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Research Paper

Effects of Fermented Soybeans in Reducing Paracetamol-Induced Liver Toxicity in Rats: A Dual Strain Study

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Abstract

The impact of fermented soybeans was assessed in rats' livers intoxicated with paracetamol. Food-derived protein and peptide hydrolysates can provide benefits that extend beyond their basic nutritional value, offering functional properties that may enhance health and well-being. The current research investigates the potential of *Lactobacillus plantarum* and *Bifidobacterium longum* fermented soybeans against paracetamol-induced liver toxicity in rats. Liver toxicity is caused by an overdose of paracetamol for seven days, followed by a 28-day intervention. The results revealed that both fermented soybean types by probiotic fermentation cause a significant reduction in the liver toxicity group. The most significant results were shown in the LFSM group. The values of liver enzymes AST, ALT, ALP, and bilirubin in the liver toxicity group were increased to 276.47±11.41 IU/L, 128.92±3.94 IU/L, 453.03±6.61 IU/L, and 3.04±0.15 mg/dl, respectively. Levels of AST, ALT, ALP and bilirubin were reduced to 214.92±15.95 IU/L, 89.34±5.93 IU/L, 380.99±23.10 IU/L, 2.04±0.06 mg/dl, respectively, in the group supplemented with LFSM. The hematological parameters also improved in supplementation groups. The antioxidant capacity showed reduced value in the liver toxicity group, while the value improved after treatment. This research indicates that soybeans fermented with *Lactobacillus plantarum* provide better protection against liver damage caused by drugs.

1. Introduction

Paracetamol, often known as acetaminophen, is a popular over-the-counter painkiller and antipyretic due to its efficiency and ease of use (Prescott et al., 2024). Nonetheless, even though paracetamol is widely used, overdosing on it is among the major reason behind abrupt liver failure worldwide (Saad et al., 2024). When taken in large quantities, the metabolism of paracetamol switches to the cytochrome P450 enzyme pathway, which produces detrimental metabolite that is N-acetyl-p-benzoquinone imine (NAPQI) (Sani et al., 2024). NAPQI builds up when glutathione supplies are low, which causes oxidative stress, mitochondrial malfunction, and hepatocellular damage (Hossen et al., 2024). The current treatments, such N-acetylcysteine (NAC), work best when given soon after an overdose, but they become less effective in more severe cases and may cause

adverse effects (Okapra et al., 2024). Alternative hepatoprotective techniques are thus desperately needed in order to supplement current therapies and offer longer-term, safer management option (Eltahir et al., 2024).

Functional foods have gained traction as viable options for complementary and alternative medicine, especially those high in bioactive chemicals (Saubenova et al., 2024). Soybeans are a highly promising food because of their rich nutritional profile, which includes vital amino acids, high-quality protein, and isoflavones which have been linked to antioxidant and anti-inflammatory (Liu et al., 2024; Rizzo et al., 2024). Fermentation breaks down complex chemicals, increases nutrient bioavailability, and introduces bioactive peptides (Farid et al., 2024) with potential therapeutic applications, all of which contribute to the health benefits of soybeans (Elhalis et al., 2024). Probiotics like *Lactobacillus plantarum* (Lactobacillales; Lactobacillaceae) and *Bifidobacterium longum* (Bifidobacteriales; Bifidobacteriaceae) are known to improve the functional qualities of fermented foods by increasing their antioxidant activity and by producing beneficial metabolites that have additional health benefits (Lin et al., 2024; Wong et al., 2024).

Because *L. plantarum* and *B. longum* have well-established probiotic benefits and roles in a variety of fermented foods, they were selected for the soybean fermentation used in this investigation (Gad et al., 2024). *Lactobacillus plantarum* is famous probiotics that contributing key role in promoting gut health and improve immunity of well beings (Yang et al., 2024). It contains antioxidant compounds containing superoxide dismutase (SOD) that prevent cell necrosis (Yasmin et al., 2024). It generates many bio peptides during fermentation process occur in intestinal tract that help reducing oxidative stress and inflammation (Hu et al., 2024). *Bifidobacterium longum* is well known probiotic showing greater benefits in the digestive system. It causes protective barrier in the gastrointestinal tract maintaining inner mucosal lining (Mora et al., 2024). Probiotics enhancing gut friendly bacteria, reducing acid reflux and inflammation (Luasiri et al., 2024). The metabolism of probiotics produced diverse bioactive compounds such as omega 3 fatty acids, peptides, polyphenols and flavonoids. (de São José et al., 2024). *L. plantarum* and *B. longum* both contain health improving properties. Limited studies are done over the comparison between these two strains against liver toxicity induced by paracetamol (Hernandez et al., 2024).

The main goal of this research is to analyze the influence of *L. plantarum* and *B. longum* on rodent liver injured with paracetamol. Fermented soybeans by probiotics cover distinct health aspects including biochemical and hematological properties of liver. In this research we also evaluate methods to ameliorate free radicals and inflammation. Hence, this study makes a beneficial addition in the field of functional foods for improving the liver health and makes a substantial research focus for future scientists.

2. Material and Methods

Soybeans were procured from local market of Multan. However, culture of *Lactobacillus* and *Bifidobacterium* were purchased from My Drug store, Lahore. Furthermore, Paracetamol (APAP) was sourced from Abbott Laboratories, Pakistan.

2.1 Preparation of fermented soybeans

Whole Soybeans around 1 kg were drenched in water for twelve hours. After soaking they were blanched in water. Then cool them at room temperature. Thereafter, four teaspoons of *Lactobacillus* and *Bifidobacterium* culture were uniformly spread above 500g cooled soybeans of each sample then stirring softly. Then shift the treated beans into perforated plastic bags and incubate at 35^{o}C for 25 hours. Then fermented soybeans were dehydrated and pulverized to dry powder after incubation period (Taya et al., 2024).

2.2 Study design and treatment protocols

A total 20 male albino rats, approximately two months old and weighing between 200 to 250 grams, were obtained from pharmacy department at Bahauddin Zakariya University in Multan. They were accommodated in wire cage at a temperature of 22±1°C and 60% dampness, with a controlled 12-hour light and dark cycle. Male albino rats were divided into four distinct groups (n=5/ group). Group 1 was given a regular diet. Group 2 was given a single oral dosage of paracetamol (1g/kg) served as the model of hepatotoxicity. Group 3 were given *Bifidobacterium* fermented soybeans meal (BFSM). Group 4 received *Lactobacillus* fermented soybeans meal (LFSM) (Table 1).

Table 1 Treatment plan and ingredients used in the study

Group of rats	**Diet**	**Dose**
Group 1: (control)	Standard diet	500 mg/kg/b. wt. distilled water
Group 2: (positive control)	Standard diet	Paracetamol 1000 mg/kg/ b. wt.
Group 3: (BFSM)	Standard diet	Paracetamol + BFSM (500 mg/kg/ b. wt.)
Group 4: (LFSM)	Standard diet	The treatment group getting Paracetamol + LFSM (500 mg/kg/ b. wt.)

BFSM= *Bifidobacterium* fermented soybean meal; LFSM= *Lactobacillus* fermented soybean meal.

2.3 Induction of liver toxicity

The dose of paracetamol used to induce liver toxicity was 1000 mg/kg body weight. It was given to rats through oral gavage for the period of seven days (Neag et al., 2020).

2.4 Biochemical analysis

The blood samples were collected after the completion of experiment. The blood was taken through cardiac puncture following anesthesia. The vials were kept in centrifuge at a speed of 3000 rpm for 15 minutes for serum separation. Liver function test (LFT), Renal function test (RFT) were evaluated using auto-analyzers (BT 1500, 80 Italy and Biolis 50i, Japan) (Chinnappan et al., 2023). Oxidative status was checked using using an assay reported by (Riane et al., 2020). This test measures the ability of superoxide radicals to react with nitroblue tetrazolium (NBT) from Sigma, resulting in the formation of formazan crystals. These crystals were quantified by measuring absorbance at 560 nm.

2.5 Serum lipid profile and serum protein analysis

Serological analysis was conducted, concentrating on lipid profiles, protein profiles and blood glucose levels. All parameters of lipid profile included measurements of total cholesterol (TC), triglycerides (TG), low density lipoproteins (LDL), very low density lipoproteins (VLDL) and high density lipoproteins (HDL). These measurements were examined using enzymatic hydrolysis, with absorbance readings taken at 456 nm (Gungor et al., 2023). The levels of serum proteins containing total proteins, globulin and albumin were analyzed using standard assay kits (Roche Diagnostics GmbH, USA) and studied using the 902 automatic analyzer (Hitachi-Boehringer Mannheim).

2.6 Hematological Analysis

Whole blood samples were used for hematological assessments, including red blood cell count (RBC), white blood cell counts (WBC), hemoglobin concentration, hematocrit, and platelet count. Additional parameters, such as mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC), were also evaluated to detect any potential hematological abnormalities. In brief, blood samples were collected in vials containing ethylenediaminetetraacetic acid (EDTA) as an anticoagulant. The samples were examined by using automated hematology

analyzer (Sysmex KX-21N, Japan). All measurements were conducted in triplicate to ensure accuracy.

2.7 Statistical Examination

The data attained from the study were showed as mean ± standard deviation (SD). SPSS software package (version 21) was used for the performance of statistical analysis. One-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons was used for significant difference among groups. A p-value < 0.05 was considered statistically significant.

3. Results

3.1 Efficacy study

In this stage of the study, albino rats' liver damage caused by paracetamol (acetaminophen) is prevented by the hepatoprotective effects of fermented soybeans. To harm the liver, the rats were given 1g/kg body weight of paracetamol for seven days in a row. They were then given LFSM 500mg/kg and BFSM 500 mg/kg for four weeks.

3.2 Effects of fermented soybeans on body and liver weight

Body weight and organ weights are important parameters of overall health and physiological status. In this research, the effects of paracetamol and subsequent treatments with LFSM and BFSM on body and organ weights were evaluated. The bodyweight of the positive control group significantly reduced to (214.00±9.16 g). However, treatment with LFSM resulted in an increase in body weight to (234.33±6.50 g), suggesting some recovery, while the BFSB group showed a moderate increase to (225.67±9.60 g). Liver and heart weight expressed normal values of (3.67±0.24 g/100g) (1.315±0.01 g) in positive control group respectively. This indicates that paracetamol did not significantly alter liver and heart weight. Spleen weights were slightly elevated in the positive control group at (0.22±0.04 g), both LFSM (0.162±0.02 g) and BFSM (0.184±0.02 g) groups showed lower spleen weights, indicating a potential reduction in splenic hyperplasia associated with liver damage. Kidney weights were significantly higher in the positive control group at (0.43±0.02 g), suggesting potential renal stress or hypertrophy due to hepatotoxicity. Treatment with LFSM reduced kidney weight to (0.378±0.01 g), while the BFSM group showed a further decrease to (0.316±0.01 g), indicating a protective effect of these treatments on kidney health as shown in Table 2.

Table 2 Effects of Fermented Soybeans on Body and Liver Weight

Parameter	Control	Positive control	LFSM	BFSM
Body Weight (g)	250.00 ± 2.00^{a}	214.00 ± 9.16^{c}	234.33 ± 6.50^{ab}	225.67 ± 9.60^{bc}
Liver Weight (g/100g)	4.11 ± 0.17^{a}	3.67 ± 0.24^{a}	4.07 ± 0.54^{a}	3.80 ± 0.67^{a}
Spleen(g)	0.21 ± 0.02^{b}	0.22 ± 0.04^{b}	0.16 ± 0.02^{a}	0.18 ± 0.02^{ab}
Heart(g)	1.33 ± 0.02^{a}	1.31 ± 0.01^{c}	1.31 ± 0.01^{ab}	1.31 ± 0.01^{b}
Kidney(g)	0.35 ± 0.02^{b}	0.43 ± 0.02^{d}	0.37 ± 0.01^{c}	0.31 ± 0.01^{a}

Values are expressed as mean ± S.E. values with different letters shows significant difference ($p<0.05$) between values.

3.3 Biochemical parameters of liver

Liver function tests (LFTs) serve as critical indicators of liver health and potential hepatotoxicity. This research indicated increased levels of liver enzymes. The bilirubin level was notably increased at (3.04±0.15 mg/dL) in the positive control group but decreased to (2.04±0.06 mg/dL) in LFSM group, indicating a reduction in liver dysfunction. Similarly, the levels of AST and ALT showed marked elevation in the positive control group, with values (276.47±11.41 U/L) and (128.92±3.94 U/L) respectively. After treatment with LFSM and BFSM, these values decreased to (214.92±15.95 U/L) (250.02±7.26 U/L) for AST, and (89.34±5.93 U/L) (112.73±3.52 U/L) for ALT respectively. The ALP level increased significantly to (453.03±6.61 U/L) in the positive control

group. The values decrease to (380.99±23.10 U/L) in LFSB group and (420.35±12.01 U/L) in the BFSB group as shown in Table 3, suggesting that both treatments may help mitigate cholestatic effects associated with liver injury.

Table 3 Biochemical parameters of liver function

Parameter	Control	Positive control	LFSM	BFSM
ALT (IU/L)	26.98 ± 1.16^{d}	128.92 ± 3.94^{a}	89.34 ± 5.93^{c}	112.73 ± 3.52^{b}
AST (IU/L)	74.52 ± 3.26^{c}	276.47 ± 11.41^{a}	214.92 ± 15.95^{b}	250.02 ± 7.26^{b}
ALP (IU/L)	142.70 ± 9.34^{c}	453.03 ± 6.61^{a}	380.99 ± 23.10^{b}	420.35 ± 12.01^{b}
Bilirubin (mg/dL)	0.59 ± 0.06^{c}	3.04 ± 0.15^{a}	2.04 ± 0.06^{b}	2.59 ± 0.08^{b}

Values are expressed as mean ± S.E. values with different letters shows significant difference ($p<0.05$) between values.

3.4 Lipid profile analysis

Lipid profiles are crucial for assessing cardiovascular health and identifying potential dyslipidemia. In this study, the effects of paracetamol and subsequent treatments with LFSM and BFSM on lipid parameters were evaluated. Total cholesterol levels were slightly elevated in the positive control group at (89.93±2.04 mg/dL). However, treatment with LFSM and BFSM resulted reduction in TC values. The values are (84.25±1.25 mg/dL) and (86.82±0.68 mg/dL), respectively. Triglyceride levels also increased in the positive control group to (94.75±1.43 mg/dL), which may reflect an adverse metabolic response due to hepatotoxicity. Triglyceride level decreased in LFSM (88.55±0.69 mg/dL) and BFSM (89.38±1.34 mg/dL) after treatment. Maintain blood lipid metabolism. The level of LDL (low density lipoprotein) cholesterol in positive control group increased to (37.98±1.07 mg/dL). Both LFSM and BFSM groups showed lower LDL levels of values (32.14±0.84 mg/dL) and (32.92±0.69 mg/dL), respectively. High-density lipoprotein (HDL) cholesterol levels were significantly lower in the positive control group at (32.99±0.79 mg/dL). The LFSM group exhibited improvement in HDL to (37.40±1.11 mg/dL), while the BFSM group had a level of (36.02±0.84 mg/dL), indicating that both treatments may enhance protective HDL levels. Lastly, very low-density lipoprotein (VLDL) cholesterol was elevated in the positive control group at (18.95±0.28 mg/dL). Treatment with LFSM and BFSM reduced VLDL levels to (17.71±0.13 mg/dL) and (17.87±0.26 mg/dL), respectively, suggesting improved lipid profiles following treatment as shown in Table 4.

Table 4 Effects of Fermented Soybeans on Lipid Parameters

Parameter	Control	Positive control	LFSM	BFSM
Total Cholesterol (mg/dL)	84.26 ± 0.49^{b}	89.93 ± 2.04^{a}	84.25 ± 1.25^{ab}	86.82 ± 0.68^{ab}
Triglycerides (mg/dL)	83.54 ± 1.51^{c}	94.75 ± 1.43^{a}	88.55 ± 0.69^{b}	89.38 ± 1.34^{b}
LDL (mg/dL)	33.01 ± 0.87^{b}	37.98 ± 1.07^{a}	32.14 ± 0.84^{b}	32.92 ± 0.69^{b}
HDL (mg/dL)	35.54 ± 0.67^{a}	32.99 ± 0.79^{b}	37.40 ± 1.11^{a}	36.02 ± 0.84^{a}
VLDL (mg/dL)	16.70 ± 0.30^{c}	18.95 ± 0.28^{a}	17.71 ± 0.13^{b}	17.87 ± 0.26^{b}

Values are expressed as mean ± S.E. values with different letters shows significant difference ($p<0.05$) between values.

3.5 Serum protein analysis

Total protein and albumin/globulin (A/G) ratio measurements are critical indicators of nutritional status and liver function. In this study, the effects of paracetamol and subsequent treatments with LFSM and BFSM on these parameters were evaluated. The total protein level significantly decreased in the positive control group to (5.63±0.44 g/dL). However, treatment with LFSM resulted in an increase in total protein levels to (6.92±0.06 g/dL), suggesting a partial restoration of protein synthesis, while the BFSM group also showed improvement with a level of (6.65±0.25 g/dL). Albumin levels were significantly reduced in the positive control group at

(3.12±0.11 g/dL). Treatment with LFSM improved albumin levels to (3.86±0.04 g/dL), while the BFSM group showed a moderate increase to (3.72±0.18 g/dL). Globulin levels were also affected, with the positive control group showing a decrease to (2.74±0.37 g/dL). The LFSM and BFSM groups exhibited improvement in globulin levels of (3.32±0.07 g/dL) and (3.05±0.10 g/dL), respectively. The A/G ratio, which reflects the balance between albumin and globulin levels, was lower in the positive control group at (1.13±0.12). Both LFSM and BFSM treatments improved the A/G ratio slightly, with values of (1.16±0.17) and (1.21±0.19), respectively, indicating a trend towards normalization. All the values are shown in Table 5

Table 5 Effects of Fermented Soybeans on Serum Proteins

Parameter	Control	Positive control	LFSM	BFSM
Total Proteins (g/dL)	7.29 ± 0.23^{a}	5.63 ± 0.44^{b}	6.92 ± 0.06^{a}	6.65 ± 0.25^{a}
Albumin (g/dL)	4.20 ± 0.14^{a}	3.12 ± 0.11^{c}	3.86 ± 0.04^{ab}	3.72 ± 0.18^{b}
Globulin (g/dL)	3.29 ± 0.08^{a}	$2.74\pm0.0.37^{b}$	3.32 ± 0.07^{a}	3.05 ± 0.10^{ab}
A/G ratio	1.27 ± 0.15^{a}	1.13 ± 0.12^{c}	1.16 ± 0.17^{a}	1.21 ± 0.19^{ab}

Values are expressed as mean ± S.E. values with same letters shows insignificant difference ($p<0.05$) between values.

3.6 Antioxidant activity and histopathological analysis

Superoxide dismutase (SOD) levels and histological scores are key indicators of oxidative stress and liver health. In this study, the SOD activity was significantly decreased in the positive control group, with a value of (54.37±4.37 μmol/L), indicating heightened oxidative stress due to hepatotoxicity. The SOD (superoxide dismutase) levels elevated in positive control group; the value is (72.66±2.51 μmol/L). The intervention groups LFSM and BFSM indicated normal antioxidant activity with values (70.33±1.52 μmol/L) and (67.39±6.02 μmol/L) respectively. The positive control group showed higher value of histology (1.62±0.13), demonstrating possible liver impairment. However, the treatment groups LFSM and BFSM indicated normal values that were (0.56±0.15) and (0.66±0.08), respectively. (Table 6).

Table 6 Assessment of SOD levels and histological scores

Parameter	Control	Positive control	LFSM	BFSM
SOD (μmol/L)	72.66 ± 2.51^{a}	54.37 ± 4.37^{b}	70.33 ± 1.52^{a}	67.39 ± 6.02^{a}
Histological Scores	0.40 ± 0.2^{b}	1.62 ± 0.13^{a}	0.56 ± 0.15^{b}	0.66 ± 0.08^{b}

The value of same letters shows insignificant difference at $p< 0.05$. Values are represented as mean ± S.E.

3.7 Hematological assessment

The determination of hematology indicates overall health status. The paracetamol group showed decreased values of hemoglobin (9.38±0.28 g/dL). While the treatment group LFSM significantly increased hemoglobin levels (10.78±0.54 g/dL). However, BFSM group also improved the hemoglobin to (9.58±0.30 g/dL). There was no significant difference observed in RBCs across all groups. Hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC) values displayed little reduction in positive control group to (33.67±0.56%), (38.77±0.76 fL), (13.42±0.19 ρg) and (29.37±0.71 g/dL) respectively. Nevertheless, the groups supplemented with LFSM and BFSM showed significant increment in MCV and MCH values to (46.51±0.29 fL), (44.56±0.63 fL), (15.68±0.19 ρg) and (14.9±0.46 ρg) respectively. The higher value of white blood cells (WBC) was seen in positive control group (13.22±0.24 U/L). LFSM and BFSM presented increased levels of WBCs to (11.08±0.28 U/L) and (12.20±0.44 U/L) respectively. Lymphocyte percentages increased

significantly in the positive control group to (68.96±2.92%), reflecting a heightened immune response, while neutrophil percentages also rose to (42.80±3.43%), indicating acute inflammation associated with liver injury. Monocyte levels were elevated in the positive control group at (7.9±0.21%), suggesting ongoing inflammation or tissue repair processes, while eosinophil percentages remained relatively stable across groups. Treatment with LFSM and BFSM improved lymphocyte, neutrophil and monocyte levels as shown in table 7.

Table 7 Hematological parameters across different experimental groups

Parameter	Control	Positive control	LFSM	BFSM
Hemoglobin(g/dL)	11.90±0.56^{a}	9.38±0.28^{b}	10.78±0.54^{a}	9.58±0.30^{b}
RBCs (million/µL)	6.95±0.44^{a}	6.17±0.32^{a}	6.71±0.39^{a}	6.36±0.23^{a}
HCT (%)	34.93±1.41^{b}	33.67±0.56^{a}	33.98±0.88^{b}	33.73±0.54^{b}
MCV (fL)	46.36±0.89^{c}	38.77±0.76^{a}	46.51±0.29^{c}	44.56±0.63^{b}
MCH (ρg)	16.00±0.43^{c}	13.42±0.19^{a}	15.68±0.19^{c}	14.9±0.46^{b}
MCHC (g/dL)	36.57±1.64^{c}	29.37±0.71^{a}	36.98±0.51^{c}	34.48±0.24^{b}
WBCs (10^3/L)	9.68±0.55^{c}	13.22±0.24^{a}	11.08±0.28^{b}	12.20±0.44^{a}
Lymphocytes (%)	59.03±4.35^{b}	68.96±2.92^{a}	60.70±2.98^{b}	64.36±0.45^{ab}
Neutrophils (%)	35.73±2.95^{ab}	42.80±3.43^{a}	36.30±2.15^{b}	39.06±2.53^{b}
Monocytes (%)	4.85±0.15^{b}	7.9±0.21^{c}	4.93±0.14^{b}	5.56±0.15^{c}
Eosinophil (%)	4.47±0.18^{c}	5.46±0.23^{c}	3.79±0.5^{b}	3.74±0.16^{ab}

Values are expressed as mean ± S.E. values with different letters shows significant difference ($p<0.05$) between values.

4. Discussion

Acetaminophen, also known as paracetamol, is a commonly used over the counter analgesic medication used to relieve pain and fever, but too much of it can damage liver. Jang et al. (2018) revealed the beneficial effects of probiotics including *L. plantarum* and *B. longum* against hepatotoxicity. The liver parameters of positive control group significantly increased during the four-week research, while the treatment groups showed reduction in liver parameters. This study provides important understanding about protective effects of LFSM compared to BFSM against paracetamol-induced liver toxicity. The findings demonstrate that LFSM treatment leads to significant improvements in various physiological parameters, indicating its potential role in improving overall gut and liver health.

Patra et al. (2018) determined significant decrease in body weight in the positive control group contrast to all other groups suggest that paracetamol cause stress, increased fat loss, metabolism disturbance, low food intake and degradation of body tissues. This study matches with the existing literature demonstrating hepatotoxicity weaken overall metabolism and immunity resulting weight reduction. Liver plays a crucial role in storage of nutrients and energy metabolism. The improvement of body weight has seen in group supplemented with LFSM. Aliouche et al. (2025) reported the same results when gave LFSM. The results indicates that LFSM increase metabolism and gastrointestinal stability throughout liver toxicity. LFSM also improved assimilation process and nutrients uptake. Fermented soybeans contain various bioacitive compounds including isoflavones that reduced weight.

The major reduction in liver enzymes (ALT, AST, ALP) and bilirubin see in the groups supplemented with LFSM and BFSM. Adawi et al. (2001) has reported the protective effects of different starins of *Lactobacillus* and *Bifidobacterium* against liver injury. The study demonstrated that alcohol induced liver toxicity increased the levels of liver enzymes that badly impairs liver

functionality. Whereas the groups supplemented with *Lactobacillus* and *Bifidobacterium* has ameliorate liver enzymes. Kim et al. (2018) has studied that *Lactobacillus* showed more significant results when compared with *Bifidobacterium.* This analysis aligns with our results that the group supplemented with LFSM showed more significant results when compared to other groups. *Lactobacillus plantarum* has recognized with its antioxidant properties, when metabolized in intestines it produces short chain fatty acids that breakdown free radicals and diminish oxidative stress (Jang et al., 2018).

Superoxide dismutase (SOD) levels were depleted in positive control group. Patra et al. (2018) has checked antioxidant activity in LFSM group and ethanol induced liver toxic group. The SOD levels were depleted in hepatotoxic group. This is due to higher disruption in liver cells causing oxidative stress. This shows that free radical chain reaction can damage the cell membranes of liver. SOD levels were significantly elevated in treatment groups, suggesting an elevated defense system. The increment of 45% SOD levels were seen in group supplemented with LFSM. However, the group supplemented with BFSM presented 35% escalation. This signifies the ability of LFSM to decrease the number of free radicals resulting decrease in oxidative stress.

Wang et al. (2021) reported the significant results of LFSM in maintaining liver histology. The Fermented soybeans with *L. plantarum* reduced inflammatory mediators that spike the TNF-α causing liver cell necrosis when compared to unfermented soybeans. Probiotics has proven for maintaining the gastrointestinal and reproductive health. The lactic acid bacteria give health superior benefits to intestinal micro flora by generating natural anti-bacterial substances like hydrogen peroxide, organic acid after metabolism. Chen et al. (2023) reported the similar finding in line with our literature that both fermented strains have proved beneficial effects when metabolized in intestinal tract by producing health promoting bio peptides. Hence, the LFSM group has showed more pronounced effects in ameliorating paracetamol induced injyry in liver cells (Palócz et al., 2016).

Gan et al. (2021) studied the variations in lipid metabolism due to ethanol exposure, resulting in an increase in LDL, total cholesterol, triglyceride and reduction in HDL levels. This is due to disturbance in liver functions that are responsible for making normal lipid production. The LFSM and BFSM treated groups showed significant effects in homogenizing lipid profile. This is due to activation of different pathways including lipoprotein lipase (LPL) and β oxidation pathways. *Lactobacillus plantarum* has the ability to bind with bad cholesterol and lowered its absorption (Kanmani et al., 2022; Raftar et al., 2021). The fermented products comprising lactic acid are able to reduce biological availability of cholesterol in intestinal cells. Kamaly et al. (1998) has described the fermentation of soy milk with *Bifidobacterium* has showed different fatty acid compositions and phytochemical profile responsible for maintaining health.

Abbasi et al. (2017) studied that fermented soy milk has reduced the GFR value in diabetic patients suggesting the positive effect on health of kidney patients. Our results indicated that albumin levels are dropped in positive control group. It is due to improper functioning of liver. While no significant differences were seen in BFSM and LFSM supplemented groups. However, the supplementation of fermented soybeans improved the bioavailability of amino acid and rejuvenation of liver functions (Raftar et al., 2021).

Hematological measurements were assessed to check the outcomes of LFSM and BFSM on the liver toxicity. Our results indicated that hemoglobin levels were significantly improved in treatment groups when compared to positive control group (9.38±0.28 g/dL). LFSM treated group showed increment to (11.90±0.56 g/dL). Our results reported anemia in Positive control group due

to malfunction of erythrocyte. This can also alter iron metabolism. Niamah et al. (2017) reported the similar effects of probiotics when given to rats increasing hemoglobin levels. Furthermore, the white blood cell count increased in positive control group indicating inflammation. Our findings represented that supplementation with LFSM for four weeks decreased the inflammatory response. The similar findings were studied by Cagno et al. (2010) showing the anti-inflammatory components found in soy milk.

Hence both treatment groups improved the hematological values to some extent. However, our study finds no serious effect of LFSM and BFSM. Mixed strains *L. plantarum* and *B. longum* fermented soybeans for the longer period could be studied for the future analysis. Moreover, gut metabolomics and the human trials should be investigated in future studies.

Conclusion

The main findings of this research comprised that LFSM and BFSM provides liver protective effects. The more significant results were showed by the group supplemented with LFSM. The ALT, ALP, AST and bilirubin significantly decreased in LFSM group. However, BFSM also showed beneficial effects against liver toxicity. These results indicated soybeans fermented with different probiotics offering various health benefits.

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