IJFANS
International Journal of Food And Nutritional Sciences

Volume 4, Issue 5, Oct-Dec 2015, www.ijfans.com e-ISSN: 2320-7876

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

IMPACT FACTOR ~ 1.021

Official Journal of IIFANS

INTERNATIONAL JOURNAL OF FOOD AND NUTRITIONAL SCIENCES

e-ISSN 2320 –7876 *www.ijfans.com*
Vol.4, Iss.5, Oct-Dec, 2015

Research Paper *Open Access*

IMPACT OF DRUMSTICK *(MORINGA OLEIFERA)* LEAF TABLET ON LIPID PROFILE OF SELECTED DIABETIC PATIENTS

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Received on: 18th November, 2015 **Accepted on: 22nd December, 2015**

ABSTRACT

The present study was conducted with the objective to formulate a dehydrated green leafy tablet using drum stick leaf followed by supplementation study and to find out the impact on lipid profile of the selected diabetic patients. The results revealed that total cholesterol, HDL, LDL, VLDL, TGL initial and final level of control group was 217 and 214 mg/dl, 43 and 45 mg/dl, 140 and 136 mg/dl, 34 and 30 mg/dl, 155 and 150 mg/dl respectively, whereas in experimental group the corresponding parameters at initial and final stage was 227 and 211 mg/dl, 42 and 47 mg/dl, 149 and 135 mg/dl, 35 and 30 mg/dl, 160 and 151 mg/dl. The results are proving drum stick leaves are suitable green leafy vegetable to improve the lipid profile of diabetic patients.

Key words- Diabetic patients**,** drumstick leaves, Low Density Lipoprotein, High Density Lipoprotein, Triglycerides

INTRODUCTION

Diabetes mellitus is fast gaining the status of a potential epidemic in India with more than 62 million diabetic individuals currently diagnosed with the disease. In 2000, India (31.7 million) topped the world with the highest number of people with diabetes mellitus followed by China (20.8 million) with the United States (17.7 million) in second and third place respectively. According to Wild et al. the prevalence of diabetes is predicted to double globally from 171 million in 2000 to 366 million in 2030 with a maximum increase in India. It is predicted that by 2030 diabetes mellitus may afflict up to 79.4 million individuals in India, while China (42.3 million) and the United States (30.3 million) will also see significant increases in those affected by the disease. India currently faces an uncertain future in relation to the potential burden that diabetes may impose upon the country. Many influences affect the prevalence of disease throughout a country, and identification of those factors is necessary to facilitate change when facing health challenges (Kaveeshwar and Cornwall, 2014).

There is increasing evidence that reactive oxygen species (ROS) play a major role in the development of diabetic complications. Oxidative stress is increased in diabetes and the overproduction of ROS in diabetes is a direct consequence of hyperglycemia. Various types of vascular cells including renal cells are able to produce ROS under hyperglycemic condition. Both NADPH oxidase and mitochondrial electron gradient play roles in hyperglycemia-induced ROS generation. In addition to their ability to directly inflict macromolecular damage, ROS can function as signaling molecules. ROS mediate hyperglycemia-induced activation of signal transduction cascades and transcription factors leading to transcriptional activation of profibrotic genes in the kidney. Furthermore, ROS-activated signaling molecules generate and signal through ROS and thus ROS act as a signal amplifier. Intensive glycemic control and inhibition of angiotensin II delay the onset and progression of diabetic nephropathy, in part, through prevention of overproduction of ROS. Conventional and catalytic antioxidants have been shown to prevent or delay the onset of diabetic nephropathy. Combination of strategies to prevent overproduction of ROS and to increase the removal of preformed ROS may prove to be effective in preventing the development and progression of diabetic nephropathy (Hunjoo *et al.*, 2008).

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 *et a*l., 2001).

Green leafy vegetables (GLV's) are rich source of vitamins such as beta carotene, ascorbic acid, folic acid and riboflavin as well as minerals such as iron, calcium and phosphorous. They also contain an immense variety of bioactive non-nutritive health promoting compounds such

as antioxidants and phytochemicals, which provide health benefits beyond basic nutrition. Green leafy vegetables have long been recognized most abundant sources of protein, vitamins and minerals (Aletor *et.al.,* 2002). Antioxidants vitamins like ascorbic acids, phenols etc. are important in human food since they function as an anticancer agent (Shibata *et al.,* 1992). Many leafy vegetables especially, amaranth, fenugreek, palak and spinach has attained commercial status and its cultivation is wide spread in India. Because of their low production cost and high yield, GLV's are considered to be one of the cheapest vegetables in the market and it could be rightly described as 'poor man's vegetables' (R.K.Yadav *et.al.,* 2013). Seeing the increasing evidence that reactive oxygen species (ROS) play a major role in the development of diabetic complications and green leafy vegetables have great potential of antioxidants and phytochemicals, hence the study was planned with the following objectives to formulate the dehydrated drumstick leaf tablet for conducting the supplementation study and to study the influence of drum stick leaf tablets on lipid profile of diabetics.

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.

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. 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 II 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 urea oral hypoglycemic agents, 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 1,800Kcal. 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

The following lipid profile parameters had been analyzed to study the impact of drumstick leaves tablets on lipid profile of the selected type II diabetic patients: Total cholesterol, High density lipo proteins (HDL), Low density lipo proteins (LDL), Very low density lipo proteins (VLDL) and Triglyceride. Commercially available kit was used for the analysis and analysis carried out in the (Hitachi-704) auto analyzer. The procedure followed based on the Tietz, 1998.

STATISTICAL ANALYSIS

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

RESULTS AND DISCUSSION

TOTAL CHOLESTEROL, HIGH DENSITY LIPOPROTEINS (HDL), LOW DENSITY LIPOPROTEINS (LDL), VERY LOW DENSITY

LIPOPROTEINS (VLDL) & TRIGLYCERIDES (TGL)

Total cholesterol level of the study group is given in Table 1. In control and experimental group, total cholesterol level was found to decrease after the study period of 90 days. Initially in control group, total cholesterol was noticed as 217 mg/dl, but after three months of study period it decreased to 214 mg/dl and this reduction was not significant. In experimental group, total cholesterol level was found to decline from the initial

value of 227 mg/dl to 211 mg/dl after 90 days of supplementation with drumstick leaf tablet. The mean difference was 16 mg/dl and it was highly significant. It confirmed that supplementation with drumstick leaf tablet had positive effect in reducing total cholesterol.

Table 1- Mean total cholesterol (mg/dl) level of control and experimental groups

Details	Initial	Final
Control group	217±18	214±16[a]
Experimental group	227±11	211±15[b,c]

a-initial Vs final p<0.678[NS], b-initial Vs final p<0.0015[**], c-control Vs experimental p<0.627[NS]

Table 2 reveals that drumstick leaf tablet had positive impact on HDL of the diabetic patients. In experimental group HDL increased from an initial of 42 mg/dl to 47 mg/dl. The mean increase was 5 mg/dl after the supplementation of drumstick leaf tablet. But in control group a slight change was noticed in HDL levels which increased from 43 mg/dl to 45 mg/dl. This increase was not statistically significant.

Table 2- Mean high density lipoprotein (mg/dl) (HDL) level of control and experimental groups

Details	Initial	Final
Control group	43±6.32	45±6.80[a]
Experimental group	42±3.71	47±5.37[b,c]

a-initial Vs final p<0.413[NS], b-initial Vs final p<0.00001[**], c-control Vs experimental p<0.544[NS]

From Table 3, it is clear that LDL level of the experimental group reduced during the study period. The mean LDL level of both groups decreased after the study period while following a standardized diet (1,500 – 1, 800 K.Cal). In control group, the mean LDL reduced from an initial value 140 mg/dl to 136 mg/dl after three months of study period. This reduction was 4 mg/dl and it was not significant. In the experimental group, a significant decrease was noticed from the initial value of 149 to 135 mg/dl after the 90 days of supplementation of drumstick leaf tablets. It shows the positive effect of drumstick leaf tablet on diabetic patients.

Table 3- Mean low density lipoprotein (mg/dl) (LDL) level of control and experimental groups

Details	Initial	Final
Control group	140±18	136±18[a]
Experimental group	149±12	135±14[b,c]

a-initial Vs final p<0.471[NS], b-initial Vs final p< 0.00036[**], c-control Vs experimental p<0.743[NS]

Table 4 depicts the very low density lipoprotein level of control and experimental group during the study period. Under a standardized diet, both experimental and control group patients showed positive VLDL reduction. This reduction was not significant in control group, but drumstick leaf tablet supplemented experimental group exhibited a significant reduction. It emphasis that drumstick leaf tablet had a positive impact on VLDL levels of diabetic patients. In experimental group, VLDL was found to be reduced to 30 mg/dl from the initial value of 35 mg/dl after supplementation with drumstick leaf tablets. In control group, mean VLDL was noticed as 30 mg/dl after 90 days study period from the initial VLDL of 34 mg/dl.

Table 4- Mean very low density lipoprotein (mg/dl) (VLDL) level of control and experimental groups

Details	Initial	Final
Control group	34±5.66	30±4.83[a]
Experimental group	35±6.21	30±3.94[b,c]

a-initial Vs final p<0.131[NS], b-initial Vs final p<0.00029[**], c-control Vs experimental p<0.648[NS]

Data on triglyceride level of the subjects is expressed in Table 5. The mean TGL of both control and experimental group showed that supplementation of drumstick leaf tablet had a positive effect on the triglyceride level. In experimental group, drumstick leaf tablet supplementation had a significant reduction on triglyceride levels, from the initial value 160 mg/dl to final value 151 mg/dl after 90 days of study. In control group, the mean value was reduced from the initial value of 155 to 150mg/dl at the end of the study period. The mean reduction noticed in the control was not significant

Table 5- Mean triglyceride (mg/dl) level of control and experimental groups

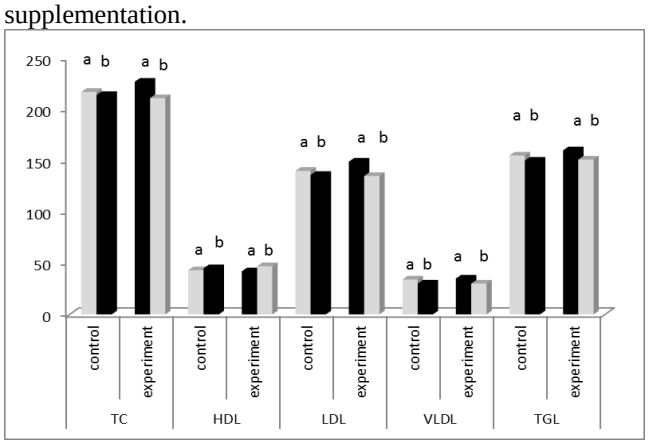
Details	Initial	Final
Control group	155±15	150±14[a]
Experimental group	160±18	151±20[b,c]

a-initial Vs final p<0.235[NS], b-initial Vs final p<0.029[*], c-control Vs experimental p<0.97[NS]

Goyle and Singhal (2004) reported that antioxidant play an important role in mitigating oxidative stress. They assessed the effect of antioxidants, vitamins E, A, C and flavonoids on serum lipid levels of coronary heart disease patients. All vitamins and flavonoids were reported to reduce total and LDL cholesterol efficiently and raise HDL cholesterol. Particularly supplementation with vitamin A lowered LDL cholesterol by about eight percent.

In the present study the diabetic experimental group after feeding the carotenoid rich drumstick leaf tablet, exhibit a significant improvement in lipid profile parameter. The previous studies are in agreement with the finding of present study.

Amirthaveni and Shrilakshmi (2003) reported that the HDL increased was from 52.8 mg/dl before supplementation to 58.8 mg/dl after 60 days of supplementation.



a- initial b-final, TC-Total Cholesterol, HDL-High Density Lipoproteins, LDL-Low Density Lipoproteins, VLDL-Very Low Density Lipoproteins, TGL-Triglycerides

Figure 1- Comparison of lipid profile between control and experimental group

Supplementation of β-carotene and ascorbic acid was effective in increasing the HDL level significantly. With regard to LDL, it was recorded as 142.3 mg/dl initially which after 60 days of supplementation reduced to 124.2 mg/dl. With regard to VLDL, it reduced to 33mg/dl from 37.2 mg/dl, after two months of supplementation.

Gorinstein *et al.* (2004) stated that consumption of 100 ml fresh Israeli Jaffa sweetie juice by coronary artery disease patients significantly improved the serum lipid levels. This was due to the presence of high levels of dietary fibres and antioxidant compounds in the fresh fruits which had positive influence on serum lipid levels and improve the serum antioxidants in coronary artery disease patients.

Amirthaveni and Thirumanidevi (2004) reported that administration of vitamin E (100g) capsules brought about a decline in total cholesterol values from 270 to 245 mg/dl which was a significant at 1 percent level. A combined administration of vitamin E capsules and vitamin C tablets brought a reduction in levels from 286 to 264 mg/dl which was significant at 1 per cent level. The effect of vitamin E capsules and vitamin E capsule plus on LDL cholesterol showed a reduction from 191 to 166 mg/dl and 204.6 to 182.8 mg/dl respectively, which was significant at one percent level. With regard to VLDL cholesterol, non-significant changes were seen in experimental group when compared with the control group. This may probably be due to the mild effect of antioxidant vitamins E and C derived from synthetic and dietary sources on VLDL cholesterol. HDL cholesterol levels on supplement with vitamin C tablet, showed an increase from 39 to 42 mg/dl. Changes were significant at 5 per cent level. Combination of vitamin E and vitamin C tablets to experimental groups showed an increase when compared to control group and the difference was significant at 5 per cent level.

Similar findings were observed in the present study also. Supplementation with drumstick leaf tablet was noticed to have a desirable effect on reducing the VLDL, LDL, total cholesterol and TGL levels besides increased the HDL levels of the experimental group significantly.

Amirthaveni and Thirumanidevi (2004) reported that all lipid values except HDL cholesterol level reduced after the supplementation with soaked fenugreek seeds. Total cholesterol level decreased from 219 to 164.4 mg/dl. The LDL level decreased from 149.1 to 127.9 mg/dl. The triglyceride level decreased from 188.8 to 167.7 mg/dl. The HDL level increased from 33.6 to 47.4 mg/dl. These findings are confirmed in the present study by proving that drumstick leaf tablets have a positive impact on lipid profile of diabetic patients.

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