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EFFECT OF SUPPLEMENTATION OF PROBIOTIC CURD ON LIPID PROFILE IN OBESE SUBJECTS

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ABSTRACT

Weight control is a significant worldwide problem. The morbid obesity has reached epidemic proportions in India affecting 5% of the country's population. To overcome such things now a days a new concept of Probiotics has been introduced in the field of obesity treatment. Probiotics helps in control of weight by reduce glucose absorption from the intestine and an increase in the metabolic use of glucose. It is often suggested that dairy calcium may also reduce the body fat. The anti - obesity activity is due to conjugated linoleic acid (CLA) produced by some of the probiotic micro flora. A total of 100 obese people were randomly selected and divided as experimental group (n=50) and control group(n=50) The experimental group was supplemented for 30days with the Probiotic curd with viz., *Lactobacillu bulgaricus (delbruki subsps)* and *Streptococcus thermophilus* (10^7 cfu/gm) mixed in a preset curd (200 gm/day/person)**.** Age and sex matched obese subjects were selected and supplemented with same amount of milk (200ml) and considered as control group. The lipid profile parameters (TC,TG,HDL) were significantly changed upon supplementation of probiotic curd in the obese subjects.i.e the Total Cholesterol levels decreased from 161.24 ±33.87 to 138.21 ± 64.93($P<0.05$) and Triglycerides from 133.59 ± 94.85 to 108.66 ± 69.97($P<0.05$) where as HDL increased significantly from 29.67 ±8.20 to 40.70 ±7.75. ($P<0.05$). This study showed the promising role of specific probiotics as food based supplements in altering the lipid levels in obese subjects.

Keywords: Probiotics, Probiotic curd, Obesity, Lipidprofile (Total cholesterol, Triglyceride, HDL).

INTRODUCTION

Human body is host to some 10^4 bacteria, majority of which reside in the gastrointestinal tract and these micro flora play an important role in health maintenance. A majority of the indigenous flora are benign and exhibit health promoting properties like Lactobacilli and Bifidobacteria while some possess the potential to cause disease like sulfate reducers and clostridia Bengmark.S (1998). Probiotics are defined as "living micro-organisms that when consumed in sufficient numbers provide health benefits to the host" (FAO/WHO, 2002). These probiotic microorganisms modulate the activity of immune system thus protecting from invading pathogenic bacteria like *Salmonella* spp, *Shigella* spp, *H. Pylori* and other disease causing organisms Salzmann *et al* (1998).

The safety of Bifidobacteria and Lactobacilli have been evaluated by several experts Salminen *et al* (1998); Van Niel, (2005) and studies done using *in vitro* and *in vivo* animal models and on human subjects indicated that the consumption of Lactobacilli and Bifidobacteria is quite safe Moreover, Lactobacilli and Bifidobacteria are well known for their survival in gastro intestinal environment and for their ability to adhere with gut wall make them potential probiotic candidates. Apart from this Lactobacilli species were known for a number of beneficial effects like their ability to produce bacteriocins Ogunbanwo *et al*,(2003) synthesis of vitamins Denter and Bisping, (1994) ability to deconjugate bile salts thus lowering cholesterol absorption Anderson and Gilliland (1999) degradation of N-nitrosamines thereby reducing colon cancers Saikali *et al,* (2004) and improve digestion of lactose in lactase deficient persons DeVrese *et al* (2001).

Probiotics could be a ray of hope and may offer exciting solution to obesity and related problems as number of studies demonstrated the role of probiotics in alleviating lipid profile The present study focuses on the effect of consumption of probiotic curd as a food supplement on the lipid profile Total Cholesterol, Triglycerides and High Density lipoproteins (TC, TG and HDL-C) in obese subjects.

MATERIAL AND METHODS

SELECTION OF SUBJECTS

INCLUSION CRITERIA

One Hundred (100) subjects with a Body Mass Index (BMI) above 25 were recruited for the study from

local population of Hyderabad city, Andhra Pradesh, India. The subjects were categorized into overweight and obese based on their Body Mass Index (BMI) status. The subjects with BMI in between 25 to 28 were considered as overweight whereas those with 28 and above as obese respectively. The institutional ethical committee approval The (IEC/NO-04/08) was obtained before the commencement of the study and the informed consent was also obtained from all subjects.

EXCLUSION CRITERIA

Those individuals having secondary health complications like Hypertension, Diabetes and Cardio vascular or Thyroid diseases etc apart from obesity were excluded from the study**.**

STUDY DESIGN

Considering 95% Confidence Interval (CI), 80 % Power, SD of BMI of overweight / obesity is 3.2 at an expected difference of 2.0 and also considering 20% drop out the required sample is 100 subjects that were recruited for each group i.e. Group A - Probiotic with 50subjects were supplemented, (experimental) Group B with 50 subjects were given same quantity of pasteurized milk i.e.200 ml /day supplemented obese are kept as controls.

INTERVENTION

The Group A individuals were supplemented with 200 gms/ per day of freshly prepared curd by fermentation of pasteurized toned milk with known mixed bacterial flora viz. *Lactobacillu bulgaricus (delbruki subsps* UBLB-38) and *Streptococcus thermophilus* (UBST-50) were obtained at free of cost from Unique Biotech Company Private Limited located in Hyderabad. Group B subjects were adviced to continue their intake of milk and considered as controls. All the participants (Group A and B) were asked to take their regular diet, and to carry out their routine work and exercise. The details of their food habits and other information have been recorded in a special structured proforma.

The subjects were asked to consume probiotic curd at a quantity of 200/gms/day/individual in one sitting ideally to take along with their lunch for a period of 30 days .The subjects were also advised not to take any fermented food products during the period of supplementation especially alcohol which may interfere in the investigations. .However subjects were not restricted to commonly consumed food items like *Idly*, *Dosa* and/or *Uthappam* as these food items do not contain any live micro flora after cooking though these are categorized as fermented foods.

PREPARATION OF PROBIOTIC CURD

The fresh milk about 2-3 liters was procured from Mother Dairy and toned using cream separator machine in the metabolic kitchen at our institute The milk was pasteurized and cool down to 37^0C to 40^0C and dispensed in to 200 ml steel cups and fermented with specific Probiotic *Lactobacillus bulgaricus and Streptococcus thermophilus* .After incubation for about 5-6 hours the curds formed was tested for its acceptability by human volunteers from NIN using a questionnaire. The sensory evaluation was carried out using hedonic scale. A total of 80 % acceptability was obtained and therefore the supplementation was initiated to obese subjects under study. Simultaneously viability was tested upon storage at 4^0 to 8^0 C and at different time point's i.e.1, 2, 3, and 4 weeks. It was found that viability was retained only up to one week time. However to avoid risk we have supplemented with freshly prepared Probiotic curd i.e., not more than one day.

Figure-1 showing the probiotic curd

LABORATORY INVESTIGATIONS

Probiotic micro flora was expressed as CFU/gm of the product and adjusted to 10^7 cfu/gm. About 3ml of overnight fasting venous blood samples were collected from all the subjects for lipid profile analysis at "0" and "30"days. Total blood cholesterol was estimated by enzymatic method of Liberman (1985) triglycerides were estimated using GPO method of Fossati and Lorenzo (1982) and HDL-C was estimated by enzymatic method of Demacker and Hifman (1980) these tests were done by commercially available kits supplied by Biosystems diagnostic pvt limited, India.

STATISTICAL ANALYSIS

The statistical analysis was carried out by Students-T test, and ANOVA using SPSS software Package (version 12.0) to find the level of significance between test results.

RESULTS

The physical examination and initial blood sample reports showed that all the subjects are free from secondary complications of overweight related problems like cardiovascular disease, diabetes and thyroid disorder etc.The characteristics of the subjects under study are shown in Table – 1. The data obtained on lipid profile are depicted in table-2 .It can be observed from table-2 the mean value of cholesterol at '0'day in group-A and group B were close by i.e. 161.24± 33.87 and 152 ± 18.0 which was not statistically significant. After supplementation there was a significant decrease in the cholesterol levels to138.21 ±64.93 ($P < 0.05$) and in controls there was a tremendous increase in the cholesterol levels from 180 ± 18.0 which were significant upon comparison.

Table I -Characteristics of the Subjects Studied

S. No.	Characteristics	Experimental group	Control group
1	Number of subjects	46	42
a	Males	23	21
b	Females	23	21
2	Age (mean value ± SD)	39.45 ± 6.2	37.26 ± 5.7
3	Weight (kgs) (mean value ± SD)	86.16 ± 0.83	87.56 ± 14.24
4	Height (cms) (mean value ± SD)	167.66 ± 10.13	165.8 ± 11.09
5	BMI (mean value ± SD)	33.64 ± 2.30	32.20 ± 2.81

Numbers declined due to dropouts during the experiment.

Table-2 -Showing changes in the biochemical parameters before and after supplementation of probiotic curd in obese subjects

Parameters (mg/dl)	Experimental group (*Lactobacillus bulgaricus* +*Streptococcus thermophilus*) (Probiotic curd) n=42 Mean±SD		Control group (Milk) n=46 Mean±SD	
	"0" Day	"30" day	"0" Day	"30" day
Total Cholesterol	161.24 ±33.87[a]	138.21±64.93[b]	152.0±28.0[b]	180±18.0[b]
Triglycerides	133.59±94.85[a]	108.66±69.97[b]	179.46±88.89[a]	190.16±23.33[b]
High density lipoproteins	29.67±8.20[a]	40.70±7.75[b]	31.74±4.9[a]	34.84±6.95[a]

*Differences in the Superscript indicates statistically significant difference of $P < 0.05$

The values for serum triglyceride levels are showed a similar trend as that of cholesterol. It is interesting to note that after supplementation of probiotic curd (experimental group) where there was a significant decrease after 30 days. On contrary in the control group, there was a significant increase in the serum triglyceride value after 30 days.

The result for the levels of HDL cholesterol showed that there was a tremendous increase in the HDL in the supplemented group at 30 days when compared to their initial values. ($P < 0.05$).

DISCUSSION

The results of the present study suggest that probiotics have promising possibilities in alleviating the hypercholesterolemia condition in overweight and obese people.

To overcome the issue of side effects associated with chemical drugs and the awareness among consumers demand search for natural alternatives. Probiotics could be helpful in controlling excess weight by reducing glucose absorption from the intestine and increase the metabolic use of glucose Brudnak, (1988) though the expected results could only be achieved with adoption of balanced diet and healthy lifestyle. Moreover, probiotic lactobacilli strains are also known to produce Conjugated Linoleic Acid (CLA), which have anti -obesity effect as reported Lee HY, et.al (2006).

There are number of reports regarding health benefits of probiotics and their promising role as safe and natural therapeutics Margreiter M (2006) The cholesterol lowering effect is due to inhibition of 3-hydroxy 3-methyl gluttony CoA reductase, which is a rate limiting enzyme endogenous cholesterol biosynthesis in the body and also by deconjugation of bile acids in the intestine which is an important mechanism in reducing the cholesterol concentrations, increased deconjugation of bile acids could also result in the greater excretion of bile salts from the intestinal tract which stimulates synthesis of replacement of bile acids from cholesterol levels in the body.

There are various reports on experimental animals inferring the role of lactobacilli in alleviating the symptoms of hypercholesterolemia by lowering down the levels of serum cholesterol to a significant level Gilliland *et al.,* (1985); Suzuki *et. al*., (1992); Nazni *et.al.*,(2014), Derodas *et al.,* (1996) Ashar and Prajapathi (1998) confirmed the hypocholesterolemic activity of *Lactobacillus acidophilus* in human beings. The lowering of triglycerides may be due to production of lipase by probiotic organisms which breaks larger molecules of fats in to simple and easily digestible substrates.

Our study is in conformity with the study of Usman & Hosono (2001)in which they supplemented the rats with *Lactobacillus gasseri* and found a significant decrease in the triglyceride levels. Kawase *et al* (2000) reported the decrease of triglyceride levels in humans by with combined supplementation of *Lactobacillus casei* and *Streptococcus thermophilus.* The increase in HDL cholesterol level is known to have protective effect on the risk of coronary heart disease. Earlier the increased HDL

cholesterol levels were reported by Hruby *et al.* (1992) after supplementation of probiotic organisms. Similar results were observed with supplementation of *Lactobacillus casei* and *Streptococcus thermophilus* in a clinical trial conducted on 20 human (Kawase *et al.* 2000) and Nazni *et.al.,* (2014).

The present results indicate that the observed hypocholestremic effect is somehow dependent on the presence of bacteria in the fermented product. An ability of certain strains of lactobacilli to assimilate cholesterol in the presence of bile acids has been demonstrated and suggests a possible association between gut micro flora and cholesterol absorption .The viability of fermenting bacterial strains in the human gut and ultimately their ability to colonize the small intestine where the most absorption of cholesterol takes place.

CONCLUSION

From the present study, it can be concluded that supplementation of promising strains of probiotic organisms may offer exciting solution to minimize the problem of high cholesterol levels in humans. However, extensive research is required to screen the potent probiotic strains and their evaluation for the effective management of good and bad cholesterol in the body and the sustainability of the desired results.

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