JJBS

Volume 13, Number 1,March 2020
ISSN 1995-6673
Pages 77 - 83

Jordan Journal of Biological Sciences

Estimation of Grape Seed Oil Alleviative Role on Cadmium Toxicity in Male Mice

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Received April 14 , 2019; Revised May 18, 2019; Accepted May 28, 2019

Abstract

Cadmium (Cd) is a non-degradable environmental pollutant, and has the ability to collect in the body organs causing humans and animals health risks. The present study was conducted to estimate the protective role of grape seed oil (GSO) against cadmium chloride ($CdCl_2$) induced toxicity in male mice. Phenolics content and antioxidant capacity of GSO were measured. Male mice had gavages of $CdCl_2$ at a dose of (7.48 mg/kg body weight (b. w.) once a day for 30 successive days, whereas they were orally administered GSO at 1 mL/kg b. w. once a day for 30 successive days either pre or plus $CdCl_2$. After the treatment, liver enzymes, sperm characteristics and organs weights were investigated. Genotoxicity was evaluated using comet assay and micronucleus test. Results revealed that $CdCl_2$ induced elevation in ALT and AST, significantly increased the relative weights of liver, decreased the testes weight, sperm count and declined motility and elevated DNA damage in tested cells compared to control group. In contrast, administration of GSO at two regimens restored the organs' weights to the normal range, ameliorated liver enzymes, improved the sperm physical characteristics and alleviated DNA damage compared to $CdCl_2$ treated mice. Furthermore, GSO pre-administration was the best regimen in the attenuation of the toxic effects of $CdCl_2$. It can be concluded that GSO may possess a protective role against the toxicity of $CdCl_2$ male mice.

Keywords: Grape seed oil, Cadmium chloride, Organs weights, Liver enzymes, Sperm physical characters, Genotoxicity.

1. Introduction

Heavy metals are a paradigm of pollutants. They are stable high-density metals present in the environment because they are one of the components of earth's crust (Tchounwou *et al.,* 2012). The heavy metals quantity in the air, water, soil and tissues of living organisms has increased because of the human activities that caused their increase in the environment (Gana and Toba, 2015). Heavy metals are toxic, but the dose, degree of exposure and the chemical form of these metals lead to differences in the extent of their toxicity (Tchounwou *et al.*, 2012).

Cadmium (Cd) is a major industrial and environmental pollutant that is principally produced from smelting, mining, electroplating, battery industrialized, paints and plastics. The wide route of exposure to Cd mostly results from smoking, air pollution and Cd contaminated foods and water utilization (Honda *et al.,* 2010). It is accumulated in the liver, kidney and testis, so it is considered as one of the common poisonous heavy metals. Cd causes a broad range of negative health hazards including hepatotoxicity (Kisok, 2012). Wang *et al.* (2017) reported that cadmium is an endocrine disruptor known to apply toxic effects on the testes, with indicated sperm dysfunction during both chronic and acute cadmium exposure. In addition, Cd is known as human carcinogen that attacks DNA directly and disturbs DNA repair mechanism (Murugavel and Pari, 2010). It is confirmed that Cd induces oxidative stress through the creation of free radicals that may damage protein, lipid, enzymes and DNA (Naskar *et al.*, 2010).

Antioxidants provide potent free radicals scavengers and thwart the incidence of disease (Islam *et al.*, 2011). In recent times, using natural foodstuffs for diseases handling has been broadly suggested due to their protective effects (Soliman *et al.,* 2013; 2015). Such findings suggest that it is necessary to identify alternative drugs for protection against toxicity and the treatment of human diseases. Grape (*Vitis vinifera*) is one of the world's major fruit crops, and grape seed extract is a multifarious matrix with approximately 40% fiber, 16% oil, 11% proteins, and 7% phenols such as tannins and mineral (Shi *et al.,* 2003). The grape seed oil (GSO) has a lot of uses for food additive, cosmetics, controlling diseases and wound healing (Shivananda *et al.,* 2011). Many researchers have mentioned plentiful health benefits of GSO, where it is considered as a potent antioxidant for its polyphenols, flavonoids, unsaturated fatty acids and vitamin E contents (Hassanein and Abedel-Razek, 2009; Kikalishvili *et al.,* 2011; Hasseeb *et al.,* 2013). Grape seed extracts (GSE) possess antimutagenic and anticarcinogenic properties by inhibiting enzymes of free radicals productions (Maier *et al.,* 2009). Moreover, the exploitation of GSE may be helpful in withdrawing the side effects of chemotherapeutic agents (Aysun *et al.,* 2008). In addition,

it is recognized that the GSO could supply protection against free radicals induced oxidative stress and cellular damage caused after exposure to heavy metals (Shinagawa *et al.*, 2015).

Consequently, the aim of this study was to estimate the potential protective role of grape seed oil against the toxicity induced by cadmium in liver and testis of mice. The study was carried out by monitoring DNA damage by micronucleus test and comet assay in male mice, by examining liver function (enzymes), sperm analysis, and by determining the body weight of animals.

2. Materials and Methods

2.1. Chemicals

Cadmium chloride ($CdCl_2$) was purchased from Sigma-Aldrich Company (St. Louis, MO, USA). Grape seed commercial oil (GSO) was purchased from EL Captin Company (Al Obour City, Cairo, Egypt). The aspartate aminotransferase (AST) and alanine aminotransferase (ALT) kits were purchased from Biodiagnostic Co., Egypt. All other chemicals were of analytical grade and purchased from standard commercial suppliers.

2.2. Animals and treatments

Male Swiss albino mice weighing 24±5 g (10-12 week old) were purchased from the Theodor Bilharz Research Institute, Giza, Egypt. Animals were housed in polypropylene cages (43cm× 30cm × 15cm, five mice per cage) with stainless steel covers in the Animal House of Environment and Bio-agriculture Department, Faculty of Agriculture, Al-Azhar University. Animals were kept under controlled temperature (23±4 °C), 50–55% relative humidity and photoperiod of 12 h light:12 h dark cycle. Throughout the experimental period, animals were maintained on standard mice pellet food (Salaam Feed Factory, El marg, Cairo) and tap water *ad libitum.* The mice were allowed to adapt to their surrounding environment for 2 weeks prior to start the experiments. All animals received human care in compliance with the guidelines of the Animal Care and Use Committee of the National Institutes of Health (NIH publication 86-23 revised 1985).

After acclimation, the animals were randomly divided into five groups (n = 10) according to approximately equal mean body weight (b.w.) and administered orally for 30 successive days with $CdCl_2$ (1/25 LD_{50}, 7.48 mg/kg b.w.) according to Yang *et al.* (2012a) and/or GSO (1mL/kg b.w.) according to Mokhtari *et al*. (2011). Experimental groups were as follows: Control group: animals were orally administered with saline. GSO group: animals were orally administered with GSO at dose of (1mL/kg b.w.). $CdCl_2$ group: animals were orally administered with $CdCl_2$ at dose of (1/25 LD_{50}). GSO pre-$CdCl_2$ group: animals were orally administered with 1 mL/kg b.w. GSO then with $CdCl_2$ (1/25 LD_{50}). GSO plus $CdCl_2$ group: animals were orally administered with 1 mL/kg b.w. GSO plus $CdCl_2$ (1/25 LD_{50}).

2.3. Grape seed oil total phenolic content

Total phenolic content (TPC) was determined using the Folin–Ciocalteau's reagent according to the method reported by Lin and Tang (2007) at 760 nm with a spectrophotometer (UV-1601; Shimadzu, Tokyo, Japan), and the quantification was done on the basis of the standard curve of gallic acid concentration ranging between 10 to 80 mg/mL (r^2= 0.99).

2.4. Measurement of antioxidant activity of grape seed oil

The ability of GSO at 50, 100 and 200µL to scavenge 2.9 mL of 1, 1'-diphenyl 1-2-picrylhydrazyl (DPPH) free radical was estimated by the method of Singh *et al.* (2002).

2.5. . Relative organs weights

At the termination of the experiments, internal organs such as liver and testes were dissected out, trimmed of excess fat and weighted. The organs weight was presented as relative organ weight as follows:

$$\text{Relative organ weight} = \frac{\text{Organ weight (g)}}{\text{Final body weight (g)}} \times 100$$

2.6. Liver function investigation

Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) liver enzymes activities were measured in serum by the method of Reitman and Frankel (1957).

2.7. Sperm collection and analysis

The epididymides from each mouse were removed and sperm was collected as quickly as possible after dissection. Epididymides was excised and minced in 1 mL of phosphate buffered saline (pH 7.2) to obtain sperm suspension; the sperms were filtered afterwards through a nylon mesh. The sperm count was assessed from right cauda epididymides while sperm motility was analyzed from the left one. Sperm count was performed according to Narayana *et al.* (2002) using a Neubauer hemocytometric chamber. Sperm motility was assessed by counting the motile sperms. Approximately 10 µL of sperm suspension was layered onto a warmed microscope slide. In each semen sample, at least 10 microscopic fields were examined with at least 100 sperm/field counted. The percentage of motile sperm was determined as described by Kvist and Bjorndahl (2002).

2.8. Bone marrow micronucleus assay

Bone marrow sampling, preparations of slide and scoring of micronucleated polychromatic erythrocytes (MNPCE) were done as described by Hayes *et al.* (2009). In brief, bone marrow was gently flushed out in fetal calf serum, centrifuged at 1000 rpm for 5 min and the medium was decanted. Pellet obtained was dispersed in 0.25 mL fetal calf serum, smeared on clean slides, fixed in 70% methanol, air dried and stained with May-Grunwald/ Giemsa protocol. Minimum of 2000 polychromatic erythrocytes/mouse were scored from treated or control group.

2.9. Single cell gel Electrophoresis in liver and testes

Single cell gel Electrophoresis (comet assay) was performed in liver and testes cells according to Bandyopadhyay *et al.* (2008). Briefly, 50 µL of cell suspension was mixed with 100 µL of 1 % low melting point (LMP) agarose and added to fully frosted slides coated with 80 µL of 1 % normal melting point (NMP) agarose. The cells were then incubated in a lysis solution (2.5 mol L^{-1} NaCl, 100 mmol L^{-1} EDTA, 10 mmol L^{-1} Tris-HCL, 1 % Triton X-100, pH 10) at 4 °C for at least 2 h, at which the slides were placed into an alkaline solution

(300 mmol L^{-1} NaOH, 1 mmol L^{-1} EDTA, pH 13) at 4 °C for 20 min so as to allow DNA unwinding, and electrophoresed at 25 V (300 mA) for 20 min. Finally, the slides were neutralized in 400 mmol/L Tris buffer (pH 7.5) for 15 min and stained with ethidium bromide (5 μg mL^{-1}). Images of 50 randomly selected nuclei per experimental group were captured using a fluorescence microscope (Eclipse 800, Nikon, Tokyo, Japan) and analyzed with image analysis software (Comet Assay IV, Perceptive Instruments, Suffolk, UK). Scored parameters included tail length, DNA percentage in tail and olive tail moment (OTM). Tail length is the maximum distance that the damaged DNA migrates from the center of the cell nucleus. DNA percentage in tail is the DNA content that migrates from the nucleus into the comet tail. OTM is the product of the tail length and percentage DNA, which gives a more integrated measurement of overall DNA damage in the cell.2.10. Statistical analyses

Statistical analyses were performed with SPSS 16 software. Experimental data was analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc test for multiple comparisons between pairs. All values were expressed as mean ± S.D. and differences were considered as significant when ($P \leq 0.05$).

3. Results

3.1. Oil analysis

GSO was found to have high level of phenolic content (42.17±0.55 mg gallic acid /100g oil) and antioxidant activity (84.35±0.35) as show in Table 1. In addition, antioxidant activity analysis of GSO revealed that its scavenging activity was raised gradually with increasing the concentration as shown in Figure 1.

Table 1. Phenolic content and antioxidant activity in grape seed oil.

Character	M ± SD
Phenolic content (mg gallic acid /100g oil)	42.17±0.55
Antioxidant activity %	84.35±0.35

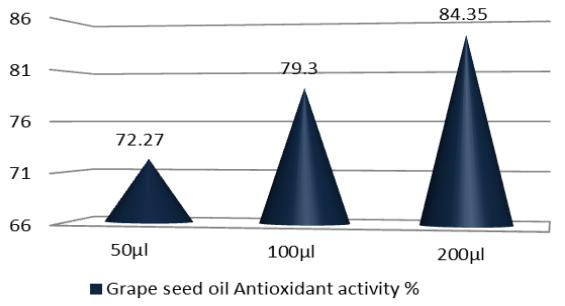

Figure 1. Grape seed oil antioxidant activity.

3.2. Relative organs weights

According to data presented in Table 2, there was no significant difference between both liver and testes relative weights of control and GSO treated groups. Oral administration of $CdCl_2$ to male mice for 30 successive days significantly increased ($P \leq 0.05$) the relative weights of liver (4.18±0.05) and decreased the testes weight (0.65±0.03) as compared to control (3.39±0.06 and 0.83±0.03), respectively. On the other hand, GSO administration either pre or plus $CdCl_2$ induced a significant decrease ($P \leq 0.05$) in the relative weights of liver (3.39±0.04 and 3.33±0.07, respectively) and increase in testes (0.77±0.02 and 0.74±0.02, respectively) compared with $CdCl_2$ treated mice.

Table 2. Relative organs weights of treated male mice with cadmium chloride ($CdCl_2$) and / or grape seed oil (GSO) for 30 consecutive days.

Treatments	Relative organs weights (g)	
	Liver	Testes
Control	3.39 ± 0.06^{bc}	0.83 ± 0.03^{a}
GSO (1 mL/kg b.wt)	3.46 ± 0.03^{b}	0.86 ± 0.02^{a}
$CdCl_2$ (1/25 LD_{50})	4.18 ± 0.05^{a}	0.65 ± 0.03^{c}
GSO pre-$CdCl_2$	3.39 ± 0.04^{bc}	0.77 ± 0.02^{b}
GSO plus $CdCl_2$	3.33 ± 0.07^{b}	0.74 ± 0.02^{b}

Data are expressed as means ± SD. Mean values in the same column within each parameter bearing the same superscript do not differ significantly ($P \leq 0.05$).

3.3. Liver function

The mean values of serum liver enzymes in $CdCl_2$ and/or GSO treated male mice are illustrated in Table 3. A significant increase ($P \leq 0.05$) in the mean values of ALT (43.50±3.31) and AST (46.25±4.11) was observed following the treatment with $CdCl_2$ for 30 successive days compared with control (24.50±1.29 and 31.00±1.83), respectively. In contrast, oral administration of GSO either pre or plus $CdCl_2$ produced a significant decrease ($P \leq 0.05$) in serum content of ALT (30.75±2.06 and 34.25±3.30, respectively) and AST (34.25±2.50 and 38.25±3.59, respectively) than that of $CdCl_2$ treated male mice.

Table 3. Serum liver enzymes of cadmium chloride ($CdCl_2$) and / or grape seed oil (GSO) treated male mice.

Treatments	ALT (U/L)	AST (U/L)
Control	24.50 ± 1.29^{c}	31.00 ± 1.83^{c}
GSO (1 mL/kg bw)	23.50 ± 1.73^{c}	29.75 ± 2.22^{c}
$CdCl_2$ (1/25 LD_{50})	43.50 ± 3.31^{a}	46.25 ± 4.11^{a}
GSO pre-$CdCl_2$	30.75 ± 2.06^{b}	34.25 ± 2.50^{bc}
GSO plus $CdCl_2$	34.25 ± 3.30^{b}	38.25 ± 3.59^{b}

Data is expressed as means ± SD. Mean values in the same column within eachparameter bearing the same superscript do not differ significantly ($P \leq 0.05$).

3.4. Sperm analysis

As shown in Figure 2, treatment with $CdCl_2$ for 30 successive days caused a significant decline ($P \leq 0.05$) in the mean values of sperm count (284.20±29.51) and motility (54.00±5.09) compared with control (465.40±22.41 and 85.00±3.53), respectively. By contrast, supplementation of GSO to male mice either before or with $CdCl_2$ significantly improved ($P \leq 0.05$) the decrease of sperm count (447.20±22.70 and 400.60±28.02, respectively) and motility (82.00±2.55 and 76.25±2.44, respectively) compared with $CdCl_2$ treated mice.

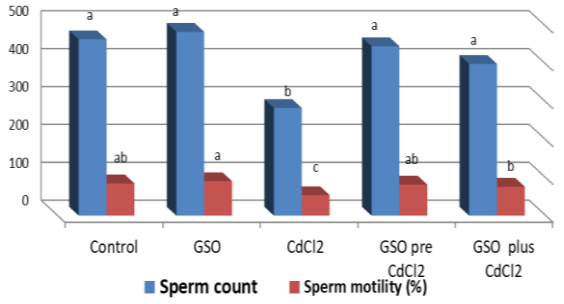

Figure 2. Sperm count and motility in cadmium chloride ($CdCl_2$) and/or grape seed oil (GSO) treated mice for 30 consecutive days. Data is expressed as means ± SD. Mean values in the same column within each parameter bearing the same superscript do not differ significantly ($P \leq 0.05$).

3.5. Effect of CdCl2 and/or GSO on micronuclei number and PCEs/NCEs percentage in bone marrow

Results in Table 4 showed that there were no statistically significant differences in the numbers of MN in the control and GSO-treated groups. While, $CdCl_2$ significantly increased ($P \leq 0.05$) the frequency of MNPCEs (40.67±6.76) as compared to control (5.33±1.33). On the other side, administration of GSO either pre or plus $CdCl_2$ decreased significantly ($P \leq 0.05$) the frequencies of MNPCEs (16.67±2.90 and 26.00±2.00) than in $CdCl_2$ group (40.67+6.76). Concerning to the PCEs/NCEs percentage, data analysis revealed that $CdCl_2$ caused marked toxicity in bone marrow cells, where it is significantly decreased ($P \leq 0.05$) the percentage of PCEs/NCEs. Along with that, GSO ameliorated the toxicity of $CdCl_2$ by increasing the PCEs/NCEs percentage .

Table 4. Frequencies of PCEs, NCEs, MN and PCEs/NCEs percentage in bone marrow cells of cadmium chloride ($CdCl_2$) and/or grape seed oil (GSO) treated mice.

Treatments	PCEs /2000Cell	NCEs /2000Cell	MNPCEs /2000Cell	PCEs/NCEs %
Control	1440.67±32.44^{b}	559.33±32.44^{c}	5.33±1.33^{c}	0.025+0.002^{b}
GSO (1 mL/kg b.w.)	1544.00±16.77^{a}	456.00±16.77^{d}	4.00±1.15^{c}	0.033+0.001^{a}
$CdCl_2$ (1/25 LD_{50})	1181.33±32.91^{d}	818.67±32.91^{a}	40.67±6.76^{a}	0.014+0.001^{d}
GSO pre-$CdCl_2$	1384.67±27.55^{bc}	615.33±27.55^{bc}	16.67±2.90^{b}	0.022+0.002^{bc}
GSO plus $CdCl_2$	1311.33±28.48^{c}	688.67±28.48^{b}	26.00±2.00^{b}	0.018+0.001^{cd}

Data is expressed as means ± SD. Mean values in the same column within each parameter bearing the same superscript do not differ significantly ($P \leq 0.05$). * PCEs: Polychromatic erythrocytes NCEs: Normochromatic erythrocytes MN: Micronuclei.

3.6. Effect of CdCl2 and/or GSO on tailed cells, tail length, tail DNA % and olive tail moment in liver and testes

The mean values of tailed, intact cells, tail length, tail DNA % and olive tail moment in $CdCl_2$ and/or GSO treated liver and testes are presented in Tables 5 and 6. There were no significant differences in all tested parameters between control and GSO treated animals in both organs cells. The values of tailed cell percentage, tail length, DNA percentage in tail and olive tail moment in liver cells of male mice treated with $CdCl_2$ were significantly increased ($P \leq 0.05$) compared to those of control. However, supplementation of GSO to male mice either pre or plus $CdCl_2$ caused a significant alleviation ($P \leq 0.05$) in the values of above-mentioned parameters than in $CdCl_2$ treated animals values. In addition, $CdCl_2$ caused a significant rise ($P \leq 0.05$) in all values of testes cells compared to control. By contrast, administration of GSO plus $CdCl_2$ with both patterns repressed significantly ($P \leq 0.05$) the increase in the all previously mentioned parameters rate as compared to those caused by $CdCl_2$.

Table 5. The mean values of intact, tailed cells, tail length, tail DNA % and olive tail moment in cadmium chloride ($CdCl_2$) and/or grape seed oil (GSO) treated liver**.**

Treatments	Intact cells (%)	Tailed cells (%)	Tail length (µm)	Tail DNA (%)	Olive tail moment (µm)
Control	86.17±0.38^{ab}	13.83±0.38^{cd}	9.04±0.57^{d}	13.04±0.37^{d}	1.18±0.07^{d}
GSO (1 mL/kg b.w.)	87.30±0.79^{a}	12.70±0.79^{d}	8.44±0.46^{d}	12.22±0.73^{d}	1.03±0.04^{d}
$CdCl_2$ (1/25 LD_{50})	70.33±3.26^{d}	29.67±3.26^{a}	19.56±0.58^{a}	21.78±0.41^{a}	4.26±0.09^{a}
GSO pre-$CdCl_2$	83.40±1.44^{bc}	16.60±1.44^{bc}	11.40±0.77^{c}	14.53±0.55^{c}	1.66±0.14^{c}
GSO plus $CdCl_{24}$	80.73±1.86^{c}	19.27±1.86^{b}	13.40±0.61^{b}	16.16±0.31^{b}	2.16±0.10^{b}

Data is expressed as means ± SD. Mean values in the same column within each parameter bearing the same superscript do not differ significantly ($P \leq 0.05$).

Table 6. Effect of cadmium chloride ($CdCl_2$) and/or grape seed oil (GSO) on Tailed cells, Tail length, Tail DNA % and Olive tail moment in testes.

Treatments	Intact cells(%)	Tailed cells (%)	Tail length (µm)	Tail DNA (%)	Olive tail moment (µm)
Control	87.23±0.65[a]	12.77±0.65[d]	7.54±0.58[cd]	10.61±0.62[d]	0.79±0.02[d]
GSO	87.70±0.36[a]	12.30±0.36[d]	6.91±0.59[d]	10.49±0.48[d]	0.72±0.05[d]
CdCl2	67.76±1.35[d]	32.24±1.35[a]	14.90±0.89[a]	18.40±0.65[a]	2.74±0.19[a]
GSO pre-CdCl2	82.43±1.02[b]	17.57±1.02[c]	8.61±0.54[c]	12.15±0.51[c]	1.04±0.03[c]
GSO plus CdCl2	77.86±2.04[c]	22.14±2.04[b]	9.87±0.48[b]	14.45±0.53[b]	1.43±0.11[b]

Data is expressed as means ± SD. Mean values in the same column within each parameter bearing the same superscript do not differ significantly ($P \leq 0.05$).

4. Discussion

Grape seed products are nutraceutical agents generally utilized as health dietary supplements. The concern about GSO as a functional food has increased because of its high content of phenolic compounds, vitamin E, unsaturated fatty acids (UFAs) and phytosterols (Karaman *et al.*, 2015). This was in line with the result of oil analysis, which confirmed that GSO possess a high antioxidant capacity due to high content of gallic acid, catechin, proanthocyanidins, procyanidins and epicatechin.

Cadmium intoxicated mice showed clear increase in liver weight, may be because the hypertrophy was induced in the liver because of cadmium toxicity. This result is consistent with a previous report indicating that $CdCl_2$ toxicity leads to increasing the weight of liver in rats (Bashir *et al.*, 2014). Otherwise, cadmium produced loss in testis weight. This was consistent with the Elgawish and Ghanem (2014) study; they indicated that the reproductive organs weight was decreased in rats exposed to $CdCl_2$ as compared to the control. The decline in testes and epididymis weight may be ascribed to the inhibition of spermatogenesis, decreased elongated spermatids and steroidogenic enzyme activity (Takahashi and Oishi, 2003). Salem and Salem, (2016) observed that cadmium induced liver weight elevations and testes weights reduction. Conversely, GSO restored the organs weight to the normal range. Our results match with Bashir *et al.* (2014); they found that cadmium decreased water and food intake followed with retardation in growth rate and increase in liver weight. Authors interpreted that Cd accumulation causes disturbances in the body and liver weight of rats due to tissue damage and reduction in their functions. All these alterations induced by Cd intoxication were significantly restored to near normal levels upon pre-administration of GSE. In our study, marked hepatic damage was observed because of the significant elevation of the serum hepatic enzymes (ALT, AST) in the $CdCl_2$ group. These characteristic features of $CdCl_2$-induced liver toxicity are comparable to those formerly reported by other investigators (Obioha *et al.*, 2009; Renugadevi and Prabu, 2010; Shati, 2011; Salem and Salem, 2016). The elevation in liver enzymes activities in $CdCl_2$ intoxicated animals had been attributed to cellular leakage in the organ and loss of functional integrity of membrane architecture of hepatocytes (Salem and Salem, 2016). On the other hand, the disturbance in the levels of the hepatic enzymes in $CdCl_2$ intoxicated mice was ameliorated by both pre- and co-administration with the GSO, as there was a significant decrease in the levels of these enzymes in mice treated with GSO pre/plus $CdCl_2$. This was in agreement with Dogan and Celik (2012) as they indicated that the grape seed extract (GSE) efficiently protected the rats against alcohol-induced hepatotoxicity, as evidenced by AST, ALT enzyme levels reduction. In addition, Abdul-Hamid *et al.* (2018) found that GSE administration with amiodarone revealed obvious improvement for liver enzyme activity compared to amiodarone-treated animals. Bishayee *et al*. (2010) suggested that polyphenol existing in grape seed could reduce hepatic enzymes through the antioxidant actions.

Our findings clarified that $CdCl_2$ administration caused significant decrease in sperm count and motility. These findings were in consent with those of earlier studies by Oliveira *et al.* (2009) and Kaur and Sharma (2015) who observed significant reduction in sperm physical characters in $CdCl_2$ exposed mice. Acharya *et al.* (2008) clarified that reactive oxtgen species (ROS) generated by cadmium can cause spermatozoa lipid peroxidation and deleterious effects on cell membrane phosphatides, inducing sperm DNA oxidation and consequently sperm abnormalities and decline in alive sperm count. The GSO caused amelioration of physical properties of semen in mice. This was in agreement with Al-Shahari and El-kott (2019); they demonstrated a defensive impact of grape seeds extract against the poisoning of monosodium glutamate, whereas Yildirim *et al.* (2011) indicated that the GSE affects sperm movement sperms number, and motility (%), with less immotile and abnormal sperm morphology when contrasted with control. Also, Al-Saeed (2016) reported that GSO caused sperm count elevation in the diazinon treated rats. This could be due to the capability of GSO to either interfere with the spermatogenic processes in the seminiferous tubules, epididymal functions of testosterone on hypothalamic release factor and anterior pituitary secretion of gonadotropins that may lead to activation of spermatogenesis.

Our data showed that $CdCl_2$ induced DNA damage measured by MN and comet assays in liver and testes cells which is considered as indicator of its genotoxicity. Likewise, Abd-El-Moneim *et al.* (2017); El-Habit and Abdel Moneim (2014) reported significant elevations in micronuclei number in murine bone marrow cells exposed to $CdCl_2$. Moreover, Mahrous *et al.* (2015) discovered highly significant frequencies of micronuclei in Nile tilapia fish treated with $CdCl_2$. Also, the results of DNA damage measured by comet assay were in consent with several studies, like Karimi *et al.* (2012) who observed significant alterations in DNA in mice kidney cells

exposed to $CdCl_2$ as compared with control mice. Abd-El-Moneim *et al.* (2017) indicated that the rates of DNA damage in liver cells of $CdCl_2$ contaminated diet fed mice were significantly increased, with significant elevation in comet parameters including (tailed cells %, tail length, tail DNA % and tail moment). In addition, Skipper *et al.* (2016) found significant elevation of DNA damage measured by comet assay in liver carcinoma cells of humans treated with $CdCl_2$. Many studies revealed that $CdCl_2$ oral administration in drinking water of rats enhanced oxidative stress and subsequently the excess of ROS, which may induce DNA damage. Literature data pointed that cadmium induced DNA damage, thus leading to the creation of single and double strand breaks (Yang *et al.*, 2012b; Virk *et al.*, 2013; De Souza Predes *et al.*, 2014). Administration of GSO before or during the treatment with $CdCl_2$ ameliorates the genotoxicity induced by $CdCl_2$ in both tested organs. These results were in agreement with study of Cavusoglu *et al.* (2014), which confirmed that oral gavages with grape seed extract significantly ameliorated the indices of hepatotoxicity, nephrotoxicity, lipid peroxidation, and genotoxicity induced by benzene. They showed a significant reduction in the frequency of MNs when compared with the group treated with benzene alone.

5. Conclusion

Grape seed oil is wealthy in phenolics, fatty acids and vitamins, with economic value to pharmaceutical and food industry. It has useful properties for health, such as anti-inflammatory, cardioprotective, antimicrobial, and anti-cancer activities, and may interact with cellular and molecular pathways. GSO showed marked alleviative role against $CdCl_2$ hepatotoxicity and genotoxicity in male mice. These activities could be attributed to high content of phenolics and antioxidant activity which is reflected in renewed liver enzymes, semen character and reduction of DNA damage almost to normal range. Therefore, the GSO may be efficient to diminish the heavy metals health risk.

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