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Hepatoprotective Effect of *Stevia rebaudiana* Bertoni Leaf Extract in CCl_4-Induced Liver Injury in Albino Rats

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ABSTRACT

The efficacy of aqueous leaf extract of *Stevia rebaudiana*, a non-calorific natural sweetener was evaluated against carbon tetrachloride (CCl_4)-induced liver damage in albino rats. Hepatotoxicity was induced by *i.p.* administration of 30% CCl_4 suspended in olive oil (1 ml/kg of body weight). After the treatment regimen animals were sacrificed and liver enzymes AST, ALT, ALP, and LDH were assayed in serum along with total protein and antioxidant status was assessed in liver tissues by determining the activities of SOD, CAT, GPx and GSH. The lipid peroxidation levels were also assessed. The observations suggested that pre-treatment of the natural sweetener could possibly protect CCl_4-induced liver damage and that the exerted effect is connected with its antioxidant and hepatoprotective nature.

Keywords: antioxidant, liver injury, natural sweetener, stevioside

INTRODUCTION

Stevia rebaudiana Bertoni, also termed 'sweet leaf' or *seeni tulsi* (in Tamil), belongs to the *Asteracea* family, native to subtropical and tropical South America (Braguini *et al.* 2003; reviewed in Meireles *et al*. 2006). The leaf extract of *S. rebaudiana* is known for its sweetness which is 300 times sweeter than table sugar and has been used as a sweetening agent for many centuries. Even today in the eastern countries *Stevia* is widely used as a food additive. The glycosides of *Stevia* were identified as the sweetening component of these leaves and stevioside is the major component (Bondarev *et al.* 2002). Clinical studies conducted earlier revealed the hypoglycemic and antihypertensive effect of this plant (Chan *et al.* 2000; Hsieh *et al.* 2003; Gregersen *et al.* 2004). These findings encouraged the use of *Stevia* by diabetic patients who crave for sweet taste and suffer from the side effects of artificial sweetening agents such as aspartame, saccharin and cyclamate (Usami *et al*. 1980). Few artificial sweeteners are carcinogenic and severally affect the normal liver functioning, although researchers raised concern over the use of *Stevia* since they believed that it might be toxic to the liver (Kelmer *et al*. 1985; Braguini *et al*. 2003).

There is thus a need to test the effect of *Stevia* on the liver. In this study, the efficacy of the aqueous leaf extract of *S. rebaudiana* was tested in CCl_4-induced liver damage in albino rats and if found to possess a hepatoprotective effect then *Stevia* would remain important for diabetics without the fear of chronic toxicity of artificial sweeteners.

MATERIALS AND METHODS

Plant material

S. rebaudiana leaves were purchased from commercial sellers, and were authenticated by The Raphinat Herbarium, St. Joseph's College, Trichy. They were shade dried at 37°C for 7 days. The dried material was powdered and stored in a dry container at room temperature.

Preparation of extract

100 g of dry leaf powder was suspended in water at a ratio of 1:600 (w/v) and stirred at 2000 rpm with a magnetic stirrer overnight at 37°C. The process was repeated in triplicate and then the extract, which was filtered using a fine muslin cloth, was stored in the refrigerator for further use.

The analytical grade chemicals used were purchased from Himedia Chemicals (India).

Animals

Male albino rats weighing about 100-150 g were obtained from the Tamil Nadu Veterinary and Animal Sciences University, Madhavaram, Chennai. The animals were housed in a well ventilated animal room of the Department of Biochemistry, St. Joseph's College, Trichy, under a 12-h light/dark cycle. The animals were fed with standard pellet diet and water *ad libitum*. The use of animals was in accordance with the rules of the Institutional animal ethical committee.

Experimental design

The animals were divided into four groups of 6 animals each and treated as follows: Group I animals served as normal control, Group II animals constituted the hepatotoxic group, which received *i.p.* administration of 30% CCl_4 suspended in olive oil (Rocchi Olive oil, Hemraj, India) (1 ml/kg of body weight) (Castro *et al*. 1974), Group III and IV animals were treated with the *Stevia* leaf extract (SLE) for 10 days and for Group IV animals CCl_4 was given as in group II on the 11^{th} day after pretreatment with the extracts. The animals were sacrificed on the 12^{th} day by cervical decapitation. Blood was collected and serum was separated and stored at 4°C for further analysis. Serum marker enzymes analyzed were aspartate transaminase (AST) (King 1965), alanine transaminase (ALT) (King 1965), alkaline phosphatase (ALP) (Kind and King 1954), lactate dehydrogenase (LDH) (Ulmer *et al.* 1956), total serum protein (TSP) (Lowry *et al*. 1951). Antioxidant status was analyzed from the levels of glutathione (GSH) (Beutler and Kelley 1963), lipid peroxidation (LP) (Stocks and Dormandy 1971) and activities of glutathione peroxidase (GPx) (Rotruck *et al*.

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1973), catalase (CAT) (Bergmeyer *et al.* 1974) and superoxide dismutase (SOD) (Roos *et al*. 1979) of the liver tissue. The results obtained were statistically analyzed by the student's *t*-test for significance ($P < 0.05$; < 0.001) between groups (Bennett and Franklin 1967).

RESULTS AND DISCUSSION

The levels of the marker enzymes ALP, AST, ALT, LDH and TSP of all the four groups experimental and control groups are shown in **Table 1**. The levels of these parameters, except for serum proteins, showed a significant increase in their levels in animals belonging to group II, the toxicity-induced group whereas the protein values decreased in the CCl_4-induced animals. The level was found to be near normal with no significant difference when compared with the control group animals and the treatment group (IV) showed marked elevation in the total protein levels and decrease in enzyme levels. The results of the antioxidant enzymes (**Table 2**) show that administration of CCl_4 decreased the antioxidant enzyme levels and a significant increase was observed in the treatment group. The lipid peroxidation assayed by the TBARS method also showed similar results and animals treated with the plant extract alone maintained normalcy to a greater extent.

The present study was conducted to determine the anti-hepatotoxic effect of *S. rebaudiana* leaf extract in albino rats. CCl_4 was used to induce hepatic damage in rats since it is known to produce acute hepatocellular injury with centrilobular necrosis and steatosis (Recknagel *et al*. 1989). It is a highly toxic chemical and its adverse effects on liver have (Recknagel 1967) been known for years (and hence it used as an ideal model to induce hepatic cirrhosis). CCl_4 metabolites such as trichloromethyl peroxyradical are also toxic to the liver (Mehandale *et al*. 1986). On induction of CCl_4 the levels of marker enzymes increased significantly compared to the normal control group I (**Table 1**). Enzyme activities decreased considerably in treatment group IV compared to the induced group II. The activities of these enzymes remained near normal and increased slightly in a few cases in Group III (**Table 1**). The increased levels of AST, ALT, ALP, and LDH in serum indicate damage to hepatic cells (Wolf 1999). Fluctuations in AST levels indicate a loss of cell integrity, while variations in the activity of ALT and LDH indicate increased permeability and cellular leakage (Sreepriya *et al*. 2001). Similarly, the activities of AST, ALT, ALP and LDH in group IV animals were found to decrease significantly compared to the induced group II when treated for experimentally induced myocardial infarction (Mohan and Robert 2007). This shows that pretreatment of *S. rebaudiana* leaf extract is capable of preventing cellular damage induced by CCl_4. The decrease in the total protein level in the induced group might be because of the decrease in the liver's ability to synthesize proteins (Ahmed *et al*. 2000). But the animals belonging to Group IV exerted a marked increase in total protein levels in serum, which is an indication of the regeneration of the damaged liver tissues and that the *S. rebaudiana* leaf extract was capable of rendering some resistance to hepatic tissues to withstand the degenerative nature of CCl_4. The antioxidant status of the *S. rebaudiana* leaf extract was evident from an increase in the levels of antioxidant enzymes (Mohan and Robert 2009). In the CCl_4-induced groups the levels of the antioxidant enzymes SOD, CAT, GPx, GSH were found to decrease and lipid peroxidation levels increased in Group II (**Table 2**). In CCl_4-induced groups lipid peroxidation was evident and might be because of changes in the cell membrane (Ohta *et al*. 1995). The decrease in the levels in group IV indicated the anti-lipid peroxidative effect of *S. rebaudiana*. SOD acts as a cellular defense element against potentially harmful effects of superoxide radicals by catalyzing the dismutation of these ions (Mohanan *et al*. 1997) (**Table 2**). GST increases the conjugation of free radicals and lipid hydroperoxides to GSH and enhances its excretability (Beckett and Hayes 1987). Catalase was also involved in ROS scavenging. The decline in the activities of SOD, CAT, GPx and GSH in the induced group and significant increase in the treated group indicates the oxidative damage produced by CCl_4 to liver (Paduraru *et al*. 1996) and the protective effect rendered by *S. rebaudiana* leaf extract to the treated animals (**Table 2**). These protective effects exerted by *S. rebaudiana* might be because of its glycosides.

CONCLUSION

The present study demonstrated the hepatoprotective and antioxidant effect of *S. rebaudiana* leaf extract in experimentally induced hepatic injury in albino rats. Further research is to be carried out to determine the active principle and dose dependent effect.

Table 1 Effect of *Stevia rebaudiana* leaf extract on liver marker enzymes in the serum of control and experimental animals.

Parameters \ Groups	Group I	Group II	Group III	Group IV
ALT (U/ml)	45.01 ± 1.9	115.67 ± 8.93^{+}	47.24 ± 1.66	80.13 ± 5.10**
AST (U/ml)	53.05 ± 3.16	165.26 ± 10.2^{+}	54.98 ± 2.10	125.30 ± 10.42**
ALP (KA units)	48.32 ± 2.11	99.65 ± 5.42^{+}	50.65 ± 3.12	63.75 ± 4.27*
LDH (IU/dl)	100.01 ± 1.02	151.68 ± 10.97^{+}	103.88 ± 3.32	140.64 ± 9.98*
TSP (mg/ml)	52.45 ± 1.48	48.12 ± 3.46^{+}	53.59 ± 2.15	50.55 ± 1.50**

Group I = Normal control, Group II = CCl_4 toxicity induced, Group III = *Stevia* leaf extract alone treated, Group IV = CCl_4 induced group pretreated with *Stevia* leaf extract. Group III were compared with Groups I and IV were compared with Group II.
Values are mean ± SEM for 6 animals in each group
* $P < 0.05$; ** $P < 0.001$; as compared with CCL_4-induced groups.
$^{+}$ $P < 0.001$ as compared with normal group.
ALT, alanine transaminase; ALP, alkaline phosphatase; AST, aspartate transaminase, LDH, lactate dehydrogenase; TSP, total serum protein

Table 2 Effect of *Stevia rebaudiana* leaf extract on antioxidant status in the liver of control and experimental animals.

Parameters \ Groups	Group I	Group II	Group III	Group IV
GSH (μg/mg of protein)	15.02 ± 1.3	10.59 ± 1.65^{+}	15.43 ± 0.2	13.72 ± 1.0*
LP (nmoles of malondialdehyde (MDA) formed/mg of protein / hr)	120.20 ± 20.1	186.32 ± 12.7^{+}	121.06 ± 0.97	140.33 ± 7.6**
GPx (μg of glutathione consumed/min/mg protein)	17.00 ± 1.0	15.12 ± 1.02^{+}	16.92 ± 0.05	16.02 ± 0.05*
CAT (unit/min/mg of protein)	34.39 ± 1.50	15.02 ± 0.38^{+}	34.06 ± 0.04	21.15 ± 0.81**
SOD (Unit/min/mg of protein)	2.45 ± 0.05	1.80 ± 0.04^{+}	2.31 ± 0.06	1.82 ± 0.09

Group I = Normal control, Group II = CCl_4 toxicity induced, Group III = *Stevia* leaf extract alone treated, Group IV = CCl_4 induced group pretreated with *Stevia* leaf extract. Group III were compared with Groups I and IV were compared with Group II.
Values are mean ± SEM for 6 animals in each group.
* $P < 0.05$; ** $P < 0.001$; as compared with CCL_4-induced groups.
$^{+}$ $P < 0.001$ as compared with normal group.
CAT, catalase; GPx , glutathione peroxidase; GSH, glutathione; LP, lipid peroxidation; SOD, superoxide dismutase

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