Cardioprotective effect of a Synbiotic Fermented Milk studied in Rat model of Isoproterenol induced Myocardial Infarction

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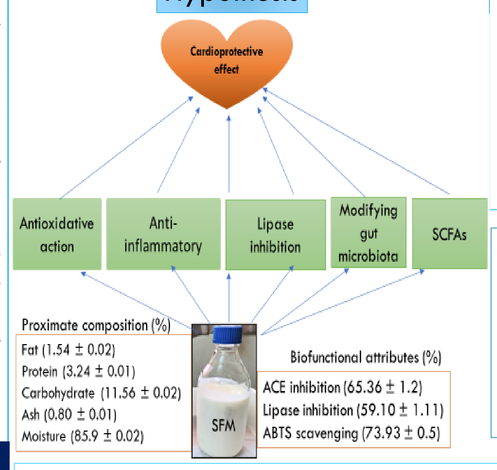
Introduction

- ✓ Myocardial Infarction (MI) remains a leading cause of mortality globally, with oxidative stress and inflammation playing pivotal roles in its pathogenesis.
- ✓ Recent research suggests that synbiotics (probiotics + prebiotics) may play significant roles in preventing MI. However, limited studies have explored this area.
- ✓ A synbiotic contributes towards beneficial gut microbiota which in turn might lower MI risk factors by controlling lipogenesis, cholesterol metabolism, and antioxidant generation (Vahed *et al.*, 2017; Wu and Chiou, 2021).
- ✓ Further the fermentation metabolites such as SCFAs, bioactive peptides, antimicrobial substances may exert cardioprotective effect (Mofid *et al.*, 2019; Mirzapour-Kouhdasht and Garcia-Vaquero, 2022).
- ✓ Milk-derived products provide essential micronutrients and protein (whey, casein and specific bioactive peptides), some of which have been associated with beneficial hypotensive effects (Mirzapour-Kouhdasht and Garcia-Vaquero, 2022; Giosue *et al.*, 2022).

Objectives

- ✓ To evaluate the effect of Synbiotic Fermented Milk (SFM) on the cardiac biomarkers [CK-MB, TNF-α concentration, Troponin I, LDH and MDA content], haematological parameters, lipid profile, histopathology, fecal bacterial count, caecal SCFAs analysis and metagenomic analysis in a rat model of isoproterenol (ISO) induced Myocardial Infarction (MI).

Hypothesis



Methods

- ✓ SFM was prepared using double toned milk supplemented with prebiotic inulin @ 2 % , honey @ 5.5 % (sweetener) and common salt @ 0.3 % (flavour enhancer) fermented using Probiotic culture [*Lactobacillus helveticus* MTCC 5463 + *Lacticaseibacillus rhamnosus* MTCC 5462 + *Streptococcus thermophilus* MTCC 5460 + *Limosilactobacillus fermentum* BM24 in equal proportion]
- ✓ The strains were selected on the basis of their bile deconjugation ability, antioxidant activity, lipase inhibitory activity, cholesterol assimilation, ACE inhibitory activity and proteolytic activity

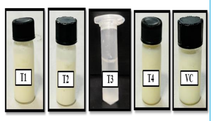
- ✓ After 28 days of oral administration of the respective test products as per the experimental design, on day 29th and 30th the rats in all groups except NC were given subcutaneous injection with ISO for induction of MI.

Institutional Animal Ethics Committee (IAEC) No. 359/VPT/2022]

Experimental Design

8 groups (n=8) of male *Wistar* rats comprising of

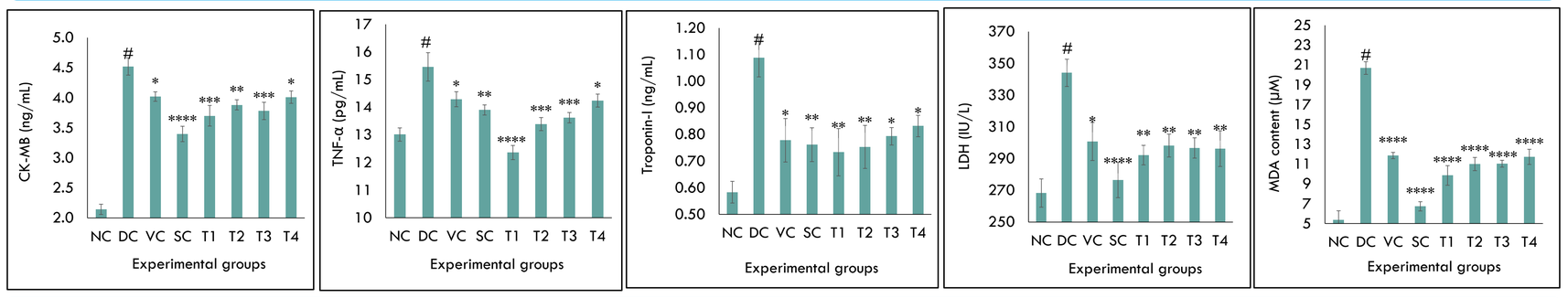
- ✓NC [RO water as sham treatment]
- ✓DC [RO water as sham treatment]
- ✓VC [milk],
- ✓SC [Std drug: Aspirin-12 mg/kg],
- ✓T1 [SFM-1 mL/day (10^8 log CFU/mL)],
- ✓T2 [Probiotic Fermented milk (PFM)-1 mL/day (10^8 log CFU/mL)]
- ✓T3 group [probiotic culture, 10^8 CFU/mL]
- ✓T4 [unfermented milk containing inulin, honey, salt]



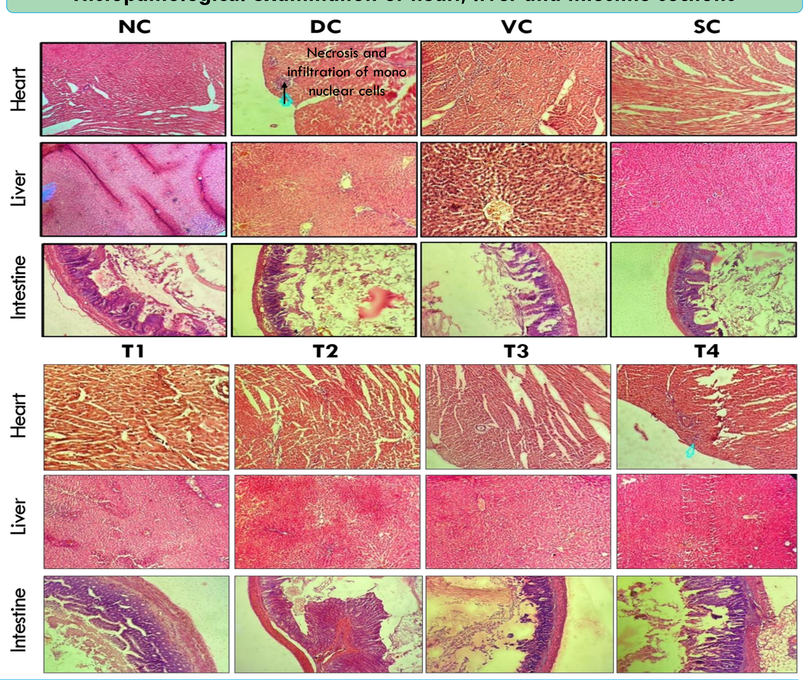
- ✓ Haematological parameters, lipid profile, cardiac profile [Creatine Kinase-MB (CK-MB), Tumour necrosis factor-alpha level (TNF-α), Troponin-I, Lactate Dehydrogenase (LDH), Malondialdehyde (MDA)] were measured using specific ELISA kits supplied by Mybiosource ELISA kit and Cayman chemical ELISA kit. Histopathology of tissues of heart, liver and intestine was carried out. Fecal counts were determined by plating technique. Caecal SCFAs (HPLC Method) and metagenomic analysis (Illumina Miseq) of rat ceca samples.
- ✓ Statistical Method: one-way ANOVA followed by Dunnet's Test using Graph Pad Prism V10.0

Results

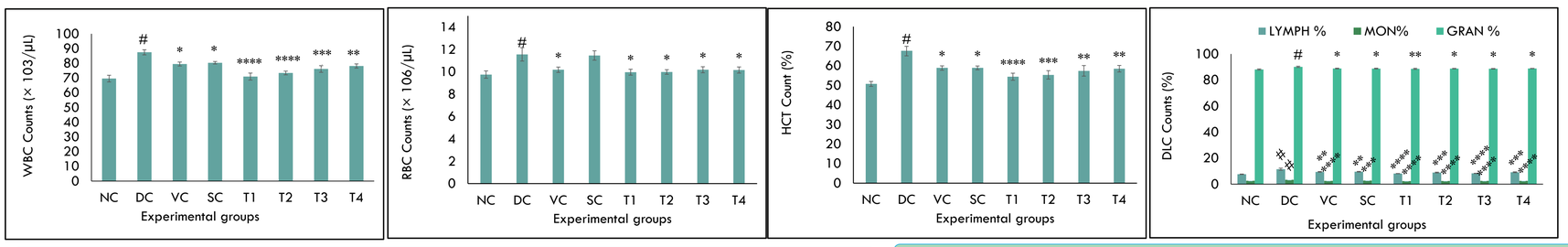
Effect of test products on serum cardiac biomarkers [creatine kinase-MB (CK-MB), tumour necrosis factor-alpha level (TNF-α), troponin-I, lactate dehydrogenase (LDH) and malondialdehyde (MDA) content] of experimental rats



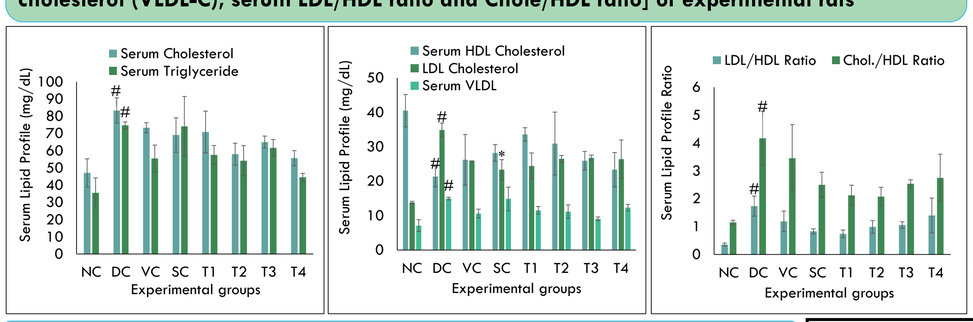
Histopathological examination of heart, liver and intestine sections



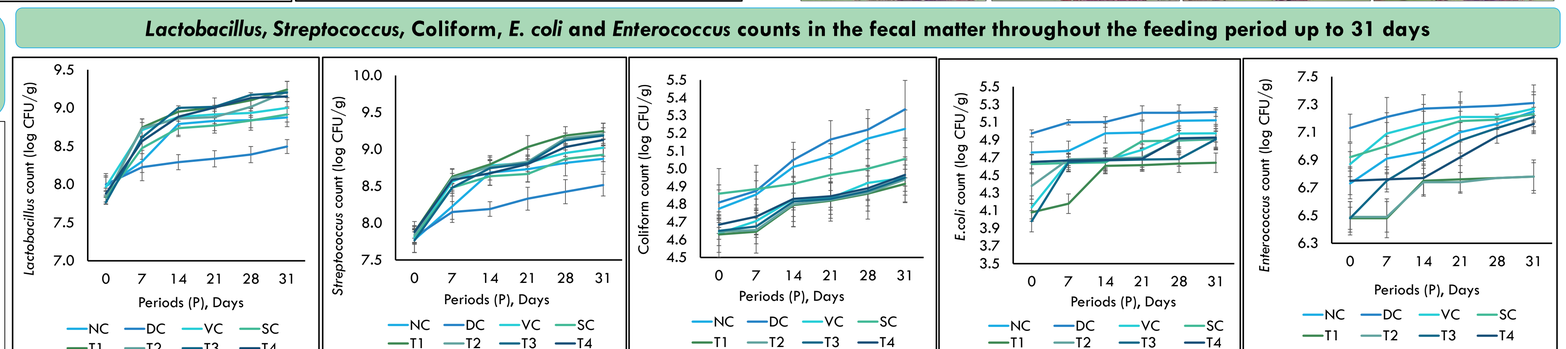
Effect of test products on serum haematological parameters [white blood cells (WBC); red blood cells (RBC); haematocrit test (HCT) and differential leukocyte count (DLC)]



Effect of test products on serum lipid profile [serum total cholesterol (TC), serum total triglyceride (TGs), serum HDL cholesterol (HDL-C), serum LDL cholesterol (LDL-C), serum VLDL cholesterol (VLDL-C), serum LDL/HDL ratio and Chole/HDL ratio] of experimental rats



***Lactobacillus, Streptococcus*, Coliform, *E. coli* and *Enterococcus* counts in the fecal matter throughout the feeding period up to 31 days**

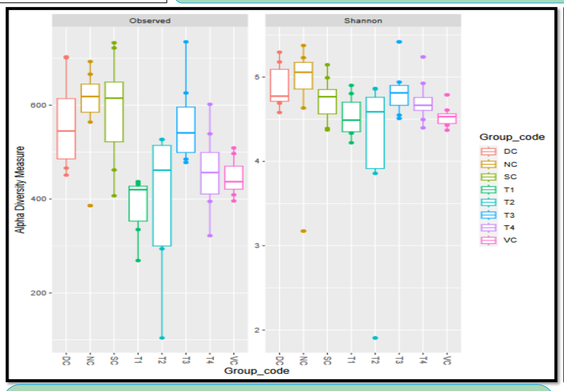


Metagenomic analysis of the caecal matter from experimental rats

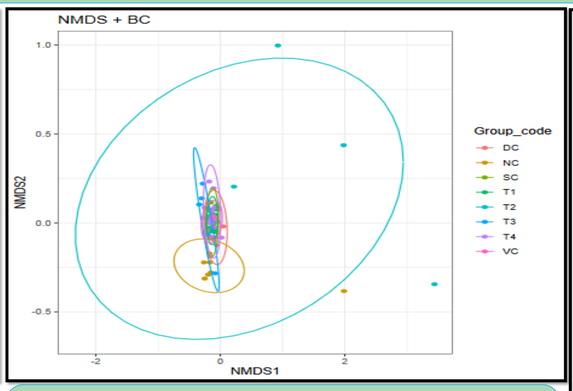
SCFAs content of the caecal matter in Experimental Rats

Treatments	Acetic acid (mg/100 mL)	Butyric acid (mg/100 mL)	Lactic acid (mg/100 mL)	Propionic acid (mg/100 mL)
NC	29.14^{a} ± 1.89	201.77^{a} ± 1.88	169.43^{a} ± 4.69	ND
DC	50.16^{a} ± 2.86	278.73^{b} ± 24.42	ND	116.06^{a} ± 2.51
VC	128.83^{b} ± 7.95	300.99^{b} ± 5.10	258.89^{b} ± 3.02	176.31^{b} ± 9.68
SC	118.76^{b} ± 7.07	287.29^{b} ± 5.17	226.01^{ab} ± 3.43	160.99^{ab} ± 7.74
T1	254.18^{d} ± 15.76	337.31^{b} ± 63.94	458.94^{d} ± 23.79	328.95^{d} ± 20.77
T2	217.22^{cd} ± 45.48	326.84^{b} ± 21.99	431.38^{cd} ± 46.26	321.01^{d} ± 45.24
T3	234.34^{cd} ± 22.29	335.71^{b} ± 51.36	421.54^{cd} ± 23.60	286.88^{d} ± 0.82
T4	192.39^{c} ± 5.07	315.88^{b} ± 23.35	394.34^{c} ± 48.38	234.19^{c} ± 24.06
SEm±	6.80	11.46	9.39	7.09
CD (0.05)	44.38	74.76	61.26	46.25
CV (%)	12.57	10.88	9.00	9.88

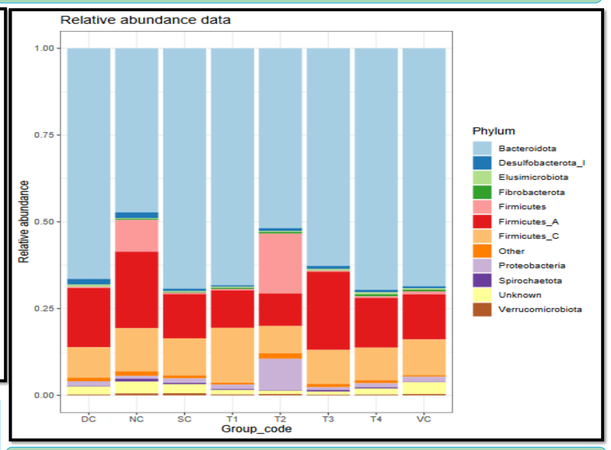
Each observation is a mean ± SD of (n=8).
Values with different superscripts in the column differ significantly (P < 0.05)



Alpha diversity measures (left: Observed ASV, right: Shannon index) of microbial community in cecal contents of different groups



NMDS plots plotted from Bray-Curtis distances calculated from the relative abundances of all samples



Bar plots showing diversity at Phylum level taxonomy

Discussion

- ✓ The elevation in cardiac biomarkers observed in DC group [CK-MB: 4.52 ng/mL, TNF-α:15.46 pg/mL, Troponin I: 1.09 ng/mL, LDH: 344.00 IU/L, MDA content: 20.69 µM] was prevented to a great extent in T1 group [CK-MB (3.70 ng/mL, P < 0.001), TNF-α (12.36 pg/mL, P < 0.0001), Troponin-I (0.73 ng/mL, P < 0.01), LDH (292.20 IU/L, P < 0.01), MDA (9.86 µM, P < 0.0001)].
- ✓ The serum antioxidant capacity of T1 group was significantly (1.23 mM, P < 0.01) higher compared to DC group (0.21 mM).
- ✓ Further, the group showed less degree of inflammatory cell infiltration and relatively well-preserved cardiac muscle fibre morphology in heart tissues and lesser micro vesicular fatty changes in liver tissues.
- ✓ The investigation did not show significant variations in the lipid profile as well as in the relative proportions of genus or phylum among various groups during metagenomic analysis of caecal samples.
- ✓ WBC and RBC counts of T1 group was at par with NC group. T1 group had higher *Lactobacillus* and *Streptococcus* counts but decreased coliform, *E. coli* and *Enterococcus* counts compared to the DC group.
- ✓ Caecal SCFA were significantly elevated in T1 group compared to DC group.

Key Message

Consumption of Synbiotic Fermented Milk could provide protection against MI. However, further preclinical and clinical studies are needed to validate the role of SFM as a new option in the proactive healthcare and the management of myocardial infarction.

Conclusion: This study concludes that synbiotic fermented milk possess cardioprotective effect against myocardial infarction

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