

## Therapeutic effects of *Cassia angustifolia* in a cadmium induced hepatotoxicity assay conducted in male albino rats

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### Abstract

The present study aims to investigate the therapeutic effects of Senna plant (*Cassia angustifolia* L.) in a cadmium induced hepatotoxicity assay by evaluating the activity of alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP) and total protein (TP) in the albino rats' serum. A total of 30 white albino rats were taken and divided into three groups; each group comprising ten rats. The group A was taken as a control group; group B was given cadmium chloride concentration of 5 mg/kg (body weight) for 42 days; and group C was given cadmium chloride 5 mg/kg body weight for first 21 days and then extract of *C. angustifolia* 100 mg/kg (body weight) was given for remaining 21 days. The analysis were performed twice i.e., on 21<sup>st</sup> day and 42<sup>nd</sup> day. Results illustrated that the concentration of cadmium was significantly elevated ( $P < 0.05$ ) at the levels of serum biochemical markers namely ALT, AST, ALP which lowered the protein levels in albino rats. Moreover, treatment with the standard extracts of *C. angustifolia* observed to reverse the effects of the cadmium significantly ( $P < 0.05$ ). It is concluded that the *C. angustifolia* had hepatoprotective effects and therapeutic potential against the cadmium induced hepatotoxicity in albino rats.

**Keywords:** *Cassia angustifolia*, Cadmium, Hepatotoxicity, Albino Rats (*Rattus norvegicus*)

### Introduction

*Cassia angustifolia*, commonly known as Senna Makki, was initially classified by Linnaeus as a single species i.e. *Cassia senna* L. under the family Leguminosae. However, later studies documented different varieties of Senna across the globe. Among them, *C. angustifolia* has been widely acknowledged for medicinal purposes (Khan et al., 2011). The historical significance of the *C. angustifolia* belongs to an ancient and blessed city Makkah where Prophet Muhammad (PBUH) firstly used it as an herbal medicine (Ahmad et al., 2010). Since then, *C. angustifolia* has been grown all over the world and is being used as a traditional folk medicine in the cure of constipation, asthma, eczema, depression, skin diseases, digestive disorders, and as a tonic (Sultana et al., 2012). The leaf extract of *C. angustifolia* contains many compounds including sennoside A, sennoside B, anthrone, rhein, palmidin A, emodin, anthraquinones and some undetermined chemicals. Sennoside A, sennoside B, and flavonoids are responsible for its medicinal action as purgative (Laghari et al., 2011). Therefore, it was found that the leaf extract of *C. angustifolia* possessed noteworthy protective effects in carbon tetrachloride stimulating liver cell damage (Ilavarasan et al., 2001).

Cadmium is a heavy metal and relatively rare element found with

zinc, copper, and lead ores, being emitted from volcanic eruptions, forest fires, generation of sea salt aerosols etc. It is present as a pollutant in our food, air, water and in the smoke of cigarette (Friberg et al., 1992). Due to high soluble characteristics, cadmium compounds are taken up readily by the plants hence get accumulated in the crops being used as a source of food for other organisms; rendering it as a stern environmental pollutant and toxicant that may can affect liver, kidney, lungs, bones, brain, testis, and cardiovascular system (Ambily et al., 2013). The toxicity to liver is of prime concern as it handles the metabolism and detoxification of drugs/xenobiotics by secretion of bile (Pradhan and Girish, 2006). Therefore, the maintenance of healthy liver is vital to overall health of an individual. However, liver is always intoxicated by environmental contaminants, poor eating habits, alcohol consumption, and usage of over the counter drugs that damages and weaken the liver, ultimately leads to hepatitis, cirrhosis and alcoholic liver diseases. The toxic effects of various toxicants such as chemotherapeutic agents, carbon tetrachloride, thioacetamide, lead, chromium, cadmium, chronic alcohol consumption and microbes are well established on liver cell injury. Moreover, it has been reported that the available synthetic drugs used for the treatment of the liver diseases may also damage the liver (Saleem et al., 2008).

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To date, there is no study available about the use of *C. angustifolia* extract to alleviate the effects of cadmium at liver because as the plant has mainly been used for constipation purposes. Therefore, the present study investigates the biochemical effects of *C. angustifolia* in cadmium provoked hepatotoxicity in Wister albino rats.

## Materials and Methods

### Experimental Animals

Thirty male albino rats of 12 to 14 weeks age and 120 to 150 g weight were purchased from the market and were placed in animal house of institute of molecular biology and biochemistry (IMBB) at the University of Lahore (UOL) Punjab Pakistan. Subsequently, the rats were placed in cages made up of stainless steel at constant temperature ( $25\pm 5$  °C) with alternating day and night cycles. Standard pellets of chicks' feed # 13 layer Crumbar and water were in free access (*ad libitum*). The appropriateness of departmental policies, guidelines, and regulations were obtained from the ethic committee of IMBB of the University of Lahore.

### Chemicals

All chemicals and reagents used were of analytical grades. The cadmium chloride ( $\text{CdCl}_2$ ) was purchased from Merck pharmaceutical company (Germany) whereas methanolic extract of *C. angustifolia* (20% sennosides) from local market (Lahore, Pakistan).

### Experimental Design

Thirty healthy male rats were divided into three groups, each having 10 rats. **Group A:** Normal control kept on normal diet and tap water. **Group B:** Rats were given  $\text{CdCl}_2$  (5 mg/kg body weight) in drinking water till the end of research for six weeks (Renugadevi and Prabu, 2009). **Group C:** Rats were given  $\text{CdCl}_2$  (5 mg/kg) in drinking water for three weeks followed by standardized extracts of leaves of *C. angustifolia* (100 mg/kg) for three weeks. Sampling and testing was done two times at 21<sup>st</sup> day and at 42<sup>nd</sup> day.

### Biochemical Analysis

Liver functioning test (LFT) including alanine transaminase (ALT), aspartate transaminase (AST), alkaline phosphatase (ALP), and total protein (TP) were measured spectrophotometrically by using standard kits of Human Diagnostics laboratories (Human

Gesellschaft für Biochemica und Diagnostica mbH, Germany). Levels of total protein were estimated by Lowry Method (Lowry et al., 1951).

### Statistical Analysis

The data were presented as the mean  $\pm$ SD values, (n=10). One-way ANOVA was carried out, and the statistical comparisons among the groups were performed with the Kruskal Wallis tests using a statistical package program (Minitab v16). The significance of data was checked at  $P<0.05$  during the analysis (Riaz et al., 2016).

## Results

The results of biochemical parameters are shown in Table 1. A significant increase in levels of ALT, AST, and ALP is observed in the blood serum of group B albino rats; which were administered with cadmium chloride as compared to the rats of control group A (Table 1). By contrast, the rats of group C which were initially treated with cadmium chloride (5 mg/kg) and then *C. angustifolia* (100 mg/kg), showed decreased levels of ALT, AST and ALP to their normal level (Table 2).

The results obtained for ALT, AST, and ALP for the group C were significantly different from that of group B in each experiment. In addition this, the levels of total protein in group B were significantly decreased by the administration of cadmium chloride at 5 mg/kg, as compared to the group A (control group) ( $P<0.05$ ). Similarly, the levels of total protein in group C were significantly increased by the administration of *C. angustifolia* 100 mg/kg as compared to the group B.

## Discussion

The therapeutic effects of *C. angustifolia* were investigated using biochemical parameters such as ALT, AST, and ALP and TP in the serum of albino rats. Results of group B showed increased levels of ALT, AST and ALP, as compared to group A. One of the important functions of liver is nitrogen metabolism i.e. urea cycle; and the enzymes (ALT, AST and ALP) are essential for proper functioning of liver cells. ALT is localized in cytoplasm of hepatocytes and AST is present in both cytosol and mitochondria of hepatocytes (Rej, 1989) and their serum levels increase in response of the toxic effects of heavy metals (Bersenyi et al., 2003). The

**Table 1:** Results of biochemical parameters at 21<sup>st</sup> day showing cadmium chloride induced hepatotoxicity

Groups	Treatment	ALT (nmol/l)	AST (nmol/l)	ALP (nmol/l)	Total Protein (nmol/l)
A	Control	34.26 $\pm$ 2.05	31.71 $\pm$ 2.24	82.65 $\pm$ 5.69	6.22 $\pm$ 0.23
B	Cadmium	106.53 $\pm$ 2.9 <sup>a</sup>	101.58 $\pm$ 3.05 <sup>a</sup>	128.08 $\pm$ 6.3 <sup>a</sup>	3.8 $\pm$ 0.5 <sup>a</sup>
C	Cadmium + <i>C. angustifolia</i> (extract)	28.29 $\pm$ 3.5 <sup>b</sup>	31.25 $\pm$ 1 <sup>b</sup>	88.02 $\pm$ 1.66 <sup>b</sup>	6.59 $\pm$ 0.58 <sup>b</sup>

Cadmium@ 5 mg/kg (body weight)  
*Cassia angustifolia* at 100 mg/kg (body weight)  
 Values are mean $\pm$ S.D for 10 replicates (n=10)  
<sup>a</sup> significantly different from controls (P<0.05)

**Table 2:** The effects of *Cassia angustifolia* on biochemical parameters in cadmium chloride induced hepatotoxicity

Groups	Treatment	ALT (nmol/l)	AST (nmol/l)	ALP (nmol/l)	Total Protein (nmol/l)
A	Control	31.22±2.0	30.51±2.04	79.65±5.69	6.20±1.02
B	Cadmium	88.03±1.8 <sup>a</sup>	92.28±2.0 <sup>a</sup>	98.08±6.3 <sup>a</sup>	4.71±1.05 <sup>a</sup>
C	Cadmium + <i>C. angustifolia</i> (extract)	82.43±2.7 <sup>a</sup>	91.11±1.8 <sup>a</sup>	99.28±4.2 <sup>a</sup>	4.55±2.15 <sup>a</sup>

Cadmium@ 5 mg/kg (body weight)

Values are mean±S.D for 10 replicates (n=10)

<sup>a</sup>significantly different from controls (P<0.05)

significant increase in serum levels of ALT, AST and ALP by the cadmium chloride showed that cadmium may have caused cell injury to hepatocytes and release of these cytoplasmic enzymes into the blood circulation (Table 1). Hence, liver dysfunction was the result of cadmium administration. These results are in agreement with those of Abdul-Moneim & Ghafeer, (2007). Afterwards, the treatment of rats in group C with extract of *C. angustifolia* may have provided the solution of cadmium caused toxicity as these rats were pre-treated with cadmium chloride (5 mg/kg) in drinking water for three weeks, i.e., 21 days. The injury may have been therefore cured with the treatment of *C. angustifolia* at 100 mg/kg (Table 2). Extract of *C. angustifolia* contain many important organic compounds but sennosides and flavenoids could have been majorly served in medicinal action of (Laghari et al., 2011). The results are similar to Ilavarasan et al., 2001 abs they found that leaf extract of *C. angustifolia* had noteworthy protective effects in CCL<sub>4</sub> stimulated liver cell damage.

Beside, liver cells also synthesize serum protein and therefore its malfunctioning may lower the serum protein level. The total protein serum levels are hence measure to confirm the liver cell dysfunction and cell injury which could be due to either hepatitis, jaundice, and/or liver cirrhosis etc. (Abatan et al., 1996). The result of total proteins of group B showed that the treatment of rats with

cadmium chloride at 5 mg/kg significantly decreased its levels as compared to that of the group A (control group). It can be therefore presumed that the presence of cadmium may have disturbed the liver cells function which ultimately resulted into disturbed protein synthesis (Abatan et al., 1996). Over and above, pre-treatment of in group C rats with cadmium chloride may have reduced the stress and therefore the serum protein was more balanced. This indicates that the *C. angustifolia* had ameliorating potential against the cadmium induced hepatotoxicity which can be seen in previous studies as well (Ilavarasan et al., 2001; Renugadevi and Prabu, 2009).

## Conclusions

Conclusively, the study reveals that the presence of cadmium in liver cell may cause cellular injury, hence increasing the serum levels of ALT, AST, ALP with decrease of total protein. However, application of *Cassia angustifolia* can alleviate the harmful impacts due to its therapeutic potential towards hepatotoxicity as indicated by the decreased levels of ALT, AST, and ALP, and increased levels of serum total protein in albino rats.

## Conflict of interest

The authors declare that they have no conflict of interest.

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