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Toxicity Studies on Chloroform Extract of *Solanum trilobatum*: Effect on Drug Metabolizing and Antioxidant Enzymes in Rats

^{1,2}H. Sini, ¹K.S. Devi and ^{1,3}K.G. Nevin

¹Department of Biochemistry, University of Kerala, Kariavattom, Thiruvananthapuram, Kerala, 695581, India

²Department of Biochemistry, Government College, Kariavattom, Thiruvananthapuram, Kerala, 695581, India

³School of Biosciences, Mahatma Gandhi University, PD Hills PO, Kottayam, Kerala, 686560, India

Corresponding Author: H. Sini, Department of Biochemistry, Government College, Kariavattom, Thiruvananthapuram, Kerala, 695581, India Tel: 914712417112

ABSTRACT

The aim of the study was to evaluate the effect of chloroform extract of *Solanum trilobatum* (CST) on drug metabolizing and antioxidant defense enzymes for assessing its chemo preventive potential and protection of tissues from oxidative damage. The CST was obtained by soxhlet extraction with chloroform. Animals (Male Sprague Dawley rats) were divided into three groups: Group I-normal control, group II-CST (200 mg kg⁻¹ b.wt.), group III-CST (800 mg kg⁻¹ b.wt.). The CST was given by oral gavage once daily for 15 days. At the end of the experimental period all the rats were sacrificed and the livers, lungs and kidneys were taken for estimating the phase I and II drug metabolizing and antioxidant enzyme activities. The gain in body weight and the levels of GSH and lipid peroxides in these tissues were also determined. The results showed that the administration of CST could modulate the phase I and phase II enzymes that are critical for cancer protection. Administration of CST at 200 and 800 mg kg⁻¹ b.wt. to animals did not cause any apparent clinical signs as survivability or visible changes caused by toxicity in the liver, lungs and kidney. Administration of CST at a dose of (800 mg kg⁻¹ b.wt.) was found to be ineffective in elevating the phase I enzymes suggesting that CST could possibly prevent the biotransformation of carcinogens. These results clearly indicate that CST with its biologically active components could be used as a chemo preventive agent in treating various cancers.

Key words: *Solanum trilobatum*, phase 1 enzymes, chemoprevention, drugs

INTRODUCTION

One of the reliable biochemical markers to assess the chemopreventive potential of a test compound is the inducibility of drug metabolizing enzymes (Prochaska and Fernandes, 1993; Banerjee *et al.*, 1995; Li *et al.*, 2000). Using experimental animal systems the induction of drug metabolizing enzymes by exogenous compounds like drugs, carcinogens and environmental chemicals is well documented (Parke, 1975; Conney, 1986; Okey *et al.*, 1986). The induction or inhibition of phase I and phase II metabolism occurs in association with drug interaction (Gibson and Skett, 2001). Chemo preventive agents exert their protective effects by inhibiting the metabolic activation of carcinogens and enhancing the enzymes involved in the detoxification of carcinogens resulting in a lower yield of the electrophilic metabolites capable of binding to DNA

(Stavric, 1994). The balance between phase I enzyme systems and phase II enzymes partly controls the susceptibility of biological systems to carcinogenesis (Talalay, 1989, 1992). It is generally understood that plant phenolic compounds can modify the metabolic activation of several carcinogens, including aromatic amines. Studies have proved that structurally diversified plant phenolics has significant modulating effect on cytochrome p450-dependent enzymes and glutathione S-transferase (GST) activities after oral administration alone or in combination with o-toluidine in rat liver and kidney (Szaefer *et al.*, 2003).

Solanum trilobatum Linn, belonging to the family Solanaceae is well known in Ayurveda medical systems since it used in herbal medicines to treat various diseases like respiratory problems, bronchial asthma cough, tuberculosis and urinary tract disorders (Ramakrishna *et al.*, 2011; Gandhiappan and Rengasamy, 2012). Previous studies in our laboratory have reported that the chloroform extractable portion of *S. trilobatum* (CST) exhibited significant antioxidant action against free radicals *in vitro* (Sini and Devi, 2004). This study is designed to evaluate the effect of CST on drug metabolizing enzymes for assessing its chemopreventive potential and monitor the levels of antioxidant defense enzymes that protect the cell from oxidative damage.

MATERIALS AND METHODS

Chemicals: All chemicals used were of highest analytical grade available. Chloroform was obtained from Merck, India.

Plant extraction: Fresh plants were collected from Nagercoil forest, Tamilnadu, India during the month of June, 2011 and taxonomically authenticated. The whole plant was cut into pieces, shade dried and powdered. The powder (100 g) was extracted exhaustively in soxhlet apparatus initially with petroleum ether [BP (60-80°C)] followed by chloroform [BP (60-62°C)]. The extracts were then concentrated *in vacuo* until the solvent was completely removed. Chloroform extract (CST) was used for further experiments (Sini and Devi, 2004).

Experimental protocol: Male Sprague Dawley rats (2 months old, 140-170 g) were used for the study. Animals were housed in well-ventilated polypropylene cages in a room maintained at 25±1°C under a 12 h light and 12 h dark cycle and given free access to standard pellet diet (Hindustan lever, India) and water *ad libitum*. Animal experiments were done according to the guide lines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Animal Welfare Division, Government of India. Animals were divided into following groups (6 animals/group): Group I-normal control (2 mL saline), group II-CST 200 mg kg⁻¹ b.wt. in 2 mL saline) and group III-CST (800 mg kg⁻¹ b.wt. in 2 mL saline). The CST was given to rats by oral gavage once daily for 15 days. At the end of the experimental period, all the rats was sacrificed by sodium pentathone injection. The livers, lungs and kidneys were removed and rinsed with cold physiological saline and stored at -80°C until analyzed.

Preparation of homogenates and subcellular fractions: Tissues were trimmed free of extraneous collagen material attached underneath by scraping and cut into small pieces and homogenized in ice-cold 0.15 M Tris-KCl buffer (pH 7.4) using a polytron homogenizer to yield a 10% (w/v) homogenate. The homogenate was then centrifuged using a refrigerated super speed

centrifuge at 10,000×g for 20 min. The resultant supernatant was transferred into pre-cooled ultracentrifugation tubes and centrifuged at 105,000×g for 60 min in Sorvall ultracentrifuge to obtain the cytosol and microsomal fraction.

Estimation of phase I enzyme activities: Microsomal fraction from the tissues were used for assaying cytochrome P₄₅₀ using carbon monoxide difference spectra according to the method described by Omura and Sato (1964), estimation of cytochrome P₄₅₀ reductase (CYR) was performed according to the method of Omura and Takesue (1970), with some modifications, by measuring the rate of oxidation of NADPH at 340 nm and NADH-cytochrome b₅ reductase was assayed according to the method of Mihara and Sato (1972) measuring the rate of reduction of potassium ferricyanide at 420 nm by NADH.

Estimation of phase II enzymes activities: Cytosol fraction from the tissues were used for assaying of Glutathione-S-Transferase (GST) by the procedure of describe by Habig *et al.* (1974). The NAD(P)H quinone oxidoreductase activity was measured by the method of Benson *et al.* (1980).

Estimation of antioxidant enzyme activities: Antioxidant enzyme activity was measured in the cytosolic fractions. Catalase was assayed by the method of Maehly and Chance (1954). The specific activity was expressed in terms of units/mg protein. Superoxide was determined by a chromogen based assay as described by Kakkar *et al.* (1984). Specific activity was expressed units/mg protein. Glutathione peroxidase activity was determined by the method of Lawrence and Burk, which is based on the oxidation of NADPH enzyme activity is expressed as nmoles of NADPH oxidized/min/mg protein (Lawrence and Burk, 1976).

Estimation of glutathione content: The glutathione content of the tissues were determined by the procedure described by Patterson and Lazarow (1955). Five hundred milligram of the tissue was homogenized in 4 mL of the precipitating solution (1.67 g of glacial phosphoric acid +0.2 g of EDTA+30 g of NaCl per 100 mL of distilled water). After mixing, the solution was allowed to stand for 5 min and filtered. To 2 mL of the filtrate, added 3 mL of phosphate solution (0.3 M Na₂HPO₄). To all the tubes, 1 mL of DTNB solution (40 mg of 5,5'-dithiobisnitrobenzoic acid in 100 mL of 1% sodium citrate) was added, mixed well and the optical density was measured at 412 nm. Blank was prepared by substituting the sample with water and following the entire procedure for test. The quantity of reduced glutathione is expressed in µg/100 g wet tissue.

Estimation of lipid peroxide content: Lipid peroxides in the tissues were assayed by the method of Ohkawa *et al.* (1979). To 0.2 mL of 10% (w/v in 1.15% KCl) tissue homogenate were added 0.2 mL of 8.1% SDS, 1.5 mL of 20% acetic acid solution adjusted to pH 3.5 with NaOH and 1.5 mL of 0.8% aqueous solution of TBA. The mixture was made up to 4.0 mL with distilled water and then heated at 95°C for 60 min using a glass ball as a condenser. After cooling with tap water, 1.0 mL of distilled water and 5.0 mL of the mixture of n-butanol and pyridine (15:1, v/v) were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken and its absorbance at 532 nm was measured. Tetramethoxy propane (TMP) was used as an external standard and the level of lipid peroxides was expressed as nmol of MDA/mg wet tissue.

Statistical analysis: Statistical analysis was determined using one way ANOVA followed by Duncan's *post hoc* test to identify the differences using SPSS 12. Differences of $p < 0.05$ was considered to be significant. Data is expressed as Mean \pm SEM.

RESULTS

No effect on body weight gain: There was any significant difference in the mean body weight in animals treated with low (200 mg kg⁻¹ b.wt.) and high dose (800 mg kg⁻¹ b.wt.) of the CST compared to control (Table 1).

Effect on phase I enzyme activities: In the liver, the level of cytochrome P₄₅₀ was found to be decreased in group II animals which received low dose of CST (200 mg kg⁻¹ b.wt.) but the reduction was not significant when compared with normal rats, whereas the animals which received high dose of CST (800 mg kg⁻¹ b.wt.) showed almost the same level as that of normal animals. The cytochrome P₄₅₀ content in the kidney and lungs of animals in all the groups were similar (Fig. 1).

Activity of CYR in the microsomal fraction of the liver, kidney and lungs analyzed was found to be significantly decreased with low 200 mg kg⁻¹ b.wt. and high dose (800 mg kg⁻¹ b.wt.) of CST treatment compared to control. When compared with the animals in the low dose treated group (group II) which received (200 mg kg⁻¹ b.wt.) of CST, those which received 800 mg kg⁻¹ b.wt. (group III) exhibited a significant decrease in enzyme activity in the liver, lungs and kidney (Fig. 1). There was significant reduction in the activity of cytochrome b₅ reductase in the liver, lungs and kidney of animals which received 200 and 800 mg kg⁻¹ b.wt. of CST (group II and III, respectively) in comparison with normal control (group I) animals. The enzyme activity was found to be markedly reduced in the liver and lungs of group III animals given CST at a dose of 800 mg kg⁻¹ b.wt. in comparison with those given CST at a dose of 200 mg kg⁻¹ b.wt. (Fig. 1).

Effect of CST on phase II enzyme activities: Activity of GST was found to be enhanced significantly in the low (200 mg kg⁻¹ b.wt.) and high dose (800 mg kg⁻¹ b.wt.) drug treated groups (II and III) relative to the level shown by group I rats. In comparison with group II animals given 200 mg kg⁻¹ b.wt. of CST the activity was significantly lowered in the lungs of group III animals whereas the activity was markedly elevated in the kidney of these animals received 800 mg kg⁻¹ b.wt. (Fig. 2). The NQOR activity was found to be noticeably elevated in the liver, lungs and kidney of group II animals given 200 mg kg⁻¹ b.wt. of CST relative to control (group I) rats. Group III animals given a higher dose of CST (800 mg kg⁻¹ b.wt.) also showed significant increase in NQOR activity in all the tissues investigated relative to control group but the enhancement was not remarkable in comparison with that of group II (Fig. 2). The specific activity of GR in the liver, lungs and kidney of all the animals treated with 200 mg kg⁻¹ b.wt. (group II)

Table 1: Effect of CST on body weight gain, total tissue GSH and lipid peroxide content

Groups	Body weight gain	Total GSH			Lipid peroxide content		
		Liver	Lungs	Kidney	Liver	Lungs	Kidney
I	22.4 \pm 1.3	4.0 \pm 0.01	1.2 \pm 0.04	0.9 \pm 0.02	15.1 \pm 0.05	25.0 \pm 0.06	10.0 \pm 0.05
II	21.0 \pm 0.8 ^{NS}	6.4 \pm 0.03*	2.1 \pm 0.01*	1.5 \pm 0.02*	12.0 \pm 0.02*	23.0 \pm 0.09*	8.5 \pm 0.03*
III	21.5 \pm 0.7 ^{NS}	6.9 \pm 0.01*	2.3 \pm 0.01*	1.6 \pm 0.01*	13.2 \pm 0.02*	24.2 \pm 0.11*	8.9 \pm 0.06*

Values are mean of 6 rats \pm SEM, Group I: Normal control, Group II: CST (200 mg kg⁻¹ b.wt.), Group III: CST (800 mg kg⁻¹ b.wt.), *Significant compared to group I, ($p < 0.05$). NS: No significant difference between group II and III

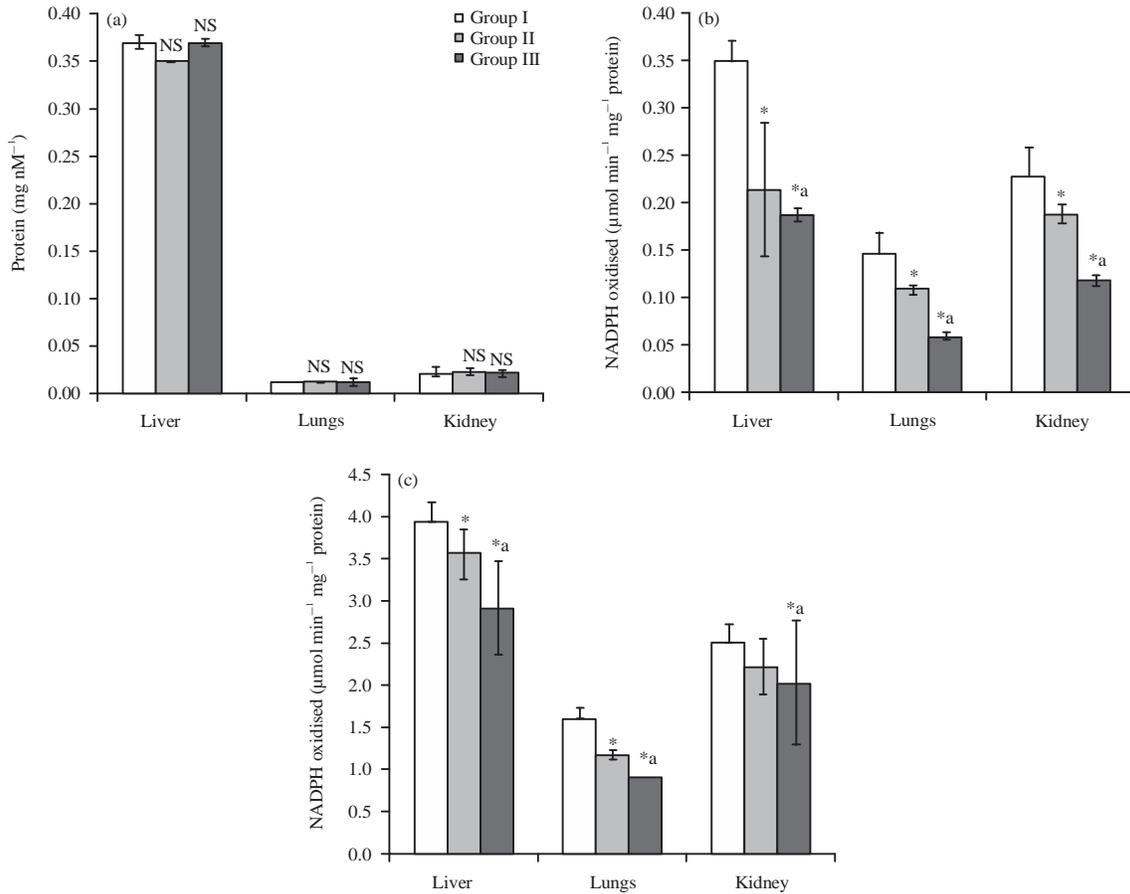


Fig. 1(a-c): Effect of CST on phase I enzyme activities, values are mean of 6 rats±SEM, group I: Normal control, group II: CST (200 mg kg⁻¹ b.wt.), group III: CST (800 mg kg⁻¹ b.wt.), NS: Non significant compared to normal rats, ^aSignificant compared to group II, (p<0.05), (a) Cytochrome P₄₅₀ content, (b) Cytochrome P₄₅₀ reductase (CYR) and (c) Cytochrome b₅ reductase

and 800 mg kg⁻¹ b.wt. (group III) of CST showed a significant elevation when compared with control rats (group I). No significant difference was observed in the liver, lungs and kidney of group II and group III animals (Fig. 2).

The GPx activity was found to be significantly augmented in the liver, lungs and kidney of low (200 mg kg⁻¹ b.wt.) (group II) and high dose (800 mg kg⁻¹ b.wt.) (group III) of CST treated animals relative to control (group I). In comparison with group II animals received (200 mg kg⁻¹ b.wt.) of CST the enzyme activity was markedly higher in group III animals given 800 mg kg⁻¹ b.wt. (Fig. 3).

Effect of CST on antioxidant status: Catalase activity was noticeably increased in the liver and kidney of animals given low (200 mg kg⁻¹ b.wt.) (group II) and high dose (800 mg kg⁻¹ b.wt.) (group III) of CST in comparison with normal control animals while the activity in the lungs was found to be significantly lowered in both the drug treated rats. Comparison between the drug

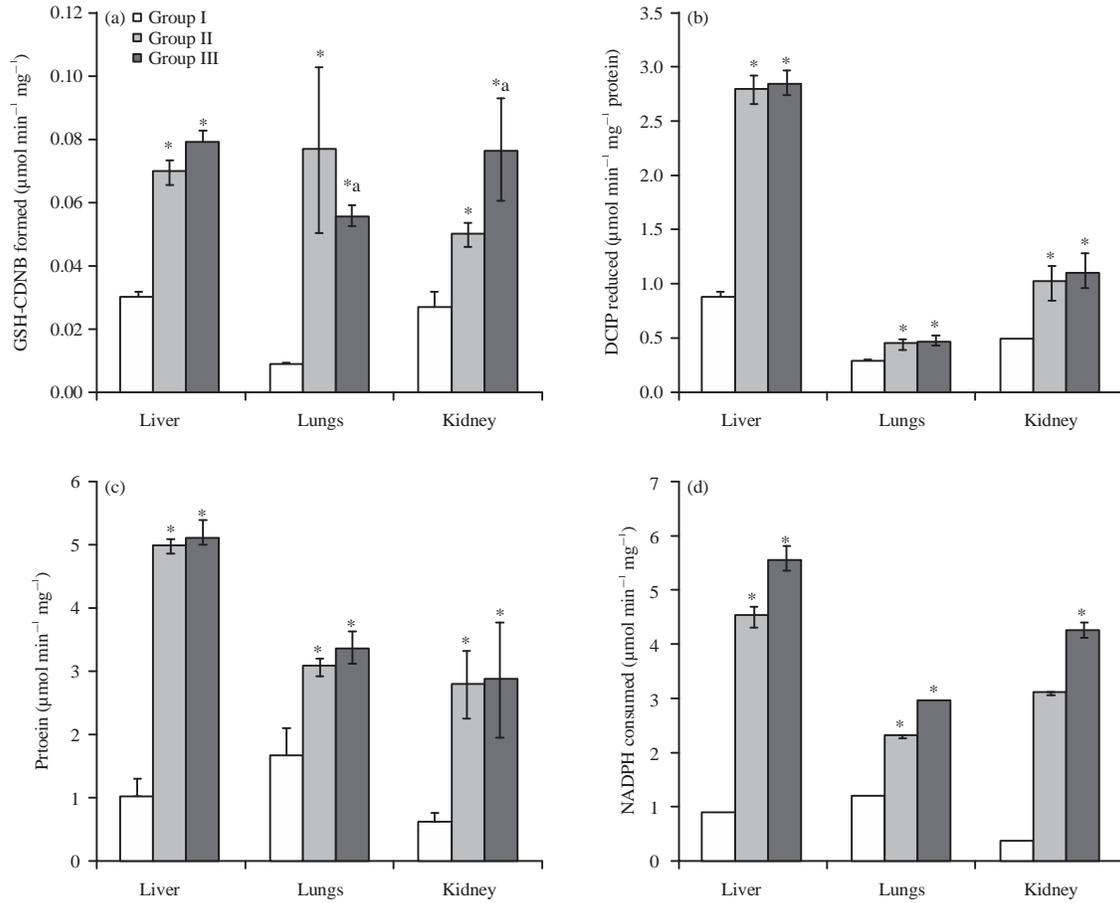


Fig. 2(a-d): Effect of CST on phase II enzyme activities, values are mean of 6 rats \pm SEM, group I: Normal control, group II: CST (200 mg kg^{-1} b.wt.), group III: CST (800 mg kg^{-1} b.wt.), *Significant compared to group I ($p < 0.05$), ^aSignificant compared to group II, ($p < 0.05$), (a) Glutathione-S-transferase (GST), (b) NAD(P)H quinone oxidoreductase (NQOR), (c) Glutathione Reductase (GR) and (d) Glutathione peroxidase (GPx)

treated groups (II and III) showed no significant difference in the activity of the enzyme (Fig. 3). Hepatic SOD activity was found to be significantly higher in 200 mg kg^{-1} b.wt. (group II) and 800 mg kg^{-1} b.wt. (group III) of the chloroform extractable portion of *S. trilobatum* (CST) received animals respectively in comparison with control (group I) whereas, the activity of the enzyme exhibited a significant elevation in group III animals given 800 mg kg^{-1} b.wt. The CST when compared with those in group II. In the kidney not much variation in enzyme activity was observed between normal and drug treated groups (II and III) (Fig. 3).

Level of GSH was found to be significantly increased in the liver, kidney and lungs of group II and III animals given 200 and 800 mg kg^{-1} b.wt. of CST, respectively compared to group I normal animals and no remarkable variation was observed between the drug treated groups (II and III) (Table 1). Group II and III animals administered CST at dose of 200 and 800 mg kg^{-1} b.wt., respectively exhibited a significant reduction in lipid peroxide content in the liver, lungs and

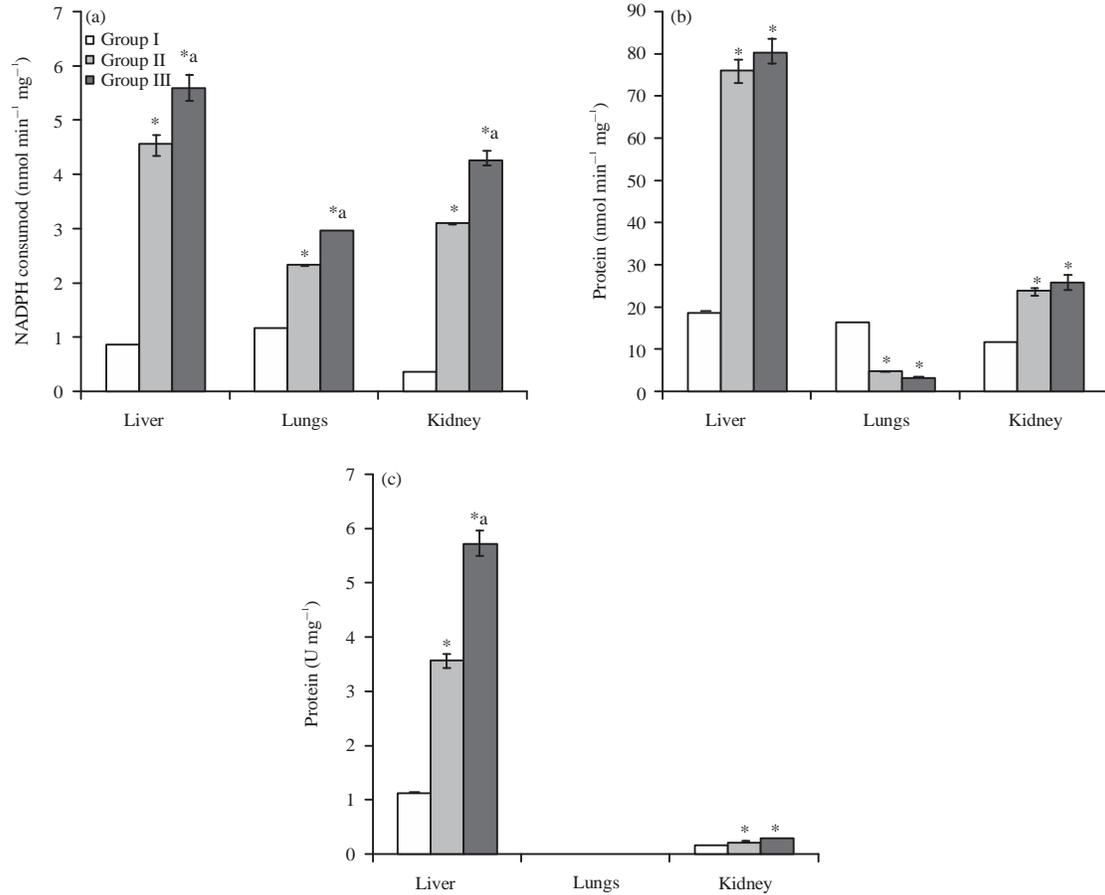


Fig. 3(a-c): Effect of CST on antioxidant enzyme activities, values are mean of 6 rats \pm SEM, group I: Normal control, group II: CST (200 mg kg⁻¹ b.wt.), group III: CST (800 mg kg⁻¹ b.wt.), *Significant compared to group I (p < 0.05), ^aSignificant compared to group II, (p < 0.05), (a) Glutathione peroxidase (GPX), (b) Catalase (CAT) and (c) Superoxide dismutase (SOD)

kidney. The high dose (800 mg kg⁻¹ b.wt.) received group (group I) showed no remarkable increase in lipid peroxide content in all the tissues investigated in comparison with group II animals (Table 1).

DISCUSSION

The results obtained in this study clearly revealed that the administration of the chloroform extractable portion of *S. trilobatum* (CST) could modulate the phase I and phase II enzymes that are critical for cancer protection. Administration of CST at 200 and 800 mg kg⁻¹ b.wt. to animals did not cause any apparent clinical signs as survivability or visible changes caused by toxicity in the liver, lungs and kidney. Administration of CST at a dose of 800 mg kg⁻¹ b.wt. was found to be ineffective in elevating the phase I enzymes suggesting that CST may be capable of inhibiting the activation of carcinogens. In the cell, cytochrome P₄₅₀ system is involved in the metabolism

of lipophilic carcinogens/compounds to more water soluble metabolites thereby phase II enzymes could convert them to more polar compounds thus assisting in their excretion (Henderson *et al.*, 2000).

Treatment with CST showed significant increase in the activity of the phase II enzyme glutathione-S-transferase (GST) in liver, lungs and kidney in comparison with control group. Based on the earlier reports we could suggest that the compounds present in CST induced the synthesis of GST enzymes which are involved in the conjugation of a variety of reactive chemicals with GSH, thus preventing reactive electrophiles from reaching cellular targets and production of cytotoxic thioether linked glutathionyl conjugate (Fahmy *et al.*, 2006). The CST treatment also elevated the activity of NAD(P)H quinone reductase (NQOR), another important phase II enzyme thus protecting the cells against Reactive Oxygen Species (ROS) generated by exogenous quinones and related compounds as reported by Talalay and Dinkova-Kostova (2004).

The CST administration at low (200 mg kg⁻¹ b.wt.) and high dose (800 mg kg⁻¹ b.wt.) significantly elevated GR activity, thereby helping the cell to maintain the basal level of GSH, which is important for many other GSH-dependent detoxification reactions. Glutathione Reductase (GR) is one of the major antioxidant enzymes that catalyses the NADPH-dependent reduction of glutathione disulfide to glutathione thereby maintaining GSH levels in the cell (Le *et al.*, 1995).

The CST was found to induce SOD activity indicating that the generation of reactive oxygen species is inhibited or CST treatment accelerated the removal of the reactive oxygen species catalyzed by SOD as per reports available (Li *et al.*, 2000). The enzyme catalase is involved in the removal of hydrogen peroxide produced by the action of SOD. The chloroform extractable portion of *S. trilobatum* (CST) administered at low (200 mg kg⁻¹ b.wt.) and high dose (800 mg kg⁻¹ b.wt.) significantly elevated the activity of catalase. The elevation in SOD along with catalase activity explains the significant reduction in lipid peroxidation caused by the chloroform extractable portion of *S. trilobatum* (CST). The GSH content was increased following CST treatment in the liver, lungs and kidney points to the fact that induction of GSH by the chloroform extractable portion of *S. trilobatum* facilitates the protection of the cell against free-radical induced damage. Reduced GSH participates in spontaneous scavenging of electrophiles or free radicals produced in reactions catalyzed by GPX and GST (Coles and Ketterer, 1990).

Solanum trilobatum contains several phytochemicals including polyphenols which showed strong antioxidant activities *in vitro* (Sini and Devi, 2004). One of the mechanisms by which plant polyphenols exhibit their anticarcinogenic effect is by modulation of enzyme activities associated with carcinogen activation and detoxification. Flavonoids like naringenin and tangeritin are potent inhibitors of microsomal 7-ethoxy resorufin-O-deethylase (EROD) which is a marker substrate for P4501A (Obermeier *et al.*, 1995). Flavone, flavanone and tangeritin in the diet increase hepatic GST activity in rats (Uda *et al.*, 1997). It was also reported that galangin, kaempferol and quercetin are all potent inducers of QR in mouse hepatoma cells.

CONCLUSION

Based on these reports it can be concluded that the chloroform extractable portion of *S. trilobatum* (CST) containing polyphenolic class compounds along with other unidentified phytochemicals could be developed as a chemopreventive agent.

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