Vitamin A-enriched probiotic carrot nectar regulates gut microbiota and reduces obesity : A leading cause of cardiovascular diseases

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Introduction

- According **to WHO, cardiovascular diseases account for 32% of deaths globally**.
- Fat accumulation and obesity are two major risk factors that need to be targeted using natural food supplements.
- Probiotic intervention through a suitable non-dairy matrix is a potential therapy for cholesterol reduction and fat accumulation.
- ***Lactiplantibacillus plantarum* MCC5231** used in probiotic carrot nectar is an **antimicrobial compound** producing probiotic bacteria with antioxidant activity **(Goel et al, 2020).**
- It also has an ***in-vitro* cholesterol-reducing ability** attributed to the production of bile salt hydrolase enzyme. Bsh is known to co-precipitate cholesterol during the deconjugation of bile salts **(Goel et al, 2023)**.

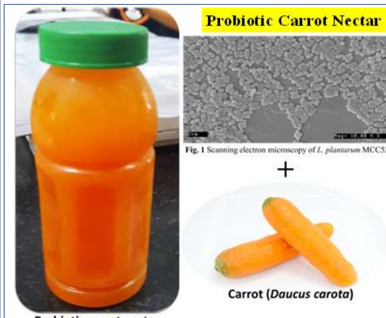
Objectives

- To analyse the changes in gut microbiota on giving probiotic treatment to HFD mice.
- To enumerate the number of probiotic bacteria in the fecal sample of mice.
- To observe the changes in the serum lipid profile of the probiotic-fed HFD.
- To observe histopathological changes after feeding probiotic bacteria for 8 weeks.
- To investigate the changes in the protein level of obesity-associated markers upon probiotic treatment of HFD-fed C57BL6 obese mice, and
- To identify the **mechanism of action of probiotic bacteria for regulating lipid metabolism**

Methods

- Eight-week high-fat diet-fed C57BL6 obese mice with an average weight of 35-37g were divided into 5 groups each with 8 mice for probiotic treatment.
  - **G1** – Normal diet control without treatment
  - **G2** – High-fat diet control without treatment.
  - **G3** – Treatment with reference probiotic bacteria
  - **G4** – Treatment with carrot nectar only (without probiotic)
  - **G5** –. Treatment with probiotic bacteria
  - **G6 -** Treatment with probiotic carrot nectar
- **Analysis of serum lipid profile** was done at the end of the study for TC, TG, LDL, HDL, SGOT and SGPT.
- **Histopathological analysis** of the liver, adipose tissue and intestine was done using H&E and Oil red staining.
- **Gut composition analysis** was done weekly for LAB, *E.coli, S. aureus*, and Bacteroidetes and probiotic bacteria using 16S rDNA-based qPCR.
- **Analysis of Obesity associated markers** in liver and adipose tissue using real-time PCR and Western blot.

Results



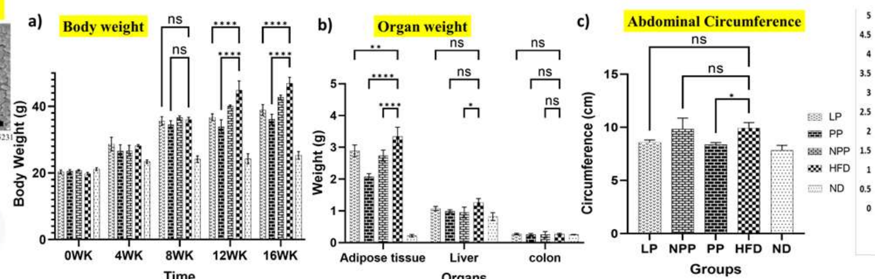


Fig 2 Changes in weekly body weight (a), organ weight (b) and abdominal circumference (c) of probiotic-fed HFD mice

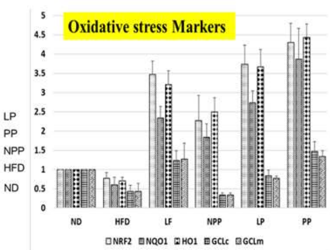


Fig.3 Changes in the gene expression level of Oxidative stress markers

Table 1 Nutritional Profile for 100gm of Probiotic Carrot Nectar

Mineral	RDA(%)	Parameter	Concentration
Sodium (Na)	0.6	Protein (%)	0.1734±0.05
Calcium (Ca)	0.365	Fats (%)	<0.1
Potassium (K)	1.01	Fiber (%)	0.8165±0.04
Magnesium (Mg)	0.80	Carbohydrate(%)	16.780±0.6
Manganese (Mn)	10	Calories	67.8164 kcal/100gm
Phosphorous (P)	1.52	Probiotic bacteria	> 8log CFU/ML
Iron (Fe)	3.25	Shelf life	75 days
Vitamin A	>50%	Estimated cost	30Rs/100ml

Table 4: Gut microbiota composition of Probiotic product-fed HFD C57BL6 Mice

Groups	LAB (%)	*Bacillus* sp. (%)	*S. aureus* (%)	*E. Coli* (%)	*Bacteroides* (%)	Total composition	*L. plantarum** (log Cfu/ml)
LP	31.8	18.40	16.90	9.87	22.90	100%	7.6±0.6
PP	32.78±0.9	18.85±1.2	17.6±0.8	10.01±0.4	20.76±1.2	100%	8.07±0.3
NPP	19.88±0.4	20.37±1.0	19.7±0.8	20.15±0.8	19.9±0.9	100%	5.39±0.7
HFD	14.6±0.2	21.25±0.4	23.2±0.2	22.7±0.3	18.15±0.5	100%	4.01±0.2
ND	20.7±0.2	20.75±0.2	22.06±0.2	12.43±0.2	22.06±0.3	100%	4.74±0.5

*Enumeration of *L. plantarum* was done using 16S rDNA-based quantitative PCR after spiking the control sample with 8log Cfu /ml of *L. plantarum* cells

Table 5: Serum lipid profile of Probiotic product-fed HFD mice

Groups	Total cholesterol (mg/dL)	Tri Glycerides (mg/dL)	High density lipoprotein (mg/dL)	Low density lipoprotein (mg/dL	SGOT (U/L)	SGPT (U/L)
LP	189.4767	410.4418	95.67032	151.6932	125.0583	103.5367
PP	175.3633	357.4297	96.05286	91.10791	109.935	94.81167
NPP	178.02	427.3896	107.8247	199.3307	145.9983	139.0183
LF	155.2067	391.9679	96.57451	166.4455	122.15	114.0067
HFD	232.7132	518.0723	120.5008	200.3129	209.4	225.6867
ND	125.2333	310.8434	85.95027	55.68052	119.8233	70.96333

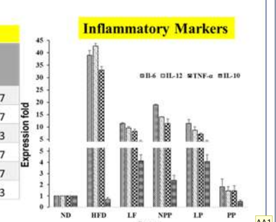


Fig.4 Changes in the gene expression level of Inflammatory markers

Table 2 Anti-microbial spectrum of *L. plantarum* MCC5231

INDICATOR BACTERIA	AU/ML
Gram positive Bacteria	
Listeria monocytogene Scott A	4000
Kocuria rhizophila ATCC9341	4000
Enterococcus faecalis A1	1200
Enterococcus faecalis JH2	1200
Bacillus cereus	4000
Leuc. mesenteroide NRRL B640	1200
L. fermentum MCC2574	1200
Multi-drug resistant Enterococcus bacteria	
Enterococcus MF0	900
Enterococcus MF2	1200
Enterococcus MF3	900
Enterococcus MF4	1200
Enterococcus CHB1	1200

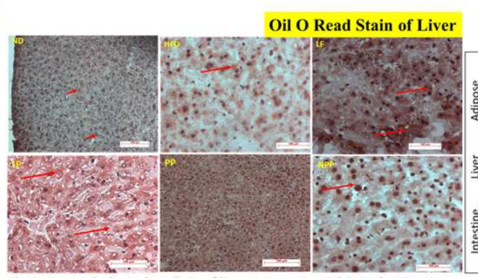


Fig.5 Histopathological analysis of liver tissue using Oil O Red stain.

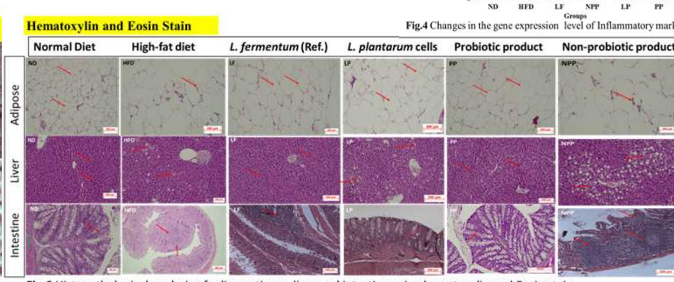


Fig.6 Histopathological analysis of adipose tissue, liver and intestine using hematoxylin and Eosin stain.

Table 3 Safety assessment of *L. plantarum* MCC5231

S. No.	Test performed	Result
1	Gram Staining	Gram Positive
2	Catalase Test	Negative
3	Hemolysis Activity	Negative
4	Lecithinase Activity	Negative
5	Virulent Factors	No threat causing factors
6	Antibiotic Resistant Genes	Non-transferable genes present
7	Mucin degradation Ability	Negative
8	Gelatinase Activity	Negative
9	Prophage Regions	Two intact + Two incomplete
10	CRISPR Regions	two

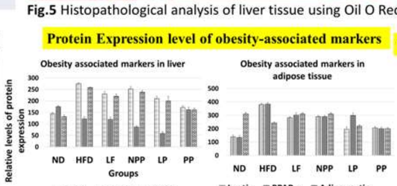
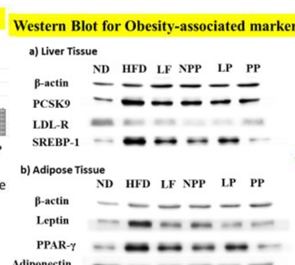


Fig. 7 Changes in protein expression in probiotic-fed HFD mice



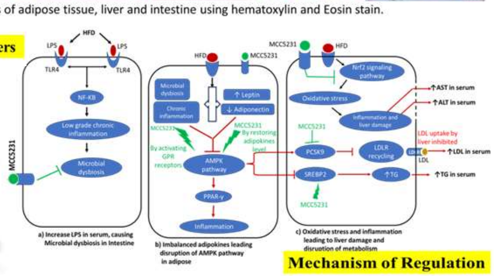


Fig.8 Mechanism of regulation of lipid metabolism by probiotic *L. plantarum* MCC5231

Discussion

- After the eight-week probiotic treatment, the **body weight was reduced to 77 per cent** and **abdominal circumference to 84 per cent** demonstrating the overall effect of the probiotic carrot nectar on obesity.
- **Serum lipid profile** also showed a corresponding decrease in the levels of TC, LDL and TG and an increase in HDL. Similar improvements were observed in **histopathological studies where the PP group showed reduced lipid droplets and inflammatory conditions**.
- Analysis of obesity-associated markers showed that abnormal levels of **Leptin and adiponectin in adipose tissue** affected the normal functioning of the **AMPK pathway**. The disturbed AMPK pathway resulted in the accumulation of LDL and TG in the liver by negatively affecting the expression level of **LDLR and SREBP in the liver.**

Conclusion

Supplementation of probiotic carrot nectar modulated gut microbiota and obesity associated markers regulating the lipid metabolism leading to reduced fat accumulation and physical parameters.

Key Message

Probiotic *L. plantarum* MCC5231 and prebiotic-vegetable matrix rich in fibre (carrot) is a **beneficial symbiotic combination** that can be used as a **natural therapy for obesity** - a **root cause of CVD.**

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