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Protective Effect of *Stevia rebaudiana* Bertoni on Cadmium-induced Nephrotoxicity in Male Albino Rats

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ABSTRACT

It is important to understand the mechanism of cadmium (Cd) toxicity and its potential risk to the health of a population exposed to Cd occupationally or environmentally. Kidneys inefficiently excrete Cd after prolonged intake and damage to the nephrons occurs, seriously affecting kidney functions. Rats treated with *Stevia rebaudiana* leaf extract could withstand Cd administered at a dosage of 6 mg/kg of body weight for 30 days. *S. rebaudiana* is a non-calorific natural sweetener considered as a food supplement. It has been widely used worldwide as a substitute for artificial sweeteners and is safe, unlike artificial sweeteners. The parameters analyzed from kidney samples were total proteins, cholesterol, GOT, GPT, acid phosphatase, alkaline phosphatase, and glutathione reductase. Histological and biochemical observations were also made.

Keywords: antioxidants, natural sweetener, nephrons, non-calorific

INTRODUCTION

Environmental pollution is a great cause of concern nowadays and the exposure of humans to heavy metals released into the environment by several sources produces deleterious and lethal effects. Many attempts have been made to overcome heavy metal poisoning. Antioxidants, herbs and drugs are used for treating heavy metal toxicity, which is the result of prolonged exposure either in an occupational or domestic environment and hence treating it immediately is not possible. Dietary substances that can considerably reduce the negative effects caused by metal toxicity are thus sought and this is the rationale that formed the baseline of this work. The leaf extract of *Stevia rebaudiana* is 300 times sweeter than table sugar, sucrose (reviewed extensively by Meireles *et al*. 2006). It was used as a sweetening agent for many centuries (Braguini *et al*. 2003) and is used in the East as a food additive. The glycosides of *Stevia* were identified as the sweetening component of these leaves of which stevioside is the major component. Clinical studies revealed the hypoglycemic, antihypertensive and Cardio protective effect of this plant (Chan *et al*. 2000; Bondarev *et al*. 2002; Hsieh *et al*. 2003; reviewed by Meireles *et al*. 2006; Karthik and Jeyachandran, 2007). These findings encouraged the use of *Stevia* by diabetic patients who crave for a sweet taste but who suffer from the side effects of artificial sweetening agents such as aspartame, saccharin and cyclamate, i.e. as a non-calorific sweetening agent. Here we tested *S. rebaudiana* for its efficacy against heavy metal poisoning produced by Cadmium (Cd). An earlier study on the structure and function of the kidney of rats intoxicated with Cd hinted that humans environmentally exposed to Cd are at risk of tubular damage (Jin *et al*. 1992; Liu *et al*. 1992; Biswas *et al*. 2001; Jeyaprakash and Chinnasawamy 2005).

MATERIALS AND METHODS

50 g of green leaves of *S. rebaudiana* were shade dried at room temperature for 7 days and powdered. The fine powder was suspended in 600 ml of distilled water and kept at room temperature for 2 days. This mixture was then filtered and the extract was evaporated to 100 ml at <40°C under reduced pressure using a rotary evaporator; the liquid part was stored at 4°C (Jeppesen *et al*. 2003).

Male albino rats weighing about 150–175 g were used as experimental animals. The animal experiments were carried out in accordance with the rules of the institutional animal ethical committee. The animals were acclimatized in laboratory conditions for 10 days and were fed normal rodent diet (Godrej commercial pelleted diet), and water was given *at libitum*.

After complete acclimatization the animals were primarily grouped into 4 groups, each containing 6 animals. Group I served as the normal control, Group II served as an experimental control (toxicity was induced with cadmium (6 mg/kg of body weight as $CdCl_2$ orally for 30 days), Group III contained animals treated with *Stevia* extract alone (2 ml/day for 30 days), and Group IV contained animals co-treated with *Stevia* extract and toxicity was induced simultaneously (i.e., with cadmium as in Group II). The extract was administered orally for 30 days.

At the end of 30 days the animals were fasted overnight, weighed and sacrificed with mild ether anesthesia. The kidney was dissected out and immediately homogenized using suitable buffers specified in the estimation procedures for biochemical analysis. Analyses of protein (Lowry *et al*. 1951), cholesterol, glutamate oxaloacetate transaminase (GOT) (Abrahams *et al*. 1982), glutamate pyruvate transaminase (GPT) (Peter 1955), acid phosphatase (ACP) and alkaline phosphatase (ALP) (King 1965), reduced glutathione (GSH) (Roos *et al*. 1979) were carried out on kidney tissue. The results obtained were tabulated and statistical analysis was carried out using the Student's *t*-test (Bennett and Franklin 1967).

Histology

After draining the blood from the kidney, it was excised and washed with normal saline and processed separately for histological observations. Initially, the materials were fixed in 10% buffered neutral formalin for 48 h and then with bovine solution for 6 h. Paraffin sections were made at 5 mm thickness, processed in an alcohol-xylene series and were stained with hematoxylin and eosin

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Short Communication

Table 1 Effect of *Stevia rebaudiana* leaf extract on various biochemical parameters of rat kidney tissue.

Groups	Total proteins (mg/100 g of tissue)	Total cholesterol (mg/100g)	GOT (IU/L)	GPT (IU/L)	ACP (IU/L)	ALP (IU/L)	Reduced glutathione (mg/g of protein)
Group I	356 ± 0.4	577.6 ± 0.3	80.71 ± 0.7	65.68 ± 0.3	65.17 ± 0.6	433.4 ± 0.7	6.30 ± 0.5
Group II	550 ± 0.6	766.6 ± 0.2	109.47 ± 0.7	86.46 ± 0.6	156.11 ± 0.5	674.7 ± 0.7	3.26 ± 0.8
Group III	362 ± 0.4*	655.5 ± 0.6*	75.14 ± 0.4*	56.78 ± 0.2*	70.77 ± 0.3*	360.4 ± 0.3*	5.89 ± 0.4*
Group IV	436 ± 0.2*	708.0 ± 0.6*	91.84 ± 0.5*	74.59 ± 0.4*	108.6 ± 0.4*	570.7± 0.4*	4.65 ± 0.3*

Group I = Normal control, Group II = Cadmium toxicity induced, Group III = *Stevia* leaf extract alone treated, Group IV = Cadmium induced group co treated with *Stevia* leaf extract. Group III were compared with Group I and Group IV were compared with Group II.
Values are expressed as mean ± Standard Deviation for 6 animals in each group. * = Significant when compared to group II ** = Not significant when compared to group II (P = <0.05)
Protein and Cholesterol (mg/100 g of tissue); GOT, GPT, ACP, ALP (IU/L); Glutathione (mg/g of protein)

(Galighter and Koyloff 1971). The sections were examined microscopically for histopathological changes.

RESULTS AND DISCUSSION

Our results show a strong correlation between these marker enzymes and biochemical parameters during acute renal toxicity produced by cadmium intoxication (**Table 1**). The tissue levels of total proteins increased significantly (P<0.05) in intoxicated rats (G II) when compared to the rats co-treated with *Stevia* leaf extract (G IV). The animals treated with *Stevia* leaf extract alone was near normal but significant difference was noticed among the control group (G I) and the intoxicated treated group (G IV). The increase in the enzyme levels might be due to the release of marker enzymes (Morales *et al*. 2005).

The cholesterol levels were found to have increased in severely intoxicated animals but following treatment the levels decreased (Yamada *et al*. 1985) (**Table 1**). The marker enzymes tissue GOT and GPT increased significantly (P<0.05) (Asaki and Yokoyama 1975) in animals belonging to Group II more than in other groups. Group IV animals showed a significant decrease in all these enzymes when compared to the experimental control group (Group II) (Mathew *et al*. 1981) (**Table 1**). The increase in tissue GOT, GPT, ALP and ACP may have been due to the production of these enzymes in the cells (Shibasaki *et al*. 1994). Similar results were obtained in Cd-intoxicated rats treated with N-benzyl-D-glucamine dithiocarbamate (BGD) and N-p-hydroxymethylbenzyl-D-glucamine dithiocarbamate where the level of the serum enzymes was found to be increased two folds in Cd-intoxicated rats (Funakoshi *et al*. 1997). Quercetin (Bauman *et al*. 1992), cisplatin and metallothionein (Dorian and Klaassen 1995) also exhibited decrease in the marker enzymes levels after the treatment in heavy metal intoxicated animals.

The co-treatment of animals with *Stevia* leaf extracts showed a considerable decrease in these marker enzyme levels which may be because of the preventive action of the glycosides of *Stevia* against toxicity (Dyrskog *et al*. 2005)

Histology of the kidney sections of the control groups showed normal glomeruli and renal tubules (**Fig. 1**). The Cd-treated group kidney sections showed cellular glomeruli congestion of blood vessels and tubular necrosis (**Fig. 2**). The kidney sections of animals administered with the *Stevia* extract alone showed normal architecture with mild residual necrosis (**Fig. 3**). Administration of *Stevia* to the Cd-intoxicated group retained normal architecture with a reversal of Cd-induced renal damage (**Fig. 4**) (Dorian and Klaassen 1995).

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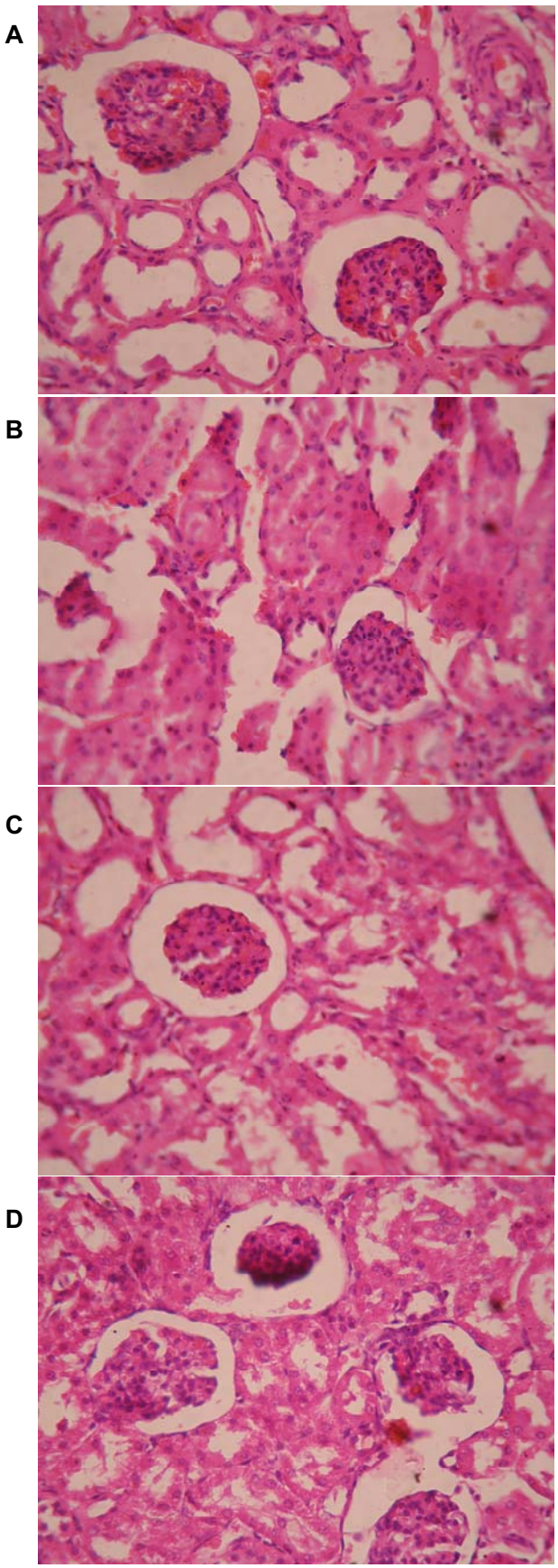

Fig. 1 (**A**) Section of rat kidney of the control group stained with hematoxylin and eosin showing normal architecture. (**B**) Section of rat kidney of the cadmium toxicity induced group stained with hematoxylin and eosin showing cellular and glomeruli tubular necrosis. Cellular necrosis of the kidney tissue. (**C**) Section of rat kidney of the *Stevia* extract alone treated group with normal architecture stained with hematoxylin and eosin. Mild residual tubular necrosis was observed. (**D**) Section of rat kidney of cadmium toxicity induced and *Stevia* extract treated group stained with hematoxylin and eosin. Reversal of cellular glomeruli and tubular necrosis was observed. All images: 100X.

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