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A diet enriched with *Pistacia atlantica* fruits improves the female rats' reproductive system

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ABSTRACT

Background and aim: Baneh (*Pistacia atlantica*) is a plant species that is commonly consumed as food and has a long-standing traditional use as a sexual enhancer. Despite its widespread use, a limited amount of academic and scientific literature is available regarding its potential impact on the reproductive system. The present research aimed to study the effect of a diet enriched with Baneh on the female rats' reproductive system.

Experimental procedure: Three groups of rats (n = 8) were subjected to the intended diet for six weeks. Subsequently, their histomorphometric parameters, sex hormone levels, as well as the expression of oxytocin (OXT) and oxytocin receptor (OXTR) genes were measured. The rats' serum vitamin D, zinc, and lipid profiles were also evaluated.

Results and conclusion: Results revealed that compared to the normal food, the diet containing 20 % Baneh significantly increased the progesterone and estradiol levels three and two times, respectively. It decreased the total body weight while increasing the ratio of ovary weight to the body weight. Furthermore, the Baneh-enriched diet raised HDL, zinc, and vitamin D levels, though it reduced the LDL and TG levels by 15 μg/dl and 24 μg/dl, respectively, and the concentration of ovary malondialdehyde decreased by 50 % in the treated group. Also, the diet increased the follicle graph, corpus luteum, the thickness of the epithelium, the number of endometrial glands, and the expression of both *OXT* and *OXTR* genes. Our findings suggested that *P. atlantica* could considerably improve the female sex hormone levels and their reproductive system.

1. Introduction

The reproductive system is one of the body organs, which in addition to the reproduction process, affects many physiological functions of the body through the secretion of sex hormones, including estrogen and progesterone.[1,2] On the other hand, different factors, such as lifestyle, nutrition, and levels of sex hormones affect the function of the reproductive system, determine reproductive health, and influence fertility.[3,4] More specifically, levels of female sex hormones play vital roles in sexual development, reproduction, and general health.[5] Oxytocin is a hormone and chemical messenger in the brain that affects human behaviors and body organs. It also controls key aspects of the reproductive system, including childbirth and lactation. It has been shown that levels of oxytocin receptors correlate with concentrations of circulating steroid hormones.[6,7] By changing the hormone secretion, many external or internal factors, such as toxic materials, oxidative stress, obesity, diabetes, and aging can affect the menstrual cycle, ovulation, and female fertility. Besides, previous studies have shown that nutritional factors and micronutrients play a vital role in the normal function of the reproductive system.

The use of herbal remedies for the prevention and treatment of a wide range of diseases is common worldwide, and many people still rely on local medications and traditional therapies.[8–11] Evidence of using herbal medicines to improve female and male fertility dates back to 200 A.D. It is argued that herbs enhance sexual potency and remedy fertility problems. For instance, Ashwagandha,[12] Pomegranate,[13] Cinnamon,[14] Dates,[15] Maca Root,[16] etc,[17,18] are herbal medicines still traditionally used as sexual enhancers in the Middle East and East Asia.[18,19] However, no reliable academic evidence is available regarding the effects of the majority of these plans and their mechanisms of action.

In recent years, researchers have focused on the investigation of the

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Abbreviation

ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
DEPC	Diethyl-Pyro Carbonate
DHT	Dihydrotestosterone
ELISA	Enzyme-Linked Immunosorbent Assay
HDL	High-Density Lipoprotein Cholesterol
H&E	Hematoxylin and Eosin
LDL	Low-Density Lipoprotein Cholesterol
MDA	Malondialdehyde
OS	Oxidative stress
OXT	Oxytocin
OXTR	Oxytocin Receptor
PUFA	Polyunsaturated Fatty Acids
TBA	Thiobarbituric acid
TG	Triglyceride

effects of plant derivatives on fertility, and invaluable results have been achieved.[20,21] One of the plants known in traditional medicine as a sexual enhancer is wild pistachio, with the scientific name of *Pistacia atlantica Deaf*. This plant species belongs to the Ancacardiaceae family and is called "Baneh" in Iran.[22] Antihypertensive, anti-inflammatory, analgesic, anti-diabetic, antiepileptic, anti-hyperlipidemia, antitumor, and antimicrobial properties of the gum, leaf, stem, and fruit of this plant have previously been studied. The fruit of this plant is consumed as a healthy food due to its impressive composition of essential nutrients, including proteins, fatty acids, and fibers[23] and Also, its essential oil contains significant amounts of tocopherol, sterols, antioxidant polyphenols, essential fatty acids, essential and non-essential amino acids, and minerals such as iron, sodium, copper, and zinc.[22] The most important fatty acids found in wild pistachio are omega-3, -6, and -9, oleic acid, palmitic acid, linoleic acid, and arachidonic acid. Of these, omega-3 and arachidonic acid are converted into prostaglandins[24] and It seems that prostaglandins as a group of endogenous acidic lipids have an important role in many reproductive processes.[25]

The current study aimed to investigate the relationship between the consumption of the wild pistachio fruit and the reproductive system of female rats. For this purpose, the animals were fed diets containing 10 and 20 % wild pistachio; then, the estrogen and progesterone levels, the weight of the body and reproductive organs, the ovary malondialdehyde (MDA) concentration, quantities of some micronutrients (zinc, vitamin D), and lipid profile, as well as the ovary and uterus histology were studied. And also The expression of oxytocin and oxytocin receptor genes was evaluated.

2. Materials and methods

2.1. Preparation of food containing P. atlantica Desf fruits (Baneh)

To prepare the food enriched with wild pistachio fruit (locally called Baneh), first, fruits of the plant were collected from the mountains near Fasa city (Fars Province, Iran), and identified by the herbarium of the Shahid Bahonar University of Kerman (associate code: 2915). Next, we washed, dried, and powdered the entire fruit of the plant, which we will now refer to as 'Baneh', including its thin green fatty husk, rigid shell, and central core. To prepare food containing 10 % and 20 % (w/w) of the fruit, the intended amounts of the powder were mixed with regular animal food, and the final composites were stored.

2.2. Animals and treatment conditions

Twenty-four healthy female Wistar rats weighing 120–150 gr (6–8 weeks old) were purchased from the Animal Center of the Neuroscience Research Center of Kerman University of Medical Sciences. Two weeks before the main experiments, the animals were allowed to acclimate and adapt to the laboratory conditions. They were kept in a dark cycle (12:12-h light) in groups of 4 rats, in standard cages; then, the animals were randomly divided into the following three groups (n = 8) and treated for six weeks; i) control, a diet with normal food, ii) a diet with food containing 10 % of Baneh, and iii) diet with food containing 20 % Baneh. All the animal treatment protocols were conducted as maintained by the guidelines prepared by the Neuroscience Research Center of Kerman University of Medical Sciences and the Neuroscience Ethics Committee (EC/KNRC/95-8A).

2.3. Hormone levels assessment

At the end of the treatment period, to analyze the effects of the diet on estradiol and progesterone hormone levels, the animals were anesthetized using ether, sacrificed, and their trunk blood was collected. To provide the serum, the blood was centrifuged at 2500 rpm for 15 min at 4 °C; then, serum levels of estradiol and progesterone were quantitatively analyzed using the Enzyme-Linked Immunosorbent Assay (ELISA; DiaSorin Inc., USA). As well, the serum levels of oxytocin were measured via the rat-specific ELISA Kit (ZellBio GmbH, Ulm, Germany). The assay sensitivity of oxytocin was 1 ng/L. Intra-assay and inter-assay coefficients of variability for oxytocin were lower than 10 % and 12 %, respectively.

2.4. Assessment of body and reproductive organ weights

Upon completion of the six-week treatment, the effects of the diet on the animals' body weight, as well as, their reproductive organ weights were evaluated. For this purpose, the animals' body weight at the beginning of the treatment period was documented and compared with their weights at the end of the treatment. Furthermore, at the end of the treatment period, after sacrificing, their reproductive organs, such as ovary and uterine, were removed and weighed. These data were compared with the control group.

2.5. Malondialdehyde (MDA) assay

For the measuring of malondialdehyde levels, as a lipid peroxidation product, ovary tissue was first homogenized by trichloroacetic acid (TCA) reagent, then centrifuged at 10,000 g for 10 min. A measurement of 1 ml of thiobarbituric acid (TBA) reagent was then added to the suspension and was heated at 95 °C for 30 min followed by cooling at room temperature. The obtained product was centrifuged at 10,000 g for 10 min. In the TBA method, lipid peroxidation produces TBA-reactive substances in the supernatant, which is measured at 532 nm. The levels of MDA were expressed as μmol/mg protein.[26]

2.6. Biochemical parameters and lipid profile assessment

To assess the impact of the administered diet, several biochemical parameters including zinc (Zn), vitamin D, kidney factors (urea, and creatine), and liver factors (aspartate aminotransferase (AST) and alanine aminotransferase (ALT)) were measured in the blood serum of the animals.

The lipid profile, including total cholesterol, high-density lipoprotein cholesterol (HDL), low-density lipoprotein cholesterol (LDL), and triglycerides (TG) were measured in the animals' serum using specific assessment kits and automatic chemistry analyzer (KONELAB 20XT, Finland).[27]

2.7. Histomorphometry analysis

To perform histomorphometry analysis, rats were sacrificed when

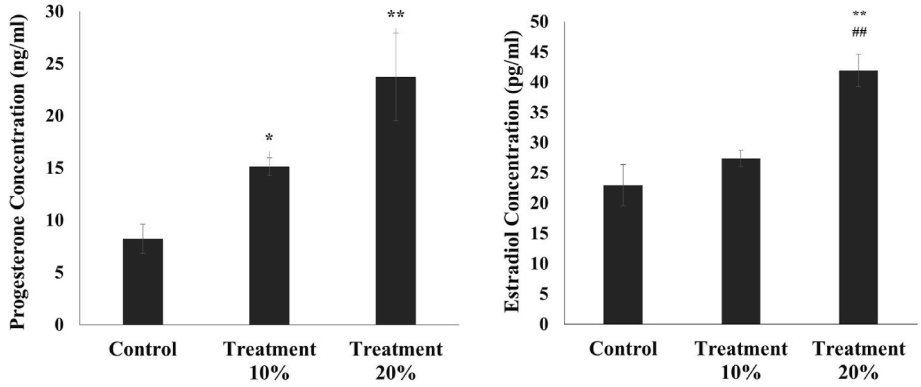

Fig. 1. The effect of the diet containing 10 % and 20 % of Baneh (treatment) on the serum levels of estradiol and progesterone in female rats. Data were expressed as mean ± SEM, **P < 0.01 significant differences than the control group. ##P < 0.01, significant differences compared to the group fed with 10 % Baneh.

they were in the proestrus phase of their estrous cycle; then, the removed ovaries and uterus were cleaned from fatty tissues, washed with normal saline, and fixed in 10 % neutral buffer formalin for 48 h to ensure proper fixation before preparing the histological slide, after that, samples underwent the tissue processing steps (dehydration by 50 %, 70 %, 80 %, 90 %, and absolute alcohol, clearing by xylene, and impregnation with molten paraffin with a tissue processor (DS 9602), respectively. Paraffin blocks were sectioned at a thickness of 5 μm using a Rotary microtome (DS 4055) and subsequently stained with hematoxylin and eosin (H&E) (Ahmadi et al., 2019). Ovarian and uterine tissue slides were examined using an optical microscope (MEDIC M – 107 BN) for histomorphometry study and measurement of the uterine horn layer's thickness, luminal epithelium layer height, types of follicles, corpus luteum, number of branches endometrial glands, and the total diameter of the uterus (μm). Histomorphometry studies were analyzed using a Dino-Lite digital camera and Dino-capture V.2 Software.[28]

2.8. Evaluation of OXT and OXTR gene expression (real-time PCR)

According to the manufacturer's instructions (CinnaGen Company) the extraction of total RNA from the rats' hypothalamus was done by the RNX-Plus reagent; then, the RNA concentration was measured by a Nanodrop, and the quality of RNA samples was evaluated by loading them on 1.5 % agarose gel. Next, cDNA was synthesized by following the instructions of the RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, Germany) and using the Oligo-dT primer and M-MuLV reverse transcriptase. To investigate the genes' expression, the real-time PCR technique was performed through the following protocol: enzyme activation at 95 °C for 15 min, followed by 40 cycles, including the denaturation phase (20 s at 95 °C), annealing phase (30 s at 60 °C), and extension phase (30 s at 72 °C). In the PCR reactions, the Real Q Plus 2X master mix (Ampliqun, Denmark) was utilized. The forward and reverse primer sequences related to OXT were 5'-CCTGGATATGCGCAAGTGTCTTC-3' and 5'- TCGGAGAAGGCAGACTCAGG -3', respectively. And, the forward and reverse primer sequences related to OXTR were 5'- GGCTGCCGAGGGGAATGAC -3' and 5'- ATGGCAATGATGAAGGCAGAAGC -3', respectively. as well, β-actin gene with the forward primer sequence of 5'- CTAGGCACCAGGGTGTGATG -3' and reverse sequences of 5'- GGTTGGCCTTAGGGTTCAGAG -3' was considered as a housekeeping gene. These genes' mRNA expression was calculated based on their ΔCT.

2.9. Statistical analysis

Statistical comparison was performed using independent T-tests and One-way ANOVA using SPSS 19.0. P-values less than 0.05 (P < 0.05) were considered as significant levels. The results are presented as mean value accompanied by the standard error of the mean (SEM).

3. Results

3.1. Estradiol and progesterone levels

The levels of estradiol and progesterone were assessed in the groups that received 10 % and 20 % of Baneh in their food. As results showed, the diet containing 10 % Baneh did not have any considerable impact on the estradiol level in treated rats while it increased progesterone levels. However, the presence of 20 % Baneh in food could significantly increase these two hormone levels in rats (Fig. 1).

3.2. The weight of the body and organs

The results of the diet impact assessment on body weight and organs are presented in Table 1. Based on the results, 10 % Baneh did not affect these parameters, while the food enriched with 20 % Baneh caused a decrease in the total body weight compared to the control group (P < 0.01). Although no significant difference was observed in the ovary and uterine weight of these groups, the ratio of the ovary/body weight in rats fed with this diet was significantly higher than in the control group.

3.3. Lipid profile assessment

The evaluation of the lipid profile revealed that the inclusion of 20 % Baneh in the diet resulted in a significant elevation of HDL levels in the

Table 1
The effects of the diet containing 10 % and 20 % of Baneh (treatment) on the body's weight and female reproductive organs (Ovary and Uterine).

Animal groups	Features						
	Initial weight (g)	Final weight (g)	Body weight difference (g)	Ovary absolute weight (g)	Uterine absolute weight (g)	Ovary/Body weight %	Uterine/Body weight %
Control	153 ± 3.3	192 ± 6.9	39	0.134 ± .004	0.416 ± .034	0.069 ± .002	0.216 ± .018
Treatment 10 %	161 ± 4.7	196 ± 6.4	35	0.133 ± .007	0.454 ± .040	0.067 ± .002	0.230 ± .019
Treatment 20 %	151 ± 1.9	178 ± 1.8**	27	0.141 ± .007	0.438 ± .047	0.08 ± .005*	0.248 ± .029

Data have been expressed as mean ± SEM, **P < 0.01, *P < 0.05, significant differences compared to the control group.

Table 2
Effects of the diet enriched with Baneh on the serum lipid profile of female rats.

Animal groups	Features			
	Cholesterol (μg/dl)	LDL (μg/dl)	HDL (μg/dl)	TG (μg/dl)
Control	55.0 ± 4.4	46.8 ± 1.6	49.6 ± 3.2	87.1 ± 8.6
Treatment 10 %	57.4 ± 1.54	40.58 ± .94	51.3 ± 1.35	71.08 ± 1.76
Treatment 20 %	60.2 ± 6.06	31.0 ± 2.2***##	58.7 ± 3.5*	63.14 ± 7.2*

Data have been expressed as mean ± SEM, ***P < 0. 001, *P < 0. 05, significant differences compared to the control group, ##P < 0. 01 significant differences compared to the Treatment 10 % group.

serum of rats. Furthermore, it markedly reduced the levels of LDL and triglycerides (TG) in the treated animals' serum by 15 μg/dl and 24 μg/dl, respectively. However, the consumption of 10 % of Baneh had no considerable effect on the rats' lipid profile. Moreover, food containing 20 % of Baneh caused an increase in HDL levels compared to 10 % of Baneh (Table 2).

Combining the results so far, we observed that the diet comprising 20 % of Baneh significantly affected the levels of estradiol and progesterone and serum lipid profile in adult female rats. Therefore, the animals that were under this treatment (food containing 20 % Baneh and six weeks of treatment) were chosen for further assessments.

3.4. Assessment of malondialdehyde (MDA) and essential ingredients/elements levels

According to the results, Baneh caused a considerable reduction in the concentration of the ovary MDA from about 0.00392 μmol/mg in the control group to about 0.0020 μmol/mg in the treated group (Fig. 2a).

The levels of elements influencing the function of the reproductive system, such as zinc (Zn) and vitamin D, also showed a significant increase in the rats treated with 20 % Baneh in their food (Fig. 2b and c).

3.5. Kidney and liver biochemical factors

The effects of Baneh on some important biochemical features related to the liver and kidney of female rats are shown in Table 3. According to the results, the Baneh administration decreased the AST levels in the serum of animals to around 182 IU/L, also this diet caused a reduction in the serum urea level. However, the effects of the diet on the ALT and creatinine levels were statistically insignificant.

3.6. Histomorphometry analysis

Histological studies revealed that feeding with Baneh can considerably affect the ovary. It caused an increase in the parameters of the healthy follicle graph and corpus luteum (Table 4, Fig. 3). Also, in uterine tissue, the thickness of the epithelium and the number of endometrial glands increased significantly in the Baneh-consuming group (Table 4, Fig. 3).

3.7. OXT and OXTR gene expression

The obtained results demonstrated that the diet containing Baneh

Table 3
Effects of the diet containing Baneh (*P. atlantica*) on the kidney and liver features of female rats.

Animal groups	kidney and liver factors			
	AST(IU/L)	ALT(IU/L)	Urea(mg/dl)	Creatinine(mg/dl)
Control	232 ± 10.4	103.4 ± 15.7	48.5 ± 3.2	0.45 ± .06
Treatment 20 %	182 ± 5.3**	88.0 ± 8.76	33.8 ± 3.3**	0.48 ± .06

Data have been expressed as mean ± SEM, **P < 0. 01, significant differences compared to the control group.

Table 4
Effects of the diet containing 20 % of Baneh (treatment) on histomorphometry parameters of the ovary and uterine tissue in female rats.

Parameters	Animal groups	
	Control	Treatment 20 %
The total diameter of the uterus(μm)	967.36 ± 25.64	998.22 ± 12.33
Epithelial Thickness(μm)	14.13 ± 1.09	19.83 ± 0.12*
Myometrial thickness(μm)	149.33 ± 7.22	156.66 ± 5.89
Endometrial thickness(μm)	290.75 ± 10.35	492.26 ± 18.11
Stromal thickness(μm)	501.07 ± 21.17	492.26 ± 18.11
Number of branches Endometrial glands	9.5 ± 0.55	12.10 ± 0.63*
Primordial follicle	219.02 ± 14.69	198.12 ± 13.09
Primary follicle	95.77 ± 6.85	101.67 ± 10.1
Secondary follicle	17.48 ± 2.33	19.97 ± 2.62
Graafian follicle	2.5 ± .25	5.25 ± .5*
Atretic follicle	3.0 ± 0.75	2.0 ± 0.50
Tertiary follicle	4.5 ± 1.00	6.5 ± 1.50
Corpus Luteum	2.5 ± .75	6.25 ± .5*

Data have been expressed as mean ± SEM, *P < 0.05, significant differences compared to the control group.

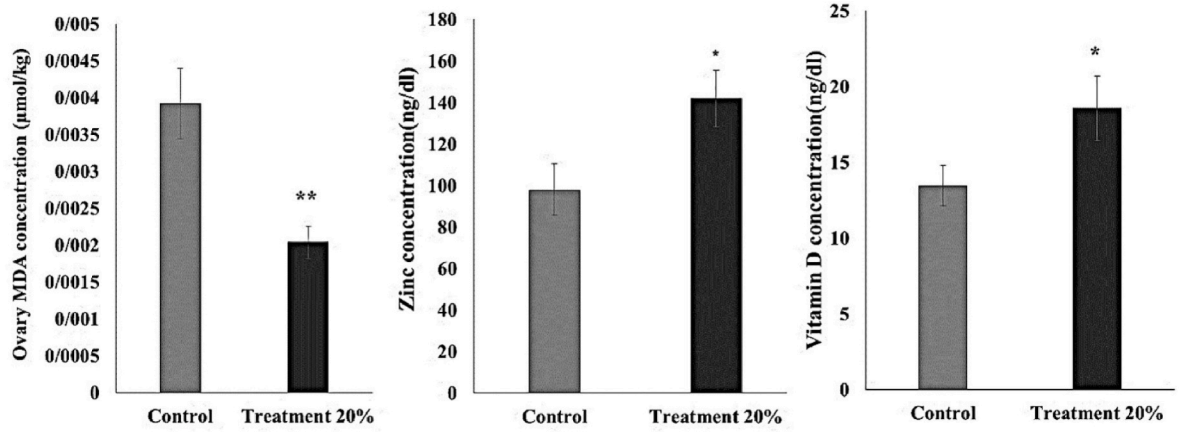

Fig. 2. Effects of the diet containing 20 % of Baneh (treatment) on ovary MDA levels (a), serum levels of zinc (b), and Vitamin D (c). Data are expressed as mean ± SEM, **P < 0. 01, *P < 0. 05, significant differences compared to the control group.

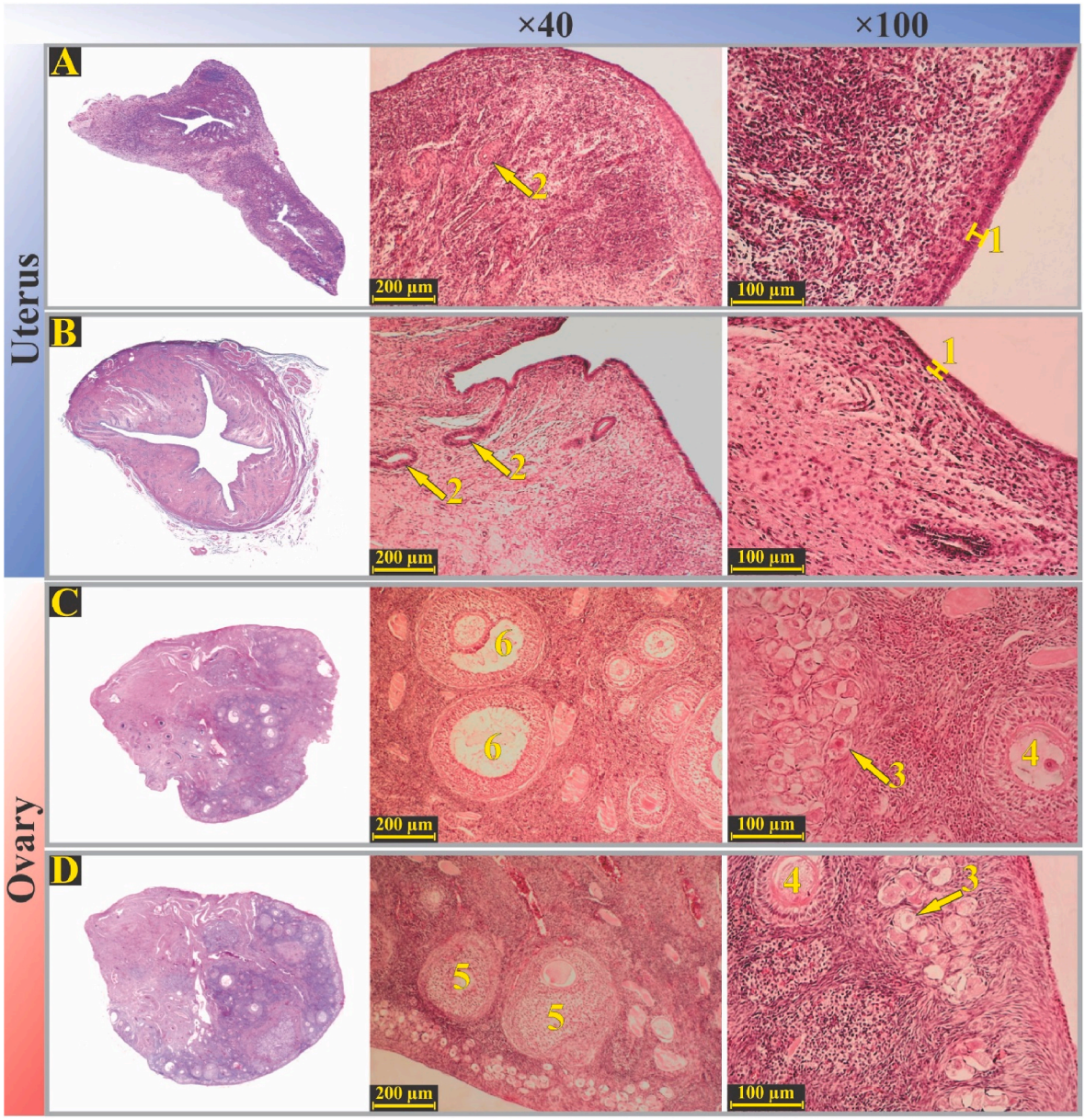

Fig. 3. Effects of the diet containing 20 % of Baneh (treatment) on histomorphometry parameters of the ovary and uterine tissue in female rats. A and B represent the uterine sections of the control and treatment groups, respectively. C and D represent the ovary sections of the control and treatment groups, respectively
No. 1: Epithelial Thickness; No. 2: Branches of endometrial glands; No. 3: Primordial Follicles; No. 4: Secondary follicles; No. 5: Tertiary follicles; No. 6: Graafian follicles.

significantly increased the expression levels of both oxytocin and its receptor genes in the rats' hypothalamus. Furthermore, the treated rats exhibited serum levels of oxytocin hormone that were twice as high as those in the control group (Fig. 4).

4. Discussion

Lifestyle and some other factors, such as diseases, injuries, and chronic health problems, can negatively affect female fertility.[29] It is widely acknowledged that nutrition, as one of the most influential factors in human health, exerts a significant impact on the reproductive system. This influence arises from its crucial role in various specific processes, including the maturation of reproductive cells, hormone production, secretions, implantation, placental growth, and angiogenesis.[30] In traditional medicine, wild pistachio (*Pistacia atlantica Desf*), known locally as Baneh, is used as a sexual enhancer food. Our findings revealed that this food could considerably improve the main features of the reproductive system of female rats.

In the present study, the results demonstrate a significant increase in progesterone and estradiol levels in the subjects who were fed with Baneh. Since these hormones are considered the main sex hormones of females and play a central role in the reproductive system, increasing their levels certainly affects the performance of this system. First of all, it should be explained how a Baneh-enriched diet may increase progesterone and estradiol levels.

In the present study, our results showed that being fed with Baneh significantly increased progesterone and estradiol levels. It has been revealed that some issues, such as diseases, injuries, and chronic health problems, can negatively affect female hormones.[31] In the present study, we evaluated the levels of sex hormones during the proestrus phase of the animals' estrous cycle. Generally, sex hormone levels change throughout this cycle. Progesterone and estradiol levels peak at the

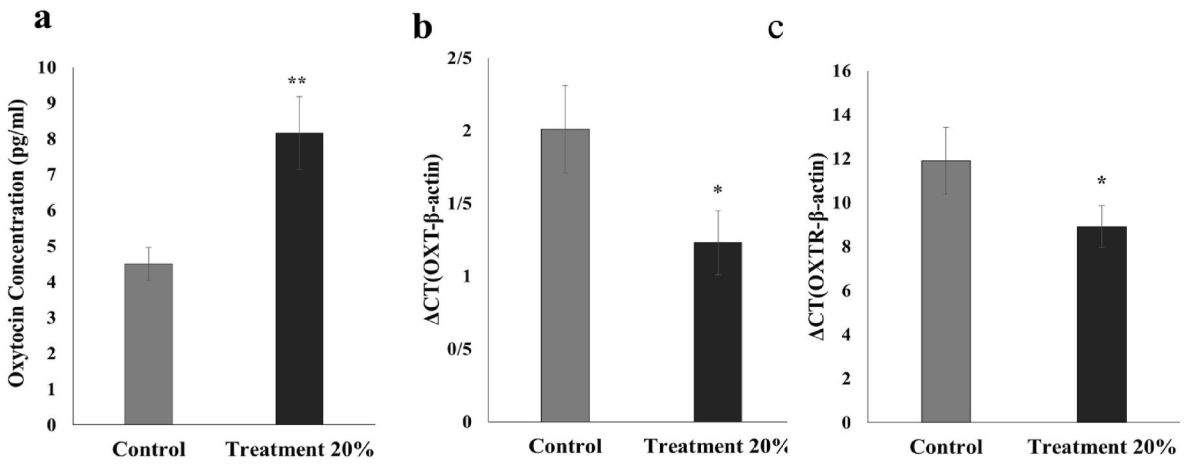

Fig. 4. Effects of the diet containing 20 % of Baneh (treatment) on the expression of OXT (a) and OXTR (b) genes in the Rats' hypothalamus and serum level of oxytocin hormone (c). Data have been expressed as mean ± SEM, **P < 0.01 *P < 0.05, significant differences compared to the control group.

proestrus and estrus stages, which correspond to the period around ovulation. On the other hand, during other stages of the cycle (metestrus and diestrus) the levels of these hormones decrease markedly or become undetectable.[32]

As previously reported, Baneh contains polyunsaturated fatty acids, which can increase progesterone levels.[33] Also, there is a strong possibility that the increased estradiol and progesterone levels can be caused by the phytoestrogens present in Baneh. These compounds have an estrogen-like structure and can affect the sex hormone receptors.[34] Studies have shown that phytoestrogens have direct estrogenic activity and increase the level of female sex hormones such as estradiol, FSH, and LH.[35] Furthermore, wild pistachio contains quercetin, a flavonoid, which converts cholesterol to pregnenolone in ovarian gonadal cells and stimulates the synthesis of progesterone and estradiol.[36] In addition, recent studies have demonstrated that phytoestrogens can bind to estradiol receptors as agonists or antagonists, affecting the hypothalamus-pituitary-gonadal axis and the peripheral reproductive system. However, their effect depends on the relative concentrations of endogenous phytoestrogen.[37]

Results of this study, also, showed that being fed with Baneh caused triglyceride and LDL levels to decrease, it also increased HDL levels in rats. These observations agree with previous reports.[38,39] Studies showed that serum LDL levels were reduced following excessive consumption of plant sterols.[40] As previously reported, estradiol caused a decrease in LDL levels and especially an increase in HDL and triglyceride levels.[41] The results of our study showed that the level of estradiol in the group fed with Baneh raised, and Baneh will likely impact the lipid profile by increasing estradiol levels.

Furthermore, our results showed that eating Baneh decreases body weight significantly. The presence of a high amount of fiber content in pistachio and its fast digestion may cause a decrease in the body weight of rats.[42] Wang et al. studies showed that pistachio consumption can help reduce plasma triglyceride levels, and body weight of obese people.[43] The present study findings also indicated that a diet containing Baneh increased the ratio of ovaries' weight to total body weight. Additionally, the increasing parameters of the healthy follicle graph, corpus luteum, the thickness of the epithelium, and the number of endometrial glands were other striking observations in the tested group. Considering the direct relationship between sex hormone levels and the functioning of the sexual organs, the significant increase in the follicle graph and corpus luteum can be attributed to the significant increase in the serum level of hormones in rats fed with Baneh. Furthermore, the alterations in sex hormone levels may be the reason for the rise observed in the thickness of the epithelium and the number of endometrial glands in uterine tissue.[1]

Oxidative stress (OS) is one of the most important influencing factors in the pathogenesis of infertility in both males and females. However, the way of action of OS on oocytes and reproductive functions has been fully understood yet. Studies have shown that weight gain and lifestyle factors can increase the production of free radicals, potentially affecting fertility. We found that in the ovary of the Baneh-consumed group, the MDA levels significantly decreased. The antioxidant properties of wild pistachios may be responsible for this effect. Reactive oxygen species (ROS) are produced during metabolic are neutralized by flavonoids and phenolic compounds in the plant.[44] Also, in our study, treatment with Baneh reduced body weight, which can be one of the reducing factors of MDA. In previous studies, it has been determined that oral administration of *P. atlantica* extract caused an increase in the level of antioxidants, such as superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase, and decreased MDA levels in the blood serum.[45]

Climate, nutrition, lifestyle, and genetic factors are among the most important factors that affect the serum level of vitamin D. According to the results, feeding with Baneh considerably increased vitamin D levels in the rats' serum. However, this vitamin is an important micronutrient for the reproductive system function in both males and females.[46] It also has the potential to modulate inflammation and hormone levels, which are critical aspects of female reproductive function. Acute exposure to vitamin D can alter estrogen and progesterone production from primary granulosa cells.[47]

Zinc, as a vital microelement, plays a vital role in crucial functions, such as the regulation of cell growth, immunological response, and hormone release. It also influences the performance of females' reproductive system, including embryogenesis and development in the body.[48] Impaired synthesis/secretion of FSH and LH, abnormal ovarian development, and estrous cycle disruption are among the complications caused by zinc deficiency in females.[49] Previous studies have determined that zinc prevents ROS production and zinc deficiency can increase oxidative stress, which subsequently increases reproductive impairment in both sexes.[50]

Results of the treatment with Baneh showed a reducing effect on AST and urea levels in our study. In a similar study, it has been found that Baneh's extract administration decreased the AST level,[51] which could be due to the presence of tocopherol, phytosterols, and unsaturated fatty acid compounds.[52]

The expression of oxytocin and its specific receptor has a direct relationship to the changing levels of sex steroids.[53] In the present study, it can be explained that the increase in the level of sex hormones in treated rats increases the serum level of oxytocin and the expression of its receptor gene in the hypothalamus. It has been observed that the increase of estradiol induces oxytocin receptors in the hypothalamus,

and the level of progesterone increases the binding of oxytocin receptors and the expansion of the area covered by these receptors in the hypothalamus. Moreover, estradiol regulates oxytocin release.[54,55]

Concerning the weaknesses of this work, it should be mentioned that serum levels of some hormones, such as luteinizing hormone (LH) and follicle-stimulating hormone (FSH) were not evaluated. These hormones play crucial roles in regulating the reproductive system and can influence female reproductive health. Measurement of LH and FSH in future studies can be helpful to provide a more comprehensive understanding of the effects of *Pistacia atlantica* on the female reproductive system.

5. Conclusion

The results of this study revealed how a diet enriched with the entire fruit of the wild pistachio (***P. atlantica Desf***), locally called Baneh, as a traditional libido booster, affects the female reproductive system's physiology and anatomy. This food can improve levels of female sex hormones (progesterone and estradiol) and oxytocin hormone. This increase causes a significant rise in the ratio of the ovary weight to the body weight and affects the histology of the uterus and ovaries. This diet considerably increases the serum level of zinc and vitamin D, improves the lipid profile, and improves the kidney and liver biochemical factors.

Data statement

We declare here that all data belonging to the represented research are reproducible and clear. All the raw data are available for sharing upon request.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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