RESEARCH PAPER

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Effect of *Majorana hortensis* leaves against lipid peroxidation

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Free radicals inducing oxidative damage of cellular lipids, nucleic acids and proteins are thought to be one of the major risks for diseases such as cancer, atherosclerosis, diabetes mellitus and various other degenerative diseases. Numerous natural free radical scavengers and antioxidants can protect biomolecules against the attack of free radicals and/or suppress the resultant injury. Three leaf extracts of candidate plant, *Majorana hortensis,* were prepared in methanol, chloroform and aqueous and subjected to test the extent of inhibition of *in vitro* lipid peroxidation by using different lipid membrane preparations, namely RBC ghosts, goat liver homogenate and goat liver slices. All the three extracts caused a substantial decline in the extent of LPO in all the three membrane preparations. The decrease in LPO was more pronounced in the liver homogenate. Among the three extracts used, the methanolic extract evinced a better protection in all three lipid preparations compared to the aqueous and chloroform extracts.

Key words : Free radicals, Reactive oxygen species (ROS), Lipid peroxidation, RBC ghosts, Membrane lipids

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INTRODUCTION

Free radicals are highly reactive atomic or molecular species that can damage vital cellular molecules like nucleic acids, lipids and proteins resulting in subsequent cell death (Pauwels *et al.*, 2007). In the biological system, lipids are the immediate targets of oxidative moieties and DNA molecules are the ultimate targets (Balakrishna *et al.*, 2009). Membrane lipids present in subcellular organelles are highly susceptible to free radical damage and cause peroxidation of polyunsaturated fatty acids in the membranes (Kim *et al.*, 2005). When reactive oxygen species attack polyunsaturated fatty acids on the cell membrane of living organisms in the presence of molecular oxygen, a chemical cascade is triggered. This eventually leads to the disintegration of fatty acids and the formation of malondialdehyde (MDA) which is called as lipid peroxidation (LPO) (Cemek *et al.*, 2006). MDA, the end product of lipid peroxidation, has also been demonstrated to be a mutagenic and genotoxic agent that can contribute to the development of human cancers (Ajith, 2010). The damage caused by LPO is highly detrimental to the functioning of the cell (Skrzydlewska *et al.*, 2005). Antioxidants are compounds which have the ability to transform reactive oxygen species into stable and harmless compounds or to scavenge both reactive oxygen and nitrogen species with a redox-based mechanism (Niki, 2009). Several epidemiological studies suggest that plants rich in antioxidants play a protective role in health and against diseases, and their consumption lowers the lipid peroxidation (Muanda *et al.*, 2009).

The present study was undertaken to test the extent of inhibition of *in vitro* lipid peroxidation by the extracts of *Majorana hortensis* using different membrane preparations. The candidate plant, *Majorana hortensis* (*M. hortensis*), is a perennial herb, belonging to family Lamiaceae (mint) and is an aromatic plant known to have many therapeutic remedies; it cures fever, digestive disorders and has antibacterial activities as well. It is commonly called majoram.

RESEARCH METHODOLOGY

The *in vitro* model systems used as an alternative to live animal models included, three different membrane model systems to analyze the extent of LPO and protection rendered by the leaf extracts. These different models were employed in order to ascertain whether the lipid

composition and the nature of the membrane influenced the effect of the leaf extracts on the extent of LPO. The extent of the inhibition of LPO was determined by quantifying the amount of TBARS (thiobarbituric acid reactive substances) formed spectrophotometrically.

Estimation of LPO in goat RBC ghosts:

RBC ghosts prepared from goat blood were used as a source of plasma membranes. Goat blood (50ml) was collected fresh from the slaughter-house, defibrinated immediately using sterile acid-washed stones and diluted 1:1 with sterile isotonic (1.15%) KCl. The RBCs were pelleted by centrifugation at 3000xg for 10 minutes at 4^0C, and the pellet was washed thrice with isotonic KCl. The washed pellet was treated with hypotonic KCl (0.5%) to lyse which was further washed with hypotonic KCl until a pale pink pellet was obtained. The pellet was then suspended in 1.5ml of TBS (tris buffered saline 10 mM, pH 7.4) and 50ìl aliquots were used for the assay as per the protocol of Dodge *et al*. (1963).

Estimation of LPO in goat liver homogenate:

A 20% homogenate of the goat liver was prepared using Tris HCl buffer (40mM, pH 7.0), which was centrifuged to remove the debris and then used as a source of internal membrane lipids. LPO assay was performed according to the method of Okhawa *et al.* (1979).

Estimation of LPO in goat liver slices:

The goat liver was collected fresh from a slaughterhouse and maintained at 4^0C till use. Using a sterile scalpel, thin slices of 1mm thickness were obtained and used for the assay. This served as a model for intact (live) cells. The extent of inhibition of LPO in goat liver slices was estimated by the method proposed by Dodge *et al*. (1963).

Preparation of leaf extract:

M. hortensis leaf extract was made with organic solvents, methanol and chloroform and evaporated to dryness. The residue was weighed and dissolved in DMSO to obtain a final concentration of 20 mg/5 ìl of DMSO. An aqueous extract was also prepared fresh.

Experimental design:

The treatment groups set up for each assay were the control groups which had no plant extract followed by aqueous, methanol and chloroform extracts and the oxidatively stressed groups containing hydrogen peroxide along with the aqueous, methanol and chloroform extracts.

RESULTS AND ANALYSIS

Extent of inhibition of *in vitro* lipid peroxidation in different membrane preparations by the extracts of *M. hortensis* leaves is shown in Table 1. The methanolic extract rendered the maximum protection as it showed the maximum inhibition of LPO in all the 3 lipid preparations compared to the aqueous and chloroform extracts. The magnitude of inhibition of LPO exhibited by the aqueous extract was considerably low compared to methanol and chloroform. The results obtained in the present study indicated that the lipid components of both plasma membrane as well as intracellular membranes can be protected from LPO by *M. hortensis* leaf extracts. It implies that the leaf extract exhibited more or less equal extent of inhibition of LPO in all the three membranes lipid systems. All three systems showed substantial decline in the peroxidation activity, though it was well evinced in the liver homogenate followed by liver slices and then RBC ghosts. The extent of protection was much better in the liver homogenate than in the other two systems. These results suggest interesting insights into the nature of the active components involved in the protection of lipids. The fact that higher protection is rendered to the system where the internal membranes are directly exposed to the antioxidants suggests that the nature of the lipids influences the extent of protection. Additionally, the observation also suggests that some component(s) in the leaf extracts may not be readily membrane permeable, as the extent of LPO in the liver slice (intact cells) was lower.

A similar finding was reported by Sumathi and Padma (2009), who postulated the involvement of an endogenous factor that acts in conjugation with the components of *Withania somnifera* to render a better protection to the intracellular membrane lipids. The ability of herbal components to inhibit LPO, as a reflection of their

Table 1: Inhibition of lipid peroxidation in different membrane preparations by *Majorana hortensis* leaf extracts

Sample	Per cent inhibition of LPO		
	RBC ghosts	Liver homogenate	Liver slices
Aqueous	42.45 ± 0.11	63.23 ± 0.14	62.10 ±0.12
Methanol	61.88 ± 0.14	87.83 ± 0.12	82.78 ±0.29
Chloroform	55.04 ± 0.18	74.67 ± 0.29	74.34 ± 0.03
Aqueous	42.45 ± 0.11	63.23 ± 0.14	62.10 ±0.12

The values are mean ± SD of triplicataes

protective antioxidant capacity has been a routine investigation in determining the antioxidant effect. Amifostine, triphosphate free oxygen scavenger showed significant inhibition of LPO in spinal cord homogenate (Chronidou *et al*., 2009). The methanol and chloroform extracts of *Lysichiton camtschatcense* (L.) were potent inhibitors of LPO induced by Fe^{2+} and ascorbate in rat kidney and brain homogenate (Takatsu *et al*., 2009). Ascorbic acid showed a dose-dependent decrease in H_2O_2-induced LPO, which was assayed by measuring the TBARS in cultured peripheral human blood lymphocytes (Siddique *et al*., 2009). Polyphenols present in *Cinnamomum zeylanicum* and *Acacia catechu* were suggested to be predominantly responsible for the LPO inhibitory action and the antioxidant activity of the plants (Yadav and Bhatnagar, 2009). Future studies - The effect of the *M. hortensis* leaf extracts can be further analysed for the protection of purified DNA preparations and proteins against oxidative damage to prove the bimolecular protective effects of the leaf extracts.

Conclusion:

The outcome of the present study enables the use of *Majorana hortensis* leaf extracts as an effective candidate to be employed in all medicinal preparations used to combat lipid peroxidation which in turn reduces the risks of chronic diseases and prevents generation of free radicals.

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