

## Original Research Article

# Inhibitory effect of *Taraxacum officinale* L (Compositae) aqueous root extract on spermatogenesis

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## Abstract

**Purpose:** To investigate if *T. officinale* root aqueous extract has anti-spermatogenic activity similar to that of the whole plant which was shown previously to inhibit spermatogenesis.

**Methods:** *T. officinale* aqueous extract was prepared by soaking 100 g of dried materials in 1 L distilled water for two days at 45 °C. Fifty adult male rats were divided into five groups and treated for 60 days. Four groups were gavaged with the whole plant or root aqueous extract in low or high doses. The male rat rats were allowed to mate with female rats. The control group received distilled water. Sperm count, motility and morphology as well as chromatin integrity were evaluated.

**Results:** Serum testosterone level, sperm parameters, pregnancy rate and average number of fetuses per pregnant females decreased significantly in the treated groups compared to control and in the root-receiving rats compared to the whole plant-receiving rats. Female rats which were mated with high dose root-receiving males did not deliver fetuses. Cross sections of seminiferous tubules of *T. officinale*-treated rats showed lesions and disorganized germinal epithelium. Late spermatogenesis maturation arrest (spermatid stage) was observed in all of the treated groups except the high dose root-receiving group which showed early maturation arrest (spermatocyte stage). In addition, the mRNA level of two spermatogonial stem cell markers responsible for self-renewal and proliferation of spermatogonia increased in high dose-receiving rats.

**Conclusion:** *T. officinale* root aqueous extract has inhibitory effects on spermatogenesis. Further studies are required to identify specific ingredient(s) in *T. officinale* that may be useful as male contraceptive(s).

**Keywords:** *Taraxacum officinale*, Dandelion, GDNF family receptor alpha 1, Macrophage Colony-Stimulating Factor, Proliferation-Inducing Myeloid Cell Protein-2, Testosterone, Sperm count

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## INTRODUCTION

Treatment of disease began long ago with the use of herbs [1]. *Taraxacum officinale* (L.) Weber ex F.H. Wigg. (Compositae) is used in folk medicine as an anti-inflammatory, anti-oxidative, anti-carcinogenic and anti-coagulatory remedy [2]. Other health-promoting activities such as diuretic, laxative, cholagogue, anti-rheumatic,

choloretic and hypoglycaemic have been attributed to the use of *T. officinale* [2].

In Jordan it is also used to enhance male fertility by enhancing sperm parameters such as concentration, motility and normal morphology. However, it has been proven recently that *T. officinale* whole plant or leaves aqueous extracts decrease male fertility instead of improving it by

causing an imbalance between spermatogonia self-renewal and differentiation [3,4].

Spermatogonial stem cells (SSCs), which are located near the basement membrane of the seminiferous tubule [5], are responsible for the maintenance of spermatogenesis in males and are capable of producing mature sperms [6].

SSC self-renewal and differentiation are controlled by several markers [7]. Glial Cell-Derived Neurotrophic Factor (GDNF) is essential for self-renewal and proliferation of SSCs [8]. GDNF works through a multi-component receptor complex composed of GDNF family receptor alpha 1 (GFR $\alpha$ -1) and Ret (tyrosine kinase transmembrane protein) [9].

Promyelocytic Leukaemia Zinc-Finger (PLZF) expression is restricted to the undifferentiated spermatogonia and it prevents SSCs differentiation [10]. Macrophage Colony-Stimulating Factor (MCSF) is another stem cell marker secreted by the interstitial Leydig and myoid cells; its effect is restricted to undifferentiated spermatogonia [11].

The current work was conducted to investigate if *T. officinale* root aqueous extract has anti-spermatogenic activity similar to that of the whole plant. In addition, the work aimed at studying the effects of the root aqueous extract on GFR $\alpha$ -1, PLZF and MCSF spermatogonial stem cells (SSC) markers.

## EXPERIMENTAL

### Plant collection and extract preparation

*T. officinale* was collected during the flowering season (February - October) of 2014 from Jubaiha region, Amman, Jordan. The plant was authenticated by a plant taxonomist at Raja'a Abu Eideh; The Hashemite University) and a voucher specimen (no. HU-42741) was deposited in the herbarium of the Department of Biology and Biotechnology, The Hashemite University, Zarqa, Jordan. The aqueous extract was prepared by soaking 100 g of air dried whole plant or root in 1 L of distilled water for two days at 45 °C. Afterwards, the mixture was filtrated by Whatman filter paper and then lyophilized [3,12].

### Screening of chemical constituents of *T. officinale*

The determination of the chemical composition of *T. officinale* was performed by high performance liquid chromatography coupled with mass spectrometer (HPLC-MS). Briefly, 0.1 mg of each

extract was dissolved in 1 ml of 20 % acetonitrile in water. Prepared samples were homogenized and centrifuged to avoid column blocking. Samples were introduced to HPLC-MS and photodiode array detector (Finigan Surveyor PDA Plus Detector, Thermo Scientific, USA). RP/C18 column (150 mm  $\times$  4.6 mm) at a flow rate of 1 ml/min was used. HPLC-MS spectra were collected for the extracts and compared to the data found in literature. As such, assignment of the chemical composition was possible only for those compounds which were already extracted and characterized for *T. officinale*.

### Animal treatment

Fifty adult Wistar male rats (*Rattus norvegicus*) weighing between 150 and 200 g were randomly selected and housed individually in cages. The animals were maintained under standard conditions; tap water and feed were provided *ad libitum*. After one week of acclimatization [12], the rats were randomly divided into five different groups, each of ten animals. The animals of the first and second groups were gavaged with 1/20 and 1/10 of *T. officinale* whole plant aqueous extract LD<sub>50</sub> and were considered the low dose whole plant receiving-group (LDWP) and the high dose whole plant-receiving-group (HDWP), respectively [3]. Rats of the third and fourth groups received 1/20 (3.68 g/kg) and 1/20 (1.84 g/kg) of the root aqueous extract LD<sub>50</sub> and were considered the low dose root-receiving group (LDR) and the high dose root-receiving group (HDR), respectively. The fifth group of animals received distilled water and was considered the control group. The doses were given orally using a gavage needle for 60 consecutive days [12].

### Fertility test

On the 55<sup>th</sup> day of treatment, each male rat was cohabitated with two adult pro-estrus female rats. The presence of a typical mating plug and/or sperms in the vaginal smear were considered indicators of successful mating. The number, weight and sex of the offspring were determined [12].

### Male rat sacrifice

Male rats were sacrificed by cervical dislocation, and the body weight was recorded. Rats were dissected and liver, testes, kidneys and seminal vesicles were removed and weighed. Relative organ weight was calculated by dividing the absolute organ weight (g) by the body weight of rat on sacrifice day (g) multiplied by 100. Blood was collected by heart puncture in a plain blood tube and centrifuged at 2000 rpm for 10 min.

## Sperm collection

Sperm samples were collected from cauda epididymis based on the method of Soleimanzadeh and Saberivand [13]. The samples were used to study sperm count, motility and morphology [14]. In addition, epididymal sperm samples were used to study chromatin integrity by acridine orange staining [15] and agarose gel electrophoresis. For acridine orange staining, two slides were prepared for each rat and at least two hundred sperms for each slide were counted [15].

## Testosterone assay

Serum testosterone level was evaluated using the Immulite 1000 immunoassay system (Siemens, Germany). Briefly, serum from control or treated rats and alkaline phosphatase-conjugated testosterone were incubated in a test tube containing polystyrene beads coated with testosterone-specific antibodies. Serum testosterone competed with the enzyme-labeled testosterone for the limited number of antibody binding sites on the beads. A chemiluminescence substrate was added to the test tube where it was hydrolyzed by the alkaline phosphatase and the absorbance was measured spectrophotometrically (Siemens, Germany).

## Histological studies

One of the testes was cut into small pieces (5 × 5 mm) and fixed in Bouin's fixative. The samples were then dehydrated in ascending alcohol series and cleared in xylene. The tissues were imbedded in pure paraffin wax and serial sections of 6 μm were made and stained with hematoxylin and eosin. Finally, the sections were cleared and mounted in DPX and then were examined by Nikon Eclipse 50i microscope.

## RNA extraction and quantitative real-time polymerase chain reaction (qRT-PCR)

For quantifying mRNA level of each SSC marker [11,16], quantitative real-time polymerase chain reaction (qRT-PCR) on Line Gene 9680 BioGR thermal cycler (Bioer Technology, USA) was carried out [17]. The SV Total RNA Isolation System (Promega, USA) was used for testicular RNA extraction and cDNA was prepared using Power cDNA synthesis kit (iNtRON Biotechnology). The quantification of the SSC markers (*mcsf*, *gfra1*, *plzf*) and *β-actin* (as a house keeping gene) was performed using the 2x Universal KAPA SYBR FAST qPCR Kit Master Mix (KAPA BIOSYSTEMS, USA). Each qRT-PCR was run as triplicate for each primer.

## Ethical approval

The study was approved by the Institute Review Board (IRB) of The Hashemite University (AM/16/10/222/140115). Animal care, handling, and the animal experiments performed were approved by The Hashemite University Institutional Animal Care and Use Committee which conforms to the guidelines of National Research Council of the National Academics [18].

## Statistical analysis

Statistical analysis of data was performed using STATISTICA 7 analysis program (StatSoft Inc., OK, USA). In order to determine differences between three or more means, one-way ANOVA with Fisher's LSD for multiple comparisons post-tests were performed. Data are presented as mean ± SD and the level of significance was set at  $p < 0.05$ .

## RESULTS

### Phytochemical constituents of *T. officinale*

HPLC-MS was used to detect the chemical compounds present in *T. officinale* aqueous extracts. Various compounds were detected in the root aqueous extract including phenols (chlorogenic acid and chicoric acid), sesquiterpenes (tetrahydroidentin B, taraxacolid-O-β glucopyranoside, ainslioside and 11β-13-dihydrolactucin), triterpene (taraxasterol and α and β amyirin) and coumarins (scopoletin and esculetin). The whole plant aqueous extract contained all of the above mentioned compounds in addition to hydroxycinnamic acid (caffeic acid) and monocaffeoyltartaric acid (phenols), luteolin 7-O-glucoside, quercetin 7-O-glucoside, luteolin 7-O-rutinoside and luteolin 7-diglucosides (flavonoid glycosides), ixerin D, taraxinic acid β-D-glucopyranoside and 11,13-dihydrotaraxinic acid (sesquiterpenes), and cichoriin and aesculin (coumarins).

### Body and organ weight of male rats

A significant decrease in body weight of the HDR-receiving rats was observed when compared to the control and whole plant-receiving rats (Table 1). In addition, there was a significant decrease in testis relative weight of the HDWP-; LDR- and HDR-receiving rats when compared to the control rats, and of the root-receiving rats when compared to the whole plant-receiving rats (Table 1). Similarly, the relative weight of the seminal vesicle showed a

significant decrease in all of the treated groups when compared to control group, and in the root-receiving groups when compared to the LDWP-receiving group (Table 1). The relative weights of the liver and the kidney did not show a significant change in any of the treated groups as compared to the control (Table 1).

### Serum testosterone level

*T. officinale* aqueous extract administration caused a significant decrease in serum testosterone level in all of the treated groups [LDWP:  $1.6 \pm 0.4$  ng/mL ( $p < 0.05$ ); HDWP:  $1.4 \pm 0.3$  ng/mL ( $p < 0.05$ ); LDR:  $0.9 \pm 0.1$  ng/mL ( $p < 0.01$ ); HDL:  $0.3 \pm 0.1$  ng/mL ( $p < 0.001$ )] when compared to the control group ( $3.7 \pm 1.2$  ng/mL), in the root-receiving groups when compared to the whole plant-receiving groups ( $p < 0.05$  for LDR and  $p < 0.01$  for HDR), and in HDR-receiving group when compared to LDR-receiving group ( $p < 0.05$ ).

### Sperm concentration, motility and morphology

Sperm concentration and progressive motility in all of *T. officinale*-treated groups decreased significantly when compared to the control, and in the root-receiving groups as compared to the whole plant-receiving groups (Table 2). A rat sperm with normal morphology has an elongated tail composed of midpiece, principal piece and

end piece, with intact hook-shaped head (Figure 1a, b). The percentage of sperm morphological abnormalities increased significantly in the treated groups when compared to the control group, and in the root-receiving groups when compared to the whole plant-receiving groups (Table 2).

### Sperm chromatin integrity

Sperm samples from cauda epididymis of the control and treated rats were stained with acridine orange. The results did not show DNA damage in either the control or treated samples as all sperms fluoresced green after excitation (Figure 1c). A single intact band was observed in all of the lanes (control and treated groups), while the ladder pattern of DNA, which is an indication of fragmented DNA, was not observed (Figure 1d).

### Effect of *T. officinale* on pregnancy parameters

A significant decrease was observed in the pregnancy rate and the average number of fetuses/pregnant females in all of the treated groups as compared to the control group, and in the root-receiving groups when compared to the whole plant-receiving groups (Table 2). None of the female rats that were mated with the HDR-receiving males delivered fetuses after the regular pregnancy period (21 days) (Table 2).

**Table 1:** Body weight gain (g) and relative organ weight (g) of control and treated rats (mean  $\pm$  SD, n = 10)

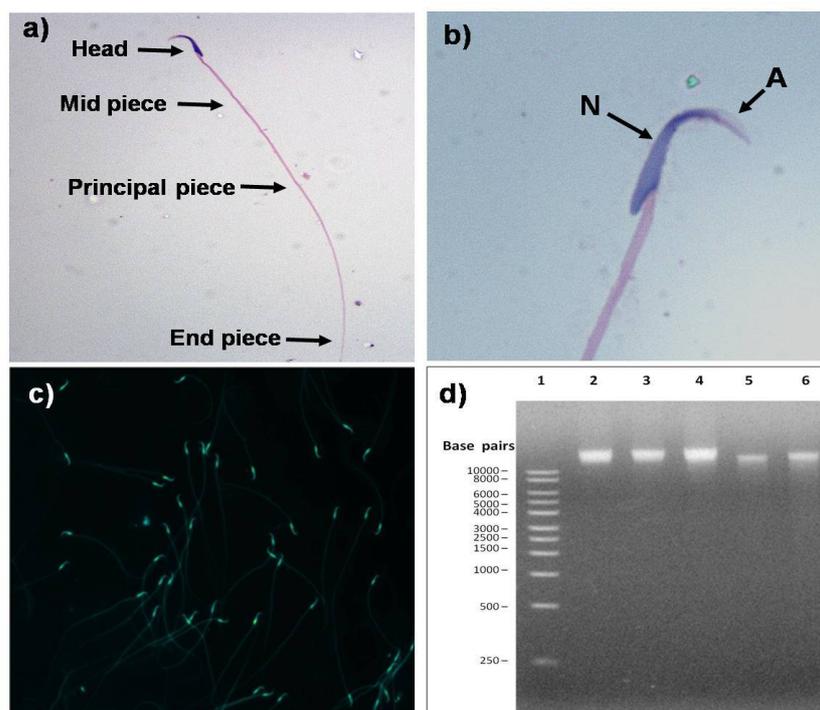
Group	Weight gain	Testes	Seminal vesicle	Liver	Kidney
Control	76.6 $\pm$ 19.8	0.69	0.32	4.7	0.37
LDWP	97.9 $\pm$ 13.5	0.70	0.23 <sup>a2</sup>	4.7	0.35
HDWP	80.6 $\pm$ 28.6	0.66 <sup>ab</sup>	0.19 <sup>a3</sup>	4.6	0.34
LDR	73.7 $\pm$ 28.7	0.51 <sup>a2b2c</sup>	0.11 <sup>a3b2</sup>	4.6	0.32
HDR	46.3 $\pm$ 23.9 <sup>ab3c</sup>	0.5 <sup>a2b2c</sup>	0.10 <sup>a3b3</sup>	4.6	0.33

*a*  $p < 0.05$ ; *a*<sup>2</sup>  $p < 0.01$ ; *a*<sup>3</sup>  $p < 0.001$  versus control, *b*  $p < 0.05$ ; *b*<sup>2</sup>  $p < 0.01$ ; *b*<sup>3</sup>  $p < 0.001$  versus LDWP, *c*  $p < 0.05$  versus HDWP

**Table 2:** Effect of *T. officinale* treatment on sperm parameters (mean  $\pm$  SD, n = 10)

Group	Concentration ( $10^6$ /ml)	Motility (%)	Morphological abnormality (%)	Pregnancy rate (%)	No. of fetus
Control	81.0 $\pm$ 8.9	67.6 $\pm$ 8.7	2.7 $\pm$ 0.9	90.0	8.3
LDWP	40.8 $\pm$ 8.3 <sup>a3d2e</sup>	26.7 $\pm$ 4.4 <sup>a3d2e</sup>	19.7 $\pm$ 5.2 <sup>a3c3d3e3</sup>	75.0 <sup>ade</sup>	6.7 <sup>ade3</sup>
HDWP	40.9 $\pm$ 4.4 <sup>a3de</sup>	30.9 $\pm$ 12.4 <sup>a2de</sup>	38.6 $\pm$ 8.9 <sup>a3b3de3</sup>	60.0 <sup>ade</sup>	5.9 <sup>ade3</sup>
LDR	25.6 $\pm$ 4.0 <sup>a3b3c2e</sup>	15.4 $\pm$ 3.6 <sup>a3b2</sup>	41.7 $\pm$ 9.0 <sup>a3b3e3</sup>	45.0 <sup>a2bc</sup>	3.8 <sup>a2bc</sup>
HDR	14.7 $\pm$ 8.1 <sup>a3b3c3d</sup>	7.8 $\pm$ 5.6 <sup>a3b3c2</sup>	60.9 $\pm$ 13.7 <sup>a3b3c3d3</sup>	40.0 <sup>a2bc</sup>	0 <sup>a3b3c3d2</sup>

*a*  $P < 0.05$ ; *a*<sup>2</sup>  $p < 0.01$ ; *a*<sup>3</sup>  $p < 0.001$  versus control, *b*  $p < 0.05$ ; *b*<sup>2</sup>  $p < 0.01$ ; *b*<sup>3</sup>  $p < 0.001$  versus LDWP, *c*  $p < 0.05$ ; *c*<sup>2</sup>  $p < 0.01$ ; *c*<sup>3</sup>  $p < 0.001$  versus HDWP, *d*  $p < 0.05$ ; *d*<sup>2</sup>  $p < 0.01$ ; *d*<sup>3</sup>  $p < 0.001$  versus LDR, *e*  $p < 0.05$ ; *e*<sup>3</sup>  $p < 0.001$  versus HDR



**Figure 1:** Effect of *T. officinale* on sperm parameters. **(a)** Normal rat sperm showing the hooked head and tail; **(b)** Sperm head showing the acrosome (A) and nucleus (N); **(c)** Evaluation of chromatin integrity by acridine orange staining. **(d)** Representative agarose gel electrophoresis analysis of sperm DNA of both the control and treated rats. Lane 1: DNA molecular weight marker; lane 2: Control; lane 3: LDWP; lane 4: HDWP; lane 5: LDR; lane 6: HDR

### Effect of *T. officinale* on rat testicular histology

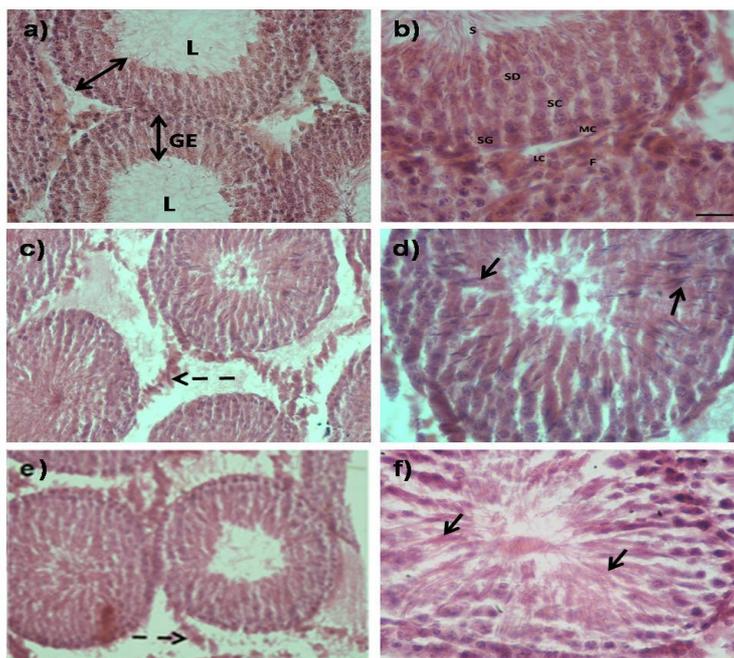
Microscopic examination of testicular cross sections of control rats showed normal histology (Figure 2a, b). The germinal epithelium (GE) was intact and the lumen (L) was wide and full of sperms (Figure 2a). In addition, different stages of sperm development were noticed (Figure 2b). Cells of the interstitial tissue such as Leydig cells (LC), myoid cells (MC) and fibroblasts (F) were also observed (Figure 2b).

However, testicular sections of LDWP-receiving group showed late maturation arrest (spermatid stage) (Figure 2c, d; arrow) in most of the seminiferous tubules (ST) with a decreased number of sperms in the lumen and reduction in interstitial tissues (dashed arrow). HDWP-receiving group testicular sections showed reduction in ST numbers, most of which showed late maturation arrest, disorganized germinal epithelium and a reduction in the interstitial tissue (dashed arrow) (Figure 2e, f). The LDR-receiving group ST cross sections showed late maturation arrest (arrows) with a reduction in germ cell number (reduced germinal epithelium thickness) and interstitial cells (dashed arrows) (Figure 3c, d). On the other hand, cross sections prepared from seminiferous tubules of HDR-receiving rats

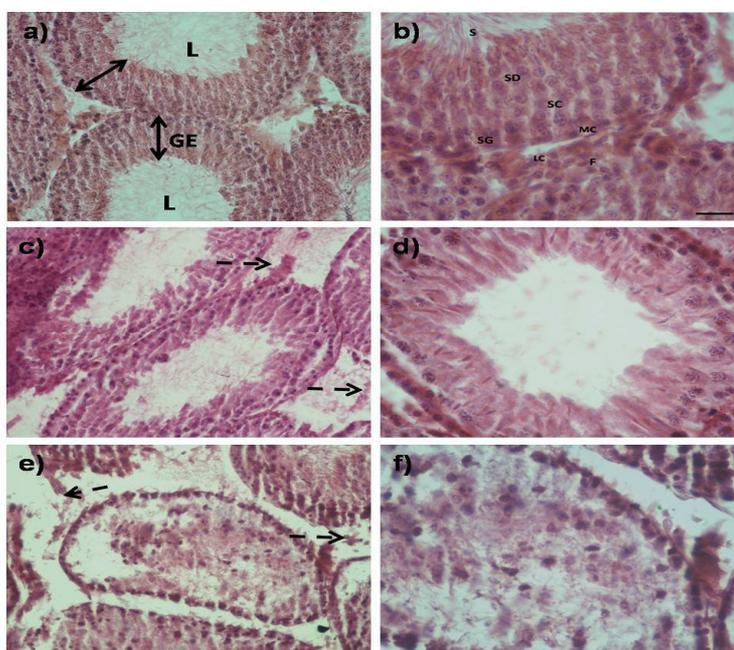
showed sever disruption in ST architecture and large spaces between germ cells. In addition, early maturation arrest (spermatocyte stage) and germ cell hypoplasia were observed with almost a complete absence of interstitial tissue (dashed arrows). One striking observation in the HDR-receiving group was the absence of a clear lumen (Figure 3e, f).

### Gene expression of SSC markers

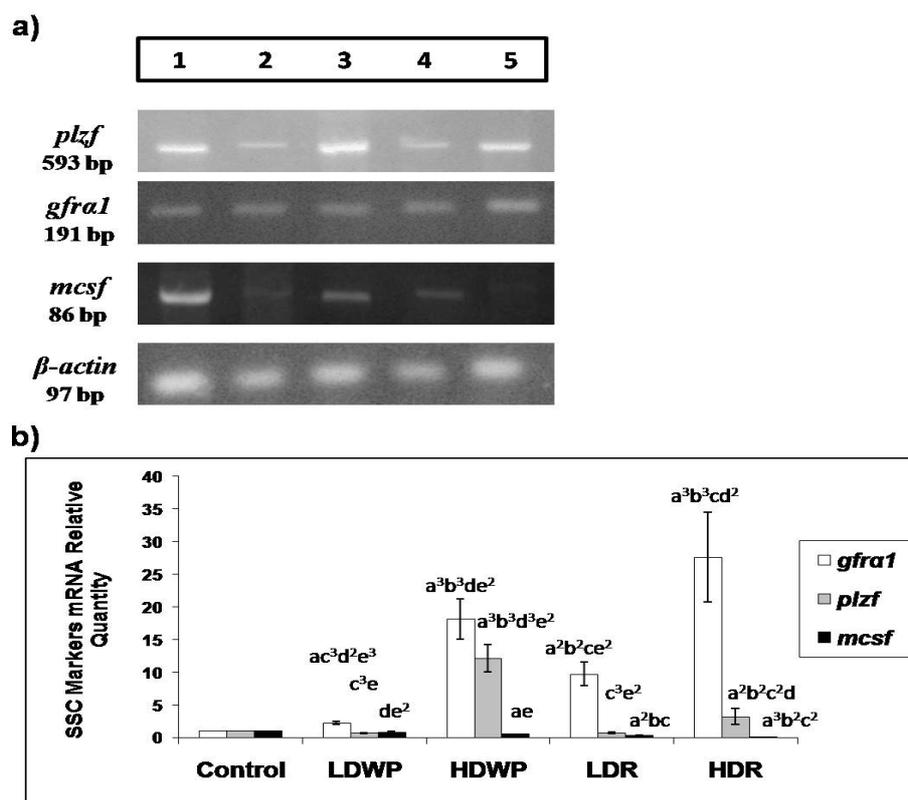
Three spermatogonial stem cell (SSC) self-renewal and proliferation genes were analyzed by qRT-PCR (Figure 4a). The mRNA levels of these markers (*gfra1*, *mcsf* and *plzf*) were normalized to  $\beta$ -actin. The level of *gfra1* mRNA increased significantly in all the treated groups when compared to the control, and in the HD-receiving groups when compared to the LD-receiving groups (Figure 4b). *plzf* mRNA level increased significantly in the HD-receiving groups when compared to the control and the LD-receiving groups, and in the HDWP-receiving group when compared to the HDR-receiving group (Figure 4b). On the other hand, *mcsf* mRNA level decreased significantly in all treated groups (except the LDWP-receiving group) when compared to the control group, and in the root-receiving groups when compared to the whole plant-receiving groups (Figure 4b).



**Figure 2:** Cross-sections of seminiferous tubules (ST) of whole plant-receiving rats. **(a-b)** Cross sections of ST of control rats showing intact spermatogenic (germinal) epithelium (GE) and lumen (L). Different spermatogenic cells: spermatogonia (SG), spermatocyte (SC), spermatid (SD) and sperm (S) were observed. Different cells of the interstitial tissue were also evident: myoid cells (MC), Leydig cells (LC) and fibroblasts (F). **(c-d)** Cross sections of ST of LDWP-receiving rats showing late maturation arrest (spermatid stage; arrows), reduction in lumen diameter and interstitial tissue (dashed arrow). **(e-f)** Cross sections of ST of HDWP-receiving rats showing disruption of GE organization, late maturation arrest (arrows) and reduction in interstitial tissue (dashed arrow). Scale bar = 50  $\mu$ m



**Figure 3:** Cross-sections of seminiferous tubules (ST) of root-receiving rats. **(a-b)** Cross sections of ST of control rats. **(c-d)** Cross sections of ST of LDR-receiving rats showing reduction in germinal epithelium (GE) and interstitial tissue (dashed arrow), and late maturation arrest. **(e-f)** Cross sections of ST of HDR-receiving rats showing severe disorganization in the architecture of the GE, the interstitial tissue was almost absent from all sections (dashed arrow), several spaces scattered among the spermatogenic cells were observed, and early maturation arrest was evident with large distribution of spermatogonia in ST. Scale bar = 50  $\mu$ m



**Figure 4:** qRT-PCR analysis of SSC markers in control and treated groups. **(a)** Representative agarose gel electrophoresis of qRT-PCR products of control and treated rats, lane 1: Control; lane 2: LDWP; lane 3: HDWP; lane 4: LDR; lane 5: HDR. **(b)** Relative quantity of *gfra1*, *plzf* and *mcsf* mRNA in treated groups in comparison to the control group, values were normalized to  $\beta$ -actin. Values are expressed as mean  $\pm$  SD, n = 10 in each experiment, three independent experiments

## DISCUSSION

*Taraxacum officinale* has been used in folk medicine for its different medicinal properties [2]. In addition to its known health benefits [1,2], *T. officinale* whole plant decoction has been traditionally used in Jordan to enhance sperm parameters such as concentration and motility [13]. However, recent studies have proved that the whole plant or leaves aqueous extracts decrease male fertility instead of improving it [3,4]. Thus, we aimed to investigate the effects *T. officinale* root aqueous extract on fertility parameters and spermatogonial stem cell (SSC) markers by comparing it to the whole plant aqueous extract.

*T. officinale* treatment caused a significant decrease in testis and seminal vesicle relative weights in treated groups (Table 1). Similar results were observed when male rats were treated with *T. officinale* whole plant or leaves aqueous extracts [3,4] and *Echinops echinatus* (Compositae) roots [19]. The decrease observed was attributed to a reduction in testosterone level.

Testosterone regulates spermatogenesis, sperm differentiation and induces the development of male accessory reproductive organs [20]. Thus, any irregularities in testosterone production and concentration would have detrimental effects on spermatogenesis. *T. officinale* aqueous extract caused a significant decrease in serum testosterone level in all the treated groups when compared to the control group and in the root-receiving rats when compared to the whole plant-receiving rats. This is correlated with the decrease in the interstitial tissue (the source of testosterone) observed in testicular sections of whole plant- or root-treated groups.

In our study, cauda epididymal sperm concentration, motility and normal morphology showed a significant decrease in all of *T. officinale*-treated groups when compared to the control group (Table 2). This result is in agreement with the results of Padashetty and Mishra [19]; their explanation was a reduction in serum testosterone level which is similar to our results.

The testicular histology of treated rats could provide another explanation for the reduction in

sperm concentration observed. Testicular cross sections showed lesions in the seminiferous tubules, disorganized germinal epithelium, spermatogenesis maturation arrest and reduction in the interstitial tissue (Figure 2 and Figure 3). The reduction in testosterone level disrupts spermatogenesis and reduces fertility parameters [21]. These results were similar to the results obtained by others [22].

Sperms with rapid swimming abilities and normal morphology have a fertilizing advantage over slower abnormal sperms [23]. *T. officinale* treatment reduced sperm parameters causing a reduction in the fertility rate and the number of fetuses/pregnant female which is in agreement with previously published results of *Calendula officinalis* (Compositae) administration [24].

The current study measured the mRNA levels of three spermatogonial stem cell (SSC) markers: *GDNF family receptor alpha 1 (gfra1)*, *macrophage colony-stimulating factor (mcsf)* and *promyelocytic leukaemia zinc-finger (plzf)* by qRT-PCR (Figure 4). These markers induce self-renewal and proliferation of undifferentiated spermatogonia rather than differentiation [7]. GDNF and its receptor GFR $\alpha$ 1 are necessary for the maintenance and proliferation of undifferentiated spermatogonia. Over-expression of these markers led to accumulation of spermatogonia that are unable to differentiate in addition to testicular atrophy [25]. Hofmann [26] suggested that mice over-expressing *gfra1* are infertile, develop testicular tumors and accumulate spermatogonia.

PLZF exerts its effects on undifferentiated spermatogonia only and it interacts with GDNF to block spermatogonia differentiation [27]. Costoya *et al.* [28] showed that testes lacking PLZF have an increased level of spermatogonia apoptosis. A nonsense mutation in *plzf* gene showed defective spermatogenic self-renewal [29]. Silvan *et al* [30] reported that MCSF interacts with GDNF and acts specifically on undifferentiated SSCs and that its effect causes spermatogonia self-renewal rather than differentiation.

The reduced expression of *mcsf* (Figure 4) could be explained by the histology of the seminiferous tubules of the *T. officinale*-treated rats - particularly the high dose root-receiving animals, as there was a lack of interstitial tissue (the source of MCSF) from the treated animals (Figure 3). On the other hand, the over-expression of *gfra1* and *plzf* (Figure 4) might have led to enhanced self-renewal of spermatogonia as was noticed in the testicular sections of HD-treated groups (Figure 3). This

enhancement of self-renewal was at the expense of differentiation induction and sperm production. In turn, this might have contributed to the disruption in spermatogenesis and the maturation arrest observed.

*Taraxacum officinale* is rich in phytochemicals. Nevertheless, few studies investigated the effects of individual compounds identified in *T. officinale* extracts on spermatogenesis. Previous studies have shown that flavonoids and amyryn inhibited spermatogenesis [31-33]. On the other hand, chlorogenic acid was found to stimulate spermatogenesis by inhibiting testosterone degradation [34]. However, the difference in the effects of the two extracts (root and whole plant) cannot be explained based on these compounds since these compounds were found in both the root and whole plant aqueous extracts. Studying the effects of individual phytochemical constituent of *T. officinale* aqueous extract on spermatogenesis is beyond the scope of this study.

## CONCLUSION

*T. officinale* root and whole plant aqueous extracts have been shown to exert inhibitory effects on spermatogenesis. More studies should be conducted to identify the specific anti-spermatogenic active ingredient(s) and whether the anti-fertility actions are reversible or not. Furthermore, additional studies are advised to investigate the specific effect of *T. officinale* aqueous extracts on spermatogonial stem cell markers' regulatory pathways. These studies could lead to the identification of specific ingredient(s) in *T. officinale* that might be used as male contraceptive.

## DECLARATIONS

### Acknowledgement

The authors are grateful to the Deanship of Research and Graduate Studies, Hashemite University for supporting the current work.

### Conflict of Interest

No conflict of interest associated with this work.

### Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them. LTH conceived and designed the study and helped to draft the

manuscript, RNA; RAA and ZAA collected and analysed the data, SRY and SIA participated in the design of the study and coordination, AZE participated in the design and performed the statistical analysis. All authors read and approved the final manuscript for publication.

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### REFERENCES

- Schulz, Volker; Hänsel, Rudolf; Tyler, Varro E. *Rational phytotherapy. A physician's guide to herbal medicine*. Berlin: Springer-Verlag; 2001; p 369.
- Schütz K, Carle R, Schieber A. *Taraxacum- A review on its phytochemical and pharmacological profile*. *J Ethnopharmacol* 2006; 107: 313-323.
- Tahtamouni L, Alqurna N, Al-Hudhud M, Al-Hajj H. *Dandelion (Taraxacum officinale) decreases male rat fertility in vivo*. *J Ethnopharmacol* 2011; 135: 102-109.
- Tahtatamouni L, Al-Khateeb R, Abdellatif R, Al-Mazaydeh Z, Yasin S, Al-Gharabli S, Elkarmi A. *Anti-spermatogenic activities of Taraxacum officinale whole plant and leaves aqueous extracts*. *Vet Res Forum* 2016; 7: 89-97.
- Goossens E, VanSaen D, Tournaye H. *Spermatogonial stem cell preservation and transplantation: from research to clinic*. *J Hum Reprod* 2013; 28: 897-907.
- Caires K, Broady J, McLean D. *Maintaining the male germline: regulation of spermatogonial stem cells*. *J Endocrinol* 2010; 205: 133-145.
- Kolasa A, Misiakiewicz K, Marchlewicz M, Wiszniewska B. *The generation of spermatogonial stem cells and spermatogonia in mammals*. *J Reprod Biol* 2011; 12: 5-23.
- de Rooij DG. *Proliferation and differentiation of spermatogonial stem cells*. *Reproduction* 2001; 121: 347-354.
- Phillips B, Gassei K, Orwig K. *Spermatogonial stem cell regulation and spermatogenesis*. *J Phil Trans R Soc B* 2010; 365: 1663-1678.
- Ketkar A, Reddy K. *Expression Pattern of OCT-4 and PLZF transcription factors during the early events of spermatogenesis in mice*. *J Cell Sci Ther* 2012; 3: e1000120.
- Ferguson L, How J, Agoulnik A. *The fate of spermatogonial stem cells in the cryptorchid testes of RXFP2 deficient mice*. *PLoS ONE* 2013; 8: e77351.
- World Health Organization (WHO) *WHO Protocol MB-50. A method for examining the effect of the plant extracts administered orally on the fertility of male rats (APF/IP, 9914E)*. Geneva: WHO; 1983.
- Soleimanzadeh A, Saberivand A. *Effect of curcumin on rat sperm morphology after the freeze-thawing process*. *Vet Res Forum* 2013; 4: 185-189.
- Jia F, Sun Z, Yan X, Zhou B, Wang J. *Effect of pubertal nano TiO2 exposure on testosterone synthesis and spermatogenesis in mice*. *Arch Toxicol* 2014; 3: 781-788.
- Tejada R, Mitchell J, Norman A, Marik J, Friedman S. *Test for the practical evaluation of male fertility by acridine orange (AO) fluorescence*. *Fertil Steril* 1984; 42: 87-91.
- Peinnequin A, Mouret C, Birot O, Alonso A, Mathieu J, Clarençon D, Agay D, Chancerelle Y, Multon E. *Rat pro-inflammatory cytokine and cytokine related mRNA quantification by real-time polymerase chain reaction using SYBR green*. *BMC Immunol* 2004; 5: 3-12.
- Morimoto H, Kanatsu-Shinohara M, Takashima S, Chuma S, Nakatsuji N, Takehashi M, Shinohara T. *Phenotypic plasticity of mouse spermatogonial stem cells*. *PLoS ONE* 2009; 4: e7909.
- Guide for the Care and Use of Laboratory Animals*. National Research Council of the National Academics. Washington: The National Academies Press; 2011. 220 p.
- Padashetty A, Mishra H. *Effect of terpenoidal fraction of Echinops echinatus roots on reproductive parameters of male rats*. *J Nat Med* 2007; 61: 452-457.
- Dohle R, Smit M, Weber F. *Androgens and male fertility*. *World J Urol* 2003; 21: 341-345.
- McLachlan I, Wreford J, O'Donnell L, De Kretser M, Robertson M. *The endocrine regulation of spermatogenesis: independent roles for testosterone and FSH*. *J Endocrinol* 1996; 148: 1-9.
- Mirhoseini M, Mohamadpour M, Khorsandi L. *Toxic effects of Carthamus tinctorius L. (Safflower) extract on mouse spermatogenesis*. *J Assist Reprod Genet* 2012; 29: 457-461.
- van der Host G, Maree L, Kotzé S, O'Riain M. *Sperm structure and motility in the eusocialnaked mole-rat, Heterocephalus glaber: a case of degenerative orthogenesis in the absence of sperm competition?*. *BMC Evol Biol* 2011; 11: 351-362.
- Agarwal M, Sharma P, Kushwaha S. *Antifertility efficacy of 50% ethanolic extract of calendula officinalis in male rats*. *Int J Pharm Pharm Sci* 2011; 3: 1-5.
- Zhou, Q; Griswold, M. *Regulation of spermatogonia*. In: Donahoe, P; Lin, H, editors. *The Stem Cell Research Community*, StemBook doi:10.3824/stembook.1.7.1. 2008.

26. Hofmann M. GDFN signaling pathways within the mammalian spermatogonial stem cell niche. *J Mol Cell Endocrinol* 2008; 288: 95-103.
27. Singh S, Burnicka-Turek O, Chauhan C, Hou S. Spermatogonial stem cells, infertility, and testicular cancer. *J Cell Mol Med* 2011; 15: 468-483.
28. Costoya A, Hobbs M, Barna M, Cattoretti G, Manova K, Sukhwani M, Orwig E, Wolgemuth J, Pandolfi P. Essential role of Plzf in maintenance of spermatogonial stem cells. *Nat Genet* 2004; 36: 653-659.
29. Buaas W, Kirsh L, Sharma M, McLean J, Morris L, Griswold D, de Rooij DG, Braun E. Plzf is required in adult male germ cells for stem cell self-renewal. *Nat Genet* 2004; 36: 647-652.
30. Silvan U, Diez-torre A, Moreno P, Arluzea J, Andrade R, Sillio M, Arechaga J. The spermatogonial stem cell niche in testicular germ cell tumors. *Int J Dev Biol* 2013; 57: 185-195.
31. Gupta R, Bhatnager A, Joshi Y, Sharma R, Sharma A. Suppression of fertility in male albino rats following  $\alpha$ -amyrin acetate administration. *Pharm Biol* 2004; 42.
32. Gupta R, Kachhawa J, Gupta R, Sharma A, Sharma MC, Dobhal MP. Phytochemical evaluation and antispermatogenic activity of *Thevetia peruviana* methanol extract in male albino rats. *Hum Fertil (Camb)* 2011; 14: 53-59.
33. Chen M, Hao J, Yang Q, Li G. Effects of icariin on reproductive functions in male rats. *Molecules* 2014; 19: 9502-9514.
34. Park J, Han K. The spermatogenic effect of yacon extract and its constituents and their inhibition effect of testosterone metabolism. *Biomol Ther (Seoul)* 2013; 21: 153-160.