

Cholesterol - Lowering Potential of Probiotic Yogurts Containing *Lactobacillus acidophilus* and *Streptococcus Thermophiles* on Mild Hypercholesterolemic Male Patients

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Abstract: Ninety mild hypercholesterolemic males aged 40 -50 years, free from serious complications were selected and divided equally into three groups viz. E₁, E₂ and C. Subjects of group E₁ were provided 150 ml of probiotic yoghurt containing only *Lactobacillus acidophilus* (MTCC-447) in the inoculum rate of 1.5% v/v and E₂ with 150 ml Probiotic yoghurt containing *Lactobacillus acidophilus* (MTCC-447) and *Streptococcus thermophilus* (MTCC-1938) in the inoculum rate of 1.0% v/v for a period of 2 months respectively, while group C was not given any supplementation. Blood lipid profile of all the subjects was recorded before and after supplementation period. A highly significant ($p \leq 0.01$) reduction in TC, LDL-C, VLDL-C and TG and significant ($p \leq 0.05$) increase in HDL -C was reported in both the experimental groups, wherein group E₁ showed the best results. The ability of the probiotics to assimilation (removal) of cholesterol and deconjugation of bile acids in the small intestine may be important in lowering the blood cholesterol concentration, consequently in reducing the risks of Coronary Heart Disease (CHD). Thus probiotic yogurt supplementation containing more than one probiotic strain can be a panacea in counteracting the problems of heart patients.

Keywords: blood lipid profile, hypercholesterolemic, inoculum, *lactobacillus acidophilus*, probiotic yogurt supplementation, *streptococcus thermophilus*.

1. Introduction

Coronary Heart Disease is one of the major causes of mortality and morbidity in population of both developed as well as developing countries. Cardiovascular disease now ranks as the world's top cause of death, causing one third of all deaths globally. The WHO has predicted that by the year 2030, cardiovascular disease will remain the leading cause of death, affecting approximately 23.6 million people around the World. Major risk factors for CHD are high LDL cholesterol, low HDL cholesterol, hypertension, diabetes mellitus, improper diet, sedentary lifestyle, obesity, physical inactivity, cigarette smoking etc. leading to hypercholesterolemia and hypertriglyceridemia.

The human gastrointestinal tract (GIT) plays an important function in overall cholesterol metabolism, as it is the site of synthesis and absorption of cholesterol. Since it is also a habitat of diversified microflora, hence it exerts some effect on the cholesterol metabolism. A dairy product containing probiotics makes a healthy "functional food package" in addition to the vitamins, calcium, other minerals, and protein obtained from milk products. Consumption of three or more servings of dairy products each day has been associated with lower levels of obesity, and hence lower incidence of hypertension and heart disease. The DASH (Dietary Approaches to Stop Hypertension) diet also recommends three servings of low fat dairy products. Considering all these findings, dairy products combined with probiotic bacteria results into improved health status (US Probiotics.org 2008).

2. Literature Survey

For the first time, Mann (1977) found that large dietary intakes of yogurt lowered serum cholesterol in humans. Since then, many reports have appeared suggesting strain specific hypocholesterolemic effect of Lactic acid bacteria (LAB), including lactobacilli. Ingestion of fermented milk containing probiotic LAB might be a natural way to decrease serum cholesterol and blood pressure in humans (Bazarre et al 1983). Many literature reports the reduction in serum cholesterol following the consumption of fermented milk and milk products containing probiotics has also been attributed to the production of Hydroxyl Methyl Glutaryl (HMG) by LAB, which inhibits HMG CoA reductases required for the synthesis of cholesterol (Shah, 2001). Studies conducted earlier have also shown that dairy foods fermented with specific probiotic bacteria can produce modest reduction in the total LDL cholesterol levels and blood pressure.

There are various experiments that suggest a range of potentially beneficial medicinal use of probiotics in CHD. Also in the year 2009, Tanaka et al demonstrated that when capsules containing heat sterilized (100 mg) of *Lactobacillus paracasei* were fed to 40 male and female subjects with borderline and mild hypercholesterolemia, it significantly decreased the serum levels of total cholesterol by 9.4% and LDL cholesterol by 13.2%. In *in vitro* study, Lye et al. (2010) evaluated the conversion of cholesterol to coprostanol by strains of lactobacilli such as *Lactobacillus acidophilus*, *L. bulgaricus* and *L. casei* ATCC 393 via fluorometric assays. The authors detected both intracellular and extracellular cholesterol reductase in all strains of probiotics examined, indicating possible intracellular and

extracellular conversion of cholesterol to coprostanol. The concentration of cholesterol in the medium also decreased upon fermentation by probiotics accompanied by increased concentrations of coprostanol.

3. Materials and Methods

Ninety male subjects aged 40-50 years at risk CHD (TG levels \geq 160 mg/dl and/or total cholesterol levels \geq 200mg/dl) were selected and divided equally into three groups i.e. 30 each.

E₁ group was given 150 ml of Probiotic yogurt/day containing *Lactobacillus acidophilus* for a period of 60 days. E₂ group was given 150 ml of Probiotic yogurt/day containing both *Lactobacillus acidophilus* and *Streptococcus thermophilus* for a period of 60 days. C group was not supplemented with any Probiotic Yogurt. The required data was collected through personal interview technique using the specially structured schedule. The subject's willingness to take part in the supplementation trial was also ascertained.

3.1 Bio-Chemical Estimation

Collection of blood samples: -Blood samples were collected early in the morning at fasting and post- fasting. Blood samples (10 ml) were collected in the beginning and at the end of the study, from antecubital arm area into a centrifuge tube by the technician using 10 ml disposable syringe disposable. About 5 ml of blood was transferred into a vial having anticoagulant (EDTA 1 mg/ml blood in the form of thin layer), which was gently to dissolve the anticoagulant. The rest 5 ml blood to clot and centrifuged at 3000 rpm for 15 minutes to separate serum which was stored in acid washed mineral free glass vials and frozen. Serum samples were analyzed for lipid profiles and glycemic index.

3.2 Analysis of Blood Samples

The serum was analyzed for Glucose, Triglycerides, cholesterol, High density lipo-protein cholesterol, Low density lipo-protein cholesterol and very low density lipo-protein cholesterol.

3.3 Estimation of Serum total Cholesterol, Triglycerides and Lipoproteins

a) Total Serum Cholesterol

Total serum cholesterol was analyzed by BIOTRON BTR 820 auto blood analyzer using enzymatic method. (Richmond, 1973).

b) Reagent Used

Buffer/ enzymes/ chromogen, Phenol, Standard: Cholesterol 200 mg/dl

Preparation of the Working Solution (code 6376) - Allowed the reagents to attain the room temperature.

Solution 1: Added 64 ml distilled water to one bottle one. Mix it gently till the contents were completely dissolved.

c) Reagent (2) Ready for use

Daily working solution: Allowed the solution 1 and 2 reagent to attain room temperature and mixed one volume of

solution 1 with one volume of reagent 2 and stored this in dark bottle.

Procedure: The sample and the daily working solution was brought to room temperature prior to use. The following general system parameters were used:

Reaction type-end point, Wave length – 505 nm (505-530), Flow cell temperature-30 degree C, Incubation – 30 min RT/5 min/37 degree C, Sample volume – 10 μ l, Reagent volume- 1.0 μ l, Standard Concentration – 200 mg/ dl, Zero setting with – 200 mg/dl

Mixed the contents of the test tube and incubated for five minutes at 37°C. Aspirated the reagent blank using aspiration switch, waited while the instrument was calculating the factor which was printed out. Finally aspirated the reacted sample number 1 and the total concentration of the total cholesterol were printed out by the instrument within five seconds. Same procedure was followed by sample number 2 and other samples.

d) Serum High Density Lipoprotein Cholesterol (HDL-C)

It was measured by using BIOTRON, BTR 820 using phototungstate method (Lopes Virella *et al* 1977).

e) Reagents (Supplied in the kit)

- HDL-Cholesterol (Buffer/Enzymes/Chromogen), HDL-Cholesterol (phenol), Precipitating reagent, HDL cholesterol Standard: 50 mg/dl
- Preparation of the working solution: Allowed the reagents to attain the room temperature:
- Solution 1- Added 29 ml of distilled water to one bottle and mixed it.
- Reagent 2 – was ready for the use.
- Precipitating Reagent – ready for use.
- Standard 50 mg/dl- ready for use.
- Preparation of Daily Working Solution (code 6680)

Allowed the solution one and reagent two to attain the room temperature. Mixed one volume of solution 1 and one volume of reagent 2.

Procedure -The sample and the reagent were brought to room temperature prior to use. A 0.20 ml sample (200 μ l) was used for the precipitation and pipette into centrifuge tubes with 0.20 ml (200 μ l) precipitating reagent. These solutions were mixed well and then centrifuged at 3500-4000 rpm for 10 minutes. Separated the cleared supernatant immediately and determined the cholesterol content as given below.

Cholesterol assay

The following general parameter systems were used: Reaction type- end point, Wave length – (505-530), Flow cell temperature- 30 degree C, Incubation – 30 min RT/5 min / 37 degree C, Sample volume – 200 μ l, Precipitating reagent volume – 200 μ l, Supernatant volume – 20 μ l, Reagent volume – 1.0 ml, Standard concentration – 50 mg/dl, Zero setting- with reagent blank.

Mix the contents of the test tubes and incubate for five minutes at 37°C. aspirated the reagent blank using the aspiration switch, waited while the instrument was calculating the factor which was printed out. Finally aspirated the reacted sample number 1 and total concentration of total cholesterol was printed out by the instrument within five seconds. This was followed by sample number 2 and other samples.

f) Serum Low density Lipoprotein Cholesterol

The value of LDL-C calculated was based on the Friedwald equation (Friedwald *et al* 1972)

LDL-Cholesterol (mg/dl) =

$$\frac{\text{Total Cholesterol} - \text{Triglyceride} - (\text{HDL} - \text{Cholesterol})}{5}$$

Serum Very Low Density Cholesterol (VLDL-C) calculation was based on

$$\text{VLDL} = \frac{\text{Triglycerides}}{5}$$

g) Serum Triglycerides:

Serum triglycerides were estimated by using auto pack reagent kit by method enzymatic DHBC colometric method (Fossati and Principle 1982).

Reagents- Enzymes/chromogen, Buffer/chromogen, Stabilizer, and Standard: 200 mg/dl ready for use.

Preparation of the Working solution (code 6630)

Allowed the reagent to attain the room temperature. Added 5.5 ml of reagent 2 and 5.5 ml of reagent 3 of the contents of one bottle of reagent 1 mixed to dissolve completely.

Procedure

The sample and working solution was brought to room temperature prior to use. The following general system parameters were used with this kit:

Reaction type- end point, Wave length- 505 nm (500-530 nm), Flow cell temperature – 30 degree C, Incubation – 15 min RT, Sample volume – 10 µl, Reagent volume – 1.0 ml, Standard concentration – 200 mg/dl, Zero setting with – reagent blank

Mixed the samples with the contents of the tubes and incubated for fifteen minutes at the room temperatures. Aspirated the reagent blank using aspiration switch, waited while instrument demanded for it. Wait again while the instrument was calculating the factor which was printed out. Finally aspirated the reacted sample number 1 and the total concentration of triglycerides were printed out by the instrument within 5 seconds. This was followed for the other samples also.

Preparation of Probiotic Yogurt

Standardization of probiotic yogurt: -Milk was standardized to 3.5-4.0 per cent fat and was heated to 70°C and then two-stage homogenized at 65°C as shown in Fig.1. The homogenized milk was then pasteurized and cooled to 43°C. Milk was then inoculated with starter culture of *Lactobacillus acidophilus* (0.5%; 1.0%; 1.5%) in case of first set of samples. In case of another set *Streptococcus thermophilus* and *Lactobacillus acidophilus* were added at

different rates (0.5:0.5; 1.0:1.0; 1.5:1.5) to the yogurt. Inoculated milk was poured into cups and incubated at 42±1°C for 3hrs and 30 mins. The prepared product was subjected to organoleptic evaluation and supplemented to the experimental group.

Organoleptic evaluation: - The samples of different probiotic yogurts were evaluated for organoleptic qualities on the basis of color, appearance, texture, taste, aroma and overall acceptability by a panel of judges. Consumer acceptance for the products was evaluated on a nine point hedonic scale. (Amerinet *al* 1965) with following scale:

Scale	Sensory Score
Like extremely	9
Like very much	8
Like moderately	7
Like slightly	6
Neither like nor dislike	5
Dislike slightly	4
Dislike moderately	3
Dislike very much	2
Dislike extremely	1

h) Probiotic yogurt supplementation

Freshly 150 ml of Probiotic yogurt was prepared and packed in disposable bowls. Feeding trials of probiotic yogurt were carried out for a period of two months to Group E₁ containing only one strain *Lactobacillus acidophilus* (MTCC-447). Group E₂ was supplemented with probiotic yogurt containing two strains *Lactobacillus acidophilus* (MTCC 48) and *Streptococcus thermophilus*(MTCC-1938). The subjects were advised to consume the probiotic yogurt along with their lunch.

i) Statistical Analysis

The data on all the parameters viz. food and nutrient intake, anthropometric measurements and blood parameters was analyzed statistically before and after supplementation. The mean standard error, percentages, analysis of variance, CD value, paired t- test and their statistical significance was ascertained using a computer programme package (Cheema and Singh 1990). Relevant coefficients of correlation were also computed.

4. Results

Ninety at risk CHD males in the age group of 40-50 yrs were selected for the present study. The subjects were equally divided into three group's viz. E₁, E₂ and C i.e. 30 in each group. Subjects of E₁ and E₂ group were supplemented with 150 ml Probiotic yogurt containing only one strain viz. *Lactobacillus acidophilus* and 150 ml Probiotic yogurt containing two stains viz. *Lactobacillus acidophilus* and *Streptococcus thermophilus* for two months, whereas C group was not given any supplementation. Subjects were advised to consume Probiotic Yogurt during lunch time. Anthropometric measurements, blood pressure and blood lipid profile, were observed before and after the supplementation period in all the three groups.

5. Organoleptic Evaluation of Probiotic Yogurts

Probiotic Yogurt containing single stain *Lactobacillus acidophilus* (Table.1): -The mean score of the acceptability trials of the formulated yogurt by the expert panel of 10 judges using nine hedonic scale. Three samples of yogurt were prepared using different inoculum concentration of the stain (*L.acidophilus*) and normal commercially available curd was used as standard. The mean score of colour was highest for A3 (1.5% v/v) was 7.87 which was liked very much. The mean scores for appearance and flavor was 8.00

and 7.60 which was highest when compared to others samples. The mean score of texture and taste was also highest with in case of A3 i.e. 7.97 and 8.13 respectively in comparison to other samples (A1 and A2) as well as control. The overall acceptability was also highest 8.37, and was liked extremely. In case of samples with lower inoculum concentration decreased the mean score than A3 and the overall acceptability also less than sample A3. Therefore the male subjects at risk CHD were supplemented with A3, containing 1.5% v/v inoculums concentration of *Lactobacillus acidophilus*.

Table1: Organoleptic Scores of Probiotic Yogurt (Mean \pm SE)

Sample	Color	Appearance Acceptability	Flavor	Texture	Taste	Overall
Control	7.50 \pm 0.17	7.60 \pm 0.13	6.77 \pm 0.14	7.73 \pm 0.13	6.30 \pm 0.19	6.87 \pm 0.12
A1	7.63 \pm 0.14	7.30 \pm 0.13	6.97 \pm 0.11	7.07 \pm 0.14	7.16 \pm 0.11	7.33 \pm 0.11
A2	7.60 \pm 0.12	7.57 \pm 0.12	7.30 \pm 0.09	7.60 \pm 0.14	7.60 \pm 0.12	7.63 \pm 0.08
A3	7.87 \pm 0.14	8.00 \pm 0.16	7.60 \pm 0.11	7.97 \pm 0.13	8.13 \pm 0.12	8.37 \pm 0.12
B1	7.87 \pm 0.12	7.67 \pm 0.12	7.16 \pm 0.11	7.80 \pm 0.13	7.30 \pm 0.10	7.37 \pm 0.09
B2	8.17 \pm 0.14	7.90 \pm 0.13	7.73 \pm 0.12	8.07 \pm 0.10	8.10 \pm 0.11	8.30 \pm
B3	8.50 \pm 0.12	8.23 \pm 0.13	7.83 \pm 0.10	8.53 \pm 0.11	7.97 \pm 0.13	0.11
F-Ratio	6.44**	5.69**	11.66**	11.85**	24.00**	8.00 \pm 0.11
CD at 5%	.39	.38	.33	.36	.38	24.77**
						.31

* Significant at 5% level of significance

Control - Normal Curd

A1 = 0.5% *Lactobacillus acidophilus*

A2 = 1.0% *Lactobacillus acidophilus*

A3 = 1.5% *Lactobacillus acidophilus*

B1 = 0.5% *Lactobacillus acidophilus* + 0.5% *Streptococcus thermophilus*

B2 = 1.0% *Lactobacillus acidophilus* + 1.0% *Streptococcus thermophilus*

B3 = 1.5% *Lactobacillus acidophilus* + 1.5% *Streptococcus thermophilus*

Probiotic Yogurt containing two stains *Lactobacillus acidophilus* and *Streptococcus thermophiles* (Table.2): -The mean score of the acceptability trials of the formulated yogurt by the expert panel of 10 judges using nine hedonic scale. Three samples of yogurt were prepared using different inoculum concentration of the stains (*L.acidophilus* and *S. thermophilus*) and normal commercially available curd was used as standard. The mean score of colour was highest for B2 (1.0% v/v each) was 8.57 which was extremely liked. The mean scores for appearance and flavor was 8.23 and 7.83 which was highest when compared to others samples. The mean score of texture and taste was also highest in case of B2 i.e. 8.57 and 8.10 respectively in comparison to other samples (B1 and B3) as well as control. The overall acceptability was also highest 8.30, and was liked extremely. In case of samples with inoculum concentration 0.5 % v/v i.e. B1 and 1.5% v/v i.e. B3 decreased the mean score when compared to B2 and the overall acceptability also less than sample B2. Therefore the male subjects at risk CHD were supplemented with B2, containing 1.0% v/v inoculums concentration of both of stains viz. *Lactobacillus acidophilus* and

5.1 Streptococcus Thermophilus.

Blood Lipid Profile of the Subjects before and After the Supplementation

Blood lipid profiles of the subjects were assessed before and after the supplementation and are presented in Table.2. The mean values of total cholesterol before and after supplementation period in all the three groups were 209.33 \pm 5.12, 212.90 \pm 5.61, 218 \pm 4.47 and 180.06 \pm 4.46, 178.36 \pm 4.54, 219.5 \pm 5.14 mg/dl. A highly significant ($p < 0.01$) decrease was reported in E₁ and E₂ groups, whereas decrease was observed in group C but it was non-significant. The cholesterol lowering effect of the probiotic yogurt could be due to assimilation of the cholesterol by the stains for their own metabolism. As depicted in the Table.2, the initial and final mean values of TG recorded in the three groups were 206.1 \pm 6.51, 202.23 \pm 7.22, 210.6 \pm 5.45 and 192.73 \pm 5.62, 186.96 \pm 5.17, 212.4 \pm 4.77 mg/dl. A highly significant ($p < 0.01$) decrease was observed in group E₁ (Fig 1) and E₂ (Fig 2), whereas a non-significant decrease was observed in group C (Fig 3). The triglycerides are important since they influence lipid deposition and clotting mechanism. The initial and final mean values of HDL-C was reported as 41.06 \pm 1.46, 43.96 \pm 1.86, 44.73 \pm 2.25 mg/dl and 42.47 \pm 1.41, 45.67 \pm 0.97, 44.9 \pm 2.23 mg/dl in all the three groups, respectively. A highly significant ($p < 0.01$) increase was reported in group E₁ and E₂ whereas, a non-significant

decrease was observed in group C. The data collected revealed that the mean initial

Table 2: Lipid profile of the subjects before and after supplementation of Probiotic Yogurts

Variables	Group	Before	After	Difference	% Change	t-value	Reference standard
TC (mg/dl)	E ₁	209.33±5.12	180.06±4.46	-29.27	13.98	7.81**	
	E ₂	212.90±5.61	178.36±4.54	-34.54	16.22	5.40**	<200 [^]
	C	218±4.47	219.5±5.14	1.5	0.68	1.97**	
TG (mg/dl)	E ₁	206.1±6.51	196.73±5.62	-13.37	6.48	7.82**	
	E ₂	202.23±7.22	186.96±5.17	-15.27	7.75	5.27**	<150 [^]
	C	210.6±5.45	212.4±4.77	1.8	0.85	1.97NS	
LDL-C (mg/dl)	E ₁	127.04±5.25	108.88±4.92	-16.16	12.02	8.08**	
	E ₂	128.44±5.72	106.44±4.94	-22	17.12	9.53**	130-160 [^]
	C	131.14±5.01	132.12±4.76	0.98	0.75	1.40(NS)	
HDL-C (mg/dl)	E ₁	41.06±1.46	42.47±1.42	1.41	3.21	5.91**	
	E ₂	43.96±1.86	45.67±0.97	1.71	3.89	3.14**	40-60 [^]
	C	44.73±2.25	44.9±2.23	0.17	0.38	1.07(NS)	
VLDL-C (mg/dl)	E ₁	41.22±1.30	39.34±1.12	-1.88	4.56	7.02**	
	E ₂	40.49±1.47	38.39±1.15	-2.1	5.18	5.36**	<40 [^]
	C	42.12±1.10	42.48±1.00	0.36	0.85	1.19(NS)	
TC :HDL-C (mg/dl)	E ₁	5.10±0.21	4.23±0.11	-0.87	17.06	11.71**	
	E ₂	4.84±0.21	3.91±0.75	-0.93	19.21	10.10**	<4.5 [@]
	C	4.88±0.23	4.89±0.23	0.01	0.21	0.92(NS)	
LDL-C:HDL-C (mg/dl)	E ₁	3.09±0.18	2.56±0.13	-0.53	17.15	10.49**	
	E ₂	2.92±0.18	2.33±0.17	-0.59	20.20	9.81**	<3 [#]
	C	2.90±0.20	2.94±0.18	0.04	1.37	0.87(NS)	

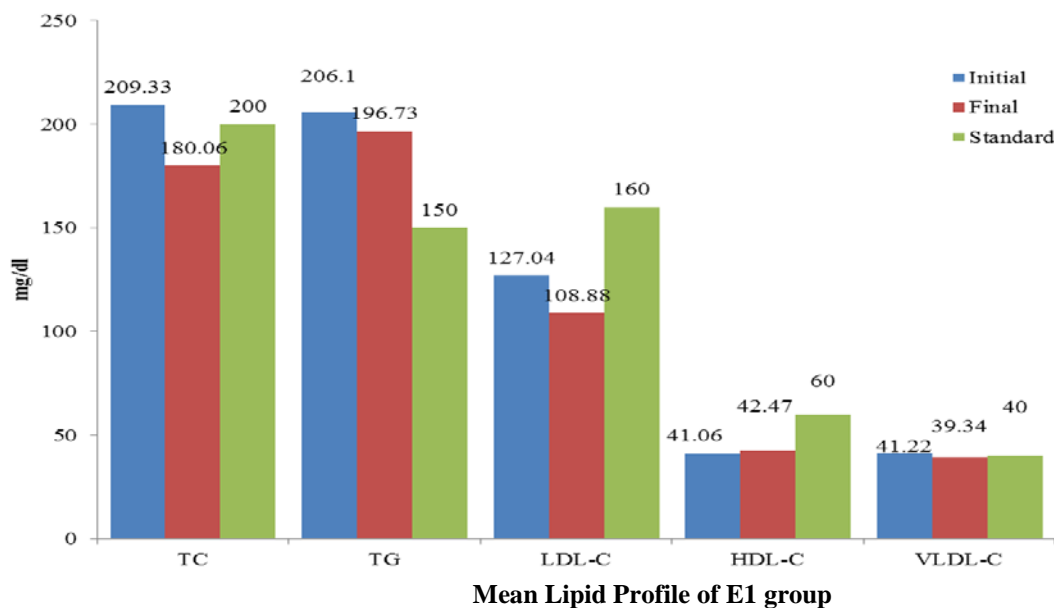
*Significant at 5% level of significance

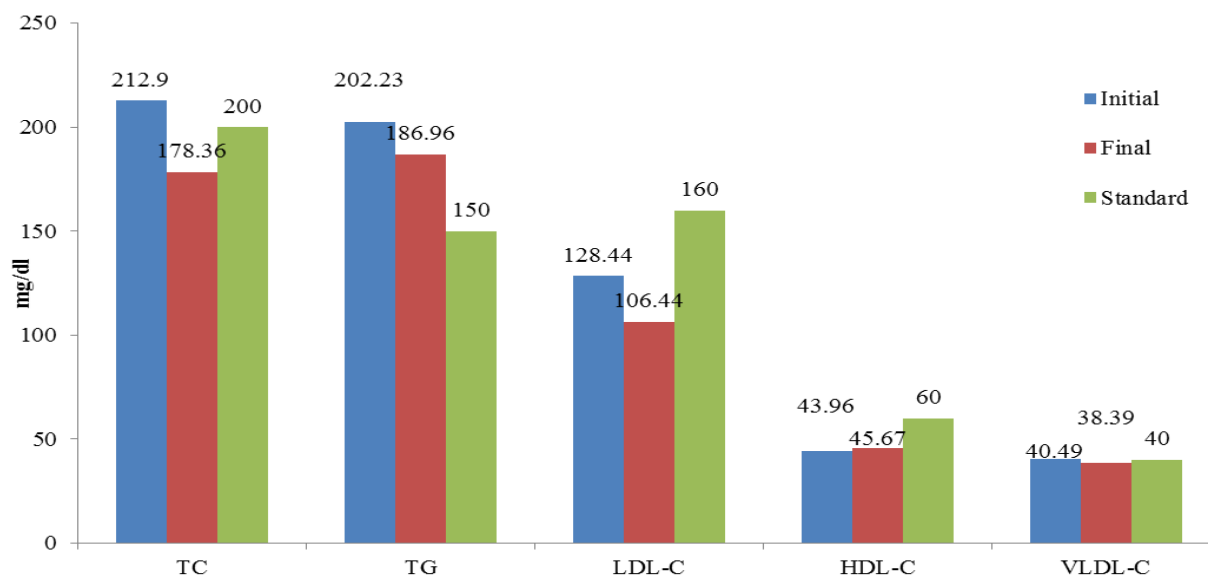
**Significant at 1% level of significance

NS (non significant)

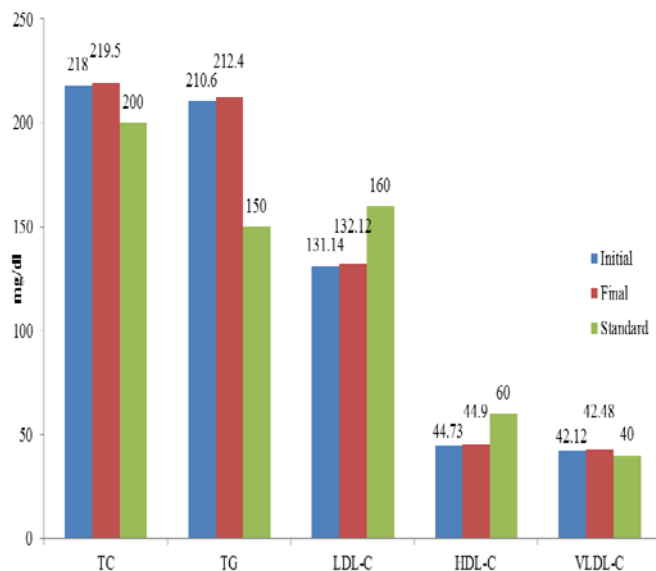
[^]ATP III (2010) [@] Anonymous (2007)

[#] Castelli *et al* (1997)





Mean Lipid Profile of E2 group



Mean Lipid Profile of C group

and final values were 127.04 ± 5.25 , 128.44 ± 5.72 , 131.14 ± 5.01 mg/dl and 108.88 ± 4.92 , 106.44 ± 4.94 , 132.12 ± 4.76 mg/dl in all the three groups respectively. A highly significant ($p \leq 0.01$) decrease was reported in group E₁ and E₂, whereas a non-significant decrease was observed in group C. The initial mean values of VLDL-C were 41.22 ± 1.30 , 40.49 ± 1.47 and 42.12 ± 1.10 mg/dl and after the supplementation period, the values decreased to 39.34 ± 1.12 , 38.39 ± 1.15 and 42.48 ± 1.00 mg/dl in all the three groups E₁, E₂ and C, respectively. A statistically ($p \leq 0.01$) significant reduction was observed in group E₁ and E₂, whereas a non-significant decrease was observed in group C.

6. Conclusion

Overall scrutiny of data indicated that maximum change was observed in the values of subjects in E₂ group followed by E₁ group. Hence, from the foregoing results, it can be inferred that 150 ml probiotic yogurt containing more than one strain supplementation for two months is an effective measure to bring favorable and significant improvements in coronary heart disease patients as compared to probiotic yogurt containing single strain and thus helps in the retardation of secondary complications. Thus probiotic yogurt is surely a panacea for patients who are at risk of CHD. A dairy product containing probiotics makes a healthy “functional food package” in addition to the vitamins, calcium, other minerals, and protein obtained from milk products. Consumption of three or more servings of dairy products each day has been associated with lower levels of obesity, and hence lower incidence of hypercholesterolemia and heart disease. The DASH (Dietary Approaches to Stop Hypertension) diet also recommends three servings of low fat dairy products. Considering all these findings, dairy products combined with probiotic bacteria results into improved health status.

7. Future Scope

Use of yogurt contains probiotic strains other than *Lactobacillus acidophilus* and *Streptococcus thermophilus* can be encouraged as it helps to improve lipid profile and could be easily incorporated in our daily diet along with meals.

People should be encouraged to consume probiotics in various other types of fermented products as it is natural, safe, has no side effects and economical alternative to the usually used hypolipidemic drugs. Dietary modifications such as increased consumption of whole cereals, whole pulses, sprouts, fresh fruits and green leafy vegetables can be incorporated to see the cumulative effect of both.

Excess intake of refined and processed food, whole milk and milk products, sugar, fats and oils, added salt and salty foods should be discouraged among at risk CHD subjects. Instead

skimmed or low fat milk and milk products should be consumed as it is rich source of calcium, potassium, magnesium and protein, which are cardio protective. Alcohol, tobacco and smoking should also be avoided. Nutritional counseling for long duration should also be imparted to risk of CHD.

Basics. [Http://www. Usprobiotics.org/basics](http://www.Usprobiotics.org/basics) Assessed on August 24th, 2010

Therapeutic lifestyle change is recommended which along with dietary modifications, includes regular exercise for 30 minutes a day for basic level of fitness. As physical activity reduces total cholesterol, triglycerides and fibrogen in the blood, increases HDL-C and lowers systolic and diastolic BP. Knowledge about the importance of regular health checkups, for identification and estimation of the risk factors especially after the age of 40 yrs can be stressed upon. As it is advisable to maintain ideal body weight and follow healthy active lifestyle.

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