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Research Article

Effect of oral administration of collagen-α® reproductive activity and growth efficiency of mature male rabbit

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Abstract: Collagen supplements are widely used for their bioactive properties, affecting cellular and tissue health, and elasticity with high repair and conditioning effects. This study was designed to evaluate the effects of oral administration of Collagen-α® on the health and reproductive performance of male rabbits. Animals were randomized into three groups (6 rabbits per group) including Group I (control group) treated with 1ml of distal water orally, Group II treated with 1ml of Collagen-α® orally for 15 days, and Group III treated with 1ml of Collagen-α® orally for 30 days. Body weight and body weight changes, organ weights, and histopathological evaluation of the testis were recorded. The study showed that the collagen-alpha treatment group had significantly lower body weight and organ weight than the control group. Furthermore, various changes in histopathology and parameters related to testicular function were found in the collagen alpha group compared to the control group.

Keywords: Collagen-α, Growth efficiency, Reproductive activity, Mature male rabbit.
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Introduction

A diet supplement is a health-beneficial product that includes a concentrated amount of nutrients or other components having favorable physiological effects. It comes in powder, capsule, powder in sachets, liquid in a bottle with a dropper, and other forms to suit the right amount. Dietary supplements are not medications, and their usage is not controlled by pharmacopeia (Wrzeniewska et al. 2007). Collagen is the primary structural protein of many connective tissues, including skin, bone, cartilage, and tendons, and accounts for about one-third of all protein in the human body. It is derived from collagen-rich substances and boiling water, which is known as gelatin. Gelatin is extracted from a variety of sources, and from different animal parts, such as pig skin (46%), bovine hide (29.4%), and bone (23.1%). However, due to faiths, cultures, and health concerns, fish gelatin has earned a lot of attention in recent years (Ali et al. 2016). Additionally, hydrolyzed collagen is the product of further enzymatic hydrolysis of gelatin (CH). Many nations and regions, including the United States, Europe, China, and Japan, have long employed CH in medicine and food. The Food and Drug Administration (FDA) center for food safety and nutrition has approved CH as Generally Recognized as Safe (GRAS). The bioavailability and absorption of CH have also received a lot of attention. CH is more easily absorbed and has better bioavailability than gelatin (Ohara et al. 2010; Hatanaka et al. 2014).

Aging and a poor diet can reduce the amount of collagen in the body. These alterations are not perceptible in the early stages of life but become apparent in the maturity period when food consumption does not reach the necessary

Table 1. Effect of collagen-α® on body weights (kg) and organs weights (g) of mature male rabbit.

Groups	Initial BW (k)	Final BW (k)	BW changes (k)	Liver weight (g)	Kidney weight (g)	Testes weight (g)
G1 (Control)	1.49±0.09^{Aa}	1.64±0.045^{Aa}	0.38±0.14^{Ab}	70.39±5.62^{Ac}	8.63±1.20 ^{Aa}	6.00±0.62^{Aa}
G2	1.51±0.06 ^{Aa}	1.25±0.048^{Aa}	-0.34±0.102 ^{Bb}	58.63±5.73^{Aa}	6.99±0.75^{Aa}	5.21±0.74^{Aa}
G 3	1.50±0.08^{Aa}	1.22±0.09^{Aa}	-0.28±0.09^{Aa}	52.67±3.93^{Bc}	6.52±0.58Aa	5.11±0.67^{Aa}
LSD	NS	0.41	0.10	12.52	1.32	0.85

Capital letters denote significant differences within groups, and small letters denote differences between groups (P<0.05) (Mean±SD).

requirements for energy. At this point, skeletal and joint diseases also increased. A balanced diet is vital not only for the prevention of chronic diseases but also for the maintenance of a healthy body and its optimal functioning (Alemán et al. 2011; Nakchum & Kim 2016). Hence, this study aimed to investigate the effects of oral administration of Collagen-α® on the health and reproductive performance of male rabbits.

Materials and Methods

Mature male rabbits were purchased from the Basrah market and acclimated for 10 days prior to the experiments and had free access to standard food and water. Mature male rabbits with weights of 900-1000gr were housed under controlled environmental conditions in the Veterinary Animal House, College of Veterinary Medicine, Basrah University. Eighteen rabbits were divided into three groups (2 animals per cage and 6 animals/group) as follows (G1) the control group was orally administered with 1ml of normal saline, (GII) (collagen-α 15) was orally administered with 1ml of collagen-α® for 15 days, and the GIII (collagen-α 30) was orally administered with 1ml of collagen-α® for 30 days.

The blood samples (5 ml) were taken by cardiac puncture from sedated animals from the heart using a 5ml sterile syringe and put into gel tubes to isolate serum. Then serum was centrifuged (3000 rpm/15 min), and kept at -20ºC till analysis. From the sacrificed animals, the testes were then removed. Testosterone hormone was measured using a special kit according to (Young 1995). Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH) were measured using an enzyme test kit (Human GmbH.53020 Wiesbaden. Germany Gesellechalf for biochemical and diagnostic mbH) (Tietz 1996).

Estimation of sperm characteristics: The sperm count was done according to (Bartels et al. 1971; Tietz 2006) and for the percentage of sperm motility, the individual motility of the sperms was performed based on (Chemineau et al. 1991). For measuring the abnormality of sperm, the percentage of abnormal spermatozoa was counted as suggested by (Evans & Maxwell 1987).

Results

Growth performance: The effects of oral collagen supplementation on the growth performance of male rabbits are shown in Table 1. GII and GIII showed a significant decrease in body weight (P<0.05). Organ weights, liver, kidneys, and testis also showed a significant decrease in the treatment groups (P<0.05).

Effect of collagen-α® on LH, FSH, and testosterone hormone in mature male rabbits: Significant differences were found between all groups. The LH, FSH, and testosterone hormones significantly (P≤0.05) decreased in group GIII more than in the other two other groups and also a significant decrease was recorded in GII compared to GI (control) (Table 2).

Impact of collagen-α® on sperm account, motility%, viability%, and abnormal sperm% of mature male rabbits: The sperm account, motility%, viability%, and abnormal sperm % decreased significantly (P<0.05) in GII and GIII compared to the control group, but these decrements in GIII significantly more than in GII (Table 3).

Histopathological examination: Figure 1 shows the

Table 2. Effect of collagen-α® on LH, FSH and testosterone hormone concentrations in themature male rabbit.

Parameters / Groups	LH (IU/L)	FSH (IU/L)	Testosterone (ng/dl)
G1 (Control)	0.30±0.08[a]	0.32±0.08 [a]	1.34±0.04[a]
G2 (Collagen-α®15)	0.20±0.05[b]	0.22±0.06[b]	1.06±1.38[b]
G3 (Collagen-α®30)	0.15±0.02[c]	0.12±7.32[c]	0.86±0.08[c]
LSD	0.175	0.10	0.21

Small letters denote differences between groups at level (P<0.05) (Mean±SD).

Table 2. Effect of collagen-α® on sperm account, motility, viability, and abnormal sperm of the male rabbit.

Parameters / Groups	Sperm account	Motility %	Viability%	Abnormality %
G1 (Control)	85.33±1.82 [a]	90.73±1.95 [a]	88.75±3.20 [a]	13.05±1.63 [a]
G2 (Collagen-α®15)	78.41±0.84 [b]	75.0±3.92 [b]	78.22±2.47 [b]	39.50±2.31 [b]
G3 (Collagen-α®30)	67.0±0.92[c]	65.44±3.43[c]	70. 43 ±3.65[c]	44.23±1.76 [b]
LSD	8.43	9.65	5.76	10.54

Small letters denote differences between groups at level (P<0.05) (Mean±SD).

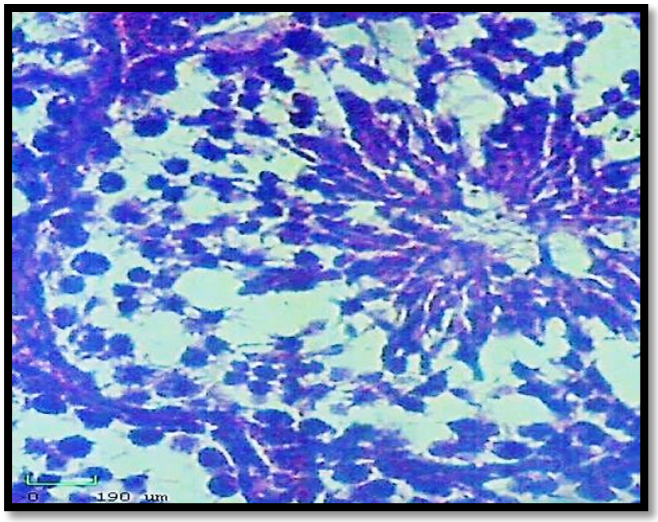

Fig.1. Histological section of testes of group I (Control group) showed normal architecture of seminiferous tubules (H&E, 400X).

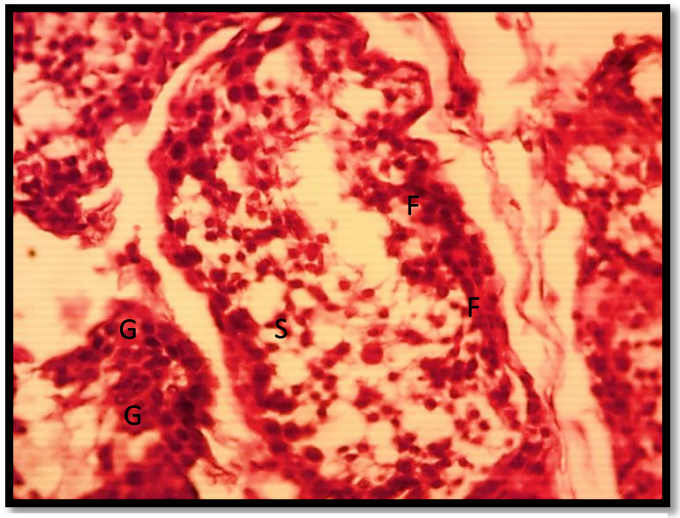

Fig.2. Histological section in testis group II (collagen-α®15) showed seminiferous tubule: there's complete suppression of spermatogenesis (S) and present of giant cells (spermatid) (G) in lumen of tubules and also there are areas of fibrosis surrounded the seminiferous tubules (F) tubules (H&E, 400X).

histological section of the testes of group I (Control group) which has a normal structure of seminiferous tubules. The histological section of the testis group II (collagen-α®15) showed complete suppression of spermatogenesis (S) and giant cells (spermatid) (G) in the lumen of tubules and also areas of fibrosis surrounding the seminiferous tubules (F) (Fig. 2). Histological section in the testis of group III (collagen-α®30) showed complete suppression of spermatogenesis (S) and present of giant cells (spermatid) (G) in the lumen of tubules and areas of fibrosis surrounded the seminiferous tubules (Fig. 3).

Discussion

The collagen-α® supplements are known as safe for human consumption as reported by FDA in the USA (Woo et al. 2018) and it has a low risk on health status but little researches are available in this regard. One of the clinical applications of collagen-α® is the treatment of skin and cartilage disorders. Little

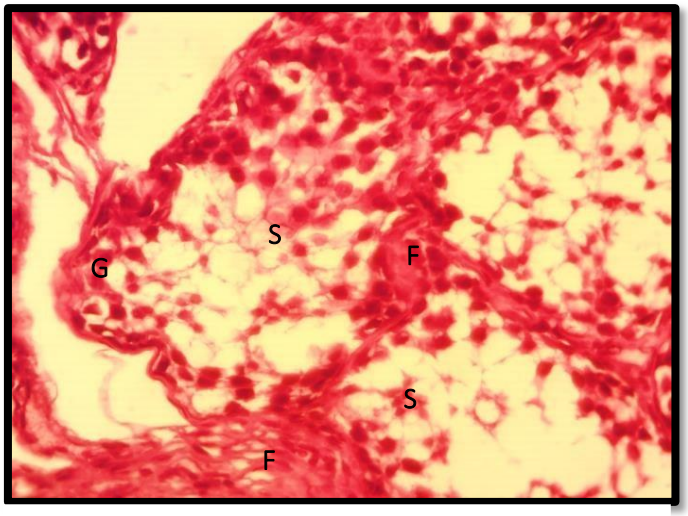

Fig.3. Histological section in testis group III (collagen-α®30) showed seminiferous tubule: there's complete suppression of spermatogenesis (S) and present of giant cells (spermatid) (G) in lumen of tubules and also there are areas of fibrosis surrounded the seminiferous tubules (H&E, 400X).

information regarding the effect of this supplement on health status and its toxicity on different organs and physiological parameters are available, therefore this study aimed to investigate the effects of collagen-α® on health and reproductive status.

In this study, a decrease in BW, BW changes, liver, kidneys, and testes weights was recorded. These may be due to its acts as anti-obesity by its regulation of lipid and protein metabolism and may be suggested its effects on appetite centers in the brain. These negative changes in BW, liver, kidneys, and testes weights agreed with the report of Kudayer et al. (2020) who used 1ml of collagen-α® supplementation for pregnant rabbits for 30 days. Jendricke et al. (2019) proposed that an increase in free fat mass leads to a reduced body fat percentage and an increase in muscular strength, demonstrating an average impact size with collagen. In addition, Kirmse et al. (2019)) pointed out a considerable increase in free fat mass with collagen. In another research, recreationally active males reported a substantial decrease in body mass and a rise in free fat mass with collagen supplementation, but no change in fat mass (FM) for either COL (Oertzen-Hagemann et al. 2019).

The histological alternations in the testes related to the changes in body weights and BW changes and also related to the weights of testes and other organs was similar to the report of Kudayer et al. (2020) that noted an increase in elasticity of muscles but decrease in muscle mass. Collagen contains a type of amino acid called hydroxyproline. Oertzen-Hagemann et al. (2019) that is converted into oxalate in the body, which may increase levels of oxalate excretion in urine (Liang et al. 2010). In a study, consuming 30 gr of gelatin derived from collagen increased urinary oxalate excretion by 43% after 24 hours (Fan et al. 2013). Several studies showed that consuming high amounts of hydroxyproline could increase oxalate levels in the urine, and may harm kidney health (Srivastava et al. 2016). Other studies suggest that these effects could be amplified in those with primary hyperoxaluria which affects oxalate metabolism and enhanced the risk of recurrent kidney stones that negatively affect the weight of the kidney. However, most of these studies used concentrated amounts of hydroxyproline. Therefore, it is unclear how the collagen found in meat or supplements may affect urinary oxalate excretion and kidney stone formation and testes function and sperm formation when consumed in normal amounts (Knight et al. 2006; Sivalingam et al. 2013; Fargue et al. 2018).

The results of reproductive hormones and semen quality are presented in Table 2. In this study, we report undesired effects of this supplement on the measured parameters that may be due to the direct effects of collagen on reproductive organs (testes and epididymis) as shown in the histological section of testes. The prolonged cytotoxic impact of the supplement Collagen-® on pregnant rabbits results in abortion and negative effects on reproductive organs (uterus and ovaries) and adverse cellular effects in target organs and reduce weight gain (Kudayer et al. 2020).

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