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Original Article

IMPACT OF ANTIOXIDANTS FROM DRUMSTICK LEAVES ON THE LIPID PROFILE OF HYPERLIPIDEMICS

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Abstract: Drumstick leaves have been reported to be rich sources of several antioxidants such as polyphenols and carotenoids which may be beneficial for the prevention of several chronic degenerative disorders (CDDs). The present study was planned to assess the impact of dehydrated drumstick leaf tablets (DDL tablets) on hyperlipidemic subjects for a period of 50 days 20 subjects formed the experimental group (serum total cholesterol >180 mg/dl and/or serum triglycerides e" 140 mg/dl), twenty subjects with similar biochemical profile formed the control group. General information, personal habits, medical history, 24-h dietary recall, anthropometric measurements like height, weight, waist, hip, BMI and waist to hip ratio was recorded at baseline. A standardized DDL tablet used for the trials contained 575 mg of DDL. While no supplementation was given to the control group of subjects, the experimental group received 8 tablets per day of the dehydrated drumstick leaves (which is equivalent to 4.6 g DDL powder) for 50 days. The DDL tablet supplementation had an overall positive impact on the lipid profile, wherein significant reduction in the non-HDL values was observed. Further efforts on developing a database on Indian fruits and vegetable antioxidant profile and their effect on various chronic degenerative diseases are warranted.

Key words: drumstick leaf tablets, hyperlipedemia, polyphenols

INTRODUCTION

Moringa oleifera Lam. (drumstick tree, horseradish tree) is indigenous to northwestern India and is often cultivated in hedges and home yards. The tree is valued mainly for the tender pods, which are esteemed as a vegetable. Our studies indicate that drumstick leaves with their high beta carotene content have a great potential not only to combat micronutrient deficiencies such as vitamin A, can be easily incorporated into our traditional diets and could be a recommended for overall health improvement [1-9].

Moringa oliefera is reported to contain alkaloids, flavonoids [8,10], anthocyanins, proanthocyanidins and cinnamates and is highly reputed in folklore and traditional system of medicine as a remedy for variety of ailments [11,12]. Ghasi et al., [13] have found that administration of the crude leaf extract of *Moringa oleifera* (drumstick leaves) along with a high-fat diet decreased the high-fat diet-induced increases in serum, liver, and kidney cholesterol levels by 14.4, 6.4, and 11.1%, respectively, in Wistar rats. Recently, Estrella et al.,[14] reported that *M. oleifera* leaves increase breast milk production day from the third postpartum day to the fifth among mothers who delivered preterm infants. In most parts of Philippines, women consume moringa leaves mixed in chicken or shellfish soups to enhance breast milk production.

Siddhuraju and Becker [15] examined the radical scavenging activities and antioxidant capacities of

water, aqueous methanol, and aqueous ethanol extracts of freeze-dried leaves of *Moringa oleifera Lam.* from different agroclimatic regions. The results revealed that among the three different *Moringa* samples, both methanol and ethanol extracts of Indian origins showed the highest antioxidant activities. By combining the knowledge of indigenous people with laboratory assessments of in vitro antioxidant activity and suitable in vivo experiments, it can be shown that nutritionally enriched *Moringa* leaves can serve as a food source and that their extracts, particularly those containing dietary polyphenolic substances, may have potential as "nutraceuticals" for improving the health of the human population. A review of literature revealed several traditional usages of these leaves in the healing of chronic degenerative diseases [16]. However, to our knowledge, there are no clinical human trials reports on the effect of *Moringa* leaves on the lipid profile of the hyperlipidemic subjects.

With the above facts in mind, the present study was planned. The major objectives of the study were to study the effect of dehydrated drumstick leaf tablets on the lipid profile of hyperlipidemic subjects.

MATERIAL AND METHODS

Effect of Dehydrated Drumstick Leaf Tablets on the Lipid Profile of Hyperlipidemic Subjects

Tablet development: In order to facilitate the clinical trials, dehydrated drumstick leaf tablets were standardized after several experiments with the help of a pharmacist in a local pharmaceutical company. A standardized DDL tablet used for the trials contained 575 mg of DDL.

Enrolment and Criteria for Selection of hyperlipidemic Subjects: For the initial selection of hyperlipidemic subjects, the records of MS University employees attending the university health center and for whom the health center records provided lipid values were screened and among the 162 such subjects, 40 hyperlipidemic subjects were selected and consent obtained. Forty hyperlipidemic subjects were enrolled and randomly divided into two groups control (n=20) and experimental where (n=20). The criteria for selection of subjects from the University Health Centre included :-

(1) Confirmed hyperlipidemics with serum total cholesterol > 180 mg/dl and/ or serum triglycerides e" 140 mg/dl by the physician.
(2) No apparent complications.
(3) Willingness to participate in the study.

The entire study was approved by the ethical committee. However due to dropouts, the final data is presented for 35 subjects, (E=17, C=18).

Supplementation: While no supplementation was given to the control group of subjects, the experimental group received 8 tablets per day of the dehydrated drumstick leaves (which is equivalent to 4.6 g DDL powder) for 50 days. Thus each subject in the experimental group received 3700 mcg beta carotene, 1775 mg total phenols and 0.28 g fiber per day (Methods reported in our earlier paper, Nambiar et al.,[7]. Four tablets were consumed by the subjects in the presence of the investigator and 4 during dinner time every day for the period of 50 days.

Parameters studied: General information, personal habits, medical history, 24-h dietary recall, anthropometric measurements like height, weight, waist, hip, BMI and waist to hip ratio was recorded at baseline. Overnight fasting and venous blood sample was drawn using disposable needle and syringes. About 5 ml of blood was drawn and collected in centrifuge tubes. The serum was separated and used for various biochemical analysis. These included estimations of FBS and lipid profile. Estimation of Serum Sugar: was done by the enzymatic kit supplied by Glaxo India Limited (GOD/POD method) [17]. Estimation of Triglycerides: The triglycerides in the serum were estimated by the GPO/POD method using the enzymatic kit [18]. Estimation of Total Cholesterol: Total cholesterol was estimated using diagnostic kit supplied by Glaxo India Ltd [19]. Estimation of High Density Lipoprotein (HDL) Cholesterol: The serum HDL-C was estimated by using the method of Warnick et al.,[20] In serum, LDL-C and VLDL-C was precipated by the addition of phosphotungstic acid and magnesium chloride using the Warnick et al., [20] method. The supernatant was used for HDL-C estimation by the Enzokit as described in total cholesterol estimation. Estimation of Very Low Density Lipoprotein (VLDL) Cholesterol: VLDL-C was calculated by dividing triglyceride values by five (TG/5). Estimation of Low

Table 1 : Socio-economic status of the hyperlipidemic subjects

VARIABLE	TOTAL %	
	MALES	FEMALES
	n=27	N=9
Age (years)		
41 – 45	20	28
46 – 50	28	36
51 – 55	40	27
56 – 60	12	9
Marital Status		
Married	100	100
Unmarried	-	-
Religion		
Hindu	100	100
Others	-	-
State		
Gujarat	57	82
Others	43	18
Occupation		
Professional	57	
Business	15	
Retired	28	
House-wife	-	100
Education		
Illiterate	-	2
Primary	-	9
High School	4	19
Graduate	32	36
Post-Graduate	21	34
Ph. D.	43	-
Type of family		
Nuclear	75	82
Joint	25	18

Density Lipoprotein (LDL) Cholesterol: VLDL-C was calculated by the difference using Friedlewald's formula. LDL-C = TC - (HDL-C + VLDL)

Post data was collected for anthropometric parameters and biochemical indices.

Statistical Analysis: The data collected was

Table 2 Anthropometric profile of the hyperlipidemic subjects (Mean±SD)

VARIABLE (n = 39)	EXPERIMENTAL		CONTROL	
	MALES	FEMALES	MALES	FEMALES
	n=14	n=3	n=12	n=6
AGE (years)	50 ± 4.27	49 ± 4.17	49 ± 4.60	47 ± 3.20
HEIGHT (cm)	165.1 ± 7.70	153.9 ± 6.89	164.4 ± 6.51	152.4 ± 2.50
WEIGHT (Kg)	67.2 ± 5.00	65.2 ± 4.69	73.2 ± 9.47	66.6 ± 3.12
BMI*	24.7 ± 2.50	27.8 ± 2.77	27.3 ± 2.65	28.8 ± 1.07
WAIST/HIP	0.93 ± 0.02	0.89 ± 0.03	0.89 ± 0.04	0.87 ± 0.01

* BMI = Body Mass Index = Wt (Kg) / Ht (m)

Table 3 Nutrient intake of the hyperlipidemic subjects (mean±SD)

NUTRIENTS	EXPERIMENTAL		CONTROL	
	MALES	FEMALES	MALES	FEMALES
	n=14	n=3	n=12	n=6
Energy (Kcal)	2083 ± 133.37	2155 ± 142.4	2271± 186	2194± 102
Protein (g)	55.9 ± 19.2	59.2± 171.	57.4± 10.8	52.3± 9.74
Fats (g)	70.1 ± 16.99	68.9± 25.5	74.4 ± 19.86	75.6± 6.22
CHO (g)	312.8 ± 24.2	327± 60.7	337.1± 27.63	321.6± 21.38
Fibre (g)	11.82± 5.15	9.97± 3.62	12.21± 3.55	10.35± 0.72
ß-carotene (µg)	3533.6± 2944.9	2008.0± 918.46	5604.5± 531.9	3983.9± 1610.3
Vitamin C (g)	187.3± 47.2	168.7± 52.7	199.1± 43.9	102.4± 60.80

subjected to statistical analysis using the MS excel. The mean along with standard deviation was calculated; paired 't' test and independent 't' test were used to determine the significant changes between the two means.

RESULTS AND DISCUSSION

Socio-Economic Background, anthropometry and nutrient intake of the Hyperlipidemic subjects: Table 1 reveals that all the subjects in both the groups were married and Hindus. Anthropometric indices of the subjects reveals that majority of the subjects were overweight. Abdominal obesity was observed both the male and female subjects, wherein the female subjects reported a greater BMI and WHR than males (Table 2).

The mean nutrient intake of the hyperlipidemic reveals that (a) the caloric intake of all the subjects of the two groups was comparable and adequate. The mean caloric intake of all the groups was approximately 2000 K cal. (b) the fat consumption was seen on the higher side than the recommended 20 gms for normal adults. The highest fat consumption was found in the control group which was 75 g/day. The percent calories coming from fats ranged from 27% to 31% which is more than the recommended allowance of d" 20% [21].

Table 4 : Impact of DDL tablets on the lipid profile of the hyperlipidemic subjects

Biochemical parameter	Experimental Group (n=17)		Control Group (n=18)	
	PRE	POST	PRE	POST
FBS mg/dl	87.83±1.12	88.98±0.9	83.9±2.5	86.5±1.12
T value	-1.04		-1.21	
TC mg/dl	187.14±2.92	184±3.14	186.14± 2.42	184.49±1.60
T value	2.42*		0.75	
TG mg/dl	180.71±3.16	179.24±3.03	173.36±3.29	173.16±2.83
	0.59		0.11	
HDL mg/dl	38.06±0.37	40.44±0.34	39.81±0.33	40.04±0.38
T value	-5.71		-0.47	
LDL mg/dl	111.28±3.01	109±3.26	106.89±2.20	104.22±1.84
T value	0.608434		1.68	
VLDL mg/dl	36.1±0.63	36.07±0.59	34.63±0.6	34.34±0.56
T value	0.05		0.77	
NON-HDL mg/dl	149.08±2.95	143.60±3.19	146.23±2.5	144.38±1.69
T value	4.74***		0.78	
TC/HDL	4.58±0.19	4.28±0.16	4.67±0.08	4.60±0.27
	4.47***		0.77	
TC/LDL	1.69±0.02	1.69±0.03	1.74±0.02	1.77±0.02
	0.10		- 1.27	
LDL/HDL	3.27±0.19	2.99±0.18	2.68±0.05	2.59±0.05
	6.52***		1.46	

Significant: * = p≤0.05, ** = p≤0.01, *** = p≤0.001

When compared to the RDA for Indians [21], the recommended % intake of calories from carbohydrates, proteins and fats is 71%, 9% and 20% respectively. (c) The fiber intake was lower than the recommended value of 20 gms/day and ranged from 7 gms to 9.8 gms/day. Fiber may alter the proportion of cholesterol into chylomicrons and lipoproteins. (d) Wide variations were seen in the ß-carotene intake in both the groups (Table 3).

Only the male subjects consumed alcohol, tobacco and cigarettes. The baseline data on the lipid profile compared with the total fat intake indicated that the control group males had higher fat intake (> 60 gms) had higher TG.

Impact of Dehydrated Drumstick Leaves Supplementation on the Lipid Profile of Hyperlipidemic Subjects : The results of the lipid profile of the subjects post DDL supplementation for 50 days reveal no particular change in the FBS levels when the experimental and control groups were compared. However a decrease in the TC values (pd"0.5), (1.65%), and marginal non-significant decreases in the TG and LDL values and a mild increase in the HDL (6.25%) values were observed in the experimental group (Table 4).

Table 4 also indicates that there was a decrease (p<0.001) in the Non- HDL-C values in the

experimental group males. Thus, the data indicates the positive beneficial impact of drumstick leaves supplementation on the Non- HDL-C values. However, the Non- HDL-C values also increased in the control group. This fact is also reflected in the results of the atherogenic indices expressed as TC/HDL, TC/LDL and LDL/HDL which are significantly lower as compared to the pre supplementation data (pd"0.001).

The NCEP (National Cholesterol Education Program) (National Institute of Health-US) in 2001 and the ATP III guidelines identifies a second lipid target called Non- HDL-C and suggests that treating Non- HDL-C should be the primary target for the prevention of CHD. Non- HDL-C is defined as the difference between TC and HDL-C (includes LDL, IDL and VLDL-C), is a strong risk factor for ischemic heart disease and a better predictor than the traditional lipid risk factors, that is, LDL-C and VLDL-C. It represents the total cholesterols carried in all the potentially atherogenic lipoprotein particles. Studies show that both LDL-C and non HDL-C are risk predictors in normo-triglyceridemic patients but in patients with hypertriglyceridemia non HDL-C is a better predictor [22].

To our knowledge, this is the first clinical human trial highlighting the impact of *Moringa Oleifera* leaves on the lipid profile. The present study suggests that drumstick leaf tablets, with a high amount of beta carotene, polyphenols as well as fiber has had a mild positive impact on the lipid profile of the hyperlipidemic subjects. Future studies with a higher dosage of DDL for a longer duration need to be conducted.

In most of the developed and developing nations, there is a rapid emergence of cardiovascular death rate in association with various risk factors like glucose intolerance, insulin resistance and hypertension. The situation on this university setup, where the present study was conducted and in the urban setting is no different [23-25]. Thus, there is an urgent and immediate need for combating against the rapid increase in the number of cardiovascular deaths[26, 27]. Therefore, WHO has designed the global strategy on diet, physical activity and control of Non-Communicable Diseases and India has also adopted the global strategy and has recommended "Settings Approach" as one of the strategy for implementation of Health Promotion Programs. "Different settings like Universities, Schools, Market Places, Hospitals and Workplaces should be identified so that large audiences can be targeted at one time" [26].

Several studies have shown that increased dietary intake of natural phenolic antioxidants correlates with reduced coronary heart diseases and longer life expectancy. Ganeshan et al., [28] reported that a higher fruit and vegetable intake was inversely associated with several risk factors for CVD such as waist circumference, BMI, total cholesterol and LDL-cholesterol concentration, independent of age, sex, smoking status, alcohol, BMI and total energy intake. Overall fruit and vegetable intake explained 48% of the protective effect against CVD risk factors. Phytochemicals are known for their properties such as free radical scavenging, strong antioxidant activity, inhibition of hydrolytic and oxidative enzymes (phospholipase A2, cyclooxygenase, lipoxygenase), and anti-inflammatory action and therefore has aroused a growing interest among researchers. Fruits and vegetables have long been recognized as healthy foods and as valuable sources of antioxidant vitamins and minerals. But their contribution to good health goes much further than this. It is now well recognized that fruits have protective effect against some of the wide range of chronic diseases affecting our society. Narasinga Rao [29] suggested that future research in the science of nutrition may be profitably directed towards evaluating the potency of phytochemicals in foods and diets and their health implications.

Nutritionist should now focus on initiation of work place wellness program in several settings and emphasize the role and use of functional foods such as drumstick leaves and robustly introduce therapeutic lifestyle changes even in the academically sound but physically inactive population.

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