Antioxidant activities of tamarind (*Tamarindus Indica*) seed coat extracts using in vitro and in vivo models

P. Sandesh · V. Velu · R. P. Singh

Revised: 4 October 2013 / Accepted: 12 November 2013 / Published online: 2 February 2014
© Association of Food Scientists & Technologists (India) 2014

Abstract *Tamarindus indica* seed coat was extracted with methanol, acetone and water and screened for DPPH radical scavenging activities. Methanol extract showed higher activity than other extracts. Treatment of albino rats (Wistar strain) with CCl_4 at 1.25 mL/kg of body weight decreased superoxide dismutase (55 %), catalase (73 %) and peroxidase (78 %), while lipid peroxidation increased nearly 2.5 fold in liver. Pretreatment of rats with methanol extract of *T. Indica* seed coat (TSCE) at 50 mg/kg (as tannic acid equivalents) followed by CCl_4 treatment, caused restoration of superoxide dismutase, catalase and lipid peroxidation to values close to control while peroxidase was restored to 67 % of the control. Histopathological studies of liver of different groups supported the protective effects of TSCE by restoring the hepatic architecture. These studies could be further extended to exploit its possible application for the preservation of food products as well as a health supplement and nutraceutical.

Keywords Carbon tetrachloride · *Tamarindus indica* · Antioxidant · Polyphenols · Lipid peroxidation · Tannic acid

Introduction

The antioxidant properties of herbs and spices are of particular interest in view of the impact of oxidative modification of low-density lipoprotein cholesterol in the development of atherosclerosis and many other degenerative ailments. As several metabolic and age-related disorders are closely associated with oxidative processes, the use of herbs and spices as a source of antioxidants to combat oxidation warrants further attention (Tapsel et al. 2006).

Tamarind (*T. indica* L.) belongs to sub family Caesalpiniaceae. India produces 300,000 MT *T. Indica* annually. In fruit, the pulp constitutes 30–50 %, the shell and fibre account for 11–30 % and the seed about 25–40 %. *T. indica* seed coat has been shown to possess epidermal wound healing property (Mohd Yusof et al. 2012), ethyl acetate and ethanol extracts of tamarind seed coat exhibit antioxidant activity (Luengthanaphol et al. 2004). Tamarind seeds inhibit snake venom enzymes which cause local tissue damage, inflammation and hypotension (Ushanandini et al. 2006). Polysaccharide from tamarind seeds has immunomodulatory effect (Sreelekha et al. 1993) and were shown to improve dry eye syndrome, to assist release of drug in human body and intraocular penetration of Rofloxacillin (Rolando and Valente 2007, Ghelardi et al. 2004). Water extract of tamarind seed has been shown to reduce blood sugar level in Streptozotocin-induced diabetic male rats (Maiti et al. 2004). Tamarind seeds are known to have high inhibitory activity against human neutrophil elastase (Fook et al. 2005).

T. indica is used as valuable ingredient in medical practice and for culinary purposes, however, not much work has been reported on the evaluation of biological potential, particularly antioxidant and hepatoprotective potential of its seed coat. The objective of the present study was to evaluate antioxidant profile of various extracts of tamarind seed coat (TSCE) by various *in-vitro* methods and to determine the hepatoprotective potential of methanolic TSCE against carbon tetrachloride (CCl_4) induced oxidative damage in animal models.

Materials and methods

Raw material

T. indica L. seeds were collected from a local market in Mysore during October–November and were separated by sieving and were thoroughly cleaned. The seeds were partially ground and the seed coats were separated to get kernel free

P. Sandesh · V. Velu · R. P. Singh (✉)
Human Resource Development Department, CSIR-Central Food
Technological Research Institute, Mysore 570 020, India
e-mail: singhrp317@gmail.com

preparation. It was ground into fine powder (particle size 0.75 mm) and stored at room temperature till further use.

Extraction

50 g seed coat powder was extracted separately with 250 ml MeOH, water and acetone in Soxhlet extractor for 8 h and was filtered through Whatman No. 4 filter paper. The extracts were evaporated under reduced pressure (34–36 kPa) using a rotary vacuum-evaporator at 45 °C and the contents were dried on hot water bath. The extracts were used directly for various analyses.

Determination of total phenolics

The concentration of phenolic compounds in the extracts was determined, as described by Jayaprakasha et al. (2001) and results were expressed as tannic acids equivalents (TAE). The extracts were dissolved in a mixture of methanol and water (6:4 v/v). Samples (0.2 ml) were mixed with 1.0 ml of ten fold diluted Folin–Ciocalteu reagents and 0.8 mL of 7.5 % sodium carbonate solution. After 30 min at room temperature, the absorbance was measured at 765 nm using Spectronic 20 spectrophotometer. The estimation was carried out in triplicate and the results were averaged.

Radical Scavenging Assay (RSA)

Radical scavenging activity of the extracts using 2,2-diphenyl-1-picrylhydrazyl (DPPH) method was determined, as described by Murthy et al. (2002). Different concentrations (25, 50 and 100 ppm) of TSCE and butylated hydroxy anisole (BHA) were taken in 100 µl with MeOH. 5.0 ml of 0.1 mM methanolic solution of DPPH was added and shaken vigorously. The tubes were kept for 20 min in dark. The control was prepared as above without any extract. The absorbance of the samples was measured at 517 nm. RSA was expressed as the inhibition percentage and was calculated using the following formula,

$$\% \text{ RSA} = (\text{Control OD} - \text{sample OD} / \text{Control OD}) \times 100.$$

Lipid peroxidation

TBA reacts with malondialdehyde (MDA) to form pink diadduct chromogen which absorbs at 532 nm (Halliwell and Gutteridge 1989). The perfused liver of normal albino rats of Wister strain was isolated and 10 % (w/v) homogenate was prepared using a Potter Elvehjem homogenizer at low temperature (0–4 °C). The homogenate was centrifuged at 1500g for 15 min and supernatant was used for the studying lipid peroxidation. Extract (25, 50, and 100 ppm, reconstituted in EtOH) were taken in test tubes and dried. One mL of 0.15 M KCl and

0.5 mL of homogenates were added and peroxidation was initiated by adding 100 µL of 0.2 mM FeCl₃. After incubation at 37 °C for 30 min, the reaction was stopped by adding 2 mL of cold HCl (0.25 N) containing 15 % trichloroacetic acid (TCA), 0.38 % TBA, and 0.5 % BHT. The reaction mixtures were heated at 80 °C for 1 h, cooled and centrifuged. The absorbance of the supernatants was read at 532 nm. Identical experiment in the absence of extract was performed to determine the amount of lipid peroxidation in the presence of inducing agents without any extract. The percentage of antilipid peroxidative activity (% ALP) was calculated by the following formula:

$$\text{Antilipid peroxidation}(\% \text{ ALP}) = 1 - (\text{sample OD} / \text{blank OD}) \times 100.$$

The results were expressed as MDA equivalents which were calculated by using an extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$. One unit of lipid peroxidation was defined as the amount of TBA that converts to TBARS. The specific activity was expressed in terms of units per mg of protein.

Ferrous ion chelating activity

The chelation of ferrous ions by TSCE and standards was estimated by the method of Elmastaşa et al. (2006) by measuring the formation of the ferrous ion-ferrozine complex. The reaction mixture containing different concentrations of TSCE in 0.25 ml was mixed with 1.75 ml of methanol, 0.25 ml of 250 mM ferrous chloride and 0.25 ml of 2 mM ferrozine was added to initiate the reaction. The mixture was shaken and left at room temperature for 10 min. The absorbance of the solution was measured at 562 nm. The percentage chelating effect of Ferrozine-Fe²⁺ complex was calculated from the equation

$$\% \text{Inhibition} = [(A_o - A_1) / A_o] \times 100$$

where A_o was the absorbance of the control and A₁ was the absorbance in the presence of TSCE and standards. The control contained FeCl₂ and ferrozine complex molecules.

Deoxyribose degradation assay

The method of Halliwell et al. (1987) was used for non-site specific deoxyribose degradation assay. The extract was mixed with Haber–Weiss reaction buffer (10 mM FeCl₃, 1 mM EDTA, 10 mM H₂O₂, 10 mM deoxyribose and 1 mM ascorbic acid) and the final volume was made to 1.0 ml. The mixture was incubated at 37 °C for 1 h and then heated at 80 °C with 1 ml of thiobarbituric acid (TBA, 0.5 % TBA in 0.025 M NaOH, 0.02 % BHA) and 1 ml of 10 % trichloroacetic acid (TCA) in a boiling water bath for 45 min. After cooling, absorbance of the mixture was measured at 532 nm. The % inhibition was calculated as described in DPPH method.

The site specific assay was done as above, except the exclusion of EDTA from the assay mixture.

Reducing power assay

The reducing power of the test samples was determined, as described by Jayaprakasha et al. (2001). Different concentrations of TSCE in 1 ml MeOH were mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1 % potassium ferricyanide. The mixtures were incubated at 50 °C for 20 min. 2.5 ml of 10 % trichloroacetic acid were added to the mixtures and centrifuged at 5000 rpm for 10 min. The upper layer (2.5 ml) was mixed with 2.5 ml water and 0.5 ml 0.1 % ferric chloride and the absorbance was measured at 700 nm. Increase in absorbance indicated the reducing power of the samples.

In-vivo analysis

Male albino rats of Wistar strain (180–220 g) were used for the studies. The required permission from competent authorities was taken for the use of animals in the experiment. The animals were placed into five groups containing six animals in each. The first group served as control, the second group was administered CCl₄ (negative control), and the third, fourth and fifth group was administered the methanolic TSCE. The extract was suspended in 0.5 % sodium carboxymethylcellulose and was fed to third group rats via oral route at 50 mg (tannic acid equivalents)/kg body weight, at 100 mg/kg body weight (IVth group) and at 200 mg/kg body weight (Vth group) for 14 days. The doses were selected on the basis of the LD₅₀ value of polyphenols (Bombardelli and Morazzoni, 1995). The animals of the first and second groups were simultaneously administered normal saline until 14th day. The animals of the second, third, IVth and Vth groups were given a single oral dose of CCl₄ (1:1 in liquid paraffin) at 1.25 ml/kg of body weight 6 h after the last dose of administration of extract / saline on the 14th day. After 24 h, animals were sacrificed, and the liver was isolated to prepare the homogenate (5 %, w/v with 0.15 M KCl) and centrifuged at 800g for 10 min. The cell-free supernatant was used for the estimation of lipid peroxidation, peroxidase, catalase, and superoxide dismutase (SOD).

Catalase

The catalase assay was carried out as per the method of Aebi (1984). One milliliter of liver homogenate was taken with 1.9 mL of phosphate buffer (50 mM, pH 7.4). The reaction was initiated by the addition of 1 mL of H₂O₂ (30 mM). Blank was prepared without homogenate with 2.9 mL of phosphate buffer and 1 mL of H₂O₂. The decrease in absorbance due to decomposition of H₂O₂ was measured at the end of 1 min against blank at 240 nm. Units of catalase were expressed as the amount

of enzyme that decomposes 1 μ M H₂O₂ per minute at 25 °C. The specific activity was expressed as units per mg of proteins.

Superoxide dismutase (SOD)

The assay of SOD was based on the reduction of nitro blue tetrazolium (NBT) to water insoluble blue formazan (Beauchamp and Fridovich, 1971). In 0.5 mL homogenate, 1 mL of 50 mM sodium carbonate, 0.4 mL of 24 μ M NBT and 0.2 mL of 0.1 mM EDTA were added. The reaction was initiated by adding 0.4 mL of 1 mM hydroxylamine hydrochloride. Zero time absorbance was taken at 560 nm followed by recording the absorbance after 5 min at 25 °C. The control was simultaneously run without homogenate. Units of SOD were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50 %. The specific activity was expressed as units per mg proteins.

Peroxidase

This assay was carried out per the method of Nicholos (1962). In 0.5 mL liver homogenate, 1 mL of 10 mM KI and 1 mL of 40 mM sodium acetate were added. 20 μ L of H₂O₂ (15 mM) was added and the change in the absorbance at 353 nm in 5 min was recorded, which indicates the amount of peroxidase. Units of peroxidase were expressed as the amount of enzyme required to change the OD by 1 unit per minute. The specific activity was expressed as units per mg proteins.

Lipid peroxidation

Lipid peroxidation was carried out as described by Buege and Aust (1978). Liver homogenate (0.5 mL) and 1 mL of 0.15 M KCl were taken and peroxidation was initiated by adding 100 μ L of 0.2 mM ferric chloride. The reaction was run at 37 °C for 30 min and was stopped by adding 2 mL of ice-cold mixture of 0.25 N HCl containing 15 % TCA, 0.3 % TBA and 0.05 % butylated hydroxyl toluene (BHT) and was heated at 80 °C for 60 min. The samples were cooled, centrifuged, and the absorbance of the supernatant was measured. The results were expressed as MDA equivalents in nanomoles per mg protein of homogenate, which were calculated as describe in materials and methods section. The specific activity was expressed as units per milligram of protein.

Determination of proteins

Protein was determined using the method of Lowry et al. (1951).

Statistical analysis

The data were expressed as mean $\pm$ SEM. The data of antioxidant activities were analyzed by one way analysis of variance

(ANOVA) followed by Dunnet's-'t' test. A *p* value less than 0.05 was considered as statistically significant.

Histopathological studies

Histopathological studies of the livers of the different groups of rats were carried out to determine the effect of protection offered by feeding TSCE against the toxic effects of CCl₄.

Results and discussion

The dried *T. indica* seed coat powder was extracted with various solvent of increasing polarity. The yield of methanolic extract was found to be higher (31.37 % w/w) than acetone (20.54 % w/w) or water (15.35 % w/w). The MeOH extract was shown to possess highest polyphenol content (83.01 ± 1.87 µg/mg TAE) than acetone (59.87 ± 1.16 µg/mg TAE) or water (78.43 µg/mgTAE) extracts. This is in accordance with the observations made by Abdille et al. (2005) indicating the superior extractability of methanol over other solvents.

DPPH radical scavenging assay

Figure 1a shows *in-vitro* RSA of various extracts, as measured by DPPH radical scavenging assay, in comparison with BHA. The DPPH scavenging potential for all three extracts was found to increase in a dose dependent manner. The MeOH extract showed higher activity than acetone or water extract which could be compared with BHA at similar concentration.

Lipid peroxidation by thiobarbituric acid (TBA) assay

MDA is the major reactive aldehyde resulting from the peroxidation of biological membrane polyunsaturated fatty acids (PUFA), and is used as an indicator of tissue damage (Pin and Yen 1997). MDA reacts with TBA to form a pink chromogen (TBARS) that is measured spectrophotometrically at 532–535 nm. The oxidation is inhibited by an antioxidant and the absorbance would be reduced (Singh et al. 2002). By virtue of higher yield, polyphenol content and RSA, MeOH extract was used for further studies. Figure 1b depicts the anti-lipid peroxidation activity of TSCE and ascorbic acid. It is obvious that TSCE possess good antilipid peroxidation activity (48.06 % at 100 ppm), however, lower than ascorbic acid at comparable concentration.

Chelating power

The chelation of Fe²⁺ by TSCE was estimated using Ferrozine, and shown in Fig. 1c. In presence of chelating agents, the complex formation is disrupted; hence the color of the complex is decreased. Thus measurement of color

reduction allows estimation of the chelating activity of the coexisting chelator (like TSCE; Yamaguchi et al. 2000). The chelating agents, which form sigma-bonds with a metal, are effective as secondary antioxidants because they can reduce the redox potential, thereby stabilizing the oxidized form of the metal ion (Gordon 1990).

Both TSCE and ascorbic acid interfered with the formation of ferrous-ferrozine complex, suggesting that by virtue of chelating activity, they capture ferrous ions before complex formation. In the absence of chelating agent, iron can stimulate lipid peroxidation by Fenton reaction, and also accelerates peroxidation by decomposing lipid hydroperoxides into peroxy and alkoxy radicals that can themselves abstract hydrogen and perpetuate the chain reaction of lipid peroxidation (Halliwell and Gutteridge 1985).

The formation of Fe²⁺-ferrozine complex is not complete in the presence of TSCE, indicating that extracts of TSCE chelate the iron and the absorbance of Fe²⁺-ferrozine complex was linearly decreased in a dose dependent manner, however, it showed a lower chelation inhibition activity than ascorbic acid at comparative concentrations (23 % and 72 % respectively at 100 ppm).

Deoxyribose degradation assay

Deoxyribose degradation in presence of H₂O₂ and Fe²⁺-EDTA complex is inhibited by an added scavenger of hydroxyl radical to an extent that depends only on the concentration of scavenger related to deoxyribose.. When OH is generated, it escapes scavenging by the EDTA itself, enters free solution and is equally accessible to deoxyribose ribose and to any added scavenger.

Site specific

2-deoxyribose is attacked by hydroxyl radicals to yield a mixture of products which on heating with TBA at low pH, form a pink chromogen that can be measured at 532 nm. If deoxyribose is incubated with H₂O₂ and Fe²⁺ in absence of EDTA, it is still degraded into product that can react to form MDA. TSCE showed concentration dependent deoxyribose degradation inhibitory activity (IA), as shown in Fig. 1d.

Non-site specific

In the non-specific deoxyribose degradation assay (in presence of EDTA), TSCE showed deoxyribose degradation inhibitory activity (IA) with the graded increase in concentration. However, the values for non site specific inhibition are higher than for specific inhibition (27.4 and 19.7 % respectively, at 200 ppm).

Fig. 1 Effect of tamarind seed coat extract (TSCE) on **a** Radical scavenging activity **b** Lipid peroxidation (significant at $p < 0.01$ level) **c** Ferrous chelating ability (significant at $p < 0.01$ level) **d** Deoxyribose degradation assay and **e** Reducing power (significant at $p < 0.01$ level)

Reducing power assay

The reductive ability serves as a good indicator of its potential as an antioxidant. The reductive capabilities of the methanolic TSCE were compared with BHA for the reduction of the Fe^{3+}

to Fe^{2+} ion. The reducing power of TSCE increased with the increasing concentration of the sample (Fig. 1e). For comparison, TSCE raised absorbance to 0.604 at 500 $\mu\text{g}/\text{ml}$, while BHA showed the absorbance of 0.578 at 100 $\mu\text{g}/\text{ml}$. TSCE offered higher absorbance value than control at all the

concentrations tested and these differences were significant. Reducing power is an indicator of electron donating activity, which is an important mechanism for testing antioxidant activity of plant extracts. Good correlation has been established between antioxidant capacity and reducing power (Yildirim et al. 2001, Yen et al. 2000)

In-vivo studies

Three doses i.e., 50, 100, and 200 mg (Tannic acid equivalent)/kg body weight were selected for the studies and the cell-free supernatant of liver (as described in materials and methods section), was used for the estimation of lipid peroxidation, peroxidase, catalase, and superoxide dismutase (SOD) activities.

Enzyme analysis

In-vivo enzyme studies were conducted with three different dose levels of methanolic extract and the results of various parameters are exhibited in Fig. 2a–d.

Superoxide dismutase

Cellular superoxide dismutase (SOD) is represented by a group of metalloenzymes with various prosthetic groups. SOD scavenges both intracellular and extracellular superoxide radical and prevents lipid peroxidation of plasma membrane. Superoxide anion is produced at a relatively high rate by the cell during normal metabolism, its low intercellular level is maintained by either spontaneous dismutation and or catalytic break down by the enzyme SOD.

The levels of SOD were significantly decreased (55 %) in CCl_4 treated group due to generation of peroxy radicals and O_2 (Fig. 2a). The SOD levels became more than twice in all the groups when compared to CCl_4 treated group. At 100 and 200 mg/kg dosage levels, the statistical significance is seen.

Catalase and peroxidase assay

The levels of catalase and peroxidase were significantly decreased (73 and 79 % respectively) in CCl_4 treated group

Fig. 2 Effect of treatment of rats with MeOH extract of Tamarind seed coat followed by feeding CCl_4 on the levels of **a** Superoxide dismutase (SOD) **b** Catalase (significant at $p < 0.01$ level) **c** Peroxidase and

d inhibition of lipid peroxidation, in rat liver (significant at $p < 0.01$ level). Each data bar label represents the mean ± SD of three replicates

Fig. 3 Histopathological slides of liver **a** Group-I, normal liver **b** Group-II, CCl₄ treated liver (arrows indicate zones of fatty degeneration)

when compared to control (Fig. 2b and c). At lower dosage levels, the statistical significance is inconsistent. Free radicals may reduce catalase, SOD, and peroxidase activities in liver, probably, due to enzyme inactivation during the catalytic cycle. TSCE extract which is rich in antioxidant potential and polyphenols, may act as free radical scavenger, reducing the levels of hydrogen peroxide and superoxide anions and, consequently, lipid peroxidation and enzyme inactivation, thus restoring enzyme activity. This may also point towards the possible *de novo* synthesis of these enzymes induced by the components of TSCE (Aruoma 1994). Pretreatment with TSCE was able to protect / recover the enzyme in a dose dependent manner against the toxic effects of CCl₄. The recovery of catalase was almost complete, however, the peroxidase could not be recovered completely in the doses of TSCE studied. At all dosage levels the statistical significance was observed.

Lipid peroxidation

CCl₄- induced lipid peroxidation is highly dependent on its bioactivation to the trichloromethyl radical and trichloromethyl peroxy radical. The results of lipid peroxidation showed that TSCE was effective in preventing the lipid peroxidation, which

may be attributed to better direct free radical scavenging activity of methanolic extract of *Tamarindus indica* L. At 200 mg/kg dosage level (Fig. 2d), the statistical significance is seen.

Histopathological examination of liver

The histopathological examination of the liver included five different groups as described in materials and methods.

Group I & II

The microscopic examination of liver of this group showed a normal portal triad, sinusoids, and cord arrangement of hepatocytes. (Fig. 3a), while Group II showed marked to moderately severe fatty change of liver with presence of large fat vacuoles in the cytoplasm pushing the nuclei at the periphery. At places, many fat vacuoles were seen united and were forming small fat cysts as well. Occasional areas in this group are also showing degeneration and necrosis of hepatocytes (Fig. 3b).

Group III, IV & V

The microscopic examination of liver revealed that TSCE at 50 mg/kg body wt. was effective in providing sufficient

Fig. 4 Histopathological slide of liver of animals pretreated with **a** Group- III, methanolic tamarind seed coat extract (TSCE) at the dose 50 mg/kg body wt. followed by exposure to CCl₄ **b** Group- IV, methanolic TSCE at the dose 100 mg/kg body wt. followed by exposure to CCl₄ **c** Group-V, methanolic TSCE at the dose 50 mg/kg body wt. followed by exposure to CCl₄

protection from fatty change in liver as the sections of liver at this dose showed moderately severe fatty change. (Fig. 4a) while Group IV showed moderate degree of fatty change in the liver; however a fair number of normal hepatocytes are seen intermixed with fat laden hepatocytes. (Fig. 4b). Histopathological examination of liver of Group V showed almost normal appearing hepatocytes and no fatty change in hepatocytes. No inflammation or necrosis was noted, only occasional fine fat vacuoles could be seen in some hepatocytes. This indicates hepatoprotection offered by TSCE at 200 mg/Kg dose. (Fig. 4c).

CCl_4 has been extensively studied as a liver toxicant, and its metabolites are involved in the pathogenesis of liver and kidney. CCl_4 causes changes around the liver central vein and other oxidative damages with the leakage of GOT and GPT in the serum (Lin et al. 1996). Histopathological studies carried out for the liver of control, CCl_4 treated, and methanolic TSCE treated are shown in Figs. 3 and 4. The massive generation of free radical in the CCl_4 -induced liver damage provokes a sharp increase of lipid peroxidation which can propagate intracellularly, thus increasing the interaction of these radicals with phospholipid structures and inducing peroxidation processes, resulting in damage of organ structure. The studies showed that in contrast to control, CCl_4 -treated liver showed total loss of hepatic architecture and areas of hemorrhage and necrosis were seen. However, in case of rats pretreated with methanolic TSCE followed by CCl_4 , the liver was shown to retain normal hepatic architecture with few areas of hemorrhage between the columns of hepatocytes. These results clearly indicate the protection provided by the methanolic TSCE. Lin et al. (1996) indicated that liver protective and antioxidative effects of certain plant extracts against CCl_4 -induced liver injury possibly involve mechanisms related to free radical scavenging effects. Singh et al. (2002) also described the hepatoprotective and antilipid peroxidative effects of ellagic acid against CCl_4 -induced hepatotoxicity in rat liver. The effects of various phenolic compounds such as caffeic acid, chlorogenic acid, cyanarin, and cyanaroside in protecting rat hepatocytes against *tert*-butyl hydroperoxide toxicity have been demonstrated

Conclusion

In the present communication, antioxidant activities of various extracts, in particular, MeOH extract, of *T. indica* seed coat were evaluated by various *in-vitro* and *in vivo* techniques and indicate the presence of compounds responsible for exhibiting these activities. The difference in activities of various extracts may indicate the differential extraction of the compounds in various solvents responsible for these properties. MeOH extract has also been shown to provide hepatoprotective effect against CCl_4 toxicity giving a scope for further studies, which

are needed to be carried out for its possible use as a nutraceutical in food formulation.

Acknowledgments The authors thank Heads, Human Resource Development and Biochemistry and Nutrition Departments and Director, CSIR-CFTRI for their help and encouragement during the course of work and preparation of manuscript.

References

- Abdille MH, Singh RP, Jayaprakasha GK, Jena BS (2005) Antioxidant activity of the extracts from *Dillenia indica* fruits. *Food Chem* 90: 891–896
- Aebi H (1984) Catalase in vitro. *Methods Enzymol* 105:121–125
- Aruoma OI (1994) Nutrition and health aspects of free radicals and antioxidants. *Food Chem* 32:671–683
- Beauchamp C, Fridovich I (1971) Superoxide dismutase: improved assay and an assay applicable to acrylamide gel. *Anal Biochem* 10: 276–287
- Bombardelli E, Morazzoni P (1995) *Vitis Vinifera* L. *Fitoterapia* 65:291–317
- Buege JA, Aust SD (1978) Microsomal lipid peroxidation. *Methods Enzymol* 52:302–310
- Elmastaşa M, Gülçin İ, Işildaka Ö, Küfrevioğlu Öİ, İbaoglu K, Aboul-Enein HY (2006) Radical scavenging activity and antioxidant capacity of bay leaf extracts. *J Iranian Chem Soc* 3(3):258–266
- Fook JMSLL, Macedo LLP, Moura GEDD, Teixeira FM, Oliveira AS, Queiroz AFS, Sales MP (2005) A serine proteinase inhibitor isolated from *Tamarindus indica* seeds and its effects on the release of human neutrophil elastase. *J Life Sci* 76:2881–2891
- Ghelardi E, Tavanti A, Davini P, Celandroni F, Salvetti S, Parisio E, Boldrini E, Senesi S, Campa M (2004) A mucoadhesive polymer extracted from tamarind seed improves the intraocular penetration and efficacy of Rufloxacin in topical treatment of experimental Bacterial Keratitis. *J Antimicrobial Agents Chemotherapy* 48: 3396–3401
- Gordon MH (1990) The mechanism of antioxidant action *in vitro*. In: Hudson BJF (ed) *Food antioxidants*. Elsevier Applied Science, London, pp 1–18
- Halliwell B, Gutteridge JMC (1985) The importance of free radicals and catalytic metal ions in human diseases. *Mol Aspects Med* 8:89–193
- Halliwell B, Gutteridge JMC (1989) *In free radicals in biology and medicine*, 2nd edn. Japan Scientific Societies Press, Tokyo
- Halliwell B, Gutteridge JMC, Aruoma OI (1987) The deoxyribose method: a simple “test-tube” assay for determination of rate constants for reaction of hydroxyl groups. *Anal Biochem* 165:215–219
- Jayaprakasha GK, Singh RP, Sakariah KK (2001) Antioxidant activity of grape seed (*Vitis Vinefera*) extracts on peroxidation models in vitro. *Food Chem* 73:285–290
- Lin CC, Lee HY, Chang CH, Namba T, Hattori M (1996) Evaluation of the liver protective principles from the root of *Cudrania cochinchinensis* var. *gerontogea*. *Phytotherapy Res* 10:13–17
- Lowry OH, Rosebrough NJ, Farr AI, Randall JL (1951) Protein measurement with Folin-phenol reagent. *J Biol Chem* 193:265–275
- Luengthanaphol S, Mongkholkhajornsilp D, Douglas S, Douglas PL, Pengsopa L, Pongamphai S (2004) Extraction of antioxidants from sweet Thai tamarind seed coat: Preliminary experiments. *J Food Engg* 63(3):247–252
- Maiti R, Jane D, Das UK, Ghosh D (2004) Antidiabetic effect of aqueous extract of seed of *Tamarindus indica* L. in Streptozotocin-induced diabetic rats. *J Ethnopharmacol* 92(1):85–91

- Mohd Yusof Bin M, Haris BA, Dinie NB (2012) Tamarind seed extract enhances epidermal wound healing. *Int J Biol* 4(1):81–88
- Murthy KNC, Singh RP, Jayaprakasha GK (2002) Antioxidant activity of grape (*Vitis vinifera*) pomace extract. *J Agri Food Chem* 50: 5909–5914
- Nicholos MA (1962) A spectrophotometric assay for iodide oxidation by thyroid peroxidase. *Anal Biochem* 4:311–345
- Pin DD, Yen GC (1997) Antioxidant activity of three herbal waxes extracts. *Food Chem* 60(4):639–645
- Rolando M, Valente C (2007) Establishing the tolerability & performace of Tamarind Seed Polysaccharide (TSP) in treating dry eye syndrome: Result of Clinical Study, *BMC Ophthalmol*
- Singh RP, Chidamabara Murthy KN, Jayaprakasha GK (2002) Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extracts using in vitro models. *J Agri Food Chem* 50:81–86
- Sreelekha TT, Vijayakumar T, Ankanthil R, Vijayan KK, Nair MK (1993) Immunomodulatory effect of a polysaccharide from *Tamarindus Indica*. *Anticancer Drugs* 4(2):209–212
- Tapsel LC, Hemphill I, Cobiac L (2006) Role of herbs and spices: the past, the present, the future. *Med J Aus* 185(4):4–24
- Ushanandini S, Nagaraju S, Harish Kumar K, Vedavathi M, Machiah DK, Kemparaju K, Vishwanath BS, Gowda TV, Girish KS (2006) The anti-snake venom properties of *Tamarindus indica* (Leguminiceae) seed extract. *Phytotherapy Res* 20(10):851–858
- Yamaguchi F, Ariga T, Yoshimira Y, Nakazawa H (2000) Antioxidant and anti-glycation of garcinol from *Garcinia indica* fruit rind. *J Agri Food Chem* 48:180–185
- Yen GC, Pin DD, Chuang DY (2000) Antioxidant activity of anthraquinones and anthrone. *Food Chem* 70:307–315
- Yildirim A, Mavi A, Kara AA (2001) Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. *J Agri Food Chem* 49(8):4083–4089