

## Effects of grape seed oil (*Vitis vinifera*) on fertility of male local rabbits

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### Abstract

The study was conducted to investigate the effect of (*Vitis vinifera*) grape seed oil on testosterone, FSH and LH hormones concentration, in addition to investigate the histopathological effects on the testis and epididymis of male local rabbits. Twenty four male rabbits weighing (1450-1550) grams were divided into three equal groups. G1 the control group was gives only (0.2) ml tap water orally, while G2, and G3 were gives grape seed oil orally (0.1) ml/kg, and (0.2) ml/kg BW respectively, once daily for (1) month. Results were found that the administration of grape seed oils produce no significance decrease ( $P>0.05$ ) in FSH, and LH concentration, while a significance ( $P<0.05$ ) decrease of testosterone levels, besides inducing too many deleterious effects in the histopathological structure of the testes and epididymis.

**Key words:** Grape seed oil, testosterone, FSH, LH.

### تأثير استخدام زيت بذور العنب (*Vitis vinifera*) في خصوبة ذكور الأرانب المحلية

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### الخلاصة

أجريت هذه الدراسة لبحث تأثير زيت بذور العنب على الهرمون الذكري والهرمون المحرض للجريبات والهرمون المحرض للإباضة إضافة إلى دراسة التغيرات النسيجية في الجهاز التناسلي في ذكور الأرانب المحلية. تم استخدام (24) أرنب بوزن (1450-1550) غرام وقسمت إلى ثلاث مجاميع متساوية ، كل مجموعة تضم (8) أرانب، وكانت المجموعة الأولى (السيطرة) وجرعت (0.2) مل بالماء العادي في حين جرعت المجموعة الثانية والثالثة بزيت بذور العنب (0.1) مل/كغم و (0.2) مل/كغم من وزن الجسم على التوالي مرة واحدة يوميا لمدة شهر واحد. أظهرت نتائج التجربة عدم وجود تأثير معنوي ( $P>0.05$ ) في مستوى الهرمون المحرض للجريبات والهرمون المحرض للإباضة مع وجود انخفاض معنوي ( $P<0.05$ ) في مستوى الهرمون الذكري عند إعطاء زيت بذور العنب إلى ذكور الأرانب ، إضافة إلى حصول بعض التغيرات النسيجية الضارة في الخصية والبربخ.

**الكلمات المفتاحية:** زيت بذور العنب ، Testosterone ، FSH ، LH.

### Introduction

Grape seed oil also called grape oil is processed from the seeds of grapes, and is thus an abundant by-product of wine making and preferred cosmetic ingredient for controlling moisture of the skin. Light and thin, grape seed oil leaves a glossy film over skin when used as carrier oil for essential oils in aromatherapy. It contains more linoleic acid than many other carrier oils (1). Grapes are one of the fruit crops grown widely in many areas of the world and 46% of the fresh grapes produced are accounted for wine

production (2, 3). In general, the use of pomace in the food industry can create some opportunities to lower production costs and to create a new food source for human consumption. Grape seeds, which comprise 20 to 26% of the pomace (4), have high protein content. They also have 10 to 20% oil, with high vitamin E content, which has very important effects on human health (5). Grape seed oil mainly consists of triglycerides (TG), which are rich in unsaturated fatty acids, such as oleic and

linoleic acids, compared to other oil –rich seeds (6). Grape seed extract have been found to have beneficial effects on health, and these compounds drawn attention because of their relative safeness and accumulated evidence of physiological properties in animals and human (7). Oil produced from grape seeds is considered a rich source of poly phenolic with strong antioxidant activity, chemopreventive, anti-inflammatory, anti-microbial and anti-cancer effects (8). Grape seed oil may provide some health benefit, a 1993 study supports the claim that grape seed oil increases high-density lipoprotein (HDL-C or "good cholesterol) levels and reduces LDL levels (9). The biological active constituents of grape seed extract proanthcyanidines which represent a variety of polymer of flavan-3-ol such as catechin and epicatechin and have a strong antioxidant effect in aqueous system (10). Medicinal and nutritional value of grapes has been heralded for thousands of years, among other beneficial effects of parts of grape seeds are believed to have a powerful antioxidant property due to its rich source of polyphenol compounds as much as 60-70 % when compared only 10 % in the fruit and 28 - 35 % in the peels (11,12). Most of the polyphenol compounds found in grape seeds are flavonoids monomeric, flavan-3ols, catechin and epicatechin, these compounds are of interest in pharmaceutical and food factories for medical treatments and health supplements (13). It is also reported that grape seed extract (GSEs) have been associated with other antioxidants to accelerate the prevention of oxidation reactions the GSE were used for inhibition of rancidity in cooked beef (14,15). Use of medicinal plants in medicine is increasing because of their widespread use and for their ability to cure various diseases. Grape seed is well known for its pharmacological and therapeutic effects such as antioxidative, anti-inflammatory, and antimicrobial activities, as well as having cardio protective, hepato-protective, and neuroprotective effects (16). Grape seed oil contains small amount of vitamin E, but safflower oil, cottonseed oil, or rice bran oil contains greater amounts (17). Grapeseed oil is high

in polyunsaturates fat and low in saturated fat, it also does not contain cholesterol or trans-fatty acids. Grape seed oil can boost the health of the heart and the cardiovascular system by lowering the level of bad LDL cholesterol. This oil can increase the level of good HDL cholesterol, which can reduce the risk of coronary diseases (18). The present study was performed in order to obtain preliminary information about the effects of oral administration of grape seed oil for one month daily on LH, FSH and testosterone levels of male rabbits.

### Materials and methods

The study was conducted on twenty four healthy male rabbits weighing 1450-1550 grams obtained from the local markets at the period between December 2015 and July 2016 in physiology department of Veterinary Medicine College of AL-Qassim Green University. Animals were kept for one week as acclimatization period before the beginning of the experiment, all rabbits were feed on concentrated food (pellets) and were given plain water, the animals room temperature was (19-23)°C, and the humidity was (45-50%), that room was washing and sterilization once a week. After acclimatization, animals were divided into three equal groups. First group (G1) was control group; it was administered tap water daily. Second group (G2) and Third group (G3); were gavages grape seed oil (Imad Company), (0.1, 0.2 ml /kg B.W) / day for one month, respectively. At the end of the experimental period, rabbits were fasted for 10hrs, anesthetized using diethyl ether and blood samples were collected by heart puncture in non-heparinized tubes. Collecting blood tubes were centrifuged at (3000) rpm for (15)min. After separation the serum from the clot, using a sampler, the samples were frozen and maintained in -20 until used to measurement in order to subsequent estimation of testosterone, FSH, LH concentration. For histopathological examinations, sections were taken from testis and epididymis tissues from different animals in each group immediately after sacrificed. These tissues were washed with the normal saline solution to remove blood, and then

fixed in 10% neutral formalin for 24 hrs, dehydrated in different concentration of alcohol, and processed for paraffin embedding. Sections of 5  $\mu$ m thickness were cut using a rotary microtome. The sections were processed and passed through graded alcohol series stained with Haematoxylin and

Eosin, cleared in xylene and examined microscopically according to (19).

#### Statistical analysis

The data were analyzed statistically by analysis of variance, for statistical significance ( $p \leq 0.05$ ) using one-way ANOVA SPSS test.

## Results

### Serum level of LH, FSH and testosterone

The effects of grape seed oil presented in (Table 1). Statistically the study showed no significant ( $P > 0.05$ ) differences was recorded during the treatment on LH, FSH among G (1,2,3), while the testosterone was significantly decrease ( $P < 0.05$ ) in the experimental group (2) and (3) in compared with control group (1), but this significant decrease was more evident in group (2) that treated with a dose of 0.1 ml/ kg BW of grape seed oil, when compared with group (3) that treated with 0.2 ml/kg BW of grape seed oil.

**Table (1): Effect of grape seed oil on serum LH, FSH and testosterone levels in male rabbits ( $M \pm SE$ ).**

Groups	LH (mIU/ ML)	FSH (mIU/ ML)	Testosterone (mIU/ML)
G1	0.103 $\pm$ 0.001 a	0.106 $\pm$ 0.003 a	5.487 $\pm$ 0. 370 a
G2	0.117 $\pm$ 0.009 a	0.100 $\pm$ 0.033 a	0.271 $\pm$ 0.036 b
G3	0.126 $\pm$ 0.100 a	0.120 $\pm$ 0.008 a	0.305 $\pm$ 0.059 c

Different letters denote significance ( $P \leq 0.05$ ) difference between groups

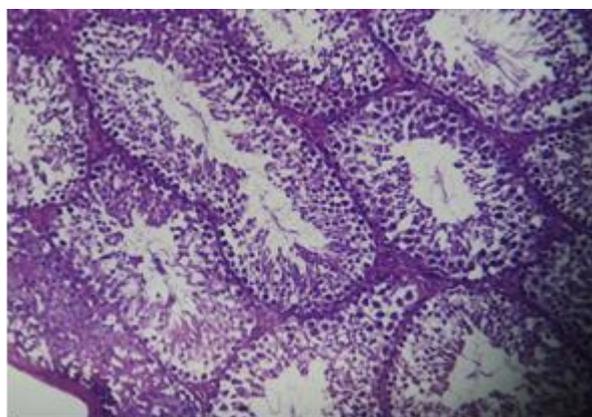
### Histopathological results of testis and epididymis sections

Histopathological sections of testis and epididymis of control rabbits revealed normal structure of the testis and epididymis (Fig.1, 2).

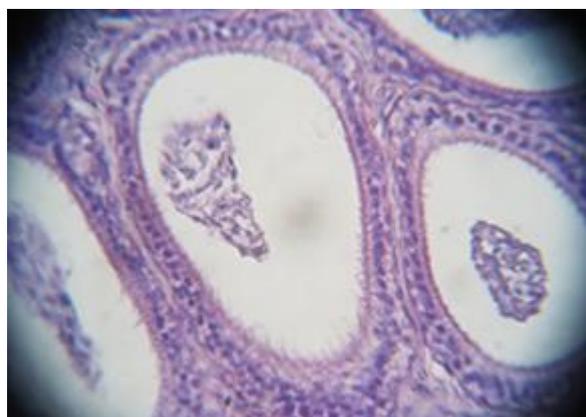
Sections of the testis of rabbits of G2 which dosage with grape seed oil 0.1 ml/ kg BW orally for one month, show necrosis of germ cell lining the seminiferous tubules, with mild interstitial edema and congestion of blood vessels (Fig. 3).

Sections of testis of G3 which dosage with 0.2 ml /kg BW grape seed oil orally had showed sloughing of tunica albugina, and extensive necrosis of germ cells and increase the space between the seminiferous tubules due to diffuse interstitial edema (Fig. 4).

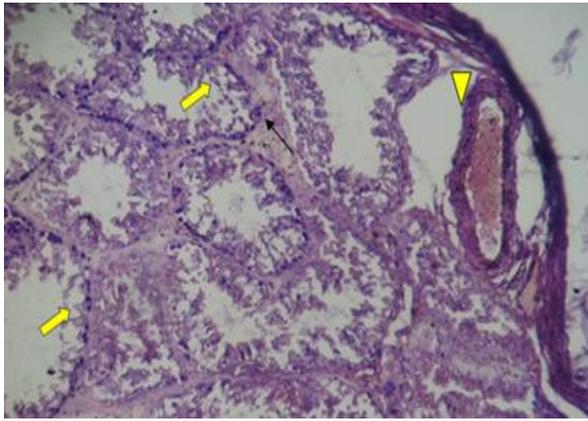
Sections of epididymis of G2 showed congestion and degenerative changes in some epididymis. (Fig. 5). Sections of epididymis of G3 showed destruction in epithelial cell lining the epididymis tubules that become filled with mature spermatozoa, and interstitial edema (Fig. 6).



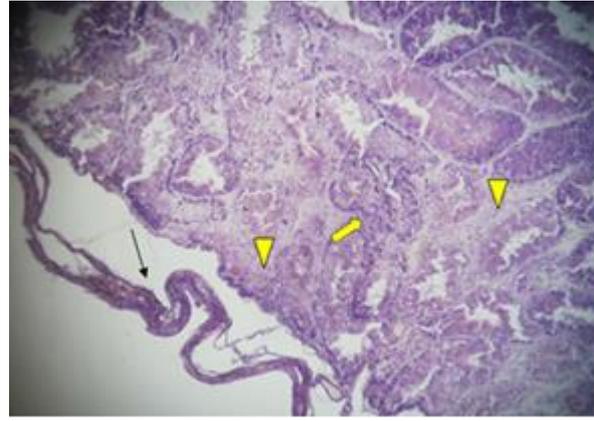
**Fig. (1): Cross section of testis of control group showing normal tissue structure (H&E, X400).**



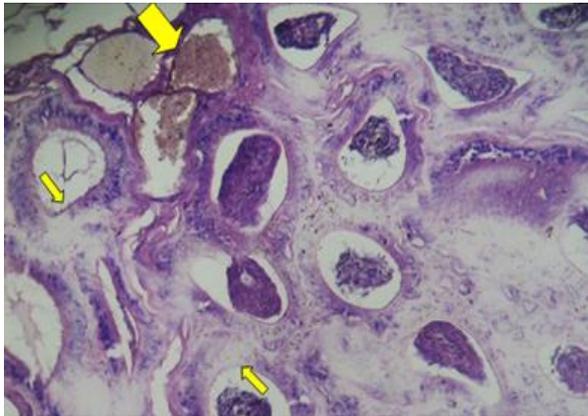
**Fig. (2): Cross section of epididymis of control group showing normal tissue structure (H&E, X400).**



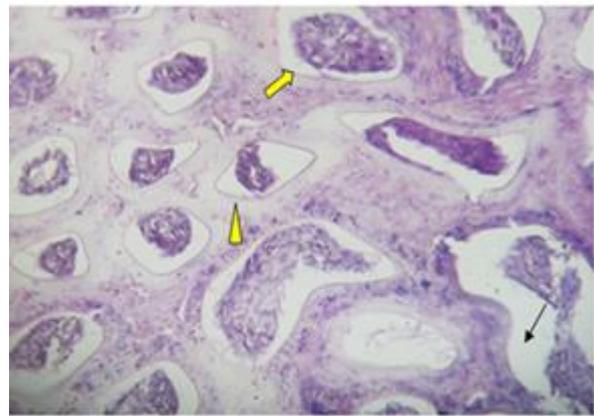
**Fig. (3):** Cross section of testis of G2 showing necrosis of germ cells lining the seminiferous tubules (yellow arrow) with mild interstitial edema (black arrow) and congestion of blood vessels (head of arrow) (H&E, X400).



**Fig. (4):** Cross section of testis of G3 showing sloughing of tunica albuginea (black arrow), extensive necrosis of germ cells (yellow arrow) increase the spaces between the seminiferous tubules due to diffuse interstitial edema (head of arrow), (H&E, X400).



**Fig. (5):** Cross section of epididymis of G2 showing congestion (thick arrow), and degenerative changes in some epididymis tubules (thin arrow) (H&E, X400).



**Fig. (6):** Cross section of epididymis of G3 showing destruction in epithelial cell lining the epididymis tubules (black arrow) that filled with mature spermatozoa (yellow arrow) and interstitial edema (head of arrow) (H&E, X400).

## Discussion

In the present study, hormonal measurement showed a significant reduction ( $P > 0.05$ ) in testosterone level, (20) observed significant decrease in plasma level of testosterone due to increased number of lipid droplets in Leydig cells after prolonged exposure to stress. Thus the increased number of lipid droplets indicates that the androgen synthesis in the Leydig cells has been suppressed. Thus the stress-induced changes in testicular function correlate well with the morphological changes in Leydig cells. (21) also found that restraint stress

causes increase in plasma level of glucocorticoids decrease in testosterone level without any effect on LH level. He suggested that increase in plasma level of glucocorticoids act via glucocorticoid receptors on testicular interstitial cells to suppress the testicular response to gonadotropins. (22) supported the primary role of glucocorticoids in stress-induced inhibition of reproductive function in rats. This is also approved by study of (23) the decrease in testosterone concentration reached statistical significance only at the

higher dose of the ethanolic seed extract of celery and can be due to the mechanisms shown for plant extracts include inhibition of gonadotropin secretion, interference with steroidogenesis at the testicular level, a fact that supports this possibility is the high content of flavonoids in celery (*Apium graveolens*) as show in our study. (24) observed significant reduction in serum concentration of testosterone when chronic administered of *Martynia annua* root extract on male rats. This is also approved by study of (25) that performed on the effects of grape seed oil and grape seed extract on reproductive system in male rat that in agreement with our findings. (25) found that administration of leave hydro-alcoholic extract effects on some of fertility parameters it has an inhibitory activity on fertile sperms and maturation of them and decreases spermatogenesis in the normal male rats. There is another study (26) done on male rats which demonstrated that treatment with grape juice had significantly reduced sperm

motility count and daily sperm production in all treatment groups. This reduction was more dramatic at higher doses. The comparative microscopic evaluation, for the concurrent administration of the grapeseed oil led to negative impact effect of the grape seed oil, through induction of lesions in the male reproductive organs of the rabbits. The histopathological changes in testes and epididymis of rabbits administered with grape seed oil are in agreement with (27) who observed deleterious effects of different concentration of grape seed extract (200, 300 mg/ kg BW) in mice which cause significant decrease in hematological and cytogenetical parameters and severe histopathological changes in brain, heart, lung, kidney, pancreas, bone marrow. In conclusion the administration of grape seed oil at dose (0.1, 0.2 ml/ kg BW) day/ orally could induced inhibition of fertility in male rabbits revealed by decreasing testosterone level and many deleterious effects in testes and epididymis tissue.

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