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Protective Effect of *Cinnamomum tejpata* on Lipid Peroxide Formation in Isolated Rat Liver Homogenate

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Abstract: Restrictions on the use of synthetic antioxidants are being imposed because of their toxic properties. The present study is the continuation of a program aimed at investigation on antioxidant activity of extracts from medicinal plants and to identify alternative natural and safe sources of food antioxidant especially from plant origin. Free radicals are involved in more than 50 diseases. Day to day researchers are adding more number of diseases to the list. In this report the anti-peroxidative effect of alcoholic extract of *Cinnamomum tejpata* has been studied in rat liver homogenate. Ferrous sulphate has been used as inducer to induce lipid per oxidation. On the basis of results, it could be concluded that TBARS production in normal condition group is very slow and in $FeSO_4$ treated groups, it is very high. Initiation of lipid peroxidation by ferrous sulphate takes place either through ferryl- perferryl complex or through OH Radical from Fenton's reaction. Effect of different concentrations of ferrous sulphate for induction of lipid peroxidation in rat liver homogenate was studied. Dose dependent increase in lipid peroxidation was found. Results revealed that at lower doses the rate of formation of TBARS is slow, which increased with dose. Significant and moderate results were found from 0.40 mM to 0.80 mM of ferrous sulphate.

Key words: Anti-peroxidative effect, cinnamomum tejpata, ferryl- perferryl complex, lipid peroxidation, TBARS production

INTRODUCTION

Free radicals are capable of inducing lipid peroxidation in biological membranes. The effects of free radicals on human beings have recently been considered as their close toxicity, diseases and ageing (Harman, 1981). Recently there has been an upsurge of interest in the therapeutic potential of medicinal plants as antioxidants in reducing such free radical-induced tissue injury (Pourmorad *et al*., 2006). Besides, well known and traditionally used natural antioxidants from teas, wines, fruits, vegetables and spices, some natural antioxidants (e.g., rosemary and sage) are already exploited commercially either as antioxidant additives or as nutritional supplements (Schuler, 1990). Reactive oxygen species can easily initiate the lipids causing damage of the cell membrane constituent i.e., phospholipids, lipoproteins by propagating a reaction cycle (Raja Sudararajan *et al*., 2006). It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds (Duh *et al*., 1999). Flavonoids are a group of polyphenolic compounds with known properties, which include free radical scavenging, inhibition of hydrolytic oxidative enzymes and anti-inflammatory action (Frankel, 1995).

The aim of this study is to examine the optimum dose and time of the protective effect of the alcoholic extract of *Cinnamomum tejpata*.

Malabathrum, also known as Malobathrum or Malabar leaf is the name used in classical and medieval texts for the leaf of the plant *Cinnamomum tejpata*. In ancient Greece and Rome, the leaves were used to prepare fragrant oil, called Oleum Malabathri, and were therefore valuable. The leaves are mentioned in the 1st century Greek text Periplus Maris Erytraei as one of the major exports of the Tamil kingdoms of southern India. The name is also used in mediaeval texts to describe the dried leaves of a number of trees of the genus Cinnamomum, which were thought to have medicinal properties.

The leaves, known as tejpat, tej pat, tejpatta, or tejpata or in Hindi and tamalpatra in Marathi, are used extensively in the cuisines of India (particularly in the Moghul cuisine of North India). They are often erroneously labeled as "Indian bay leaves," though the bay leaf is from the Bay Laurel, a tree of Mediterranean origin in a different genus, and the appearance and aroma of the two are quite different.

Devi *et al*., (2007) investigated the perturbation of oxidant-antioxidant balance in brain synaptosomes of diabetic rats and determined the antioxidant and free radical-scavenging property of the Indian bay leaf. Brain synaptosomes were isolated from control and streptozotocin-induced diabetic animals and oxidative stress parameters were assayed. A methanolic extract of

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bay leaf (BLE) was tested for the polyphenolic content and antioxidant activity by *in vitro* assays. A significant increase in the levels of lipids and lipid peroxidation products and a decline in antioxidant potential were observed in diabetic rat brain synaptosomes. The total polyphenolic content of BLE was found to be 6.7 mg gallic acid equivalents (GAE)/100g. BLE displayed scavenging activity against superoxide and hydroxyl radicals in a concentration-dependent manner. Further, BLE showed inhibition of Fe (2+)-ascorbate induced lipid peroxidation in both control and diabetic rat brain synaptosomes. Maximum inhibition of lipid peroxidation, radical scavenging action and reducing power of BLE were observed at a concentration of 220 microg GAE. These effects of BLE *in vitro* were comparable with that of Butylated Hydroxyl Toluene (BHT), a synthetic antioxidant. It can be concluded that synaptosomes from diabetic rats are susceptible to oxidative damage and the positive effects of bay leaf *in vitro*, could be attributed to the presence of antioxidant phytochemicals.

MATERIALS AND METHODS

The given study was performed in the year 2007-09 at Indraprastha Engineering College, Ghaziabad, INDIA.

Preparation of alcoholic extract: One kg of *Cinnamomum tejpata* was dried, powdered and the material was extracted with ethanol by cold percolation (material was dipped into ethanol for 7 days and ethanol was collected. The extract was freed from solvent under reduced pressure to give a red brown, highly viscous syrup. The yield was 21.4%. The ethanolic extract of *Cinnamomum tejpata* was tested for its anti peroxidative property in animal system. This extract was further fractioned by chromatography on silica gel column (80-120 mesh).

Preparation of tissue homogenate: Rats were fixed on the operation table with ventral side up and then dissected. Liver was perfused with normal saline through hepatic portal vein. Liver was harvested and its lobes were briefly dried between filter papers (to remove excess of blood) and were cut thin with a heavy-duty blade. These small pieces were then transferred to the glass Teflon homogenizing tube to prepare homogenate (1 g, w/v) in phosphate buffer saline (pH 7.4) in cold condition. It was centrifuged at 2000g, for 10 min. Supernatant was collected and finally suspended in PBS to contain approximately 0.8-1.5 mg protein in 0.1 ml of suspension to perform the in vitro experiment.

Estimation of lipid peroxidation in terms of TBA-RS:
Principle: This method is based on determination of TBA-RS, the end product of lipid peroxidation, which can react with thiobarbituric acid to yield a pink coloured trymethionine complex exhibiting an adsorption maximum at 530-535 nm.

Procedure: 0.1 mL of reaction mixture (5% homogenate with or without toxin treated/drug treated) was transferred to a tube containing 1.5 mL of 10% trichloroacetic acid (TCA). After 10 min tubes were centrifuged and TCA soluble fraction was fully separated to develop the colour reaction.Now the tube containing TCA soluble fraction was added to 1.5 ml thiobarbituric acid (TBA) in 50% acetic acid and mixed well. It was heated in boiling water bath for 30 min, to complete the reaction. The tubes were cooled to determine the absorbance at 535 nm. The values were evaluated on the standard curve using 1,1,3,3, tetra ethoxy propane (TEP).

Statistical evaluation: The results, given here are the mean±SD of six separate experiments. Level of significance has been evaluated by using Student's t test.

RESULTS

Effect of different concentrations of ferrous sulphate for induction of lipid peroxidation in rat liver homogenate: This experiment was aimed to determine the optimum dose of ferrous sulphate for induction of lipid peroxidation in our experimental conditions. 3 mL of rat liver homogenate (5% in phosphate buffer saline, pH 7.4) was taken to each 35 mm petridish. To these plates, different concentrations of ferrous sulphate were added as given in Table 1. Plates were mixed gently and incubated for 30 min. At the end of incubation time, 0.1 mL of aliquots was taken out from each plate to estimate TBARS, produced. Results were compared with the normal control value, obtained under similar conditions.

Results-Dose dependent increase in lipid peroxidation has been seen (Table 1). Results shows that at lower doses the rate of formation of TBARS is slow, which increases with dose. Significant and moderate results have been found from 0.40 to 0.80 mM of ferrous sulphate.

Effect of incubation period on ferrous sulphate induced lipid peroxidation in Rat liver homogenate: This experiment was aimed to determine the effect of the

Table 1: Effect of different concentration of ferrous sulphate for induction of lipid peroxidation in rat liver homogenate

S.No.	$FeSO_4$ (mM)	TBA-RS (n mole/100 mg protein)
1	0.00	72.97±10.17
2	0.10	123.60±12.68
3	0.20	220.82±13.38
4	0.30	310.40±10.18
5	0.40	405.69±20.28
6	0.60	430.52±14.09
7	0.80	575.23±16.42
8	1.25	660.26±18.37

Values are mean±SD of six different experiments

Table 2: Effect of Incubation period of ferrous sulphate for induction of lipid peroxidation in rat liver homogenate

		TBA-RS (n mole/100 mg protein)			
		Time (min)			
Sr.No.	$FeSO_4$ (mM)	15 (A)	30 (B)	45 ©)	60 (D)
1	0.00	69.62 ±10.13	72.97 ±16.13	77.29 ±23.13	81.09 ±10.12
2	0.10	150.60 ±14.63	123.24 ±12.18	325.50 ±16.64	340.24 ±16.24
3	0.20	190.07 ±19.51	220.62 ±13.28	330.24 ±8.74	440.64 ±11.84
4	0.30	213.74 ±18.74	310.78 ±10.13	380.44 ±9.79	450.24 ±10.18
5	0.40	240.24 ±24.20	405.62 ±12.28	479.58 ±12.13	570.50 ±10.11
6	0.60	280.34 ±14.13	430.52 ±15.90	609.62 ±10.09	716.08 ±9.13
7	0.80	325.93 ±16.28	575.20 ±8.13	610.24 ±22.13	650.74 ±19.45
8	1.25	421.24 ±18.14	660.26 ±9.14	750.05 ±20.13	839.15 ±13.14

incubation time on ferrous sulphate induced lipid peroxidation in Rat liver homogenate.

For this experiment, 8 petridishes containing 3 mL of liver homogenate (5% in phosphate buffer saline, pH 7.4) were taken. Different concentrations of ferrous sulphate ranging from 0.10 to 1.25 Mm were taken in dishes no.2 to 8 as per protocol. Dish no.1 was taken as control which is devoid of ferrous sulphate. All the plates were mixed gently and at the time of 15,30,45 and 60 min.0.1 mL of aliquots were withdrawn from each dish in a tube containing 1.5 mL of 10% TCA separately to stop reaction. Lipid peroxidation was measured in terms of TBARS by noting down the absorbance at 535 nm.

Table 2 indicates that in control conditions, 69.63 nmoles of TBARS is formed at the time of 15 min, which increases to 81.09 nmoles at 60 min. In ferrous sulphate treated dishes formation of TBARS increases 2 to 3 folds over the control value at the same time points.The optimum point was selected as 30 min.

Effect of *Cinnamomum tejpata* on ferrous sulphate induced lipid peroxidation in RAT liver homogenate: This experiment was aimed to determine the optimum dose and time of the protective effect of the alcoholic extract of Cinnamomum tejpata.

Experiments were conducted in 3 different groups. 3 ml of homogenate (5% in phosphate buffer saline, pH 7.4) was used. In the first group, drug vehicle (tween 80: water, 1:9) was added in different concentrations and value of TBARS was estimated for varied doses ranging from 0 to 500 µL. In the second group, only alcoholic extract of *Cinnamomum tejpata* suspended in the drug vehicle, was added and TBARS was monitored for various doses ranging from) 0 to 666 µg/mL. In the third group, various doses of *Cinnamomum tejpata* and ferrous sulphate (0.5 mM) were added. At the end of experiments 0.1 mL aliquots were withdrawn to estimate TBARS.

Table 3 indicates that only ferrous sulphate treated group, produced 405.69 unit of TBARS, which gradually decreases from 400.09 to 250.68 n mole/ 100 mg protein in the dose dependent manner in the presence of *Cinnamomum tejpata* The TBARS values were similar to the control, in case of drug vehicle, *Cinnamomum tejpata* and treated plates. This indicates the non–toxic effect of drug and the vehicle on the above-mentioned concentrations.

DISCUSSION

Peroxidation of lipid is a natural phenomena and occurs on its exposure to oxygen. Recently, free radical induced lipid peroxidation has gained much importance because of its involvement in several pathologies such as ageing, wound healing, oxygentoxicity, liverdisorders, inflammation etc. Many plants are known to have beneficial therapeutic effects as noted in the traditional Indian system of medicine, ayurveda. Many natural and synthetic antioxidants are in use to prevent lipid peroxidation.In this report, the alcoholic extract of *Cinnamomum tejpata* has been investigated for its protective response. Antilipid peroxidative property of *Cinnamomum tejpata* might be either due to chelating or redox activity. The specific ratio of ferrous to ferric is important for induction of lipid peroxidation.It has been reported that at least 1:1 ratio of ferrous to ferric is critical for initiation of lipid peroxidation.Therefore, antioxidant activity of *Cinnamomum tejpata* may result from multiple factors involving hydrogen or electron transfer, metal chelating activity and synergistic activity and appear to be

Table 3: Effects of alcoholic extract of *Cinnamomum tejpata* on ferrous sulphate induced lipid peroxidation in rat liver homogenate (A dose response study)

				TBA-RS (n mole / 100 mg protein)	
S.No.	Drug vector (µL)	TBA - RS (n mole/ 100 mg protein) (A)	C. tejpata (mg/ml)	(B)	$FeSO_4$ (C)
1.	0	72.48±8.21	00	75.63 ± 6.21	405.69 ± 20.18
2.	50	79.54±6.43	0.33	81.01 ± 10.2	400.09 ± 18.11
3.	100	81.48±10.11	0.66	85.00 ± 8.25	382.12 ± 20.48
4.	200	89.01±7.21	1.20	92.54 ± 9.34	370.64 ± 10.76
5.	500	79.61±8.77	2.40	80.48 ± 7.49	323.46 ± 10.05
6.	-	-	4.80	79.25 ± 7.49	280.49 ± 12.57
7.	-	-	5.60	81.63 ± 9.70	250.68 ± 10.28

Values are mean±SD of six different experiments, Statistical comparison by student‘t’ test shows that values are highly significant

the result of many different activities. Considering the activities of free radicals and concentrations of substrates, the phenolic compounds from natural sources are promising candidates for drugs for atherosclerosis, depending on their reactivity towards free radicals, localization, mobility in lipoprotein and fate of its radicals.

CONCLUSION

This study supports the hypothesis that the inclusion of cinnamon compounds in the diet could reduce risk factors associated with production of free radicals and can act as a good antioxidant.

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