Effect of a yogurt drink combination from jack bean (*Canavalia ensiformis* L.) extract and kidney bean (*Phaseolus vulgaris* L.) extract on the lipid profile of Sprague Dawley rats fed with a high-fat diet

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Abstract. High-fat-diets (HFD) associated with obesity and impaired lipid metabolism. Fermented legume-based yogurt has gained attention due to its bioactive compounds and Lactic Acid Bacteria (LAB). This study evaluated effects of yogurt from jack bean and kidney bean extract on lipid profiles in HFD-fed male Sprague Dawley (SD) rats. In vivo experimental study with randomized block design was conducted using 31 male SD rats in five groups (n=6 for baseline and n=5/group); normal control, HFD control, standard yogurt control, and two treatment groups receiving yogurt containing jack bean:kidney bean extract (60%:20%) at doses of 1.5 mL and 3 mL/day for eight weeks. Total Cholesterol (TC), triglycerides (TG), HDL-C, and LDL-C were analysed at baseline, week-4, and week-8. Serum lipid was analysed by blood biochemical analysis using semi-automatic photometric analyser. Data were analysed using one-way ANOVA. The 3 mL yogurt intervention showed greater reductions in TC, TG, HDL-C, and LDL-C than HFD control, although differences were not statistically significant ($p>0.05$). Notably, HFD administration significantly increased body weight in obese rats compared normal control. Overall, this yogurt demonstrated potential lipid-modulating effects and may serve as supportive functional food, warranting further studies with longer interventions or dietary control.

1 Introduction

The global prevalence of obesity continues to rise and is closely associated with dyslipidaemia and cardiovascular disease [1-3]. High-fat-diets (HFD) play a central role in the development of obesity by promoting excessive energy intake and adiposity, and disrupting lipid metabolism, typically increasing total cholesterol (TC), triglycerides (TG),

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and LDL while reducing HDL, thereby elevating cardiometabolic risk [4]. In recent years, functional foods have attracted growing attention as nutritional strategies to support metabolic health.

Fermented foods have been widely studied due to their probiotic content and bioactive metabolites, which may modulate lipid metabolism through mechanisms such as bile salt hydrolase activity, inhibition of cholesterol synthesis, and modulation of gut microbiota [5-6]. Plant based fermented products are particularly promising as functional foods because they provide not only probiotic effects but also bioactive compounds such as plant protein, dietary fibre, resistant starch, and phenolic compounds that may synergistically contribute to lipid regulation. Jack bean (*Canavalia ensiformis* L.) and kidney bean (*Phaseolus vulgaris* L.) are legumes with potential lipid modulating effects. Jack bean contain saponins that reduce cholesterol absorption and enhance bile excretion, while kidney bean is rich in dietary fibre and polyphenols that improve lipid metabolism and gut microbiota regulation [7-8]. Fermentation into yogurt drink may enhance their bioactivity and sensory acceptability [9-10]. Despite the growing interest in fermented legume products, evidence on the combined formulation of jack bean and kidney bean extract and their effects on lipid metabolism remains scarce. Given their complementary bioactive profiles and the potential of fermentation to enhance functionality, this study aimed to evaluate the effects of yogurt drink from jack bean and kidney bean extract on lipid profiles in HFD-fed male Sprague Dawley (SD) rats. This preclinical approach aims to clarify its physiological relevance and lipid-modulating potential under obesogenic condition and strengthen the scientific basis for developing novel plant-based functional beverages targeting cardiometabolic risk.

2 Materials and methods

2.1 Study design and animals

This in vivo experimental study used a randomized block design. A total of 31 male SD rats were acclimatized for 14-days and then assigned to five groups: (n=6 baseline: 3 normal and 3 obese; n=5/group); normal control (standard diet), HFD control, standard yogurt control (1.5 mL/day standard yogurt+HFD), and two treatment groups receiving yogurt drink containing jack bean:kidney bean extract (60%:20%) at doses of 1.5 mL/day+HFD (TrG1) and 3 mL/day+HFD (TrG2) for 8 weeks.

2.2 Preparation of yogurt drink

Yogurt drink was prepared using jack bean and kidney bean extracts (60%:20%), supplemented with 10% skim milk (Greenfields®) and sugar (5%), and inoculated with 5% Lactic Acid Bacteria (LAB) starter cultures (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*). Fermentation was conducted at 40^0C for 12 h. Standard yogurt control was made with 90% full cream milk (Greenfields®) and sugar (5%), and inoculated with 5% LAB starter cultures. Fermentation process was similar to that described previously.

2.3 Outcome measurement

Primary outcomes include serum lipid parameters which are TC, TG, HDL-C, and LDL-C. Blood samples were collected after 12 h fasting at baseline, week-4, and week-8. Serum lipid levels were measured using enzymatic colorimetric methods via biochemical blood analysis with a semi-automatic photometric analyser. Body weight was measured weekly to confirm obesity induction and evaluate the body weight changes during intervention.

2.4 Statistical analysis

Data were analysed using one-way ANOVA to compare differences among groups and paired t-test to compare differences among midline data (week-4) and endline data (week-8). Statistical significance was set at p-value<0,05.

3 Results

Data in Table 1 shows that high-fat-diets effectively induced obesity, with rats in HFD groups demonstrating significantly greater body weight compared with the normal control ($p<0.001$). At baseline (week-0), the obese group had a markedly higher body weight compared with the normal group (333±7g vs 239.3±25.4 g).

Table 1. Body weight, total cholesterol (TC), triglycerides (TG), HDL-C, and LDL-C at midline data (week-4) and endline data (week-8).

Groups	Body weight (Means±SD)	TC (Means±SD)	TG (Means±SD)	HDL-C (Means±SD)	LDL-C (Means±SD)
Baseline data (week-0)					
Normal	239.3±25.4	55.86±10.2	90.9±40.3	64.5±5.5	17±2.2
Obese	333±7	40.3±14.5	126.8±81.6	66.82±20.4	19.9±7
Midline data (week-4)					
NC	235±18.2	37.6±16.9	85.9±25.3	53±14.1	22.7±4
HFDC	311.4±23.5	69.5±17.9	107.5±30.8	72.6±12.6	24.6±4.7
SYC	300.6±12.8	69.9±16.9	147.3±51.8	74.6±6	21.8±3.9
TrG1	327.6±14.4	63.1±6	111.6±20.4	65.6±12.3	23.8±4.8
TrG2	313.4±20.7	73.1±30.7	153.3±45.3	66.5±19	27.3±8.8
p-value	<0.001*	0.135	0.106	0.246	0.874
Endline data (week-8)					
NC	245.2±21.7	36±8	64.7 ±27.5	43.8±9.8	18.9±4.4
HFDC	339.8±31.9	51.3±9.1	115.2 ±38.4	54.7±16.5	23.7±8.7
SYC	312.6±22.4	58.5±23.1	110.9±28.6	64±13.8	16.7±5.2
TrG1	351.4±16	51.6±17.9	112.7±54.1	56.6±17.1	22.1±5.5
TrG2	335±5.4	53.4±8.8	110.9± 47.6	54.1±17.6	19.5±9.2
p-value	<0.001*	0.171	0.122	0.181	0.285
p-value	<0.001†	0.008†	0.142	<0.001†	0.017†

*: significant difference at $p<0.05$ based on one-way ANOVA among groups; †: significant difference at $p<0.05$ based on paired t-test between week-4 and week-8; normal: normal baseline; obese: obese baseline; NC: standard diet; HFDC: HFD control; SYC: fed with standard yogurt 1.5 mL+HFD; TrG1: yogurt from jack bean and kidney bean extract 1.5 mL+HFD; TrG2: yogurt from jack bean and kidney bean extract 3 mL+HFD

The analysis showed that body weight increased consistently throughout the intervention period, confirming the obesogenic effect of the diet. Baseline lipid parameters were generally comparable between groups, although triglyceride levels were descriptively higher in the obese group (126.8±81.6 mg/dL) than in the normal group (90.9±40.3 mg/dL). Total cholesterol, HDL-C, and LDL-C values showed overlapping ranges between groups, indicating relatively similar lipid status at study initiation.

At week 4, body weight remained highest in the TrG1 group (327.6±14.4 g) and the other intervention groups, compared with the normal control group (235±18.2 g). By week 8, body weight had further increased in all HFD-exposed groups, whereas the normal control group remained substantially lower (245.2±21.7 g). Statistical analysis confirmed a significant

effect of HFD on body weight ($p<0.001$ for one-way ANOVA; $p<0.001$ for t-test), indicating successful induction of obesity. Serum TC showed a downward trend by week 8 among the treatment groups ($p=0.008$). The 3 mL intervention group demonstrated the greatest reduction compared with the HFD control, although between-group differences were not statistically significant ($p>0.05$). TG levels increased after 4 weeks of HFD exposure in some groups and subsequently decreased by week 8, with the greatest reduction observed in the higher-dose yogurt group (TrG2), whereas the HFD control maintained relatively elevated TG concentrations; however, the reduction was not significant (one-way ANOVA $p>0.05$; t-test $p>0.05$). HDL levels fluctuated over time and declined in most groups by the end of the study. Although the intervention did not significantly increase HDL levels ($p>0.05$), the decline was less pronounced in the yogurt intervention groups than in the HFD control group, suggesting a potential protective effect of the yogurt intervention. LDL concentrations increased during HFD feeding and declined modestly in the intervention groups. The 3 mL dose showed the greatest descriptive reduction compared with the HFD control group, although the difference was not statistically significant between groups ($p>0.05$). Overall, lipid changes were influenced primarily by time rather than by treatment.

4 Discussion

This study demonstrated that a yogurt drink derived from jack bean and kidney bean extracts did not significantly modify serum lipid parameters, as indicated by non-significant differences between the intervention and control groups ($p>0.05$). The observed changes in TC, TG, and LDL-C appeared to be driven primarily by time-dependent effects rather than by the intervention. Although the formulation contained bioactive compounds and fermentation-derived components associated with lipid metabolism, these mechanisms did not produce significant changes in serum lipid levels. This limited response may be related to the absence of dyslipidaemia at baseline and sustained exposure to a high-fat diet, which may have overridden the potential benefits of the intervention.

As the yogurt drink was fermented using LAB, its potential effects may be partly related to LAB activity, such as bile salt hydrolase activity and cholesterol assimilation. At the same time, the legume extracts provide bioactive compounds relevant to lipid metabolism, including saponins, dietary fiber, and polyphenols. Therefore, the formulation may exert a combined effect derived from both the legume matrix and LAB fermentation.

Although HFD significantly increased body weight ($p<0.001$), lipid parameters remained relatively stable, indicating a dissociation between adiposity and lipid impaired. This finding is similar with previous studies showing that HFD can induce adiposity without necessarily causing dyslipidaemia [11]. The dissociation between increased adiposity and preserved lipid profiles may reflect metabolically healthy obesity (MHO), a phenotype characterized by relatively normal metabolic status despite excess adiposity. This condition may involve adaptive mechanisms such as efficient lipid storage and preserved insulin sensitivity, which limit lipid spillover and delay metabolic dysfunction, potentially explaining why lipid changes were driven more by time than by the intervention [12].

In addition, evidence indicates that metabolic responses to dietary fat depend on overall diet composition. High-fat, high-cholesterol diets induce dyslipidaemia more readily than high-fat diets alone, suggesting that fat intake alone may be insufficient to markedly alter circulating lipid levels. In the present study, the HFD did not include additional cholesterol, which may have contributed to the lack of significant changes in serum lipid profiles. These findings highlight the complexity of diet-induced metabolic responses and the importance of dietary context in interpreting the effects of fermented legume yogurt [11].

5 Conclusion

Yogurt drink derived from jack bean and kidney bean extracts did not significantly improve lipid profiles under high-fat-diet condition, although the higher dose showed a modest lipid modulating tendency. HFD exposure increased body weight without inducing overt dyslipidaemia, indicating that adiposity may occur without lipid impairment. The limited response may reflect the absence of dyslipidaemia and continuous high-fat feeding, which may have attenuated the yogurt's effect. Future studies should employ dyslipidemic models fed high-fat, high-cholesterol diets, with longer intervention periods and stricter dietary control.

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