The Effect of Grape Seeds Ethanolic Extract On Telomeres Activation Against Cadmium - Induced Sexual Dysfunction in Male Rats

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Abstract

Background: Telomeres are protective DNA sequences at chromosome ends, essential for maintaining genomic stability and reproductive function. Cadmium (Cd), a toxic environmental pollutant, induces oxidative stress, telomere shortening, and testicular dysfunction. Grape seeds extract GSE, rich in oligomeric proanthocyanidins, possesses potent antioxidant properties and may counteract Cd-induced reproductive damage**. Aim of the study**: The current study aimed to evaluate the protective role of GSE on telomere length and their function on the testes of male rats exposed to cadmium. **Materials and Methods**: Forty adult male albino rats were divided into five groups (n=8): first group as negative control fed on basal diet only, group 2 as positive control and from group 3 to group 5 fed on basal diet and were injected intraperitoneal with a single dose of 1 mg/kg bw of CdCl2 dissolved in 0.9% saline for one month to induce sexual dysfunction in male rats. After 4 weeks' positive groups (with testicular dysfunction) fed on basal diet only and from (3-5) groups with testicular dysfunction fed on basal diet and giving orally grape seeds extract at 200 mg/kg.bw, 400 mg/kg.bw and 800 mg/kg.bw, for 4 weeks. At the end of the experimental period (2months), several parameters were assessed. **Results:** Cadmium exposure caused significant, decreased in testosterone levels, impaired sperm quality, shortened telomeres., on the other hand groups with testicular dysfunction fed on basal diet and given orally grape seeds extract at 400 and 800 mg/kg, had significant improvement in liver enzymes (ALT, AST), lipid profiles (TG, TC, LDL-c, LDL-c, and HDL-c levels), testosterone level and sperm count. Interestingly groups (with testicular dysfunction fed on basal diet and given orally grape seeds extract at dose (400and800mg/ kg.bw) showed significant decrease Casepase3 gene while increase in STAR gene improvement in the sperm morphology analysis compared to positive group (with testicular dysfunction) and fed on only basal diet. C**onclusion:** Grape seed extract exhibits protective effects against cadmium-induced testicular dysfunction, likely through its

antioxidant properties, ability to modulate telomerase activity and gene expression. GSE may serve as a valuable preventive nutraceutical in mitigating environmental toxin-related reproductive damage and promoting genomic stability.

Keywords; Telomere, Ethanolic Extract of Grape seeds, Cadmium, Testicular dysfunction, Testicular toxicity and Gene expressions.

1.INTRODUCTION

The primary function of the male reproductive system is to produce androgens such as testosterone that maintain male reproductive function and to promote spermatogenesis and transport into the female reproductive system for fertilization **Gurung *et al.*, (2023).** The human reproductive system is made up of the primary and secondary organs, which helps to enhances reproduction. The male reproductive system is designed to produce male gametes and convey them to the female reproductive tract through the use of supportive fluids and testosterone synthesis **Obukohwo *et al*., (2021),** The paired testis (site of testosterone and sperm generation), scrotum (compartment for testis localization), epididymis, vas deferens, seminal vesicles, prostate gland, bulbourethral gland, ejaculatory duct, urethra, and penis are the parts of the male reproductive system. The auxiliary organs aid in the maturation and transportation of sperm. Semen is made up of sperm and the secretions of the seminal vesicles, prostate, and bulbourethral glands (the ejaculate) **Das *et al*., (2023).** Ejaculate is delivered to the female reproductive tract by the penis and urethra **Puppo and Puppo., (2016).** Male reproductive dysfunction, poor sexual performances and loss of libido are common public health disorders as well as a major health challenge among humans in many part of the world (**Lotti and Maggi 2018).** Cadmium is a heavy metal posing severe risks to human health. On reproductive system it induces testicular necrosis and sexual hypo function. **Bhardwaj *et al*., (2021).** Cadmium Cd is a non-essential toxic heavy metal, an environmental toxicant, and toxic at a low concentration, and it has no known beneficial role in the human body. Its exposure induces various health impairments including reproductive health **(Shalaby *et al*., 2015).** Telomeres are the protective end caps of chromosomes and shortens with every cell division **(O'sullivan and Karlseder,2010)**. Telomere length has been proposed as biomarkers of biological age and risker factor of age related chronic diseases as testicular dysfunction (**Fasching ,2018),** It is known as elixir of life as it has a link with antioxidant activity. Telomerase is a chromosome-stabilizing enzyme, active at the end of a mitotic cycle and its main purpose is to add the missing section of the DNA on the delayed strand. It works on telomeres located

at the 3′ ends of eukaryotic chromosomes, whose role is to protect the DNA against possible damage during copying **Bartoszewska *et al.,* (2024),** Telomerase inhibition has a link with age related diseases as sexual hypo function. Numerous plants and their products that have significant antioxidant activities can use as supportive treatments during several diseases. Grape seeds contain many nutrients and active ingredients such as proteins, sugars, minerals, salts, flavonoids, coumarins, and tannins **(Kim *et al*.,2006 and Alotaibi *et al*., 2021).** In addition, it is a rich source of polyphenol substances, including proanthocyanidins (89%), dimmers (6.6%), trimers (5.0%), tetramers (2.9%), and oligomers (74.8%) **(Schelz *et al*., 2016).** Also, they are rich in epicatechin, anthocyanins, catechins, and epicatechin- 3-o-gallate. Thus, grape seed extract (GSE) has powerful antioxidant properties empowering to be utilized for therapy of numerous sicknesses. Telomerase is a chromosome-stabilizing enzyme, active at the end of a mitotic cycle and its main purpose is to add the missing section of the DNA on the delayed strand. It works on telomeres located at the 3′ ends of eukaryotic chromosomes, whose role is to protect the DNA against possible damage during copying. Telomerase inhibition has a link with age related diseases as sexual hypo function.

2.MATERIALS AND METHODS

2.1. Materials:

Rats: A total number of forty rats of Sprague Dawley strain weighing about 225± 10 g body weight and 8 months' age were purchased from the Agriculture Research Center, Ministry of Agriculture, Egypt.

Diet: Casein, vitamins, minerals, calcium phosphate, ethanol, L. cysteine, choline tartrate were obtained from El-Gomhorya Pharmaceutical Company, Cairo, Egypt. Starch, corn oil, and sucrose were obtained from the local market.

Grape seeds: were purchased from Plants Research Laboratory - Horticultural Research Institute - Agricultural Research Center.

Cadmium chloride CdCl2: in crystalline form was obtained from Sigma-Aldrich (St. Louis, MO, USA) and dissolved in 0.9% saline to the desired final volume used in the study.

Chemical kits: for serum biochemical analysis were purchased from local distributer of Sigma Chemical Company, Cairo branch, Egypt.

2.2. Methods:

2.1. Preparation of Basal Diets: The basal diet (AIN-93M) was consisted of protein (14%), corn oil (5%), minerals mixture (3.5%), vitamins mixture (1%), fiber (5%), sucrose (10%), choline chloride (0.25%) and the remainder was corn starch up to 100%. These constituents were thoroughly mixed and formulated according to **Reeves *et al*., (1993).**

2.2. Preparation of Grape seeds extracts: Grape seeds extracts were prepared by ethyl alcohol 70 % **(Jayaprakasha *et al*.,2003).**

2.3. Induction of testicular dysfunction; Cadmium chloride CdCl2 was intraperitoneal injected a single dose of 1 mg/kg CdCl2, for 4 weeks then daily fed basal diet according to **Kini *et al,* (2009).**

2.4. Experimental design: Forty Sprague Dawley strain rats were housed in well ventilated cages at room temperature (22 ±4 °C), 45% ± 5% humidity with a 12 hr dark/light cycle. Rats were adapted for one week on AIN-93 basal diet and received water ad libitum.

2.5. Rats: were assigned into five groups each of 8 rats as follows: Group1: Negative control group fed on basal diet (AIN 93) only. Group2: Positive control group rats with testicular dysfunction and fed on basal diet. Group3: Rats with testicular dysfunction, given orally grape seeds extract at 200 mg/kg.bw and fed on basal diet. Group 4: Rats with testicular dysfunction, given orally grape seeds extract at 400 mg/kg.bw and fed on basal diet. Group 5: Rats with testicular dysfunction, given orally grape seeds extract at 800 mg/kg.bw and fed on basal diet. At the end of the experimental period (2 months), the rats will be fasted overnight and anesthetized by sodium pentobarbital (Nesdonal®) at a dose of 40 mg/kg bw. Blood samples will be collected from orbital plexus of eye of each rat by microcapillary tubes. Blood samples will be centrifuged at 9000 rpm for 15 minutes to obtain clear serum for biochemical analysis.

2.6. Collection of Blood Samples: Blood samples were collected from orbital plexus of eye of each rat by micro capillary tubes. Blood samples were centrifuged at 9000 rpm for 15 minutes to obtain clear serum for biochemical analysis.

2.7. Biochemical analysis: After anesthesia of rats, the blood was withdrawn from orbital plexus of eye and blood samples were collected for determination of activities of serum aspartate aminotransferase (AST) and

alanine aminotransferase ALT according to the method of **Bergmeyer *et al.*, (1978).** Total cholesterol TC was calorimetrically determined according to **Allain *et al.*, (1974)** and triglycerides TG according to **Wahlefeld (1974).** High density lipoprotein cholesterol HDL-c was calorimetrically determined according to **Rajagopal *et al.*, (2012**). Very low density lipoprotein cholesterol VLDL-c and low density lipoprotein cholesterol LDL-c were mathematically calculated. Total serum testosterone levels**)** were determined using a radioimmunoassay method RIA and a diagnostic kit to measure quantitatively the total testosterone and sperm count according to **(Wilke and Utley 1987).**

2.8. Morphological analysis of sperm: Rats in each group, semen samples were collected from cuda epididymis and examine under microscope. Sperm cell count, sperm motility percent, alive / dead sperm percent and sperm abnormalities were be recorded.

2.9. Quantitative real-time PCR: Quantitative real-time PCR assay was applied to total RNA extracted from testicular tissue by a TRIzol reagent, according to the structure of the manufacturer to assess changes in the mRNA level of the inducible nitric oxide synthase genes expression in testicular tissues. The integrity of total RNA was investigatedby1%agarose gel electrophoresis. To detect the change in caspase-3(Casp3), steroidogenic acute regulatory protein (StAR), cDNA was synthesizedusing BioRad SYBER Green PCR Master Mix on Rotorgene RT-PCRsystem. The primer sequences were caspase-3 forward primer (F) 5′ CGATTATGCAGCAGCCTCAA- 3′ and reverse primer (R) 5′- AGGAGATGCCACCTCTCCTT-3; StAR (F) 5′-GCAGCAGGCAACCTGGTG-3′and (R) 5′-TGATTGTCTTCGGCAGCC-3 **(Hassen *et al.*, 2021).**

Statistical Analysis: All obtained data were presented as mean± standard deviation (SD). Analysis of Variance (Prism) test was used for determining the significances among different groups according to **(Motulsky,2007)**. All differences were considering significant if P-values at $P< 0.00$.

3-RUSLTS AND DISCUSSION

3.1. The effect of grape seeds ethanolic extract in Gene expression (STAR gene) and (Caspase3 gene) of telomeres against cadmium - induced sexual dysfunction in testicles tissue of male rats:

Table (1) illustrated effect of grape seeds ethanolic extract in gene expression (star gene) and (Caspase3 gene) of telomeres against cadmium - induced sexual dysfunction in testicles tissue of male rats. The positive control group showed a very highly significant decrease ($P<0.001$) in the mean value of testicles tissue STAR gene fold (0.3011±0.05336) compared to the negative control group (1.002±0.05333) respectively. On the other hand, a group of rats with testicular dysfunction, fed on basal diet and given orally grape seeds extract at dose 200mg/kg.bw had a non-significant differences in the mean value of testis STAR gene (0.5521±0.05332) compared to the positive control group (0.3011±0.05336) respectively.

Moreover, a group of rats with testicular dysfunction, fed on basal diet and given orally grape seeds extract at dose 400mg/kg.bw, observed a highly significant increase ($P<0.01$) in the mean value of testicles tissue STAR gene fold (0.6417±0.05335) compared to the positive control group (0.3011±0.05336) respectively. Rats with testicular dysfunction, fed on basal diet and given orally grape seeds extract at dose 800mg/kg.bw, observed a very highly significant increase ($P<0.001$) in the mean value of testicles tissue STAR gene fold (0.7981±0.05337) compared to the positive control group (0.3011±0.05336) respectively.

Regard to testicles tissue Caspase3 gene fold change in rats. results illustrated that positive control group showed a very highly significant increase ($P<0.001$) in the mean value of testicles tissue Caspase3 gene fold (5.303±0.2448) compared to the negative control group (1.005±0.2452 mg/dl) respectively. On the other side group of rats with testicular dysfunction, fed on basal diet and given orally grape seeds extract at dose 200mg/kg.bw, showed a non- significant differences in the mean value of testicles tissue Caspase3 gene fold (3.754±0.2449) compared to the positive control group (5.303±0.2448) respectively. Moreover, a group of rats with testicular dysfunction, fed on basal diet and given orally grape seeds extract at dose 400mg/kg.bw, observed a highly significant decrease ($P<0.01$) in the mean value of testicles tissue Caspase3 gene fold (2.444±0.2452) compared to the positive control group (5.303±0.2448) respectively.

Also, a group of rats with testicular dysfunction, fed on basal diet and given orally grape seeds extract at dose 800mg/kg.bw, showed a very highly significant decrease ($P<0.001$) in the mean values testicles tissue Caspase3 gene fold (1.917±0.2444) as compared to the positive control group (5.303±0.2448) respectively.

In the current study the positive control group showed a very highly significant decrease of testicles tissue STAR gene fold rats with testicular dysfunction by cadmium. This result integrated with **(Zhu *et al*., 2022)** who found that STAR PCR analysis demonstrated significant telomere shortening in the testicular tissues of Cd-exposed male rats, which correlates with increased oxidative damage, testicular atrophy, and impaired spermatogenesis. These findings are consistent with previous studies reporting that Cd disrupts telomere maintenance by inhibiting telomerase activity and promoting DNA strand breaks**.** The results revealed a significant reduction in telomere length in the Cd-exposed group, reflecting enhanced oxidative stress and accelerated cellular aging. This telomere attrition was associated with impaired spermatogenesis and testicular dysfunction, in line with previous findings linking shortened telomeres to reduced fertility and disrupted reproductive processes. This result agreement with **(D'Angelo, 2023)** who found that administration of grape seed ethanolic extract, rich in potent antioxidants such as proanthocyanidins, quercetin, and vitamin E, effectively preserved telomere length, as evidenced by STAR gene. This suggests that the extract may activate telomerase activity or indirectly protect telomeric DNA by reducing ROS levels and DNA strand breaks exposure to cadmium, a potent environmental toxicant, has been shown to accelerate telomere shortening through the generation of reactive oxygen species (ROS), which induce oxidative damage to DNA, particularly guanine-rich telomeric regions **(Iakovou and Kourti,2022).** Also, the preservation of telomere length in the treated group highlights a fertility-preserving effect of natural antioxidants under oxidative stress conditions **Jourabchi *et al*., (2025).** These findings support the hypothesis that targeting telomere dynamics through antioxidant intervention may offer a novel strategy to mitigate cadmium-induced testicular toxicity cadmium is a heavy metal known for inducing reproductive toxicity through mechanisms involving oxidative stress, inflammation, and DNA damage. One of the most significant consequences of Cd-induced oxidative stress is telomere attrition, which reflects cellular aging and loss of genomic stability **(Keeler *et al*.,2017).** Telomeres, composed of repetitive DNA sequences (TTAGGG)n, are highly susceptible to oxidative damage due to their guanine-rich structure. Single telomere length analysis PCR (STAR gene) has emerged as a sensitive and reliable method for assessing telomere length at the level of individual chromosome ends, enabling

researchers to study telomere dynamics in response to toxic insults such as cadmium exposure **(Cawthon, 2002).** On the otherwise, gene analysis demonstrated significant telomere shortening in the testicular tissues of Cd-exposed male rats, which correlates with increased oxidative damage, testicular atrophy, and impaired spermatogenesis. These findings are consistent with previous studies reporting that Cd disrupts telomere maintenance by inhibiting telomerase activity and promoting DNA strand breaks **(Lv *et al*., 2024).**

Our results corroborated that the groups of rats fed on grape seeds extract at levels (400 and 800 mg/ kg.bw) had a significant increase in STAR as compared to the positive control group these findings at the same line with **Tian *et al*., (2018),** Treatment with grape seed ethanolic extract, rich in bioactive antioxidants like proanthocyanidins, catechins, and quercetin, was found to preserve telomere length in testicular tissue**.** It's clear that the antioxidant properties of the extract protect telomeric DNA from oxidative damage, indirectly support telomerase activity and reduce its inhibition under stress conditions **Jacczak *et al*., (2021).** These protective effects contribute to the activation or stabilization of telomeres, thereby maintaining chromosomal integrity and reproductive function in the face of cadmium toxicity **Khan *et al*., (2022).** This protective action may stem from the extract's ability to scavenge reactive oxygen species (ROS), reduce DNA strand breaks, and enhance the expression of genes involved in oxidative defense **Apostolou *et al.,* (2013)**. As a result, grape seed extract not only attenuated telomere shortening (as shown by STAR gene), but also contributed to the restoration of testicular function and spermatogenic activity, ultimately counteracting cadmium-induced sexual dysfunction. These findings support the potential of natural antioxidants in maintaining telomere integrity and reproductive health under toxicological stress. Overall, the use of STAR gene in this context highlights the critical role of telomere biology in male fertility and supports the therapeutic potential of natural antioxidants in protecting reproductive cells from environmental toxins **Matzkin *et al*., (2021).**

Exposure to cadmium can occur through industrial processes, cigarette smoke, contaminated water, and food sources. Its toxicological effects are primarily mediated through oxidative stress, lipid peroxidation, and induction of apoptotic pathways, particularly via the activation of caspase enzymes such as Caspase-3 **(El-Demerdash *et al*., 2004).** In recent years, the search for natural antioxidants that can mitigate cadmium-induced damage has gained momentum **Unsal *et al*., (2020).** It is well-known that Cd toxicity induces oxidative stress via the production of free radicals, which are harmful to cells **Branca *et al*., (2020).** Free radicals may damage protein, lipid, enzymes and

DNA, and therefore must be neutralized by antioxidants before entering cells **Mathew *et al.*, (2011)**. The current study showed that intake of grape seeds extract at levels (400 and 800 mg/kg.bw) showed significant decrease in casepase3 gene expression compared to the positive control group. These findings were supported by the results of previous studies which proved that GSE is one of the most powerful antioxidants, which contains high levels of bioflavonoids, vitamin C and vitamin E **Cuevas *et al.*, (2011)**. GSE protects cells from damage by regulating cell oxidative damage, reducing organ injury, improving the balance between oxidants and antioxidants, and reducing the release of inflammatory mediators. Therefore, the present study was designed to examine the possible ameliorative and protective effects of GSE against chronic Cd-induced testicular dysfunction **Liu *et al., (*2022).**

Table (1) Effect of grape seeds ethanolic extract on Gene expression (STAR gene) and (Caspase3 gene) of telomeres against cadmium - induced sexual dysfunction in testicles tissue of male rats:

Parameter / Group	STAR gene	Caspase3 gene
Control (-ve) group	1.002±0.05333	1.005±0.2452
Control(+ve) group	0.3011±0.05336***	5.303±0.2448***
Group3 (Grape seed extract 200mg)	0.5521±0.05332ns	3.754±0.2449ns
Group4 (Grape seed extract 400mg)	0.6417±0.05335**	2.444±0.2452**
Group5 (Grape seed extract 800mg)	0.7981±0.05337***	1.917±0.2444***

- Results are expressed as mean ± SD
- Means with different superscript stars in the column are significantly different at ** highly significant ($p < 0.01$); *** very highly significant ($p < 0.001$) and Ns. none significant as compared to group 2 positive group.

3.2. The effect of grape seeds ethanolic extract on Semen testosterone level in telomeres activation rats with cadmium-induced toxicity:

Table (2) illustrated the effect of grape seeds ethanolic extract on Semen testosterone in telomeres activation on rats with testicular dysfunction. positive control group (rats with testicular dysfunction) and fed on basal diet showed a very highly significant decrease ($P<0.001$) in the mean value of semen testosterone concentration (5.1976±0.095nmg/dl) compared to the negative control group (24.316±0.009nmg/dl).

On the other hand, a group of rats with testicular dysfunction fed on basal diet and given orally grape seeds extract at dose 200mg/kg. bw. showed a non-significant differences in the mean value of semen testosterone level (12.279±0.094nmg/dI) compared to the positive control group (rats with testicular dysfunction) (5.1976±0.095nmg/dl) respectively.

Moreover, a group of rats with testicular dysfunction fed on basal diet and given orally grape seeds extract at dose 400mg/kg. bw. showed a high significant increase ($P<0.01$) in the mean value of semen testosterone level (17.629±0.101nmg/dl) compared to the positive control group (rats with testicular dysfunction) (5.1976±0.095nmg/dl) respectively. Also, the highest level of semen testosterone level in rats with testicular dysfunction fed on basal diet and given orally grape seeds extract at dose 800mg/kg. bw, (group5) in the mean value (21.276±0.106nmg/dl) compared to the positive control (rats with testicular dysfunction) (5.1976±0.095nmg/dl) respectively.

Our study showed that Cd-induced toxicity effected on different body organs the exceptionally testes, in positive control group. many experimental and epidemiologic studies have been carried out to ascertain Cd-induced toxicity in different body organs in different species across the world. However, among the various organs, the testes are exceptionally sensitive to the Cd toxicity **Siu *et al*., (2009**). Also other studies showed that Cd can interfere with reproductive processes in males, including impairment of parental fertility, effects on offspring development and fertility, and has been identified as an endocrine disruptor due to its ability to interrupt the production and regulation of reproductive hormones in males **Ji *et al*., (2010).** Cd also diminishes fertility by causing damage such as decreased testicular weight, testicular hemorrhage, edema, necrosis and atrophy in mice, in addition to decreasing sperm count and motility **(Alharthi *et al*., 2020)**. Cd negatively affects sperm viability, quality, and quantity as well as sperm production, and hormones **(Kumar and Sharma, 2019).** One proposed mechanism is Cd-induced vascular damage in the testes, which may interfere with the utilization of essential elements like zinc **Souza et al *et al*., (20٢١)**. Since zinc, manganese, and selenium are crucial for testicular

health, the substitution of zinc by toxic metals like Cd can impair fertility **Maciejewski *et al*., (2022).** It is well-known that Cd toxicity induces oxidative stress via the production of free radicals, which are harmful to cells. Free radicals may damage protein, lipid, enzymes and DNA **Valko *et al.,* (2006).**

This study concluded that GSE had beneficial protective effects against the deleterious effects of CdCl2 on the testis. Cd effect Several studies have indicated that cadmium disrupts male reproductive function by impairing hormone production and testicular structure, classifying it as an endocrine disruptor **Arteaga *et al.,* (2020).** CdCl2 toxicity induced a significant downregulation in the mRNA expression levels of cytochrome P450 cholesterol side-chain cleavage enzyme, cytochrome P450 17A1, 3β-hydroxysteroid dehydrogenase (3β-HSD), 17β-HSD, androgen receptor, steroidogenic acute regulatory protein, and follicle-stimulating hormone receptor **(Arukwe *et al*.,2008).** Our study showed that intake of grape seeds extract at levels (400 and 800 mg/kg.bw) showed significant increase semen testosterone level compared to the positive control group and this result agreement with **Naskar *et al*., (2010)** who found that antioxidants serve as potent scavengers for free radicals and prevent the occurrence of disease Co-treatment with GSE and CdCl2 exhibited ameliorative effects on the immunoreactivity of B-cell lymphoma 2-associated X protein **Alkhedaide *et al*., (2016),** GSE administration exhibited a stimulatory effect on steroidogenesis-associated enzymes, and co-treatment with GSE and CdCl2 normalized and upregulated the mRNA expression levels of these examined genes.

Table (2): Effect of grape seeds ethanolic extract on semen testosterone level on telomeres activation against cadmium - induced sexual dysfunction in male rats:

Parameter / Groups	**Testosterone nmg /ml**
Control (-ve) group	24.316±0.009
Control (+ve) group	5.1976±0.095***
Group 3 (Grape seed extract 200mg)	12.279±0.094 ns
Group4 (Grape seed extract 400mg)	17.629±0.101**
Group5 (Grape seed extract 800mg)	21.276±0.106***

- Results are expressed as mean ± SD
- Means with different superscript stars in the column are significantly different at ** highly significant ($p < 0.01$); *** very highly significant ($p < 0.001$) and Ns. none significant as compared to group 2 positive group.

3.3. The effect of grape seeds ethanolic extract on sperm count in telomeres activation against cadmium - induced sexual dysfunction in male rats.

Table (3) illustrated the effect of grape seeds extract ethanolic on sperm count in telomeres activation against cadmium - induced sexual dysfunction in male rats. The positive control group (group with testicular dysfunction) showed a very highly significant decrease ($P<0.001$) in the mean value of semen Sperm count (7.37 ±0.051) compared to the negative control group (22.49±0.011) respectively. On the other hand, a group of rats with testicular dysfunction fed on basal diet and given orally grape seeds extract at dose 200mg/kg. bw showed non-significant differences in the mean value of semen Sperm count (13.07±0.008) compared to the positive control group (7.37 ±0.051) respectively. Moreover, a group of rats with testicular dysfunction fed on basal diet and given orally grape seeds extract at dose 400mg/kg.bw, as showed a highly significant increase ($P<0.01$) in the mean value of semen Sperm count_(16.08±0.004) compared to the positive control group with testicular dysfunction (7.37 ±0.051) respectively. Rats with testicular dysfunction fed on basal diet and given orally grape seeds extract at dose 800mg/kg. bw had a very highly significant increase ($P<0.001$) in the mean value of semen Sperm count level (18.626±0.009) compared to the positive control group (7.37 ±0.051) respectively.

Besides sperm concentration, sperm motility in the current study administrated that the positive control group (rats with testicular dysfunction) showed a very highly significant decrease. In the same line **Wang *et al*., (2017)** found that men with low sperm motility had significantly higher serum Cd levels than did men with normal sperm motility. In a single low dose of Cd (0.05 or 1.0 mg/kg body weight), administered to adult rats, has resulted in failure of spermiation, the final phase of sperm differentiation and also to reduced sperm concentration and motility.

Sperm motility is recognized to be more sensitive to this trace element, as the reduced sperm motility has been observed at a dose far below the dose affecting sperm production **Benoff *et al.,* (2009)** the data show that while animal experiments support an adverse effect of low Cd exposure on semen parameters, more research is needed to clarify this relationship in male rats.

This study concluded that GSE had beneficial protective effects against the deleterious effects of CdCl2 on the testis interestingly the intake of grape seeds extract at levels (400 and 800 mg/kg.bw) showed significant increase sperm count level compared to the positive control group. This result was in the same line as the result of several studies have demonstrated that grape seeds extract

GSE, grape seeds proanthocyanidin extract, and grape juice concentrate act as effective protective agents against cadmium-induced toxicity. These compounds have been reported to improve histopathological outcomes by increasing seminiferous tubule diameter, testicular weight, and Johnsen's mean testicular biopsy score, while also reducing the apoptotic index **(Sönmez *et al*., 2016**; **Alkhedaid *et al*., 2016; Evcimen *et al.,* 2020).**

Table (3) Effect of grape seeds ethanolic extract in sperm count on telomeres activation against cadmium - induced sexual dysfunction in male rats:

Parameter / Groups	**Sperm count x 10**
Control (-ve) group	22.49±0.011
Control (+ve) group	7.37 ±0.051***
Group 3 (Grape seed extract 200mg)	13.07±0.008 ns
Group 4 (Grape seed extract 400mg)	16.08±0.004**
Group 5 (Grape seed extract 800mg)	18.626±0.009***

- Results are expressed as mean ± SD
- Means with different superscript stars in the column are significantly different at ** highly significant ($p < 0.01$); *** very highly significant ($p < 0.001$) and Ns. none significant as compared to group 2 positive group.

3.4. The effect of grape seeds ethanolic extract on liver functions in telomeres activation against cadmium - induced sexual dysfunction in male rats. Table (4) showed the effect of grape seeds extract on alanine aminotransferase ALT and aspartate aminotransferase AST in rats with cadmium-induced toxicity. The positive control group (testicular dysfunction group), showed a very highly significant increase ($P < 0.001$) in the mean values of ALT and AST (98.25 ±1.980 and 113 ± 2.393 u/ml) respectively compared to the negative control group (32.51±1.977 and 45.69±2.399 u/ml) respectively. On the otherwise, a group of rats with testicular dysfunction, fed on basal diet and given orally grape seeds extract at dose 200 mg / kg. bw showed non-significant differences in the mean values of ALT and AST (73.87±1.981 and 84.99±2.398 u/ml) respectively compared to the positive control group (98.25 ±1.980 and 113 ± 2.393 u/ml) respectively. The obtained results in the group of rats with testicular dysfunction, fed on basal diet and given orally grape seeds extract at dose 400mg / kg.bw had a highly significant decrease ($P<0.01$) in the mean values of ALT and AST levels (59.61±1.979 and 71.34±2.394 u/ml)

respectively compared to the positive control group (98.25 ±1.980 and 113 ± 2.393 u/ml) respectively. On the otherwise the group of rats with testicular dysfunction fed on basal diet and given orally grape seeds extract at dose 800mg /kg. bw had a very highly significant decrease ($P<0.001$) in the mean values of ALT and AST levels (47.3±1.974 and 58.2± 2.395 u/ml) respectively compared to the positive control group (rats with testicular dysfunction) (98.25 ±1.980 and 113 ± 2.393 u/ml) respectively.

In this study, the positive control group with testicular dysfunction exhibited a significant increase in liver enzymes alanine aminotransferase ALT and aspartate aminotransferase AST compared to the negative control group. These findings are consistent with those reported by **Shaikh *et al*., (1999),** who observed elevated serum ALT, and AST levels after nine weeks of cadmium administration, indicating hepatic damage these results support the hypothesis that cadmium-induced liver dysfunction may indirectly contribute to exacerbated testicular damage. Our results corroborated that the groups of rats fed on grape seeds extract at levels (400 and 800 mg/ kg.bw) had a significant improvement in ALT and AST as compared to the positive control group these findings at the same line with **Hassan *et al.,* (2020)** who indicating that that grape seed extract (GSE) exerts protective effects against cadmium induced testicular toxicity in rats, who funded in their experiment after 90 days in the intoxicated cadmium group, serum AST activity significantly increased by about 9% as compared to the control group. Co-administration of GSE plus cadmium group, however, resulted in AST activity improvement. The serum level of ALT was markedly increased by 2.2 fold after 90 days in the cadmium treated group, compared with the control group. Co-administration of GSE plus cadmium, on the other hand, decreased ALT operation by about 34% as compared to cadmium alone and 51% after 90days as compared to standard regulation.

Table (4): Effect of grape seeds ethanolic extract on liver functions in telomeres activation against cadmium - induced sexual dysfunction in male rats.

Parameter / Groups	ALT (u/ml)	AST (u/ml)
Control(-ve) group	32.51±1.977	45.69±2.399
Control(+ve) group	98.25±1.980***	113.2±2.393***
Group3 (200mg/grape seeds extract)	73.87±1.981ns	84.99±2.398ns
Group4 (400mg/grape seeds extract)	59.61±1.979**	71.34±2.394**
Group5 (800mg/grape seeds extract)	47.3±1.974***	58.2±2.395***

- Results are expressed as mean ± SD
- Means with different superscript stars in the column are significantly different at ** highly significant ($p < 0.01$); *** very highly significant ($p < 0.001$) and Ns. none significant as compared to group 2 positive group.
- ALT (alanine transaminase) and AST (aspartate transaminase)

3.5. The effect of grape seeds ethanolic extract in lipid profile on telomeres activation against cadmium - induced sexual dysfunction in male rats LDL, HDL, TG and Cholesterol: Table (5) presented the effect of grape seeds extract on total serum cholesterol, triglycerides, LDL and HDL levels in rats with testicular dysfunction. The positive control group with testicular dysfunction showed a very highly significant increase ($P<0.001$) in the mean value of serum total cholesterol, triglycerides, LDL levels (117.9±3.545mg/dl, 166.8±3.241mg/dl and 68.99± 1.534 mg/dl) respectively compared to the negative control group (64.05±3.544, 84.47±3.246, and 15.46±1.544 mg/dl) respectively. Otherwise the positive control group with testicular dysfunction group had a very highly significant decrease ($P<0.001$) in the mean value of HDL level (10.09±1.23 mg/dl) compared to the negative control group (40.2±1.29 mg/dl) respectively. On the other hand, a group of rats with testicular dysfunction fed on basal diet and given orally grape seeds extract at dose 200 mg/kg bw showed a non-significant differences in the mean values of serum total cholesterol, triglycerides and LDL (91.69±3.543 mg/dl, 122.7±3.242mg/dl and 42±1.553mg/dl) respectively compared to the positive

control group (group with testicular dysfunction) (117.9±3.545mg/dl, 166.8±3.241mg/dl and 68.99± 1.534 mg/dl) respectively. Otherwise rats with testicular dysfunction fed on basal diet and given orally grape seeds extract at 200 mg/kg bw showed a non-significant differences in the mean value of HDL level (17.06±1.25 mg/dl) compared to the positive control group with testicular dysfunction (10.09±1.23 mg/dl) respectively. Moreover, rats with testicular dysfunction fed on basal diet and given orally grape seeds extract at dose 400mg/kg. bw. showed a highly significant decrease ($P<0.01$) in the mean values of serum total cholesterol, triglycerides and LDL (82.72±3.58mg/dl, 102±3.230 mg/dl and 31.72±1.576mg/dl) respectively compared to the positive control group with testicular dysfunction (117.9±3.545mg/dl, 166.8±3.241mg/dl and 68.99± 1.534mg/dl) respectively. Otherwise rats with testicular dysfunction fed on basal diet and given orally grape seeds extract at dose 400 mg/kg bw had a highly significant increase ($P<0.01$) in the mean value of HDL level (24.373±1.22 mg/dl) compared to the positive control group with testicular dysfunction (10.09±1.23 mg/dl) respectively. Also, the lowest serum total cholesterol, triglycerides and LDL levels values in rats with testicular dysfunction fed on basal diet and given orally grape seeds extract at dose 800 mg/kg.bw (group5) in the mean values (76.4±3.535, 93.46±3.243mg/dl and 22.62±1.555mg/dl) compared to the positive control group with testicular dysfunction (117.9±3.545mg/dl, 166.8±3.241mg/dl and 68.99± 1.534mg/dl) respectively.

Otherwise rats with testicular dysfunction fed on basal diet given orally grape seeds extract at dose 800 mg/kg, bw showed a very highly significant increase ($P<0.001$) of HDL level (30.523±1.26mg/dl) compared to the positive control group with testicular dysfunction (10.09±1.23 mg/dl) respectively. After the experimental period (2 months), Cd administration significantly increase serum levels of TC, TG and LDL, and significantly decreased the serum HDL level versus the control group Cd exposure produced significant changes in oxidative stress parameters in the serum of rats these results agreement with **Samarghandian *et al.*, (2015),** reported that the serum Cd level in the Cd-exposed group was significantly increased compared to the control group. The results of the present study indicate that oral administration of Cd significantly affected the adverse metabolic effects in rats after 3 months Cd exposure for 3 months resulted in higher TC, TG, LDL and Cd levels in the serum of exposed rats compared with non-exposed animals (control). In addition, Cd administration significantly decreased HDL-C and GSH compared to control rats **Azimi-Nezhad *et al.*, (2015)**. Also, considerable increases in TC, TG and LDL-C fractions were observed in rats exposed to dose of Cd as compared with the control group. Other studies have demonstrated similar increases in serum levels of TC, TG and LDL-C after high dose administration

of Cd to rats **(Prabu *et al*., 2010)**. Our results corroborated that the groups of rats grape seeds extract at levels (400 and 800 mg/ kg. bw), had a significant improvement in HDL and decrease levels of (T C, LDL, TG) as compared to the positive control group this result agreement with **Dinicola *et al*., (2014)** reported that GSE contains numerous compounds called polyphenols which contain dimers, trimers, and other oligomers (procyanidins) of catechin and epicatech. All of these compounds are types of proanthocyanidins. These compounds protect the body against atherosclerosis. Moreover, ethanolic grape seed extract GSE, rich in proanthocyanidins, has demonstrated protective effects against cadmium (Cd)-induced alterations in lipid profiles in rats. Cd exposure often leads to dyslipidemia, characterized by increased total cholesterol (TC), triglycerides TG, low-density lipoprotein cholesterol LDL, and very low-density lipoprotein cholesterol VLDL, alongside decreased high-density lipoprotein cholesterol HDL **(Ali *et al*., 2021).**

Table (5); **Effect of grape seeds ethanolic extract on lipid profile in telomeres activation against cadmium - induced sexual dysfunction in male rats:**

Parameters / **Groups**	**HDL** (mg /dl)	**LDL** (mg /dl)	**T G** (mg /dl)	**Cholesterol** (mg /dl)
Control (-ve)	40.2±1.29	15.46±1.544	84.47±3.246	64.05±3.544
Control (+ve)	10.09±1.23***	68.99±1.534***	166.8±3.241***	117.9±3.545***
Group 3 grape seeds extract (200mg/kg. bw)	17.06±1.25ns	42.14±1.55ns	122.7±3.242ns	91.69±3.543ns
Group 4 grape seeds extract (400mg/kg. bw)	24.373±1.22**	31.72±1.576**	102±3.230**	82.72±3.538**
Group 5 grape seeds extract (800mg/kg. bw)	30.523±1.26***	22.62±1.555***	93.46±3.243***	6.4±3.535***

- Results are expressed as mean ± SD;
- Means with different superscript stars in the column are significantly different at ** highly significant ($p < 0.01$); *** very highly significant ($p < 0.001$); Ns. none significant as compared to group 2 positive group.
- Total cholesterol (TC), Triglycerides (TG), High density lipoprotein cholesterol (HDL-c), and low density lipoprotein cholesterol (LDL-c).

3.6. Effect of grape seeds extract on the sperm Morphology analysis changes.

1. Group 1:

Representative Figures: 1, 2

- Overall Morphology: Normal sperm architecture with preserved structural integrity
- Head: Smooth, oval-shaped heads with intact acrosomes. Uniform chromatin condensation observed.

- Midpiece: Cylindrical and symmetrical connection between head and tail.

- Tail: Uniform, slender flagella with smooth surfaces and linear orientation.

- Abnormalities: None detected (<1% abnormality rate).

Histological Significance: Represents baseline physiological spermiogenesis.

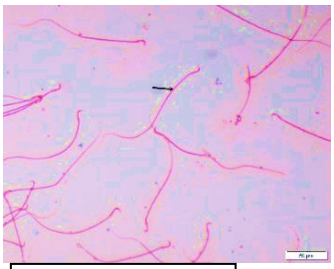

Figure (1)

Figure (2)

2. Group 2:

Representative Figures: 3,4

- Overall Morphology: Severe teratozoospermia with multiple structural defects
- Head:

- 43% incidence of bent/curved heads

- 28% decapitated sperm

- Midpiece: Frequent cytoplasmic droplets noted

- Tail:

- Coiling defects (67% incidence)
- Rough membrane surfaces (82% incidence)
- Hairpin loops observed
- Abnormalities: 100% of sperm showed ≥1 major defect.

Histological Significance: cadmium-induced oxidative damage disrupts spermiogenesis.

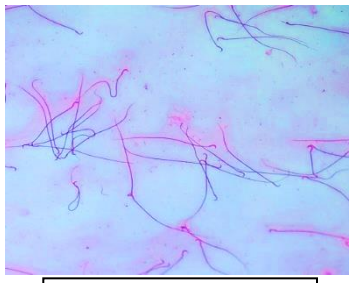

Figure (3)

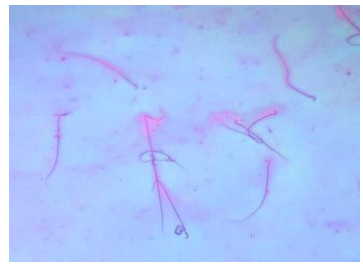

Figure (4)

3. Group 3:

Representative Figures: 5,6

- Overall Morphology: Moderate improvement in tail defects
- Head:
  - 18% bent heads (mild improvement vs G2)
  - 9% decapitation
- Tail:
  - Persistent rough surfaces (55% incidence)
  - Reduced coiling (32% incidence)
  - Abnormalities: 76% defect rate.

Histological Significance: Partial remediation of tail defects but persistent head abnormalities.

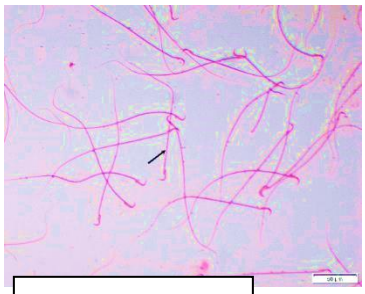

Figure (5)

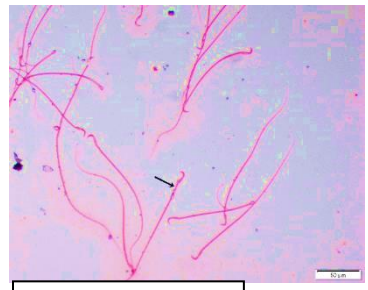

Figure (6)

4. Group 4:

Representative Figures: 7,8

- Overall Morphology: Significant head recovery with residual tail defects
- Head:

  - Near-normal morphology (92% normal heads)

  - Minor bending in 8%

Figure (7)

- Tail:

  - Focal roughness (37% incidence,

  - Occasional coiling (15%)

  - Abnormalities: 42% defect rate (primarily tail).

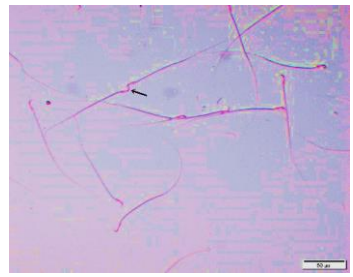

Figure (8)

Histological Significance: Effective head protection with moderate tail improvement.

5. Group 5:

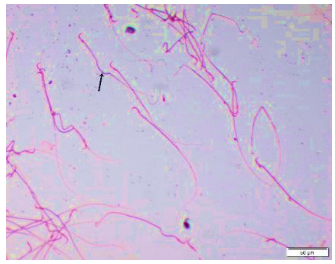

Figure (9)

Representative Figures: 9,10

- Overall Morphology: Near-normal morphology
- Head:

  - 97% normal oval configuration

  - Intact acrosomal caps

- Tail:

  - Mild surface irregularities (22% incidence)

  - Minimal coiling (8%)

  - Abnormalities: 28% defect rate (mild tail defects only).

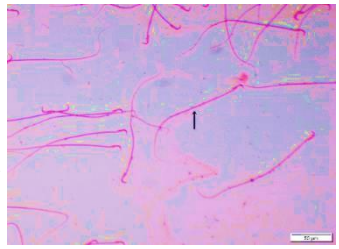

Figure (10)

Histological Significance: Synergistic restoration of sperm architecture

approaching control levels.

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تأثير المستخلص الايثانولي لبذور العنب علي تنشيط التولوميرات ضد العجز الجنسي المحدث بالكادميوم في ذكور الفئران

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المستخلص

التيلوميرات هي تسلسلات حماية من الحمض النووي DNAعند أطراف الكروموسوم، وهي ضرورية للحفاظ على الاستقرار الجيني والوظيفة الإنجابية. الكادميوم (Cd) وهو ملوث بيئي سام، يسبب الإجهاد التأكسدي، وتقصيرطول التيلومير، وخللًا وظيفيًا في الخصيتين. يتميز مستخلص بذور العنب (GSE) الغني بالبروأنثوسيانيدينات، بخصائص مضادة للأكسدة قوية وقد يُقلل الضرر الناتج عن الكادميوم الذى يؤثر علي القدرة الإنجابية .هدفت هذه الدراسة إلى تقييم الدور الوقائي لمستخلص بذور العنب ((GSEعلى طول التيلوميرات ووظيفتها في خصيتي ذكور الفئران المعرضة للكادميوم. تم تقسيم أربعين جرذًا ذكرًا بالغًا من الفئران البيضاء إلى خمس مجموعات (عددها٨): المجموعة الأولى كمجموعة تحكم سلبية تتغذى على النظام الغذائي الأساسي فقط، والمجموعة الثانية كمجموعة تحكم إيجابية، ومن المجموعة الثالثة إلى المجموعة الخامسة تتغذى على النظام الغذائي الأساسي وحقنت داخل الصفاق بجرعة واحدة مقدارها ١ملغم/كغم من وزن الجسم من كلوريد الكادميوم المذاب في محلول ملحي ٠,٩٪ لمدة شهر واحد لتحفيز الخلل الجنسي لدى ذكور الفئران. بعد ٤ أسابيع، تتغذى المجموعات الإيجابية (المصابة بخلل في الخصية) على النظام الغذائي الأساسي فقط، ومن (٣-٥) مجموعات مصابة بخلل في الخصية تتغذى على النظام الغذائي الأساسي مع إعطاء مستخلص بذور العنب عن طريق الفم بجرعات ٢٠٠ ملغم/كغم من وزن الجسم، و٤٠٠ ملغم/كغم من وزن الجسم، و٨٠٠ ملغم/كغم من وزن الجسم، لمدة ٤ أسابيع. في نهاية الفترة التجريبية (شهرين).تم تقدير عددة قياسات: هرمون التستوستيرون في السيرم، وكفاءة السائل المنوي. النتائج: أظهرت النتائج أن التعرض للكادميوم أدي الي انخفاضا ملحوظا في مستويات التستوستيرون، وتدهورملحوظا في كفاءة الحيوانات المنوية، وقصرا في طول التيلوميرات. علي العكس أظهرت النتائج الخاصة ، بالمجموعات التي تعاني من خلل وظيفي في الخصية وتغذت على نظام غذائي أساسي ومستخلص بذور العنب وخاصةً بجرعتي ٤٠٠ و٨٠٠ ملغم/كغم، تحسنًا ملحوظًا في نشاط إنزيمات الكبد (ALT وAST)، وصورة الدهون (TG وTC وLDL-c و HDL-c)، ومستوى التستوستيرون الكلي في السيرم وعدد الحيوانات المنوية. من المثير الاهتمام ان المجموعات التي تعاني من خلل وظيفي في الخصية وتغذت على نظام غذائي أساسي ومستخلص بذور العنب٨٠٠،٤٠٠ملغم/كغم من وزن الجسم أظهرت انخفاضًا كبيرًا في جين Casepase3 وزيادة كبيرة في جين STAR و شكل الحيوانات المنوية مقارنةً بالمجموعة الضابطة الموجبة (التي تعاني من خلل وظيفي في الخصية) وتغذت على النظام غذائي أساسي فقط.**الخلاصة**: مستخلص بذور العنب ربما يكون له تأثيرات وقائية ضد الخلل الوظيفي في الخصية الناجم عن الكادميوم، ويرجع ذلك إلى خصائصه المضادة للأكسدة وقدرته على تحسين نشاط التيلوميرات و جين اكسبريشين و الذي قد يكون مرتبطا بالسموم البيئية.

الكلمات المفتاحية: التيلومير،المستخلص الإيثانولي لبذور العنب، الكادميوم، خلل وظيفي في الخصية، سمية الخصية وجين اكسبريشين.