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Therapeutic efficacy of water grape seed extract against lead-induced hepatotoxicity in rats

Maha Moustafa[1]*, Omayma M.Hassanin[2]

[1]Biochemistry Department, Faculty of Sciences, Ain Shams University, (EGYPT)

[2]Clinical Pathology, Molecular Biology Department, Medical Research Center, Ain Shams University, (EGYPT)

E-mail : mmkmahmed@yahoo.com

ABSTRACT

Lead toxicity is a worldwide health problem due to continuous exposure of to lead in the environment especially workers in industries The study was planned to evaluate the efficacy of water grape seed extract (WGSE) in preventing lead induced oxidative stress in liver tissue of rats. 75 male Wistar rats were randomly divided into 5 groups: Group (1) control group in which rats were administrated distilled water orally for 6 weeks. In group (2) rats were given lead acetate (0.2%) in drinking water for 6 weeks. In groups (3, 4 and 5), rats were given lead acetate (0.2%) in drinking water for 5 weeks followed by grape seed extract in a dose of 50, 100, 150 mg/kg body weigh/ day (by oral gavage), respectively for one week. Lead acetate treatment caused hepatic injury as evident from increased activities of plasma alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), depleted hepatic reduced glutathione (GSH) and elevated hepatic malondialdhyde (MDA) concentration. Lead-induced oxidative stress in liver was evident from increased levels of lipid peroxidation and reduced level of GSH. The decreased activity of glutathione peroxidase (GPx) indicates possible accumulation of ROS. The changes in AST and GPx activities and MDA concentration were found to be mitigated when the rats were treated with WGSE. Also, WGSE treatment of lead acetate-exposed rats was found to enhance superoxide dismutase (SOD) activity. Results indicate that WGSE has the potential to ameliorate lead-induced hepatic injury due to oxidative stress in rats. Grapes may exert its protective actions against lead-induced hepatotoxicity in rats possibly through its antioxidant mechanisms and may have future therapeutic relevance.

KEYWORDS

Hepatotoxicity;
Grape seed;
Oxidative stress.

INTRODUCTION

Exposure to lead continues to be a widespread problem in many countries. Lead is a persistent metal which is present in the environment – in water, brass plumbing fixtures, soil, dust, and imported products manufactured with lead[1] Lead-exposure occurs through the respiratory and gastrointestinal systems and lead

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which is ingested and absorbed is stored mainly in soft tissues and bone[2]. Autopsy studies of Pb-exposed humans indicate that liver tissue is the largest repository (33%) of Pb among the soft tissues. Pb can cause liver damage and may disturb the normal biochemical process. Several antioxidant molecules such as glutathione (GSH) and glutathione disulphide (GSSG) and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and glutathione reductase (GR) are the most common parameters used to evaluate lead induced oxidative damage[3]. Medicinal plants are commonly used for the treatment of various ailments, as they are considered to have advantages over the conventionally used drugs that are much expensive and known to have harmful side effects. Grape- vine (*Vitisvinifera*), is cultivated today in all temperature regions of the world[4]. Its seeds contain several active components including flavonoids, poly phenols, anthocyanins, proanthicyanidins and procyanidines, grape seeds extract (GSE) contain 70% - 95% standardized proanthocyanidins[5]. A variety of naturally occurring grape seed extracts have been found to have beneficial effects on health, and these compounds have drawn attention because of their relative safeness and accumulated evidence of physiological properties in animals and human[6]. Oil produced from grape seeds is considered a rich source of polyphenolics with strong antioxidant activity, chemopreventive, anti-inflammatory, anti-microbial and anti-cancer effects[7]. Therefore, the present study aimed to investigate whether the administration of grape seeds extract to rats could protect against lead-induced hepatotoxicity.

MATERIALS AND METHODS

Chemicals

Lead acetate was purchased from Sigma Chemical Co., St. Louis, MO, USA. Lead acetate as a powder was dissolved in double distilled water prior to use.

Preparation of grape seed extract (GSE)

Grape seed extract was prepared from red grape seeds using the procedure described by[8,27] with some modifications as follows: Grape seeds were separated from the grapes manually, air dried (in shade, 25-30°C) for one week and milled to fine powder. To prepare water grape seeds (WGSE), 0.2 g of grape seed powder was macerated in 20 ml of distilled water (DW) for 24 h at 5°C and was stirred three times. The mixture was filtered with cheese cloth and the resulting filtrate was used as WGSE.

Animals and experimental design

The study was carried out on 75 healthy adult male Wistar rats, weighing 220-250 gm. Rats were fed with a standard rat diet and had free access to water. The rats were housed under standard conditions of humidity and controlled temperature (20- 26°C) with 12 hours light and 12 hours dark exposure.

After 1 week of acclimatization, rats were randomly distributed into five groups, fifteen rats in each group:

- Group 1: (control) received distilled water orally for 6 weeks.
- Group 2: received lead acetate (0.2%) in drinking water for 6 weeks.
- Group 3: received lead acetate (0.2%) in drinking water for 6weeks and from the fifth week they were also treated daily with a single oral dose of GSE (50 mg/kg body weight/day) for one week.
- Group 4: received lead acetate (0.2%) in drinking water for 6weeks and from the fifth week they were also treated daily with a single oral dose of GSE (100 mg/kg body weight/ day) for one week.
- Group 5: received lead acetate (0.2%) in drinking water for 6weeks and from the fifth week they were also treated daily with a single oral dose of GSE (150 mg/kg body weight/ day) for one week.

At the end of the experimental period, blood samples were collected from all animals from the retro-orbital venous plexus. The blood samples were collected into heparinized tubes. The plasma obtained after centrifugation (3000 rpm for 10 min at 4°C) was used for various biochemical assays.

Following the collection of blood samples, all animals were sacrificed, the liver from each rat was removed, washed using chilled saline solution. Tissue was minced and homogenized in appropriate buffer and then

centrifuged, according to the instructions of the biochemical assay. The resulting clear supernatant was collected and stored at -20°C till further analysis and was used for various enzymatic and non-enzymatic biochemical assays.

BIOCHEMICAL ASSAYS

1- Blood lead measurement

It was estimated using atomic absorption spectrophotometer/flame emission spectrophotometer (Shimadzu- model AA-630-02- Japan) using an air acetylene flame and hollow cathode lamp. The wave length was adjusted to 283.3 nm and the used current was 6 mA. It was expressed as μg/dl[9].

2-Liver functions, MDA, and antioxidant enzymes

All kits used for biochemical analyses were purchased from the Biodiagnostic Company, Cairo, Egypt. Plasma ALT and AST activities were determined spectrophotometrically by the method of Reitman and Frankel (1957). The pyruvate or oxaloacetate formed by transaminases is measured in its derivatives form 2, 4-dinitrophenylhydrazine. The absorbance of the colored product was measured at 505 nm. Plasma ALP activity was determined spectrophotometrically by the method of Belfield and Goldberg (1971). The principle of this method relies on conversion of phenyl phosphate by ALP into phenol and phosphate. The liberated phenol was measured in the presence of 4- aminophenazone and potassium ferricyanide at 510 nm. Hepatic reduced GSH concentration was determined spectrophotometrically by the method of Beutler *et al*. (1963). The method was based on the reduction of 5,5 dithiobis (2-nitrobenzoic acid) (DTNB) with GSH to produce a yellow compound. The reduced chromogen directly proportional to GSH concentration and its absorbance can be measured at 405 nm. Hepatic MDA concentration was determined spectrophotometrically by the method of Satoh (1978). Thiobarbituric acid (TBA) reacts with MDA in acidic medium at temperature of 95°C for 30 min to form TBA reactive product. The absorbance of the resulting pink product can be measured at 534 nm. Hepatic SOD activity was determined spectrophotometrically by the method of Nishikimi *et al*. (1972). The principle of this assay relies on the ability of the enzyme to inhibit the phenazine methosulphate-mediated reduction of nitroblue tetrazolium dye. The percent inhibition directly proportional to SOD activity was calculated, depending on the increase in absorbance at 560 nm for control and sample, respectively. Hepatic GPx activity was determined spectrophotometrically by the method of Paglia and Valentine (1967). The assay is an indirect measure of the activity of cellular GPx (c-GPx). Oxidized glutathione (GSSG), produced upon reduction of organic peroxide by c-GPx, is recycled to its reduced state by the enzyme glutathione reductase (GR). The oxidation of NADPH to $NADP^+$ is accompanied by a decrease in absorbance at 340 nm (A340) providing a spectrophotometric means for monitoring GPx enzyme activity. To assay c-GPx, tissue homogenate is added to a solution containing GSH, GSH reductase, and NADPH. The enzyme reaction is initiated by adding the substrate, hydrogen peroxide and the A340 is recorded. The rate of decrease in the A340 is directly proportional to the GPx activity in the sample.

STATISTICAL ANALYSIS

Statistical analysis was done using one way analysis of variance (ANOVA) which was used to compare between the control and other treated groups. The data were presented in the form of mean± standard error (SE). When only two groups were compared, Student's t-test was used. The difference was insignificant at $p > 0.05$, significant at $P < 0.05$ and highly significant at $P < 0.01$.

RESULTS

1-Lead concentration in blood

The mean of blood lead concentration was highly significantly increased in lead treated group ($P < 0.001$) as compared to control group. Compared to lead treated group, there was a significant decrease in the mean blood lead level on treatment with WGSE 150 mg/Kg b.w ($P < 0.05$). There was no significant decrease ($p<0.05$) in blood lead in the groups treated with WGSE 50 and 100 mg/Kg b.w when compared to lead treated group as shown in TABLE 1.

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TABLE 1 : Blood lead concentration in different rat groups

Group	Blood lead concentration (µg/dl) (Mean ±SE)
G.1: control	1.059±0.76
G.2: lead only	27.68±1.9••
G.3: lead + WGSE (50mg/Kg b.w)	26.05± 2.57
G.4: lead + WGSE (100mg/Kg b.w)	24.12±1.22
G.5: lead + WGSE (150mg/Kg b.w)	22.54±0.8*

••Highly significant at P< 0.001 compared to control (t- test); *Significant at P< 0.05 compared to lead treated group (ANOVA); ** Highly significant at P< 0.001 compared to lead treated group (ANOVA).

TABLE 2 : Hepatic MDA concentration (nmol/g tissue) in different rat groups

Groups	hepatic MDA (nmol/mg tissue) (Mean±SE)
G.1: control	19.08±0.36
G.2: Lead only	85.21±2.51••
G.3: lead + WGSE (50 mg/Kg b.w)	81.03±3.42••
G.4: lead + WGSE (100 mg/Kg b.w)	72.95±1.06*
G.5: lead + WGSE (150 mg/Kg b.w)	62.85±4.23**

••Highly significant at P< 0.001 compared to control (t- test); * Significant at P< 0.05 compared to lead only group (ANOVA); ** Highly significant at P< 0.001 compared to lead only group (ANOVA).

TABLE 3 : Plasma liver function tests in different rat groups

Groups	ALP (IU/l) (Mean±SE)	AST (IU/l) (Mean±SE)	ALT (IU/l) (Mean±SE)
G.1: control	136.6±4.5	56.5±2.8	22.8±4.22
G.2: Lead only	215.6±9.06••	143.1±9.51••	119.2±5.826••
G.3: lead + WGSE (50 mg/Kg b.w)	208.1±10.72	137.7±9.03	111.38±7.25
G.4: lead + WGSE (100 mg/Kg b.w)	189.7±10.02*	121.2±6.02*	87.6±8.34*
G.5: lead + WGSE (150 mg/Kg b.w)	148.4±6.2**	76.57±3.54**	58.01±6. 67**

•• Highly significant at P< 0.001 compared to control (t- test); * A significant at P< 0.05 compared to lead treated group (ANOVA); ** Highly significant at P< 0.001 compared to lead treated group (ANOVA)

2-Hepatic lipid peroxidation (MDA) level

The mean MDA level was highly significantly increased in lead only treated group as well as in the lead group treated with WGSE 50 mg/Kg b.w (P< 0.001) as compared to control group. A significant decrease in MDA was observed in the WGSE 100 mg/Kg b.w (P < 0.05) when compared to lead only group. There was a highly significant decrease in the mean hepatic MDA level in treatment with WGSE 150 mg/Kg b.w (P< 0.001) as shown in TABLE 2.

3-Liver function tests

The mean plasma levels of ALP, AST and ALT were highly significantly increased in lead treated group (P< 0.001) as compared to control group. There was no significant decrease in the elevated plasma levels of ALP, AST and ALT on treatment with WGSE 50 mg/Kg b.w as compared to the lead only group. There was a highly significant decrease (P< 0.001) in the elevated serum levels of ALP, AST and ALT when WGSE was given at a high dose of 150 mg/Kg b/w when compared to lead treated group, while only a slightly significant decrease was observed in the group treated with 100 mg/Kg b.w. of WGSE as shown in TABLE 3.

4-Reduced glutathione (GSH) liver content

The concentration of the antioxidant GSH has highly significantly decreased (P < 0.001) in the lead only group as compared to control group. Treatment of lead-exposed rats with WGSE 50 mg/Kg b.w did not significantly (P < 0.05) change GSH concentration as compared to the control group, but it was significantly in-

TABLE 4 : Liver GSH content in different rat groups

Groups	GSH (mg/g tissue)
G.1: control	212.867± 51.56
G.2: Lead only	21.064±3.681••
G.3: lead + WGSE (50 mg/Kg b.w)	46.662±10.458 *
G.4: lead + WGSE (100 mg/Kg b.w)	127.32±36.014**
G.5: lead + WGSE (150 mg/Kg b.w)	148.4±6.2**

•• Highly significant at P< 0.001 compared to control (t- test); * A significant at P< 0.05 compared to lead treated group (ANOVA); ** Highly significant at P< 0.001 compared to lead treated group (ANOVA)

creased compared to lead only group (P < 0.05). On the other hand, treatment with WGSE 100 and 150 mg/Kg b.w has highly significantly (P< 0.001) increased hepatic GSH concentration as compared lead only group TABLE 4

5-Hepatic antioxidant enzyme activities

The results of antioxidant enzymes SOD and GPx activities in liver tissue homogenate of all groups are shown in TABLE 5. Treatment with lead only without WGSE treatment had highly significantly (P < 0.001)

TABLE 5 : Hepatic antioxidant enzymes in different groups

Groups	SOD (U/g tissue)	GPx (U/g tissue)
G.1: control	2892.70± 32.1	65.463± 7.496
G.2: Lead only	2120.87± 54.5 ••	30.201±4.107••
G.3: lead + WGSE (50mg/Kg b.w)	2136.15±39.07	32.078±2.174
G.4: lead + WGSE (100mg/Kg b.w)	2207.02±17.37*	40.76±2.899*
G.5: lead + WGSE (150mg/Kg b.w)	2581.26±12.78 **	52.38±4.695**

•• Highly Significant at P< 0.001 compared to control (t- test); * A significant at P< 0.05 compared to lead treated group (ANOVA); ** Highly significant at P< 0.001 compared to lead treated group (ANOVA).

reduced both liver GPx activity and SOD activity as compared to control rats. Treatment with WGSE 50 mg/Kg b.w of lead-exposed rats had no significant effect on neither SOD nor GPx activities as compared to lead only group. However, GPx and SOD activities of groups treated with WGSE 100 mg/Kg b.w have significantly increased (P < 0.05) as compared to lead only group, while treatment with WGSE 150 mg/Kg b.w. cause a highly significant effect on both SOD activity and GPx (P < 0.001).

DISCUSSION

Liver is largest organ and it is target for toxicity because of its role in clearing and metabolizing chemicals through the process called detoxification. Drug induced liver disorders occurred frequently can be life threatening and mimic all forms of liver diseases[16].

Lead (Pb) exposure is considered to be a major public health problem, therefore it has paid attention by researchers in probing further into its toxicity. This heavy metal has been found to induce a wide range of behavioral, biochemical and physiological effects.The liver, kidneys, and brain are considered to be the target organs for the toxic effects of lead[17]. Toxicity of lead is mainly attributed to the induction of oxidative stress by elevation of reactive oxygen species (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and lipid peroxides, therefore, increased interest among phytotherapy investigators to use medicinal plants with antioxidant activity for protection against metal, especially lead, toxicity has been noted[18].

Lead is known to adversely affect many organs, where the liver being one of the major organs involved in the storage, biotransformation and detoxification of toxic substances, is of interest in heavy metal poisoning. Lead produces oxidative damage in the liver by enhanc- ing lipid peroxidation and cause liver dysfunction and increase free radical damage[20].

Grape seeds extract (GSE) contains polyphenols including proanthocyanidins and procyanidins which have antioxidant and free radical scavengers, being more effective than either ascorbic acid or vitamin E[21]. Their effects include the ability to increase intracellular vitamin C levels, decrease capillary permeability and fragility and scavenge oxidants and free radicals. Several studies have indicated that extracts obtained from grape seed inhibit enzyme systems that are responsible for the production of free radicals[22]. Red grape seed extract also blocks cell death signaling[23]. Many studies have

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provided evidence that proanthocyanidin has potent radical scavenging ability, antioxidant properties and significant neuroprotective as well as cardiovascular protective effect[24]. There is great evidence that GSE prevents oxidative injury by modulating the expression of anti oxidant enzyme systems[25].

In this study, lead acetate treatment caused hepatic injury as evident from increased activities of plasma ALT, AST and ALP and elevated hepatic MDA concentration. Lead-induced oxidative stress in liver was evident from increased levels of lipid peroxidation and reduced level of GSH. The decreased activity of GPx indicates possible accumulation of ROS. The changes in liver enzymes and antioxidant enzyme activities and MDA concentration were found to be minimized when the rats were treated with high doses of WGSE.

Compared to lead acetate control group which had a blood lead level of 27.68±1.9 ìg/dl, WGSE could significantly ((P< 0.05) lower blood lead level to 22.54±0.8 ìg/dl at a WGSE dose of 150 mg/Kg b.w., while lower WGSE doses of 50 and 100 mg/Kg b.w. had no significant effect. These results are in contrast to Badavi *et al*., 2008 who claimed that GSE administration for 45 days had no effect on blood lead level.

Liver enzymes ALP, AST, and ALT, were highly increased (215.6±9.06, 143.1±9.51 and 119.2±5.826 IU/L, respectively) in the lead acetate group. High dose of WGSE (100 mg /Kg body weight) was effective in significantly (P< 0.05) decreasing ALP, AST and ALT to 189.7±10.02, 121.2±6.02, and 87.6±8.34 IU/L, respectively. Moreover, a higher WGSE dose of 150 mg/Kg b.w. had a highly significant (P< 0.001) effect lowering ALP, AST and ALT to 148.4±6.2, 76.57±3.54, and 58.01±6. 67 IU/L, respectively.

In contrast, a low WGSE dose of 50mg/Kg b.w was ineffective in ameliorating the effect of lead acetate on liver enzymes. These results are in accordance with Waggas *et al*., 2012, who found that oral administration of WGSE at a dose of 100 mg/Kg b.w. could decrease AST, ALT activities after being increased following lead acetate toxicity.

Increased hepatic MDA levels as much as 85.21±2.51 nmol/mg tissue, in the lead acetate exposed rat group, were significantly (P< 0.05) decreased to 72.95?1.06 nmol/mg tissue after treatment with WGSE 100mg/Kg b.w, and then to 62.85?4.23 nmol/mg tissue after treatment with a higher dose of WGSE (150mg/Kg b.w). Furthermore, all doses of WGSE had significantly increased hepatic GSH content which were decreased following lead acetate administration. These results are in aggrement with Feng *et al*. (2005) reported that, treatment with grape seed extract suppresses lipid peroxidation in rats. However, Waggas *et al*., 2012 suggedted that it may be the presence of proanthocyanidins and procyanidins which have antioxidant and free radical scavenging activities.

The results of antioxidant enzymes SOD and GPx activities in liver tissue homogenate of all groups have shown that treatment with lead only without WGSE treatment had highly significantly (P < 0.001) reduced both liver GPx activity and SOD activity as compared to control rats. Treatment with WGSE 50 mg/Kg b.w of lead-exposed rats had no significant effect on neither SOD nor GPx activities as compared to lead only group. However, GPx and SOD activities of groups treated with WGSE 100 mg/Kg b.w have significantly increased (P < 0.05) as compared to lead only group, while treatment with WGSE 150 mg/Kg b.w. cause a highly significant effect on both SOD activity and GPx (P < 0.001).

Cetin *et al.* (2008) reported that grape seed extract may be promising as a therapeutic option in RTx-induced oxidative stress in the rat liver. El-Ashmawy *et al.* (2010) concluded that grape seed extract is a useful herbal rem edy, especially for controlling oxidative damages and is considered as a potent protective agent against hepato toxicity. Several lines of evidence demonstrated that, grape seed proanthocyanidins exhibited *in vivo* hepato- protective and anti-fibrogenic effects against liver injury and act as free radicals scavengers and protective liver damage[30].

ABBREVIATIONS

WGSE: water grape seed extract; ALT:alanine aminotransferase; AST:aspartate amonitransferase; ALP: alkaline phosphatase; MDA:malondialdehyde; SOD: superoxide dismutase; GSH: reduced glutathione; GPx: glutathione peroxidase; IU: international unit; b.w: body weight; GR: glutathione reductase; CAT: catalase.

CONCLUSION

Results of this study indicate that treatment of rats

with the present dose of lead acetate brings about oxidative stress-induced hepatotoxicity due to alterations in the balance between antioxidant/pro-oxidant systems and affecting the antioxidant enzymes. Grape seed extract may exert its protective actions against lead-induced hepatotoxicity in rats possibly through its antioxidant mechanisms. The results raise the possibility of grape seed extract being considered as an important nutritional supplement for people in the areas, where they may have chances of exposure to lead occupationally or environmentally.

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