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Effects of Tolterodine on Depression, Anxiety, Learning and Memory in Mice

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Authors' contributions

This work was carried out in collaboration among all authors. Authors MHT and PT designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors PT and MEB managed the analyses of the study. Authors RKK and AO performed the experiment. Authors RKK, SNBB, DO and OM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: Overactive bladder (OAB) is characterized by urinary symptoms such as frequent urination, urgency, urinary incontinence, and nocturia. Tolterodine is a drug specially developed for the treatment of overactive bladder. The aim of present study is to evaluate the effects of tolterodine on depression, anxiety, learning and memory to understand if tolterodine may be effective in OAB caused mood and cognitive disorders.
Methodology:
Study Design: All the drugs were given intraperitoneally (i.p.), 30 min before the experiment. Here, we investigated the effects of tolterodine (0.3, 1, 3 mg/kg) on depression, anxiety, learning and memory by using forced swimming test, elevated plus maze test, passive avoidance, and Morris water maze, respectively in mice. Locomotor activity was evaluated by open field test.

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Place and Duration of Study: Department of Pharmacology and Department of Urology, Sakarya University, Animal Research Center, between August 2019 and September 2020.
Results: All doses of tolterodine dose-dependently reduced immobility time, compared to saline group. Tolterodine (1, 3 mg/kg) prolonged the time spent in open arms compared to saline group. Tolterodine (3 mg/kg) significantly increased the number of entries into the open arms. Tolterodine had no effect on learning and memory performance of normal mice; however, tolterodine (3 mg/kg) significantly ameliorated learning and memory disruption induced by scopolamine.
Conclusion: Our results demonstrate that tolterodine prevented experimentally induced depression and anxiety, improved memory and learning of naive animals, and reversed memory and learning impairment with scopolamine. Further preclinical and clinical studies with tolterodine should be done to support all these hypothesis and patients with OAB who need antidepressant and anxiolytic therapy may be treated with single drug instead of more than one drug in the future.

Keywords: Tolterodine; learning; memory; anxiety; depression; overactive bladder.

1. INTRODUCTION

Overactive bladder is characterized by urinary symptoms such as frequent urination, urgency, urinary incontinence, and nocturia as a result of excessive contraction of the detrusor during bladder filling. These contractions are mostly under the control of the parasympathetic system [1-3]. Antimuscarinic drugs suppress the contraction of the detrusor by preventing acetylcholine from binding to muscarinic receptors [4,5].

Antimuscarinic drugs are used in the first step at the accommodation. Antimuscarinic drugs exert a muscarinic blocking effect on the detrusor muscle [6]. There are 5 types of muscarinic receptors. M3 receptors are responsible for the contractions of the detrusor muscle [7,8]. Also, muscarinic receptors are found in the central nervous system other than the bladder; plays an important role in learning and memory [7,9].

The first antimuscarinic drug used in overactive bladder was oxybutynin. However, oxybutynin has side effects such as dry mouth and constipation that limit its clinical use [10]. These side effects are less common with the use of second generation antimuscarinic agents such as tolterodine [11]. Tolterodine is a drug specially developed for the treatment of overactive bladder; have more specific effects on M3 receptors [12].

Use of medicines in an unapproved indication, age group, dose or administration route is defined as off-label drug use. Off label drug use provides new opportunities for existing approved drugs, and reduces the time and cost involved in drug discovery. Muscarinic receptors are also found in the central nervous system other than the bladder; based on this information we want to understand if tolterodine may be effective in OAB caused mood and cognitive disorders.

Depression and anxiety are associated with OAB; can affect their life qualities. With all these background, we investigated the effects of antimuscarinic drug tolterodine on depression in forced swimming and on anxiety in elevated plus maze test in mice and also imipramine as positive control for depression, and diazepam as positive control for anxiety. Besides these, we investigated the effects of antimuscarinic drug tolterodine on learning in passive avoidance and on memory in morris water maze test in mice and also on learning and memory in scopolamine-treated mice, which is used as model showing deficits in cognitive performance.

2. METHODOLOGY

2.1 Animals

Male inbred BALB/c ByJ mice (Animal Research Center, Sakarya-Turkey) aged 7 weeks upon arrival to the laboratory were used in this study. Female animals mostly don't be used in behavioral tests because they have menstrual cycle which may cause false positive or negative results. For this reason, we used male animals similar to our previous studies [13,14]. Animals (4–5 per cage) were kept in the laboratory at 21 ± 1.5 ◦C with 60% relative humidity under a 12 h light/dark cycle (light on at 8.00 p.m.). Tap water and food pellets were available ad libitum. All experiments were performed between 10.00 and 12:00 a.m. All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were

followed, as well as specific national laws where applicable.

2.2 Drugs

Tolerodine, imipramine hydrochloride, diazepam and scopolamine were purchased from Sigma Chemicals (St Louis, Mo, USA). Drugs were dissolved in saline. Saline (%0.9) was used as the vehicle controls. All the drugs were given intraperitoneally (i.p.) in a volume of 0.1 ml per 10 g body weight of mice. The doses were chosen based on previous behavioural studies [13,14]. Drugs were prepared freshly on the day of experiment.

2.3 Experimental Design

We investigated the effects of tolterodine on depression, anxiety, learning and memory by using forced swimming test, elevated plus maze test, passive avoidance and morris water maze, respectively, in mice. Additionally, the locomotor activity was evaluated by measuring the total distance traveled in the open field test.

2.3.1 Forced Swimming Test (FST)

The mice were dropped individually into plexiglas cylinders (height 25 cm, diameter 10 cm) containing 10 cm of water maintained at 23-25°C and left there for 6 min. The duration of immobility (in seconds) was recorded during the last 4 min of the 6-min testing period. The absence of hind leg movement was recorded as immobility by stopwatch cumulation by a single observer who was aware of the treatments during the exposures.

Forty male inbred BALB/c ByJ mice were used in the Forced Swimming Test. Mice were randomly divided into experimental groups (n=8 mice): saline, imipramine 30 mg/kg (Imip), tolterodine 0.3, 1 and 3 mg/kg, respectively. All experiments were performed between 10.00 and 12:00 a.m. All drugs or saline were given 30 min before the experiment.

2.3.2 Elevated Plus-Maze (EPM) test

Anxiety-related behavior was measured by the EPM test. The experiments were conducted in a dimly lit, semi-soundproof room, illuminated with table lamp (80 lux). Maze was made of wood and consisted of two open (29 cm long × 5 cm wide) and closed arms (29 cm × 5 cm with 15 cm high walls) forming a square cross with a 5 cm square center piece. In order to avoid falls the open arms was surrounded by a short (1 cm) plexiglass edge. The maze was elevated 40 cm above the floor. The open arms and central platform were painted white and enclosed arms were painted black.

Each mouse was placed at the center of the maze facing one of the open arms and allowed to explore the maze. During a 5-min test period, the number of entries into both open and enclosed arms of the maze (defined as the entry of all four limbs into the arms) and the time spent in the open arms was recorded. The observer was present always in the same position towards to the open arms and behind the animals. The open arm activity was evaluated as the following: 1) time spent in the open arms relative to the total time spent in the plus-maze (300 s), expressed as a percentage; 2) number of entries into the open arms relative to the total number of entries into both the open and closed arms, expressed as a percentage. These values were used as indices of anxiety in mice. Any animal that fell off the maze was excluded from the experiment.

Chart 1. Schematic design of experiments

TESTS/ GROUPS	FST (Forced Swimming Test)	EPM (Elevated Plus Maze)	PA (Passive Avoidance)	MWM (Morris Water Maze)	LA (Locomotor Activity)
Saline %0.09	N=8	N=8	N=8	N=8	N=8
İmipramin 30	N=8				N=8
Diazepam 2		N=8			N=8
Scopolamine 0.6			N=8	N=8	N=8
Tolterodine 0.3	N=8	N=8	N=8	N=8	N=8
Tolterodine 1	N=8	N=8	N=8	N=8	N=8
Tolterodine 3	N=8	N=8	N=8	N=8	N=8
Scop 0.6+tol 3			N=8	N=8	N=8

Elevated plus-maze is one of the tests used to evaluate anxiety in animals. In the normal cases, the animals prefer to stay within the closed arms instead of open arms owing to feel more safe. Drugs with anxiolytic properties increase the time spent on the open arm. As the values for both of the measured parameters changed in the same direction compared to control values (i.e., if both the time spent in the open arms and the number of open arm entries was increased or if both were decreased) and the change in one of the parameters was statistically significant, then an effect on anxiety was considered to have occurred. The time spent in the open arms and the numbers of open arm entries were always observed to change in the same direction.

Thirty-eight male inbred BALB/c ByJ mice were used in the study. Mice were randomly divided into experimental groups in EPM: saline, diazepam 2 mg/kg (Dzm), tolt 0.3 mg/kg, tolt 1 mg/kg and tolt 3 mg/kg. Each experimental group consisted of 7-8 mice. All experiments were performed between 10.00 and 12:00 a.m. All drugs or saline were given 30 min before the experiment.

2.3.3 Passive Avoidance (PA) test

Animals were trained in a one-trial, step-through PA apparatus to evaluate memory based on contextual fear conditioning and instrumental learning. A decrease in retention latency indicates an impairment in memory in the PA task. The apparatus consisted of a box with an illuminated part (L 7 × 12.5 × h 14 cm) and a dark part (L 24 × 12.5 × h 14 cm), both equipped with a grid floor composed of steel bars (0.3 cm diameter) spaced 0.9 cm apart. The inhibitory avoidance task consisted of two trials. On the first day of training, the mice were individually placed into the light compartment and allowed to explore the boxes. The intercompartment door was opened after a 10 second acclimation period. In the acquisition trial, each mouse was placed in the illuminated compartment, which was lit by a bright bulb (2000 lux). If the mouse stepped into the dark compartment (2/3 of the tail in the dark compartment), the door was closed by the experimenter, and an inescapable foot shock (0.3 mA/1 second) was delivered through the grid floor of the dark compartment. A cut-off time of five minutes was selected. The time taken to enter the dark compartment (training latency) was recorded. Immediately after the shock, the mouse was returned to the home cage. The retention trial started 24 hours after the end of the acquisition trial. The animals received drugs prior to retention training. Each mouse was placed in the illuminated compartment as in the training trial. The door was opened after a 10 second acclimation period. The step-through.

Latency in the retention trial (with a maximum 300 seconds cut-off time) was used as the index of retention of the learned experience. A shock was not applied during the retention trial.

Forty eight male inbred BALB/c ByJ mice were used in the study. Mice were randomly divided into experimental groups in PA test: saline; scopolamine 0,6 mg/kg (Scop), tolt 0.3 mg/kg, tolt 1 mg/kg, tolt 3 mg/kg and Scop+tolt 3 mg/kg. Each experimental group consisted of 8 mice. All experiments were performed between 10.00 and 12:00 a.m. All drugs or saline were given 30 min before the experiment.

2.3.4 Morris Water Maze (MWM) test

The MWM comprised a circular pool (90 cm diameter) filled with water (22°C) and rendered opaque by addition of small black balls. The pool was located in a dimly lit, soundproof test room with various visual cues, including a white/ black colored poster on the wall, a halogen lamp, a camera, and the experimenter. The maze was divided into four quadrants, and three equally spaced points served as starting positions around the edge of the pool. The order of the release positions was varied systematically throughout the experiment. A circular escape platform (6 cm diameter and 12 cm high) was located in one quadrant 1 cm above the water surface during the familiarization session and 1 cm below the water surface during the other sessions. Video tracking was conducted with a video camera focused on the full diameter of the pool. Navigation parameters were analyzed using the Ethovision 8.5 video analysis system (Noldus Ethovision XT). Mice were trained in MWM five times per day (familiarization session, S1, S2, S3, and S4). One familiarization and four acquisition sessions were carried out using the MWM. During the familiarization session and acquisition phase of experiment, each mouse underwent three trials. The delay between trials was 60 seconds, and a 1-day interval was used between each session. For each trial, the mouse was removed from the home cage and placed in the water maze at one of three randomly determined locations with its head facing the center of the water maze. After the mouse had found and climbed onto the platform, the trial was

terminated, and the escape latency was recorded. If the mouse did not climb onto the platform in 60 seconds, the trial was terminated, and experimenter guided the mouse to the platform; an escape latency of 60 seconds was recorded. Twenty-four hours after the final acquisition session, a "probe trial" was used to assess the spatial memory retention of the location of the hidden platform. During this trial, the platform was removed from the maze and the mouse was allowed to search the pool for 60 seconds. The percent of time spent in each quadrant was recorded.

Forty-eight male inbred BALB/c ByJ mice were used in the study. Mice were randomly divided into experimental groups in MWM test: saline; scopolamine 0,6 mg/kg; tolterodine 0.3 mg/kg; tolterodine 1 mg/kg; tolterodine 3 mg/kg; scopolamine 0,6 mg/kg+tolterodine 3 mg/kg. Each experimental group consisted of 8 mice. All experiments were performed between 10.00 and 12:00 a.m. All drugs or saline were given 30 min before the probe trial of MWM test.

2.3.5 Open field test

Since compounds altering motor activity may give false positive/negative effects in FST, elevated plus maze test, passive avoidance test and Morris water maze test, spontaneous locomotor activity of mice was evaluated by monitoring the activity of the animals in an open field. The animals were placed in the center of the apparatus and behaviors were recorded for a period of 5 min using the Ethovision-XT video tracking system. The locomotor activity was evaluated by measuring the total distance traveled in the apparatus and the speed of the animals.

2.4 Statistics

All data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using GraphPad Prism 6.0® software (GraphPad Software, Inc., San Diego, CA). Groups of data were compared with one-way analysis of variance (ANOVA) and Tukey post-hoc test. Values were considered significantly different at $p < 0.05$.

3. RESULTS

3.1 Forced Swimming Test

One-way ANOVA posthoc Tukey test showed a significant effect of tolterodine and imipramine treatment upon immobility time in FST [$F(40,4) = 38,402$, $p<0.0001$]. Post-hoc comparisons revealed that imipramine ($p<0.001$) and tolterodine (0.3, 1 and 3 mg/kg) dose dependently reduced immobility time, compared to saline group ($p<0.01$, $p<0.001$, $p<0.001$; respectively, Fig. 1).

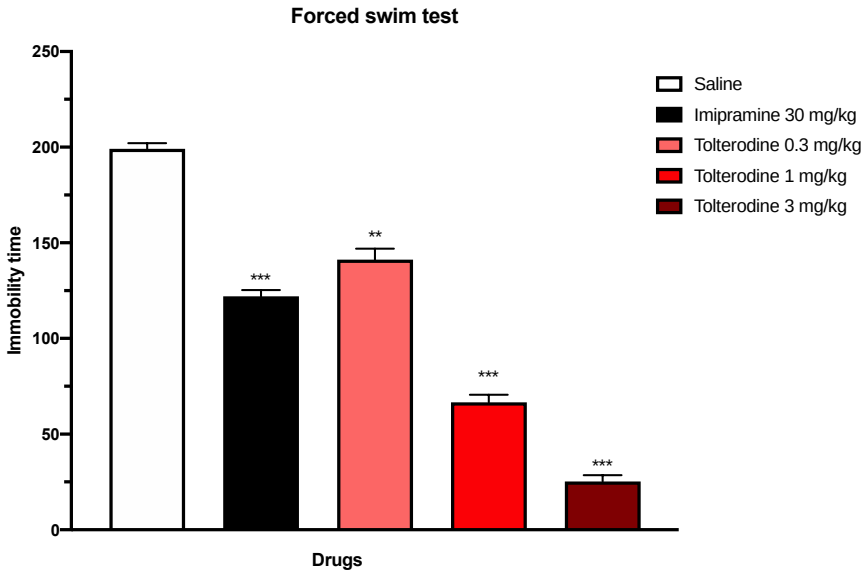

Fig. 1. Immobility time (seconds) in the forced swim test (n=8)

****: p<0.001 compared to Saline group, by ANOVA (Tukey test), **: p<0.01 compared to Saline group, by ANOVA (Tukey test). Mean ± S.E.M = Mean values ± Standard error of means*

3.2 Elevated plus Maze Test

One-way ANOVA posthoc Tukey test showed a significant effect of drug treatment upon the time spent in open arms in EPM test [F (37,4) =13,634, p<0.0001; Fig. 2a]. Post-hoc comparisons revealed that diazepam (2 mg/kg) significantly increased the time spent in open arms compared to saline group (p<0.001). Tolterodine (0.3 mg/kg) did not have any effect on time spent in open arms while tolterodine (1 and 3 mg/kg) prolonged the time spent in open arms (p< 0.05) (Fig. 2a).

One-way ANOVA posthoc Tukey test displayed an important effect of drug treatment upon the number of entries to the open arms in EPM test [F(37,4)=21,546, p<0.0001; Fig. 2b]. Post-hoc comparisons revealed that diazepam (2 mg/kg) significantly increased the number of entries to the open arms compared to saline group (p<0.001) and also tolterodine (3 mg/kg) increased the number of entries into the open arms (p< 0.05) (Fig. 2b). However, Tolterodine (0.3 and 1 mg/kg) did not have any effect on the number of entries in open arms.

3.3 Passive Avoidance Test

There was no significant difference in first day latency between the groups. The second day latency (retention latency) significantly differed between the groups [F (5,47)=3,284, p=0,0136 (Fig. 3). Scopolamine significantly shortened the second day latency compared to the saline group (p<0.001). On the other hand, tolterodine (0.3, 1 and 3 mg/kg) did not significantly have any effect on latency compared the saline group. Furthermore, cognitive performance impaired with scopolamine has been significantly improved with 3 mg/kg tolterodine (p<0.05).

Table 1. Immobility time (in seconds) in the forced swim test.(n=8)

Drugs	Immobility time (s) *Mean ± S.E.M*	Number of mice	p value
Saline	199.2±8.19	8	
Imip 30 mg/kg	122±9.53	8	p<0.001 (saline & imip)
Tolt 0.3 mg/kg	141.25±16.03	8	p<0.01 (saline & tolt 0.3)
Tolt 1 mg/kg	66.62±10.96	8	p<0.001 (saline & tolt 1)
Tolt 3 mg/kg	25.25±9.21	8	p<0.001 (saline & tolt 3)

p<0.001 compared to Saline group, by ANOVA (Tukey test),
p<0.01 compared to Saline group, by ANOVA (Tukey test)
Mean ± S.E.M = Mean values ± Standard error of means

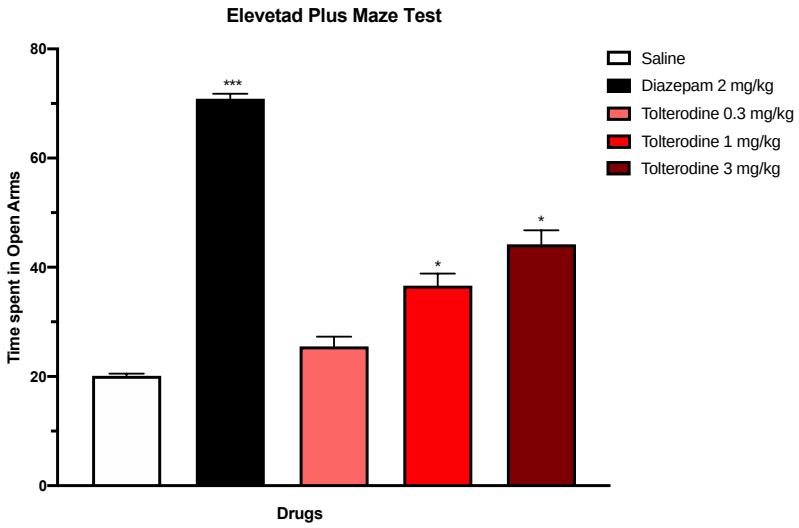

Fig. 2a. Percentage of time spent in the open arms (2a) in elevated plus-maze test. (n=8)
****: p<0.001 compared to Saline group, by ANOVA (Tukey test), *: p<0.05 compared to Saline group, by ANOVA (Tukey test).Mean ± S.E.M = Mean values ± Standard error of means*

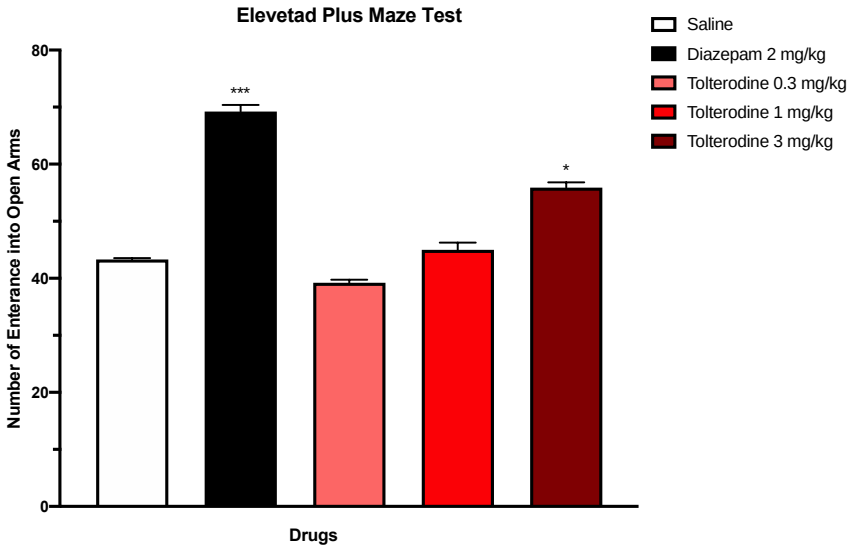

Fig. 2b. Percentage of number of entries into the open arms (2b) in elevated plus-maze test. (n=8)

****: p<0.001 compared to Saline group, by ANOVA (Tukey test), *: p<0.05 compared to Saline group, by ANOVA (Tukey test).Mean ± S.E.M = Mean values ± Standard error of means*

Table 2a. Percentage of time spent in the open arms. (n=8)

Drugs	Percentage of time spent in the open arms (%) *Mean ± S.E.M*	Number of mice	p value
Saline	20.14±1.15	8	
Diaze 2 mg/kg	70.87±2.66	8	p<0.001 (saline & diaze)
Tolt 0.3 mg/kg	25.52±5.03	8	p>0.05 (saline & tolt 0.3)
Tolt 1 mg/kg	36.65±6.25	8	p<0.05 (saline & tolt 1)
Tolt 3 mg/kg	44.23±7.17	8	p<0.05 (saline & tolt 3)

p<0.001 compared to Saline group, by ANOVA (Tukey test), p<0.05 compared to Saline group, by ANOVA (Tukey test) Mean ± S.E.M = Mean values ± Standard error of means

Table 2b. Percentage of number of entries into the open arms (n=8)

Drugs	Percentage of number of entries into the open arms (%) *Mean ± S.E.M*	Number of mice	p value
Saline	43.31±0.63	8	
Diaze 2 mg/kg	69.25±3.07	8	p<0.001 (saline & diaze)
Tolt 0.3 mg/kg	39.22±1.51	8	p>0.05 (saline & tolt 0.3)
Tolt 1 mg/kg	44.97±3.63	8	p>0.05 (saline & tolt 1)
Tolt 3 mg/kg	55.92±2.57	8	p<0.05 (saline & tolt 3)

p<0.001 compared to Saline group, by ANOVA (Tukey test), p<0.05 compared to Saline group, by ANOVA (Tukey test) Mean ± S.E.M = Mean values ± Standard error of means

3.4 Morris Water Maze Test

There was a significant difference between drug groups or their combination [One- way ANOVA post-hoc Tukey test; (F (47,5) =12,250; p<0.0001; Fig 4] in the time spent in the target quadrant during the probe trial of the MWM test when tolterodine groups were evaluated. Tolterodine (0.3, 1 and 3 mg/kg) had no effect on the time spent in the target quadrant in naïve

mice. Scopolamine (0.6 mg/kg) significantly decreased the time spent in the target quadrant (p<0.01) but tolterodine (3 mg/kg) significantly prolonged the time spent in the target quadrant in scopolamine-treated mice (p<0.01) (Fig. 4a).

There was a significant difference between drug groups or their combination [One- way ANOVA post-hoc Tukey's test; F (47,5) =18,502; p<0.0001; Fig 4b] in the mean distance to the platform in the probe trial of the MWM test when tolterodine groups were evaluated. Tolterodine (0.3, 1 and 3 mg/kg) had no effect on the mean distance to the platform in naïve mice. Scopolamine significantly increased the mean distance to the platform (p<0.001). Tolterodine (3 mg/kg) significantly decreased the mean distance to the platform in scopolamine-treated mice (p<0.001) (Fig. 4b).

3.5 Effects of drugs on Locomotor Activity in the Open Field Test

The influence of all the above treatments on the locomotor activity was concurrently evaluated. Neither tolterodine (0.3, 1 and 3 mg/kg) nor other drugs modified the total distance traveled [F(63,7)=0,5777; p>0,05 Fig. 5] in the open field test.

Table 3. Effects of tolterodine on latency (n=8)

Drugs	Latency (s)	Number of mice	p value
Saline	114.03±10.69	8	
Scop 0.6 mg/kg	19.96±1.69	8	p<0.001 (saline & scop)
Tolt 0.3 mg/kg	90.9±28.72	8	p>0.05 (saline & tolt 0.3)
Tolt 1 mg/kg	124.44±35.83	8	p>0.05 (saline & tolt 1)
Tolt 3 mg/kg	147.97±32.62	8	p>0.05 (saline & tolt 3)
Scop 0.6 + tolt 3 mg/kg	85.82±16.36	8	p<0.05 (scop & scop+tolt 3)

p<0.001 compared to Saline group, by ANOVA (Tukey test),
p<0.05 compared to scopolamine group, by ANOVA (Tukey test)
Mean ± S.E.M = Mean values ± Standard error of means

Table 4a. Effects of tolterodine on time spent in target quadrant (n=8)

Drugs	Time Spent in Target Quadrant (s)	Number of mice	p value
Saline	17.75±0.49	8	
Scop 0.6 mg/kg	7.25±1.72	8	p<0.001 (saline & scop)
Tolt 0.3 mg/kg	23±2.81	8	p>0.05 (saline & tolt 0.3)
Tolt 1 mg/kg	24.5±2.19	8	p>0.05 (saline & tolt 1)
Tolt 3 mg/kg	24.75±1.96	8	p>0.05 (saline & tolt 3)
Scop 0.6 + tolt 3 mg/kg	21±1.3	8	p<0.01 (scop & scop+tolt 3)

p<0.001 compared to Saline group, by ANOVA (Tukey test),
p<0.01 compared to scopolamine group, by ANOVA (Tukey test)
Mean ± S.E.M = Mean values ± Standard error of means

Table 4b. Effects of tolterodine on mean distance to platform (n=8)

Drugs	Mean distance to platform (cm)	Number of mice	p value
Saline	42.37±0.53	8	
Scop 0.6 mg/kg	56.62±2.05	8	p<0.001 (saline & scop)
Tolt 0.3 mg/kg	41.875±1.125	8	p>0.05 (saline & tolt 0.3)
Tolt 1 mg/kg	38.5±1.50	8	p>0.05 (saline & tolt 1)
Tolt 3 mg/kg	37.75±2.22	8	p>0.05 (saline & tolt 3)
Scop 0.6 + tolt 3 mg/kg	42.375±1.51	8	p<0.01 (scop & scop+tolt 3)

p<0.001 compared to Saline group, by ANOVA (Tukey test),
p<0.01 compared to scopolamine group, by ANOVA (Tukey test)
Mean ± S.E.M = Mean values ± Standard error of means

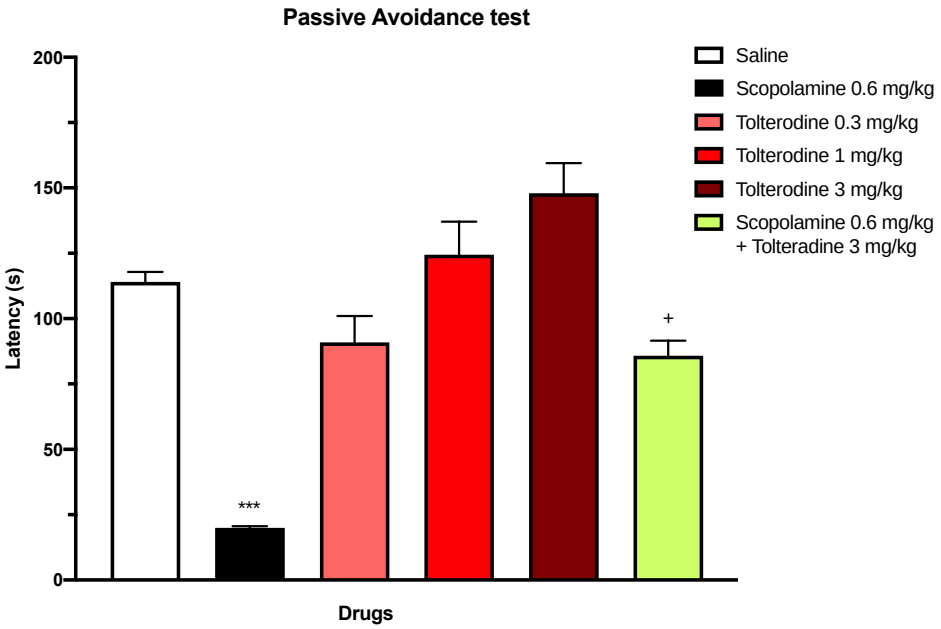

Fig. 3. Effects of tolterodine on latency in passive avoidance test. (n=8)

****: p<0.001 compared to Saline group, by ANOVA (Tukey test), +: p<0.05 compared to Scopolamine group, by ANOVA (Tukey test). Mean ± S.E.M = Mean values ± Standard error of means*

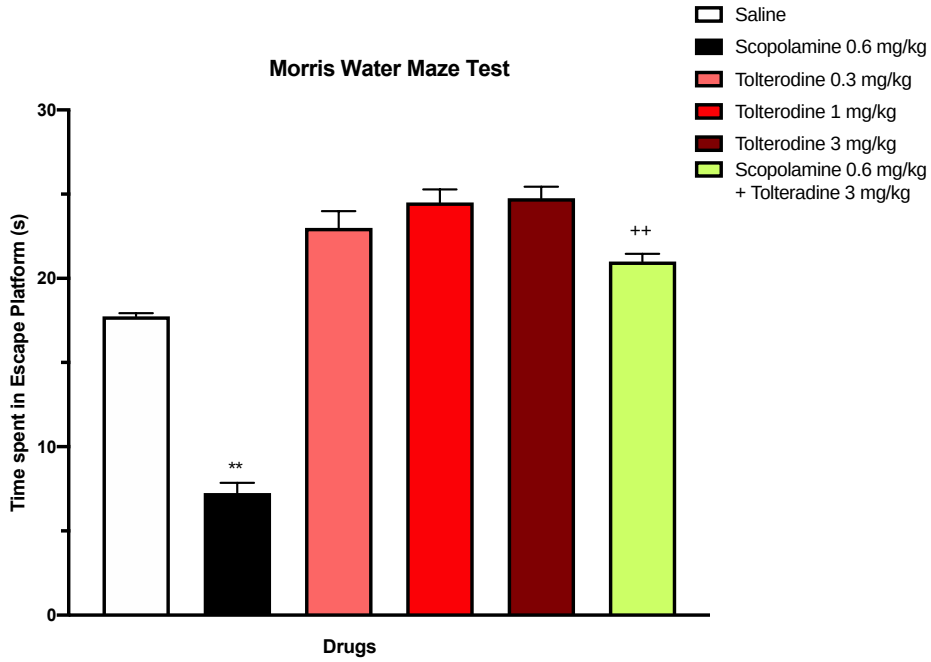

Fig. 4a. Effect of tolterodine on the time spent in target quadrant in the probe trial of Morris water maze test. (n=8)

***: p<0.001 compared to Saline group, by ANOVA (Tukey test),*
++: p<0.01 compared to Scopolamine group, by ANOVA (Tukey test)
Mean ± S.E.M = Mean values ± Standard error of means

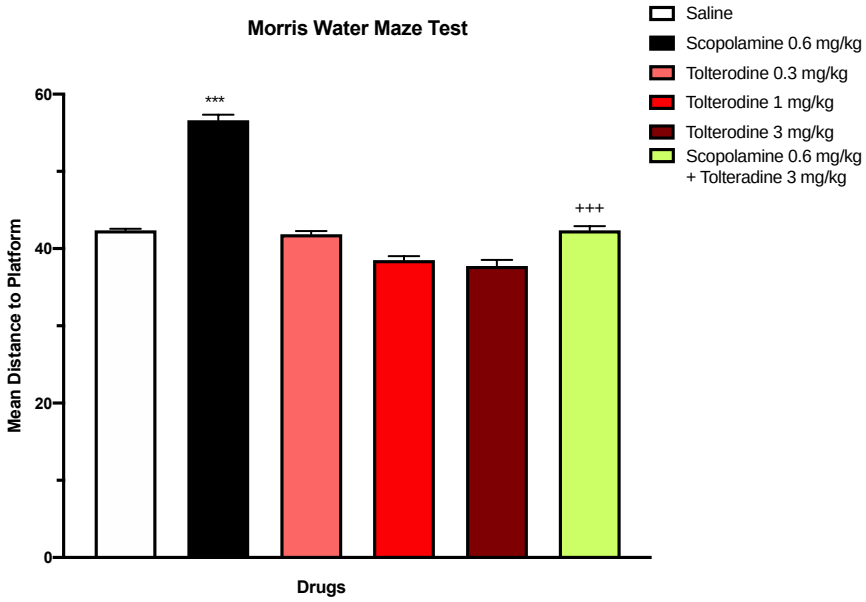

Fig. 4b. Effect of tolterodine on mean distance to platform in the probe trial of Morris water maze test. (n=8)

****: p<0.001 compared to Saline group, by ANOVA (Tukey test),*
+++: p<0.001 compared to Scopolamine group, by ANOVA (Tukey test)
Mean ± S.E.M = Mean values ± Standard error of means

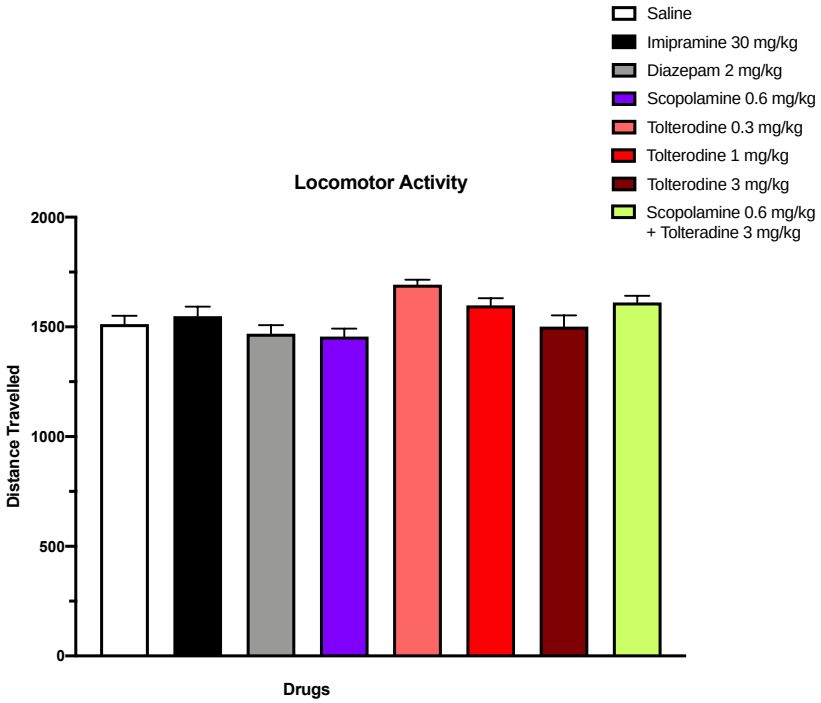

Fig. 5. Effect of drugs on total distance traveled in locomotor activity test. (n=8)

Mean ± S.E.M = Mean values ± Standard error of means

Table 5. Effects of tolterodine on distance travelled (n=8)

Drugs	Distance Travelled (cm)	Number of mice	p value
Saline	1512.62±107.28	8	
Imip 30 mg/kg	1549.12±121.34	8	p>0.05 (saline & imip 30)
Diaze 2 mg/kg	1469±111.03	8	p>0.05 (saline & diaze 2)
Scop 0.6 mg/kg	1456±102.85	8	p>0.05 (saline & scop 0.6)
Tolt 0.3 mg/kg	1692.25±65.33	8	p>0.05 (saline & tolt 0.3)
Tolt 1 mg/kg	1598.87±89.96	8	p>0.05 (saline & tolt 1)
Tolt 3 mg/kg	1501.25±144.66	8	p>0.05 (saline & tolt 3)
Scop 0.6 + tolt 3 mg/kg	1611,37±86.65	8	p>0.05 (saline & scop+tolt 3)

p<0.001 compared to Saline group, by ANOVA (Tukey test),
p<0.01 compared to scopolamine group, by ANOVA (Tukey test)
Mean ± S.E.M = Mean values ± Standard error of means

4. DISCUSSION

OAB is mostly characterized by urgent urinary symptoms that can cause frequent urination, nocturia, and sometimes urinary incontinence [15]. These symptoms greatly impair the person's daily life activities such as sexual function, working life, sleep patterns, and sports activities [16]. Many previous studies have shown that there is a relationship between OAB and depression [17]. It has been observed that the frequency of OAB symptoms such as urge incontinence and nocturia is increased in individuals with high levels of anxiety and depression [18]. The opposite is also true. Especially in patients with urge incontinence, the frequency of depression was found to be higher [19]. It has been understood that fatigue in patients whose sleep is interrupted due to nocturia also predisposes them to depression [20].

The aim of the pharmacological treatment of OAB is primarily to prevent excessive and involuntary contraction of the detrusor [21]. There are 5 types of muscarinic receptors in human bladder smooth muscle, the M2 and M3 receptors being the most abundant [22]. By blocking these muscarinic receptors, it is aimed to prevent excessive and frequent contraction of the detrusor [23]. The first bladder-selective anticholinergic agent developed for the treatment of OAB is tolterodine. Tolterodine is bladder-selective and not selective for any of the 5 muscarinic receptor subtypes. Many in vivo or in vitro experiments have shown that the degree of inhibition of detrusor contraction by tolterodine is much greater than the degree of inhibition of the salivary gland [24]. It produces less dry mouth compared to other anticholinergic drugs, making it an antimuscarinic agent with a higher safety profile than others [25,26].

Tolterodine is metabolized in the liver and its active metabolite is 5 hydroxymethyl. Most of the anticholinergic agents cross the blood-brain barrier and bind to muscarinic receptors in the brain, thus causing dysfunction in the central nervous system [27].

The Forced Swimming Test (FST) is one of the tests we use to determine the level of depression. We use imipramine as a positive control in this test. Imipramine reduces immobility time due to its antidepressant effect. In our study, imipramine and tolterodine (0.3, 1 and 3 mg/kg) significantly shortened the immobility time compared to the saline group. This shows that tolterodine has an antidepressant effect like imipramine. Also, in previous studies they are reported that muscarinic M1 and M2 receptor antagonists showed antidepressant-like effect in animal models in FST [28,29].

Elevated Plus-Maze (EPM) Test is also used to determine the level of anxiety, just like the FST. We use diazepam as a positive control in this test. Diazepam increases the time spent outdoors by decreasing the level of anxiety in rats. In our study, while Tolterodine did not show any effect at the dose of 0.3 mg/kg, it increased the number of transitions to the open area and the time spent in the open area compared to the saline group at 1 mg/kg and 3mg/kg doses. So, tolterodine showed anxiolytic effect like diazepam. In a previous study, muscarinic receptor antagonist such as cyamemazine demonstrated anxiolytic-like activity in the light/dark exploration test [30].

Passive Avoidance (PA) test (in other words, inhibitory avoidance) is one of the simplest and cheapest methods to evaluate learning and memory levels in experimental animals. We use it as a negative control in this test, as scopolamine impairs learning. Compared to the saline group, tolterodine had no effect on the latency period, while tolterodine used at a dose of 3 mg/kg improved the cognitive function impaired by scopolamine. Similarly, in their study, Cappon et al. showed that tolterodine alone did not affect learning and memory levels [31]. Therefore, in the treatment of overactive bladder in diseases such as Alzheimer's, it may be a good alternative to tolterodine as it does not affect cognitive function [32].

The M2 receptors are prevalent in the cortex, basal forebrain, hippocampus, and striatum [33,34,35]. In a previous study they suggested that selective M2 receptor antagonists may be beneficial for cognition [33]. And also in another study, M3 antagonism does not impair cognitive function [36]. Both scopolamine and tolterodine are anticholinergic. However; when tolterodine blocks M2 auto receptors, acetylcholine release increases and it may improve memory by reversing the acetylcholine-reducing and accordingly memory-impairing effect of scopolamine.

Morris Water Maze (MWM) test is used to evaluate memory level in experimental animals. As in the passive avoidance test, we use scopolamine as a negative control in this test. Tolterodine (0.3, 1 and 3 mg/kg) had no effect on time spent in the target quadrant in naive mice. Scopolamine (0.6 mg/kg) significantly reduced time spent in the target quadrant ($s<0.01$), but tolterodine (3 mg/kg) significantly prolonged time spent in the target quadrant ($s<0.01$) in scopolamine-treated mice. Tolterodine (0.3, 1 and 3 mg/kg) had no effect on the mean distance to the platform in naive mice. Scopolamine significantly increased the mean distance to the platform ($s<0.001$). Tolterodine (3 mg/kg) significantly reduced the mean distance to the platform in scopolamine-treated mice ($p<0.001$). An anticholinergic drug scopolamine impaires memory via acetylcholine-reducing effect. But, tolterodine blocks M2 autoreceptors, acetylcholine release increases and this may improve memory.

Open field test is used to evaluate locomotor activity in experimental animals. In this study, neither the other drugs nor the doses of tolterodine had an effect on the total distance traveled by the mice.

5. CONCLUSION

In conclusion, tolterodine prevented experimentally induced depression and anxiety, improved memory and learning of naive animals, and reversed memory and learning impairment with scopolamine. Further preclinical and clinical studies with tolterodine should be done to support all these hypothesis that in the future patients with OAB who need antidepressant and anxiolytic therapy may be treated with single drug instead of more than one drug. We think that the use of tolterodine can reduce the use of antidepressants and anxiolytics, and thus, the use of low-dose antidepressants and anxiolytics can reduce the side effects of these drugs. More preclinical and clinical studies with tolterodine should be conducted to support all these hypotheses.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments have been examined and approved by the appropriate ethics committee. Ethical approval was granted by the Sakarya University Ethics Committee (04.04.2018, Number = 12, Sakarya/Turkey).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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