The Effects of Urtica Dioica Root and Rosa Damascena Mill Petal Extracts on Andropause symptoms and Reproductive Characteristics of Middle-age Male Rats

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Abstract

Background

Andropause starts in middle-aged men and affects hormonal balance and behavioral/sexual functions. The aim of the present study was to determine the effects of two *Rosa Damascena Mill.* (*Rosaceae, Rosa*) and *Urtica dioica* L. (*Urticaceae, Urtica*); in relieving andropause symptoms due to their antioxidant and reproductive properties.

Methods

Animals were allocated into five groups including the young, control vehicle, *Rosa*, *Urtica* and *Rosa + Urtica* groups. Behavioral tests were performed. Sperm parameters and sex hormones were also assessed.

Results

Both extracts, especially in combined form increased preference index and muscle strength and decreased the level of depression significantly. Semen quality increased in the extract-treated groups. Testosterone level was increased significantly in the *Rosa + Urtica* group in middle-aged animals after 50 days of treatment. luteinizing hormone (LH), follicle-stimulating hormone (FSH), and the level of sex hormone binding globulin (SHBG) also changed in the extract-treated groups.

Conclusions

Rosa Damascena Mill and *Urtica dioica* can change testosterone level in the middle-aged animals and also ameliorate andropause symptoms. Mood, muscle strength and cognition would improve following administration of these herbs. The herbal nature of these extracts and their worldwide use in traditional medicine make them more appropriate for human studies and applications.

Introduction

In men, unlike women, age-related changes of sex hormones occur gradually. Andropause, or male menopause, is a result of gradual decline in the male sex hormones, particularly, testosterone (1, 2). Androgens level alterations in elderly men are associated with mental, physical, and sexual health disturbances (3). Testosterone administration can partially ameliorate the adverse effects of andropause, including decreased energy level, decreased skeletal muscle strength, depression, cognitive impairment, and sexual disorders such as decreased libido and erectile dysfunction (1, 2). On the other hand using synthetic testosterone suppresses LH secretion and affects Leydig cells and spermatogenesis (4), enlarges prostate, increases chance of prostate cancer, and cardiovascular disorders.

Accordingly, treatment with natural products is believed to obviate or otherwise, decline the adverse effects of testosterone therapy and can be used as an alternative treatment (1, 5). Researchers suggested herbal

medications can increase fertility and prevent complications such as hormonal imbalance, impotence, prostatitis, varicocele, etc (6). Some examples of these plants are *Urtica dioica*, *Nigella sativa*, Fenugreek (7), ginger, ginseng, *Tribulus terrestris*, and *Rosa Damascena Mill* (8). Among there herbs scopoletin, caffeic acid, fatty acids, flavanonol (9), Lignan and flavonoids in *Urtica dioica* (10) have been shown to increase testosterone levels in young adult rats (11). According to the literature, no study has yet investigated the effects of *Urtica dioica* on middle-age rats, and its probable influence on andropause symptoms.

Rosa Damascena Mill. (*Rosaceae*) is also widely used as an herbal medicine and as a flavoring agent in various industries (8). The petals contain flavonoids, anthocyanins, vitamin C, tannin, terpenes, fatty and organic acids (12). It has antimicrobial, antidiabetic and antioxidant properties that may alter reproductive system, increase male fertility, and improves memory and learning (13) .

Andropause, which affect millions of men worldwide, is on the rise, especially in polluted industrialized societies. On the other hand, since the use of chemical drugs, especially in old age, should be done with caution due to the side effects, the use of effective herbal drugs with less side effects can be a promising way as an alternative. Also, it is valuable to introduce natural products to ensure the protection of Leydig cells and maintain testosterone production to prevent andropause symptoms (14). Therefore, the present study was designed to investigate the possible effects of *Urtica dioica, Rosa Damascena Mill*, and their combination, on cognitive behavior, muscle strength, sex hormone levels and sperm parameters in middle-aged rats as a model of andropause.

Materials and methods

Extract Preparation

Urtica dioica root and *Rosa Damascena Mill.* petals were purchased from a traditional medicine center at Kerman, were identified and approved by a botanist (Dr. M. Mehrabani at Department of Pharmagnosy, Faculty of Pharmacology, Kerman University of Medical Sciences, Kerman, Iran). These specific part of herbs (Pharmacognosy Herbarium voucher at the Faculty of Pharmacology; *Urtica dioica*: KF 1293; *Rosa damascene*: KF 1362) were hot-air-dried, powdered, and were then soaked in 70% ethanol for 72h. The solids in the extract were filtered out, condensed using an rotary evaporator, and the crude extracts were stored at 4 C for future use (8).

Animals and study design

The study was approved by ethics board committee for the care and use of animals at Kerman University of Medical Science, Kerman, Iran (KMU.1396.1523). As andropause starts at middle age, 12-month-old male Wistar rats were used as middle-age model (15, 16). A young group (4-month-old) of rats (young group) was used to compare and approve the characteristics of middle-age animals. The animals were kept in the animal room with a temperature of 23 ± 3 C, a light-dark cycle of 12/12 h and unlimited access to food and water. Testosterone level less than 4 ng/mL was considered as the inclusion criterion for middle-age groups (17). The animals were allocated into the following groups (n = 8): young group without treatment, and the middle-age animals as follows: a control vehicle group receiving 1 ml distilled water; *Rosa* group, receiving 40 mg/kg *Rosa damascena* hydro alcoholic extract (8); *Urtica* group, receiving 50 mg/kg *Urtica dioica* root hydroalcoholic

extract (18); and *Rosa + Urtica* group, received combined 40 mg/kg *Rosa damascena* + 50 mg/kg *Urtica dioica* root hydroalcoholic extracts.

The extracts were dissolved in distilled water on a daily basis, and 1 ml was gavaged for 50 consecutive days. On days 1, 25 and 50, animals were subjected to behavioral tests (see below).

Novel object recognition (NOR) test

The NOR task evaluates the episodic memory. The animals were allowed to explore an empty well-lit plastic cage (40 × 60 × 60 cm) for a “habituation session” of 10 min. Twenty-four h later, the animals were allowed to explore identical objects for 5 min (training session). Forty-five min later, one of the objects was randomly replaced by a novel object and the time spent to explore the novel object (test session) was recorded by a camera for 3 min.

Open Field Test

The animals were placed inside a pre-cleaned 50 × 80 × 80 cm box, with a camera on the box. The animal’s level of locomotor activity and exploration including rearing, grooming and crossing was recorded for 5 min to be analyzed later (19).

Muscle Strength Test

This test was started with the rats hanging from a metallic wire (80 cm wide and 7 mm thick) by their front legs. As the animal began to grab the bar, the latency to fall was recorded using a stopwatch.

Forced swimming Test

Rats were separately immersed in a container (8 × 12 × 25 cm) filled with 25 ºC water for 15 min (habituation session). Twenty-four h later, rats were placed in water for 5 min. Periods of lack of paw movement was considered as immobility time. The immobility episode, as well as climbing time was recorded by a stopwatch. Climbing was defined as active movement of front paw on the tank wall (20).

Blood Sampling

Animals were anesthetized with 50 mg/kg ketamine and 13 mg/kg xylazine at 8 to 9 a.m. Then, blood samples were collected from the retro-orbital sinus of rats and centrifuged at 4000 rpm for 15 min. Serum was stored at -20 C for further analysis.

Measurement of Total and Free Serum Testosterone, LH, SHBG, and FSH Levels

Free testosterone levels were measured by the Chemiluminescent Assay Kit (IBL, Germany) and total testosterone, luteinizing hormone (LH), follicle-stimulating hormone (FSH), and sex hormone binding globulin (SHBG), by the Rat Elisa Kit, according to the manufacturer recommendations (EASTBioPHARM, China)(2).

Sperm Analysis

The right testis was removed and weighted. The vas deferens and epididymis tail were isolated and placed in a plate containing one ml culture medium (human tubal fluid culture medium (HTF) + 12 mg/mL bovine serum

albumin (BSA) + 2.4 mg/mL (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) (HEPES), they were minced using scissors to release the spermatozoa. Samples were kept in a 37 C incubator for 30 min before sperm count, motility and viability were recorded as described elsewhere (21).

Statistical analysis

Statistical analysis was performed by SPSS software version 16 for Windows. Normality of the data was assessed using the Kolmogorov-Smirnov test. One-way analysis of variance (ANOVA) followed by post hoc Bonferroni's test was used where applicable. Repeated measure followed by Bonferroni's test was also used when several measurements at different time points was carried out. Also paired t test was used to compare before and after treatment levels of testosterone in each group. Percentage interpretation was undertaken using chi-squared test. The values were reported as mean ± SEM. Differences at $P < 0.05$ were considered significant.

Results

Novel Object Recognition (NOR) Test

A significant difference ($P < 0.001$) between the young and middle-age groups was detected at day one. NOR score increased in the treatment groups at day 25 reaching a significant level in *Rosa* ($P = 0.033$) and *Rosa + Urtica* ($P = 0.029$) groups compared with the vehicle group. It also increased on day 50 in all treatment groups compared with the vehicle group, and it was highest in the *Rosa + Urtica* group ($P \leq 0.001$). Interestingly, on day 25 and 50 there was no significant difference between the young and any of the middle-age groups under treatment (Fig. 1).

Wire grip test

Muscle strength was significantly ($P \leq 0.05$) weaker in the middle-age rats compared with young group at any time point, while it was comparable in the middle-age groups at day 1, 25 and 50. However, some nonsignificant increase was noted at day 50 in all treated groups compared with the control group (Fig. 2).

Activity in open field box

The number of rearing in vehicle and *Urtica* groups was significantly higher than that of young group ($P \leq 0.05$). This value also decreased in all groups tested at day 50, reaching a significant difference ($P \leq 0.05$) between the vehicle and *Rosa* group. Grooming was also higher in the middle-age groups compared with young group, but comparable among the middle-age groups at day one. It decreased at day 50 and reached a significant difference ($P \leq 0.05$) between *Rosa + Urtica* group compared with the vehicle group. Crossing was comparable among the middle-age groups while it was significantly ($P \leq 0.001$) lower than the young group. At the end of experimentation period crossing value decreased in all groups. Treatment with *Urtica* resulted in a significantly ($P \leq 0.05$) lesser decrease compared with the vehicle group (Table 1).

Table 1
Rearing, grooming and crossing number in open field test in different groups

Group	Rearing (N/S)			Grooming (N/S)			Crossing (N/S)		
Day of measurement	**1**	**25**	**50**	**1**	**25**	**50**	**1**	**25**	**50**
Young	1.43 ± 0.4	1.28 ± 0.7	1.14 ± 0.4	1.86 ± 0.5	1 ± 0.4	1.14 ± 0.5	20.8 ± 1.8	14.7 ± 1.5	15.3 ± 1.3
Vehicle	5 ± 1.1*	3.75 ± 1	2.62 ± 0.6	4.25 ± 0.7	3.62 ± 0.6*	3.37 ± 0.7	13.25 ± 0.6***	5.25 ± 1.2***	6.37 ± 1.5**
Rosa	2.22 ± 0.5	0.22 ± 0.1#	0.44 ± 0.2#	3.67 ± 0.6	2.33 ± 0.5	1.44 ± 0.4	13.63 ± 0.7***	3.12 ± 0.7***	5.87 ± 1.5***
Urtica	2.52 ± 0.9**,	1.66 ± 0.5	1.11 ± 0.5	4.33 ± 0.4	3.22 ± 0.5*	2.78 ± 0.4	13.5 ± 0.8***	10.75 ± 1.7#,	9.25 ± 1.3*
Rosa/Urtica	2.88 ± 0.4	0.55 ± 0.2##	0.78 ± 0.4	4.22 ± 0.6	2.89 ± 0.4	1.11 ± 0.4#	13.13 ± 0.6***	5.12 ± 0.8***, +	5.5 ± 0.9***

Values are mean ± S.E.M. N/S: Number per session. Compared with the young group; * P < 0.05, ** P < 0.01, *** P < 0.001. Compared with the vehicle group; # P < 0.05, ## P < 0.01. Compared with the *Rosa* group; Θ P < 0.05, ΘΘ P < 0.01. Compared with the *Urtica* group; + P < 0.05

Swimming Retention Test

On the first day of treatment immobility time in the middle-age groups was significantly ($P \leq 0.001$) more than the young group, and no significant difference was detected among the middle-age groups. Immobility time in all groups decreased after 50 days, and reached a significant level ($P \leq 0.001$) in all treated groups compared with the vehicle group. The lowest immobility time was observed in *Rosa + Urtica* group which was also significantly ($P \leq 0.001$) better than *Rosa* group. Interestingly, it was close to the value in the young group, without any significant difference. We found that the slope of the immobility, climbing and swimming values changed in a regular pattern. The highest inclination was observed in the *Rosa + Urtica* group followed by *Urtica* group, and the lowest inclination was observed in the young and vehicle groups (Fig. 3). It was true also for climbing index which was higher in *Rosa + Urtica* group followed by *Urtica* group, and the lowest in the vehicle and young groups. At the beginning of the examination, climbing time was comparable in the middle-age groups, while it was significantly ($P \leq 0.01$) lower than young group. Following 50 days treatment it increased in all middle-age groups with a higher significant level in the *Rosa + Urtica* group compared with the vehicle ($P \leq 0.01$) and *Rosa* ($P \leq 0.05$) groups. Swimming time was comparable among the groups at the beginning of the examinations which remained significantly unchanged following 50 days treatment (Table 2).

Table 2
Immobility, climbing and swimming time in force swimming retention test in different groups

Group	Immobility (sec)			Climbing (sec)			Swimming (sec)		
Day of measurement	**1**	**25**	**50**	**1**	**25**	**50**	**1**	**25**	**50**
Young	9.36 ± 1.7	10.3 ± 2.9	2.38 ± 0.5	110.7 ± 9.8	114.4 ± 6.4	99.6 ± 4.4	179.9 ± 10.4	175.3 ± 6.8	198 ± 5
Vehicle	58.8 ± 5.4***	42.45 ± 2.2***	27.2 ± 1.7***	72.51 ± 5***	69.22 ± 5.1**	79.56 ± 5.1	168.7 ± 7.1	188.32 ± 5.6	193.2 ± 4
Rosa	56.95 ± 4.7***	36.9 ± 3.4***	16.35 ± 1.7***, ###	79.23 ± 4.3**	74.38 ± 2.8**	91.67 ± 4.9	163.81 ± 3.7	188.71 ± 5.6	191.98 ± 4.8
Urtica	53.53 ± 5***	25.56 ± 2.7**, ###,	10.64 ± 2.1*, ###	58.35 ± 2***	99.41 ± 6.1#	106.32 ± 9.5	188.12 ± 5.8	175.03 ± 6.6	183.03 ± 9.7
Rosa + Urtica	51.9 ± 4.5***	20.11 ± 3###,	4.93 ± 1.1###,	72.31 ± 4.2***	117.4 ± 11.1###,	123.23 ± 7.6##,	175.8 ± 5.3	162.49 ± 10.8	171.84 ± 7.4

Values are mean ± S.E.M. Swimming time was calculated as: total time - (immobility time + climbing time). Compared with the young group; * P < 0.05, ** P < 0.01, *** P < 0.001. Compared with the vehicle group; # P < 0.05, ## P < 0.01, ### P < 0.001. Compared with the *Rosa* group; Θ P < 0.05, ΘΘ P < 0.01, ΘΘΘ P < 0.001.

Body weight, testis weight and gonadosomatic index

The body weight of the animals among the treated groups was comparable, and higher than the young group as it was anticipated. Testes weight was also comparable among different groups. Neither the body weight nor the testis weight was significantly different among the groups on each day of experiments.

However, gonadosomatic index ([testis weight/ body weight] × 100) was significantly lower in the middle-age groups than the young group (P < 0.001) and did not improve following treatments (Table 3).

Table 3
Body weight, testis weight and gonado-somatic index in the different groups

	B.W of day 1	B.W of day 25	B.W of day 50	Testes weight	GSI
Young	225.14 ± 11	248.14 ± 9.5	255.85 ± 9.6	1.41 ± 0.01	0.55 ± 0.03
Vehicle	332.25 ± 9.8	331.25 ± 9.7	331.25 ± 10.8	1.41 ± 0.03	0.42 ± 0.01***
Rosa	330.89 ± 9.8	327.11 ± 26.5	327.33 ± 10.5	1.41 ± 0.1	0.43 ± 0.02***
Urtica	334.28 ± 9.4	336.44 ± 9.7	337.55 ± 9.1	1.37 ± 0.1	0.40 ± 0.01***
Rosa + Urtica	343.75 ± 8.6	341.37 ± 28.7	338.25 ± 11	1.4 ± 0.1	0.42 ± 0.01***

Gonado-somatic index was calculated as: testis weight/ body weight × 100. Values are mean ± S.E.M., B.W: Body weight, GSI: Gonado-Somatic Index. Compared with the young group; *** P < 0.001

Sex hormones

At the beginning of the study, testosterone level of the middle-age animals was 2.56 ± 0.29, and in the young group it was 4.1 ± 0.4(P = 0.033). It was also comparable among the middle-age groups. By the end of the treatment period, the testosterone level increased significantly in the *Rosa + Urtica* compared with the vehicle and *Rosa* groups (P < 0.01) (Fig. 4). Nonetheless, comparison of the vehicle group at the beginning and at the end of the study revealed a significant (P < 0.01) constant decline in the untreated condition. Furthermore, the testosterone level at the beginning and at the end of the treatment period indicated a greater significant decline in the *Rosa* group (P < 0.001) compared with the vehicle group (Table 4). The level of free testosterone in the young animals was significantly higher than the vehicle group (P = 0.013). Free testosterone increased nonsignificantly in the *Urtica* and *Rosa + Urtica* groups at the end of the treatment period (Table 5). Totally, the values of LH, SHBG and FSH were higher in the *Rosa + Urtica* group but no significant difference was detected among the groups (Table 5).

Table 4
Changes in the total testosterone levels in different groups before and after treatment

group	Mean	t-value	P-value
Young	Before 4.15 ± 0.05	-2.45	0.092
	After 4.25 ± 0.1		
Vehicle	Before 2.57 ± 0.3	10.42	< 0.001
	After 1.5 ± 0.4		
Rosa	Before 2.56 ± 0.3	6.1	< 0.001
	After 1.19 ± 0.1		
Urtica	Before 2.6 ± 0.3	0.046	0.964
	After 2.57 ± 0.5		
Rosa + Urtica	Before 2.65 ± 0.3	-1.53	0.169
	After 3.47 ± 0.4		

The data was analyzed by paired t test. Significant level is 0.05 (mean ± S.E.M.).

Table 5
Free testosterone, SHBG, LH and FSH concentration in serum of different groups after 50 days of treatment

	Free testosterone (ng/ml)	SHBG (ng/l)	LH (mIU/ml)	FSH (mIU/ml)
Young	17.55 ± 2.9	87.5 ± 7.8	15 ± 0.6	14.3 ± 1.4
Vehicle	1.13 ± 0.5*	75 ± 17	11.94 ± 0.8	10.66 ± 1
Rosa	0.66 ± 0.1*	90.9 ± 10.4	12.94 ± 0.5	13.03 ± 0.6
Urtica	1.54 ± 0.8*	108.6 ± 8.3	15.98 ± 2.3	14.5 ± 1.6
Rosa + Urtica	1.97 ± 0.5	104 ± 3.9	16.11 ± 2.4	12.36 ± 0.9

The data for free testosterone and LH were analyzed by Kruskal Wallis test. Values are mean ± S.E.M., F.T: Free testosterone, SHBG: Sex hormone binding globulin, LH: Luteinizing hormone, FSH: Follicle stimulating hormone. Compared with the young group; * P < 0.05

Sperm number, motility and viability

A significant (P = 0.022) decline was observed in the sperm count of vehicle group compared with the young group. It increased in all treated groups to a higher level following 50 days treatment than that the vehicle group, but it did not reach a significant level (Table 6).

Table 6
Sperm count, motility and viability in different groups after 50 days treatment

	Count (×106)	Motility (%)	Viability (%)
Young	56.34 ± 3.2	54.25 ± 2.7	54.5 ± 2.2
Vehicle	16.08 ± 1.01*	20 ± 1***	24 ± 1.7***
Rosa	20.42 ± 3.6	25.8 ± 2.9***	33.22 ± 3.9***
Urtica	21.38 ± 4.6	28.16 ± 2.6***	29.06 ± 1.7***
Rosa + Urtica	18.78 ± 4.6*	29.81 ± 5.1**	31.87 ± 2***

Data related to sperm count were analyzed by Kruskal Wallis test. Values are mean ± S.E.M. Compared with the young group; * P < 0.05, ** P < 0.01, *** P < 0.001.

Motility and viability were also significantly lower in the middle-age vehicle group compared with the young group (P < 0.001). Treatment of middle-age animals with either of the extracts and their combination increased motility and viability to a higher level than that of the vehicle group but the difference was not statistically significant (Table 6).

Discussion

Andropause usually appears gradually in middle-aged people and affects many aspects of life, including mood, libido, sperm count, sperm motility, muscle strength, depression level, memory and learning, which are most likely associated with change in the level of androgens. We were able to successfully demonstrate the positive effects of *Urtica* root and *Rosa* petals extracts in reducing andropautic symptoms. Although not significantly different, overall, combination of these herbs did not reduce the protective potential of each herb against andropause signs and symptoms, especially anxiety, muscle strength and sperm parameters, suggesting the probable synergistic effect of these herbs. A closer examination of the results indicates that the combination therapy of *Urtica* and *Rosa* extracts significantly increases testosterone level, while testosterone secretion reduces after the administration of *Rosa* alone; the decrease in testosterone level is probably due to the phytoestrogenic compounds in *Rosa*, which can affect testosterone level (8). However, the improvement of sperm parameters in *Rosa*-treated animals may be due to high antioxidant levels in *Rosa* extract, which requires further research. Contrary to our findings in middle-age rats, an Increase in the level of testosterone, FSH and LH in young rats has been reported following the administration of *Rosa* extract (22). Whether the age of the animals or the design of the experiments could have affected the outcome is not clearly known, but the strong phytoesterogenic properties of the *Rosa* should be considered when analyzing the outcomes. Moradi et al and Chrubasil et al worked on *Urtica* root extract and reported that this extract inhibited the activity of 5a-reductase, thereby preventing the conversion of testosterone to dihydrotestosterone (9, 10). Comparing the level of free testosterone in the young animals with the middle-age animals indicates that free testosterone levels decrease with ageing. Comparable level of free testosterone between *Urtica* and vehicle group along with the increase in SHBG and total testosterone level indicates that *Urtica* by having 5a-reductase inhibitor activity increases the level of testosterone and, on the other hand, phytoestrogens in the *Urtica* root stimulate production of SHBG in the liver (23).

Spermatogenesis, the sperm count and sperm with normal motility, will be affected in middle-aged and elderly people (24). Mammalian spermatozoa contain unsaturated fatty acids, which are very sensitive to free ROS. Excess ROS will reduce sperm motility (25). In our study, although we did not analyze ROS levels in seminal plasma, sperm count, mobility, and viability decreased significantly with age. In contrast, administration of *Urtica* resulted in a significant increase in sperm motility, which indicates the protective role of *Urtica*. This finding is consistent with the results of previous studies (25). Our results are close to the results of Jalili which investigated the effect of *Urtica* extract on the damage caused by nicotine on sperm and hormonal parameters (11). Also, our results confirm the Inic et al findings that *Urtica* can be used as an antioxidant agent against oxidative stress (26).

A gradual change in muscle mass and strength, as well as a decrease in memory, learning and motivation are common disorders that occur after the onset of andropause. In our study rats that received *Rosa + Urtica* combined treatment showed higher muscle strength in the swimming retention test, and stronger memory and learning ability in NOR test. Testosterone improves skeletal muscle performance and can increase physical ability (2). The higher level of testosterone in *Urtica* and *Rosa + Urtica* groups and the presence of antioxidant compounds in the *Urtica* and *Rosa* could have reduced sarcopenia (27) and increased muscle strength in middle-age male rats. Higher level of testosterone increases the synthesis of myofibril proteins and also affects muscle strength (28). Whether greater muscle strength in the treated animals could solely be attributed to the rise in testosterone level, requires further studies.

Testosterone can reduce anxiety and improve depression, as well as increase quality of life in men with hypogonadism (29). Decreased androgenic hormones can affect cognitive function through various mechanisms. Changes in cognitive memory may be due to the interaction between the dopamine system and the hormone testosterone, which is involved in cognitive memory. Suppression of testosterone secretion can also reduce memory and motility (30). This can explain increased mobility value in *Urtica* and *Rosa + Urtica* groups, as well as the increase in the recognition index in the *Rosa + Urtica* group.

The flavonoids and kaempferol in *Rosa damascene* mimic imipramine, which is an anti-depressant drug (31). In our study, *Rosa* administration alone or in combination with *Urtica* decreased the immobilization time. This effect is comparable to imipramine, which inhibits the absorption of monoamines. Studies on rodents have shown that orchiectomy reduces monoamines in the brain and leads the animal to passive behavior. Testosterone boasts central dopaminergic metabolism in male rats (32). Reports indicate that flavonoids and kaempferols have an inhibitory effect on the monoamine oxidase enzyme. As a result, it can be suggested that flavonoids and kaempferols in *Rosa* are attributed to the anti-depressant properties of this plant (31). Increase in the crossing index in the *Urtica* group is consistent with previous findings that *Urtica's* quercetin has anti-anxiety and anti-depressant properties (33).

One of the interesting outcomes of the current study was the use of the normal andropause model (elderly animals with low serum testosterone levels) compared to the orchiectomy model and sex cells depression model of andropause, that can be easily extended to middle-aged males. Another advantage of this model is that all animals are certainly exposed to andropause at the same time, which is important in animal experimentations. Our study has some limitations such as the inability to examine animals' fecundity. In

addition, administrating testosterone to a positive control group as a common treatment in andropause is of value.

Conclusion

We conclude that *Rosa Damascena Mill*. and *Urtica dioica* can affect andropause complications by direct or indirect mechanisms. In addition to the testosterone alteration, the useful compounds of these plants can also directly affect the symptoms of non-sexual signs of andropause. These novel findings could be used as a solution to reduce andropause complications. The herbal nature of these extracts and their common use in traditional medicine make them more appropriate for future human applications.

Declarations

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Declaration of interest statement

The authors declare that there is not any conflict of interest regarding publication of present manuscript.

Ethical Approval and Consent to participate

The study was approved by ethics board committee for the care and use of animals at Kerman University of Medical Science, Kerman, Iran (Approval code, KMU.1396.1523).

Availability of supporting data

Supporting data will be available upon written request to the corresponding author.

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Authors' contributions

Conceptualization of the study: SNNM. Study design: MAE, TH and SNNM. Performed the experiments: MAE, MRA, Contributed reagents/materials/analytical tools: MAE, TH, SNNM and VM. Statistical analysis: MAE and TH. Wrote the first draft: MAE and VM, Edited the manuscript: TH, VM and SNNM. Approved the submission for publication: MAE, TH, MRA, VM and SNNM

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Figures

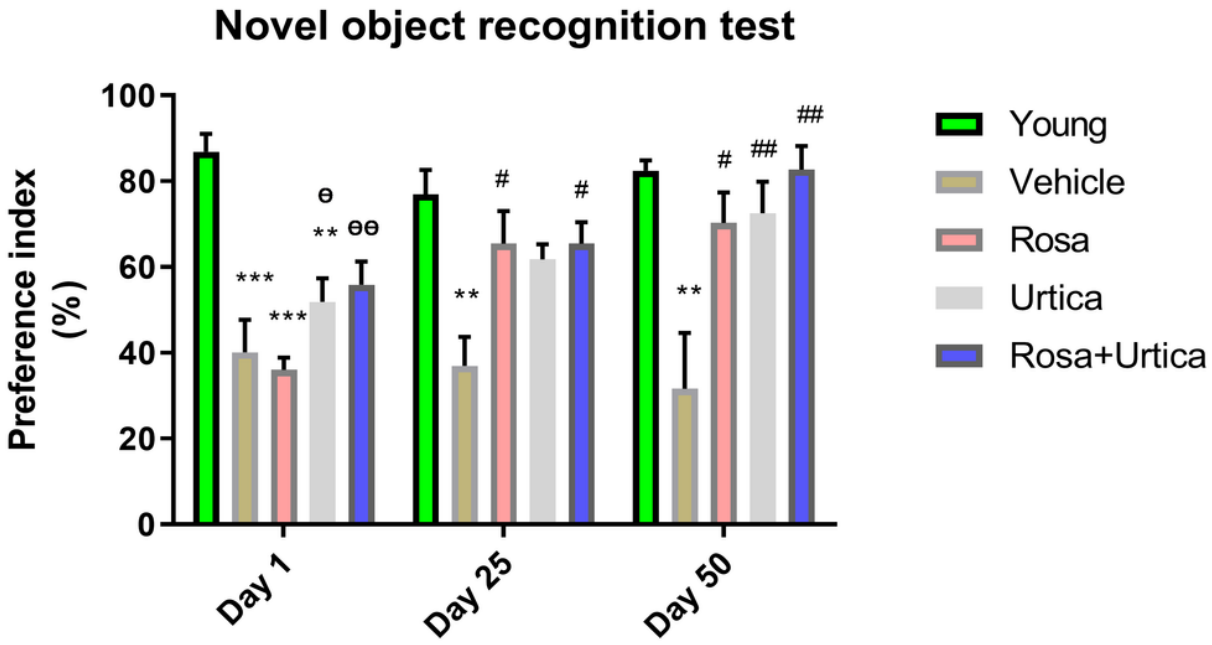

Figure 1

Preference index in novel object recognition test in different groups. Preference index was calculated as: time of attention to new object/ total time of attention to old and new object ×100 (mean± S.E.M.). Compared with the young group; ** P<0.01, *** P<0.001, Compared with the vehicle group; # P<0.05, ## P<0.01, compared with the *Rosa* group; Θ P< 0.05, ΘΘ P<0.01.

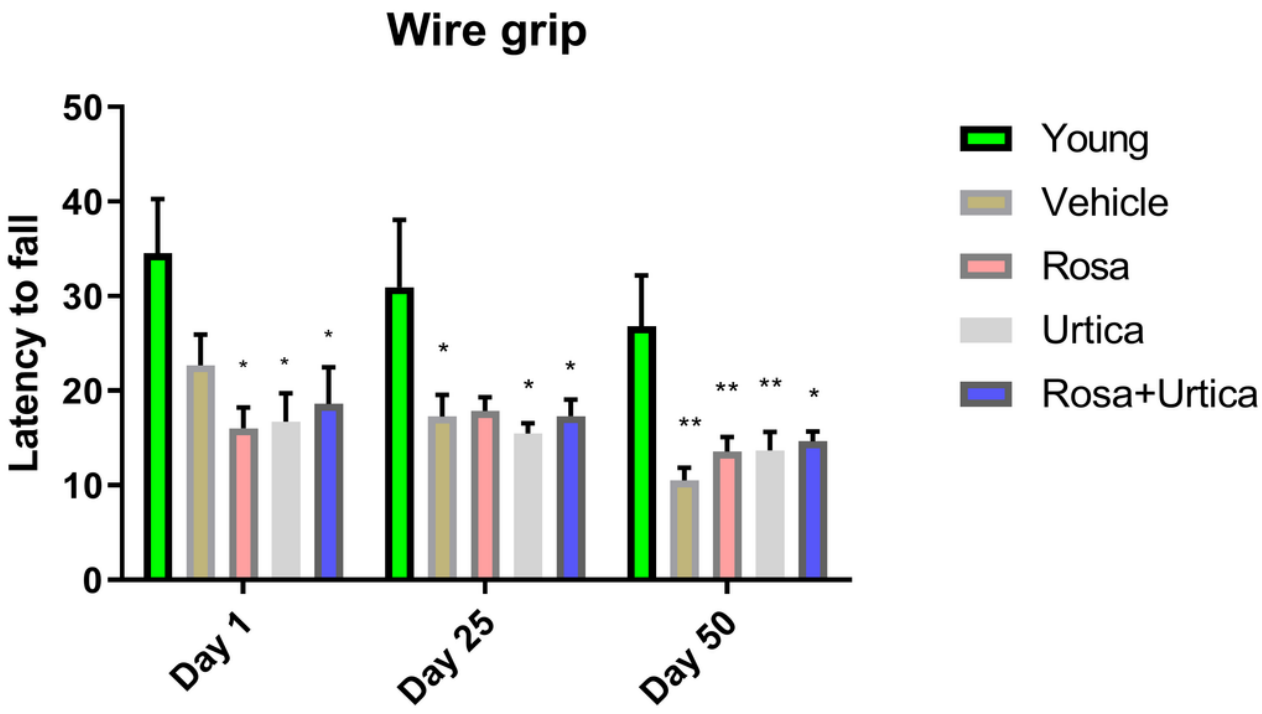

Figure 2

Latency time to fall in wire grip test in different groups. (mean± S.E.M.).

Compared with the young group; * P<0.05, ** P<0.01.

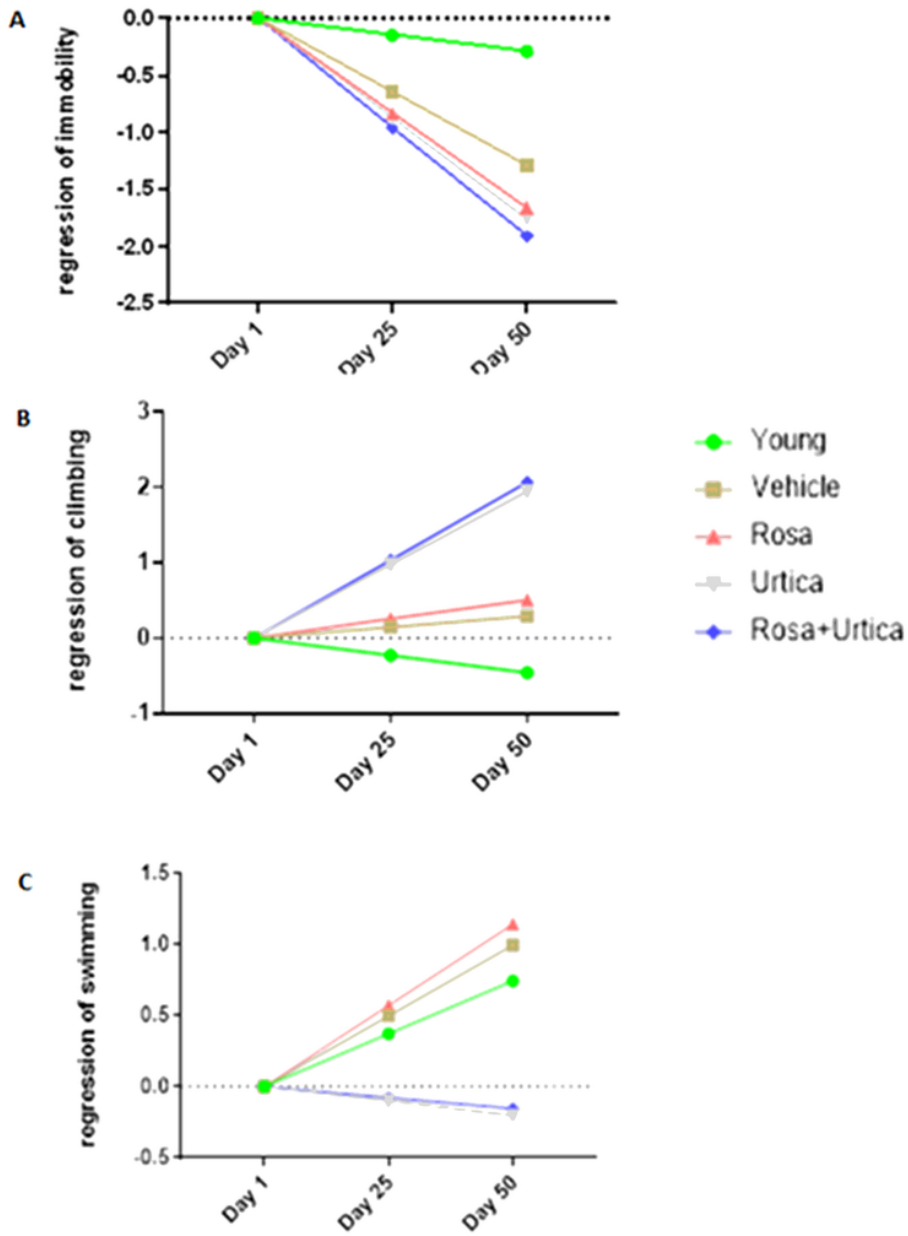

Figure 3

A, B, C Trend of forced swimming retention test in different groups at immobility, climbing and swimming time. Slopes in different groups; 3A; Young: -0.143, Vehicle: -0.644, *Rosa*: -0.828, *Urtica*: 0.873, *Rosa+Urtica*: -0.956. 3B; Young: -0.229, Vehicle: 0.145, *Rosa*: 0.257, *Urtica*: 0.972, *Rosa+Urtica*: 1.034. 3C; Young: 0.372, Vehicle: 0.498, *Rosa*: 0.571, *Urtica*: -0.101, *Rosa+Urtica*: -0.077. Analyzed by repeated measure test followed by Bonferroni's test.

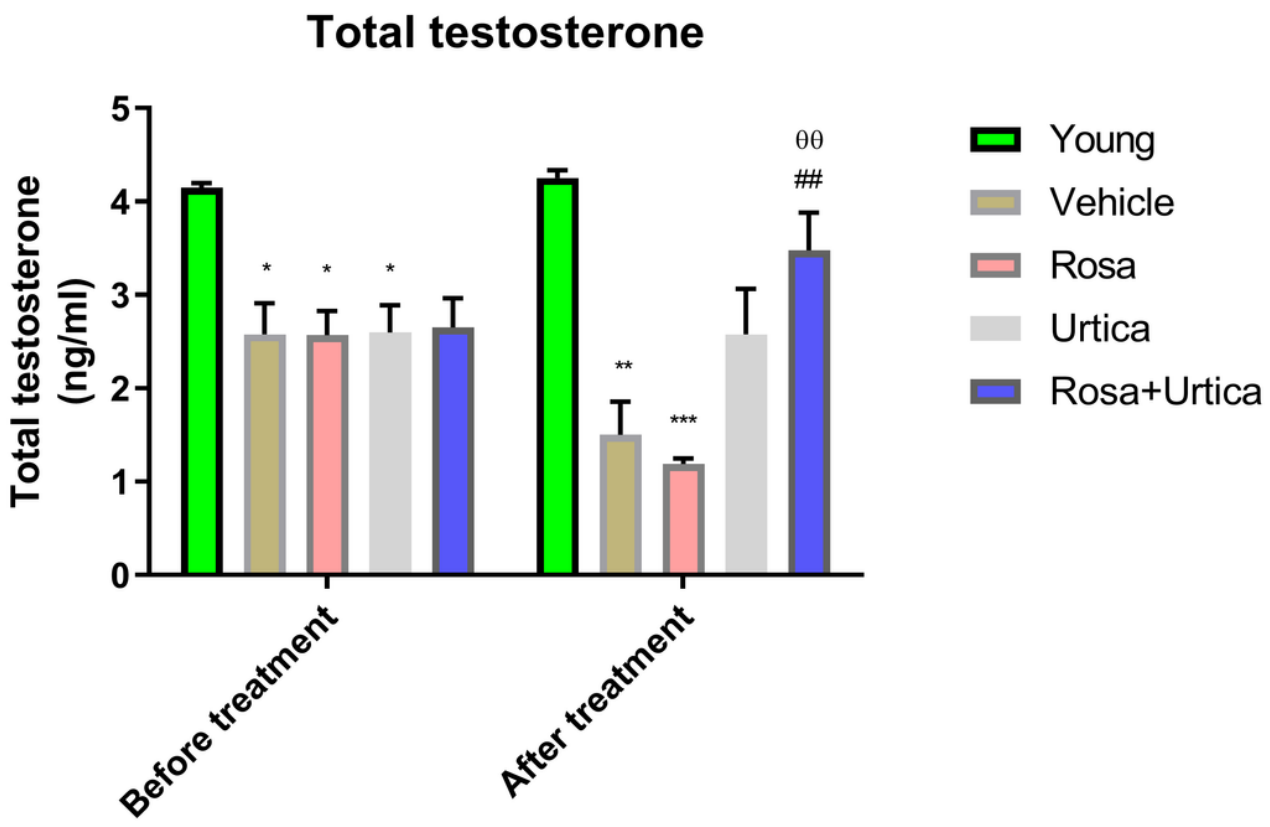

Figure 4

Total testosterone in serum of different groups before and after 50 days of treatment period. (mean± S.E.M.). Compared with the young group; * P<0.05, ** P<0.01, *** P<0.001. Compared with the vehicle group; ## P<0.01. Compared with the *Rosa* group; ΘΘ P<0.01.