of testosterone and flutamide on haloperidol-induced catalepsy: As shown in Fig. 1C, the duration of gripping the bar in the group receiving testosterone and flutamide simultaneously for 7 days, and then haloperidol on the final day at all times of 15, 60, 120 and 180 minutes ((145±22), (148±25), (152±17) and (150±14), respectively) was significantly ($p<0.05$) higher than the preventive group. In addition, acute flutamide injection with testosterone before haloperidol significantly ($p<0.05$) neutralized the protective effects of testosterone and increased the duration of animal's bar-gripping ((168±11), (172±8) (160±15) and (156±22), respectively), compare with the protective group (Fig. 1c).

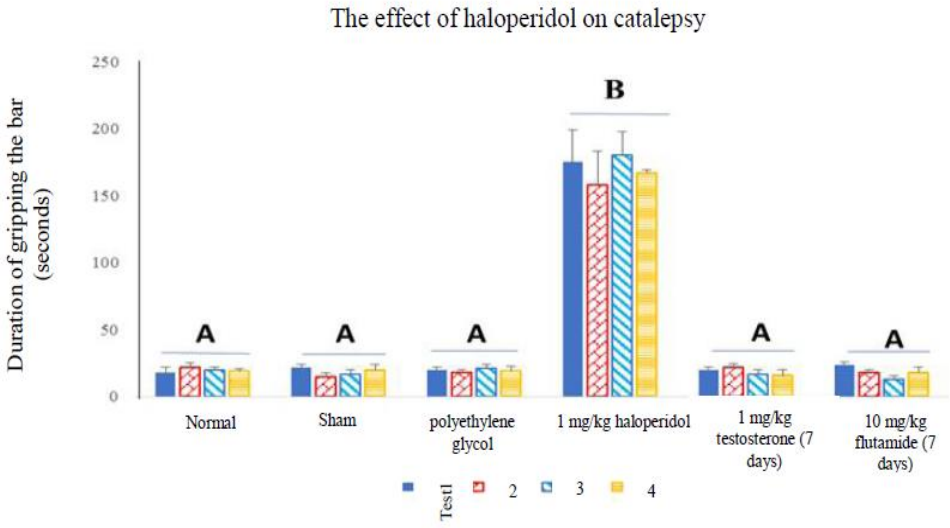

Figure 1 a. The effect of intraperitoneal injection of haloperidol on catalepsy in bar test; data were shown as Mean±SEM; $p<0.001$ [B]: the haloperidol group compared to control, sham, polyethylene glycol, testosterone alone and flutamide alone groups; $p<0.001$ [A]: compared to the haloperidol group. There were no significant differences between different test times (tests 1, 2, 3 and 4) within different groups.

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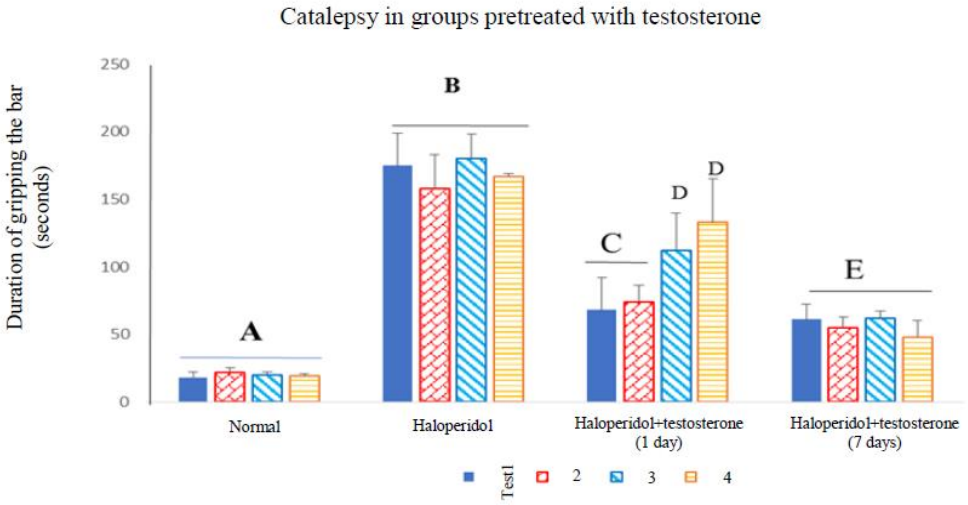

Figure 1 b. The effect of premedication and acute treatment with testosterone on haloperidol-induced catalepsy; data were shown as Mean±SEM; p<0.001 [B]: comparison between the haloperidol group and the normal group; p<0.001 [E]: the group pre-treated with testosterone compared to the haloperidol group, p<0.001 [C]: acute testosterone injection compared to the haloperidol group; p<0.001 [A]: compared to the haloperidol group; [D]: no significant difference with the haloperidol group. There were no significant differences between different test times (tests 1, 2, 3, 4) within different groups.

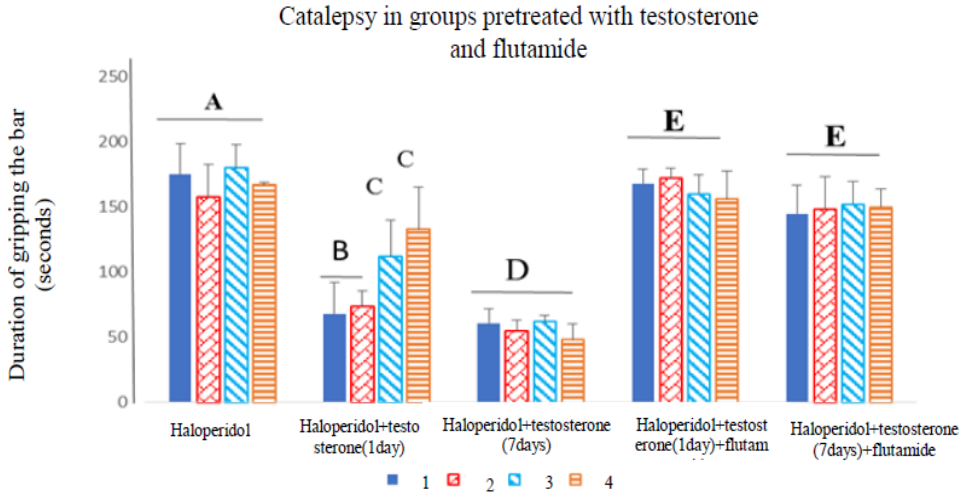

Figure 1 c. The effect of simultaneous injection of testosterone and flutamide on haloperidol-induced catalepsy; data were shown as Mean±SEM; p<0.001 [A]: the haloperidol group compared with the normal group, p<0.001 [D]: the group pre-treated with testosterone compared to the haloperidol group; p<0.001 [E]: the group receiving the acute testosterone injection compared to the haloperidol group, p<0.001 [B]: the group receiving flutamide and testosterone simultaneously compared to testosterone alone, [C]: no significant difference with the haloperidol group. There were no significant differences between different test times (tests 1, 2, 3 and 4) within different groups.

The effect of intraperitoneal injection of haloperidol on motor coordination: The results showed that the mean duration that the animal was able to stand and keep its balance on the rod at 15, 60, 120, 180 minutes after receiving haloperidol ((49±14), (52±8), (45±18), and (61±3), respectively) decreased significantly ($p < 0.001$) compared to the control group, solvent and sham ((702±18), (710±10), (716±4), and (718±2) respectively), respectively). Intraperitoneal injection of testosterone or flutamide alone for 7 days had no effect on the duration that the animal was able to stand and keep its balance on the rod and compared to the normal and sham groups, no significant difference was observed (Fig. 2a).

The preventive / protective effect of testosterone on haloperidol-induced motor disorder: comparing the rotarod results of the haloperidol group with the preventive group showed that the duration of standing

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on the rod in the prevention group at all times of 15, 60, 120, and 180 minutes after administration of haloperidol ((172±16), (178±8), (181±11), and (169±22), respectively) were significantly ($p < 0.05$) higher than those receiving haloperidol alone ((49±14), (52±8), (45±18), and (61±3), respectively).

However, the acute injection of testosterone in the protective group ((58±24), (74±12), (62±17), and (72±15), respectively) showed no significant difference compared to haloperidol group in standing on the rod (Fig. 2b).

The effect of simultaneous injection of testosterone and flutamide on haloperidol-induced motor disorder: Duration that the animal was able to stand and keep its balance on the rod in the group receiving testosterone and flutamide simultaneously for seven days, and then haloperidol on the last day at all times of 15, 60, 120, and 180 minutes ((70±11), (75±8), (81±9), and (79±11), respectively) decreased significantly ($p < 0.05$) compared to the preventive group ((172±16), (178±8), (181±11), and (169±22), respectively). In addition, the acute injection of flutamide and testosterone simultaneously before haloperidol reduced the duration of standing on the rod ((45±11), (55±5), (38±11), and (46±8), respectively) compared with the protective group ((58±24), (74±12), (62±17), and (72±15), respectively), although no significant difference was observed (Fig. 2c).

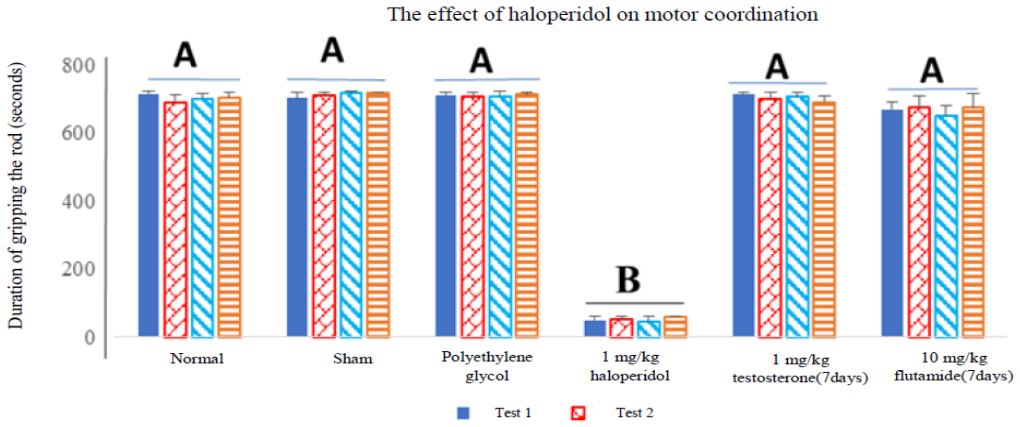

Figure 2 a. The effect of intraperitoneal injection of haloperidol on motor coordination in rotarod; data are shown as Mean±SEM; $p<0.001$ ^{B}: the haloperidol group compared to control, sham, polyethylene glycol, testosterone alone and flutamide alone groups; $p<0.001$ ^{A}: compared to the haloperidol group. There were no significant differences between different test times (tests 1, 2, 3, 4) within different groups.

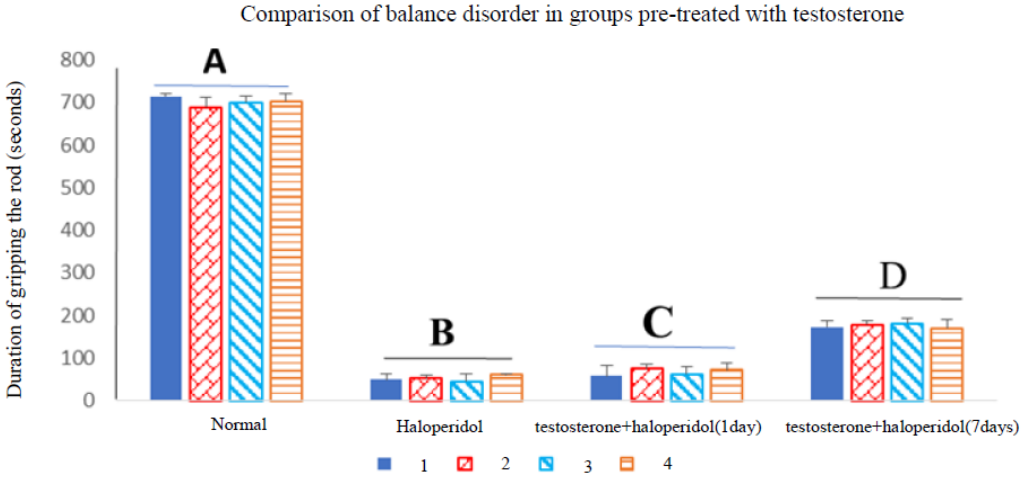

Figure 2 b. The effect of premedication and acute treatment with testosterone on haloperidol-induced tor disorder; data are shown as Mean±SEM; $p < 0.0001$ ^{B}: comparison between the haloperidol group and the normal group; $p < 0.001$ ^{D}: the group pre-treated with testosterone compared to the haloperidol group; $p < 0.001$ ^{A}: compared to the haloperidol group; C: no significant difference with the haloperidol group. There were no significant differences between different test times (tests 1, 2, 3, 4) within different groups.

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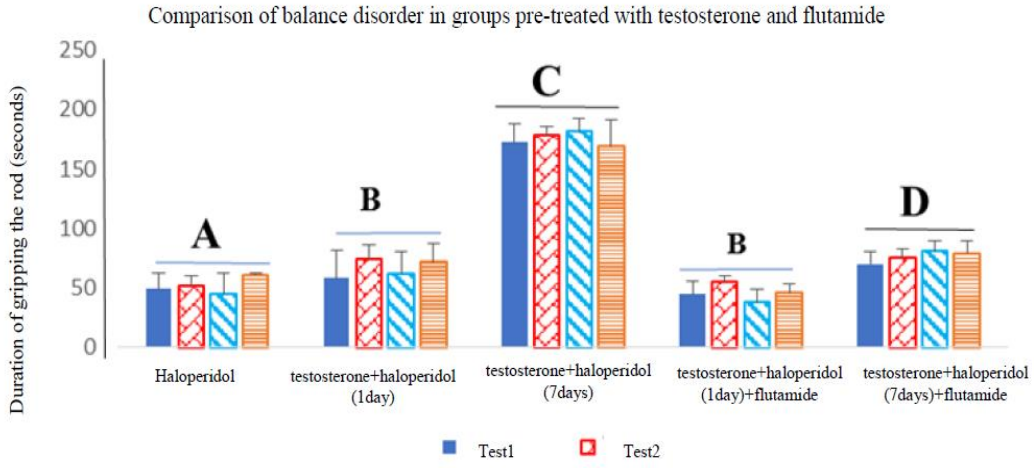

Figure 2 c. The effect of simultaneous injection of testosterone and flutamide on haloperidol-induced motor disorder; data are shown as Mean±SEM; $p < 0.05$ [D]: simultaneous flutamide and testosterone group compared to testosterone alone, $p < 0.05$ [C]: group pre-treated with testosterone compared to haloperidol group; $p < 0.05$ [A]: compared to haloperidol plus testosterone group (7 days). [B]: There was no significant difference with haloperidol group. There were no significant differences between different test times (tests 1, 2, 3 and 4) within different groups.

Discussion

The results of our study showed that intraperitoneal injection of haloperidol can significantly cause extrapyramidal disorder in rats. The study of the effect of testosterone on motor disorders induced in rats showed that seven-day pre-treatment with testosterone could reduce haloperidol-induced catalepsy and coordination disorders, and acute injection of testosterone improved these symptoms to some extent. As previously mentioned, Parkinson's disease is a progressive disorder of the nerves that occurs due to the destruction of dopaminergic neurons in the substantia nigra pars compacta region and affects the pathways of the basal nuclei. Some common symptoms of this disease are tremor, bradykinesia, muscle stiffness, imbalance, and catalepsy (1, 6).

Gender differences play an important role in the sensitivity and pathogenesis of many motor disorders such as Parkinson's disease (19). In this study, testosterone has preventive and protective effects on haloperidol-induced extrapyramidal disorders, which improves haloperidol-induced extrapyramidal disorders in rats. In the present study, intraperitoneal administration of haloperidol caused extrapyramidal symptoms (catalepsy and imbalance) in animals, which is consistent with previous studies (20).

In addition, pre-treatment with testosterone and acute injection could have preventive and protective effects on haloperidol-induced catalepsy. The results of previous studies have shown that testosterone deficiency in castrated animals increases the complications of catalepsy and its replacement in castrated animals improves the complications of catalepsy, which is in line with the results of the present study (21).

Although some studies indicate the anti-cataleptic effects of acute injection of testosterone, some other studies achieved contrary results (4). In this study, acute injection of testosterone at 15 and 60 minutes after receiving haloperidol was able to reduce the haloperidol-induced catalepsy, but its therapeutic effects were reduced with time, so there was no significant improvement at 120 and 180 minutes, which is probably due to reduction in blood and brain levels. Moreover, in the present study, acute injection of testosterone did not show any improvement in rat's motor coordination in any of the test times. However, its pretreatment has shown to have preventive effects on haloperidol-induced balance disorders, which confirms its effects on extrapyramidal disorders.

In order to determine the mechanisms of testosterone action and to examine the role of androgenic receptors in anti-cataleptic effects and increasing the balance, flutamide was simultaneously injected as an antagonist of testosterone receptors. According to the results of this study, flutamide resulted in the blockage of the protective and preventive effects of testosterone.

Flutamide blocks the androgen receptors, and this has a direct relationship with the exacerbation of extrapyramidal complications, including balance disorders. Since testosterone injections were able to relieve these symptoms as a pretreatment, it seems that

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testosterone has anti-cataleptic effects and improves balance by affecting androgenic receptors. The results of this study showed that administration of testosterone can improve haloperidol-induced extrapyramidal disorders in rats, and the anti-cataleptic and balance-improving effects of this hormone take place by affecting androgenic receptors, although more investigations and further studies are required for explaining its mechanism precisely. More clinical studies are needed to prove the positive effects of testosterone on extrapyramidal disorders caused by haloperidol and other neuroleptic drugs.

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