Effectiveness of the *Mentha spicata* leaves in reducing testosterone levels and aggressive behaviour in female Wistar albino rats treated with testosterone propionate

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**Abstract**  
High testosterone hormone levels play an important role in exhibiting aggressive behaviour and several disorders in female rodents and women. The present study aimed to determine the protective role of the alcoholic extract of *Mentha spicata* leaves (MSL) in the reduction of the level of testosterone and aggressive behaviour of female rats that suffer from high levels of testosterone. A total of 30 female Wistar albino rats were divided into 6 Groups. Group 1: Control rats received sesame oil (0.5 ml). Group 2: Rats injected with testosterone propionate (TP) alone. Group 3: Rats received MSL alone (100 mg/kg) orally. Groups 4, 5, and 6: Rats received TP + MSL, 6 mg/rat of TP followed by 200, 400, and 600 mg/rat, respectively) for 60 days. The testosterone, dopamine, and aggressive behaviour were measured using specialized ELISA test kits. The results showed that testosterone and dopamine levels in the serum had a significant decrease (P<0.05) (4.82 (ng/ml) in testosterone and 272.83 (pg/ml) in dopamine) in animals treated with MSL only compared to Group 2, which found a significant rise (P<0.05) (16.52 (ng/ml) in testosterone and 607.59 (pg/ml) in dopamine) in the levels of testosterone and dopamine. The results exhibited a significant rise (P<0.05) in the aggressive behaviour in Group 2 of rats compared to the control and other Groups. In comparison, aggressive behaviour was significantly decreased (P<0.05) (7.40 (ng/ml) in testosterone and 263.49 (ng/ml) in dopamine) in Groups 2, 4, 5, and 6. Thus, the study revealed the protective role of the alcoholic extract of MSL in reducing levels of testosterone and aggressive behaviour in female rats suffering from higher levels of testosterone.

**Keywords**: Aggressive behaviour, Dopamine, Female rats, *Mentha spicata*, Testosterone

**INTRODUCTION**

Testosterone is a sex hormone created by males and females. In males, it is created and released from the Leydig cells in the testes, while in females produced by the placenta and ovaries. In both sexes, testosterone is secreted by the adrenal cortex about 25% (Banihani, 2019). The testosterone levels differ significantly in both sexes, with males having higher testosterone levels than females. However, the typical serum testosterone levels in females are generally 10 to 20 times lower. Testosterone has physiological impacts on reproductive tissues in women (Parish *et al*., 2021). A woman of reproductive age produces three to four times as much testosterone as estrogen every day from her ovaries (Yang *et al*., 2010). But these androgen levels decrease with age, about in the mid-30s onward, with no clear significant rise in the speed of decrease at menopause due to the ova continuing to produce hormones (Donovitz, 2021). Testosterone is present in blood either unbound (free) or bound with albumin proteins. Moreover, inactive testosterone hormones are bound to globulin sex hormones-binding globulin (SHBG), carrier proteins created in the liver that have a high affinity to the testosterone hormones. Free testosterone hormone is the active portion of the androgens Group and hence free testosterone could connect more to the risk of detection aggressiveness (Regis *et al*., 2021).
High levels of testosterone have an impact on the brain areas and may be the cause of some behavioural disorders in both sexes. Numerous studies have been conducted on testosterone’s effects, particularly in models of anxiety and aggression (Domonkos et al., 2018). The neurophysiological mechanism of aggression is the same in all vertebrates, and aggression is a social activity that occurs naturally in mammals to protect their area, preserve resources, and increase the likelihood of fertile mating. However, when levels of aggression become excessive, harmful, disturb social order, and have a cost that is significantly greater than its benefit, the behaviour is no longer adaptive and is referred to as maladaptive or pathologic aggression (Ishola, 2020).

Aggressive behaviour is a complex social behaviour and is commonly involved with intellectual disability and psychiatric disorders; it has received huge research attention for more than five decades. Two types of aggression have been identified, the first sub-type with automatic control and the interactive-impulsive sub-type. The second is considered impulsive, usually associated with anger, while the first is more objective and directed towards the goal. Neurotransmitters refer to particles in the nervous system that function as signaling particles on specific receptors for each neurotransmitter such as dopamine. These molecules are major cofactors in a wide variety of molecules (Trifu et al., 2020). Aluja et al. (2015) studies in healthy men and women confirm the hypothesis that high testosterone levels impact risk-taking or impulsive behaviour. In a later study, it was exhibited that early-onset users under testosterone rage are more impulsive and show deficits in behavioural disinhibiting, affective processing, and planning.

Spearmint leaves are medical plants that are widely used as anti-bronchitis, antispasmodic, stomach pain-relieving agents, and carminative. The major compounds isolated from mint leaves include terpenoids, flavonoids, and phenols, and rosmarinic acid in essential oils of spearmint was found to have hypoglycemic, antimicrobial, hypocholesterolemic, insecticidal, and antioxidant properties (Bayani et al., 2017). A study shows that menthol in mint lowers testosterone levels in men, which ultimately leads to a decrease in libido. In addition, mint may reduce sperm production if consumed in excess, which causes impotence. So, spearmint may be advantageous in reducing free testosterone levels and hirsutism in women with PCOS, and its adverse histopathological impacts on the liver, kidney, and uterine tissues in animals were noticed (Alaae et al., 2020). Mentha spicata has a lethal effect on the testis by causing a significant in experimental rats. Past reports showed structural changes in testicular tissue, morphological distortions and spermatogenesis inhibition of different mammals treated with spearmint (Mohammed et al., 2021). Another study displays that female students who received M. spicata in capsule shape show dysmenorrhea with lesser acuteness compared to placebo. The ethanolic extracts of M. spicata possess antidepressant-like features in the rat. These responses might be recognized as interactions with the serotonergic, noradrenergic, and dopaminergic receptors. Furthermore, the enhancing impact of the M. spicata extract with conventional antidepressants looks promising and its potential as a complementary agent for depression management (Abbasi-Maleki et al., 2017). In the present study, a female rat model with elevated testosterone levels was used to examine the significance of utilizing a mixture of spearmint alcoholic extracts and its impact on reduced testosterone levels and aggressive behaviour in female Wistar albino rats.

MATERIALS AND METHODS

Ethical approval
The University of Babylon, the College of Sciences, and the Department of Biology approved the experimental protocol. (1522/3-6-2022), and the National Committee for Research Ethics in Science and Technology (NETNT) reports that the studies were conducted as per approved procedures and ethical standards.

Preparation extract of Mentha spicata leaves (MSL)
The alcoholic extract of M. spicata leaves was prepared by washing them well and then drying them at room temperature. The leaves were ground until they became a powder, dissolved in 70% ethanol, and put in the shaker for an hour, then filtered with gauze. At a temperature of 45°C, put it in the oven until it hardens and the pieces are ground (González-Montelongo, et al., 2010).

Testosterone propionate (TP) preparation
Testosterone was purchased from China (FedEx) and was prepared by taking 6 mg/rat of testosterone propionate powder and dissolving it in 0.5 ml of sesame oil.

Study design
In this study, 30 female Wistar albino rats were divided into six Groups, 5 rats in each.
Group 1: Negative control- Animals received sesame oil (0.5 ml) orally.
Group 2: Positive control- Animals were injected subcutaneously with TP (6 mg/rat).
Group 3: Animals were orally treated with an alcoholic extract of MSL (100 mg/rat).
Group 4: Animals were injected subcutaneously with TP (6mg/rat)+ MSL (200 mg/rat) orally.
Group 5: Animals were also injected subcutaneously with TP (6mg/rat)+MSL (400 mg/rat) orally.

(2021). Another study displays that
Group 6: Animals were injected subcutaneously with TP(6mg/rat) + MSL (600 mg/rat) orally. All Groups were treated for 60 days.

Physiological assay
Serum testosterone and dopamine were measured according to the manual procedure of Elabscience, a company based in the USA that offered specialized ELISA test kit sets.

Aggressive behaviour (Resident-intruder test)
The Resident-intruder test model was used to understand aggressive behaviour in which one rat (intruder) was permitted to build a territory (resident) in its home cage, another rat was in the resident’s home cage and two animals were permitted to interact with each other for a fixed period. The duration and frequency of resident rats were determined by the following behavioural parameters: Attack latency: (the time between the introduction of the intruders and the first clinch attacks) (Rammal et al., 2010). Before this test, drops of 40% zinc sulfate were put in the nose of the intruder male prior to encounters to disrupt the sense of smell.

Statistical analysis
Data for parameters represent a mean ± SE and data of all behaviour tests represent a mean ± SD. A one-way analysis of variance (ANOVA) and the Duncan test for Post Hoc multiple comparisons were used to analyze the variation between the values statistically. SPSS statistical software version (23). A value of p<0.05 was considered statistically significant for all tests. Analysis of the correlation between parameters of Groups was done by Pearson test.

RESULTS AND DISCUSSION

Physiological study
Effect of Mentha Spicata leaves on the testosterone and dopamine levels
Table 1 illustrates data on the testosterone and dopamine levels in the serum of female rats treated with alcoholic extract of mint leaves. Rats treated with TP showed a significant rise (p<0.05) in the levels of testosterone hormone and dopamine compared to the Control Group and the other Groups, whereas the animals in the 4, 5 and 6 Groups treated with TP (6mg/rat) + MSL (200, 400 and 600 mg/kg) respectively exhibited a significant decline (P<0.0) (Sin the levels of testosterone and dopamine as compared to the Group 2. While no significant differences appeared in the levels of these hormones (p>0.05) between Groups 1 and 5, 6 of the rats were treated with TP + AML. It may be because mint leaves contain terpenoid compounds, namely carvone and menthol, which cause low testosterone levels and triglyceride (Ashkar et al., 2020).

Correlation between physiological parameters and extract of mint leaves
Correlation between the testosterone and dopamine levels in the female rats that were treated with TP showed that there was a significant positive linear relationship between the levels of testosterone and dopamine hormones; the equation was linear between them y = 34.82x - 39.217 and the correlation coefficient r = 0.810, as noted in Fig. 1.

Table 1. Effect of alcoholic extract of Mentha Spicata leaves on the testosterone and dopamine levels in the serum of female rats treated with testosterone propionate for 60 days. (n=5)

<table>
<thead>
<tr>
<th>Parameters Groups</th>
<th>Testosterone (ng/ml) Mean±S.E</th>
<th>Dopamine (pg/ml) Mean±S.E</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>4.74±0.58</td>
<td>125.73±13.70</td>
</tr>
<tr>
<td>Group 2</td>
<td>16.52±1.20a</td>
<td>607.59±62.88</td>
</tr>
<tr>
<td>Group 3</td>
<td>4.82±0.42b</td>
<td>272.83±24.28a</td>
</tr>
<tr>
<td>Group 4</td>
<td>7.40±0.65ab</td>
<td>263.49±20.49b</td>
</tr>
<tr>
<td>Group 5</td>
<td>6.02±1.40b</td>
<td>135.43±13.92ab</td>
</tr>
<tr>
<td>Group 6</td>
<td>6.81±1.16b</td>
<td>131.33±2.786b</td>
</tr>
</tbody>
</table>

a:Mean significantly different at the P≤0.05 as compared to Group 1 (controls);
b:Mean significantly different at the P≤0.05 as compared to Group 2;
c:Mean significantly different at the P≤0.05 as compared to Group 3;
d:Mean significantly different at the P≤0.05 as compared to Group 4;
The study exhibited a significant negative linear correlation between the levels of testosterone and dopamine in the female rats and the concentration of the alcoholic extract of mint leaves (Mentha Spicata) (200, 400 and 600 mg/rat). The correlation coefficient for testosterone was \( r = -0.720 \). For dopamine, it was \( r = -0.847 \), as shown in Fig. 2. There are positive correlation between testosterone and dopamine and testosterone injections cause a high dopamine response in rats. Based on these results, it is suggested that mint leaves led to lower testosterone levels, which led to lower dopamine levels. Research on the effects of testosterone hormones on midbrain dopamine circuitry in adult male rats varies. Some studies in rats suggest that neuron release of dopamine is inhibited by testosterone (Aubele and Kritzer, 2011). Other studies suggested the opposite, where testosterone can raise dopamine levels and dopamine turnover in animals (Owens et al., 2018).

**Aggressive behaviour study**

**Effect of alcoholic extract of Mentha spicata on the aggressive behaviour of the rats**

The effect of the alcoholic extract of *M. spicata* on the aggressive behaviour of the female rats is shown in Table 2. Injection of TP into rats in Group 2 led to a significant decrease (\( P<0.05 \)) in aggressive behaviour parameters of all mean of latency to the first threat and thrust (second) and latency to the first attack (sec.), while the rats in Groups 3, 4, 5 and 6 were treated with MSL (100, 200, 400, 600 mg/kg) showed a significant increase (\( p<0.05 \)) in these parameters compared to Groups 1 and 2.

The result showed a significant increase (\( P<0.05 \)) in all means the number of threats and thrusts, and the number of attacks and keep down in the Group 2 treated with TP compared to Group 1 as well as the rats in the Group 3 treated with MSL (100 mg/kg) that showed a significant decrease (\( P<0.05 \)) in these parameters. The number of threats and thrusts in Group 6 significantly decreased in Group 1. No significant (\( p>0.05 \)) difference was seen between control Group 1 and Groups 3, 4 and 5.

Aggressive behaviour is usually measured by the sum of the number of threats, thrusts, attacks and keep down. The pie chart demonstrates the effect of alcoholic extract of mint leaves (Mentha Spicata) on the female rats' decreased aggressive behaviour, as shown in Fig. 3. Injection of testosterone propionate (6 mg/kg) into rats in Group 2 for 60 days led to a significant rise (\( P<0.05 \)) in aggressive behaviour. *Mentha* has an anti-fertility effect; therefore, it inhibits the levels of testosterone and will inhibit sexual and aggressive behaviour in men. Testosterone triggers dopamine liberat in the medial preoptic areas of the hypothalamus, which is the area controlling aggressive and sexual behaviour. Testosterone profoundly affects the brain circuits involved in threat and aggression, and this study agrees with (Geniole et al., 2020 and Njoroge et al., 2015) that a single dose of testosterone increased aggressive behaviour in female rats. This study agrees with Estuman et al. (2019), who showed that one injection of the hormone testosterone was sufficient to raise functional connectivity between and within the hemispheres, modify physiology, addiction and behaviour, and encourage hostility and aggression. The study showed that short-term oral testosterone administration to women shows a range of alterations, including traits...
associated with aggressive behaviour in female rats (Carré and Archer, 2018). The experiment on female rats' results support the effectiveness of the alcoholic extract of mint leaves in lowering the aggressive behaviour in female rats that suffer from a high testosterone level compared to the Control Groups and when comparing the Groups with each other. This is because of its soothing mint scent. This study agrees with Abbasi-Maleki et al. (2017) and Moss et al. (2023) that spearmint's ambient aroma may effectively decrease aggressive behaviour. It was hypothesized that spearmint aroma would decrease aggressive behaviour and negative feelings such as stress and aggression while raising calmness and alertness. Since the alcoholic extract of mint leaves reduced testosterone, we conclude from our study in this research that the alcoholic extract of mint leaves also reduced aggressive behaviour. Pheromone information processed by the accessory olfactory bulbs is necessary for appropriate social behaviour in rats, including sexual and aggressive behaviour. Disrupting the activation of either the olfactory or the vomeronasal organs causes males to evade aggressive behaviour and severely affects male sexual behaviour in many rodents (Quadros et al., 2020). Therefore, experiment animals treated with zinc sulfate (%4 Zinc sulfate) by putting one drop in the nose of the intruder rat for the destruction of the nasal epithelium to eliminate participant behaviour both in the experienced and inexperienced males (Angoa-Pérez and Kuhn, 2021).

Clinical signs

Animals in all Groups were carefully examined daily before and after the experimental period for likely clinical signs due to testosterone injections, and these signs are:

- Hair loss and appearance of pimples in TP-injected animals compared to the Control Group and animals treated with TP+ ALMP are shown in Fig. 4 A and C.
- Slight hair loss in the Group of animals that were given TP+ ALMP is shown in Fig. (4 B).
- Female hair loss may be due to many factors like genetics and age but is mostly due to hormonal alters and imbalances, some of the hormones associated with hair loss, such as the cortisol and thyroid hormones. Stressful cases and anxiety can cause an increase in cortisol levels, which can then influence hair growth. Females produce testosterone in small amounts. An imbalance of testosterone can cause females to lose hair on their scalp or grow rough body.

### Table 2. Effect of alcoholic extract of mint leaves (*Mentha spicata*) on the aggressive behaviour of the rats treated with testosterone propionate for 60 days (n =5)

<table>
<thead>
<tr>
<th>Parameters/Groups</th>
<th>Latency to the first threat and thrust (sec.)</th>
<th>Number of threat thrust</th>
<th>Latency to the first attack (sec.)</th>
<th>Number of attack</th>
<th>Number of keep down</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>120.82±13.12</td>
<td>11.00±1.87</td>
<td>176.07±8.99</td>
<td>3.40±1.14</td>
<td>2.40±1.14</td>
</tr>
<tr>
<td>Group 2</td>
<td>15.48±3.68a</td>
<td>45.00±7.97a</td>
<td>31.69±5.59a</td>
<td>17.60±2.07a</td>
<td>22.40±3.65a</td>
</tr>
<tr>
<td>Group 3</td>
<td>306.18±16.69ab</td>
<td>8.40±1.52b</td>
<td>359.79±60.45ab</td>
<td>5.60±1.14bcd</td>
<td>1.20±0.45a</td>
</tr>
<tr>
<td>Group 4</td>
<td>213.06±21.83abc</td>
<td>14.00±3.39bc</td>
<td>256.01±5.63abc</td>
<td>9.40±1.82bcd</td>
<td>12.40±3.05abc</td>
</tr>
<tr>
<td>Group 5</td>
<td>238.21±14.76ab</td>
<td>7.00±1.87cd</td>
<td>285.11±7.07abc</td>
<td>8.00±1.58abc</td>
<td>8.20±1.48abcd</td>
</tr>
<tr>
<td>Group 6</td>
<td>278.16±27.20abc</td>
<td>4.20±1.64bcd</td>
<td>350.98±11.97abcd</td>
<td>6.60±2.64bcd</td>
<td>4.40±1.14bcde</td>
</tr>
</tbody>
</table>

a: Mean significantly different at the P ≤0.05 as compared to Group 1 (control); b: Mean significantly different at the P ≤0.05 as compared to Group 2; c: Mean significantly different at the P ≤0.05 as compared to Group 3; d: Mean significantly different at the P ≤0.05 as compared to Group 4; e: Mean significantly different at the P ≤0.05 as compared to Group 5;
hair on the chest or face (Iamsumang et al., 2020).

**Conclusion**

The present study indicated the protective role of the alcoholic extracts of mint (M. spicata) leaves in reducing testosterone levels in the serum of Wistar female rats treated with testosterone propionate. The extract of mint leaves also improved aggressive behaviour in female rats suffering from high testosterone.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

**REFERENCES**


**Fig. 4.** Clinical signs noted in rats Groups: A- hair loss of rat in TP Group, B- Slight loss of hair rats in TP+ALMP Groups, C- appearance of blood pimples