



The Potential Effect of Grape Seeds Extract against Lead toxicity That Induces Infertility to Male Rats

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Introduction

Grape vine (*Vitis vinifera*), is cultivated today in all regions with high temperature of the world [1]. Seeds of grape were, containing many active components including poly phenols, flavonoids, proanthocyanidins and procyanidines. Grape seeds extract (GSE) contain 70%-95% proanthocyanidins[2-3]. Proanthocyanidins are widely located in vegetables, flowers, fruits, seeds, and grape seed. The medicinal properties of proanthocyanidins have been widely reviewed [3-4]. In addition to antioxidant activity, proanthocyanidins possess anticarcinogenic, vasodilatory, immune-stimulating, cardioprotective, antiinflammatory, antiviral and estrogenic activities, as well as being inhibitors of enzymes such as cyclooxygenase, phospholipase A2, and lipoxygenase [5-6]. Lead is a ubiquitous environmental and industrial pollutant that may have its toxic effects on the male [7]. Lead has known to induce over production of reactive oxygen species(ROS) and enhance lipid peroxidation. Lead may inhibit spermatogenesis and reduce a mature spermatids, spermatocytes, and mature spermatids [8-9]. So, the aim of this study is to know the role of grape seed extract against lead toxicity that induced infertility of male rats.

Abstract

The present study was designed to find the role of grape seed extract against lead toxicity. Study was used 25 male rats that divided randomly to five groups (5 rats in each group) and the treatment continue four week in each group, control group administrated with normal saline, rats group treatment with (orally) lead (Pb), third group received lead (Pb) 100mg/kg and 50mg/kg seed extract, fourth group received lead (Pb) and 100mg/kg seed extract, fifth group received lead (Pb) and 200mg/kg seed extract. Rats group received lead show significant ($P<0.05$) decrease in counts, motility of sperm and increase the deformity of sperms. Other wise, rats group received lead show significant ($P<0.05$) decrease in glutathione (GSH), catalase (CAT) and increase levels of malonedialdehydied (MDA) compared with control group. The treated group with seed extract at different doses, sperm parameters and oxidative stress show gradual recovery. In 200mg/kg dose of seed extract, all parameters of study back to the normal ranges. It was concluded from this study that grape seed extract has important role against toxicity of lead in male rats.

Materials & methods

Animal model

25 adult male rats, (wt: 225-250mg with age: 4-6 Mon) obtained from general company for manufacture pharmaceuticals and medical requirements, Samara/Iraq, and kept on standard pellet diet and water for two weeks before experiment.

Preparation of the extract

Grape seed extract was purchased by isolated the seed from grape and homogenized by machine grinder. A total of 250 g of dried grape seed extract were extracted from 5 kg of fresh grape fruit. The extract was evaporated to dryness in oven at 60 °C for 40 minute. The extract was suspended in distilled water and used in this study in concentrations as in recommendation [10].

Experimental design

25 adult male rats were used and distributed in five groups (five rats in each group) as following and administrated treatments orally:

A. Control group received normal saline and normal diet for seven days.

B. Second group administrated lead (orally, 100mg/kg) for four weeks, and then killed.

C. Third group administrated lead (orally, 100mg/kg) and 50mg/kg seed extract for four weeks, and then killed.

D. Fourth group administrated lead (orally, 100mg/kg) and 100mg/kg seed extract for four weeks, and then killed.

E. Fifth group administrated lead (orally, 100mg/kg) and 200mg/kg seed extract for four weeks, and then killed.

Prepare of blood solution

2 ml of blood collected by cardiac puncture under anesthesia and put in test tubes. Then using centrifugation 5000 cycle/min for 15 min. Sera were taken and stored by deep freezing to estimate the biochemical measurement.

Semen Collection

Under anesthesia, testes and epididymides were removed. epididymides of each rat was divided in two parts. The caudal part was separated from the testes. Collected semen diluted with normal saline and put it in the tubes. Semen plasma was obtained by using centrifuge to study some parameters [11].

Sperm analysis

The content of the caudal part of epididymis was put in tube contain sodium citrate (1.9%) at (37° C). then, one drop put on slide and mixed with one drop of eosin - nigrosin stain to determining the percentage of live/dead and to abnormal/normal [12]. Also, sperm forms, sperm count, motility of sperm was done.

Statistical analysis

Data of study were analyzed by using a statistical program known as Minitab. data Means were compared using Duncan's Multiple Range test. Probability levels of more than 0.05 were regarded as statistically non-significant, whereas values less than 0.05 were considered as significant [13].

Results

Sperm counts and normality

Lead treated group showed significant ($P < 0.05$) decrease in number of sperm counts (3.4 ± 0.3) compared with control (5.6 ± 0.2). The sperm counts still show significant decreased in group treated with lead and seed extract (50mg/kg) and lead and seed extract (100mg/kg) (3.9 ± 0.5 and 4.7 ± 0.3 respectively) compared with control group, but better than the lead group. The sperm counts in group treated with lead and seed extract (200mg/kg) group showed non-significant changes in the counts of sperm. The percent of normal and deformity of sperm (60 ± 7 and 40 ± 5 respectively) in lead group showed significant decrease compared with control (93 ± 5 and 7 ± 2 respectively). The percent of normal and deformity of sperm still show significant decrease in lead and seed extract (50mg/kg) group and lead and seed extract (100mg/kg) (69 ± 2 and 4.7 ± 0.3 respectively) compared with control group, but better than the lead group. The percent of live and deformity of sperm in 200mg extract group (88 ± 3 and 10 ± 3 respectively) showed non-significant changes as show in table (1).

Table (1): counts and form of sperm in all study groups

Groups	Parameters	Sperm count ($\times 10^6$)	Normal (%)	Abnormal (%)
Control		5.6 ± 0.2 a	93 ± 5 a	7 ± 2 d
Lead		3.4 ± 0.3 c	60 ± 7 c	40 ± 5 a
Lead + seed extract (50mg/kg)		3.9 ± 0.5 bc	69 ± 2 c	31 ± 4 b
Lead + seed extract (100mg/kg)		4.7 ± 0.3 b	78 ± 4 a	22 ± 6 c
Lead + seed extract (200mg/kg)		5.3 ± 0.2 a	88 ± 3 a	10 ± 3 d

Sperm counts and normality

Lead treated group showed significant ($P < 0.05$) decrease in motility of sperm (Mot: 26 ± 4 , Slu: 32 ± 6 , Non-mot: 42 ± 7 respectively) compared with control (Mot: 69 ± 5 , Slu: 18 ± 4 , Non-mot: 13 ± 2 respectively). The sperm motility still show significant decrease in lead and seed extract (50mg/kg) group (Mot: 38 ± 3 , Slu: 32 ± 4 , Non-mot:

30 ± 2 respectively) and lead and seed extract (100mg/kg) group (Mot: 43 ± 7 , Slu: 37 ± 2 , Non-mot: 20 ± 3 respectively) compared with control group, but better than the lead group. The sperm motility in lead and seed extract (200mg/kg) group (Mot: 61 ± 5 , Slu: 21 ± 5 , Non-mot: 14 ± 5 respectively) showed non-significant changes in the counts of sperm compared with control group as show in table (2).

Table (2): Motility of sperm in all study groups

Groups	Motility	Motile (%)	Sluggish (%)	Non-motile (%)
Control		69 ± 5 a	18 ± 4 b	13 ± 2 d
Lead		26 ± 4 c	32 ± 6 a	42 ± 7 a
Lead + seed extract (50mg/kg)		38 ± 3 bc	32 ± 4 a	30 ± 2 b
Lead + seed extract (100mg/kg)		43 ± 7 b	37 ± 2 a	20 ± 3 c
Lead + seed extract (200mg/kg)		61 ± 5 a	21 ± 5 b	14 ± 5 d

Sperm deformity

The microscope examination show normal form of sperm in control group (fig:1). Lead treated group showed a deformity in the tail of sperm, that appear as ring in some and it's disappear in other (fig: 2-3). Lead and seed extract (50mg/kg) group and lead and seed extract (100mg/kg) groups showed a deformity in the tail of sperm that also appear as ring in some and it's disappear in other (fig: 4-5). Lead and seed extract (200mg/kg) group show decreased in deformity of sperm tail and appear near to the normal state (fig: 6).

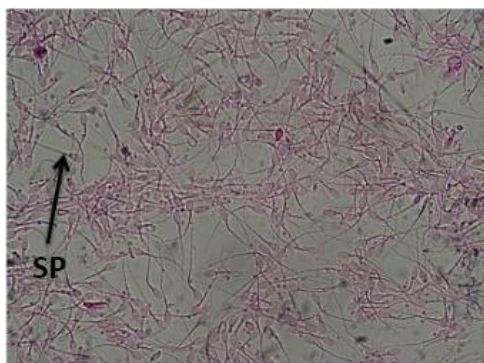


Figure (1): control group show normal sperm (SP) E&N X400.

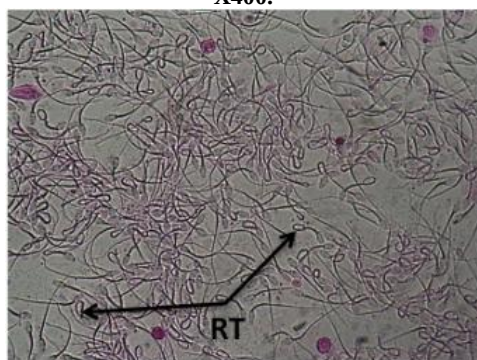


Figure (2): Lead group show ring tail of sperm (RT) E&N X400.



Figure (3): Lead group show ring tail (RT) and Non tail (NT) of sperm E&N X400.

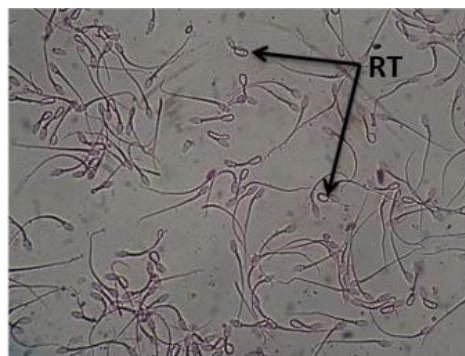


Figure (4): Lead and seed extract 50mg/kg group show ring tail of sperm (RT) E&N X400.

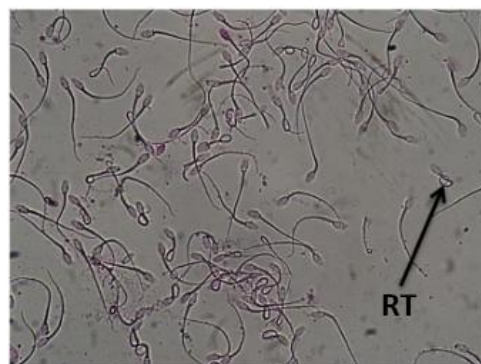


Figure (5): Lead and seed extract 100mg/kg group show ring tail of sperm (RT) E&N X400.



Figure (6): Lead and seed extract 200mg/kg group show ring tail of sperm (RT) E&N X400.

Oxidative stress

Lead treated group showed significant ($P < 0.05$) increase in MDA (Serum: 2.62 ± 0.23 , testis: 1.24 ± 0.16) compared with control (Serum: 1.51 ± 0.12 , testis: 1.24 ± 0.16). MDA still show significant increased in lead and seed extract (50mg/kg) group (Serum: 2.05 ± 0.15 , testis: 1.92 ± 0.16) and lead and seed extract (100mg/kg) group (Serum: 1.8 ± 0.083 , testis: 1.62 ± 0.12) compared with control group, but lower than the lead treated group. MDA levels in lead and seed extract (200mg/kg) group (Serum: 1.8 ± 0.083 , testis: 1.62 ± 0.12) showed non-significant changes compared with control group as show in table (3).

Lead group showed significant ($P < 0.05$) decrease in GSH and catalase (Serum: 0.42 ± 0.089 , 0.83 ± 0.054 and testis: 0.31 ± 0.063 , 0.9 ± 0.043 respectively) compared with control (Serum: 0.93 ± 0.054 , 1.2 ± 0.067 and testis: 0.72 ± 0.028 , 0.57 ± 0.035).

respectively). GSH and catalase still show significant decrease in lead and seed extract (50mg/kg) (Serum: 0.59 ± 0.067 , 0.94 ± 0.04 and testis: 0.45 ± 0.057 , 0.76 ± 0.29 respectively) and lead and seed extract (100mg/kg) group (Serum: 0.78 ± 0.062 , 1.01 ± 0.3 and testis: 0.61 ± 0.073 , 0.89 ± 0.37 respectively)

compared with control group, but lower than the lead treated group. GSH and catalase levels in lead and seed extract (200mg/kg) group (Serum: 0.9 ± 0.043 , 1.22 ± 0.042 and testis: 0.78 ± 0.051 , 1.01 ± 0.048 respectively) showed non-significant changes compared with control group as show in table (3).

Table (3): MDA, GSH and catalase in all study groups

Parameters Groups	MDA (mmol/l)		GSH (mol/l)		Catalase (mmol/l)	
	Serum	Testis	Serum	Testis	Serum	Testis
Control	1.51 ± 0.12 c	1.24 ± 0.16 d	0.93 ± 0.054 a	0.72 ± 0.028 a	1.2 ± 0.067 a	0.9 ± 0.043 a
Lead	2.62 ± 0.23 a	2.38 ± 0.19 a	0.42 ± 0.089 d	0.31 ± 0.063 d	0.83 ± 0.054 c	0.57 ± 0.035 c
Lead + seed extract (50mg/kg)	2.05 ± 0.15 b	1.92 ± 0.16 b	0.59 ± 0.067 c	0.45 ± 0.057 c	0.94 ± 0.04 b	0.76 ± 0.29 b
Lead + seed extract (100mg/kg)	1.8 ± 0.083 bc	1.62 ± 0.12 c	0.78 ± 0.062 b	0.61 ± 0.073 b	1.01 ± 0.3 ab	0.89 ± 0.37 ab
Lead + seed extract (200mg/kg)	1.43 ± 0.13 c	1.29 ± 0.2 d	0.9 ± 0.043 a	0.78 ± 0.051 a	1.22 ± 0.042 a	1.01 ± 0.048 a

Discussion

The results of previous studies reported that the exposure of human to lead (Pb) can reduce human semen quality. Where, they suggest lead induce decrease in sperm counts and motility and alter morphology [14-15]. Also, studies reported that exposure to lead (Pb) has side effects on sperm parameter (decreased counts, motile and increase sperm abnormality) [16-17], that is in agreement with results of present study. Also, in study carried by Ramah *et al.* (2015) referred that the lead acetate caused oxidative stress. They found that the lead acetate cause decrease in level of GSH and GST and significant increase in level of MDA in rats group administrated with lead acetate [9]. Other wise, Salawu *et al.* (2009) referred that the lead (Pb) has effect on antioxidant factors in mice. They found that the lead caused significant ($P < 0.05$) decrease in both plasma and testicular catalase of animals treated with Pb only compare with control group [18], that is in agreement with results of present study.

The results of current study showed a protective effect of grape seeds extract against the toxicity of lead (Pb). Where, In study of Khattab *et al.* (2010) referred that the grape seeds extract has a potential effects on sperm activity. Where in their study rats administrated with aluminum chloride ($AlCl_3$), they found $AlCl_3$ induced highly significant decrease in

sperm count, motility (%) and viability (%), with increase in dead and abnormal sperm count as compared to both control groups but treatment with grape seed extract showed potential role of seed extract against the toxicity of $AlCl_3$. Also, they found that $AlCl_3$ decrease the total antioxidant capacity in testicular tissue and serum of rats, but after treatment with grape seed extract, levels of total antioxidant capacity back to normal ranges [19]. Also, the results of present study agreed with Aysun *et al.* (2008) who reported that oral intake of grape seed extract reduced the oxidative stress [20]. Other wise, grape seed extract treatment increased the formation of antioxidant products which may be regarded to the phenolic constituents of GSE and its antioxidant activity [21]. Hafsa *et al.* (2016) referred to the protective effect of grape seed extract against the toxicity lindane in male rabbits. They found that lindane decrease the sperm counts, sperm concentration, total sperm output, sperm motility and increase in percentage of abnormal sperm, dead sperm. After treatment with grape seed extract, all sperm parameters back to normal ranges, suggest the positive effects of dietary grape seed extract on both sperm count and sperm motility and the reduced percentage of dead sperm could be linked to the antioxidative properties of grape seed extract [22].

Reference

1. Rice-Evans, C.A.; Miller N.J. and Paganga G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *J. Free Radic Biol Med* 20: 933–956.
2. Hasseeb, M. M.; Fahad A. and Yehia A. H. (2011). A Histopathologic Study of the Protective Effect of Grape Seed Extract Against Experimental Aluminum Toxicosis in Male Rat. *J. Sci. King Fai. Uni.* 12(1): 283-300.
3. Bagchi D.; Bagchi M.; Stohs S.; Ray S.D.; Sen C.K. and Preuss H.G. (2002). Cellular protection with

proanthocyanidins derived from grape seeds. *Ann NY Acad Sci* 957: 260–270.

4. Belviranlı, M.; Hakkı G.; Nilsel O. and Sadık B. (2015). Effects of grape seed extract on oxidative stress and antioxidant defense markers in streptozotocin-induced diabetic rats. *Turk J Med Sci* 45: 489-495.
5. Yamakoshi, J.; M. Saito, S. Kataoka and M. Kikuchi. (2002). Safety Evaluation of Proanthocyanidin - Rich Extract from Grape Seeds. *J. Food Chem. Toxicolo.* 40(5): 599-607.

6. Jia, Z.; Z. Song, Y. Zhao, X. Wang and P. Liu. (2011). Grape Seed Proanthocyanidin Extract Protects Human Lens Epithelial Cells from Oxidative Stress via Reducing NF- κ B and MAPK Protein Expression. J. Molec. Vis. 17: 210-217.
7. Garu, U.; Sharma, R. and Barber, I. (2011). Effect of lead toxicity on developing testis of mice. IJPSR. 2(9): 2403-2407.
8. Kaushal, D.; Bansal, M.R. and Bansal, M.P. (1996). Cell kinetics of the rat seminiferous epithelium following lead acetate treatment. J Trace Elem Exp Med 9, 47-56.
9. Ramah, A.; Ragab M.; Nabila M.A. and Elham A. (2015). The effect of lead toxicity on male albino rats reproduction with ameliorate by vitamin E and pumpkin seeds oil. J. Ben. Veter. Med. 28(1):43-52.
10. Majeed, H. M.; Khatam, J. S. and Rashad, F.G. (2008). Role Of Grape Seed Extract As Anti Hyperglycemia And Antioxidant In Experimental Diabetic Rats. J. Missan Res. 4(8): 1-25
11. AL-Saeed, M. H. and Nahla S. H. (2015). Study the effect of isoflavonoid extract of punica rinds on fertility efficiency and semen fluid prosperities in male rabbits. J. Vet. Res. 14(2):17-30.
12. Alsadi AA. (2001). Fertility and Artificial Insemination. 2nd ed College of Veterinary Medicine, University of Mosul.
13. Beth, D.; Robert, G. and Trapp. (2004). Basic and clinical biostatistics, 4th ed. Lange Medical Books/Mc Graw-Hill Medical Publishing Division. New York. PP: 83-154.
14. Alexander, B.H.; Checkoway, H.; Van Netten, C.; Muller, C.H.; Ewers, T.G.; Kaufman, J.D.; Mueller, B.A.; Vaughan, T.L. & Faustman, B.M. (1996). Semen quality of men employed at a lead smelter. Occup. Environ. Med., 53: 411-416.
15. Viskum, S; Rabjeng, L.; Jorgensen, P.J. & Grandjean, P. (1999). Improvement in semen quality associated with decreasing Occupational lead exposure. Am. J. Ind. Med., 35: 257-363.
16. Kumar, K. (2004). Occupational and exposure associated with reproductive dysfunction.. J. Occup. Health., 46: 1-19.
17. Hussein J.; Jasim M. S.; Hannan J. and Fawzia M. A. (2011). Toxic Effect of Lead on Reproductive System in Male Albino Mice. J. Baby. Uni. Pur. App. Sci. 19(3): 894-899.
18. Salawu, E. O.; Olusola A. A.; Olutunde P. F.; Uthman A. Y.; Abiodun O. and Anthony A. A. (2009). Tomato (Lycopersicon esculentum) prevents lead-induced testicular toxicity. J Hum Reprod Sci. 2(1): 30–34.
19. Khattab, H. A.H.; Inas, Z.A. A. and Gehan, M. K. (2010). Grape seed extract alleviate reproductive toxicity caused by aluminium chloride in male rats. J. Amer. Sci. 6(12): 1200-1209.
20. Aysun, C., Leylagul, K., Ismail, K., Sibel, H., Recep, S., Ahmet, O., Okan, O. and Osman, S. (2008). The effect of grape seed extract on radiation-induced oxidative stress in the rat liver. Turk J. Gastroenterol., 19 (2): 92-98.
21. Perez, D., Strobel, P. and Foncea, R. (2002). Wine, diet, antioxidant defenses and oxidative damage. J. Ann NY Acad. Sci., 957: 136-145.
22. Hafsa, S. H.; Hassan A. A.; Assadi S. E.; Agatha P. and Dorina M. (2016). Grape seed (vitis vinifera l.) Alleviate reproductive toxicity caused by lindane in new zealand white male rabbits. J. Econo. Engine. Agricu. Rur. Develop. 16(3): 17-27.

التأثير الفعال لمستخلص بذور نبات العنب ضد سمية الرصاص الذي يحث على العقم لذكور الجرذان

احمد حمد صالح

قسم تقنيات التحليلات المرضية ، كلية القلم الجامعة ، كركوك ، العراق

الملخص

صممت الدراسة الحالية لايجاد دور مستخلص بذور العنب ضد سمية الرصاص. استخدمت الدراسة 25 جرذ قسمت الى خمس مجموعات (5 جرذان لكل مجموعة) واستمرت المعالجة لمدة اربع اسابيع في كل المجاميع. جرعت مجموعة السيطرة بالمحلول الملحي، مجموعة جرذان الثانية واعطيت (فمويًا) رصاص 100mg/kg، مجموعة جرذان الثالثة اعطيت (فمويًا) رصاص ومستخلص البذور لمدة اربع اسابيع 50mg/kg، مجموعة جرذان الرابعة اعطيت رصاص ومستخلص البذور 100mg/kg، مجموعة جرذان الخامسة اعطيت رصاص ومستخلص البذور 200mg/kg. اظهرت مجموعة الجرذان المعطاة رصاص 100mg/kg انخفاض معنوي ($P < 0.05$) في اعداد وحركة النطف وزيادة في تشوهات النطف مقارنة مع مجموعة السيطرة. من جهة اخرى، اظهرت مجموعة الجرذان المعطاة رصاص 100mg/kg انخفاض معنوي ($P < 0.05$) في GSH و CAT وزيادة في MDA مقارنة مع مجموعة السيطرة. وادت المعالجة بمستخلص البذور وبجرعات مختلفة ادى الى تحسن تدريجي بمعايير النطف والجهد التأكسدي. وقد عادت مع الجرعة 200mg/kg من مستخلص البذور كل معايير الدراسة الى الحدود الطبيعية. يستنتج من هذه الدراسة بان مستخلص بذور العنب لعب دورا مهما ضد سمية الرصاص في ذكور الجرذان.