Effects of Hydro-alcoholic Extract of Pumpkin Seeds on Oogenesis Pathway, Liver, and Kidney of Female Rats

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Abstract

Background: Pumpkin seed extract can be a good alternative to hormone replacement therapy since it is rich in phytoestrogens.

Objectives: The present research aimed to investigate the effect of hydro-alcoholic extract of pumpkin seeds on the oogenesis pathway, liver, and kidney of female rats.

Methods: This experimental study was performed on 64 Wistar female rats (including 32 adults and 32 immature rats). The adult rats were randomly divided into three experimental and one control groups (n=8 per group). Moreover, the immature rats were allocated to groups in a similar manner. The experimental groups 1, 2, and 3 received a hydro-alcoholic extract of pumpkin seed in doses of 20, 50, 100 mg/kg, respectively, via intraperitoneal injection for 21 consecutive days. For the purposes of the study, blood samples were taken one day after the last injection to determine the serum levels of female hormones as well as renal and hepatic factors. The ovaries, livers, and kidneys of the rats were also separated for histological tests.

Results: Based on the results, significant increases were observed in the bodyweight of all immature rats; estrogen levels in the adult experimental group 3 and the immature experimental groups 1 and 2; follicle-stimulating hormone in the adult experimental group 3 and the immature experimental groups 1 and 2; luteinizing hormone and Graafian follicles in the adult experimental group 3 and the immature experimental groups 1 and 2; areteic follicles in the immature experimental group 3; aspartate aminotransferase, total protein, unstable angina (UA), and the renal diameter in the immature experimental groups 1 and 2; and renal diameter in the immature experimental group 3; diameters of proximal and distal tubule as well as Henle’s loop in all immature rats; diameter of glomerular in the immature experimental groups 1 and 2; diameter of the renal cortex, glomerular, and Bowman’s capsule in the adult experimental groups 2 and 3; secondary follicles in the adult experimental group 1, immature experimental groups 1 and 3; and primitive and early follicles in all the adult rats, compared with the control group (P<0.05).

Conclusion: Based on the findings, it can be concluded that the pumpkin seeds provide the nutritional needs of the body at the onset of sexual maturity, prepare the body for sexual maturity, and regulate female sex hormones without having adverse effects on the hepatic tissues. However, it must be noted that its consumption at the onset of sexual maturity requires dosage determination and further studies.

Keywords: Estrogen, Extract, Female, Hormone, Kidney, Liver, Rat

1. Background

Chemical medicines used for disease treatment can have various side effects. For example, hormone replacement therapy used for the treatment of sexual maturation disorders or genital anomalies is associated with hyperplasia, endometrial cancer, breast cancer, or thromboembolism that can also affect renal and hepatic functions (1). In general, due to the side effects of chemical medicines, researchers have decided to focus on the use of herbal products in a variety of medical fields (2). It should be noted that herbal medications, which have been used in traditional medicine, are made from a group of herbs with active ingredients used to treat or prevent various diseases (3).

According to the review of the related literature, pumpkin is also known as Cucurbita maxima and it is originally from South America. It belongs to the family Cucurbitaceae and has been widely cultivated around the world since its discovery. Pumpkin seeds are rarely used by people around the world and may even be considered as a waste. However, the extract of these seeds have numerous benefits for human health and can be used as nutrient enrichment of food products or an alternative to oil (4-6).

Pumpkin seed is an excellent source of minerals, including phosphorus, potassium, and selenium, which help the body fight various diseases, such as arthritis, inflammation, and prostate cancer. In addition, it is said to be rich in various elements, namely calcium, magnesium, and zinc (6). According to the previous research, pumpkin seeds extract contains chemical compounds, such as linoleic acid and oleic acid, which reduce blood cholesterol (7-9). It is also a good source of unsaturated fatty acids and phytosterols (β-sitosterol) that can prevent chronic diseases.

Pumpkin seeds can also effectively reduce the risk of bladder-stone disease and treat lower urinary tract symptoms and benign prostatic hyperplasia (10). It should be noted that β-Sitosterol can reduce cholesterol, affect estrogen, and have anticancer activities (11). Based on the findings of previous
studies, pumpkin has potential antioxidant activity. Reactive oxidants in the follicles are necessary for the ovulatory response; therefore, the ablation of the ovarian oxygen species hinders the ovulation and the whole repertoire of essential preovulatory responses (12, 13). It should be noted that more changes were observed in sex hormones at the onset of sexual maturation in comparison to after the completion of sexual development. Abnormal levels of sex hormones, especially estrogen and progesterone, can affect the kidney and liver (13-15).

Renal and hepatic failure is common among women who take steroid-containing contraceptives (14). According to previous studies, the use of certain amounts of synthetic estrogen and progesterone for the treatment of female hormone disorders may affect renal and hepatic functions in certain circumstances (13-15). For example, tamoxifen is a selective estrogen receptor modulator that can lead to cholestasis (16). Moreover, ethinyl estradiol, which is used for treating ovarian insufficiency and menopausal symptoms, can have side effects, such as changes in hepatic function and jaundice associated with biliary obstruction. In addition, HD pills, used for menstrual regulation, can increase the risk of biliary disease and gallstone formation (17).

It is noteworthy that peliosis hepatitis is a form of hepatic disease that can be associated with the use of anabolic-androgenic steroids, oral contraceptives, azathioprine, tamoxifen, and danazol. This disease can be asymptomatic or manifest itself as hepatomegaly, hepatic failure, and intraperitoneal hemorrhage or even cause death (16-18). Infection, inflammation, and removal of the gallbladder have been reported in women taking oral estrogen, which may be due to the hepatic effects of oral administration of estrogen. Estrogens increase biliary cholesterol secretion and saturation which is associated with decreased gallbladder motility and increased crystallization of cholesterol in bile (19, 20).

Given the side effects of chemical medications, pumpkin seed extract can be used as an alternative sex hormone regulator. However, it can replace chemical medications only after it is proved that this extract does not have any adverse effects on the kidney and liver at the onset or end of sexual maturation (21).

2. Objectives

The present research aimed to investigate the effect of hydro-alcoholic extract of pumpkin seeds on the oogenesis pathway, liver, and kidney of female rats.

3. Methods

3.1. Subjects and group allocation

This study was performed on immature and adult female Wistar rats. All ethical issues regarding how to work with laboratory animals and the principles of laboratory animal care were considered in the present research (ethics code for immature and adult rats were IR.HUMS.REC.1398.426 and jums.REC.1393.071, respectively). All the selected immature rats were at the onset of puberty. The adult and immature rats were kept in an animal breeding room under similar conditions for a week to adapt. They were maintained in a 12/12 h light/dark cycle and the humidity was about 50-55% (22-25).

For the purposes of the study, 32 adult Wistar female rats that were 60 days old and weighted 180±10 g were randomly divided into three experimental and one control groups (n=8 per group). Moreover, 32 immature Wistar female rats that were 30-35 days old and weighted 100±80 g were randomly divided into three experimental and one control groups (n=8 per group).

The sample size was calculated using the information extracted from a study performed by Solomon et al. and based on the formula

\[
N = \left(\frac{2(1-p)+z^2\alpha + z^2\beta}{\frac{p}{2}}\right)^2
\]

in which \(\alpha=0.05\) and \(1-\beta=0.9\). Accordingly, the formula \(N=\frac{10.49(0.51^2 + 0.52^2)}{(6.4-0.5)^2}\) was considered in each experimental group (26).

Both the experimental and control groups received the right doses of solutions via insulin syringe through intraperitoneal injection for 21 successive days on a daily basis, at 10 a.m. and once a day (27). The details are described below:

Adult rat groups:

Group 1: received 20 mg/kg bw of hydro-alcoholic extract of pumpkin seeds (0.2 ml in each daily intraperitoneal injection) (27).

Group 2: received 50 mg/kg bw of hydro-alcoholic extract of pumpkin seeds (0.2 ml in each daily intraperitoneal injection) (27).

Group 3: received 100 mg/kg bw of hydro-alcoholic extract of pumpkin seeds (0.2 ml in each daily intraperitoneal injection) (27).

Group 4 (Control): received no medicine.

Immature rat groups:

Group 1: received 20 mg/kg bw of hydro-alcoholic extract of pumpkin seeds (0.1 ml in each daily intraperitoneal injection) (27).

Group 2: received 50 mg/kg bw of hydro-alcoholic extract of pumpkin seeds (0.1 ml in each daily intraperitoneal injection) (27).

Group 3: received 100 mg/kg bw of hydro-alcoholic extract of pumpkin seeds (0.1 ml in each daily intraperitoneal injection) (27).

Group 4 (Control): received no medicine.

On day 22, after weighing the rats, blood samples were taken directly from their hearts using a 5 cc syringe (under anesthesia induced by Ketamine hydrochloride and Xylazine). Thereafter, their
abdomens were cut and their livers, kidneys, and ovaries were removed from the surrounding adipose tissue by scalpel and pence for histological studies.

3.2. Preparation and extraction of plant materials

Extraction was performed using the Soxelle method (28). Dry pumpkin seeds (100 g), (Cucurbita maxima genus, “Cucurbitaceae” family, and C. maxima [danhobak] species) (4, 5) were comminuted and combined with 500 mL of 80% ethanol and stored in a percolator. Finally, the extract drops were collected after three days. When all of the drops were collected, 80% ethanol was poured into the machine to clear the color of the extract. Moreover, in order to thicken it, the resulting mixture was passed through a filter and evaporated in a rotary evaporator at 40°C. In addition, the remaining material was under vacuum in a desiccator for 24 h to lose its moisture. Afterward, the obtained dry material was weighed and evaluated its efficiency which indicated that 16 g of dry extract was obtained from 100 grams of pumpkin seeds powder and the remaining was waste. At last, a 16% pumpkin seed extract was acquired through this procedure. Finally, the researchers determined lethal, maximum, moderate, and minimum doses of this extract (27).

3.3. Dose determination

Several various concentrations of pumpkin seed extract were randomly selected and injected in rats (four experimental groups of 8). The lethal dose 50 (LD50) of a group was found to be 400 mg/kg and the maximum, moderate, and minimum doses were specified as well. There was 400 mg of pure extract in each liter and for LD50 determination, 1 ml of it was injected into 5 rats that weighed 200 g. To obtain a dose of 100 mg/kg bw, the basic extract was diluted four times using normal saline (group 1), and to provide a dose of 50 and 20 mg/kg bw, the extract was diluted twice and five times using normal saline, respectively (groups 2 and 3) (27).

3.4. Measurement of plasma biochemical parameters

Blood samples were used in order to measure the serum levels of blood urea nitrogen (BUN), creatinine, unstable angina (UA), alanine transaminase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), total protein (TP), bilirubin, albumin (Alb), estrogen, and progesterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH). The blood biochemical factors were measured based on the colorimetric method using biochemical test kits, and Selectra XL fully-automated analyzer (manufactured in Netherlands).

3.5. Microscopic examination of renal, hepatic, and ovarian tissues

After the bleeding ended, the abdomens of the rats were cut, and their kidneys, livers, and ovaries were separated from their surrounding tissues. Afterward, they were weighed, washed with normal saline, and stored in a 10% formalin solution for 14 days. For the preparation of microscope slides, the kidneys, ovaries, and livers were sent to the histology laboratory. The slides were used to study the tissue since they were separately prepared from different parts of renal and ovarian tissues.

In each kidney, the diameters of the cortex, medulla, glomerular Bowman capsules, urinary space, proximal tubule, distal tubule, and Henle loop (in millimeters) were determined. Moreover, each liver was evaluated regarding congestion, hyperemia, atresia, and mean value of liver tissue destruction which included disruption of the polarized arrangement of hepatocytes, vasodilation, hyperemia, hepatic necrosis, Kupffer cell aggregation, inflammatory cell infiltration, and portal space changes. Furthermore, in each ovary, hyperemia, vacuolization of the cells of ovarian tissue and follicular atresia, as well as the mean value of primitive, early, secondary, Graafian, atretic, and yellow follicles were measured by an optical microscope with a magnification of 400X in 10 fields of view (a total of 50 fields of view for each animal). Thereafter, their mean values were calculated and the groups were compared (27).

3.6. Statistical analysis

The collected data were analyzed in SPSS software (version 23) using descriptive statistical tests (e.g., mean and standard deviation), one-way ANOVA, and Chi-squared test. It should be noted that a p-value of 0.05 was considered statistically significant. Healthy adult and immature female Wistar rats were included, while the dead rats were excluded from the study.

4. Results

Effect of hydro-alcoholic extract of pumpkin seeds on:

4.1. Bodyweight

Comparison of the bodyweight of the rats on the first and last days of pumpkin seed extract injection revealed a significant increase in the three immature experimental groups, compared to the control group (P<0.001). However, no significant alterations were observed in the bodyweight of the adult experimental groups (P=0.014).

4.2. Serum concentrations of BUN, Creatinine, and UA

There were a significant increase and decrease in the level of creatinine (P<0.001) and UA (P=0.04), respectively, in the three immature experimental groups, compared to the control group. Moreover, a significant increase was observed in the UA in the immature experimental groups 1 and 2 in comparison to the control group (P=0.04) (Figure 1). However, no other significant changes were found in
the blood test results of the adult (P=0.016) and immature groups (P=0.05) in this regard.

A significant increase was observed in the UA in the immature experimental groups receiving doses of 20 and 50 mg/kg in comparison to the control group (P<0.05). No significant difference was observed between the columns with at least one letter in common (P>0.05).

4.3. Serum concentrations of ALT, AST, ALP, OT, PT, T.p.r.t, and Alb

The ALP in the adult experimental group 3 underwent a significant decline in comparison to the control group (P=0.023). In addition, there was a significant increase in AST in the immature groups 1 and 2, compared to the control and the immature group 3 (P=0.04). Furthermore, a significant increase was observed in the total protein level in the immature groups 1 and 2 in comparison with the control group, while the immature group 3 experienced a significant decline in this regard, compared to the control and other immature groups (P=0.023). No significant alterations were observed in other blood test results of the adult and immature groups (P>0.05).

4.4. Serum concentrations of female sex hormones

According to the results, there was a significant increase in the level of FSH in the adult experimental group 3 in comparison to other adult experimental groups and the control group (P=0.002). Moreover, FSH underwent a significant increase in the immature experimental groups 1 and 2 in comparison to the control group (P<0.001). In addition, there was a significant increase in LH in the adult experimental group 3, compared to the control group (P=0.046).

Based on the results of the estrogen test in different adult and immature groups, there was a significant increase in the estrogen level of the adult group 3 in comparison to the control group (P=0.003). Similarly, the estrogen level of immature experimental groups 1 and 2 underwent an increase in comparison to the control group (P=0.02) (Table 1). Moreover, a significant increase was observed in the progesterone level in the immature experimental group 3 in comparison to the control group (P<0.001). However, there was no other significant alteration regarding the other ovarian serum data (P>0.05).

4.5. Renal tissues

Based on the results, the pumpkin seed extract significantly decreased the diameters of the renal cortex (P=0.04), glomerular capsule (P<0.001), and Bowman’s capsules (P=0.023) in the adult experimental groups 2 and 3 in comparison to the control group. Moreover, a significant decrease and increase were observed in Bowman’s capsule and urinary space dimensions in the immature experimental groups 2 and 1, respectively, in comparison to the control group (P<0.001).

There was also a significant increase in the renal diameter in the immature groups 1 and 2, compared to the control group, while there was a significant diminution in this regard in the immature group 3 in comparison to the control and other immature groups (P=0.023). Furthermore, the diameters of the proximal tubule (P<0.001), distal tubule (P=0.008), and Henle loop (P<0.001) underwent a significant diminution in all the immature experimental groups in comparison to the control group. Additionally, there was a significant diminution in the diameter of glomerular in the immature groups 1 and 2 in

![Figure 1. Comparison of unstable angina in the immature rats after the injection of pumpkin seed extract](image-url)

Table 1. Comparison of estrogen levels in the adult and immature groups after the injection of hydro-alcoholic extract of pumpkin seeds

| Variable                        | Group 1 (g/ml) | Group 2 (g/ml) | Group 3 (g/ml) | Group 4 (control) (g/ml) |
|---------------------------------|---------------|---------------|---------------|------------------------|
| Estrogen levels in the adult    | 58.5±47.93    | 68.3±50.59    | 79.3±75.75    | 53.2±25.32            |
| Estrogen levels in the immature | 50.5±42.00    | 44.2±26.02    | 76.2±26.20    | 37.5±15.30            |

* Group 1 (receiving a dose of 20 mg/kg); Group 2 (receiving a dose of 50 mg/kg); Group 3 (receiving a dose of 100 mg/kg); Group 4 (control) (P=0.05).

** No significant difference was observed in the mean values of the rows with at least one letter in common according to Duncan’s test (P>0.05).
comparison to the control (P=0.008). No more significant alterations were observed in other renal tissue results in the adult and immature groups (P>0.05).

4.6. Hepatic Tissues
Based on the results, the pumpkin seed extract had no significant effect (including hyperemia, inflammatory cell infiltration, necrosis, vacuolation, clearance of cytoplasm, and portal space changes) on the hepatic tissues in the adult and immature experimental groups (P>0.05) (Figure 2).

4.7. Ovarian Tissues
According to the findings, pumpkin seed extract was able to significantly increase and decrease the atretic and secondary follicles, respectively, in the immature groups 1 and 3 (P=0.008), compared to the control group and immature group 2 (P=0.023). Moreover, a significant increase was observed in the Graafian follicles in the adult experimental group 3. Besides, there was a significant diminution in the early (P=0.01) and primitive follicles in the three adult experimental groups in comparison with the control group (P=0.013). Furthermore, the secondary follicles underwent a significant decrease in adult group 1, compared to adult groups 2 and 3 (P=0.038) (Table 2). Meanwhile, there was no significant difference between the mean values of the rows with at least one common letter according to Duncan’s test. It is noteworthy that no change was observed regarding the other variables (P>0.05).

Table 2. Changes in ovarian tissue variables in immature female rats after the injection of hydro-alcoholic extract of pumpkin seeds

| Ovarian tissue variables | Group 1 | Group 2 | Group 3 | Group 4 (control) |
|--------------------------|---------|---------|---------|-------------------|
| Number of secondary follicles (percent) | 3.0±75.0.478a | 6.0±25.0.478b | 3.0±75.0.250a | 6.0±25.0.103a |
| Number of atretic follicles | 1.0±50.0.025b | 0.2±50.0.075b | 1.0±50.0.040b | 0.0±50.0.040a |

* Group 1 (receiving a dose of 20 mg/kg body weight); Group 2 (receiving a dose of 50 mg/kg body weight); Group 3 (receiving a dose of 100 mg/kg body weight); Group 4 (control)
** No significant difference was observed in the means between the rows with at least one common letter according to Duncan’s test (P>0.05).

5. Discussion

Cucurbita maxima are cultivated worldwide for both food and medicinal purposes. It is one of the most popular vegetables that are also affordable and easy to grow (29); in addition, its seeds have significant quantities. According to the previous research, the highest mean weight of its seed mass per fruit is 110.7 g (30, 31). Pumpkin seeds contain water (94 g), protein (1.1 g), fat (0.1 g), starch (6.3 g), calcium (28 mg), phosphorus (30 mg), iron (0.4 mg), potassium (2.2 mg), and vitamins (i.e., A: 400 units, B1: 0.05 mg, B2: 0.09 mg, B3: 1 mg, and C: 22 mg). They are also good sources of zinc, unsaturated fatty acids, phytosterols (β-sitosterol), antioxidants, carotenoids, tocopherols, and fatty acids (i.e., palmitic, stearic, oleic, and linoleic acids) which have positive effects on body tissues.

However, bezoars are one of the disadvantages of pumpkin seeds. Bezoars are foreign bodies or undigested masses of material that accumulate in the digestive tract. Nevertheless, based on previous research, pumpkin seed extract has had no adverse effects on the liver and kidney so far (32, 33). In this study, there was a significant increase in the body weight in all of the immature experimental groups due to the nutrient composition of pumpkin seed extract as it fulfilled the nutritional requirements of the rats at the onset of their sexual maturation when their body underwent potential changes.

According to previous studies, pumpkin seeds contain phytoestrogens which are estrogen-like molecules that can have complex effects on non-reproductive behaviors, such as anxiety and movement. This means that they can exert anxiolytic effects that, in turn, increase motor activity and exploratory behaviors. These effects are associated with hormone action and sex steroids. Increased mobility, which can lead to weight loss (34), was observed in the groups that received moderate and maximum doses of pumpkin seed extract. Moreover, no weight gain was observed in the adult experimental groups which may be due to the increased mobility; however, this finding requires further investigation.

The phytoestrogens present in the human...
foodstuffs are isoflavones that are classified as flavonoids. Isoflavones are phytoestrogens that are able to bind to estrogen receptors in different organs (35). Today, synthetic estrogens are used for the treatment of female hormone disorders and infertility. According to the findings of a study performed by Siano (2016), only certain amounts of synthetic estrogens in special circumstances can affect the liver (36). According to the findings of another study conducted by Sangiovanni et al. (2020), hepatic dysfunction was observed in women using estrogen-containing steroids for contraceptive purposes (37).

In the present study, the hydro-alcoholic extract of pumpkin seeds had no harmful effects on the hepatic tissues in the adult and immature experimental groups, while the estrogen levels significantly increased in the adult and immature experimental groups 3 and in the immature experimental group 2 in comparison with the control group. Hence, this extract can be used at the onset of puberty and in the menstruation period. However, there is a need for further analysis and subsequent tests.

A significant increase was also observed in progesterone in the immature group 3 in comparison with the control group. However, the adult and immature groups underwent no significant alterations regarding the albumin level. It should be noted that estrogens and progesterone are transported in the blood by binding to albumin and specific sex hormone-binding globulins. The attachment of the hormones to this protein are so weak that they are released to the tissues over a period of approximately 30 min (38). Hence, it can be concluded that in the present study, liver function was normal in terms of albumin pathway.

The estrogen found in the contraceptive pills can lead to cholestasis; moreover, this effect can be exacerbated by the progestin. Furthermore, the consumption of anabolic steroids and androgens has been associated with the development of hepatocellular adenoma, hepatocellular carcinoma, and hepatic angiosarcoma (39). The sex hormone disorders that reduce testosterone levels and elevate estradiol levels are symptoms of hepatic cirrhosis (40). Important effects of estrogens on the liver include cholestasis, liver tumors, Budd–Chiari syndrome, and dose-dependent jaundice. It should be noted that medication-induced cholestasis is completely reversible after medication withdrawal (39, 40).

A significant diminution was observed in ALP in the adult group 3 in comparison with the control group. Since this hepatic serum change was observed only in adult groups, it can be said that pumpkin seed extract can be the best choice for hormone replacement therapy in adult groups. Moreover, there was a significant increase in the AST level in immature groups 1 and 2 in comparison with the control group and the immature group 3. In addition, there was a significant increase in the total protein level in the immature group 1 and 2 in comparison with the control group. Moreover, there was a significant diminution in the total protein level in the immature group 3 in comparison with the control and the other immature groups; however, this finding requires further studies.

According to previous studies, the route of estrogen administration may affect its function. More specifically, the effect of exogenous estrogens on the liver depends on the type and dose of estrogen as well as the route of administration. Based on previous studies, oral consumption of synthetic estrogen has a great effect on proteins (plasma and coagulation), lipoproteins, and triglycerides, while estradiol topical has minimal effect on the liver function.

Clinically, oral steroids are effective for the treatment of prostate cancer; however, they are associated with severe cardiovascular side effects and changes in liver metabolism (41). Infection, inflammation, and removal of gallbladder are more common in women taking oral estrogen, probably due to its hepatic effects. Estrogens enhance biliary cholesterol secretion and saturation, increase cholesterol in bile, and decrease gallbladder movement through the increase of crystallization in the bile (41–43).

Transdermal (e.g., gels) or subcutaneous (implants) administration of estrogens maintains blood estrogen as they are transported in the blood without leaving a high concentration of conjugated estrogens in the liver (44). In this study, the intraperitoneal injection was performed due to better absorption caused by the high number of arteries and veins in the peritoneal cavity (45).

Results of previous studies have indicated that pumpkin seed drops can be used to treat prostate diseases (46). According to the findings of a study performed by Sun et al. (2018), there are only three types of estrogen in human plasma (i.e., β-estradiol, estriol, and estrone) while β-estradiol is the major estrogen produced in the ovaries. Moreover, they found that the secretion of estrone is limited and it is mainly derived from the conversion of androgens secreted by ovarian theca cells and adrenal gland in peripheral tissues (47).

Witorsch et al. (2016) in their study found that estradiol is metabolized in the liver and converted to estrone and estriol which are excreted in the urine as glucuronide and sulfate conjugates (48). Therefore, in addition to the hepatic pathway, its metabolism has a renal pathway. In the present study, renal serum results indicated a significant increase in the creatinine level and a significant diminution in UA in the immature group 3 in comparison with the control group. Moreover, there was a significant increase in
UA in immature groups 1 and 2 in comparison with the control group.

Furthermore, Bowman's capsule and urinary space dimensions underwent a significant decrease and increase in the immature experimental groups 2 and 1, respectively, in comparison with the control group. Besides, a significant increase was observed in the renal diameter in the immature experimental groups 1 and 2 in comparison with the control group. However, the renal diameter underwent a significant decrease in the immature experimental group 3 in comparison with the control and other immature groups.

According to the results, the diameters of the proximal tubule, distal tubule, and Henle loop underwent a significant diminution in all the immature experimental groups in comparison with the control group. Additionally, there was a significant decrease in the diameter of the glomerulus in the immature groups 1 and 2, compared to the control group. No other significant alterations were observed in other results about kidney tissues in all of the adult and immature experimental groups (P<0.05).

Based on the results, most of the changes occurred in immature experimental groups. Nevertheless, there was also a significant diminution in the adult groups regarding the diameters of the renal cortex, glomerular capsule, and Bowman's capsule in groups 2 and 3 in comparison with the control group. According to the results of this study and the review of the literature, it can be said that there is a need for caution in the use of this extract in immature groups. This may be due to the younger age range of immature rats in comparison with the adult ones.

The sex hormone activity and body weight were strongly affected by steroids. Moreover, successful differentiation of follicles pertains to the steroids and follicular growth factors. Growth and differentiation of ovarian follicles take place via proliferation and differentiation processes of the granulosa cells (49). Adequate amounts of both FSH and LH are required for oocyte growth and development and meiotic division (50).

In the present study, FSH significantly increased in the adult group 3 in comparison with the other adult experimental and control groups. The FSH also increased in immature groups 1 and 2, compared to the control group. There was also a significant increase in the LH levels in the adult group 3 in comparison with the control group. It can be said that the extract had a significant positive relationship with these two hormones (i.e., FSH and LH). Deiman and Chaola (2008) in their study also found that androgen production increased with the increase of LH, which is in line with the results of the present study (51, 52). In this research, the estrogen increased in the adult group 3, while the immature groups require more investigation in this regard.

Follicles trigger ovulatory cascade and fatty acids cause the growth of Graafian follicles and increase their number and size. As previously mentioned, pumpkin seeds are also a good source of unsaturated fatty acids and flavonoids (phytoestrogens). It is also noteworthy that estrogen is a potent stimulus of follicular growth in rodents (53, 54).

According to the results of the present study, there was a significant diminution in the secondary follicles in adult group 1 and immature groups 1 and 3 in comparison with the control groups. Moreover, the atretic follicles increased in immature groups 1 and 3; hence, caution is advised regarding the use of the extract in immature groups. There was also a significant diminution in the early and primitive follicles in all the three adult experimental groups, compared to the control group, which is inconsistent with the results of the previous research. This inconsistency may be due to some agents, such as breed, hormone, and treatment duration.

It has been found that pumpkin seeds also have antioxidant activities. It is necessary to remember that reactive oxidants in the follicles are essential for ovulation and their ablation hinders proper ovulation (55, 56). The increase in the Graafian follicles in the adult experimental group 3 is consistent with the results of a previous study conducted by Forouzan Fard et al. (57).

Pumpkin seeds also contain calcium (28 mg) and phosphorus (30 mg). Average level of phosphorus in the blood is 3 to 4 mg per 100 ml. Since each 1 kg of pumpkin seeds contains 30 mg of phosphorus, it enters the blood faster than calcium and replenishes the reserves of the body. Excessive phosphorus intake reduces calcium absorption since these two elements compete to enter intestinal cells. Moreover, it can cause the excretion of excessive serum phosphorus in the urine. It is essential to know the amount of phosphorus and calcium intake to evaluate the effect of pumpkin seeds on the kidney since phosphorous precipitation in the kidneys can cause renal dysfunction (43). Therefore, future studies should investigate the effect of pumpkin seed extract on phosphorus and calcium intake and compare the results in adult and immature groups.

6. Conclusion

In conclusion, it can be said that the nutrients found in pumpkin seeds provide the nutritional needs at the onset of sexual maturity. They affect female sex hormones and provide conditions for sexual maturity without any adverse effects on the hepatic tissues and also regulate female sex hormones after puberty. However, the use of this extract at the onset of sexual maturity requires caution and accurate dosage determination. Moreover, in adults, it requires caution in terms of renal factors and tissues.
Therefore, further molecular biology studies are suggested in this regard.

Footnotes

Authors’ Contribution: Study concept and design: Samaneh Motamed Jahromi and Sadegh Niami. Analysis and interpretation of data: Samaneh Motamed Jahromi. Drafting of the manuscript: Samaneh Motamed Jahromi. Critical revision of the manuscript for important intellectual content: Samaneh Motamed Jahromi. Statistical analysis: Sadegh Niami.

Conflict of Interests: The authors have no conflict of interest to declare.

Ethical Approval: All of the experiments were reviewed and approved by the Ethics Committee of Hormozgan and Jahrom Universities of Medical Sciences (code of ethics for immature rats groups: IR.HUMS.REC.1398.426 and adult rats groups: jums.REC.1393.071).

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