Int J Health Nutr 2011 2 (1): 1-5

International Journal of Health & Nutrition

www.academyjournals.net

Orjinal Article

Anti diabetic property of drumstick *(Moringa oleifera)* leaf tablets

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Received: 30.09.2010 ***Accepted: 28.01.2011*** ***Published: 22.02.2011***

Abstract

The objective of this study was to formulate dehydrated green leafy tablets using drum stick leaves followed by supplementation study and to find out the anti diabetic property on the selected diabetic patients. The results showed that post prandial blood glucose of experimental group initially was 210 mg/dl and it reduced to 191, 174 and 150 mg/dl respectively after the first, second and third month of supplementation. In control group post prandial blood glucose level was reduced to 169, 167, 163 mg/dl respectively, after first, second and third month of study from the initial value of 179 mg/dl. Glycated hemoglobin in experimental group was initially 7.81 and decreased to 7.4 per cent after the supplementation period; but in the control group it decreased to 7.36 from the initial value of 7.38 per cent. The results indicated that drum stick leaves are suitable source of green leafy vegetable to reduce the diabetic complications in diabetic patients.

Key words: Drum stick leaves, diabetic patients, green leafy tablets, post prandial blood glucose, glycated hemoglobin

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INTRODUCTION

Diabetes mellitus (DM) is one of the most common non-communicable diseases in the world. Diabetes affect about 135 million people in 1995 and the prevelance is expected to rise to 300 million in the year 2025 (King *et al.*, 1998). Evidence showing that Indian migrants have a high tendency to develop diabetes that lead to several national epidemiological studies in India. Availability of uniform criteria for diagnosis and classification of diabetes made comparison between the possible studies (WHO 1985). These surveys showed that diabetes was as common in urban India as among migrant Indians (Ramaiya *et al*., 1990). Studies from different parts of India showed an increasing trend in the prevalence of diabetes. A series of epidemiological studies carried out by the Diabetes Research Centre in Chennai showed that the prevalence of diabetes had steadily increased among urban Indian adults from 5.2 per cent in 1984 to 13.9 per cent in 2000 (Ramachandran *et al*., 2002).

Oxygen free radicals and other "reactive oxygen species" are constantly produced in the human body. Multiple studies have shown that type 2diabetes is accompanied by increased oxidative damage to all biomolecules in body. Diabetes produces disturbances of lipid profiles, especially an increased susceptibility to lipid peroxidation. An increased oxidative stress has been observed in diabetic patients as indicated by high free radical production (Giugliano *et al.*, 1996). Oxidative damage due to free radicals was associated with vascular disease in people with types 1 and 2 diabetes mellitus (Oberley, 1988). There are several potential resources of free radical production in diabetics including autoxidation of plasma glucose (Piper *et al.*, 1995), activation

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of leucocytes, and increased transition metal bioavailability (Wolff *et al.,* 1991). The Total Antioxidant Status (TAS) in type 1 or 2 DM was lower than that of age-matched controls, and this might be attributed to lower levels of vitamin C, vitamin E (Maxwell *et al.,* 1997), or other factors including micronutrients (Mooradian *et al.,* 1994, Anderson, 1995 Anderson *et al.,* 1997 and Cunningham, 1998) in blood.

The increased susceptibility of tissues such as the liver and kidney of diabetic animals to diabetic complications may be due to increased lipid peroxidation. In addition, increased lipid peroxidation under diabetic conditions resulted due to excessive oxidative stress. From this view point, prevention of oxidative damage was considered to play a crucial role in diabetes and / or its complications resulting from lipid peroxidation (Stanely and Menon, 2001).

Green leafy vegetables (GLV) offer a cheap but rich source of a number of micronutrients and other phytochemicals having antioxidant properties. The potential of 30 GLV in the raw and cooked form as natural antioxidant supplements for vegetarian diets was assessed. There was a large variability in the values of antioxidant activity of various GLV extracts in the lipid micelles (1.5-5.6 mM vitamin E/100 g for raw samples and 1.6-3.8 mM vitamin E/100 g for cooked samples). Similar to thiobarbituric acid reactive substances values, the superoxide scavenging ability values also exhibited large variation (10.6-55.9), with significantly higher values in the raw state than the cooked state ($P<0.001$). Omum leaves, radish leaves and lettuce had high values for this index. The range of values for ferrous iron chelating activity was from 9.3 to 65.7 mM EDTA/100 g food material, indicating again a large variability in this assay. Leaves of coriander, *Amaranthus viridis*, colcasia green and drumstick showed high values, while Colocasia black and amaranthus red exhibited low values. Differences between raw and cooked values were highly significant for all the three indices ($P<0.001$) (Tarwadi and Agte, 2003). Hence the study was planned with the following objectives to formulate the dehydrated green leafy tablet for conducting the supplementation study and to study the anti diabetic property of drum stick leaf tablets.

MATERIALS AND METHODS

Tablet Formulation

Drumstick leaf powder

Leaves were separated from the stalks, cleaned, washed and dried in microwave power level of 7 for 10 minutes and powdered by the researchers.

Ingredients used for tablet formulation

For making tablet the following ingredients were used: Drumstick leaf powder (98.34%), Carboxy Methyl Cellulose Sodium (1.30%), Methyl Paraben Sodium (MPS) (0.18%), Propyl paraben sodium (PPS) (0.02%), Bronopol (0.01%), and Tale (0.16%).

Method of making the tablet

The preservatives MPS, PPS and Bronopol were added in boiling water and mixed thoroughly. To this, carboxy methyl cellulose sodium was added little by little with continuous stirring using a mechanical stirrer, till a fine paste was obtained. This paste was added to the active ingredient in small quantities and mixed well. Manual granulation was done using a sieve. The granules were dried at 50°C for one hour. Tale was added to lubricate the granules, which were then pressed into tablets in a punching machine at the pharmaceutical company, Paris Dakner, Thirumangalam, Madurai, under the supervision of the researchers. The nutrients were analyzed after preparing the Tablet and found to contain crude fibre-3.48 g per cent, vitamin C-27.70 mg/g, carotenoid- 156 mg/100g and antioxidant activity - 85 per cent.

Selection of patients

One hundred type 2 diabetic patients visiting private clinic, Madurai, were selected for the study. Information on socio economic background of selected patients was elucidated using a framed questionnaire. Among hundred diabetics studied, sixty subjects were selected for further study. The subjects selected were on sulfonylurea (Oral Hypoglycemic Agents) users, BMI between 20-25 kg/m^2 and who were involved in sedentary activity and age group between 40-58 years. They were divided into control and experimental groups each containing 30 subjects.

Administration of tablets to the selected diabetes

The subjects were given a pack of 30 tablets for every 15 days of the study period. They were asked to take two tablets daily with one tablet each after breakfast and dinner for a period of 90 days. Both control and experimental group subjects were advised to take a standardized diet which had calories restricted between 1,500 to 1,800 Kcal. This was achieved by giving awareness about the food exchange list, the subjects and their diet intake was verified once in every fifty days.

Bio chemical analysis

To study the anti diabetic property of drumstick leaves tablets on the selected type 2 diabetic patients Glycated hemoglobin (%) (HbA_{1c}) and Post Prandial Blood Glucose (after two hours of the diet) had been studied**.** Commercially available kit was used for the analysis and analysis carried out in the auto analyzer. The glycated hemoglobin procedure followed based on the Nathan *et al.,* 1984.

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Statistical Analysis

The following statistical tools were used in this study for analysis and interpretation of data (Rangaswamy, 1995): Independent T-Test, F-Test, Correlation, and Factorial Completely Randomized Design (FCRD).

RESULTS AND DISCUSSION

Glycated hemoglobin

Glycated hemoglobin also known as glycol-hemoglobin, glycosylated hemoglobin, HbA_{1C} or HbA_1, refers to a series of stable hemoglobin components formed by the combination of glucose and hemoglobin. HbA_{1C} level provides an average indication of the overall blood glucose level, for a period of two or three months.

Table 1, gives the mean glycated hemoglobin level of experimental and control group. The initial value of glycated hemoglobin in control group was 7.38 and a slight decrease (7.36%) was noticed after 90 days of study. This reduction was not significant statistically. In experimental group, in which the glycated hemoglobin was 7.81 initially and the values were found to decrease to 7.4 per cent after the supplementation for the period of three months. The mean reduction was 0.41 per cent after feeding drumstick leaf tablets for a period of three months. This reduction was highly significant, which emphasis that supplementation with drumstick leaf tablet had positive effect in reducing glycated hemoglobin.

According to Boeing *et al.* (2000) obesity, high energy and saturated fat intake and increasing in age was found to be significantly associated with high HbA_{1c} values, whereas high intake of alcohol, vitamin C and Vitamin E were inversely associated with high HbA_{1c} values.

In this study also similar observations were made, because, majority of them belonged to the age group of more than 40 years.

Dandona *et al.* (1996) observed inverse relation between vitamin C and vitamin E and hemoglobin glycation.

Keenoy *et al.* (1999) studied the effect of supplementation of flavonoid based antioxidant medication to 28 diabetic patients while following a standardized 1,800 - 2,000 calorie diet. The results showed that decrease in initial and final values of glycated hemoglobin was significant in experimental group, whereas in control group the decline was non-significant. The above study stated that supplementation of antioxidants and flavonoids control the glycation process, so that decrease in glycated hemoglobin was seen in experimental group. Similar observation was found in present study due to feeding of drumstick leaf tablet to experimental group.

Table 1 Mean glycated hemoglobin (%) (HbA_{1c}) level of control and experimental groups

	Initial	Final
Control group	7.38±0.60	7.36±0.59[a]
Experimental group	7.81±0.51	7.40±0.63[b, c]

a, initial vs final $p<0.088^{NS}$; b, initial vs final $p<0.0076^{**}$; c, control vs experimental $p<0.092^{NS}$

Table 2 Mean post prandial blood glucose (mg /dl) level of the control and experimental groups

	Initial	**1st. month**	**2nd. month**	**3rd. month**
Control group	179±36.09	169±36.73	167±35.44	163±34.08
Experimental group	210±48.83	191±48.64[a]	174±36.77[b]	150±21.10[c]

a, initial vs 1st. month $p<0.0088^{**}$; b, 1st. month vs 2nd. month $p<0.035^{*}$; c, 2nd. month vs 3rd. month $p<0.0098^{**}$

Table 2, gives the picture about the blood glucose level of diabetic patients during the study period. Post prandial blood glucose level showed a reducing trend in both control and experimental groups. But the reduction in post prandial blood glucose levels in drumstick leaf tablet supplemented experimental group was significant. Initially in experimental group, post prandial blood glucose level was 210 mg/dl and it reduced to 191, 174 and 150 mg/dl respectively after the first, second and third month of supplementation. In control group post prandial blood glucose level was reduced to 169, 167, 163 mg/dl respect after first, second and third month of study from the initial value of 179 mg/dl, Drumstick leaf tablet had a significant role in reducing the blood glucose level of diabetics.

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Ford *et al.,* (1999) analyzed the data from Phase I of the Third National and Nutrition Examination Survey (NNES) (1988-1991) to examine carotenoids concentration in 40 to 70 year old subjects with normal glucose tolerance (1010 subjects), impaired glucose tolerance (277 subjects) and newly diagnosed diabetic cases (148 subjects). All serum carotenoids were found to be inversely associated with the fasting serum insulin levels. Moreover, serum lycopene and β-carotene level in the diabetic patients were significantly lower when compared to the levels in persons with impaired glucose tolerance and also found to be lower than the levels in subjects with normal glucose tolerance. They also stated inverse relations of serum β-carotene, cryptoxanthin and lycopene concentrations with glucose concentrations and an inverse relation was also observed between serum carotenoids concentrations and fasting serum insulin concentration

The above mentioned study explain that there was negative relation between antioxidant especially carotenoids and blood glucose. It proves that there was a positive role of antioxidants in glucose metabolism.

Oral administration of the antioxidant fraction to diabetic rats was observed to reduce dose – dependently, the serum levels of glucose and glycosylated protein, indicated improvement in the impaired glucose metabolism. Therefore, the antioxidant fraction would be expected to prevent diabetic damage by controlling the glucose metabolism (Sato *et al.,* 1979).

According to Opara, (1996), chromium and the antioxidant vitamins C and E have been found to be efficacious in controlling blood glucose levels in type 2 diabetes and in the prevention of diabetic complications.

Yokozawa *et al.* (2003) reported that oral administration of the mustard leaf fraction to diabetic rats reduced, dose-dependently, the serum levels of glucose and glycosylated protein, indicating improvement in the impaired glucose metabolism. Therefore, the mustard leaf fraction would be expected to prevent diabetic damage by controlling the glucose metabolism.

Meral *et al.* (2004) inferred that serum glucose concentration increased significantly in diabetic rabbits supplemented *N. Sativa.*L treatment, elevated glucose concentration significantly ($P \leq 0.05$) in diabetic rabbits. However, their glucose concentrations was still significantly higher ($P \leq 0.05$) than those of the control group.

Anuradha and Vidhya (2001) stated that there was significant reduction in blood glucose levels (fasting, postprandial and random) in the experimental when compared to the control group supplemented with spirulina.

Ylonen *et al.,* (2003) conducted study on dietary intake and plasma concentrations of carotenoids and tocopherols, in relation to glucose metabolism in subjects at high risk of type 2 diabetes. They revealed that population at high risk of type 2 diabetes had low dietary intakes of α- and β-carotene. Lycopene, as well as plasma β-carotene concentration showed beneficial associations with glucose metabolism in the men ; an inverse association with fasting plasma glucose concentrations was observed in the former, and an inverse association with insulin resistance was observed in the later.

The above studies pointed out that antioxidants like carotenoids, vitamins C and E, and flavonoids had an important role in reducing the blood glucose of the diabetic patients. This is due to the improvement in the impaired glucose metabolism and decrease in insulin resistance. The results observed in the present study were on par with above studies.

The findings showed that an inverse association between plasma β-carotene concentrations and insulin resistance in men, but women had a direct relation between plasma β-carotene concentrations and fasting plasma glucose concentrations which was in contrast with the aforementioned findings (Ylonen *et al.,* 2003).

The present study results were inverse to the Ylonen *et al.* (2003) study. The results did not show any deviation between the male and female of the feeding group. Both male and female volunteers, blood glucose level had reduced after the administration of drumstick leaf tablet. It concluded that drum stick leaf tablet have a significant impact on anti diabetic property of the selected patients.

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