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Hypolipidemic effects of *Lysinibacillus sphaericus* fermented tomato and carrot juices in high-fat diet-fed albino Wistar rats

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ABSTRACT

We have conducted the present study to evaluate the effect of probioticated tomato juice (PTJ), probioticated carrot juice (PCJ), and their mixed juices (PCT) containing *Lysinibacillus sphaericus* on high-fat diet (HFD)-fed albino Wistar rats. Eight groups consisting of 6 rats each were designed in the experiments and orally administered the above probioticated juices (at 1–2 mL per rat) to HFD-fed rats for 28 days. This included a group maintained on normal control diet (NCD), HFD, and an experimental control group maintained using lovastatin (at 5 mg/kg body weight). After feeding the rats, the lipid profile of the blood serum was studied in all the eight groups. HFD-fed rats showed elevated lipid profile levels as compared to NCD-fed rats. Probioticated juices reversed the elevated lipid profile levels to near normal in HFD-fed rats. This hypolipidemic effect was more significant in PCJ-fed (2 mL/rat) groups than the other groups. On treatment with the PTJ, PCJ and PCT showed the lowering of body weights when compared to HFD group.

1. INTRODUCTION

The definition of probiotics is supplementation of living microorganisms, which benefits the host by improving its intestinal microbial balance [1]. These are supplemented with food products and provide several health benefits that include controlling of intestinal infection, serum cholesterol levels, improving lactose utilization, and having anticarcinogenic activity [2]. The probiotic bacteria have broad application in foods for promoting health benefits. The increase in demand of functional food is in response to the consumer demand for health foods [3]. The major industrial probiotic foods are dairy products, which may inconveniences to consumers due to the presence of lactose and cholesterol [4]. Hence, the developments of fruit and vegetable juice based on functional beverages with probiotics have real interest, based on the taste profiles that are appealing to all age groups [5]. Probiotics have potent characteristics features such as resistance to acid, bile, epithelial cell attachment, and stomach colonization [6].

Fruit and vegetable juices have beneficial nutrients such as vitamins, antioxidants, dietary fibers, and minerals. For cultivating probiotics, the juices are one of the ideal media suggested by several researchers [7]. In general, the fruits and vegetables do not contain any dairy allergens that prevent usage by certain groups of population [8]. The juices may be considered as vehicles for the incorporation of probiotics into human intestine [9]. The amounts of sugars present in the fruit and vegetable juices also encourage probiotic growth. For the above reasons, fruit and vegetable juices are being examined for their ability to support probiotic delivery in humans.

One of the lipid-lowering drugs are statins, but all have hazardous side effects [10]. Oxidative stress inhibition is considered an important therapeutic approach. Efforts have been made to identify potential antioxidant compounds from medicinal plants [11]. However, all the tested antioxidants failed to confirm significant antiatherosclerotic effects in humans [12]. Two research teams, Shaper *et al.* [13] and Mann and Spoerry [14] observed that tribal men of Samburu and Maasai of Africa after consumption of fermented milk with Lactobacillus showed reduced serum cholesterol. The cholesterol-lowering bacteria include *Lactobacilli*, *Bifidobacteria,* and *Enterococci* [15].

Due to immunomodulatory and metabolic function, the probiotics have beneficial effects when they are administered for prophylactic and nutritional purposes [11,16]. Probiotics do not cause accumulation of toxic substances in the body, but they can induce little side effects. They can restore normal intestinal microflora and exclude the acquired pathogens by decreasing the intestinal permeability [17,18]. After unveiling the benefits in treating diseases such as atherosclerosis, diabetes, hypertension, neoplasm, and HIV infections, the interest

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in probiotics is increased [19-21]. *In vivo* studies have reported that some Lactobacilli can lower the levels of total cholesterol (TC) and low-density lipoproteins (LDL) [11,22]. The regulation of the gut microbiome influences the metabolic health, atherosclerosis, and obesity. This also helps in the alternative strategy for the treatment of cardiovascular diseases. There are several reports on hypolipidemic and antioxidant potential of dairy probiotics [23,24].

Lysinibacillus is found in plants, animals, and soil. *Lysinibacillus sphaericus* the first identified species in the genus *Lysinibacillus*, classified on the basis of cell wall and peptidoglycan. Ahmad *et al.* [25] reported that the *Lysinibacillus* has potential antimicrobial properties against foodborne bacterial and fungal pathogens. The present study aimed to evaluate probioticated tomato juice (PTJ) and probioticated carrot juice (PCJ) (individually and mixed) for their usefulness in lowering the cholesterol levels in high-fat diet (HFD)-fed rats.

2. MATERIALS AND METHODS

2.1. Probiotic Culture

L. sphaericus isolate (KR140152) of our Laboratory (Department of Biochemistry, S.V. University, Tirupati) was maintained using MRS agar medium (HiMedia, Mumbai) [26] and used in the present study.

2.2. Processing of Juices

Tomatoes and carrot were procured from the vegetable market of Tirupati city [Figure 1a and b]. The vegetable stored in a wooden box for further maturation and were washed with running tap water to remove impurities. Air dried at room temperature before use and treated with steam for 3–4 min for sterilization and peeling. The juice was prepared by using a laboratory grinder and filtered through a sieve (0.8–1.1 mm pore size) [27]. Finally, the juice samples subjected to probiotication.

2.3. Preparation of Inoculum

L. sphaericus (isolate) pure culture grown into two successive broth cultures at 37°C for 24 h. The culture in the active condition inoculated into the MRS broth and incubated at 37°C for 24 h and used as the inoculum for probioticating the juices.

2.4. probiotication of Tomato and Carrot Juices

A 100 mL of tomato and carrot juices were taken separately into a conical flask and autoclaved for 15 min at 121°C. Probiotic culture *L. sphaericus* inoculated into these flasks and incubated at 37°C for 72 h [7].

2.5. Probiotication of Mixed Juice (PCT)

A 100 mL of 1:1 ratio sample (tomato and carrot) was taken into a conical flask and autoclaved for 15 min at 121°C. Probiotic culture *L. sphaericus* inoculated into the flask and incubated at 37°C for 72 h.

2.6. PTJ, PCJ and PCT Physicochemical Analysis

The digital pH meter (Eutech, Japan) was used for measuring the pH of probioticated juices. Hand refractometer (Erma, Japan) was used for determining the total soluble solids (TSS) and the values expressed as Bx. Pour plate method was used for determining the total viable count at 37°C for 72 h and expressed as colony-forming unit per mL [28].

2.7. Experimental Animal Design and Treatments

6-month-old male albino Wistar rats (180–200 g) were procured from Sri Raghavendra Suppliers, Bengaluru. The rats were maintained in a dry polypropylene cages and provided pellet diet and water. As per the guideline of the CPCSEA, the animals kept in a ventilated house with 12 h light and 12 h dark. The experimental animal work was approved by the Animal Ethical Committee of our university with a resolution No.39/2012.2013/i/a/CPCSEA/IAEC/SVU/OVS-SNS/Dt.08-07-2012.

2.8. Induction of Hyperlipidemia

By feeding the HFD, the rats were made hyperlipidemic for 21 days. Table 1 represents the composition of HFD. Forty-eight rats divided into eight experimental groups of 6 each as per the design of Arola *et al*. [29], and PTJ, PCJ, and their mixed juices (PCT) were orally administered for 28 days [30].

Group-1: Normal control diet

Group-2: HFD

Group-3: HFD+PTJ (1 mL) (HFD+T1)

Group-4: HFD+PTJ (2 mL) (HFD+T2)

Group-5: HFD+PCJ (1 mL) (HFD+C1)

Group-6: HFD+PCJ (2 mL) (HFD+C2)

Group-7: HFD+PTJ plus PCJ (2 mL) (HFD+MX)

Group-8: HFD+Lovastatin (5 mg/kg body weight) (HFD+STD)

2.9. Determination of Body Weight and Biochemical Parameters

Throughout the experimental period, the weight gain of rats was monitored weekly. Blood samples were collected from overnight fasting normal and treated HFD-fed rats through retro-orbital puncture. Estimation of serum cholesterol was carried out using the method of Zlatkis *et al.* [31]; triglycerides (TG) were carried out by the method of Foster and Dunn [32]; high-density lipoprotein (HDL) levels were

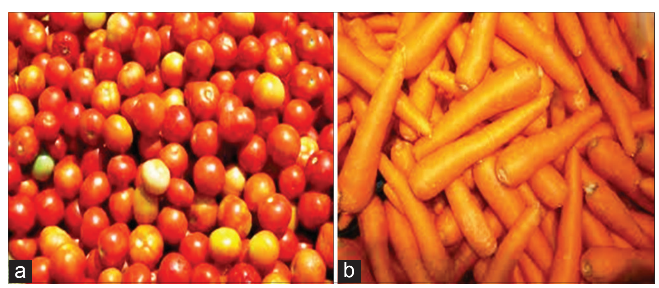

Figure 1: (a) Tomato, (b) carrot

Table 1: Composition of HFD

Content	g/kg pellet diet
Cholesterol	25
Cholic acid	13.6
Dalda	250
Coconut oil	15
Ghee	15

HFD: High-fat diet

carried out by the method of Burstein *et al.* [33]; LDL and very LDL (VLDL) were carried out by the method of Freidwald *et al.* [34].

2.10. Histopathological Studies

The liver and adipose tissues were collected from the sacrificed animals and fixed in 10% formalin solution. Later, the tissues embedded in paraffin and cut into 5-µm thick slices followed by fixing on slides. Both hematoxylin and eosin used for staining the slides. Finally, stained slides were observed under light microscope fitted with charge-coupled device camera [35].

2.11. Statistical Analysis of the Experimental Data

The data were expressed as mean and standard deviation and analyzed by one-way analysis of variance using SPSS version 16.0.

3. RESULTS

L. sphaericus isolate has shown probiotic nature when subjected to acid and bile tolerance tests [26]. Hence, the above culture applied in the PTJ, PCJ, and PCT in the present study.

3.1. Analyses of PTJ, PCJ, and PCT for Physicochemical Changes

The pH and TSS of juices were decreased during probiotication of tomato, carrot, and tomato plus carrot at 37°C during the period of 72 h, whereas the cell viability was increased in the first 24 h and later gradually decreased due to the consumption of nutrients. The better cell viability was shown at acidic pH [Table 2]. *L. sphaericus* growth was better in tomato, carrot, and tomato plus carrot juices even at acidic pH during the period of 72 h. However, 24 h PCJ, PTJ, and PCT have shown better cell viability, and therefore, they were used in experiments of hypolipidemic activity.

3.2. Effect on Serum Lipid Profile

Post-treatment with probioticated juices in rats was found to reduce levels of serum cholesterol, TG, LDL, and VLDL and increase levels of HDL [Figure 2]. Among oral administration of probioticated juices, the PCJ (2 mL/rat) has shown decreased levels of serum cholesterol, TG, LDL, and VLDL and increase of HDL, when compared to the HFD-fed rats, followed by tomato plus carrot (2 mL/rat), tomato (2 mL/rat), carrot (1 mL/rat), and tomato (1 mL/rat). The supplementation of carrot juice (2 mL/rat) has shown similar results that are nearer to standard statin drug (lovastatin)-treated rats.

3.3. Effect of Probioticated Juices on the Body Weights in HFD-Fed Rats

Oral administration of PTJ, PCJ, and PCT fruit juices containing *L. sphaericus* significantly lowered the body weights of HFD-fed rats, when compared with their corresponding control (Group 2). The results definitely proved that PCJ has a better effect than the tomato and mixed juices in lowering hyperlipidemic activity [Table 3].

Table 2: Physicochemical analyses of PTJ, PCJ, and mixed juices

Vegetable juice	Incubation (h)	TSS (Brix)	pH	Viable count ($\times10^8$ CFU/mL)
Tomato (T)	24	6.8±0.6	4.2±0.04	7.6±0.04
	48	5.7±0.8	3.7±0.04	6.6±0.1
	72	3.9±1.0	3.1±0.08	6.2±0.08
Carrot (C)	24	8.6±0.47	4.8±0.04	8.6±0.04
	48	6.8±0.61	4.2±0.04	7.4±0.09
	72	5.2±0.47	3.6±0.04	7.1±0.1
T plus C	24	7.2±0.31	4.3±0.02	7.8±0.1
	48	6.6±0.23	3.9±0.06	7.4±0.2
	72	4.9±0.11	3.3±0.10	6.8±0.3

PTJ: Probioticated tomato juice, PCJ: Probioticated carrot juice, TSS: Total soluble solids, CFU: Colony-forming unit

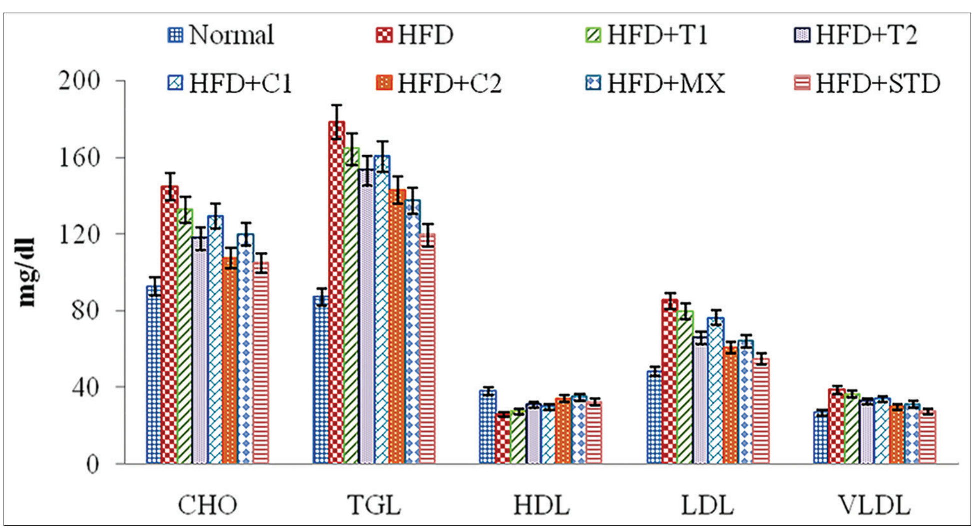

Figure 2: The effect of probioticated tomato, carrot, and mixed juices on serum lipid profile

Table 3: Effect of probioticated supplemented juices on body weights of rats

Body weight (g)	NCD	HFD	HFD+T1	HFD+T2	HFD+C1	HFD+C2	HFD+MX	HFD+STD
Initial weight	175.63±2.17	183.26±2.16	175.16±2.72	175.18±1.37	179.33±3.77	173.62±2.14	174.83±1.16	177.16±4.24
After HFD fed	224.26±3.23	362.81±7.16	354.26±4.94	363.4±4.94	359.42±2.69	344.57±1.26	348.26±2.8	368.37±2.14
After treatment	257.38±3.12	427.36±2.25	325.37±2.33	296.5±2.34	309.33±3.77	262.16±4.28	302.46±2.18	252.83±4.26

NCD: Normal control diet, HFD: High-fat diet

3.4. Effect of Probioticated Juices on Histopathological Changes in Rats

Oral administration of probioticated tomato, carrot, and tomato plus carrot fruit juices containing *L. sphaericus* significantly modulated the changes of histopathological architecture. Figure 3 shows the architecture of normal and treated liver tissue of rats, whereas Figure 4 shows that architecture of normal and treated adipose tissue of rats. In these, Figures 3a and 4a refer to normal diet-fed rat liver and adipose. Figures 3b and 4b refer to HFD-fed rat liver and adipose, and some architectural variations were found in HFD-fed rats. The recovery of architecture found in probioticated juice-treated rats (Figures 3c and 4c refer to HFD+C1; Figures 3d and 4d refer to HFD+C2; Figures 3e and 4e refer to HFD+T1; Figures 3f and 4f refer to HFD+T2; and Figures 3g and 4g refer to HFD+MX), whereas lovastatin (HFD+STD)-treated rats, tissue architecture was similar to normal diet-fed rats in architecture [Figures 3h and 4h].

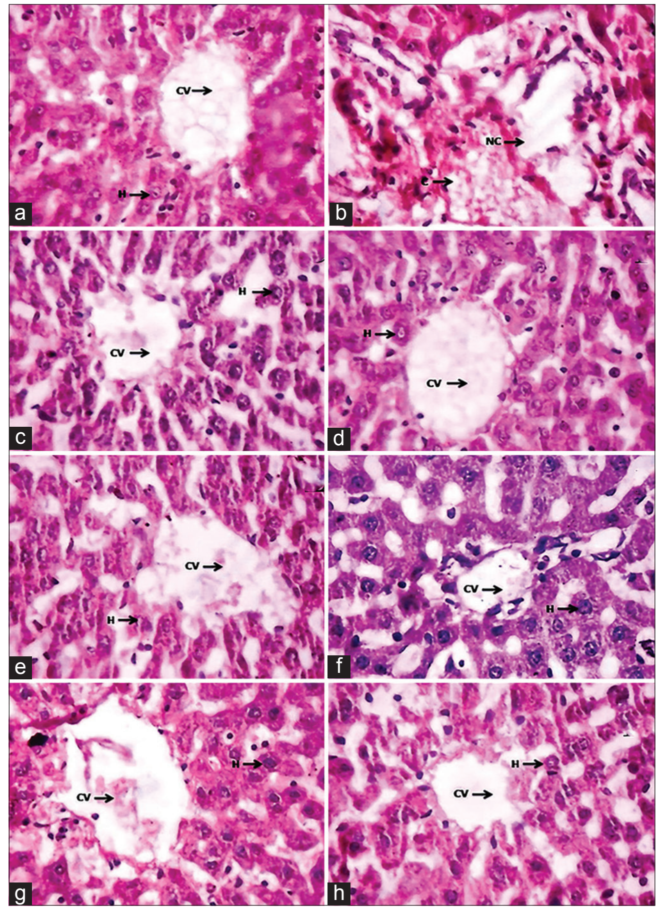

Figure 3: Liver histopathological changes of normal and probioticated juice supplemented rats (a) normal; (b) HFD; (c) HFD+C1; (d) HFD+C2; (e) HFD+T1; (f) HFD+T2; (g) HFD+MX; (h) HFD+STD

4. DISCUSSION

A promising cost-effective approach in lowering serum and plasma levels of different lipids is through the probiotic dietary intervention in the management of several diseases. Probiotic bacteria are considered normal intestinal microflora in humans and animals associated with various health promoting properties [36].

The PTJ, PCJ, and PCT have shown low pH when increased time duration and also observed that a decreased cell viability is due to consumption of nutrients or lack of nutrients. The above results are similar to Sivudu *et al.* [37] and Yoon *et al.* [7]. The cell viability of probiotic bacteria plays an important role in the health benefits [38]. The main factor affecting the cell viability is due to accumulation of organic acids that result in decrease in pH [39]. Babu *et al.* [40] reported that the addition of substrate increases the cell viability. On the contrary, the decreased cell viability was observed in the present study due to lack of nutrients.

According to Bhattacharya *et al.* [41], the reduction of serum and tissue lipid levels was observed in *Emblica officinalis*-treated hyperlipidemic rats due to the presence of good antioxidants. The phytochemicals such as alkaloids, flavonoids, saponins, and tannins have a great responsibility in the reduction of pathological conditions such as hypoglycemic and hypolipidemic activities [42]. Lee *et al.* [43] reported that the tomato juices contained the hypolipidemic activity and da Silva Dias [44] reported that carrot possessed the property of lowering cholesterol due to the presence of phytoconstituents. Kumar *et al*. [45] reported that the probioticated sapota and mango have better hypolipidemic activity than non-probioticated juices.

Several researchers proposed mechanisms for the reduction of total lipid content by the probiotic bacteria. These include deconjugation of bile acids through bile salt hydrolase while the cholesterol act as precursor for bile acid synthesis in blood [46]. The probiotics have other mechanisms for cholesterol removal and these include assimilation of cholesterol by growing cells, binding of cholesterol to cell surface, and incorporation of cholesterol into cell membrane [47,48]. The probiotic cultures convert cholesterol into coprostanol [49] and short chain fatty acids such as propionate, and they also involved in the redistribution of cholesterol from plasma to liver [50]. Ooi and Liong [51] reviewed the lowering cholesterol effects of probiotics and prebiotics in both *in vitro* and *in vivo* conditions.

In the present study, PTJ and PCJ containing *L. sphericus* have been tested in rats fed with HFD for their lowering ability of TC, TG, LDL, and VLDL. The results indicated that the probioticated juices did potentially possess the hypolipidemic activity in the rat model system. The above results are in agreement with the previous reports on the hypolipidemic activity of dairy probiotic foods [52,53].

LDL plays a vital role in the transportation of fat from the liver to periphery. The increased levels of LDL lead to deposition of fat in artery and aorta, and hence, it leads to coronary heart diseases [54]. The decreased levels of LDL levels were found in supplementation

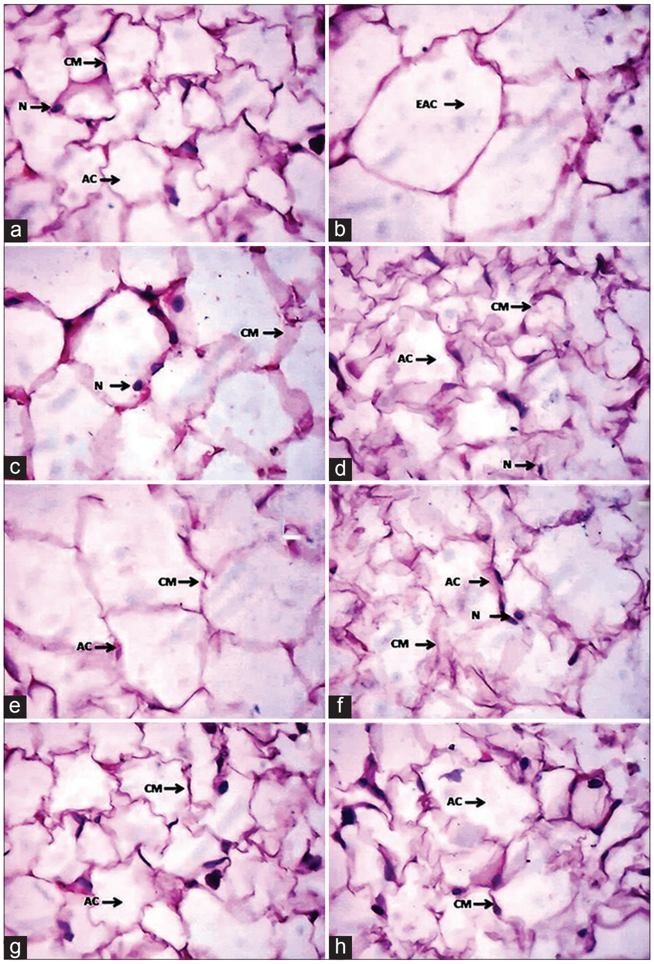

Figure 4: Adipose histopathological changes of normal and probioticated juice supplemented rats (a) normal; (b) HFD; (c) HFD+C1; (d) HFD+C2; (e) HFD+T1; (f) HFD+T2; (g) HFD+MX; (h) HFD+STD

of probioticated tomato and carrot to HFD-fed rats. A significant increase of HDL levels was observed in PTJ-, PCJ-, and PCT-treated hyperlipidemic rats. Furthermore, partly increased levels of HDL cholesterol were observed in hyperlipidemia rats treated with PTJ and PCJ due to the presence of phytochemicals in the tomato and carrot with probiotic strains that are responsible for the hypolipidemic activity.

The HFD-induced obesity is a popular model due to the similarity of mimicking obesity in humans. Buettner *et al.* [55] reviewed the metabolic data obtained with different HFD approaches. The present study revealed that significant increase in animal body weight when exposed to HFD for 3 weeks. In the body weights of HFD and normal diet-fed rats, we have observed a significant difference, but no difference was observed in the daily intake of food. The above observation provides that body weight increase is independent on the amount of food consumed by the rat. The supplementation of probioticated juices (PCJ, PTJ, and PCT) along with HFD showed the reduction in body weights when compared to its corresponding control. These results have suggested that PTJ, PCJ, and PCT are preventing the weight gain and helping in maintaining the correct body weight.

The swelling of hepatocytes together with excessive cytoplasmic vacuolation and vascular congestion was observed in the liver histopathological changes in HFD group rats (Group 2). The recovery of hepatocytes was observed in probioticated juice-supplemented rats, and these results were in agreement with El-Moneim and El-Mouaty [56]. There was also an increase in the amount of white adipose tissue surrounding the kidney, and an increased size of adipocytes was observed in HFD rats compared with the rats received PCJ, PTJ, and PCT together with HFD. These results were in agreement with Nanna *et al.* [57] and they reported that histological changes were observed in aqueous leaf extract of *Tiliacora triandra* administrated to HFD mice.

5. CONCLUSIONS

The *L. sphaericus* has probiotic nature and shown better growth in both tomato and carrot juices even at low pH and 72 h incubation. The PCJ has better growth than PTJ. A number of fat removal mechanisms by probiotics have been proposed, by several researchers. Both PTJ and PCJ were found to possess hypolipidemic activity. The PCJ showed better action on hyperlipidemia than the PTJ. A 2 mL concentration of PCJ has shown an action similar to lovastatin (statin drug)-treated rats. Hence, beverages made from tomato and carrot with probiotics may provide better health benefits to the consumers.

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