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Original article

Healing effects of *Ziziphus jujuba* hydroalcoholic extract with exercise training on histopathological changes of male wistar rats testicular tissue in response to Boldenone steroid administration

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Abstract

Background and objectives: Anabolic steroids are routinely consumed by athletes. Therefore, this study was conducted to investigate the effect of Boldenone (BOL) on testicular tissue and the healing effect of jujube (Ziziphus jujuba) hydroalcoholic extract in addition to physical exercise.
Methods: In the present case–control study, 42 male wistar rats were purchased and divided into 3 experimental groups. 28 rats were subjected to BOL injection and subsequently divided into 4 groups. The control group, Sham and 8-week BOL group which was also divided into 4 subsequent groups of controlling BOL complications after 8 weeks, untreated control, jujube extract and jujube extract along with physical exercise.
Results: In the present study we found that jujube extract exerted healing effects on all groups pf treated rats in addition to the exercise training groups.
Conclusion: Jujube extract along with physical exercise may exert healing effects on testicular tissue after administration of anabolic BOL steroid.
Keywords: Boldenone, Ziziphus jujuba, Hydroalcoholic extract, Testicular tissue, Wistar rat

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Introduction

Androgenic-anabolic steroids (AAS) are compounds derived from testosterone and the main male hormone (1). Androgenics are associated with sexual performance stimulation and are responsible for the development of male traits and the growth of the musculoskeletal system of the body (2). AAS are often used by athletes as an anabolic agent to improve their athletic performance (2). Application of these drugs has been rapidly expanding since the end of Second World War among athletes (3). Many steroids naturally exist in various types of hormones and vitamins and can be ingested through oral consumption (4). Drugs known as anabolic steroids are manufactured in industrial laboratories and have the same chemical composition of steroids found in testosterone (3-4). AAS are usually formed in the interstitial or Leydig cells of the testicles and secreted by the adrenal glands (5). The role of these drugs in the construction of muscle tissue (anabolic effect) and male characteristics (the effect of androgenetic) makes it interesting for athletes bodybuilders (6). Steroids are consumed in both medicinal and sport applications. It is consumed in a dosage of 2 mg per Kg of body weight medicinally, while taken 5 mg per kg for sport (6-7). These drugs are rarely prescribed for therapeutic approaches. However, they are sometimes prescribed in the treatment of late puberty, severe weight loss, AIDS and other severely debilitating diseases (8). Steroid supplements are converted into testosterone or other steroidal compounds in the body (9). High doses of steroids increases the risk of heart muscle enlargement and abnormalities, high blood pressure, blood clots, heart attacks and stroke. Invasion and aggressiveness or depressions which can lead to suicide, as well as water and salt retention in the organs are the other side effects of these drugs (10-11). Nowadays, doping and consuming the energizing drugs have become a complex problem in sport with the undeniable role of AAS (12). The uncontrolled use of these compounds may lead to irreversible alterations in the body including the reduction of sperm production and testicular resolution (13). Several experiments have shown that high and low doses of steroids (including BOL steroid) have reduced the quality and quantity of sperm in rats (10). Due to the extensive use of these drugs by endurance athletes and in particular bodybuilders and the fact that they are widely administered to unrestrained volunteers by unskilled persons, and also lack of adequate knowledge of the side effects of these drugs, we aimed to investigate the effects of anabolic BOL steroid effects in addition to eight weeks of aerobic physical exercise on testicular tissue alterations in male wistar rats.

Findings of other researchers including Aoki and Boada reveled injuries to the skeletal and liver systems in the anabolic steroids group (12-13). Previous studies have investigated the clinical relevance of anabolic steroids, while there is limited information about the effect of physical activity, while the effect of resistance training has not been studied on the metabolism of steroids including BOL. On the other hand, it is clear that resistance training may result in extensive physiological changes during and after exercise in the human body and animals and affect the metabolism of various substances such as medications. Resistance training has also a significant effect on elevating testosterone levels, luteinizing hormone (LH) and follicular stimulatory hormones (FSH) in men (14-15). Thus, it could be expected that

accompanying resistance training with injectable BOL may be effective in altering the potential negative effects of this drug on the liver, nervous system, genital system, etc. (15). Although the negative effects of these drugs have been clinically studied, athletes and consumers still have opposing opinions against doctors, often use anabolic steroids and recommend them to others to achieve better performance and ignore possible injuries. Considering the wide application of these drugs by athletes including bodybuilders, we studied the effect of BOL on structural changes in the testis of male wistar rats in an eight-week period in addition to resistance training along with the use of jujube hydroalcoholic extract. This study was performed on male rats due to lack of access to human testicular cells in order to meet ethical issues and also to control the effective factors.

Materials and Methods

The present study is an experimental and randomized study in which all ethical principles in handling laboratory animals have been applied. 42 male rats (2 months old) weighing 200 ± 20 g were purchased and kept at the animal research center of Shahrood Islamic Azad University. Handling and keeping characteristics were identical for all animals including a temperature of 22 ± 2 °C, a relative humidity of 55%, and a period of 12 hours of light and 12 hours of darkness. Animals were kept in polycarbonate transparent cages and cleaned daily and disinfected. After one week of adaptation, male rats were randomly divided into 3 groups of control (n = 7), sham (n = 7) and BOL (n = 28). The control group did not undergo any treatment from the beginning until the end of the period. The sham group received olive oil injection meanwhile. The BOL group received BOL injection for 8 weeks (5 mg / kg) by insulin syringe as a deep intramuscular injection at the back of the thigh. In the end of week 8, the rats were randomly allocated into four groups (groups of 7). The first group was then sacrificed and subjected to sampling. The second group did not receive any exercise and/or medicine and was used as a comparison with other groups. The third group received a jujube hydroalcoholic extract at a dose of 600 mg/kg for 5 weeks. The fourth group received juvenile hydroalcoholic extract at a dose of 600 mg/kg body weight along with endurance exercises (Table 1).

Endurance sports schedule included daily use of treadmill with specified protocol. In all phases of the sampling, the rats were initially anesthetized by intraperitoneal injection of a combination of ketamine and xylazine, the abdominal cavity was cut in a subsequent surgical operation and their testicles were removed and weighed. The specimen was then kept in the containers containing 10% formalin to fix the tissue. After 24 hours, the formalin solution was replaced and the specimen was sent to the pathology laboratory for histological sections. After preparation of histologic sections and tissue staining using hematoxylin and eosin method, the slides were visualized under optical microscopy to evaluate the histological changes of each tissue.

Table 1. The specification of physical practice

Period	Five-day orientation	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6	Week 7
Speed (m/min)	15	20	20	25	25	30	30	30
Time (min)	20	20	25	25	30	30	35	35

The hydroalcoholic extract of jujube contained 98% of the effective material which was provided by the food industry center of Tarbiat Modares University, Tehran, Iran. Boldenone anabolic steroid was purchased from Meditech, Germany and applied at a dose of 5 mg/kg. A 12-hour daily and a 12-hour night period was considered for rats physical training. This study was designed and conducted according to the ethics of animals' research.

Results

The specifications of the control group represented ease in the samples and the tissue components were in normal morphology (Fig. 1 and 2).

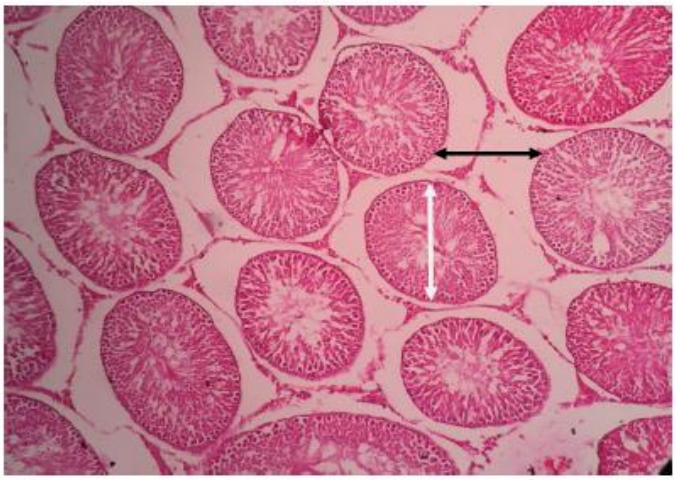

Figure 1. Histological section of the rat testicular tissue in the control group. Seminiferous tubules (two-way white arrow). Interstitial texture (double-armed black arrow). (Hematoxylin and Eosin staining 100X magnitude)

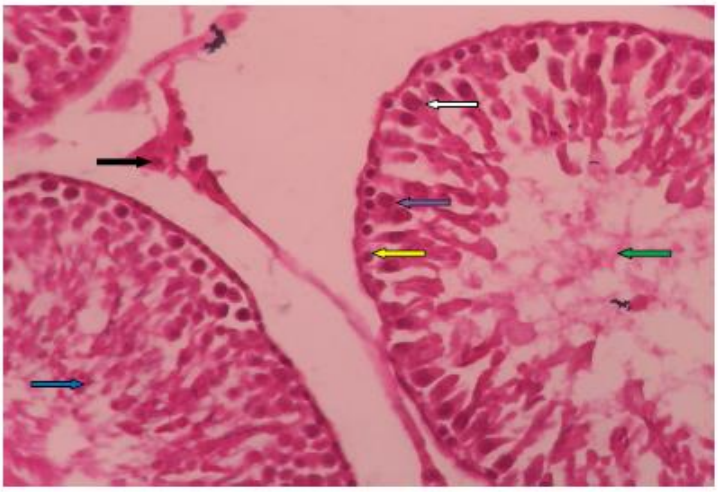

Figure 2. Histological section of the rat testicular tissue in the control group. (Hematoxylin and Eosin staining 400X magnitude)

Regularity and uniformity was observed throughout the tissue. The diameter of the seminiferous tubules (double-armed white arrow) was appropriate and isometric, while the interstitial space (double-armed black arrow) had wide and diffused texture. Leydig cells (black arrow) have explicit nuclei and cytoplasm with normal count in interstitial tissue. In the interior seminiferous tubules, the sertoli cells (white arrow) are quite normal in size and morphology. The spermatogenic cells are visible at all stages of division in this region. Spermatogenic cells (yellow arrow), spermatocyte (purple arrow), spermatid (blue arrow) and spermatozoids (green arrow) with the appropriate number and normal cellular features were demonstrated. Tissue specifications in the sham group were similar to the control group. However, some vascular expansion and congestion in addition to the decrease in spermatozoids count of the seminiferous tubules and darkening of the

nuclei of spermatogonial cells was demonstrated. Microscopic evaluation of the 5 mg/kg BOL group samples revealed that tissues were undergoing degeneration process and various pathologic alterations in size and order (Figures 3 and 4).

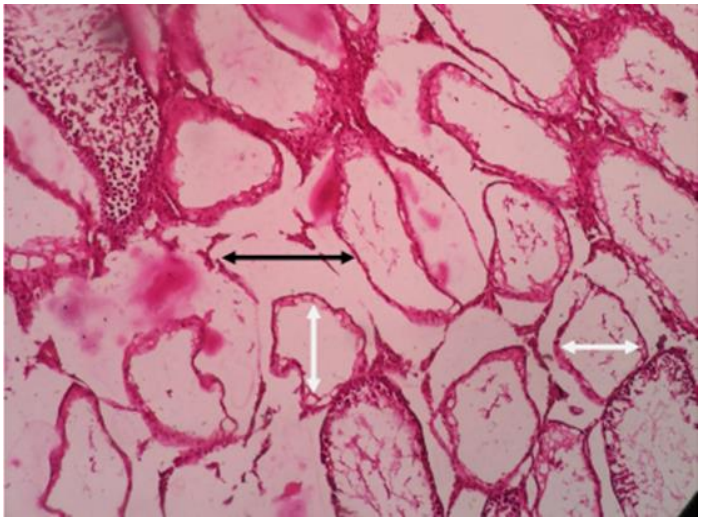

Figure 3. Histological section of the rat testicular tissue in group (dose of 5 mg/kg). (Hematoxylin and Eosin staining 100X magnitude)

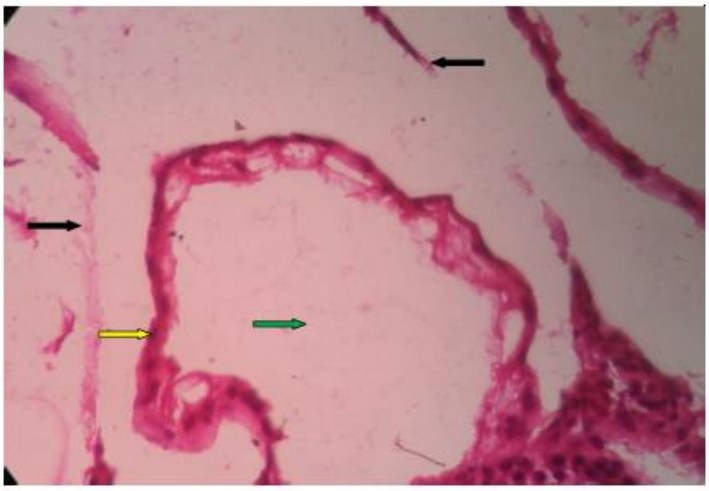

Figure 4. Histological section of the rat testicular tissue in BOL group (5 mg/kg). (Hematoxylin and Eosin staining 400X magnitude)

Seminiferous tubules were in different sizes and diameters, while the interstitial tissue was not demonstrated in most intertubular areas and the count of Leydig cells were decreased accordingly. The number and mass of spermatogenic cells were obviously reduced in tubular walls and merely consisted of spermatogonial cells (yellow arrow). The number of spermatozoids is also very low and often seen empty in the lumen of the tubes (green arrow).

The tubes had a broken and irregular wall in tissue samples taken from untreated group. Interstitial tissue is also less than normal. The spermatogonial cells had a heterochromatin nucleus and there were few sertoli cells.

As shown in figures 5 and 6, the tissue structure is ordered and consistent and the size and diameter of most tubes are equal in the histology section of the jujube extract receiving group. The interstitial tissue was expanded appropriately in the space between the tubes, while the count of Leydig cells (black arrow) was acceptable. Moreover, size and morphology of the sertoli cells (white arrow) was appropriate in the wall of seminal tubes. The minor visible damage was the presence of a mass of spermatogenic cell lineages in the margins of tubular walls which were demonstrated as empty spaces (blue arrows). The interiors of the tubes showed acceptable numbers of spermatozoids (green arrow).

Figure 5. Histological section of the rat testicular tissue in the jujube group. (Hematoxylin and Eosin staining 100X magnitude)

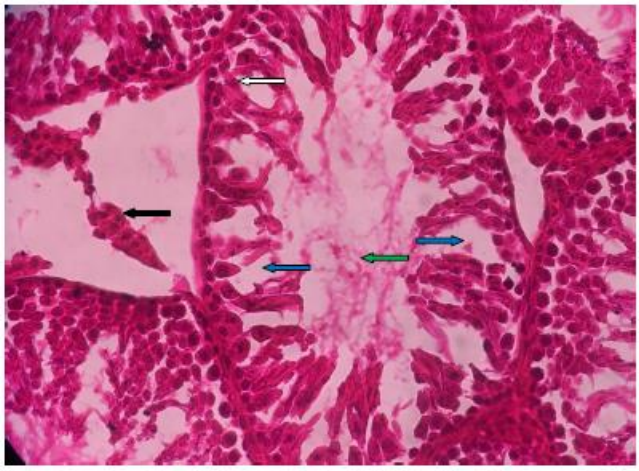

Figure 6. Histological section of the testicular tissue in the jujube group (Hematoxylin and Eosin staining 100X magnitude).

The histological specimens obtained from the jujube extract and the endurance training group revealed the characteristics of the normal tissue (Figures 7 and 8). The size of tubes was equal and the amount of the interstitial tissue was desirable. The spermatogonial (yellow arrow) and sertoli (white arrow) cells were in normal numbers and morphology and the wall of tubes was completely healed. The volume of spermatid and spermatozoid (green arrow) cells was less than the control group in the space inside the tubes.

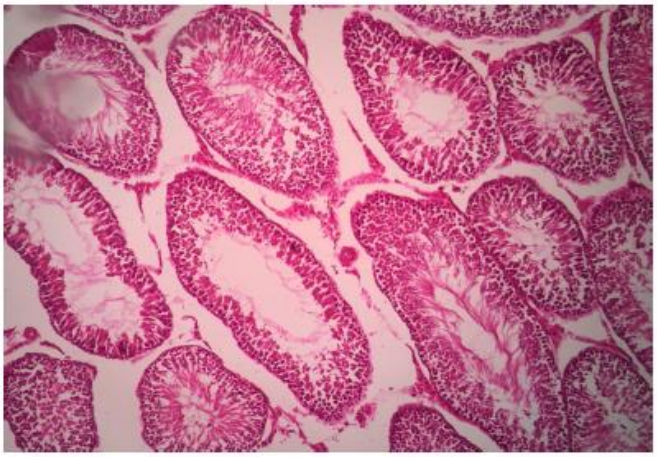

Figure 7. Histological section of the testicular tissue in the jujube and training group (Hematoxylin and Eosin staining 100X magnitude).

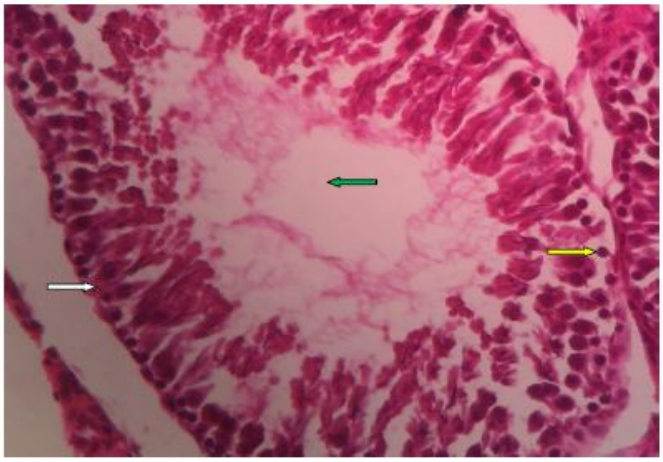

Figure 8. Histological section of the testicular tissue in the jujube and training group (Hematoxylin and Eosin staining 400X magnitude).

Discussion

The results of the current study showed that the application of Boldenone anabolic steroid was associated with various degrees of changes in the structure of testicular tissue in experimental groups compared with the control group, while aerobic exercise and jujube extract had inhibitory effects on these changes. Sadeghi et al. investigated the effect of 6 weeks of resistance training in addition to Boldenone supplementation on the expression of 5-alpha reductase and aromatase genes in testicular tissues of 30 male wistar rats (12 weeks). It was revealed that 6 weeks of resistance training in addition to Boldenone supplementation increased the levels of 5-alpha reductase and aromatase in the testicular tissue of wistar rats (16). They concluded that exercise training could reduce the effects of anabolic steroids which were consistent with our findings. The mechanism of action of androgenic anabolic steroids may be different in various compounds due to alterations in the steroid molecule and their affinity to androgen receptors. Several molecular pathways have been introduced including 5-α-reductase enzyme which plays

an important role by the conversion of androgenic anabolic steroids to dihydrotestosterone that acts on the nuclei of target cells such as male prostate gland. 5-α-reductase is produced in many tissues including testicular and genital organs of both males and females (17).

Another mechanism involves aromatase enzyme which mediates the conversion of androgenic anabolic steroids in female sex hormones (Estradiol and Estrone). This enzyme exerts antagonistic effects on estrogen and is known as a competitive antagonist for glucocorticoid receptors (17). 5-α-reductase mediates the irreversible conversion of testosterone to dihydro testosterone (18, 19). Dihydro testosterone could be involved in cancer initiation, benign prostatic hyperplasia, loss of male and female hair follicles and alteration in the puberty of boys and girls (20). The high incidence of these disorders in elderlies is due to increased estrogen (caused by elevated adipose tissue).

Karbalaeidoost, et al. (2007) found that a 14-week injection of low and high doses of Nandrolone could reduce the quality and quantity of sperm in rats. These parameters were improved after the removal partially of the nandrolone (5). The mentioned study suggested that discontinuation of steroids administration would reduce the amount of damaging effects to some extent which were in accordance with our findings after discontinuation of the BOL injection. Ahmed et al. (2013) identified that the administration of nandrolone even in therapeutic doses could induce testicular tissue damage and also cause temporary or permanent infertility (1). According to the results obtained in our study, the injection of BOL as an anabolic steroid could also lead to a cellular deformity and disorder in the process of spermatogenesis. Shen et al., found that jujube could modify the effects of carbon tetrachloride which causes liver damage (21). The jujube aqueous extract could prevent lipid peroxidation and reduce free radicals caused by hyaluronic acid and depolymerization. The researchers attributed these effects to jujube polysaccharides (22).

According to previous studies, the effect of aerobic exercise training on tissue healing is likely to be due to increased growth hormone and consequently an increase in IGF1. Increased IGF1 could also reduce LDH and its complications. In accordance with our findings, Ranjbar et al. showed that consumption of soluble alcoholic jujube extract with resistance training could significantly decrease tissue damage markers and liver enzymes after BOL administration (23).

Conclusion

The hydroalcoholic extract of jujube (Ziziphus jujuba) could be effective in healing almost all of the defects caused by Boldenone on testicular tissue. Moreover, jujube extract administration and training exercise simultaneously was also able to heal almost all the changes and injuries caused by Boldenone anabolic steroid on testicular tissue.

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اثرات الیتام بخشی عصاره ی هیدروالکلی عناب همراه با تمرین ورزشی بر تغییرات هیستوپاتولوژیک بافت بیضه پس از مصرف استروئید بولدنون

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چکیده:

زمینه و هدف: استروئیدهای آنابولیک همواره توسط ورزشکاران مورد استفاده قرار می گیرد. لذا این تحقیق با هدف بررسی اثر بولدنون بر بافت بیضه و تأثیر التیام بخشی عصاره ی عناب و تمرینات ورزشی پس از مصرف بولدنون مورد مطالعه قرار گرفت.

روش بررسی: مطالعه حاضر از نوع مطالعات تجربی و شاهددار تصادفی می باشد که در آن ۴۲ سر موش صحرایی نر خریداری و به ۳ گروه مساوی تقسیم شدند. به ۲۸ سر موش صحرایی بولدنون تزریق گردید و سپس به ۴ گروه تقسیم شدند. گروه کنترل، شم، گروه بولدنون ۸ هفته که سپس موش صحرایی این گروه، به ۴ گروه کنترل عوارض بولدنون پس از ۸ هفته، شاهد فاقد تیمار، عصاره عناب، عصاره عناب همراه با تمرینات ورزشی تقسیم شدند. گروه کنترل بدون مصرف دارو، گروه شم روغن زیتون، گروه بولدنون به مدت ۸ هفته داروی بولدنون دریافت و سپس موش صحرایی این گروه به ۴ گروه تقسیم شدند. گروه اول معدوم و نمونه گیری شد، گروه دوم هیچ تمرین و دارویی دریافت نکرد، گروه سوم عصاره هیدروالکلی عناب با دوز ۶۰۰ میلی گرم و گروه چهارم شروع به دریافت عصاره هیدروالکلی عناب با دوز ۶۰۰ میلی گرم بر کیلوگرم همراه با تمرینات ورزشی استقامتی خواهد کرد. برنامه ورزشی استقامتی شامل استفاده روزانه از تردمیل بود.

یافته ها: مطالعه هیستوپاتولوژی نشان داد که در گروه دریافت کننده بولدنون دوز ۵ میلی گرم شمای میکروسکوپیک بافت کاملاً در روند دژنراسیون قرار داشته و تغییرات مختلف همراه با بی نظمی دیده می شود به طوری که تغییرات دژنراتیو سلول های روند اسپرماتوژنیک، سرتولی و لیدیگ بیش از ۶۰ درصد و ۳ درجه تغییرات کیفی رتبه ای بود. اما در گروه دریافت کننده عصاره ی عناب به تنهایی و همراه با تمرینات ورزشی تغییرات سلول های سرتولی و لیدیگ در حدود صفر درصد و تغییر سلول های روند اسپرماتوژنیک کمتر از ۲۰ درصد مشاهده گردید.

نتیجه گیری: عصاره عناب به تنهایی و همراه با تمرینات ورزشی اثرات التیام بخشی بهتری را بر بافت بیضه پس ازمصرف استروئید آنابولیک بولدنون دارد.

کلمات کلیدی: بولدنون، عصاره ی هیدروالکلی عناب، بافت بیضه، موش صحرایی

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