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***Research paper***

# Cadmium induced hepatic intoxication and amelioration by grape seed extract

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**Abstract**

Cadmium (Cd) is wide-spread toxic metal that pollutes most of the vegetables, which eaten by numerous populations all over the world. The aim of the current work is to evaluate the protective and prophylactic effects of the antioxidant materials in the grape seed extract (GSE) on the hepatic intoxication induced by cadmium chloride toxic material in male Wistar rats. Male adult Wistar rats were divided into four groups. Control group fed on balanced diet and given drinking water. Group two (control positive) given CdCl2 in the dose of 0.44 mg/kg body weight (BW) by stomach tube daily. Group three given grape seed extract GSE in the dose of (100 mg/kg BW) daily by stomach tube. Finally, the fourth group gave mixture of (GSE and CdCl2) by stomach tube in the recommended dose. Blood and liver tissues were collected for further biochemical and histopathological studies. CdCl2 significantly increased the serum levels of malondialdehyde, ALT and AST. Cadmium administration decreased levels of antioxidants (Catalase, GSH-R and GSH-Px). The liver of the control positive groups which given CdCl2 showed degenerative changes in the form of vacuolar and hydropic degeneration. Congestion was evident in the central vein and proliferation of the kupffer cells. These hepatic biochemical and degenerative changes were ameliorated by the co addition of GSE.

***Keywords***: *Cadmium, Grape seed extract, Intoxication, Liver*

## 1. Introduction

Most of the heavy metals including cadmium which was naturally located in the rocks and earth crust. It interfered with the antioxidant system of the cells via the membrane lipids in the tissues and altered its function (Renugadevi &Prabu 2010). Cadmium can accumulate in the liver and kidney via drinking and eating. It causes injury to the liver, kidney and testis (Suzuki et al. 1989). Liver necrosis and cytotoxicity can induced after exposure to large dose of cadmium in rats and mice (Dudley et al. 1982 , Theocharis et al. 1991). This was done through the induction of oxidative stress (Pathak &Khandelwal 2006, Suru 2008) and establishment of reactive oxygen species (ROS) which leads to lipid peroxidation, membrane protein damage and malfunction of anti-oxidant system. The main causes of liver lesions can come from the interaction between the hepatotoxic noxious agents with the main cellular components (Grattagliano et al. 2009). When the hepatic cells were exposed to severe damage. It lead to apoptosis or cell death (necrosis), this was happened due to DNA damage (Thévenod 2003, Cuypers et al. 2010). Grape seed extract (GSE) is a natural extract from the seeds of Vitisvinifera, rich in flavonoids, mainly flavan-3-ols and proanthocyanidins (Ferreira &Li 2000). It has wide spread therapeutic purpose against oxidative stress and free radicals. It also has pharmacological, biological and chemo protective effects (Bagchi et al. 2000).They constitute a large group of low molecular weight polyphenolic phytochemicals found in plants. Berries, grapes and cherries are recognized as fruits with a high content of antioxidants. The antioxidant properties of these fruits are believed to be due to a high content of flavonoids mainly the proanthocyanidins. Also, the grape juices with high content of phenolic compounds exhibit antioxidant effects (García-Alonso et al. 2004). Proanthocyanidins, sometimes referred as "condensed tannins" are responsible for astringency in many foods and medicinal herbs. These flavonoids occur naturally in black and green teas (Kris-Etherton &Keen 2002) chocolate and cacao (Wan et al. 2001), red rice (Oki et al. 2002) and many fruits: blueberries, blackberries, strawberries, elderberries, and other red/blue/purple colored plant parts (Gu et al. 2002).Natural antioxidants strengthen the endogenous antioxidants defenses from reactive oxygen species and restore the optimal balance by neutralizing the reactive species (Ho et al. 1994).The aim of the current work is to evaluate the protective and prophylactic effects of the antioxidant materials in the grape seed extract (GSE) on the hepatic intoxication induced by cadmium chloride toxic material in male Wistar rats.

## 2. Material and methods

### 2.1. Chemicals and reagents

Cadmium chloride was purchased from Sigma (St. Louis, MO, USA). Grape seed extract (GSE) was obtained from (Mepaco Arabian Pharmaceutical Company, Egypt)

## 2.2. Animals

60 male mature wistar albino rat's weighting 200–250 g was used. They were fed standard pellet diet and given access water in the animal house of Faculty of Veterinary Medicine, Benha University. All Rats were kept for 2 weeks in the house to check them for diseases or infection before the experiment.

## 2.3 Experimental design

The animals were divided into four groups (15 rats per group) the first group (control) was fed on balanced diet and was used as a control for two months.

The second group (control positive) was administered cadmium chloride orally at a dose of 4.4 mg/ kg bw/ day (1/20 of LD50) that was dissolved in distilled water for 2 months. Each 8.8 mg cadmium chloride was freshly prepared, dissolved in 10 ml distilled water, and each rat was administered 0.5 ml of the solution orally. This means that each rat will receive 0.44 mg CdCl2 per day. Oral rat LD50 for Cadmium Chloride anhydrous is 88 mg/kg body weight (AFDOAQ, 1951).

The third group was administered GSE 100 mg/ kg body weight/day dissolved in distilled water for two months (Cetin et al. 2008).

The fourth group was given mixture 100 mg GSE diluted in distilled water in addition to 0.44 mg cadmium chloride /day dissolved in distilled water for two months.

## 2.4. Methods

At the time of sacrifice, rats were anaesthetized with ether then blood samples were collected from the retro-orbital plexuses. The liver of each rat was dissected out carefully.

### 2.4.1. Collection of blood samples

Sera were separated by centrifugation at 3000 rpm and stored frozen at -20$^{o}$C until the time of biochemical analysis. Preparation of liver homogenate immediately after blood sampling, animals was sacrificed by cervical dislocation under ether anesthesia and livers were collected for biochemical and histopathological examinations. Liver tissues were rapidly removed, washed in ice-cooled saline, plotted dry and weighed. Then it was homogenized by electric homogenizer. The homogenate was centrifuge at 3000 rpm for 5 minutes. Then the homogenate centrifuged again in cooling centrifuge at 4c for excluding of the debris from the homogenate.

### 2.4.2. Determination of LPO and antioxidant enzymes

Measurement of Lipid peroxidation (LPO): Malondialdehyde MDA, as a marker of lipid peroxidation, was measured calorimetrically in liver homogenate (nmol / gram. tissue) according to the method of (Ohkawa et al. 1979) using commercial available kit (Biodiagnostic, Cairo, Egypt). Thiobarbituric acid reacts with MDA in acidic mediums at 95 $^{o}$C for 30 min to form thiobarbituric acid reactive product, and the absorbance of the resultant pink product can be measured at 534 nm.

Measurement of reduced glutathione: Reduced glutathione GSH was determined according to the method of (Beutler et al. 1963) using commercially available kit (Biodiagnostic, Cairo, Egypt). GSH determination is based on the reduction of 5, 5`-dithiobis (2-nitrobenzoic acid) with GSH to produce a yellow compound. The reduced chromogen is directly proportional to GSH concentration, and its absorbance can be measured at 405 nm.

Determination of Antioxidant Enzymes:

Determination of catalase (CAT) activity: CAT activity (in U/g tissue) was assessed liver homogenate by means of the method of (Aebi 1984), as catalase reacts with a known quantity of hydrogen peroxide and the reaction is stopped after 1 min with the catalase inhibitor. In the presence of peroxidase, the remaining hydrogen peroxide reacts with 3,5-dichlorvos-2-hydroxybenzene sulfonic acid and 4-aminophenazone to form a chromophore with a color intensity inversely proportional to the amount of catalase in the sample. The absorbance was measured at 510 nm and the activity was expressed as unit/mg protein.

Estimation of glutathione peroxidase (GPx):GPx activity was assessed in liver homogenate (U/gm. tissue) according to (Paglia &Valentine 1967) using commercially available kit (Biodiagnostic, Cairo, Egypt).The assay is an indirect measure of the activity of GPx. Oxidized glutathione (GSSG), produced upon reduction of organic peroxide by 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.

**Biochemical analysis**

Alanine aminotransferase (ALT), aspartate aminotransferase (AST) are measured in serum according to the methods described by Huang et al. (2006).

### 2.4.3. Histopathological examination

Different parts of the liver were taken from the different groups. The specimens were fixed in Bouin's solution. After fixation, the specimens were dehydrated in ascending grades of ethanol, cleared in xylene and embedded in paraffin wax. Paraffin sections were stained according to (Bancroft &Gamble 2002).

### 2.4. 4. Immunohistochemical examination

Paraffin sections (5µm thickness) of the liver tissues were collected on positive charged slides. After deparaffinization and rehydration, sections of the liver were treated with 3% hydrogen peroxide for 10 minutes to reduce peroxidase activity. Then after, sections were incubated with the following primary antibodies; mouse monoclonal anti-Bax (Clone 2D2, Neomarkers, USA) .The sections were then stained with immunoperoxidase technique employing commercially available reagent ABC kit (Labvision, Fermont, CA, USA). For demonstration of binding sites, DAB chromogen was applied. Phosphate-buffered saline was used for rinsing between each step and finally all sections were counterstained with Mayer’s hematoxylin (Kiernan 2008).

### 2.4.5. Statistical analysis

The values were presented as means ± SD of different groups. One way ANOVA was used to estimate the differences between the mean values. The results were significant when $p < 0.01$.

# 3. Results

## 3.1. Biochemical changes

**Table 1:** Biochemical Parameters of the Serum in the Wister Rats

| Parameters | Control | Cadmium chloride (CdCl2) | Grape seed extract | CdCl2+GSE |
|---|---|---|---|---|
| Catalase (U/g protein) | 32.2±2.8 | 17.9±2.8a | 36.6±2.3a | 26.1±1.7b |
| GSH-R (U/g protein) | 73.1±6.7 | 25.3±4.1a | 76.9±10.3a | 43.9±4.1b |
| GSH-Px (U/g protein) | 76.9±6.9 | 31.9±11.1a | 59±4.3a | 42±4.7b |
| MDA (nmol/g protein) | 28.1±3.2 | 84.6±7.7a | 34.3±5.5a | 54.5±8.1b |
| ALT (U/L) | 62.82 ± 4.43 | 132.11± 10.9a | 84.27± 2.7a | 91.5 ± 3.94b |
| AST (U/L) | 94.58± 6.72 | 152.75± 8.34a | 111.38± 1.39a | 121.6± 2.97ab |

All these values are expressed as mean± standard deviation (SD) of n = 15 animals; a significant as compared with control. Group; b significant as compared with Cd Cl2 –treated group. MDA: malondialdehyde; GSH-R: reduced glutathione; CAT: catalase; GPx: glutathione peroxidase. ALT: alinineaminotransferease. AST: Aspartate aminotransferase

Administration of CdCl2 lead to oxidative stress that can evaluate by a significant increase in lipid peroxidation product MDA in liver compared to control group ($P < 0.0001$) as well as increase in the serum level of ALT and AST. Meanwhile, marked depletion was noticed in tissue levels of CAT, GSHPx activity and GSH-R contents (Table.1).

Co treatment with GSE significantly attenuated oxidative stress by reducing MDA and improving GSH content as well as antioxidant enzymes activities in addition to improving serum level of ALT and AST as compared to CdCl2 treated group ($P < 0.0001$).

### 3.2 Histopathological changes

The hepatic tissue was consisted of hepatic cords around the central vein (Fig.1). Some hepatocytes showed positive immunostaining for BAX (Fig.2). The histopathological changes in the hepatic tissues showed congestion in the central vein, degenerative changes in the hepatocytes in the form of hydropic, vacuolar degeneration and proliferation of kupffer cells (Fig.3). Numerous hepatocytes showed necrosis and take faint BAX immunostaining reaction (Fig.4).Co administration of GSE made regeneration of the hepatocytes although some blood vessels still congested (Fig.5), and the collagen fibers around the central vein appeared thick. Faint positive BAX immunostaining reactions were noticed in the hepatocytes (Fig.6).

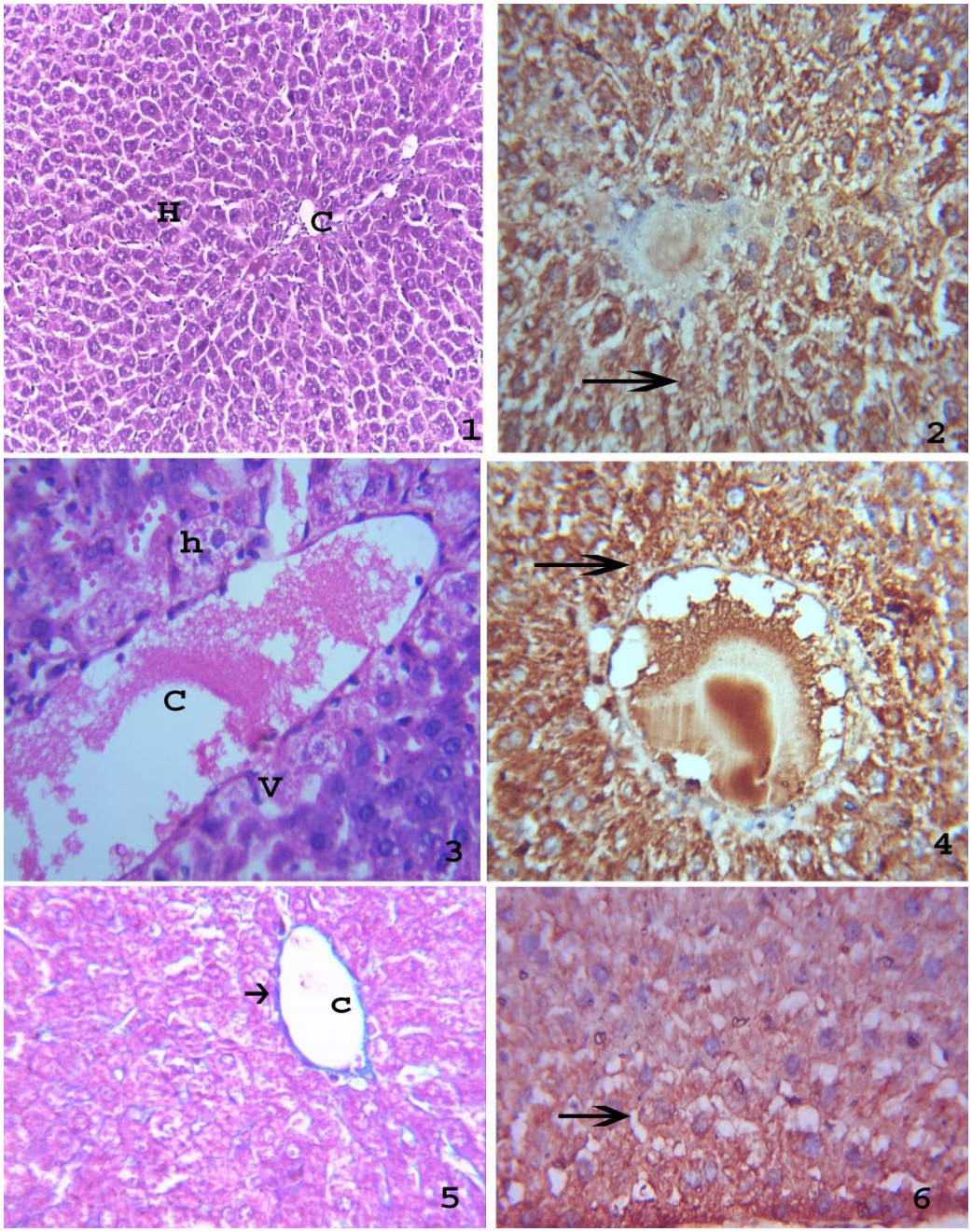


**Plate 1:** Photomicrography of the liver tissue showing; Hepatic cords (H) and central vein (C) Fig.1 H&E. Faint positive immunostained cells was demonstrated in the hepatic tissues (Arrow) Fig.2 BAX Immunostaining. The liver tissue showing congestion (C), hydropic degeneration (H), vacuolar degeneration (V) and Kupffer cells Proliferations Fig.3 H&E. The hepatic tissues showed faint positive BAX Immunostaing (Arrow) Fig.4 BAX Immunostaining. The co-administration of GSE showed regeneration in the hepatic tissue while the central vein wall showing thickening (Arrow) Fig.5 Masson Trichrome X20.The liver tissue showed faint BAX positive reaction Fig.6 BAX Immunostaining.

## 4. Discussion

The pollution was worldwide and spread in different environments. Heavy metals are one of the most dangerous one which are toxic and persist for long time in the environment(Al-Alawi &Mandiwana 2007)

Cadmium is toxic heavy metal to both plants and animals(El-Sharaky et al. 2007). It was spread in the environment in the form of combined with other minerals associated with zinc and lead(Hwang &Wang 2001) not in the metallic state (Wynne 1999). Cadmium was transferred from the contaminated earth crust to the plants and crops which grown in the contaminated land (Eisler 2000) The nonsmoking population take the cadmium via eating the contaminated crops with cadmium(Goyer 1986).

The cadmium mainly concentrated in the liver and kidney in high concentration (50-75% of the total cadmium contaminant),(Reeves &Rossow 1996), and also it concentrated in other organs as testis, spleen (Alkhedaide et al. 2016), pancreas , thyroid and nervous system (El-Tarras et al. 2016).

Intoxication with cadmium chloride interferes with the antioxidant defense system in the hepatic tissue. It causes increase in lipid peroxidation product MDA in liver, ALT and AST. It also causes marked depletion in tissue levels of CAT, GSHPx activity and GSH-R contents. These findings was augmented by the results of (Zikic et al. 1996, Bagchi et al. 1997).

Cadmium intoxication to the hepatic tissues occurred via interruption of the cell integrity which intern lead to freedom of reactive oxygen that directly make oxidative damage to the lipid structural content of the cell membrane and disturbance in the cytotoxic and inflammatory mediators in the hepatocytes (Koyu et al. 2006).

The histopathological changes in the hepatic tissues showed congestion in the central vein, degenerative changes in the hepatocytes in the form of Hydropic degeneration which done due to marked disturbance in the hepatocytes permeability and vacuolar degeneration which is primary response to hepatocytes injury(Robbins &Angell 1976). Most of the hepatocytes undergoes apoptosis due to cadmium injury to the hepatocytes. (Gathwan et al. 2012). Co administration of GSE made regeneration of the hepatocytes although some blood vessels still congested. These findings were supported by the results of (El-Sokkary et al. 2010, Mahran et al. 2011, Albasha and Azab 2014).

The grape seed extract (GSE), now is widely used as pharmacological products which contains flavonoids which is considered strong antioxidant. It contains numerous compounds called polyphenols that contain dimers, trimers, and other oligomers of catechin and epicatechin and their gallate derivatives. All these compounds are called proanthocyanidins. GSE used to overcome the oxidative stress and also may use as anti-inflammatory and anticancer effects (Katiyar 2008) It also used as drugs which improve the physical activity and keep the people in health condition(Bashir et al. 2014)

Grape seed extract (GSE) has a protective effect on oxidant-induced production and deposition of extracellular matrix components, which results in hepatic fibrosis (Dulundu et al. 2007). It also improves hepatic ischemia-reperfusion injury and reduces the size of the emboli in cardiac ischemia in the rat (Sehirli et al. 2008).

Grape seed in addition with chemotherapy can give good results due to it inhibit enzyme systems that are responsible for the production of free radicals, and that they are antimutagenic and anticarcinogenic (Halpern et al. 1998, Li et al. 2002,Alkhedaide2015).

Oxidative stress induced via over production of ROS. This is done due to the depletion of antioxidants or to the direct action of Cd on peroxidation reaction and iron-mediated peroxidation (Pillai &Gupta 2005).

Binding of Cd to sulphydryl groups in mitochondria lead to primary injury of the cells and secondary injury of the cells initiated by the activation of kupffer cells have also been mentioned as possible mechanisms of toxic effect of Cd on the liver (Rikans &Yamano 2000). Inactivation of sulphydryl groups causes oxidative stress, mitochondrial permeability transition and mitochondrial dysfunction (Jurczuk et al. 2004). It is also suggested that kupffer cells release pro-inflammatory cytokines and chemokines which stimulate the migration and accumulation of neutrophils and monocytes in the liver (Dudley et al. 1984). It also suggested that hepatocytes injury may be caused by ischemia due to sinusoidal endothelial cell dysfunction. Necrosis of the cells occurred due to accumulation of the cadmium on the wall of the endothelium of the hepatic blood sinusoids. The activation of hepatic cytochrome P 450 leads to formation of toxic metabolites in the liver cells by the cadmium(Wong et al. 1981).

Serum levels of ALT and AST were elevated by administration of CdCl2.These elevations were due to hepatic injury as mentioned by (Renugadevi &Prabu 2010). These elevations attributed to the degenerative changes and necrosis in the liver due to cadmium toxicity (Adebajo et al. 2009).

Administration of CdCl2 leads to oxidative stress that can evaluate by a significant increase in lipid peroxidation product MDA in liver. This finding was augmented by the results of (KARA et al. 2005).

GSH forms complexes with several heavy metals and thus might function in protection of cells against metal toxicity(Swiergosz-Kowalewska 2001).

Some toxic effects of Cd seem to be indirect and due to oxidative stress promoted in response to this ion. As a result, lipid peroxidation can occur. This is a chain reaction in which polyunsaturated fatty acids of cell membranes are oxidized through C and O-centered radicals and hydroperoxy-intermediates to yield various products, including epoxy-fatty acids, alkanes, alkenes and aldehydes (e. g. MDA). Determining the level of MDA is usually the most practical and reliable method for detecting and screening oxidative stress (Nair et al. 2008).

Marked depletion in tissue levels of CAT, GSHPx activity and GSH-R contents were noticed in the current work. Cd ions decreased the GSH content in mice liver and red blood cells after 14 days of injection (Renugadevi &Prabu 2010). Cd ions cause an increase in reactive oxygen species (ROS), which cause irreversible damage to various biomolecules. ROS cause a substantial decrease in GSH content along with depletion of other defenses, such as super oxide dismutase and catalase (Swiergosz-Kowalewska 2001, Waisberg et al. 2003).

## 5. Conclusion

The cadmium can pollute the food and water and caused severe hazard to the hepatic tissue. These hazards can ameliorate by the co addition of GSE.

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