ELSEVIER

Journal of Ethnopharmacology 47 (1995) 59–67

Journal of ETHNO-PHARMACOLOGY

The anti-oxidant activity of turmeric (*Curcuma longa*)

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Received 1 February 1992; revision received 2 March 1995; accepted 17 March 1995

Abstract

The turmeric anti-oxidant protein (TAP) had been isolated from the aqueous extract of turmeric. The anti-oxidant principle was found to be a heat stable protein. Trypsin treatment abolished the anti-oxidant activity. The anti-oxidant principle had an absorbance maximum at 280 nm. After gel filtration, the protein showed a 2-fold increase in anti-oxidant activity and showed 2 bands in the SDS-PAGE with approximate molecular weight range of 24 000 Da. The protein showed a concentration-dependent inhibitory effect on the promoter induced lipid peroxidation. A 50% inhibitory activity of lipid peroxidation was observed at a protein concentration of 50 μg/ml. Ca^{2+}-ATPase of rat brain homogenate was protected to nearly 50% of the initial activity from the lipid peroxidant induced inactivation by this protein. This protection of Ca^{2+}-ATPase activity was found to be associated with the prevention of loss of –SH groups.

Keywords: Turmeric antioxidant protein; Lipid peroxidation; Thiols

1. Introduction

One of the processes involved in the adaptation of organisms to live in an aerobic environment has been the development of mechanisms for defence against damage induced by oxygen and activated oxygen species, such as hydroxy radicals and superoxide anions (Halliwell and Gutteridge, 1985). Peroxidation of unsaturated lipids in biologic membranes has been implicated in a wide range of diseases including ageing and cancer (Lunec, 1990), diabetes and cardiovascular diseases (Baynes, 1991) and rheumatoid arthritis (Blake et al., 1989). Current interest has focused on the potential role of anti-oxidants and anti-oxidant enzymes in the treatment and prevention of certain diseases. Dimethyl sulfoxide has been shown to have a beneficial effect on amyloidosis and butylated hydroxy anisole and santoquin have been found to inhibit the carcinogenicity of benzapyrene and 7,12-dimethylbenzanthracene (Wattenberg, 1972).

Turmeric (*Curcuma longa*), one of the major spices, is being consumed in India and other Asian countries. *Curcuma longa* is often quoted in medical literature as possessing potential anti-cancer,

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SSDI 0378-8741(95)01250-H

anti-coagulative and anti-hepatotoxic principles (Yoshinobu, 1983; Ramadasan et al., 1985; Takuo et al., 1988). In addition to its role in dissolving urinary calculus (Leskovan et al., 1983) and the control of diabetes (Janjua, 1988), turmeric at 0.5–1.0% levels in ground nut oils is shown to considerably reduce the formation of peroxides during the accelerated stability tests and addition of turmeric has been shown to increase the shelf-life of oils (Rimpler et al., 1970). The active anti-oxidant principle in *Curcuma longa* has been identified as curcumin (Choiu et al., 1983; Moken et al., 1984). The aqueous and alcoholic extracts isolated from turmeric have been shown to be as effective as butylated hydroxy anisole in their anti-oxidative activity (Shalini et al., 1987; Hirahara, 1975). However, the nature of the principle involving the anti-oxidant property in the aqueous extract remains obscure. This communication presents evidences for a heat stable protein with anti-oxidant properties from turmeric *Curcuma longa* (TAP).

2. Materials and methods

Fresh pure cod liver oil supplied as 'Seven Seas' by Universal Generics Pvt. Ltd., Bombay and a male Wistar rat brain (body weight 120–150 g) 10% homogenate in Tris HCl buffer (0.01 M, pH 7.4) were used as substrates for the study of lipid peroxidation.

2.1. Preparation of turmeric extract

Turmeric tubers obtained from the local market were finely powdered. About 1.5 g of turmeric powder was dissolved in 75 ml of boiling distilled water. The solution was vortexed, centrifuged (1500 rev./min, 10 min, 25°C) and the clear supernatant was collected.

2.2. Isolation of the turmeric anti-oxidant/protein (TAP)

The aqueous turmeric extract obtained was concentrated to a final volume of 10 ml by evaporation on a boiling water bath and was dialyzed overnight against water at 25°C. The insoluble material was centrifuged out. About one fifth of the extract containing approximately 15 mg protein was then loaded on a Sephadex-G-200 column (20 × 1.5 cm) previously equilibrated with Tris-HCl buffer (0.01 M, pH 7.4) and eluted with the same buffer. Three millilitre fractions were col-

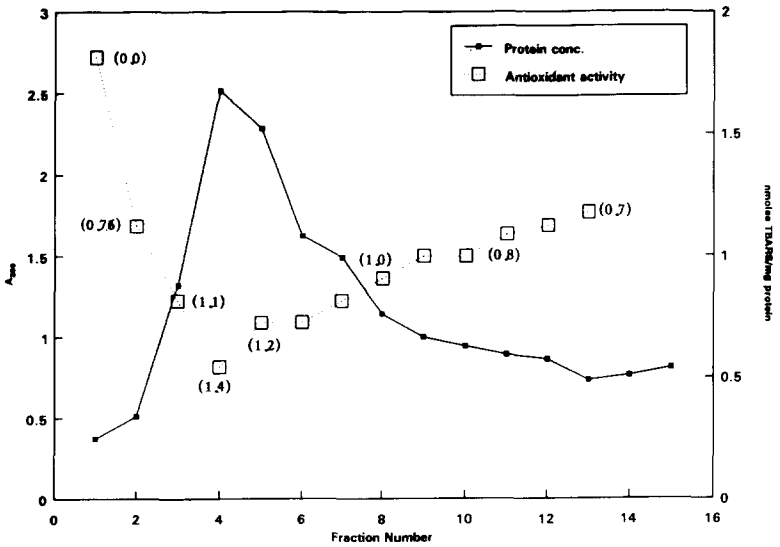

Fig. 1. Elution profile of TAP on Sephadex-G-200 column. Values within parenthesis indicate anti-oxidant activity of TAP/ml eluate on rat brain lipid peroxidation. One anti-oxidant unit = 50% inhibition of brain tissue lipid peroxidation in terms of TBARS released in presence of TAP; control being taken as 100%.

Table 1
Separation of TAP on Sephadex-G-200 column

S. No.	Particulars	Volume (ml)	Activity (anti-oxidant activity in units*/ml)	Total units	Protein (mg/ml)	Specific activity	% Yield
I.	Aqueous extract	75	12.12	909	1.4	8.66	100.00
II.	Concentrate	10	78.80	788	8.75	9.0	86.68
III.	Sephadex-G-200 column effluent (4th and 5th fraction)**	6	6.6	39.8	0.42	16.47	21.99[a]

* One anti-oxidant unit = 50% inhibition of brain tissue lipid peroxidation in presence of TAP; control being taken as 100%.
** Only one fifth of the concentrate was loaded on the column and the protein recovery in the 4th and 5th fractions was 65%.
[a]Computed for the total concentrate.

lected and A_{280} was measured in Kontron-Uvikon-930 spectrophotometer. The protein was also simultaneously assayed by the method of Lowry et al., (1951) which correlated with the A_{280} curve. The elution profile is given in Fig. 1.

Anti-oxidant activity of TAP expressed in terms of anti-oxidant unit (1 anti-oxidant unit = 50% inhibition of brain tissue lipid peroxidation in terms of nmoles of thiobarbituric acid reactive substances (TBARS) released/mg protein in the

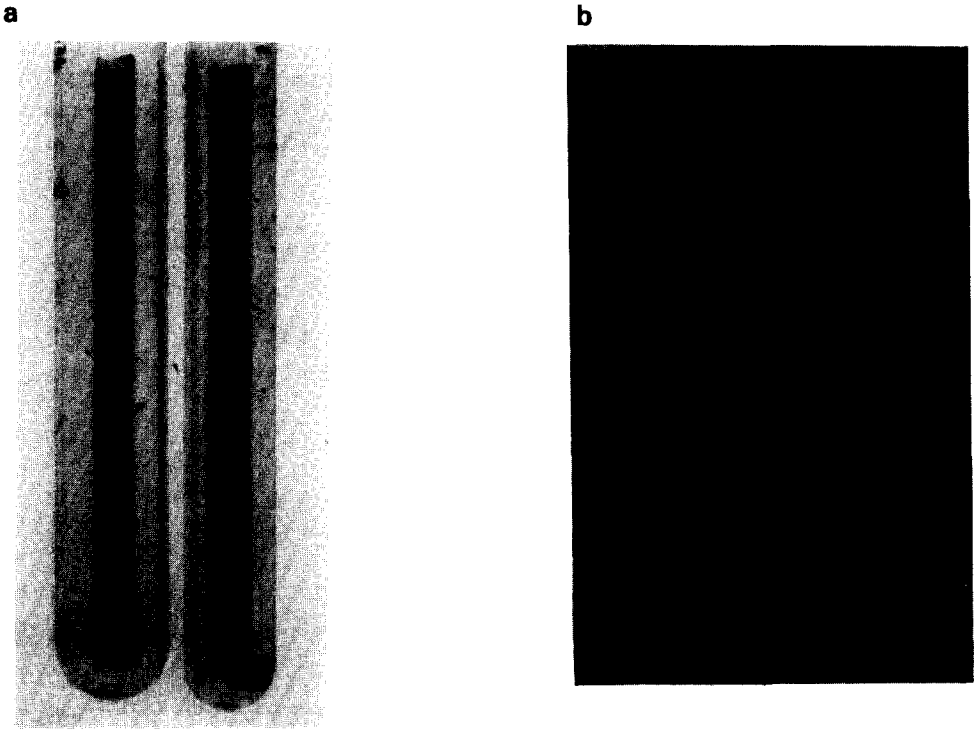

Fig. 2. (a) Native polyacrylamide gel (7.5%) electrophoretic pattern of TAP: A, TAP from column eluate (50 μg protein); B, turmeric extract (100 μg protein). (b) SDS-PAGE pattern of TAP. Twelve percent acrylamide was used. Coomasie Blue R-250 was used for staining. A, molecular weight markers: from bottom to top: 1, α-lactalbumin (14 kDa); 2, trypsin inhibitor (20 kDa); 3, trypsinogen (24 kDa); 4, carbonic anhydrase (29 kDa); 5, bovine serum albumin (66 kDa). B and C, TAP from column eluate, 25 and 50 μg, respectively.

presence of TAP; control being taken as 100%) was determined in each fraction. Peak fractions 4 and 5 containing the maximal protein and maximal anti-oxidant activity were pooled. The yield was 22% with a 2-fold increase in specific activity. The purity of TAP was checked by both native as well as SDS-PAGE, (Laemmli, 1970). The molecular weight of TAP was determined using marker proteins on SDS-PAGE.

2.3. Assay of lipid peroxidation using brain tissue or cod liver oil

Lipid peroxidation in brain tissue was assayed by the method of Brogan et al., (1981) and was expressed in terms of nmoles of thiobarbituric acid reactive substances (TBARS) produced/mg protein. 1,1,3,3-Tetraethoxypropane (malaondialdehyde bis (diethyl acetal)) was used as a standard after acid hydrolysis.

Lipid peroxidation in cod liver oil was assayed according to the method of Luotola et al., (1985) using the thiobarbituric acid reactivity method. Oil was dissolved in chloroform-methanol (1:1 v/v) (50 mg/ml) in vacuum tubes. Five-hundred microlitres of the mixture was taken and the volume made up to 700 μl by the addition of chloroform-methanol. The samples were incubated at 30°C for 30 min.

Samples were also incubated in the presence of Fe^{2+}/ascorbate-Fe^{2+} (1 mM each) with or without TAP. After incubation for 30 min, the solvents were evaporated.

TBA reactivity was measured by treating 500 μl of 2-TBA (2% w/v) and 4 ml of 10% trichloroacetic acid to the tubes and heating at 100°C for 15 min. The tubes were cooled to room temperature and centrifuged (2000 *g*, 10 min). The TBA-chromogens were measured in the supernatant at A_{532} against an appropriate blank.

2.4. Assay of Ca^{2+} ATPase activity

Ca^{2+}-ATPase activity of the rat brain tissue was determined according to the method of Evans (1969) in the presence of Fe^{2+}/ascorbate-Fe^{2+}/TBH (tertiary butyl hydroperoxide) (1 mM each) with or without TAP. The effect of sodium fluoride and diamide (5 mM each) on Ca^{2+}-ATPase activity, both in the presence and absence of TAP (100 μg), and reduced glutathione (40 μg) were determined. The enzyme activity was expressed as μmoles of phosphorous liberated/min per mg protein at 37°C.

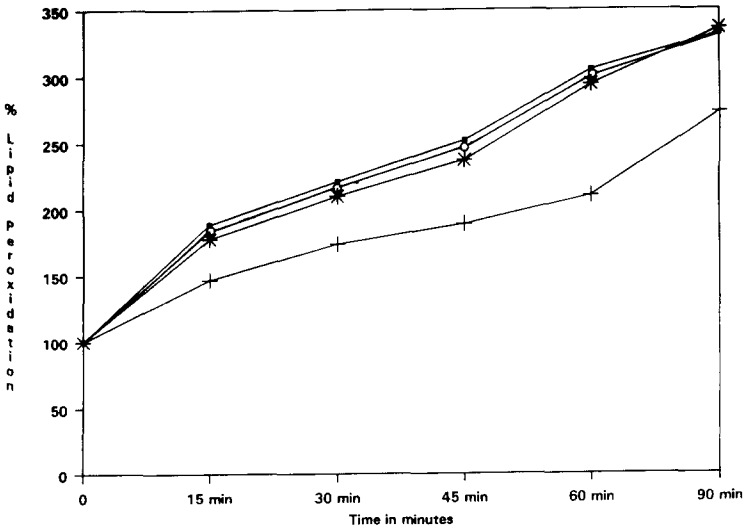

Fig. 3. Effect of trypsin on TAP. The homogenates were pre-incubated with TAP (100 μg) or TAP (100 μg) + trypsin (1 mg) for the requisite time after which lipid peroxidation was assayed as given in Materials and Methods. (■) None; (+) TAP (100 μg); (*) TAP (100 μg) + trypsin (1 mg); (○) trypsin (1 mg).

2.5. Determination of total sulphydryl content

The total sulphydryl content of the brain tissue in the presence of Fe^{2+}/ascorbate-Fe^{2+}/TBH with or without TAP (50 μg) was estimated by the method of Sedlack and Lindsay (1968) using reduced glutathione as the standard.

Protein was estimated by the method of Lowry et al. (1951) using bovine serum albumin as standard.

3. Results

The anti-oxidant principle was found to be heat stable and no loss of activity was seen during extraction. The TAP was found to have a specific activity (specific anti-oxidant activity) of 8.66 units/mg protein and the activity was further increased by 2-fold after column chromatography (Table 1).

Native and SDS-PAGE of the TAP after gel filtration showed at least 2 protein bands (Figs. 2a,b). Further purification was not attempted.

The absorption maxima of TAP was found to be at 280 nm. The approximate molecular weight of the proteins were in the range of 24 000 Da. Treatment of TAP with trypsin (10 mg/ml) abolished the anti-oxidant activity (Fig. 3) suggesting that the anti-oxidant principle was a protein. All further studies were carried out using the TAP obtained from column chromatography.

3.1. Inhibition of lipid peroxidation by TAP

A 50% inhibitory activity of lipid peroxidation was observed at a protein concentration of 50 μg/ml in both the rat brain and cod liver oil systems studied. A 70% protection from peroxidation in both systems was noticed at the highest concentration of protein (300 μg/ml). The TAP had a concentration-dependent inhibitory effect on the Fe^{2+}/ascorbate-Fe^{2+}/TBH-induced lipid peroxidation. A 50% inhibitory effect was brought about at 100 μg concentration of protein in the ascorbate-Fe^{2+}/TBH-induced systems in rat brain.

Peroxidation studies using oil as the substrate are given in Table 2. The TBARS released was 10.6 nmoles/50 mg oil, in 30 min and in the presence of 40 μg of TAP, this release was reduced to 6.38 nmoles/50 mg oil, showing about 40% inhibition. In the presence of Fe^{2+}/ascorbate-Fe^{2+}/TBH-induced systems, the same amount of protein inhibited TBARS released by 26%, 27% and 44%, respectively.

3.2. Influence of TAP on Ca^{2+}-ATPase activity in rat brain homogenate in relation to lipid peroxidation

Ca^{2+}-ATPase is known to be inhibited by lipid peroxidation reactions. Hence, studies were undertaken to see whether TAP could protect Ca^{2+}-ATPase activity.

Fig. 4 shows the relationship between the lipid peroxide formation and inactivation of Ca^{2+}-ATPase of brain homogenate. When the homogenate was subjected to lipid peroxidation, the release of TBARS was increased with the time of incubation. A 5.5-fold increase in TBARS was noted in 30 min. However, in the presence of TAP the release of TBARS was curtailed by 50%. In contrast, Ca^{2+}-ATPase activity was found to be decreased by 40% with increase in TBARS release. It is interesting to note that TAP protected the enzyme from inactivation. Similarly, in the presence of 100

Table 2
Effect of TAP on non-enzymatic lipid peroxidation of cod liver oil

Particulars	Without TAP	With TAP (40 mg)	% inhibition
Oil	10.63 ± 1.76	6.38 ± 0.92	40.00
Oil + 1 mM Fe^{2+}	21.25 ± 2.45	15.75 ± 1.37	25.88
Oil + 1 mM Ascorbate — Fe^{2+}	15.00 ± 2.04	10.88 ± 1.02	27.50
Oil + 1 mM TBH	34.95 ± 3.42	19.47 ± 2.14	44.29

Values are mean ± S.D. of 6 determinations and are expressed as nmoles TBARS released/50 mg oil in 30 min.

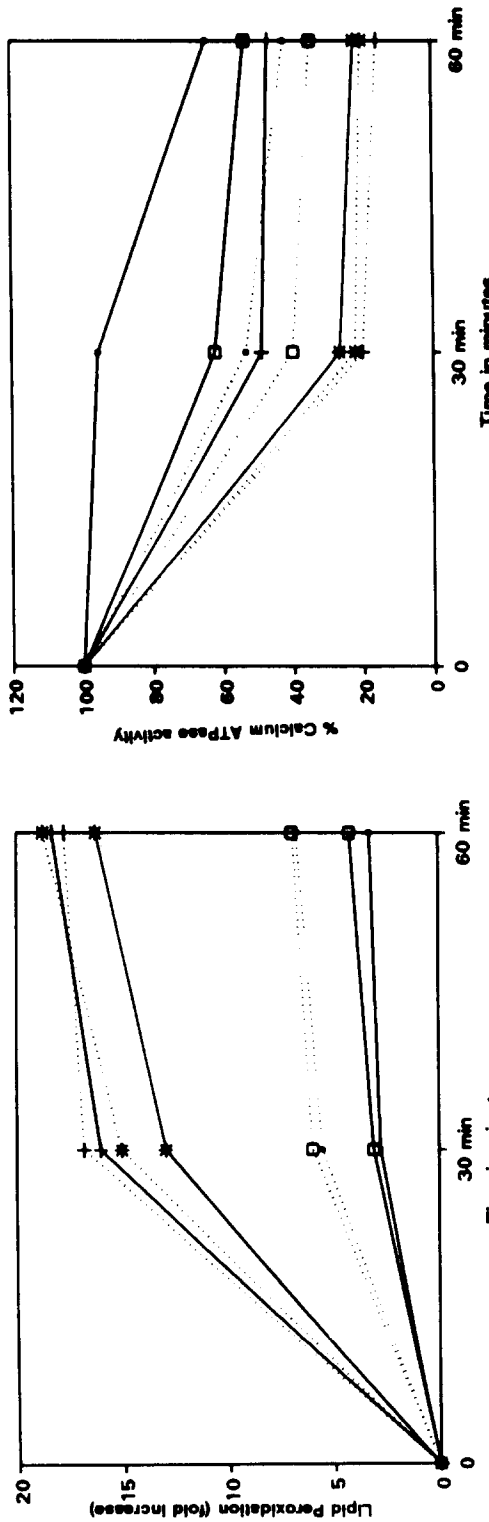

Fig. 4. Influence of lipid peroxidation on Ca^{2+}-ATPase activity and effect of TAP. The brain homogenates were pre-incubated with TAP for 10 min after which Fe^{2+}/ascorbate — Fe^{2+}/TBH (1 mM each) were added and incubated with vigorous shaking for requisite time (■) basal; (+) 1 mM Fe^{2+}; (✱) 1 mM ascorbate-Fe^{2+}; (□) 1 mM TBH. (...) without TAP; (—) with TAP (100 μg).

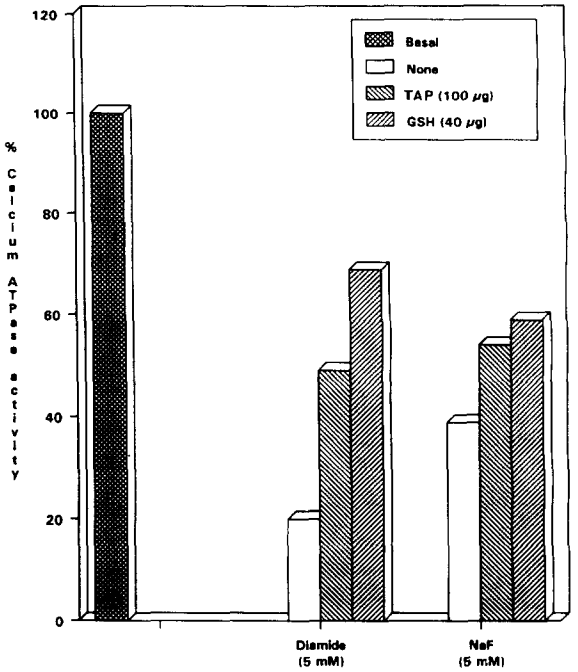

Fig. 5. Effect of TAP and GSH on Ca^{2+} — ATPase activity in the presence of thiol oxidizing agents. The brain homogenates were pre-incubated with TAP/GSH for 10 min and then 5 mM diamide/sodium fluoride (NaF) were added and incubated for 30 min.

μg of TAP both the lipid peroxidation as well as the inhibition of enzyme activity were significantly reduced even in the presence of promoters of lipid peroxidation. Nearly 50% of the enzyme activity remained in the presence of TAP.

Sodium fluoride and diamide which are known thiol reacting agents inhibited Ca^{2+}-ATPase activity (Fig. 5) by 60% and 80%, respectively when compared to the initial activity (100%). But, in presence of TAP or a known thiol reducing agent, reduced glutathione, 50% activity was retained showing that TAP could protect –SH groups of the enzyme and thereby protect the activity.

3.3. Total sulphydryl content changes with lipid peroxidation — effect of TAP

Further studies were undertaken to determine the effect of TAP on protecting the total sulphydryl content during lipid peroxidation. The total –SH content of brain homogenate decreased by 10% and 45% at 30 and 60 min, respectively (Fig. 6). In the presence of TAP only 38% depletion was seen even after 60 min. In the presence of promoters of lipid peroxidation, TAP protected

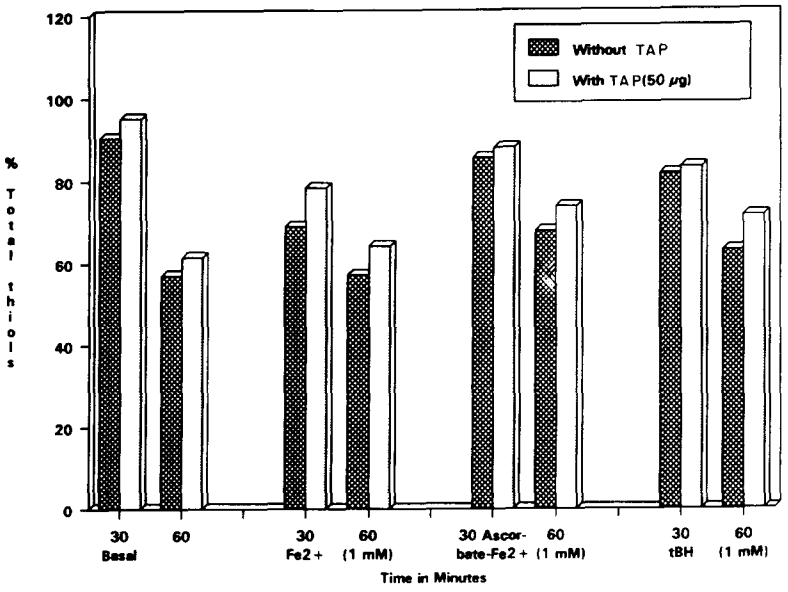

Fig. 6. Effect of TAP on the total thiols of rat brain homogenate. The brain homogenates were pre-incubated with TAP (50 μg) for 10 min after which 1 mM Fe^{2+}/ascorbate-Fe^{2+}/TBH were added and incubated with vigorous shaking.

the total –SH content from depletion by 10% when compared to that in its absence.

4. Discussion and conclusions

An anti-oxidative principle from the aqueous extract of turmeric has been isolated and has been found to be heat stable. It is purified 2-fold when subjected to Sephadex-G-200 column chromatography. The anti-oxidant principle is protein in nature by its maximal absorbance at 280 nm and loss of its anti-oxidant activity on trypsin treatment. PAGE of TAP has shown 2 protein bands, with approximate molecular weight range of 24 000 Da. The partially purified protein is found to be effective in inhibiting the formation of lipid peroxides, even in the presence of promoters of lipid peroxidation. The protein at 50–100 μg concentration is found to produce a 50% inhibition of lipid peroxidation.

TAP effectively prevents Ca^{2+}-ATPase from inactivation, in the presence of promoters of lipid peroxidation or thiol reagents. In addition it prevents the depletion of cellular –SH content during peroxidation. This suggests that the anti-oxidant activity may be mediated through the protection of the –SH group of the enzyme. Similar observation of impairment of the function of the sarcoplasmic reticulum by oxidation of sulphydryl groups of Ca^{2+}-ATPase by oxidative stress and partial restoration of the activity in presence of reduced thiol reagents has been reported by Scherer and Deamer (1986). Rafatullah et al. (1990) have reported anti-ulcer activity of crude turmeric extract by the restoration of the non-protein sulphydryl content of the glandular stomachs of rats.

Dietary anti-oxidants like levamisole, butylated hydroxy toluene, 2-mercaptoethanol, α-tocopherol acetate and santoquin are shown to enhance cellular and humoral immunity with age (Harman et al., 1977). Butylated hydroxy anisole is used in the preservation of food products. However, a high concentration of butylated hydroxy anisole is found to be toxic (Watts, 1985; Ito et al., 1985). It is very interesting to note that consumption of high concentrations of turmeric has been reported to be non-toxic (Vijayalakshmi and Chandrasekara, 1982). Therefore, the presence of a heat stable anti-oxidant protein in the aqueous extract of turmeric is highly significant in relevance to its dietary consumption in Asia. This study also opens newer vistas on the potential efficacy of the turmeric anti-oxidant protein in protecting tissues from peroxidative damage.

Acknowledgement

One author, Lalitha Subramanian is extremely grateful to Rameshwardas Birla Smarak Kosh Hospital Trust, Bombay, India, for providing financial assistance.

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