

## The Effect of *Gundelia Tournefortii* Hydro-Alcoholic Extract on Sperm Motility and Testosterone Serum Concentration in Mice

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Article information	Abstract
<p>Article history: Received: 20 Non 2011 Accepted: 15 Jan 2012 Available online: 30 Dec 2012 ZJRMS 2013; 15(8): 18-21</p> <p>Keywords: <i>Gundelia tournefortii</i> extract Sperm Testosterone Male mice</p> <p>*Corresponding author at: Department of Biology, Payam-e-noor university, Iran E-mail: s_nasri1@pnu.ac.ir</p>	<p><b>Background:</b> <i>Gundelia tournefortii</i> was used as a food and medicinal in human life from past time. The present study has been investigated the effects of <i>Gundelia tournefortii</i> on number and motility of sperms and testosterone serum concentration.</p> <p><b>Materials and Methods:</b> In this experimental study, male mice were divided into control and 4 experimental groups (<i>Gundelia tournefortii</i> extract at doses of 100, 200, 400, 800 mg/kg was administered intraperitoneally for 14 days). One week after the last injection, blood samples were collected for hormonal assay. Also weight of testes, motility rate and number of sperms were assessed. Data analysis was performed using one-way ANOVA followed by Tukey test.</p> <p><b>Results:</b> Studies showed the number of sperms increased significantly at dose of 400 mg/kg (<math>2.88 \pm 0.4580</math>) (<math>p=0.001</math>). The percentage of sperm motility (<math>p=0.001</math>) and the testicular weight (<math>p=0.012</math>) significantly increased at doses of 200 (<math>36.83 \pm 1.2506</math>) and 400 mg/kg (<math>57.51 \pm 2.1113</math>) (<math>p=0.001</math>). Testosterone serum level significantly increased at doses of 100 (<math>p=0.05</math>), 200 (<math>p=0.05</math>) and 400 mg/kg (<math>p=0.001</math>) as compared to control group.</p> <p><b>Conclusion:</b> <i>Gundelia tournefortii</i> extract increases the number, motility of sperm and testosterone level because of antioxidant components for example Quercetin presumably.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

### Introduction

From the beginning of human creation, infertility was medical and social problem that endanger psychological health of individual and society. In order to resolve this problem some researches done. Today infertility that formerly was in superstitions, divine punishment and unscientific domain change into scientific subject and now is in the field biology and is in seeking and strange subject [1]. *Acanthus* with scientific name: *Gundelia tournefortii* is in Chicory family. *Acanthus* is permanent vegetable covered with wool hair down and lots of blade its stem is thick, simple or branching with short branch and in the form of corymb [2].

*Gundelia tournefortii* family grows in Mediterranean area and Middle East and in most part of Iran. In traditional medicine this vegetable is mentioned for vitiligo, diabetes, epilepsy and stomach and intestine disease. Nooraei has written in his book that this plant increase libido and contain plants hormones that they reinforce sexual libido [2- 6].

Results show that this vegetable has antioxidant component that lead to many therapy effects [7]. Another research in Iran proves the protection effect of this vegetable on liver cells [8]. *Gundelia tournefortii* increases lipid level of plasma [9]. *Gundelia tournefortii* contain phenol compound such as Quercetin, this substance has strong antioxidant effects [10, 11].

Antioxidant components protect sperm cells from free radicals and improve quality of sperm. Antioxidant therapy protects against oxidative stress and improves fertility parameters [12, 13].

According to antioxidant effect of *Gundelia tournefortii* and the fact that it wasn't any research about the effect of *Gundelia tournefortii* on sperm parameters and sexual hormones, this research study effect of this vegetable on sperm parameters and level of testosterone hormone.

### Materials and Methods

This study is experimental study and done in 2010-11 in Payam-e-Noor University (East Tehran branch laboratory). Seventy five mice collected in Iran Pastor Institute and their average weight was  $25 \pm 5$ . They divided accidentally in 3 experimental groups and one control group that in every group 15 mice examined. Animal were kept in animal room on a 12 hours light and 12 hours darkness cycle,  $22 \pm 2^\circ\text{C}$  temperature and free access to water and food. All of experiment were conducted in accordance with the principles of laboratory animal care. In this research leaf and stem of *Gundelia tournefortii* were used and collected from Anjedan area (Markazi province) and identified by pharmacy university of Tehran university with voucher number 6721-THE. Leaf

and stem were dried in standard situation (without light, infection and humidity), then extract with the method of percolation. Solvent evaporate by vacuum in 40°C [14, 15].

Selected dose of leaf and stem was according to lethal dose (LD50) of extract that was calculated by 6.28 g/kg of animal weight. Experimental dosage was assigned 100, 200, 400, 800 mg/kg. Control group received normal saline [14].

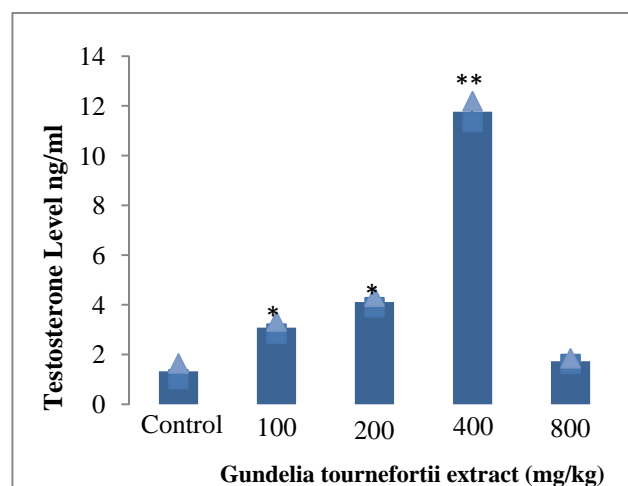
Experimental Groups received one dose of extract that solve in 1 ml normal saline for 14 days peritoneally. One week after last injection mice unconscious by ether, then by opening their abdomen bleed from their liver vein (because of blood mass and simple availability) [16]. Blood sample during 20 minutes centrifuge with 3000 round per minute and serum separated and send to laboratory in order to measuring testosterone hormone in blood serum with RIA method by testosterone kit (Kavoshyar company) [16]. Then mice were killed by cutting spinal cord and their epididymis and testis removed and weight testis. Their epididymis washed in normal saline for cleaning from blood. Epididymis tissue was sliced into 2 ml normal saline and volume of normal saline added to 5 ml and mixed 10 minutes and then incubated in 37°C temperature. Average of normal sperm number was assigned. For that one drop of sample (sample mass is 5 ml that include epididymis sperm) was putted on hemocytometer and counted on squares of white blood cell of hemocytometer. Then achieved number multiply in  $10^6$  (epididymis/ $\times 10^6$ ) in order to reach to whole sperms [17].

Ten microliter of solution (Sample mass is 5 ml that include epididymis sperm) put on microscope slide in order to examined sperm movement percent. In order to achieve sperm movement percent, we observed 10 microscopic squares on glass slide and then total average of moving sperms study in 10 point of view of microscope squares in the shape of percent of movement was studied [17]. Results of movement power, whole number of sperms, testis weight, testosterone concentration were compared between experimental and control group by using SPSS-17 software and ANOVA

test and following post test of Tukey.  $p < 0.05$  was considered statistically significant.

## Results

Comparison of number of sperms in experimental and control group showed meaningful increase in third group (400 mg/kg) ( $p=0.001$ ) (Table 1). According to statistical analysis sperm movement percent in second (200 mg/kg) and third (400 mg/kg) experimental group in comparison with control group had significant increase ( $p=0.001$ ) (Table 1). Average of testis weight had meaningful increase in second group (200 mg/kg) ( $p=0.012$ ) and third (400 mg/kg) ( $p=0.014$ ) (Table 1). Concentration of testosterone serum in control group was  $1.47 \pm 0.3160$  and in first experimental group was  $3.08 \pm 0.2450$  ( $p=0.05$ ) and in second experimental group was  $4.42 \pm 0.2101$  ( $p=0.05$ ) and in third experimental group was  $11.76 \pm 0.4179$  ( $p=0.001$ ) and in fourth experimental group was  $1.73 \pm 0.1118$ . According to statistical analysis first, second and third group in comparison with control group had meaningful increase (Fig. 1).



**Figure 1.** The effect of different doses of *Gundelia tournefortii* extract on plasma levels of testosterone of control and experimental groups in the mice

\*The mean difference is significant at the 0.01 level

\*\*The mean difference is significant at the 0.05 level

**Table 1.** The effect of different doses of *Gundelia tournefortii* extract on weight of testes, motility rate and number of sperms of control and experimental groups in the mice

Parameters		Control Group	Group 1 (100 mg/kg)	Group 2 (200 mg/kg)	Group 3 (400 mg/kg)	Group 4 (800 mg/kg)
Testicular weight Mean±SD		0.0170± 0.07	0.0077±0.33	0.0165±0.09**	0.0112±0.09**	0.0162±0.08
CI95%	Lower bound		-0.0288	-0.0347	-0.0351	-0.0221
	Upper bound		0.0002	-0.0055	-0.0059	0.0070
Number of sperm×10 <sup>6</sup> Mean±SD		0.58±1.91	0.50±2.43	0.34±2.50	0.45±2.88***	0.30±1.97
CI95%	Lower bound		1.173-	-1.236	-1.623	-0.7113
	Upper bound		0.1238	0.0613	-0.3261	0.5863
Motility rate of sperm Mean±SD		3.33±29.14	1.98±29.80	1.25±36.83***	2.11±57.51***	1.85±27.27
CI95%	Lower bound		-3.847	-10.873	-31.554	-1.310
	Upper bound		2.519	-4.506	-25.187	5.055

\*\*\*The mean difference is significant at the 0.001 level

\*\*The mean difference is significant at the 0.01 level

## Discussion

Results from this experimental study for the first time show the positive effect of *Gundelia tournefortii* hydro alcoholic extract on sperm parameters and testosterone concentration. Most effect dose is 400 mg/kg in compared to control group. In different study, phenolic compounds such as Quercetin refined from *Gundelia tournefortii* extract [10]. Quercetin is the most numerous natural flavonoid in vegetable and plant, but it is a group of flavonoid without glycoside. Flavonoids without glycoside more powerful antioxidants related to their glycoside flavonoid [18-20]. Other study shows the powerful antioxidant effect of Quercetin [11]. So *Gundelia tournefortii* has high capacity of antioxidant [7]. Oxidative stress, lipid peroxidation and change in membrane characteristics cause death of generative cell in different stages of growth and cause decrease of sperm amount and peroxide of hydrogen is able to immobile sperms. Antioxidant could protect sperms by free radical and improve quality of sperms [12, 21, 22]. Antioxidant therapy, is a protection defense against oxidative stress and improving fertility parameters [13]. Quercetin that is available in *Gundelia tournefortii* has protective effect on spermatogonia Cells under oxidative stress and by giving electron to active oxygen types (ROS) decrease destruction DNA [23, 24]. Quercetin decreases destructive effect of 2, 3, 7, 8 tetra chlorodibenzo-p-dioxin (TCDD) on testis tissue and sperm parameters and testosterone hormones [25]. Also this substance has anti-apoptosis effect that protect mice's testis from poisonous effect of cadmium [26].

Care of diabetic mice with Quercetin increase sperm population, ability to living and sperm movement percent because of decrease ROS level in serum [27]. So according to these reasons it is possible to increase number and movement of sperms via antioxidant effect of quercetin of *Gundelia tournefortii*. Increasing of testis weight probably is because of Quercetin, this increasing maybe is because of developing of seminifer tube or increasing number of cells such as spermatozoid in testis [28].

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Antioxidant not only protect lipids peroxidation and prevent oxidative stress of testis but also play important role in production of steroid in testis [29]. Positive role of Quercetin on testosterone level of men is proof and it is clear that Quercetin increase level of testosterone in men [30, 31]. Aktoz demonstrated increasing of testosterone on rats with varicocele [32]. Meaningful increase in testosterone in first experimental group (100 mg/kg) and second group (200 mg/kg) and third (400 mg/kg) could result from Quercetin in *Gundelia tournefortii*. Quercetin in *Gundelia tournefortii* could decrease production of nitric acid, this compound (NO) could inhibit production of steroid. So extract of *Gundelia tournefortii* could increase steroid production in leydig cells of testis and so increase testosterone concentration [32, 33].

Result of this study show that probable properties of antioxidant of leaf and stem of *Gundelia tournefortii* on sperm parameter has protective effect against free radical and help improve sperm parameter situation especially number and movement of sperms and could increase testis weight and testosterone hormone. This vegetable is native in our country and we can use that for increasing fertility of males. Result limitation of this research is related to seasonal collection of this vegetable and also complete research on human. In addition effect of different fraction of this vegetable prepare and study their effect on Pituitary-Gonadal axis and spermatogenesis.

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## Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

## Conflict of Interest

The authors declare no conflict of interest.

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