Protective Effect of Alcoholic extract of Black Current in Male Reproductive System of Methionine Overload Rats

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Summary

This study was undertaken to investigate the deleterious- effect of methionine overload on male reproductive system of adult male rats. It was also aim to studying the beneficial protective effect of alcoholic extract of black current. Eighteen adult male rats (175-200 gm) were used, animals were randomly divided into three groups (6/group) and were treated daily for 42 days as follows: 1-Group C (control); 2- group I(GI), the rats in this group were orally intubated by gavages needle DL-methionine (100 mg / Kg BW.) 3- animals in group II(GII) were administered orally alcoholic extract of black current (60 mg /Kg BW.) in addition to methionine. At the end of the experiment blood samples were collected for measuring serum testosterone (T) hormone and follicular stimulating hormone (FSH) concentrations. As well as histological section, for testis was taken for histopathological study the results revealed that oral intubation of 100mg /kg BW. of methionine for 42 days caused significant decrease - in serum testosterone (T) hormone and follicular stimulating hormone (FSH) concentrations in groups (GI and GII) comparing to control ,while oral intubation of alcoholic- extract of black current (GII) caused significant elevation in the concentration of these two hormones comparing to methionine treated group (GI). Furthermore, methionine intubation caused significant decrease in diameter and thickness of seminiferous tubules with cellular degeneration of Sertoli cells, while black current intubation caused significant increase in the previous criteria and regeneration of Sertoli cells. In conclusion, this study pointed to the deleterious effect of methionine overload and assures protective effect of alcoholic extract of black current on adult male rats reproductive system.

Keywords: Methionine, testosterone, FSH, male reproduction, black current.

التأثير الوقائي للمستخلص الكحولي للزبيب الأسود في الجهاز التناسلي الذكري للجرذان المعاملة بفرط المثيونين

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الخلاصـــــــــــــة

صممت هذه الدراسة لمعرفة التأثير الضار لفرط المثيونين على الجهاز التناسلي الذكري للجرذان ،بالأضافة الى معرفة التأثير الوقائي للمستخلص الكحولي للزبيب الأسود .تم أستخدام ثمانية عشر من ذكور الجرذان البالغة وقسمت عشوائياً الى ثلاثة مجاميع متساوية وعوملت كالاتي لمدة 42 يوم 1-مجموعة السيطره (C)جرعت محلول الدارىء ؛2- مجموعة المعاملة الأولى (GI) جرعت الجرذان في هذه المجموعة 100 ملغم /كغم من وزن الجسم مثيونين بأستخدام اللي المعدي 3- جرعت حيوانات المجموعة الثانية (GII) 60ملغم /كغم من وزن الجسم بالمستخلص الكحولي بالأضافة الى150 ملغم/كغم وزن الجسم من المثيونين . جمعت عينات الدم في نهاية التجربة لقياس تركيز هرموني التستوستيرون وهرمون محفز الجريب في مصل الدم ،فضلاً عن أخذ مقاطع نسيجية من الخصية لدراسة التغيرات النسجية المرضية. أظهرت النتائج حدوث انخفاض معنوي في تركيز هرموني التستوستيرون و محفز الجريب في المجاميع المعاملة بفرط المثيونين (GI وGII) مقارنة مع مجموعة السيطرة ،في حين أدى التجريع الفموي للمستخلص الكحولي للزبيب الأسود (GII) الى حصول ارتفاع معنوي في هذين الهرمونين مقارنة مع مجموعة(GI) .فضلا عن ذلك فقد تسبب فرط المثيونين الى حصول انخفاض معنوي في سمك وقطر النبيبات المنوية مع حدوث تنكسات خلوية في خلايا سرتولي مقارنة مع المجموعة المجرعة بالمستخلص الكحولي للزبيب الأسود الذي تسبب في حدوث ارتفاع معنوي في سمك وقطر النبيبات المنوية مع انكفاء التنكس الحاصل في خلايا سرتولي .نستنتج من هذه الدراسة وجود التأثير الضار لفرط المثيونين والذي يمكن الوقاية منه بأستخدام المستخلص الكحولي للزبيب الأسود في الجرذان البالغة.

الكلمات المفتاحية: الزبيب الاسود ،الجرذان ، فرط المثيونين ، الجهاز التناسلي الذكري.

Introduction

Methionine is an essential for maintaining proper growth and development in mammals and domestic animals, such as pigs and chicken ,contributes to better production efficacy(1). Lynch and Strain, (2) found that excess L-methionine intake induce the production of oxidative metabolites and cause defect in the enzymatic antioxidant defense system due to induction of homocysteine (Hcy). Hcy is an endogenous sulfur-containing amino acid found during the metabolism of methionine and is not obtained from diet (3).

Hyperhomocysteinemia (HHcy), is a state of elevated plasma homocysteine level (4). Methionine is believed to exert several toxic effects, it has been implicated in retrospective and prospective studies, as a risk factor for atherosclerosis which includes venous thrombosis (5, 6 and 7), coronary artery disease (8), myocardial infarction, stroke (9 and10), primary hypertension (11), rheumatoid arthritis and osteoporosis (12) as well as hypertrophy and fatty change in the liver (13). HHcy exerts its toxicity by increasing H2O2 production and promoting lipid peroxidation (LPO) (14 and 15). Besides, HHcy induced endothelial dysfunction through the attenuating nitric oxide (NO) bioavailability and injury involves oxidative damage (16 and17).

Reactive oxygen species (ROS) including oxygen ions, free radicals and peroxides are generated by sperm and seminal leukocytes within sperm produce infertility (18 and19) by damaging the sperm membrane or by alter sperm DNA (20).Today, grape seed extract use to treatment of a range of health problem related to free radicals damage including aging, DNA damage, cancer and heart disease (21). Shi *et al.*, (22) pointed that polyphenol in different grape species potentially inhibited reactive oxygen species .Also procyanidins (PCOS) found in grape juice are highly antioxidant effects than vitamin E and C(23) . Recently, it has been shown that natural honey (24) and Nigella sativa (25) caused almost protection against methionine - induced hyper homocysteinemia in rats.

Current interest is focused upon modulating the methionine over load through natural preventive strategies. The study aims to investigate the effect of induced methionine over load on some testicular functions in adult male rats and treated by using black current.

Materials and Methods

Seventy percent of alcoholic extract of black current *Vitis Venifera L*. belong to family Vitaceae was prepared according to the procedure described by Harbom (26). Eighteen adult male rats weighed (175-200gm) were randomly divided into three groups (6/group) and were treated daily for 42 days as follows: Group C; rats in this group were administered daily buffer solution 0.1M, PH 7 by oral intubation using gavage needle and served as control group; group I (GI) the rats in this group were orally by gastric gavage tube DL.methionine (100 mg / Kg BW.) (27). Diluting in buffer 0.1M, PH7 and animals in groupII (GII) were administered orally alcoholic extract of black current (60mg /Kg BW.) (28) in addition to methionine as described in GI, using gastric gavage tube .

At the end of the experiment blood samples were drawn via cardiac puncture technique and serum was collected by centrifugation and frozen at -20 until analysis. Testosterone (T) hormone and follicular stimulating hormone (FSH) concentrations was measured in serum by radioimmunoassay (RIA) kits (Beckman-France).The testis was excised and a portion of it was immersion fixed in 10% neutral buffered formalin .Sequential five sections of testis were prepared and stained with hematoxylin and eosin (H&E) to evaluate the histological changes of seminifereous tubules (29). The results were analyzed using one-way analysis of variance (ANOVA) .The level of statistic was set at $P<0.05$(30).

Results and Dissuasion

Table (1) the mean value of testosterone concentration and FSH in control and treated groups .The results showed a general tend for testosterone values to decrease in two treated

groups (GI and GII) as compared to control group .This decrement reached statistical significance (P<0.05) after 42days of experiment where the mean values of serum testosterone concentration in GI and Gil after 42days of experiment are 2.68±0.05 and 2.91±0.06 respectively, as compared to control. From other side oral intubation of alcoholic extract of black current seeds (GII) caused significant elevation of this hormone comparing to methionine treated group (GI). A significant decrease (P<0.05) in serum FSH concentration in methionine treated group (GI) were observed at the end of the experiment comparing to control group .While, oral intubation of alcoholic extract of black current with methionine (GII) superesed the decrease of FSH concentration (P>0.05) compared with GI group.

The effect of oral intubation of alcoholic extract of black current and methionine to adult male rats on mean value of diameter of seminiferous tubules were clarified in table (2). A significant decrease (P<0.05) in the mean value of this parameter in group GI (192.16±2.50) was observed as compared to control (210.63+1.67).Moreover, the value of diameter of seminiferous tubules tend to increase significantly (P<0.05) in group Gil (204.78+2.96) comparing to GI and the value tend to normalize that of the control . Depending on the results clarified in table (2), there was significant decrease (P<0.05) in thickness of seminiferous tubules in GI(72.0±1 .47) and GII(80.20±1.59)comparing to control group (86.51±2.05) while oral intubation of alcoholic extract of black current caused significant elevation in thickness of seminiferous tubules comparing to methionine treated group.

Histological section of rat testis treated with (100mg/kg BW.) of methionine for 42 days showed acute cellular degeneration in the Sertoli cells with low spermatogenesis and presence of protenious materials in the lumen of seminiferous tubules (figure-1) comparing to normal section of testis in control group (figure-2). Oral intubation of 60mg/kg BW. of alcoholic extract of black current concurrently with 100mg/kg BW. Methionine caused regression of pathological lesions and black current preserved the histological structure of testis with regeneration of testicular tissue (figure-3).

Table (1): Effect of alcoholic extract of *Black Current* and methionine overload on serum testosterone (T), follicular stimulating hormone (F.S.H) in adult male rats.

Group Parameters	(C) Control	(GI) Methionine (100mg/kg. BW)	(GII) (Methionine150mg/kg.BW +black Current 60mg/kg.BW)
Testosterone (ng/ml)	**3.51 ± 0.06 A**	**2.68 ± 0.05 B**	**2.91 ± 0.06 C**
F.S.H (I U/ml)	**1.77 ± 0.03 A**	**1.52 ± 0.02 B**	**1.69 ± 0.03 A**

Values are expressed as mean ± SE; n= 6 / group.
Different capital letters denote between groups differences, P< 0.05 Vs. control.

Table (2): Effect of alcoholic extract of *Black Current* and methionine overload on diameter and thickness of seminiferous tubules in adult male rats.

Group Parameters	(C) Control	(GI) Methionine (100mg/kg. BW)	(GII) (Methionine 150mg/kg.BW +black Current 60mg/kg.BW)
Diameter (µM)	210.63 ± 1.67 A	192.16 ± 2.50 B	204.78 ± 2.96 A
Thickness (µM)	86.51 ± 2.05 A	72.00 ± 1.47 B	80.20 ± 1.59 C

Values are expressed l as mean ± SE; n= 6 / group. Different capital letters denote between groups differences, P< 0.05 vs. control.

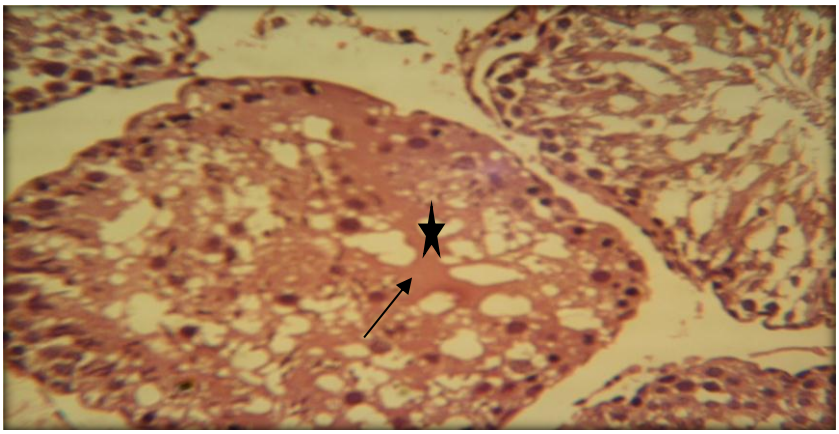

Figure (1): Histological section of rat testis treated with methionine overload (GI).Show degeneration in Sertoli cell (→) and proteineous material in lumen of seminiferous tubule (★) (H&E X40).

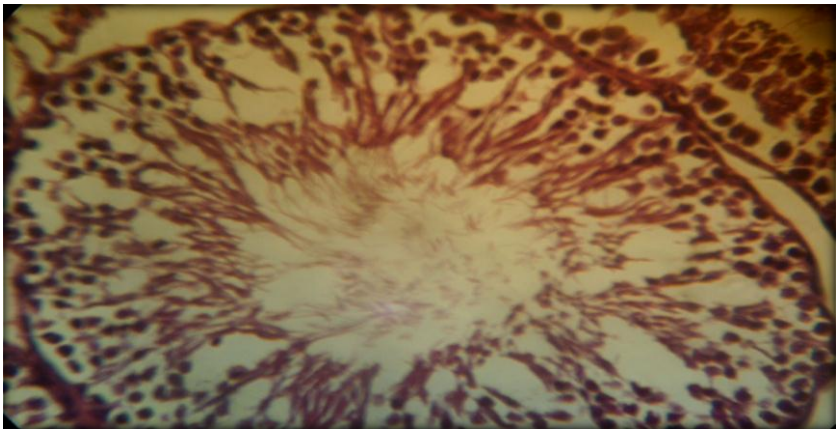

Figure (2): Histological section of testis of rat from control group .Show normal structure of seminiferous tubule (H&E X40).

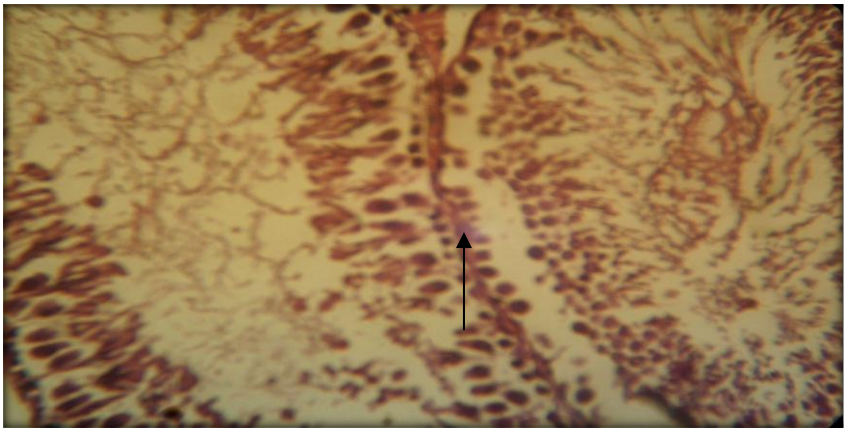

Figure (3): Histological section of rat testis treated with methionine overload and alcoholic extract of black current (GII).Show regression of pathological lesion (→) (H&E X40).

Evidence now suggests that ROS — mediated oxidative damage to sperm is a significant contributing to pathology of 30-80% of male infertility (31, 32, 33 and 34). Inductions of

HHcy and oxidative stress after methionine overload have well documented (35, 36, 37 and 38). Accordingly, it can be hypothesized that oxidative stress and HHcy induced after methionine overload may be responsible for such detrimental damage of male rat reproductive system. The toxic accumulation of homocysteine may cause reproductive dysfunction and oxidative stress within the testis (39 and 40) ; abnormal spermatogenesis and male infertility (41).Furthermore, epididymal sperm concentration , percentage of progressive sperm motility and plasma testosterone concentration were significantly decreased in Hcy — treated rats (40), It has been well documented that inflammatory cytokines ,such as those induced by HHcy ,have been associated with impaired sperm parameters and male fertility(42).Furthermore, nitric oxide (NO) signaling are involved in penile erection ,spermatogenesis ,dynamic of blood tests barrier ,sperm motility , capacitation ,acrosomal reaction and fertilization (43 and 44) .Thus any alteration of NO bioavailability by HHcy may have direct consequences on male reproductive functions. The major methyl donor, SAM stimulated human chorionic gonadotropin (hCG) - mediated testosterone synthesis in purified rat leydig cells in vitro, whereas, SAH produced due to methionine overload (methylation defect) had opposite effect (45).

The suspected decrease in number of leydig cells, which is responsible for synthesis and secretion of testosterone after methionine overload necrosis and atrophy of Sertoli cells, may lead to decrease in testosterone concentration (46). Selective suppression of FSH in this experiment could result from action of methionine overload on induction of hepatic cytochrom P450 enzymes and expression of substantial level of non-steriodogenic P450 inciting local cell land tissues damage (47) leading to inactivation of cell responsible for secretion of hormone including FSH hormone. Overall, this scenario is consistent with histological picture of methionine overload group exhibited cellular degeneration of Sertoli cells and proteineous materials in lumen of seminiferous tubules.

The results also showed that the alcoholic extract of *Black Current* caused significant correction to male function through elevation of testosterone and FSH concentration in addition to increase thickness and diameter of seminiferous tubules. Several studies have reported that the levels of ROS within semen can be reduced by augmented the scavenging capacity of seminal plasma using oral antioxidant supplementation like a astaxanthine (48) ,combination of vitamins E and C (49) zinc and selenium (50). Antioxidant supplementation like folate which possessed the capacity to increase the enzymatic efficacy of the Mther and cystathionin glutathione B-synthase enzyme responsible for removing of Hcy from circulation (51) has beneficial effect (antioxidant) on spermatogenesis and enhancement sperm quality (50).Accordingly, supplementation of grape seed extract may restore antioxidant activity of the body, repair the case of oxidative stress and improve seminiferous tubule function. The beneficial effect of grape seeds against toxic effect of methionine may be attributed to the its active compound like resveratrol and proanthocyanidin (52) .These compound are considered as antioxidant which remove the free radicals produced after methionine overload and restoring male reproductive function . Increase in FSH and testosterone concentration in animal received grape seeds extract, may increase testicular weight and volume concomitant with increasing diameter of seminiferous tubules (53 and 54). On the other hand, testosterone supports spermatogenesis and increase maturation; facilitating round to elongated spermatid and may lead to increase lumen and diameter of seminferous tubules (55 and 56).

Vitamin E is one of the major membranes protective against ROS and lipid peroxidation and improved sperm viability in cattle under oxidative stress (57).As well as, vitamin E as antioxidant can prevent adverse effect of homocystein on testosterone level, epididymal sperm count and motility in male rats (40). Antioxidant compound of grape seeds extract may increase plasma vitamin E (58), which is play important role in decreasing the toxicity of methionine and returning the diameter of seminiferous tubules near to normal.

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