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Evaluate the effect of Alcoholic Extraction of Walnut on Gonadal Hormones of Treated Rats

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ABSTRACT

Infertility, which is typically defined in the scientific context as the failure to conceive after 12 months of frequent unprotected intercourse, is one occurrence that has become increasingly characterized as a medical disease. Walnut (*Juglanse persica*), a significant crop that produces nutritious nuts, and a vital components of good diets have been linked to a longer lifetime. Some walnut by-products, such as leaves or green husks, have been described and shown to contain beneficial chemicals such as tocopherols and phenolic compounds. The samples included 3-month old adult male white rats with an average weight of 150-250 grams. The experiment employed 60white adult male rats for 60 days, from May 15 to July 15th, 2021. The rats were split into three groups at random, each with 12 rats. After receiving the appropriate food and drink, the first group returned to the control group. The results showed significant increases in LH, FSH and Testosterone levels ($p<0.05$) of blood serum in rats treated with walnut extract compared to those in the control group. Finally, walnut intake and delivery of walnut seeds resulted in the increased LH, FSH, and Testosterone levels, as well as elevations in the viability and sperm production of male albino rats.

Key Words: Rat, Walnut, Testosterone, LH, FSH.
Article type: Research Article.

INTRODUCTION

Walnut is the world's oldest tree food, having been around for thousands of years. It is first cultivated in ancient Persia, hence known as the Persian walnut (Bostani *et al.* 2014). It is high in antioxidants, omega-3 fatty acids, and vitamin E, as well as minerals such as iron, sodium, calcium, magnesium, manganese, copper, potassium, and phosphorus, along with protein and fiber, making it a varied and healthy meal (Adelakun *et al.* 2019). Walnut is a member of the Juglandaceae family and has long been utilized in traditional medicine across the world. The genus Juglandaceae gets its name from the presence of 5 hydroxyl-1 and 4- naphthoquinonein in its leaf, fruit shell, wood, and root. Walnut oil is also high in omega-3 fatty acids, which are important for human health (Kamoun *et al.* 2021). Linoleic, oleic and linoleic acids are the three main fatty acids present in walnut oil. Monounsaturated and polycyclic unsaturated fatty acids (MUFA and PUFA) have been found to play a function in the prevention and lowering the risk of cardiovascular disease (Chijoke *et al.* 2017; Mohammed & Hussein 2020). Walnut intake (both kernel and oil) has been shown to decrease blood cholesterol levels (Hassan *et al.* 2021). Its oil contains antioxidant qualities and has been proven to lower the risk of coronary heart disease, inflammation, and is beneficial in the treatment of skin disease and high blood pressure in studies (Kara *et al.* 2019). Its kernels are being used to lower blood lipids by increasing high density lipoprotein, while decreasing

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low density one (Ghorbani *et al.* 2014). It is also useful for treating type 2 diabetes and improving cardiovascular flexibility (Kalgaonkar *et al.* 2011). It has been claimed to provide protection against some forms of cancer due to its high content of natural antioxidants. Infertility, which is typically defined in the scientific context as the failure to conceive after 12 months of frequent unprotected intercourse, is one occurrence that has become increasingly characterized as a medical disease. The medicalization of infertility began in earnest in the United States in the 1950s with the invention of fertility medications, but it has been accelerated with the advent of assisted reproductive technologies (ART) such as in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI; Thompson 2005). Infection, damage, toxin exposures, anatomic variations, chromosomal abnormalities, systemic illnesses, and sperm antibodies are all causes of male infertility. Smoking, alcohol usage, obesity, and advanced age are all possible risk factors; however, the evidence is limited by a paucity of pregnancy-related outcomes (Lindsay & Vitrikas 2015). A semen analysis is the first step in the laboratory examination. Abstinence from ejaculation for 48 to 72 hours should be part of the sample collection instructions. Because the time it takes for sperm to mature is slightly over two months, it is best to wait three months before sampling again (Pacey *et al*. 2014). Ovulation problems, uterine abnormalities, tubal blockage, and peritoneal issues are all causes that contribute to female infertility. Cervical factors are considered to have a modest part in the development of cervical cancer, but they are seldom the only cause. Cervical mucus evaluation is unreliable, therefore investigations are ineffective in the treatment of infertility (Practice Committee of the American Society for Reproductive Medicine 2012). Normal spermatogenesis, or the process by which immature spermatogonia in the testis divide and differentiate into the mature elongated spermatid form, which is then discharged from the seminiferous epithelium, is required for normal male fertility (Nishimura & Hernault 2017). The advancement and maturation of sperm through the excurrent duct system and the epididymis is also necessary for the discharged spermatozoa to reach its full fertilizing potential. Spermatogenesis is the division, differentiation, and meiosis of immature germ cells in order to produce haploid elongated spermatids. This occurs in the testis seminiferous tubules, in close proximity to the Sertoli cells, which are somatic cells of the seminiferous epithelium (Carreau & Hess 2010). The most essential biological aim of humans is to have children. Male factor infertility is a causal agent in half of the instances. There is evidence that certain male reproductive issues are linked to issues with the spermatogenesis process (Purkayastha & Mahanta 2012). Proper pituitary secretion of folliculostimulating (follicle-stimulating hormone – FSH), luteinizing hormone (LH), and testicular secretion of testosterone are required for optimal spermatogenesis. Pituitary gonadotropins regulate hormonal testicular function: LH increases the synthesis of sex steroids via Leydig cells, while FSH and testosterone operate on seminiferous tubules via Sertoli cells to sustain and maintain spermatogenesis (Mares *et al.* 2012). Hormones indirectly affect spermatogenesis by regulating the activity of somatic cells, particularly Sertoli cells. Spermatogenesis is governed directly by the local regulatory system's function. FSH and androgen receptors are absent in gametogenic cells. Sertoli cells, being the epithelium's only receptor cells for these hormones, provide the signal transmission system necessary at various phases of spermatogenesis (Wdowiak *et al*. 2014). Testosterone is an androgen-like steroid hormone present in mammals, reptiles, birds, and other animals (Kelly & Jones 2015). Testosterone is predominantly produced by males' testes and females' ovaries in animals; however, tiny quantities are also produced by the adrenal glands (Nieschlag *et al.* 2012). It is the primary male sex hormone as well as an anabolic steroid. Testosterone plays an important function in the development of male reproductive tissues such as the testis and prostate in males. Furthermore, it enhances secondary sexual characteristics including muscle gain, bone mass, and hair growth (Snyder *et al.* 2016). Testosterone is also necessary for good health and the prevention of osteoporosis (Kloner *et al.* 2016). Testosterone is a kind of androgen produced by the sterol C-19. Testosterone, like all other steroid hormones, is predominantly produced in the testes' Leydig cells from cholesterol. The testicular Leydig cells are affected by the central nervous system (Ohlander *et al.* 2016). The pituitary gland is controlled by the brain, which produces luteinizing hormone (LH) and follicle-stimulating hormone (FSH) in both sexes. Females, on the other hand, mostly generate testosterone, whereas men primarily create estrogen (Kumar & Sait 2011). Different hormonal responses can also be created depending on the kind of receptor present in cells. The hypothalamus generates gonadotropin releasing hormones (GnRH), which drive FSH and LH from the pituitary gland to enter the bloodstream and then travel to the Leydig cells, where they make testosterone (Mitri *et al*. 2014). Walnut, on the other hand, can be considered as an effective drug for impotency because of its compounds such as niacin, which helps the adrenal gland produce steroid hormones; arginine and aspartic acid, which stimulate GnRH and LH; oleic acid, which inhibits 5-alpha reductase; and bromine (Br), which increases

estrogen in postmenopausal women (Akomolafe & Oboh 2018). Because there have been few studies on the effects of walnut oil on the reproductive system. The present study was conducted to investigate the effect of walnut oil on hormonal changes in the reproductive system of male rats, its findings could be useful in the field of walnut oil pharmaceutical applications, particularly in the reproductive system.

MATERIALS AND METHODS

Animals

Adult male white rats, 3-months in age and 150-250 g in weight, were employed in this study. It was taken from the College of Veterinary Medicine's animal home at the University of Kufa. They were kept in plastic cages and provided access to all of the required food and water. In addition to replacing the floor of the cages twice a week to keep the animals clean, the animals were given a month to adapt before examinations.

Preparation of the alcoholic extract of walnut pulp

The walnut pulp was crushed and then steeped for 48 h at room temperature in alcohol (ethanol 75%), with 5 L ethanol per kilogram of walnut pulp. The mixture was strained using a filter cloth after 48 h to remove the walnut hulls. The mixture was then placed in a 5-liter Pyrex and baked for 24 h at 45 °C, where the alcohol was evaporated and a dark brown extract with a soft dough-like consistency was formed. A sensitive scale was used to weigh the extract, yielding 75 g of extract per kg. The extract was kept in a firmly-sealed glass jar and stored in the freezer for further examinations.

Blood sample collection

On days 30 (Treatment 30 = T_{30}) and 60 (Treatment 60 = T_{60}), from three groups (G) including G1 (which treated without alcoholic extract of walnut), G2 (treated with 10 mg/kg alcoholic extract) and G3 (treated with 20 mg kg^{-1} alcoholic extract), blood samples were collected by drawing 3-mL blood from the heart after anesthetizing the animal, placing the samples in test tubes containing a gel, then centrifuging at 3000 rpm.

Measuring the level of some hormones

The enzyme linkage immunosorbent assay (ELISA) method was used to measure the hormonal blood parameters including LH, FSH, testosterone and progesterone (ng dL^{-1}).

RESULTS AND DISCUSSION

Table 1 depicts the impact of alcoholic extract of walnut on LH concentration, where there is variance in values across examined groups that were treated with alcoholic extract of walnut in male rats, where the total LH concentration for groups was calculated. The mean concentration was higher in T_{30} compared to control, which might be related to the walnut action against citalopram antidepressant, whereas the mean of LH was higher in T_{60} compared to control and T_{30}.

Table 1. Serum LH levels (ng dL^{-1}) of male rats treated with alcoholic walnut extract.

Parameters	**LH concentration**		p-values
Treatments	**Treated groups (Mean ± SD)**	**Control group (Mean ± SD)**	
T_{30}	23.37 ± 2.79	16.8 ± 1.25	0.03
T_{60}	13.62 ± 4.3	16.4 ± 1.25	0.07

Fig. 1 illustrates the mean concentration of serum LH in T_{30} for each group (G), where the mean (± SD) in G2 (treated with 10 mg kg^{-1} alcoholic extract of walnut) was17.7 ± 3.21, while in G3 (treated with 20 mg kg^{-1} alcoholic extract) was 33.5 ± 3.4 and in control group (G1) was 16.3 ± 3.15. These results show the positive effect of alcoholic extract of walnut by elevated doses. Our results were in agreement with Mokhtari *et al.* (2012) who worked on the concentration of LH, FSH and testosterone.

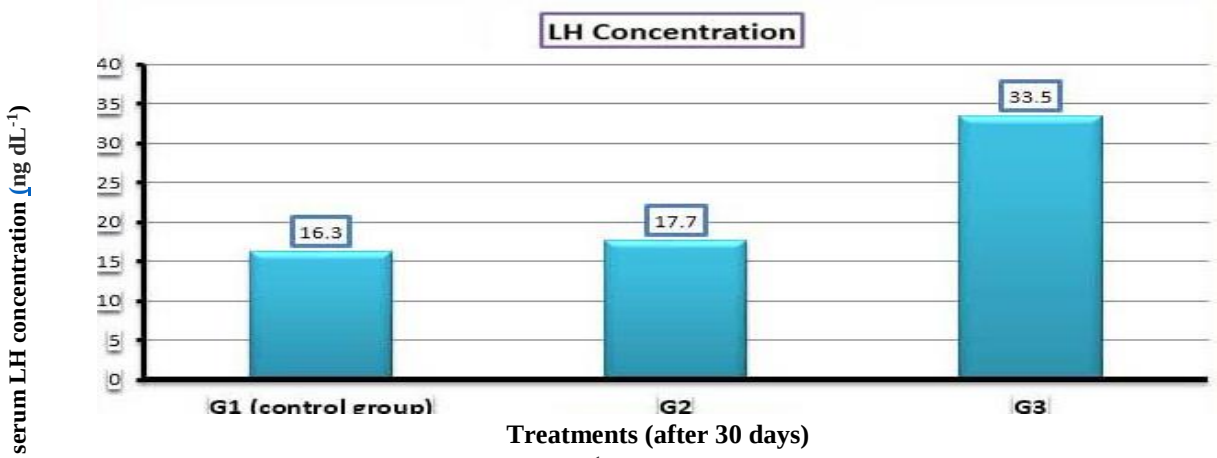

Fig. 1. The mean serum LH concentration (ng dL^{-1}) in male rats treated with walnut extract among groups of T_{30}.

Fig. 2 show the mean concentration of serum LH in T_{60} for each group, where the mean (± SD) in G2 (treated with 10 mg kg^{-1} alcoholic extract) was 18.66 ± 2.7, while in G3 (treated with 20 mg kg^{-1}) was 18.3 ± 2.52 and in G1 or control group was 17.2 ± 2.25. The results of T_{60} significantly decreased in comparison with T_{30}. Plant-based medication has been man's ultimate therapeutic agent over the years and is still in the frontline for improving human health.

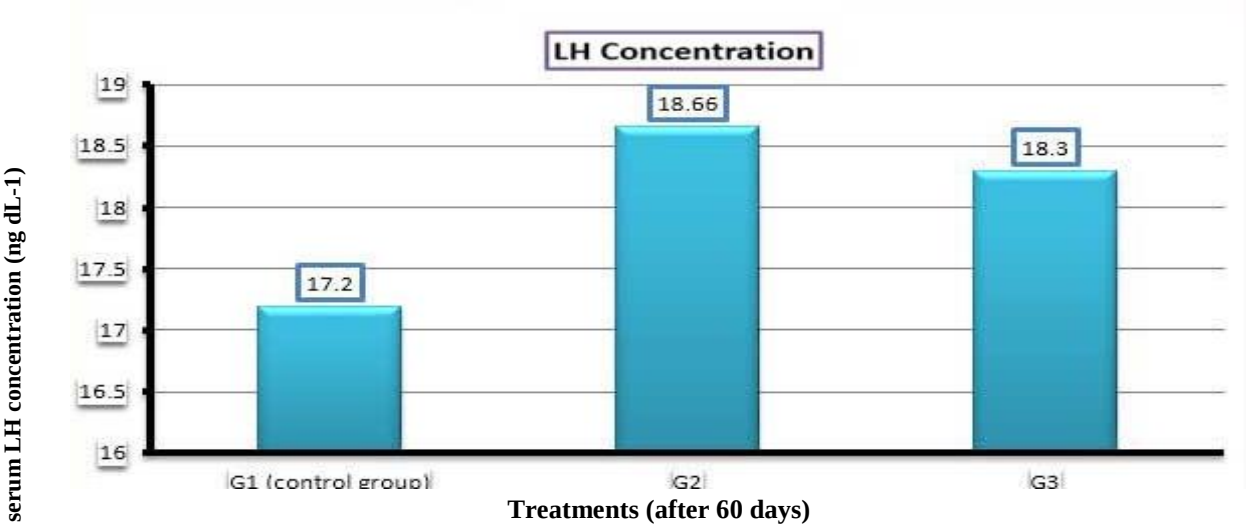

Fig. 2. The mean serum LH concentration (ng dL^{-1}) in male rats treated with walnut extract among different groups in T_{60}.

Table 2 depicts FSH concentration, where there is variation in values among studied groups that was treated with alcoholic extract of walnut in male rats. The total FSH concentration in all groups (Gs), was high for T_{30} in comparison with control, which may be due to the walnut effect on hormones, while on the other hand, the mean of FSH in T_{60} was higher than in control and in T_{30}.

Table 2. Serum FSH levels (ng dL^{-1}) of male rats treated with alcoholic walnut extract.

Parameters	**FSH Concentration**		**p-values**
Treatments	**Treated groups (Mean ± SD)**	**Control group (Mean ± SD)**	
T_{30}	217.5 ± 9.24	121.81 ± 1.7	0.02
T_{60}	271.5 ± 12.5	123.53 ± 2.9	0.03

Fig. 3 show the mean concentration of serum FSH in T_{30} for each group. where the mean (± SD) in G2 was 189.5 ± 26.4, while in G3 and G1 were 197.7 ± 74.5 and 125.4 ± 2.8 respectively. These results show the positive effect of alcoholic extract of walnut by increasing the dose.

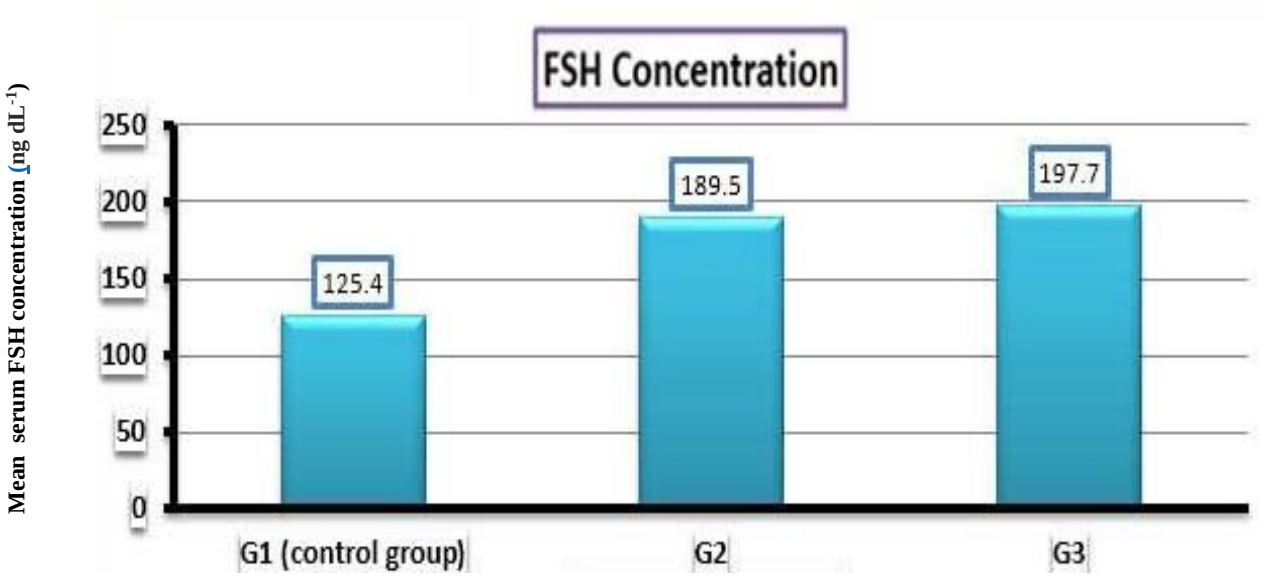

Fig. 3. The mean serum FSH concentration (ng dL^{-1}) in male rats treated with walnut extract among different groups in T_{30}.

Fig. 4 depicts the mean concentration of serum FSH in T_{60} for each group (G), where the mean (± SD) in G2 was 188.7 ± 20.4, while in G3 and G1 were 201.5 ± 25.7 and 127.5 ± 2.7 respectively.

Fig. 4. The mean serum FSH concentration (ng dL^{-1}) in male rats treated with walnut extract among groups in T_{60}.

Table 3 shows the effect of walnut on testosterone concentration, where there are some variation in values among studied groups treated with alcoholic extract of walnut in male rats. The total testosterone concentration in all groups of control was 2.9 ± 0.15, whereas the mean concentration was high in T_{30} (3.5 ± 1.81) in comparison with control, which may be due to walnut effect on the hormone secretion. On the other hand, in T_{60}, we found that the mean of testosterone was higher (4.7 ± 1.38) than in control and in T_{30}.

Fig. 5 depicts the mean concentration of serum testosterone in T_{30} for each group (G), where the mean in G2 (± SD) was 3.5 ± 0.4, while in G3 and G1 were 6.9 ± 1.5 and 3.29 ± 1.8 respectively. These results show the positive effect of alcoholic extract of walnut by increasing the doses.

Table 3. Serum testosterone levels (ng dL^{-1}) of male rats treated with alcoholic walnut extract.

Parameters	Testosterone concentration		p-values
Treatments	Treated group (Mean ± SD)	Control group (Mean ± SD)	
T_{30}	3.5 ± 1.81	2.9 ± 0.15	0.78
T_{60}	4.7 ± 1.38	3.12 ± 0.17	0.52

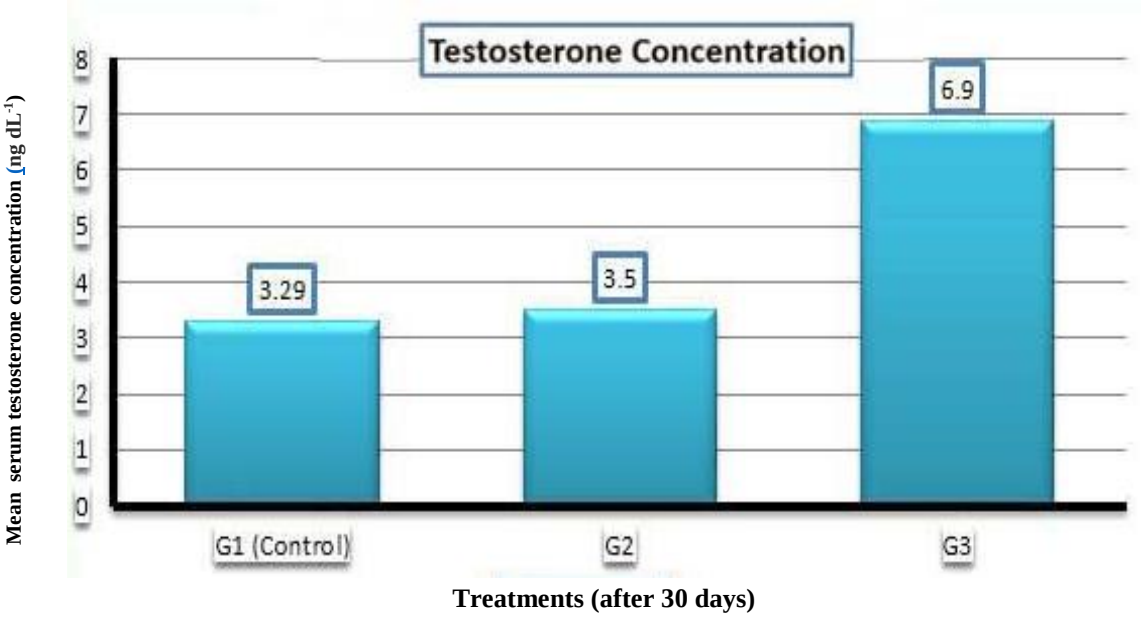

Fig. 5. The mean serum testosterone concentration (ng dL^{-1}) in male rats treated with walnut extract among differet groups in T_{30}.

Fig. 6 illustrates the mean concentration of serum testosterone in T_{60} for each group (G), where the mean (± SD) in G2 was 2.93 ± 0.54, while in G3 and G1 were 3.9 ± 1.7 and 3.29 ± 2.7 respectively.

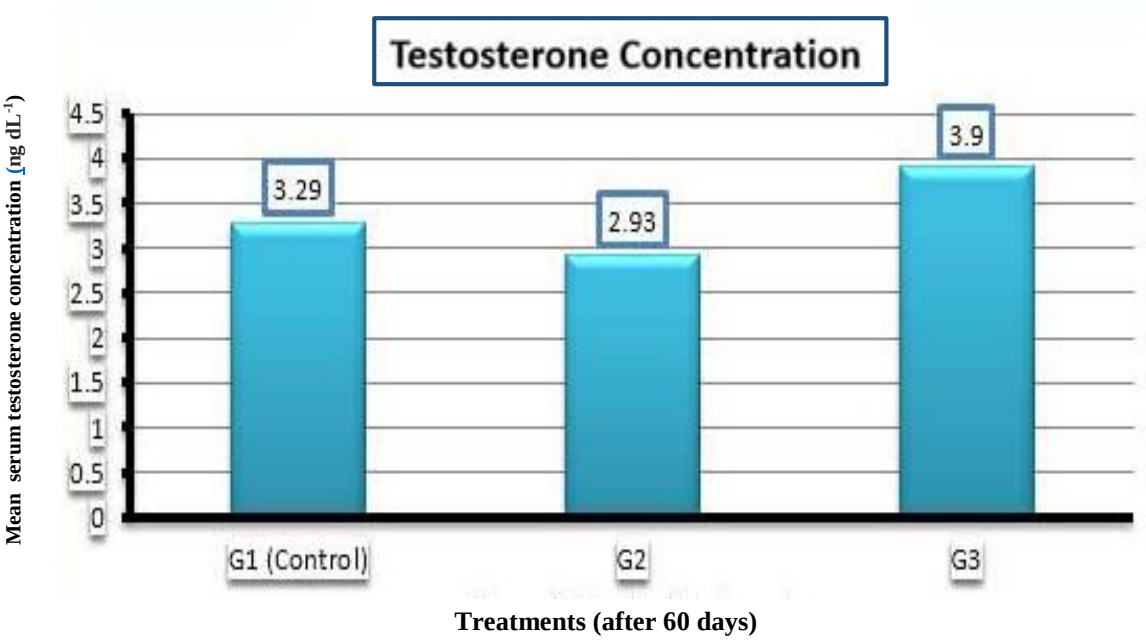

Fig. 6. The mean serum testosterone concentration (ng dL^{-1}) in male rats treated with walnut extract among different groups in T_{60}.

Table 4 indicates the effect of walnut on progesterone concentration, where there are some variations in values among studied groups treated with alcoholic extract of walnut in male rats. The total progesterone concentration for all groups in control was 0.9 ± 0.05, whereas the mean concentration was higher in T_{30} (1.2 ± 0.07) in comparison with control, which may be due to walnut effect. On the other hand, in T_{60}, we found that the mean of progesterone was higher (1. 87 ± 0.09) than in control and in T_{30}. Fig. 7 presents the mean concentration of serum progesterone in T_{30} for each group (G), where the mean in G2 was 1.2 ± 0.04, while in the G3 and G1 were 1.55 ± 0.08 and 0.7 ± 0.04 respectively, exhibiting that the positive effect of alcoholic extract of walnut by increasing the doses.

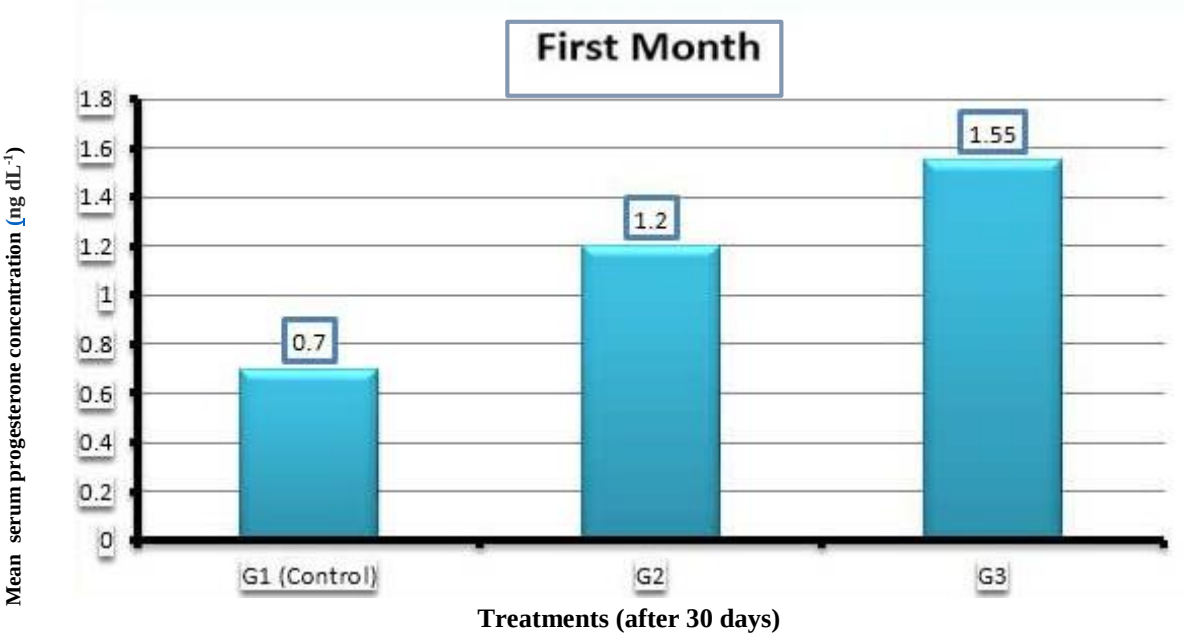

Fig. 7. The mean serum progesterone concentration (ng dL^{-1}) in male rats treated with walnut extract among different groups in T_{30}.

Fig. 8 depicts the mean concentration of serum progesterone in T_{60} for each group (G), where the mean in G2 (± SD) was 1.34 ± 0.03, while in G3 and G1 were 1.9 ± 0.07 and 0.98 ± 0.007 respectively.

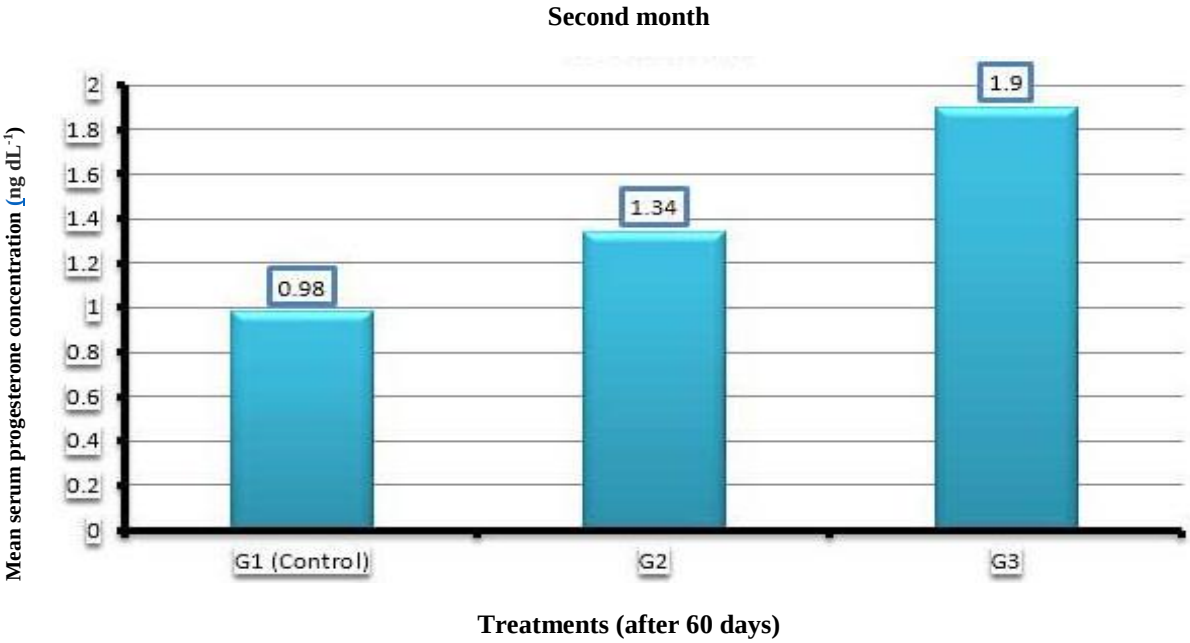

Fig. 8. The mean serum progesterone concentration (ng dL^{-1}) in male rats treated with walnut extract among groups in T_{60}.

Table 4. Serum progesterone levels (ng dL^{-1}) of male rats treated with alcoholic walnut extract.

Parameters / Treatments	Progesterone concentration		p-values
	Treated group (Mean ± SD)	Control group (Mean ± SD)	
T_{30}	1.2 ± 0.07	0.9 ± 0.05	0.73
T_{60}	1. 87 ± 0.09	0.78 ± 0.03	0.04

CONCLUSION

In conclusion, walnut intake and delivery of walnut seeds resulted in an increase in the levels of LH, FSH and Testosterone, as well as an increase in the viability and sperm production of male albino rats.

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