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Positive Effects of Almond Seeds in Raising Fertility in Subfertility Male Rats

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Abstract

In accordance with new research released by the World Health Organization (WHO) today, infertility affects a significant fraction of the world's population. There is an urgent need to enhance access to high-quality fertility care, as 17.5% of adults (1 in 6) globally struggle with infertility. The goal of this inquiry was to examine the positive effects of almond seed powder found in the al-Baha area in raising the level of fertility in subfertility rats. Caged animals were used for the experiment in this investigation. The rats were housed in groups of six and fed a baseline diet for a week before the experiment was conducted. As a control, the first group of rats, designated as C-ve, received nothing more than the standard diet for the entire period of twenty-eight days. Cadmium chloride (CdCl2) was administered to the remaining rats (n=24). One group was diagnosed with the sickness and disease in addition was not provided the trial diet, while the other three groups were fed varied quantities of almond seed (5%, 10%, 15%). Group 3 (almond seeds at a concentration of 5 percent) fared better in terms of testosterone hormone treatment compared to the control group (+). Group 5 (15% almond seeds) also had the most successful outcome in lowering FSH levels. D almond seeds are indicated for those who are experiencing infertility issues.

Keywords: Almond seeds, Subfertility male rats, Positive effects, Fertility

Introduction

The capacity of a person to conceive through regular sexual activity is referred to as fertility. Normal fertility requires the production of enough healthy sperm by the male. Nutrients in almonds may aid in cancer prevention, heart health, bone health, fertility, as well as other areas. Almonds provide enough protein to satisfy one-eighth of a person's daily needs with just one ounce (Arthur *et al.,* 2018). The almond tree is a member of the Rosaceae family. They are classified as either members of the Prunoideae or Amygdaloideae subfamily. Due to the widespread practice of assigning almonds to their own family (Prunaceae or Amygdalaceae), it is now often assumed that prunus evolved from the Spiraeoidae subfamily. The average height of an almond tree is 4-10 meters, as well as its trunk is roughly 30 centimeters in diameter. Young twigs are born a green tint, but as they age and are exposed to more sunlight, they turn purple. The average leaf length is three to five inches. The five-petaled, baby-pink blossoms are delicate and lovely. Once planted, it takes 5–6 years for the tree to achieve maturity, which occurs in the fall (Lee *et al*., 2017). The capacity to cultivate aesthetically pleasing Almond domestication made it one of the earliest fruit trees to be domesticated. By the early Bronze Age, around 2000 B.C., almonds had been domesticated. To remove toxicity, wild almonds were likely leached or roasted before being gathered for human consumption. Before there were farms and homes. However, cultivated Almonds in their sweet form are not poisonous. According to Diamond, early farmers grew a mutant characterized by a lack of glycoside amygdalin "at first unintentionally in the garbage heaps then more intentionally in their orchards" due to a common genetic mutation (Markiewicz-Zukowska *et al.,* 2022). Two distinct varieties of almonds exist. Bitter almonds as well as sugared almonds. The sweet variety of almonds can be eaten, while the bitter variety is deadly. When compared to its sweet counterpart, the bitter almond is longer and wider. Half of the oil in a sweet almond is solid. Hydrogen cyanide may be extracted from Bitter Almond. The kernel, often known as the almond's meat, the middle shell, as well as the outer green shell make up an almond. The kernel coat, brown skin, or seed coat is a thin, leathery coating. The kernel provides most of the plant's calories. Vitamins, protein, minerals, and fiber can all be found in abundance in almonds. They can be consumed either raw or roasted. They're technically a drupe rather than a nut (Ozcan, 2023). Seed extract, skin, and hull phenolic chemicals have been isolated from almonds over the past few decades. Polyphenols, an abundant micronutrient in the human diet, have been revealed to protect against cancer also cardiovascular disease. In addition to a beneficial association between almonds and male fertility, the health benefits of polyphenols rely on the amount taken as well as their bioavailability (Creedon *et al.,* 2023). Several phenolic compounds isolated from almonds and almond products are discussed, along with their antioxidant characteristics as well as potential applications as dietary antioxidants (Rajaram *et al.,* 2023).

Aim of the Study

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The main focus of this investigation is to ascertain the positive impacts of almond seeds found in the Al-Baha area in raising the level of fertility in subfertility rats.

Materials and Methods

Materials

Preparation of Almond Seeds

Almond seeds were obtained from a local market in the Al-Baha area and then properly cleaned, sliced into little pieces, in addition, oven-dried for three days at 50 degrees Celsius, before crushing and and grinding into a powder form.

Cadmium Chloride

was acquired with no modifications from the German chemical firm Merk and put into immediate service.

Experiential Animals

Thirty mature male Sprague Dawley rats weighing (175-170 g) were utilized in this investigation. The Medical Insects Research Institute in Doki, which is located in Cairo, is where we received these rats.

Rats were acclimated to laboratory life for a week by keeping them in wire cages and feeding them standard laboratory food. Rats were fed from specialized food cups to prevent spillage, and water was offered in glass tubes propped up to one side of the cage; both were available ad libitum and were examined regularly.

Biological Experiment

Rats' Normal Diet

The diet based on basil included vitamin mixture (1%), corn starch (69.5%) 10% casein, 10% maize oil, 5% cellulose, 0.25% choline chloride, 0.35% methionine, and 4% salt mixture (Morsi, 1992).

Test diet baseline components included $Fe(C_6H_5O_7)$ $26H_2O$ (55 mg), $MgSO_4.2H_2O$ (20_4 mg), $CaCO_3$ (600 mg), NaCl (334 mg), $CaHPO_4.2H_2O$ (150 mg), K_2HPO_4 (645 mg), $ZnCl_2$ (0.5 mg), $CuSO_4.5H_2O$ (0.06 mg), $MnSO_4.4H_2O$ (10 mg), and K_1 (1.6 mg) (Hegsted *et al*., 1941).

The experimental baseline diet included Calcium pantothenic acid (0.40 mg), Vitamin K (0.50 Iu), Vitamin B12 (2.00 g), Vitamin E (10 Iu), Pyridoxine (1.00mg), Thiamin (0.50 mg), Folic acid (0.02 mg), Vitamin D (100 Iu), Niacin (4.00 mg), Para-amino – benzoic acid (0.02 mg), Vitamin A (200 Iu), Inositol (24 mg), Choline chloride (200 mg), (Campbell, 1963).

Diet Experiment

Table 1 illustrates the trial diet, which is ten percent of the basic diet enriched with plant powders.

Table 1. The basic and experimental diets' compositions.

(g) Component	Basal diet	5% Almond seeds	10% Almond seeds	15% Almond seeds
Test ingredients	---	5	10	15
Casein	20	20	20	20
Corn oil	4.7	4.7	4.7	4.7
Mineral mix	3.5	3.5	3.5	3.5
Vitamin mix	1	1	1	1
Cellulose	5	5	5	5
Cholin chloride	2	2	2	2
Sucrose	10	10	10	10
Corn starch	**Up to 100**	**Up to 100**	**Up to 100**	**Up to 100**

Induced Subfertility for Rats

Cadmium chloride (0.1%) injections at 1ml/kg body weight caused male subfertility in rats (Kini *et al*., 2009).

Rats

Albino Sprague-Dawley male rats of adulthood, with a body weight (B.Wt) of 150-160 g. The animals were taken from the Animal Laboratory when they were fourteen to sixteen weeks old. The samples were housed in neat, well-maintained plastic cages with stainless steel tops. Before the experiment, rats were fed the basal diet for one week to allow them to adjust. Ad libitum water was available from a small-mouth bottle attached to a metallic tube and a plastic tubing at the mouth. For seven days before we began with the research, the rodents were trained to a schedule of twelve hours of sunlight followed by the same hours of darkness, as was previously mentioned.

Experimental Design

The rats were kept in standard conditions, in wire cages at 25 degrees Celsius. Rats were separated into the following groups:

Group 1: Neutral control group consisting of six healthy rats. The rats in this group were fed the typical rodent diet and given only tap water.

Group 2: Subfertility induced rats (24 rats). These rats subdivided into 4 subgroups for eating up the diets in trials for (four) weeks consistent with the following:

Group 2: Six rats: Positive control group (untreated group).

Group 3: Six rats: Infertility rats fed on basal diet +5% almond seeds powder.

Group 4: Six rats: Infertility rats fed on a basal diet of +10% almond seeds powder.

Group 5: Six rats: Infertility rats fed on basal diet +15% almond seeds powder.

Biological Evaluation

Each day for the 28-day study, the amount of food consumed was noted, and body weight was recorded each week. The feeding efficiency ratio (F.E.R.), the body weight growth (B.W.G. %), and the organ weight were all calculated (Chapman *et al.*, 1959).

Blood Sampling

After the trial, samples from the blood were taken from the participants after they had fasted for twelve hours. After taking blood samples with highly specialized glass tubes using the retro-orbital approach, the blood samples were left to clot at room temperature (37 degrees Celsius) for a period of half an hour. Serum was extracted from blood samples by centrifuging them for 10 minutes at 3000 rpm before glucose testing. To prepare for analysis, we carefully aspirated the residue, transferred it to sterile polypropylene tubes with secure caps, and then froze it at -20 degrees Celsius.

The kidney, liver, heart, and, spleen were collected, rinsed in a salt solution, measured, as well as stored in ten percent formalin as instructed (Drury & Wallington, 1980).

Biological Evaluation

Body weight gain % (BWG%), Feed efficiency ratio (FER), food consumption, and feed efficiency ratio. Using the equation following (Chapman *et al*., 1959):

$$BWG\% = \frac{Final\ weight - Initial\ weight}{Initial\ weight} \times 100 \quad (1)$$

$$FER = \frac{Gain\ in\ body\ weight\ (g/day)}{Food\ Intake\ (g/day)} \quad (2)$$

$$The\ relative\ weight\ of\ organs = \frac{Organ's\ weight}{Animal\ body\ weight} \times 100 \quad (3)$$

Analytical Biochemistry

- *Measurement of Liver Enzyme Activity*
- *Measurement of (AST) Aspartate Aminotransferase Activity*

According to Reitman and Frankel (1957), the aspartate aminotransferase enzyme was measured with a spectrophotometer along with specific kits (BioMerieux).

- *Measurement of Serum (ALT) Alanine Aminotransferase Activity*

Reitman and Frankel (1957) established a calorimetric method for evaluating alanine aminotransferase enzyme activity.

- *Evaluation of Serum (ALP) Alkaline Phosphatase Activity*

Based on Roy's method from 1970, the colorimetric measurement of alkaline phosphatase was completed.

- *Determination of Follicle-Stimulating Hormone (FSH)*

Follicle-stimulating hormone was identified colorimetrically according to the method of (Fahim *et al*., 1982).

- *Determination of Luteinizing Hormone (LH)*

Luteinizing hormone was determined colorimetrically following the method of (Fahim *et al*., 1982).

- *Determination of Testosterone Hormone*

The levels of the hormone testosterone were measured colorimetrically using the technique of (Pardelles *et al*., 1985).

Histopathological Examination

After the time of the experiment had passed, the animals were sacrificed, then liver samples were collected, after which they were fixed in neutral formalin at a concentration of ten percent, dried in ethyl alcohol, purified in xylene, and at last embedded in paraffin wax. Hematoxylin staining, in addition, was carried out on slices that were around four and six microns thick (Carleton & Maycock, 1978).

Statistical Analysis

The statistical analysis was performed with a one-way classification as the basis for the computations. Snedecor and Cochran (1967) proposed an analysis of variance (ANOVA) that made use of the Least Significant Difference (LSD).

Results and Discussion

The initial goal of this trial was to estimate the positive effects of almond seeds found in the Al-Baha area in raising the level of fertility in subfertility rats.

Biological Changes

Influence of Almond Seeds on the Feed Efficiency Ratio, Body Weight Gain (g), and Feed Intake (g/d) in Subfertility Rats

Statistics offered in **Table 2** indicate the mean values of (FER) in addition to (FI) (g/d) for the negative control, (BWG) (g/d), positive control, and other diverse groups of subfertility rats fed on different levels of almond seeds. It could be observed that a decrease in mean values of body weight gain and feed efficiency ratio of the control (+) group took place which were (0.11±0.034g and 0.004±0.001) respectively, while (FI) value was increased (30.87±2.93 g/d). Percent increase of control (-) for BWG and FER were (609.09% and 850%) respectively, with values being; (0.78±0.089g and 0.35±0.005) respectively, but a 33.95% decrease in (FI) occurred being; (20.39±1.08) (g/d).

Table 2. Shows the Influence of almond seeds on the feed efficiency ratio, BWG (g), and feed intake (g/d) in subfertility rats.

Groups	Parameters					
	BWG(g)	% change of C (+)	FI (g/d)	% change of C (+)	FER	% change of C (+)
G1 C (-)	0.781±0.089 [a]	+609.09	20.39±1.08 [a]	-33.95	0.038±0.005 [a]	+850
G2 C (+)	0.11±0.034 [f]	--	30.87±2.93 [f]	--	0.004±0.001 [f]	--
G3: almond seeds Powder 5%	0.77±0.026 [b]	+600	24.06±2.37 [b]	-22.06	0.032±0.004 [c]	+700
G4: almond seeds Powder 10%	0.89a±0.067 [c]	+709.09	26.11±3.11 [c]	-15.42	0.034±0.003 [b]	+750
G5: almond seeds Powder 15%	0.35±0.022 [d]	+218.18	I3.09±2.44 [d]	-57.59	0.027±0.005 [d]	+575
LSD	0.104		4.S19		0.0064	

Effect of Almond Seeds Powder on Liver Enzymes (AST, ALT, ALP, and AST/ALT) (U/L) in Hepatic Rats

Data from **Table 3** showed a significant rise in aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase activity, and AST/ALT for control positive groups which were (105.08±1.59 U/L, 50.93±2.47 U/L, 269.55±1.67 U/L and 2.06±0.082) respectively, compared to control negative group which were (76.23±1.28 U/L, 43.0±1.99 U/L, 171.5±2.32 U/L and 1.773±0.01) respectively.

Due to feeding on almond seeds powder (5%, 10%, and 15%) the significant decreases of (AST, ALT, ALP also aspartate aminotransferase/alanine aminotransferase) for rats were evident compared with the control (+ve)- group. Maximum decrease of (AST, ALT, and ALP) recorded to G4 (10% almond seeds) respectively. While for AST/ALT ratio, the lowest value was recorded for G3 (5% almond seeds) which was (1.25±0.019) but revealed nonsignificant differences between G5 (15% almond seeds) for AST/ alanine aminotransferase ratio which were (1.586±0.012).

Table 3. Effect of almond seeds powder on liver enzymes (AST, ALT, ALP, and aspartate aminotransferase/alanine aminotransferase) (U/L) in subfertility rats

Groups	Parameters							
	AST (IU/L)	% change of C(+)	ALT (IU/L)	% change of C(+)	ALP (mg/dl)	% change of C(+)	AST/ALT	% change of C(+)
G1 C (-)	76.23b±l.28 [a]	-27.46	43.0 ±1.99 [a]	-15.57	171.5J±2.32 [a]	-36.38	l.77b±0.01 [a]	-13.93
G2 C (+)	105.08±1.59[f]	…	50.93±2.4[b]	…	269.5±1.67 [b]	…	2.06 ±0.082 [b]	…
G3: almond seeds Powder 5%	50.93±2.47 [d]	-58.60	34.80d±1.68	-31.67	156.57±1.99c	-41.91	1.25±0.019 [c]	-39.32
G4: almond seeds Powder 10%	69.55±1.67 [b]	-63.84	26.49c±1.55 [c]	-47.99	143.8±l .25d	-46.65	l.435±0.0 [d]15	-30.34
G5: almond seeds Powder 15%	60±0.082 [c]	-39.63	40.01±0.88 [f]	-21.44	186.22±2.07f	-30.91	1.58±0.012 [f]	-23.01
LSD	2.264		3.269		3.557		0.0636	

Effect of Almond Seeds Powder on Sexual Hormones of Subfertility Rats

1. Testosterone Hormone (ng/ml)

Data in **Table 4** illustrate the mean value of (testosterone) hormone (ng/ml) of subfertility rats fed on several diets. It could be observed that the mean value of (Testosterone) of the control () group was higher than control (+) group, being 2.35±0.01 and 0.38±0.05 respectively, designated significant variance with percent of increase +518.4% when compared to the control (+) group. All infertility rats fed on various diets revealed significant increases in mean values as compared to the control (+) group. The values were 0.86±0.02, 0.39±0.01 and, and 0.73±0.01 (ng/ml) for (5, 10 and and 15% almond seeds powder). The percent of increases were +126.3, +2.6, and +92.1 for groups 3, 4, and 5 respectively. Group 3 (5% almond seeds) recorded a better treatment of testosterone hormone as compared to the control (+) group.

2. Follicle Stimulating (FSH) Hormone (MLU/ ml)

The mean value of (FSH) of the control (-) group was higher than control (+) group, being 0.11±0.03 and0.27±0.01 respectively, **Table 4** suggested significant alteration with a percent of increase +62.96% when compared to the control (+) group. Groups 3, 4, and 5 for (5, 10, and 15% almond seeds powder) revealed significant decreases in mean values as compared to the control (+) group. The measurements were 0.18±0.05 and 0.12±0.01 (MLU/ml). The percent of increases were +80.00, +20, and +50 for groups 3, 4, and 5 respectively. Group 5 (15% almond seeds) documented the best treatment of FSH hormone

3. Luteinising (LH) Hormone

The average level of luteinizing hormone (LH) hormone (MIU/ml) in subfertility rats fed the various diets is displayed in **Table 4**. It was discovered that there was a significant distinction between the control (+) as well as control (-) groups, with the control (-) group

having a lower mean value of (LH) than the control (+) group (0.11±0.009 vs. 0.17±0.002), indicating a reduction of 35.30 percent. All subfertility rats fed on various diets revealed significant decreases in mean values as compared to the control (+) group. The values were 0.14±0.005, 0.115±0.0009, and 0.11±0.003 (MIU/ml) for (5, 10 and 15% almond seeds powder) respectively. The percent of decreases were -17.7, -32.4 and-35.3 for groups 3, 4, and 5 respectively. Group 5 (15% almond seeds) was found to be the better treatment of LH hormone.

Table 4. Effect of almond seeds powder on sexual hormones (Testosterone hormone, (FSH) hormone, and (LH) hormone) in subfertility rats.

Groups	Parameters					
	(Testosterone) hormone(ng/ml)	% change of C(+)	(FSH) hormone (MLU/ml)	% change of C(+)	(LH) hormone (mLU/ml)	% change of C(+)
G1 C (-)	2.35a ±0.01	+518.4	0.27±0.01 [a]	+62.96	0.11 ±0.009 [c]	-35.3
G2 C (+)	0.38f ±0.05	-	0.1±0.03 [e]	-	0.17 ±0.002 [a]	-
G3: almond seeds Powder 5%	0.86b ±0.02	+126.3	0.18±0.05 [b]	+80	0.14 ±0.005 [b]	-17.7
G4: almond seeds Powder 10%	0.39e ±0.01	+2.6	0.12±0.01 [c]	+20	0.11 ±.0009 [c]	-32.4
G5: almond seeds Powder 15%	0.73c±0.01	+92.1	0.15±0.07 [b]	+50	0.11 ±0.003 [c]	-35.3
LSD	0.02	-	0.01	-	0.009	-

- *Histopathological Results*

Microscopic examination of the testicular tissue of the control rats showed control rat screening normal histology of the seminiferous tubules with normal spermatogoneal cells layers with mature sperms filling the lumens While the testes of control-positive rats presented marked diffuse coagulative necrosis of the seminiferous tubules which showed nuclear ruminants of the necrotic spermatogonial cells, degenerated sperms local calcium deposits **(Figure 1)**. Regarding the treated groups. Almond seed powder (5%) significantly reduced damage to the testicles and stimulated spermatogenesis in the majority of seminiferous tubules in control-positive males **(Figure 2)**. Seminiferous tubules demonstrate coagulative necrosis in the testes of a control-positive rat subjected to treatment (ten percent almond seeds powder)**.** Treatment with fifteen percent almond seed powder led to a thickening of the blood vessel wall and coagulative necrosis of the seminiferous tubules in the testes of control-positive rats **(Figure 3)**.

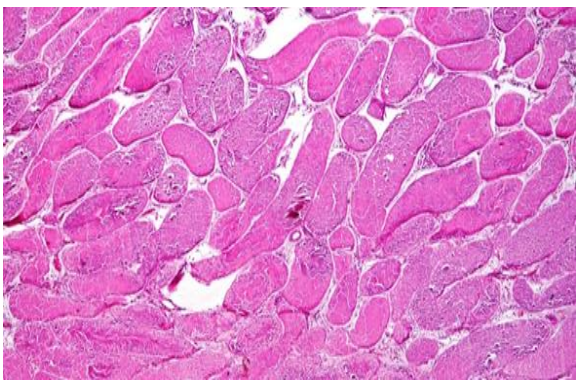

Figure 1. Testis of control positive rat showing marked diffuse coagulative necrosis of the seminiferous tubules (arrow). (H and E x 100)

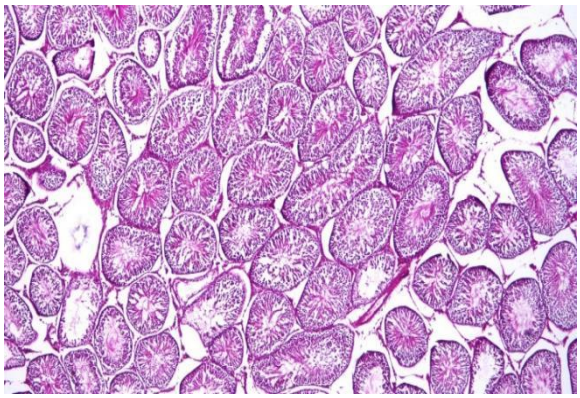

Figure 2. Testis of control positive rat treated with (5 % almond seeds powder) showing marked protection of the seminiferous tubules with active spermatogenesis (arrow) in most of them and active sperms in their lumen. (H and E x100)

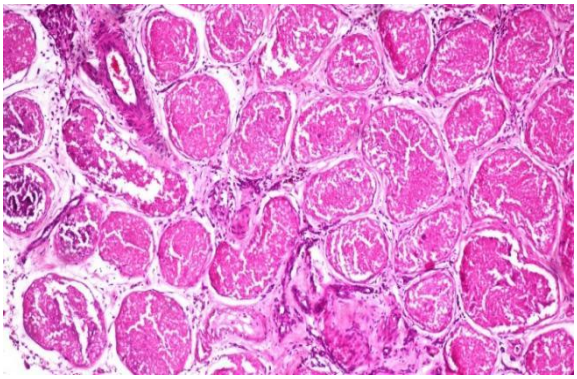

Figure 3. Seminiferous tubules in the testes of a control-positive rat treated with (15%) almond seeds powder revealed coagulative necrosis (CN), with some tubules exhibiting dystrophic calcification (dotted arrow) and a small number of inflammatory cells infiltrating the interstitial spaces (arrow) with swelling (Ed). (H x 100) "E"

Our results in **Tables 2-4** are in the same line as those obtained by Keser *et al*. (2000) who discovered that relative to a control group, consumption of almond seeds enlarged sperm count as well as luteinizing hormone, testosterone, and follicle-stimulating hormone concentrations in blood serum. Based on the findings of this trial, almond seeds can operate as a moderating agent for male reproductive potential by altering reproductive activity as well as positively impacting testes to boost testosterone density. (Berryman *et al.,* 2015) reported that almond seed extract caused a significant increase in serum levels of testosterone in serum. (Kernel, 2014) It is a micronutrient with the potential to improve sperm quality via antioxidant and anti-apoptotic effects. Zinc is required for spermatogenesis because it catalyzes the reactions of metalloenzymes that transcribe DNA as well as synthesize proteins. Semen volume and serum testosterone were both lowered due to severe zinc depletion when compared to normal zinc consumption. Linoleic acid and the fat-soluble vitamins calciferol, tocopherol, and retinol, along with phylloquinone are delivered by almond seed oil (Azimi Zadeh & Ahmadi*,* 2018; Rusu *et al.,* 2019). As well as the bioactive macronutrients already mentioned, almond seeds contain a plethora of additional nutrients that may improve metabolic and cardiovascular health. They often have a high L-arginine content and are a great protein source (accounting for around 25% of the calories). Intake of nuts may assist in enhancing vascular responsiveness because they include a precursor to the endogenous vasodilator nitric oxide (NO), as will be explained below. These findings corroborate those of (Bowen *et al*. 2019), who discovered that, in their less mature forms, almond seeds have a high enough soluble fiber content to help prevent diabetes-related damage. It is widely established that insulin resistance is caused by a diet high in excess calories. Almond seeds have been lauded for their health benefits as well as antioxidant properties, especially their impact on biochemical markers, enzyme activities, and lipid peroxidation (Azimi Zadeh & Ahmadi, 2018). Almonds may help reduce blood pressure due to their potassium, vitamin E, and calcium content.

Recommendations

1. For subfertility cases, different almond seed levels are recommended.
2. Almond seeds at dissimilar concentrations, especially 15%, can improve subfertility in male rat

Conclusion

The results showed that almond seed has a strong effect in Increasing the fertility of subfertility rats and the improvement rate increased in the group containing 15% almond seed, because it contains sterol and phenolic acids which consider antioxidant that might increase antioxidant enzyme activities such as superoxide dismutase, catalase, and glutathione peroxidase which improves fertility.

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Ethics statement: None

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