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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 provides protection against foreign substances by detoxifying and eliminating them [3].

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 *vivo* [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 CdCl2 @ 5 mg/kg B.Wt in drinking water till the end of research for six weeks [21]. Group III: Rats were given CdCl2 @ 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 Okhawa 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 compared 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.

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 compared to that of group A indicated that cadmium cause their release from the hepatocytes (Table1). Lowered total protein (TP) of group B as compared to that of group A was might be due to stoppage of protein synthesis and increased excretion 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.

| GROUPS | MEAN ± SD (LSD=9.23) | MEAN ± SD (LSD=5.85) | MEAN ± SD (LSD=5.23) | MEAN ± SD (LSD=1.05) |
|--------|-----------------------|-----------------------|-----------------------|-----------------------|
| A      | 101.58 ± 3.05a        | 82.65 ± 5.69c        | 86.22 ± 0.23a         |
| B      | 105.63 ± 22.90a       | 82.65 ± 5.69c        | 86.22 ± 0.23a         |
| C      | 105.63 ± 22.90a       | 82.65 ± 5.69c        | 86.22 ± 0.23a         |

Statistical Analysis

SOD CAT and ALP were improved by decreasing the process of lipid per-oxidation.

Duncan’s multiple range tests.

Statistical Significance

- Level of significance =0.05

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

| 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          | 45.58 ± 1.52c         | 32.58 ± 3.19a         |
| B      | 4.28 ± 0.41c          | 91.16 ± 9.14a         | 17.03 ± 3.36c         |
| C      | 5.93 ± 0.58b          | 58.61 ± 3.79b         | 24.59 ± 3.51b         |

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