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PROTECTIVE EFFECT OF *MURRAYA KOENIGII* 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. In this report the anti-peroxidative effect of alcoholic extract of *Murraya koenigii* 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. 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.

KEYWORDS

Murraya koenigii, anti-peroxidative effect, lipid peroxidation, ferryl- perferryl complex, TBARS production.

INTRODUCTION

Free radicals are capable of inducing lipid peroxidation in biological membranes[1]. Lipid peroxidation induced damage is involved in ageing pathological disorders, atherosclerosis, neuronal ceroid lipofuscinosis, intermittent claudication, oxygen toxicity and liver injury caused by oratic acid and ethanol[2-9].The effects of free radicals on human beings have recently been considered as their close toxicity, diseases and ageing[10-11]. 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[12]. 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[13]. Active oxygen species can easily initiate the lipids causing damage of the cell membrane constituents i.e. phospholipids, lipoproteins by propagating a reaction cycle[14]. It has been mentioned that antioxidant activity of plants might be due to their phenolic compounds[15]. Flavonoids are a group of polyphenolic compounds

with known properties which include free radical scavenging, inhibition of hydrolytic oxidative enzymes and anti-inflammatory action[16].

Murraya koenigii, belonging to the family Rutaceae, commonly known as curry-leaf tree, is a native of India, Srilanka and other south Asian countries. Leaves are rich in minerals, vitamin A, vitamin B, and are a rich source of carbohydrates, proteins, amino acids and alkaloids[17-18].The plant has also been used in traditional Indian medicine systems for a variety of ailments[19-20].

It was found that reduction in total serum cholesterol and an increase in the HDL and lower release of lipoproteins into the circulation take place when rats were fed with a standard diet along with curry leaves[21]. Curry leaves also exhibited strong antioxidant property on liver and heart. It was found that phenolic antioxidant is present in *Murraya koenigii* and other herbs[22]. Hypoglycemic activity of *Murraya koenigii* on normal and diabetic rats was found[23]. The beneficial effects of *Murraya koenigii* leaves on antioxidant defense system and ultra structural changes of pancreatic beta cells in experimental diabetes in rats was studied[24].

MATERIAL AND METHODS

(i) Preparation of Alcoholic Extract

One kg of *Murraya koenigii* was dried, powdered and the material was extracted with ethanol by cold percolation method (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 *Murraya koenigii* was tested for its anti peroxidative property in animal system. This extract was further fractioned by chromatography on silica gel column (80 – 120 mesh).

(ii) 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 gm, w/v) in phosphate buffer saline (pH 7.4) in cold condition. It was centrifuged at 2000g, for ten minutes. 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.

(iii) 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 absorption 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 minutes tubes were centrifuged and TCA soluble fraction was fully separated to develop the colour reaction as described by Yagi et. al.,(1987) with slight modification. 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

1.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 minutes. 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.

Result

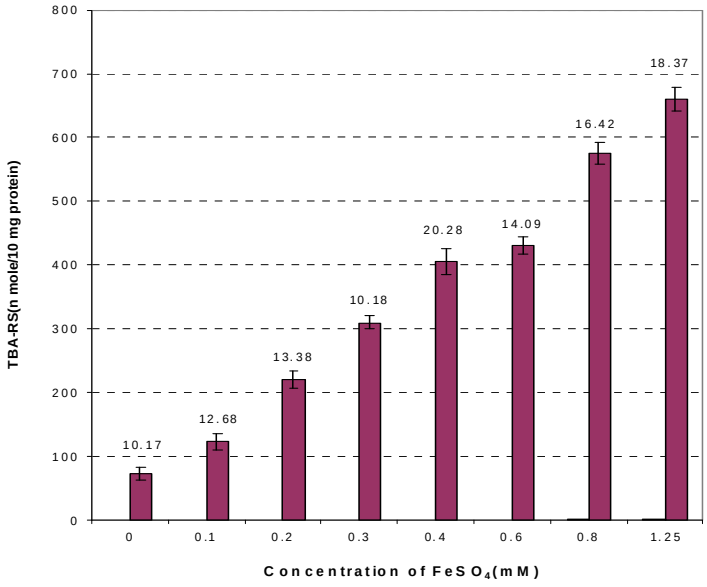
Dose dependent increase in lipid peroxidation has been seen (Table 1). Results shows that at lower doses the rate of formation of TBARS is slow, which increases with dose. Significant and moderate results have been found from 0.40 mM to 0.80 mM of ferrous sulphate

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/ 100mg 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.



Graph 1. Effect of different concentration of ferrous sulphate for induction of lipid peroxidation in rat liver homogenate.

2. Effect of *Murraya koenigii* on ferrous sulphate induced lipid peroxidation in rat liver homogenate

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 µl to 500 µl. In the second group, only alcoholic extract of suspended in the *Murraya koenigii* drug vehicle, was added and TBARS was monitored for various doses ranging from 0 µg/ml to 1000 µg/ml. In the third group, various doses of *Murraya koenigii* and ferrous sulphate (0.5 mM) were added. At the end of experiments, 0.1 ml aliquots were withdrawn to estimate TBARS.

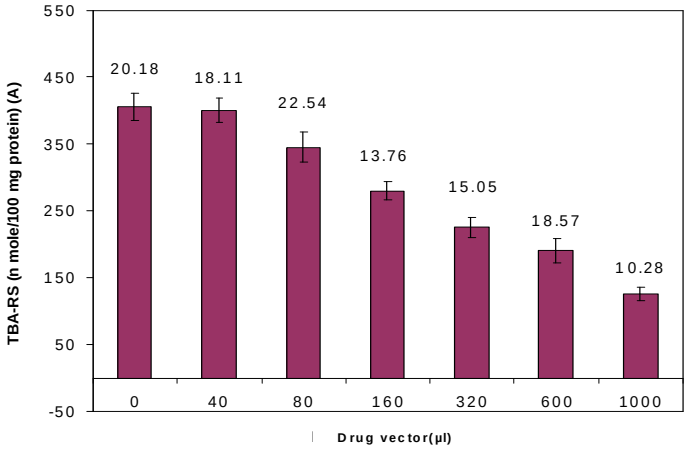
Result-Only ferrous sulphate treated group, produced 405.69 unit of TBARS, which gradually decreases from 400.09 to 125.66 n mole/ 100 mg protein in the dose dependent manner in the presence of *Murraya koenigii* (Table II). The TBARS values were similar to the control, in case of drug vehicle, *Murraya koenigii* and treated plates. This indicates the non–toxic effect of drug and the vehicle on the above-mentioned concentrations.

Table 2

Effects of alcoholic extract of *Murraya koenigii* on ferrous sulphate induced lipid peroxidation in rat liver homogenate (A dose response study)

S. No.	Drug vector (µl)	TBA –RS (n mole/100 mg protein) (A)	*M.koenigii* (µg/ml)	TBA –RS (n mole / 100 mg protein)	
				(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	40	81.01 ±10.2	400.09 ±18.11
3.	100	81.48 ± 10.11	80	85.00 ±8.25	345.12 ± 22.54
4.	200	89.01 ± 7.21	160	92.54 ± 9.34	280.65 ±13.76
5.	500	79.61 ± 8.77	320	80.48 ± 7.49	225.46 ±15.05
6.	-	-	600	79.25 ± 7.49	190.49 ±18.57
7.	-	-	1000	81.63 ± 9.70	125.66 ± 10.28

Values are mean ± SD of six different experiments.
Statistical comparison by student's t test shows that values are highly significant



Graph 2 .Effects of alcoholic extract of *Murraya koenigii* on ferrous sulphate induced lipid peroxidation in rat liver homogenate (A dose response study)

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, oxygen toxicity, liver disorders, 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 *Murraya koenijii* has been investigated for its protective response. Plant extracts can be characterized by polyvalent formulations and interpreted as additive, or, in some cases, potentiating[25]. Antilipid peroxidative property of *Murraya koenijii* 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 *Murraya koenijii* may result from multiple factors involving hydrogen or electron transfer, metal chelating activity and synergistic activity and appear to be 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.

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