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Hepatoprotective and Antioxidative Effects of *Terminalia Arjuna* against Cadmium Provoked Toxicity in Albino Rats (*Ratus Norvigicus*)

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Abstract

The extract of bark of *Terminalia arjuna* was investigated for its hepatoprotective and antioxidative effects on cadmium provoked toxicity. It was found that cadmium (Cd) significantly ($P<0.05$) elevated the serum levels of following biomarkers alanine amino transferase (ALT), aspartate amino transferase (AST), alkaline phosphatase ALP, and malondialdehyde (MDA) simultaneously, it lowered the protein and depleted the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) upon administration of cadmium chloride (5 mg/kg) to albino rats. Study results indicated that the treatment of these rats with extracts of *Terminalia arjuna* (200 mg/kg) significantly reversed the effects of cadmium and proved that it has hepatoprotective, and antioxidative potential. The results also suggested that the phytochemicals present in the extract of bark of T. *arjuna* have potential therapeutic value.

Keywords: Cadmium chloride; Stress; Toxicity; *Terminalia arjuna*; Hepatoprotective; Antioxidative

Introduction

The liver performs an array of functions and the most important one is its role in metabolism so no other organ is more important for healthy metabolism than the liver. It is accountable for detoxifying the poison or any foreign substance by converting and excreting waste and toxin [1]. Other major functions of liver are the metabolism of carbohydrates, lipid, protein and secretion of bile. Thus the maintenance of healthy liver is vital for overall health [2]. It is considered as one of the most vital organs due to the handling the metabolism and excretion of drugs and other xenobiotics thus it provides protection against foreign substances by detoxifying and eliminating them [3]. It is frequently abused by the environmental toxins, heavy metals, poor eating habits, alcohol, prescription and the counter drug use thus it is damaged and weakened ultimately leads to the hepatitis, cirrhosis and alcoholic liver disease. Various toxicants, chemo-therapeutic agents, carbon tetrachloride (CCl_4), thioacetamide, lead, chromium, cadmium, chronic alcohol consumption and microbes are well studied for liver cell injury. Many synthetic drugs are used for the treatment of liver diseases also damage the liver [4].

Human health is under the serious threat of heavy metal pollution which is a gift of modern age of industrialization [5]. Cadmium is environmental pollutant and toxicant that can affect liver, kidney, lungs, bones, brain, testis and cardiovascular system [6]. It became commercial in 20th century due to its agricultural and industrial applications and then these actions cause the entry of cadmium into the soil and drinking water. Extremely soluble characters of compounds of cadmium make its entry into the plants and crops that are used to get food and feed [7]. Other sources of cadmium are smoking, occupational disclosure and house dirt [8]. Respiratory tract and gastrointestinal tract are two main ways of absorption of cadmium. It competes with iron during absorption so iron status of individual is more important thus iron deficiency increase its absorption [9]. Although there is no specific transporter for its absorption but divalent metal ion transporters are used to take up free cadmium ion from cadmium [10].

As cadmium enters the blood circulation taken up by the red blood cells or get attach with albumin in blood plasma. Within first six hours cadmium is taken into the hepatocytes and makes new complex with metallothionein [11], with other proteins or peptides and glutathione (GSH) [12]. From liver it is send to kidney for excretion. While passing through the proximal tubules, it is reabsorbed and stored in these cells [13].

Oxidative stress is the basic mechanism of cadmium toxicity but cadmium is incapable to induce the production of reactive oxygen species (ROS) directly because of being a non-fenton metal. Indirectly it provokes stress via dislodgment of metal ions e.g. Fe+2, reduction of ROS scavengers, denaturation of enzymes and rollout of ETC (electron transport chain) cause the production of ROS [14,15]. The important way of production of hydroxyl radical is fenton reaction [16]. Hydroxyl radical is most reactive and damaging to lipids proteins and DNA, it damages membranes by initiating lipid peroxidation (LPO) results in production of malondialdehyde (MDA) from the breakdown of polyunsaturated fatty acids [17].

Medicinal plants are the major source of drugs and have been proven for the presence of hepatoprotective potential. Extracts of such plants are widely used for the treatment of liver diseases like hepatitis, cirrhosis, and loss of appetite and the *Terminalia arjuna* is one of such plants that are used for liver diseases [18]. *Terminalia arjuna* is an ever green, 20-30m long, South Asian plant, generally known as 'Arjuna. Antibacterial and antioxidant nature of T. arjuna has been well explored *in vitro* [19]. T. *arjuna* has antioxidant properties due to presence flavonoids, tannins and oligomeric proanthocyanidins [20].

Aims and Objectives

The present work was aim to determine the hepatoprotective and antioxidative effects of *Terminalia arjuna* against the Cd-provoked hepatotoxicity and oxidative stress in albino rats.

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Material and Methods

Animals

Fifteen albino rats were selected from the population of 50 rats and placed in animal house of institute of molecular biology and biochemistry (IMBB) the University of Lahore (UOL). They were placed in cages made up of stainless steel at constant 25 ± 5°C temperature with alternating day and night cycles, and standard pellets and water were in free access (*ad libitum*). This project was approved by the ethic committee of IMBB the University of Lahore.

Chemicals

All chemicals and regents were of analytical grades. Cadmium chloride (CdCl2) was purchased from Merck Pharmaceutical Company Germany.

Experimental design

Out of 50 total rats only 15 male healthy rats were chosen and were separated in three groups each having 5 rats. Group I: Normal control kept on normal diet and tap water. Group II: Rats were given $CdCl_2$ @ 5 mg/kg B.Wt in drinking water till the end of research for six weeks [21]. Group III: Rats were given $CdCl_2$ @ 5 mg/kg B.Wt in drinking water for three weeks then they were given standardized extract of bark *Terminalia arjuna* @ 200 mg/kg B.Wt. for next three weeks.

Biochemical analysis

LFT (Liver functioning test include AST, ALT, ALP) was measured by using spectrophotometric method as described by Anonymous, 1996 [22]. Levels of total protein were estimated by Lowry Method [23]. SOD activity was measured by Kakkar method [24]. TBARS was measured by the method of Ohkawa et al. [25]. Catalase activity was measured by Aebi's method [26]. Glutathione was measured by method described by Moron et al. [27]

Statistical Analysis

The values were reported in mean ± SD (n=5). Experimental results were statistically analyzed by using analysis of variance (ANOVA) by Duncan's multiple range tests.

Results

Table1 showed that Cadmium had a significant effect on alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and total protein (TP) level in serum. The administration of Cadmium 5mg/kg in rats provokes significant ($P<0.05$) increase in ALT, AST, ALP levels as compared to group A denoting the presence of liver dysfunction. The treatment with *Terminalia arjuna* 200 mg/kg group C significantly ($P<0.05$) decreased the toxic effect of Cadmium by decreasing the ALT, AST, ALP and by increasing TP levels in serum.

Table 2 showed that Cadmium had significant deleterious effects on serum antioxidative status. Cadmium (Cd) at the doses of 5 mg/kg (Group B)had significantly ($P<0.05$) decreased the serum levels of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) as compare to positive control (group A) whereas melondialdehyde (MDA) in serum had increased by the administration of cadmium alone indicating stress mediated lipid per-oxidation. The rats received *Terminalia arjuna* 200 mg/kg (Group C) had significantly reversed the situation as compared to group B, depicted that *Terminalia arjuna* alleviated the toxic effects of cadmium and the levels of glutathione, SOD and CAT were improved by decreasing the process of lipid per-oxidation.

Liver profile	**ALT** (nmol/ml)	**AST** (nmol/ml)	**ALP** (nmol/ml)	**TP** (mg/dl)
GROUPS	**MEAN ± SD (LSD=19.23)**	**MEAN ± SD (LSD=5.55)**	**MEAN ± SD (LSD=8.23)**	**MEAN ± SD (LSD=1.05)**
A	34.26 ± 2.05 b	31.71 ± 2.24b	82.65 ± 5.69c	6.22 ± 0.23a
B	106.53 ± 22.90 a	101.58 ± 3.05a	128.08 ± 6.38a	3.8 ± 0.50b
C	30.92 ± 1.16 b	28.26 ± 1b	104.54 ± 4.92b	6.24 ± 1.01a

Significance level =0.05

Table 1: Estimation of serum ALT, AST, ALP and total Protein

Stress profile	GSH (µg/ml)	SOD (µg/ml)	MDA (nmol/ml)	CAT (µmol/mol)
GROUPS				
	MEAN ± SD (LSD=1.15)	MEAN ± SD (LSD=2.89)	MEAN ± SD (LSD=8.72)	MEAN ± SD (LSD=4.96)
A	8.68 ± 0.54a	79.87 ± 1.62a	46.58 ± 1.52c	32.58 ± 3.19a
B	4.28 ± 0.41c	42.26 ± 2.64e	91.16 ± 9.14a	17.03 ± 3.36c
C	5.93 ± 0.58b	62.28 ± 1d	58.61 ± 3.79b	24.59 ± 3.51b

Significance level=0.05

Table 2: Estimation of serum GSH, SOD, MDA and Catalase

Discussion

It is well known that both aminotransferases (ALT and AST) are highly concentrated in the liver; ALT is localized solely in the cytoplasm, whereas AST is present both in the cytosol and mitochondria of hepatocytes [28]. Increased serum level of ALT, AST and ALP of rats of group B as compare to that of group A indicated that cadmium cause their release from the hepatocytes (Table1). Lowered total protein (TP) of group B as compare to that of group A was might be due to stoppage of protein synthesis and increased execration of proteins with cadmium (Table 1). Similar results were discussed by [29,30]. Our study showed that cadmium provoked apoptotic cell death in liver hepatocytes that was inverted by the phytochemicals in extract of *Terminalia arjuna* group C (Table1) in agreement with [31]. Administration of cadmium could cause cell lysis and release of cytoplasmic enzymes into the blood circulation, thereby leading to increased levels of these enzymes in the serum. This property is often used to assess the extent of cadmium-induced cellular damage. In the current study, elevated levels of AST and ALT were noted in response to cadmium induced toxicity. However, the level of enzymes significantly declined following concomitant administration of *Terminalia arjuna* extract. In the present study, we found that lipid per oxidation level was significantly elevated in plasma, of rats treated with cadmium as compared to control group thus significance increased oxidative stress. Similar results were supported by [32,33] who described that LPO is an early and sensitive effect of Cd exposure. Hassoun and Stohs [34] established that oxidative stress was induced following oral administration of cadmium chloride to rats. A parallel data had been described by Jurezuk et al. [35]. Additionally, Elizabeth et al, Jahangir et al and Eybl et al. [35-38] demonstrated that cadmium is considered to provoke lipid per oxidation and this has frequently been believed to be the main cause of its deleterious influence on membrane-dependent function.

An antioxidant should be fundamental component of an effective management of cadmium poisoning [39] as the results of present research showed that the mechanism of cadmium provoked damage, like other heavy metals, include the creation of reactive oxygen species and free radicals that alter the mitochondrial activity and genetic information [40,41]. Heavy metals cause their toxic effects directly or

indirectly by producing cellular stress. Present research work and many previous studies have shown that cadmium metal has the capacity to produce free radicals and reactive oxygen species (ROS) consequential in depletion of enzyme activities, damage to lipid bilayer and DNA oxidation [42]. The reactive and free radical species include oxygen, carbon, sulfur and nitrogen radicals that are originating from super oxide radical, hydrogen peroxide and lipid peroxide [43].

Cd is unable to induce ROS directly because it cannot catalyze the fenton reaction but Cd induces oxidative stress indirectly. A number of studies have revealed the capability of Cd to replace Fe which is an active metal and run the fenton reaction thus increase in concentration of free Fe in cells enhance oxidative stress and lipid peroxidation by producing highly damaging hydroxyl radicals (°OH) [14]. Many reports in animal models have illustrated that cadmium intoxication greatly increase the malondialdehyde (MDA) a product of lipid peroxidation [44]. MDA levels were found significantly high in plasma of rats treated with cadmium alone as compared to control group thus signifying increased oxidative stress. Manca et al, Abdul-Moniem and Ghafeer [31,33] who described that LPO is an early and sensitive effect of Cd exposure.

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