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Efficacy of mint (M*entha spicata* L.) leaves in combating oxidative stress in type 2 diabetes

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ABSTRACT

In diabetes, hyperglycemia leads to an imbalance between prooxidants and antioxidants resulting in oxidative stress characterized by lipid peroxidation, protein oxidation and decreased activities of defense enzymes. Administration of mint leaf powder (5g/day) containing a no. of bioactive compounds to type 2 diabetes patients for 60 days corrected these abnormalities i.e. controlled oxidative stress as shown by decreased lipid peroxidation, protein oxidation and decreased activity of catalase (CAT) in erythrocytes, increased serum β carotene, vitamin A, E and C levels. In addition, activity of erythrocyte antioxidant enzyme i.e. glutathione–S–transferase (GST) and reduced glutathione content (GSH) were significantly improved in mint leaves-treated diabetics. This study reveals amelioration of oxidative stress in diabetics due to the supplementation of mint leaf powder. In conclusion, antioxidant property exhibited by mint leaves is a result of synergistic action of antioxidant phytochemicals, carotenoids, flavonoids etc. present in the leaves. The findings from this study suggest that the leaves may be consumed for combating oxidative stress that causes chronic diseases viz. diabetes.

Key words: *Diabetes; Oxidative stress; Lipid peroxidation; Protein oxidation; Defense enzymes; Mint leaves.*

INTRODUCTION

Oxygen free radicals (OFRs) cause damage to cellular biomolecules and consequently may adversely affect immune functions (Nilsson et al., 2004) either due to the deficiency of natural protective substances or excess intake of chemicals that can stimulate free radical production such as iron (Bothwell & Charlton, 1982). A variety of natural antioxidants exist in living species to scavenge OFRs and to prevent oxidative damage to biological membranes. One group of those antioxidants is enzymatic (intracellular) viz. copper-zinc superoxide dismutase (Cu-ZnSOD), selenium – dependent glutathione peroxidase (GPx) and catalase (Halliwell & Gutteridge, 1985). In addition to the enzymatic antioxidants, there are non-enzymatic antioxidants (extracellular) which include vitamin A (retinol), vitamin C (ascorbic acid) and vitamin E (α-tocopherol) (Thompson & Godin, 1995). *In vivo*, thiols (especially GSH) are often regarded as antioxidant agents, since they protect protein-SH groups against oxidation and can scavenge oxygen radicals and some other reactive species (Halliwell & Gutteridge, 1999). β-carotene, α-tocopherol and ascorbic acid act as potent antioxidants in protecting membrane lipids against free radical damage. Hence, antioxidant principles from natural resources are multifaceted in their multitude/magnitude of activities and provide enormous scope in correcting the imbalance through regular intake of proper diet (Kamat, 2007).

Oxidative stress may be increased in diabetes patients since persistent hyperglycemia causes an increased production of oxygen free radicals (OFRs) through autoxidation of glucose and non-enzymatic protein glycation (Baynes, 1991). The efficiency of enzymatic and non-enzymatic defense mechanism is altered in diabetes (Wohaib & Godin, 1987). Oxygen free radicals (OFRs) have been implicated in the pathology of diabetes mellitus through destruction of pancreatic β-cells or insulin resistance as well as initiations of atherosclerosis, retinopathy and other diabetic complications (Maxwell et al., 1997).

Nature is bestowed with a variety of foods and herbal products, frequently considered to be less toxic and free from side effects than synthetic ones (Valiathan, 1998). Spices are used throughout the

world to flavor foods and beverages and India is truly called as 'Spice bowl' of the world as spices are indispensable in the daily diet of Indians. The bulk of the dry matter of spices consists of carbohydrates, volatile oil, fixed oil, protein, tannin, resins, pigments and minerals (Subblakshmi & Nayak, 2002). Since each of the spices possesses more than one health beneficial property and there is also a possibility of synergy among them in their action, liberal consumption of spices is not only proved to be safe, but may even offer beneficial effects on the antioxidant status (Srinivasan, 2005). Keeping these facts in consideration, the present study was under taken to evaluate the *in vivo* antioxidant efficacy of mint (*Mentha spicata*) leaves in ameliorating oxidative stress in type 2 diabetes.

MATERIALS AND METHODS

Procurement of mint leaves and preparation of powder

Mint leaves were procured from the local market of Anantapur, thoroughly cleaned and washed in fresh water to free from extraneous matter, shade dried and finely powdered using electric blender and packed in polythene covers to be used for clinical trial. The powder was prepared fresh every week and supplied to the subjects.

Experimental subjects and experimental design

Both male and female non insulin dependent (type 2) diabetes patients in the age group of 40-60yrs. with no other specific complications were selected from local Diabetes Hospital on the basis of a specific questionnaire. Out of the selected subjects, 20 served as control and 40 served as experimental. The experimental group received mint leaf powder 5g per day in 2 equal doses for a period of 60 days. All the subjects were given dietary guidelines and were under the supervision of a diabetologist.

Clinical analysis

At the initial and final stages of the experiment, fasting blood was drawn for the assay of various parameters. Fasting blood glucose (Trinder, 1969), lipid peroxidation in plasma (Buege & Aust, 1978) and erythrocytes (Stocks & Dormandy, 1971), protein oxidation (Levine et al., 1990), vitamin A and β-carotene (Henry et al., 1995), vit. C (Roe, 1961) and vit.E (Desai, 1984) in serum were estimated. Activities of catalase (Chance, 1954), glutathione–s–transferase (Raghuramulu et al., 1983) were assayed and reduced glutathione (GSH) content (Beutler et al., 1963) was determined in erythrocytes. Mean, standard error of means and paired difference't' test were conducted(Gupta, 1995) to assess significant difference between the data obtained before and after treatment.

RESULTS

A decrease of 24% ($p<0.01$) was noted in fasting blood glucose in mint–treated group while a 2% increase was seen in control diabetics. A 4% increase in protein oxidation in the control and 23% ($p<0.01$) decrease in experimental group were observed. A 19% ($p<0.05$) increase in plasma lipid peroxidation in control where as a 25% ($p<0.01$) decrease in mint-treated group were observed. The erythrocyte lipid peroxidation before and after experimental period in control and experimental groups indicated 20% ($p<0.05$) increase in control group and 51% ($p<0.001$) decrease in the treated group **(Table 1)**. **Table 2** shows a 47% ($p<0.05$) increase in the activity of catalase in control, a 40% ($p<0.01$) decrease in the treated group as compared to initial values. Activity of GST was elevated by 49% ($p<0.001$) in mint–treated diabetics while11% ($p<0.05$) decrease was seen in control group (**Table 2)**. **Table 2** depicts 51% ($p<0.01$) increase in GSH in the treated group and 13% ($p<0.01$) decrease in control group. Serum vitamin A levels indicate 4% decrease in control and 10% ($p<0.01$) increase in the treated group. **Table 3** presents serum β-carotene levels i.e. 13% ($p<0.05$) decrease in control group and 15% ($p<0.01$) increase in mint-treated group. A 2% decrease in serum vitamin C levels in control group and 10 % ($p<0.05$) increase in the treated group were observed. A slight increase (3%, $p<0.05$) was seen in serum vit. E in mint–treated group and a slight decrease (1%) was seen in control group as indicated in **Table 3**.

Table 1. Influence of mint leaves on fasting blood glucose, protein oxidation in serum, lipid peroxidation in plasma and erythrocytes

Parameter	Control		Experimental	
	Initial	Final	Initial	Final
Fasting blood glucose (mg/dl)	137.3± 3.7	140.0± 3.3 (2)	138.3±3.4	105.3±2.1** (24)
Protein oxidation (nmol/ml)	48.0±0.01	50.0±0.04 (4)	53.0± 0.02	41.0±0.03** (23)
Plasma lipid peroxidation (nmol MDA/dl)	401.5±27.2	476.33±14.2* (19)	416.5±17.3	310.7±17.8** (25)
Erythrocyte lipid peroxidation (nmol MDA/gHb)	6.88±0.9	8.28±1.1* (20)	19.95±1.4	9.78±1.7*** (51)

Values are mean ± SEM of 20 subjects in control group and 40 in mint leaves-treated group. ***The figures in parentheses indicate per cent increase/decrease over respective initial values. Comparison between initial and final: *p<0.05; * * p<0.01; *** p<0.001***

Table 2. Influence of mint leaves on erythrocyte antioxidant enzymes and reduced glutathione

Parameter	Control		Experimental	
	Initial	Final	Initial	Final
Catalase (K/gHb)	6.72± 0.8	9.89± 0.4* (47)	9.95± 0.4	6.01 ± 0.1** (40)
Glutathione-s-transferase (IU/gHb)	24.37± 1.3	21.72± 0.2* (11)	21.77± 0.9	32.53± 2.8*** (49)
Reduced glutathione (μmol/gHb)	9.57± 3.41	8.35± 4.2** (13)	16.85± 4.64	25.41± 3.9** (51)

Values are mean ± SEM of 20 subjects in control group and 40 in mint leaves-treated group. ***The figures in parentheses indicate per cent increase/decrease over respective initial values. Comparison between initial and final: *p<0.05; * * p<0.01; *** p<0.001***

Table 3. Influence of mint leaves on serum non-enzymatic antioxidants

Parameter	Control		Experimental	
	Initial	Final	Initial	Final
Vitamin A (μg/dl)	24.42±0.7	23.37±0.8 (4)	22.17±0.8	24.42±0.4** (10)
β carotene (μg/dl)	158.3±16.9	138.3±11.2* (13)	166.6±14.1	191.6±16.1** (15)
Vitamin C (mg/dl)	1.93±02	1.88±0.2 (2)	2.00± 0.3	2.20± 0.2* (10)
Vitamin E (mg/dl)	3.14±0.0	3.10±0.13 (1)	3.02±0.1	3.11±0.1* (3)

Values are mean ± SEM of 20 subjects in control group and 40 in mint leaves-treated group. ***The figures in parentheses indicate per cent increase/decrease over respective initial values. Comparison between initial and final: *p<0.05; ** p<0.01***

DISCUSSION

Mitochondrial glucose over load results in increased electron transfer to oxygen and formation of free oxygen radicals. Acute glucose fluctuations induce oxidative stress and these fluctuations were suggested to be valuable predictors for the risk of diabetic complications (Monnier et al., 1963). In the present study, mint treated group showed a significant decrease in fasting blood glucose which indicates that mint leaves controlled hyperglycemia. This result can be supported by significant decrease in lipid peroxidation (a marker of oxidative stress) in erythrocytes and plasma and decreased protein oxidation in the treated diabetics **(Table 1).** In the present study, decreased glucose levels indicate control over oxidative stress as Turko et al., (2001) reported that hyperglycemia can directly cause increased generation of reactive oxygen species because glucose undergoes autooxidation and generates OH radicals.

Extracellular proteins are more susceptible to functionally critical oxidative damage due to their very low turn over rates and a much lower degree of protection from oxidative damage compared to intracellular proteins. Elevated levels of oxidized tryptophan, tyrosine and methionine residues have been reported in cardiac proteins of streptozotocin (STZ) – diabetic rats and in plasma proteins of diabetic patients (Hamblin et al., 2007). In the present study, hyperglycemia persisted in the subjects reflected in protein oxidation. Treatment with mint leaves showed significant decrease in protein oxidation as compared to initial values. This data indicates antioxidant potential of mint leaves by virtue of a number of antioxidant phytochemicals present in the leaves (www.ars_grin.gov/duke).

In diabetes, significant changes in lipid metabolism and structure also occur, particularly in patients with vascular complications (Sato et al., 1981). Lipid peroxides might be formed by nonenzymatic reactions of unsaturated lipids with superoxide radical, hydrogen peroxide and metal ions in the circulation or extravascular space or at the surface of endothelial and phagocytic cells (Baynes, 1991). The result of the present study, showed significant decrease in lipid peroxidation in the treated group which shows decreased oxidative stress as lipid peroxidation is a marker of oxidative stress. This is also supported by controlled hyperglycemia **(Table 1)** as hyperglycemia results in increased production of ROS leading to increased lipid peroxidation. Decreased lipid peroxidation in this study, is a result of synergistic action of the antioxidant vitamins, free radical scavengers reported to be present in the leaves (www.ars_grin.gov/duke).

Peroxidation in the RBC membrane may cause changes in membrane function and stability leading to decreased survival of RBCs (Cooper, 1977). Lipid peroxidation induced by H_2O_2 in erythrocytes and its membrane was normalized under dietary spice treatment, probably due to decreased cholesterol (Kempaiah & Srinivasan, 2004). Remarkably decreased erythrocyte lipid peroxidation in the present study, strongly supports controlled oxidative stress in treated subjects and antioxidant potential of

mint leaves and is further supported by increased vit. C and E levels **(Table 3)** in the treated subjects.

Increased activity of catalase is a reflection of increased ROS production especially hydrogen peroxide as catalase acts to detoxify H_2O_2 to form water (Kornatoustaka & Luciak, 1998). Decreased activity of catalase in the present study in mint treated group, is a positive sign of controlled oxidative stress and is strongly evidenced by decreased lipid peroxidation in erythrocytes and plasma **(Table 1).** This is also supported by decreased blood glucose levels because hyperglycemia leads to the formation of ROS (Hunt et al., 1988).

Glutathione-S-transferases exert protective effects because they are able to catalyze the conjugation of GSH with oxidation end products and represent a second line of defense against the highly toxic spectrum of substances produced by ROS-mediated reaction (Masella et al., 2005). In the present study, significantly and remarkably increased GST in the treated group indicated protection against ROS. This is further supported by significantly increased GSH **(Table 2)** and decreased erythrocyte lipid peroxidation **(Table 1).**

Glutathione (GSH), a tripeptide with the capacity to reduce hydrogen peroxide (H_2O_2) and lipid peroxides was observed to decrease in diabetes which is probably due to its increased utilization by the tissues. In diabetes, GSH may be decreased by different pathways – 1) increased sorbitol synthesis causing NADPH depletion there by decrease of GSSG to GSH, 2) decreased activity of HMP shunt enzymes which generate NADPH and 3) transport of GSSG through erythrocyte membranes due to oxidative stress induced membrane damage (Konukoglu et al., 1999). Treatment with mint leaves significantly increased GSH levels in erythrocytes probably by countering any of the mentioned effects in diabetes mainly by the antioxidant principles and free radical scavengers present in the leaves.

β-carotene is shown to be an effective antioxidant in scavenging certain reactive oxygen species (ROS), especially peroxyl radical and singlet oxygen and this antioxidant activity appears to be greatest at low oxygen tension (Halliwell & Gutteridge, 1999). β-carotene especially has been shown to protect isolated lipid membranes from peroxidation (Bendich & Olson, 1989). Unlike antioxidants that prevent the initiation of lipid peroxidation, β-carotene stops the chain reactions by trapping the free radicals. β-carotene in mint leaves would have significantly decreased lipid peroxidation in the treated subjects as the leaves have substantial amount of carotene (1,620 mg%) (Gopalan et al., 2007).

Because of their structure, vitamin A and carotenoids can autoxidise when O_2 tension increases, and thus are most effective antioxidants at low oxygen tension, that are typical of physiological levels found in tissues (Palace et al., 1999). In the present study, significantly raised vitamin A levels (**Table 3**) are due to the carotenoids present in mint leaves as they are supplemented for 60 days to diabetics. The oxygen quenching nature of carotenoids and vitamin A would have resulted in decreased lipid peroxidation in erythrocytes and plasma observed in mint-treated group in this study (**Table 1).**

Diabetes mellitus results in decreased plasma ascorbic acid and this decrease could be due to decreased transport of ascorbic acid as glucose and ascorbic acid share the same transport system and hyperglycemia also could be responsible for decreased transport of ascorbic acid. The decrease in the levels of ascorbic acid, thus may also be due to its consumption during the free radical scavenging process as diabetes is associated with increased oxidative stress (Wolff, 1987). Mint leaves by possessing certain compounds which scavenge free radicals (β-carotene, flavonoids, carotenoids etc.), controlled oxidative stress and protected vitamin C from oxidation, resulting in elevated serum vitamin C levels in the treated subjects while such effect was not seen in control subjects.

Vitamin E is a potent lipid-soluble antioxidant in biological system with the ability to directly quench free radicals and functions as a membrane stabilizer (Rokitzki et al., 1994). Hence, the increase observed in serum vitamin E in the present study (**Table 3)**, is a reflection of controlled oxidative stress resulted due to significantly decreased blood glucose, erythrocyte and plasma lipid peroxidation as presented in **Table 1**. The rise in vitamin E is probably because of the protection of tocopherol from oxidation to tocopheroxyl due to the scavenging of free radicals by the scavengers present in the leaves and/or the regeneration of tocopherol from tocopheroxyl by the oxidation of ascorbic acid to dehydroascorbic acid as reported by Garg et al., (1997) in a study on experimental diabetes.

CONCLUSION

Mint leaves controlled oxidative stress in type 2 diabetes. The leaves elevated serum non-enzymatic antioxidants and improved activities of erythrocyte antioxidant enzymes and very effectively decreased

lipid peroxidation in erythrocytes and plasma in type 2 diabetes patients which is a result of synergistic action of phytochemicals, vitamins and minerals present in mint leaves. Further investigations on the mechanism of action of active principles in mint leaves are in progress.

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