HYPOLIPIDEMIC ACTIVITY OF PROBIOTICATED MANGO AND SAPOTA FRUIT JUICES IN ALBINO WISTAR RATS

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Abstract
The study was designed to evaluate the effect of probioticated mango (PMJ) and sapota (PSJ) fruit juices containing Lactobacillus plantarum NCDC LP20 as well as normal juices on high fat diet (HFD) fed albino Wister rats. Eight groups of rats (each group consisting of six rats) were fed with experimental diets: a normal control diet (NCD), HFD, HFD + PMJ, HFD + mango juice (MJ), HFD + PMJ + MJ, HFD + PSJ, HFD + sapota juice (SJ), HFD + PSJ + SJ. Probioticated fruit juices were administered orally (10 mL/kg bw/day for 28 days) to HFD fed rats. After treatment, the lipid profile of the blood serum was studied in all the groups. HFD fed rats showed elevated lipid profile levels as compared to NCD fed rats after 21 days. These HFD fed rats were orally administrated with probioticated mango and sapota fruit juices and normal mango and sapota juices (10 mL/kg body weight/day) for 28 days. Probioticated juices reversed the elevated lipid profile levels to near normal in HFD fed rats. This hypolipidemic effect is more significant in PMJ + MJ and PSJ + SJ treated groups than the other groups. On treatment with the probiotic mango and sapota fruit juices, the body weights of the treated groups significantly lowered in HFD fed rats. The results unequivocally establish an alternative dietary approach to reduce serum lipid levels as well as body weights in HFD fed rats by probioticated mango and sapota juices, better than the normal juices.

Key words: Probioticated fruit juices, Lactic acid bacteria, Serum lipid profiles

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1. INTRODUCTION

Probiotics have been defined as “live microbial food supplements which beneficially affect the host by improving the intestinal microflora balance”, or more broadly as “living microorganisms, which upon ingestion exert health benefits beyond the inherent general nutrition”. Prebiotics have been defined as a non - digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or the activity of one or a limited number of bacteria in the colon, and thus improves host health (Mishra et al., 2012). Probiotic benefits include: Increased resistance to infectious diseases, particularly of the intestine, decreased duration of diarrhea, reduction in blood pressure and serum cholesterol concentration, reduction in allergy, stimulation of phagocytosis by peripheral blood leucocytes, modulation of cytokine gene expression, regression of tumors and reduction in carcinogen or co-carcinogen production (Jamila et al., 2011).

Cholesterol plays a major role in human heart health. Cholesterol can be both good and bad. High-density lipoprotein (HDL) is good cholesterol and low-density lipoprotein (LDL) is bad cholesterol. Excess cholesterol in the blood stream can form plaque (a thick, hard deposit) in artery walls. The cholesterol or plaque build-up causes arteries to become thicker, harder and less flexible, slowing down and sometimes blocking blood flow to the heart. When blood flow is restricted, angina (chest pain) can result. A heart attack will result when blood flow to the heart is severely impaired and a clot stops blood flow completely. When there is too much LDL cholesterol in the blood, it is deposited inside the blood vessels, where it can build up to hard deposits and cause atherosclerosis, the disease process that underlies heart attacks (Ma, 2006). Hyperlipidemia is an elevation of lipids (fats) in the bloodstream. These lipids include cholesterol, cholesterol esters (compounds), phospholipids and triglycerides. They are transported in the blood as part of lipoproteins.

These are the five major families of blood (plasma) lipoproteins: (1) chylomicrons, (2) very low-density lipoproteins (VLDL), (3) intermediate-density lipoproteins (IDL), (4) low-density lipoproteins (LDL), (5) high-density lipoproteins (HDL). When hyperlipidemia is defined in terms of class or classes of elevated plasma lipoproteins, the term hyperlipoproteinemia is used (de Jongh et al., 2002). Obesity is said to be an abnormal metabolism of lipids. Hyperlipidemia/hyperlipo-proteinemia is the condition of abnormally elevated levels of any or all lipids and/or lipoproteins in the blood (Dorland, 2007). It is the most common form of dyslipidemia (which also includes any decreased lipid levels). High lipid levels cause atherosclerosis. Atherosclerosis is a condition in which fatty material collects along the walls of arteries. This fatty material thickens, hardens (forms calcium deposits) and may eventually block the arteries. Although elevated low density lipoprotein cholesterol (LDL) is thought to be the best indicator of atherosclerosis risk, dyslipidemia can also describe elevated total cholesterol (TC) or triglycerides (TG), or low levels of high density lipoprotein cholesterol (HDL). South Asian countries have a high prevalence of hyperlipidemia in line with their economic development. India, in particular, has a high burden of coronary heart disease. Hyperlipidemia associated lipid disorders are considered to cause atherosclerotic cardiovascular disease (Saravanan et al., 2003). Among these, hyper cholesterolemia and hyper triglyceridemia are closely related to ischemic heart disease. Coronary artery disease (CAD) is one of the major causes of premature death globally and it is expected to be the most important cause of mortality in India by the year 2010 (Ma Angeles Zulet et al., 1999). It has been well established that nutrition plays an important role in the etiology of hyperlipidemias and atherosclerosis. Several animal and human studies have confirmed the hyper-cholesterolemic properties of saturated fatty acids and cholesterol which include increasing total cholesterol and altering lipoprotein pattern and whose mechanisms remain under study.

However, cholesterol is essential to life. It is a precursor of several steroid hormones, including the sex hormones estrogen, progesterone, and testosterone as well as the corticosteroids. The major portion of cholesterol in the body is the precursor of bile acids. Bile acid is necessary for the absorption of fat in intestine. The important clue to cholesterol metabolism is that most of the bile acids are not lost as feces but are reabsorbed from the lower intestine and recycled to the liver. Cholesterol is widely distributed in animal and human tissues as cholesterol esters. The predominant form of cholesterol in the plasma is cholesterol ester. Three reactions, each catalyzed by a distinct enzyme or class of enzymes, are responsible for the formation of cholesterol esters in the body. Two of these, acyl-CoA: cholesterol O-acyltransferase (ACAT) and cholesterol ester hydrolase (Cholesterol esterase) are present in most, if not all, tissues. The third enzyme, lecithin: cholesterol acyltransferase (LCAT) is thought to be active only in the plasma and peripheral lymph. The fatty acyl esters of cholesterol can be hydrolyzed by enzyme to free cholesterol. Enzymes catalyzing the hydrolysis of cholesterol ester are presented in pancreatic juice and in many animals and human tissues (Myant, 1981).

Emulsification of dietary fat is an intermediate process in fat absorption. Bile salts together with phospholipids and cholesterol form micelles which help in the absorption of cholesterol. Lactobacilli de-conjugate the bile salts in the intestine to form bile acids and thereby inhibit micelle formation. This leads to decreased absorption of cholesterol. Cholesterol entering the intestine through the enterohepatic circulation is similarly treated. Lactobacilli elaborate the enzyme conjugated bile acid hydrolase (CBH), which hydrolyzes bile salts, and hydroxy steroid dehydrogenase (HSDH) which degrades bile acids and interrupts the enterohepatic circulation of bile acids. Another factor thought to be elaborated by lactobacilli is hydroxy methyl glutarate CoA

(HMG CoA), which inhibits HMG CoA reductase, the rate limiting enzyme in endogenous cholesterol synthesis.

Earlier we have studied the suitability of mango and sapota fruit juices for probiotication using *Lactobacillus plantarum*. Further we have shown that the probioticted juices have better health quotients compared to raw juices and showed significant antibacterial activity against test cultures (Vijaya Kumar et al., 2015). The present study is designed to evaluate the effect of probioticated mango and sapota fruit juices containing *Lb. plantarum* as well as normal juices for hypolipidemic activity in albino Wister rats.

2. MATERIAL AND METHODS

Microorganism

The *Lactobacillus plantarum* (NCDC LP 20) was obtained as a gift from Dr. R. Hemalatha, National Institute of Nutrition, Hyderabad, India. It was grown at 37^{o}C for 24 h in MRS broth and was used as inoculum (3×10^6 cells/mL).

Fruits and their processing

Good quality of mango (Cv. *Banginapally*) and sapota (Cv. *Kalipatti*) fruits were purchased from a local market in Tirupati. The fruits were allowed to ripen for a period of 5-7 days (30ºC). After ripening; the fruits were washed with tap water and later with sterile water for removal of surface contamination. Then fruits were peeled off with the help of a sharp knife, then cut into small pieces and then made into pulp with the help of laboratory blender. After pulping, 0.1 g/L potassium meta-bisulphate was added as a preservative and kept in a freezer at -4°C till further use.

Probiotication of fruit juices

The experiments were conducted in 3-L fermenter (Bio-Age, Punjab, India) with 2 L of pasteurized (at 85ºC for 15 min) mango and sapota juices, individually. All fermenter batches of juice were inoculated with 24 h old culture (10^5 CFU/mL) and incubated at 30ºC for 72 h. After fermentation/probiotication, the fruit juices were stored at -4°C for further analysis.

Experimental animals and treatments

Six-month old male albino Wister rats obtained from Sri Raghavendra Suppliers, Bangalore, (~180-230 g) were used in the present study. The rats were maintained on standard pellet diet and provided access to water. They were housed in clean, dry polypropylene cages and maintained in a well-ventilated animal house with 12 h light and 12 h dark cycle, as per the guidelines of the National Institute of Nutrition, Indian Council for Medical Research, Hyderabad, India and approved by the Animal Ethical Committee of the Sri Venkateswara University (Resolution No.39/2012. 2013/(i)/a/CPCSEA/IAEC/SVU/OVS-BVK/Dt.08-07-2012). Forty eight animals were randomly divided into eight experimental groups of 6 animals each and probioticated as well as non-probioticated mango and sapota juices 10 mL/kg body weight /day were orally administered for 28 days (Kujawska et al., 2011).

Group-1: Normal rats with control diet (NCD)
Group-2: High fat diet (HFD)
Group-3: High fat diet + Probiotic mango juice (HFD + PMJ)
Group-4:High fat diet+Mango juice (HFD+MJ)
Group-5: High fat diet + Probiotic mango juice + Mango juice (HFD + PMJ + MJ) (together 10 mL)
Group-6: High fat diet + Probiotic sapota juice (HFD + PSJ)
Group-7: High fat diet + Sapota juice (HFD + SJ)
Group-8: High fat diet + Probiotic sapota juice + sapota juice (HFD + PSJ + SJ) (together 10 mL)

Induction of hyperlipidemia

The rats with average body weights of 180 - 200 g were made hyperlipidemic by feeding them with the high fat diet (HFD) for 21 days. The HFD contained fat (2%), cholic acid (1%), dalda (20%) and coconut oil (6%), used as major constituents.

Biochemical parameters

Blood samples were collected from overnight fasting normal and treated HFD fed rats through retro orbital puncture. The following parameters were analyzed by adopting standard methods.

Total fat (TF)

Estimation of cholesterol was carried out by the method of Zlatkis et al. (1953).

Triglycerides (TAG)

Serum triglycerides were measured by the method of Foster and Dunn (1973).

HDL

Estimation of serum HDL cholesterol was carried out by the method of Burstein et al. (1970).

Statistical analysis

The analysis on the same sample was made in three replications and the results were expressed as mean value ± standard deviation.

3. RESULTS AND DISCUSSION

1. RESULTS

Probiotics, with regard to animal applications, are defined as a live microbial feed supplements that beneficially lowered lipid profile levels, which are elevated in HFD fed rats (Ibrahim et al., 2010). As indicated in Table 1, in the present study, HFD fed rats (Group-2) showed higher lipid profile levels than those rats fed with NCD (Group-1). Oral administration of probioticated mango or sapota fruit juices (10 mL/kg bw /28 days) to HFD fed Groups 3 and 6 and along with normal mango or sapota juices to Groups 5 and 8 significantly decreased the elevated lipid profiles such as TC, TAG, LDL and VLDL and increased HDL cholesterol levels to near normal when compared to the rats fed with normal mango or sapota juices alone (Group 4 and 7). On oral administration of probioticated mango and sapota fruit juices containing *Lb. plantarum* NCDC LP 20 significantly lowered the body weights in HFD fed rats when compared with normal mango or sapota fruit juices fed rats (Table 2). Further reduction in body weight of HFD fed rats was noticed in both probioticated and normal mango or sapota juice fed rats. The results definitely proved that probioticated juices are better than the normal juices for lowering hyperlipidemic activity.

In one such study, the effect of *Lb. acidophilus* and dietary yogurt on mice plasma lipids and TAG levels were examined and significant decrease was observed in total and LDL cholesterol levels in the *Lb. acidophilus* fed group (Akalin et al., 1997). In a similar study, Xiao et al. (2003) reported the lowering of serum concentrations of total cholesterol, LDL cholesterol and TAG in rats fed a diet supplemented with lyophilized powder of fermented milk with yogurt starters and *Bifidobacterium*, whereas no change in HDL cholesterol concentration was observed.

A probiotic dietary intervention could be a promising and cost-effective approach in lowering serum/plasma TC, TG, LDL and VLDL cholesterol in the management of cardio-vascular diseases. Probiotic lactobacilli are considered normal components of the intestinal microflora in humans and animals and have been associated with various health-promoting properties.

2. DISCUSSION

Coronary heart disease is linked to increased levels of total serum cholesterol. The use of probiotic bacteria *Lb. plantarum* NCDC LP 20 in reducing serum cholesterol level has attracted much attention. Probiotic fruit juices are given orally because they must be acid and bile tolerant to survive in the human gastrointestinal tract. A number of fat removal mechanisms by probiotics have been proposed, which include assimilation of fat by growing cells, binding of fat to cellular surface, incorporation of fat into the cellular membrane, de conjugation of bile via bile salt hydrolase, and co precipitation of fat with de conjugated bile. However, the exact mechanisms remain unclear and controversial. Much work has already been accomplished to help us to understand.

Table 1: Effect of experimental feed on serum lipid profile of Wister rats

Lipid profile (mg/dl)	NCD	HFD	HFD + PMJ	HFD + MJ	HFD + PMJ + MJ	HFD + PSJ	HFD + SJ	HFD + PSJ + SJ
Serum TC	147.96±0.72^{a}	348.95±0.18^{h}	243.03±0.40^{d}	268.08±0.50^{f}	201.06±0.60^{b}	254.0±0.28^{e}	288.68±0.79^{g}	212.0±0.69^{c}
Serum TAG	78.05±0.84^{c}	119.15±0.56^{h}	83.83±0.25^{b}	96.0±0.56^{f}	80.03±0.50^{a}	86.01±0.33^{e}	107.98±0.36^{g}	81.85±0.60^{d}
HDL	68.38±0.48^{h}	51.01±0.55^{d}	58.2±0.35^{a}	53.01±0.53^{c}	63.98±0.33^{f}	56.06±0.31^{b}	52.1±0.35^{e}	59.03±0.51^{g}
LDL	53.96±0.41^{a}	148.08±0.46^{g}	97.88±0.51^{d}	108.08±0.46^{e}	76.11±0.30^{b}	99.0±0.41^{d}	112.9±0.54^{f}	83.96±0.60^{c}
VLDL	17.91±0.37^{d}	23.98±0.41^{g}	20.05±0.30^{b}	21.0±0.28^{e}	18.03±0.39^{a}	20.91±0.43^{d}	21.33±0.35^{f}	19.05±0.32^{c}

Normal Control Diet (NCD); High Fat Diet (HFD); Probiotic Mango Juice (PMJ); Mango Juice (MJ); Probiotic Sapota Juice (PSJ); Sapota Juice (SJ); Total Cholesterol (TC); Tri acyl glycerol (TAG); High Density Lipoprotein (HDL); Low Density Lipoprotein (LDL); Very Low Density Lipoprotein (VLDL). Results are expressed as Mean ± S.D (n=6), Means with in a row with different superscript letters are significantly different (P<0.05).

Table 2: Effect of experimental diets on body weight of Wister rats

Body weight (g)	**NCD**	**HFD**	**HFD + PMJ**	**HFD + MJ**	**HFD + PMJ + MJ**	**HFD + PSJ**	**HFD + SJ**	**HFD + PSJ + SJ**
Initial weight	174.33±2.87	182.66±2.16	177.66±2.87	175.5±1.37	179.33±3.77	172.16±2.04	175.83±1.16	181.0±4.0
After HFD feeding	220.42±3.23	364.81±7.16	352.81±4.94	363.4±4.94	349.2±2.69	341.17±1	338.42±2.8	368.17±2.6
Final weight	255.83±3.12	425.33±2.25	275.33±2.33	328.5±2.34	269.33±3.77	282.16±4.91	306.16±2.40	312.83±5.38
Weight loss/gain	+35.5±4.46 ^{a}	+61.0±3.16^{d}	-73.66±3.55^{c}	-31.66±3.98^{d}	-80.0±3.94^{b}	-60.0±5.40^{d}	-20.33±2.94^{d}	-56.83±6.85^{c}

Normal Control Diet (NCD); High Fat Diet (HFD); Probiotic Mango Juice (PMJ); Mango Juice (MJ); Probiotic Sapota Juice (PSJ); Sapota Juice (SJ); Results are expressed as Mean ± S.D (n=6), Means with in a row with different superscript letters are significantly different (P<0.05).

The present study was designed to evaluate the beneficial effect of the combination of probiotic mango or sapota fruit juices as well as their normal fruit juices to decrease lipid profiles in experimentally induced hyperlipidemia by feeding high fat diet to rats. Probioticated juices of mango and sapota, as well as their combinations of with non-probioticated juices have been tested for their TC, TG, LDL and VLDL cholesterol lowering potential. Furthermore, HDL cholesterol levels were partly increased in hyperlipidemia wistar rats fed with PMJ, MJ, PSJ, SJ, PMJ + MJ and PSJ + SJ due to the presence of phytochemicals in them responsible for the anti-hyperlipidemic activity.These observations are consistent with previously published studies on the anti-hyperlipidemic effect of probiotic fermented foods (Akalin et al., 1997; Xiao et al., 2003).

The above results were also similar with *in vitro* and *in vivo* studies on flavonoids from *Emblica officinalis* which revealed reduction in serum and tissue lipid levels of hyperlipidemic rats. Besides they also possess good anti-oxidant and cardio-protective properties (Anila et al., 2002; Bhattacharya et al., 2002). Aqueous alcoholic extract of *Phyllanthus amarus* contains alkaloids, flavonoids, saponins and tannins which are responsible for the application in wide range of pathological conditions and these include hypoglycemic and hypolipidemic (Olapade, 1995; Umbar et al., 2009). Preclinical evaluation with ginger has revealed antioxidant and hypolipidemic activities (Ahmed et al., 2000; Bhandari et al., 1998). Previous studies have also confirmed that the presence of phytoconstituents like flavonoids, alkaloids, saponin and tannins in extract or as isolated compounds might contribute towards hypolipidemic activity (Olagunju et al., 1995). These reports led to prepare the PMJ, MJ, PSJ, SJ, PMJ + MJ and PSJ + SJ and evaluate them scientifically for their hypolipidemic and antioxidant potentials using wistar rats as experimental animal models. Significant reduction in serum cholesterol level in albino rats after feeding the probiotic fermented dairy products (dahi and yoghurt) for 30 days, indicating hypocholesterolaemic effect of the probiotic cultures (*Lb. acidophilus* and *Bifidobacterium bifidum*) was reported (Vijayendra and Gupta, 2012).

The presence of tannin, flavanoids and vitamin C might be responsible for the reducing capacity of the formulations. Joris et al. (1983) reported that rats were hyporesponsive to dietary fat alone. So cholic acid is added in order to induce hyperlipidemia (Guido and Joesph, 1992). The high fat diet (HFD) administered in the present study includes HFD for effective hyperlipidemia induction. The significant ($p<0.05$) change in lipid profile noticed in the experimental animals confirmed the induction of hyperlipidemia in HFD fed rats. High fat diet increased triglycerides level and led to hardening of arteries (Joris et al., 1983). The present study showed that HFD significantly ($p<0.05$) increased TG level as compared to standard pellet fed rats.

HDL is a beneficial lipoprotein synthesized in intestine and liver which protects the system from the pathogenesis of atherosclerosis (Xu et al., 2005). In the present study, it is noticed that HDL fat level in serum increased significantly ($p<0.05$) in PMJ, MJ, PSJ, SJ, PMJ + MJ and PSJ + SJ treated hyperlipidemic rats. Increase in LDL level causes deposition of fat in the arteries and aorta and hence, it leads to CHD (Coronary heart disease). LDL transports fat from the liver to the periphery (Boden and Pearson 2000; Pedersen, 2001). The fortification of LDL from oxidation and decrease in oxidative stress might therefore be useful for prevention of atherosclerosis associated CVD (Cardio vascular disease) (Witzum, 1994). In the present study administration of PMJ, MJ, PSJ, SJ, PMJ + MJ and PSJ + SJ effectively reduced LDL fat content of hyperlipidemic rats. For a good lipid lowering therapy, a drug should be able to significantly lower LDL and increase HDL fat concentration and this appreciably decreases the fatty cytoplasmic vaculated cells in liver parenchyma and prevents hepatic necrosis and this correlates with the present study.

Reduced LDL and increased HDL concentration were observed in the present study, thereby suggesting that these probioticated juices could be used as good lipid lowering therapeutic agents. VLDL particles contain less protein and are rich in triglycerides. VLDL is less harmful but still can damage the arterial lining and their production in body is directly related to the body fat, as their elevation in blood leads to hyper fatemia. The level of VLDL of hyperlipidimic rats increased significantly ($p<0.05$) compared with standard diet fed rats. After treatment with PMJ, MJ, PSJ, SJ, PMJ + MJ and PSJ + SJ, the VLDL level reduced significantly ($p<0.05$).

Obesity is a major risk factor for augmented morbidity and mortality and is associated with various medical ailments (Wang and Lobstein, 2006). High fat diet-induced obesity has been considered as the most popular model among researchers due to its high similarity of mimicking the usual route of obesity episodes in human (Buettner et al., 2007) and hence, it is considered as a reliable tool for studying obesity as they will readily gain weight when fed high-fat diets (Gajda, 2009). Human studies have revealed that increased fat intake is associated with body weight gain, which can lead to obesity and other related metabolic diseases. This study thus proved that rats exposed to high-fat diet for 3 weeks cause a significant increase of animals' body weight, thus verifying the obese status (Neyrinck et al., 2009). Although there was a significant difference in the body weights between the high-fat and normal diet groups, no significant difference was observed in the daily food intake of animals. This observation provides us with the fact that an increase in body weight is independent of the amount of food consumed by the animals. Treatment of HFD rats with PMJ, MJ, PSJ, SJ, PMJ+MJ and PSJ+SJ conversely causes a remarkable reduction of body weights as compared to the HFD administered rats. The results also suggest the said juices are capable of preventing body weight gain, concomitantly helping in maintaining the correct body weight. Feeding albino Wister rats with fermented dairy products (yoghurt and dahi) containing *Lb. acidophilus* and *B. bifidum* led to increase in body weight gain (Vijayendra and Gupta, 2012).

4. CONCLUSIONS

Probiotics have been found to be clinically effective for a large number of disorders which include hyperlipidemia. The use of any probiotic and prebiotic substances for the enrichment of fermented products provides their delivery into the human gastrointestinal tract and hence, a stimulation of beneficial health effects. The probioticated mango and sapota juices were found to have hypolipidemic activity. Thus these probiotic juices showed protective action and did not show much negative effects in rat model system. Hence, beverages made from mango and sapota fruits with probiotics may provide better health benefits to the consumers.

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