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Galactose-specific lectin from bean sprouts induces oxidative stress, inflammation, and apoptosis in the mouse liver

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ABSTRACT

BS-Gal, a galactose-specific lectin isolated from bean sprouts and highly resistant to digestive degradation, caused significant hepatocellular and hepatobiliary injury in BALB/c mice after 20 days of oral exposure. Plasma levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), and bilirubin were markedly elevated, indicating impaired liver function. Antioxidant defences were disrupted, with reduced activities of superoxide dismutase (SOD) and catalase (CAT) and compensatory increases in glutathione peroxidase (GPx) and glutathione reductase (GR), accompanied by elevated hepatic hydrogen peroxide (H_2O_2), nitric oxide (NO), and malondialdehyde (MDA), reflecting pronounced oxidative and nitrosative stress. This oxidative imbalance was associated with strong activation of nuclear factor kappa B (NF-κB) and increased production of proinflammatory cytokines IL-6, IL-1β, and TNF-α. Moreover, BS-Gal shifted apoptotic signalling toward cell death by upregulating proapoptotic proteins BAD and BAX and downregulating the antiapoptotic protein Bcl-2. Collectively, these findings demonstrate that BS-Gal induces coordinated oxidative, inflammatory, and apoptotic hepatic damage, emphasising potential health risks linked to the consumption of raw bean sprouts containing biologically active lectins.

KEYWORDS

lectins, hepatotoxicity, bean sprouts, inflammation, raw vegans, vegetarian diet

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1. INTRODUCTION

Lectins are carbohydrate-binding proteins widely present in plants and animals, where they mediate functions such as cell signalling, immunity, and defence (Bah et al., 2012). Plant lectins, especially those from legumes and those that bind galactose residues, can show immunomodulatory, proinflammatory, or cytotoxic effects, and some remain active after ingestion because they resist gastrointestinal degradation (Mishra et al., 2019). Although lectin toxicity is well known for raw or improperly processed legumes, its relevance has increased with the rise of raw vegan diets that rely heavily on uncooked sprouts, which may still contain biologically active lectins (Zhgenti et al., 2025b). Despite frequent dietary exposure, the safety of consuming raw legume sprouts remains insufficiently studied.

BS-Gal, a galactose-specific lectin isolated from Roman bean sprouts and first described by Zhgenti et al. (2024), displays high resistance to digestive breakdown. In mice, oral BS-Gal induces marked neuroinflammatory and neurodegenerative changes, indicating that it remains active beyond the intestinal lumen (Zhgenti et al., 2024, 2025b). This is relevant for individuals consuming raw or minimally processed legume-based foods, who may be exposed to elevated lectin levels.

The liver, a major site of detoxification, is particularly vulnerable to toxic compounds absorbed from the gut. Several plant lectins have been shown to provoke hepatotoxicity through oxidative stress, inflammation, and mitochondrial dysfunction (Gu and Manautou, 2012). Oxidative stress contributes to hepatic injury by promoting lipid peroxidation and impairing antioxidant defences, while inflammatory cytokines such as IL-6 and TNF-α activate NF-κB–dependent pathways and recruit immune cells (Chen et al., 2017; Allameh et al., 2023). Oxidative stress and inflammation can also induce mitochondrial apoptosis, which involves Bcl-2 family proteins such as Bcl-2, Bad, and Bax (Wang and Youle 2009). However, the hepatic effects of galactose-specific lectins derived specifically from bean sprouts remain poorly understood.

This study investigates the hepatic impact of a bean sprout–derived galactose-specific lectin in mice by assessing markers of oxidative stress, inflammation, and apoptosis. Clarifying these mechanisms will help evaluate the safety of dietary lectins and their potential systemic toxicity.

2. MATERIALS AND METHODS

2.1. Study design

The study used 50 male BALB/c mice (8 weeks old, 22 ± 2 g), which were housed, cared for, and tested according to the "Guide for the care and use of laboratory animals" (National Research Council, 2011) in the vivarium of the Department of Biology at Tbilisi State University under standard conditions: free access to food and water, 22 ± 2 °C temperature, a 12-h light–dark cycle, and a 7-day acclimatisation period. After acclimation, the mice were divided into two groups of 25. Group G1 received drinking water for 20 days, while Group G2 received water containing lectins from bean sprouts (BS-Gal) for the same period. The lectin was isolated and characterised as described by Zhgenti et al. (2024), and its concentration was adjusted to provide an average activity of 10 mg kg^{-1}. Each mouse consumed about 5 mL of solution daily, which was freshly prepared. On day 20, all animals were euthanised with CO_2 and decapitated according to AVMA guidelines (2020), after which blood plasma and liver samples were collected, with livers stored at −80 °C.

2.2. Liver function tests

To determine liver function tests (LFTs), plasma samples from experimental animals were used, and assay kits were provided by MyBioSource: alanine aminotransferase (ALT, MBS2540581), aspartate aminotransferase (AST, MBS2540582), alkaline phosphatase (ALP, MBS822355), gamma-glutamyl transferase (GGT, MBS8305387), and total bilirubin (MBS2801623).

2.3. Antioxidant system and oxidative stress in the liver

To assess liver antioxidant defences, we measured the activity of key enzymes neutralising reactive oxygen species. Superoxide dismutase (SOD) was quantified using the ELISA kit MBS265351 (MyBioSource), catalase (CAT) with MBS704962 (MyBioSource), glutathione peroxidase (GPx) with MAK437-1 KT (Sigma-Aldrich), and glutathione reductase (GR) with MAK535-1 KT (Sigma-Aldrich). All assays followed manufacturer's protocols.

Oxidative stress markers were also evaluated. Hydrogen peroxide (H_2O_2) was measured using MAK166-1 KT (Sigma-Aldrich), nitric oxide (NO) with MBS430190 (MyBioSource), and lipid peroxidation (MDA levels) using the colorimetric kit MBS822354 (MyBioSource).

2.4. Proinflammatory factors and cytokines in the liver

Oxidative stress is known to trigger inflammatory responses, particularly through the activation of the proinflammatory transcription factor NF-κB. Upregulation of NF-κB expression leads to increased production of proinflammatory cytokines (Hoffmann et al., 2025). Therefore, in the next phase of the study, we analysed the levels of NF-κB p65, IL-6, IL-1β, and TNF-α using Western blotting and ELISA kits provided by MyBioSource (Cat# MBS3806539, MBS2023471, MBS2021142, and MBS825075).

2.5. Proapoptotic and antiapoptotic factors in the liver

Proinflammatory signalling can induce apoptosis, particularly *via* the mitochondrial pathway (Zhgenti et al., 2025c). We analysed pro- and antiapoptotic proteins in liver tissue by Western blotting. Samples were run on 10% SDS-PAGE, transferred to nitrocellulose membranes, and probed with specific primary antibodies (Bad sc-8044 HRP, Bax sc-23959 HRP, Bcl-2 sc-509 HRP; Santa Cruz Biotechnology) following standard protocols (Zhgenti et al., 2025a). Bands were visualised with enhanced chemiluminescence (ECL), scanned densitometrically for quantification, and normalised to β-actin.

2.6. Chemicals and reagents

All reagents were purchased from Sigma-Aldrich (Sigma-Aldrich Inc., St. Louis, USA) unless otherwise specified. All assay kits were provided by MyBioSource unless otherwise specified.

2.7. Statistical analysis

Data are presented as mean ± standard error of the mean (SEM), and statistical analysis was performed using GraphPad Prism 9.0 software (GraphPad Prism 9.0, USA). To assess the assumption of normality, the Shapiro–Wilk test was applied. Only data that met the normality assumption were subjected to further analysis using a one-way ANOVA to determine statistical

significance, followed by Tukey's post hoc test for multiple comparisons. *P*-values of 0.05 or less were considered statistically significant.

3. RESULTS AND DISCUSSION

The aim of this study was to assess the hepatotoxic potential of the galactose-specific lectin BS-Gal following oral administration in mice. To achieve this, we evaluated classical liver function indicators, oxidative stress parameters, inflammatory mediators, and apoptotic markers. The results consistently demonstrate that BS-Gal induces profound disturbances in hepatic homeostasis, involving oxidative imbalance, inflammation, and activation of intrinsic apoptotic pathways.

3.1. Liver function tests

Administration of BS-Gal produced significant elevations in plasma ALT, AST, ALP, and GGT activities, as well as total bilirubin (Fig. 1). ALT ($F(1,48) = 8.22$; $P < 0.01$) and AST increases ($F(1,48) = 10.03$; $P < 0.01$) indicate hepatocellular injury, while ALP ($F(1,48) = 12.62$; $P < 0.001$) and GGT elevations ($F(1,48) = 4.82$; $P < 0.05$) suggest impaired bile duct function (Mei et al., 2019). The rise in total bilirubin ($F(1,48) = 20.77$; $P < 0.001$) further supports disruption of hepatobiliary transport. These combined alterations reflect damage to both hepatocytes and biliary epithelium, consistent with patterns observed in chemical- and toxin-induced hepatic injury. Comparable enzyme elevations have been reported for other

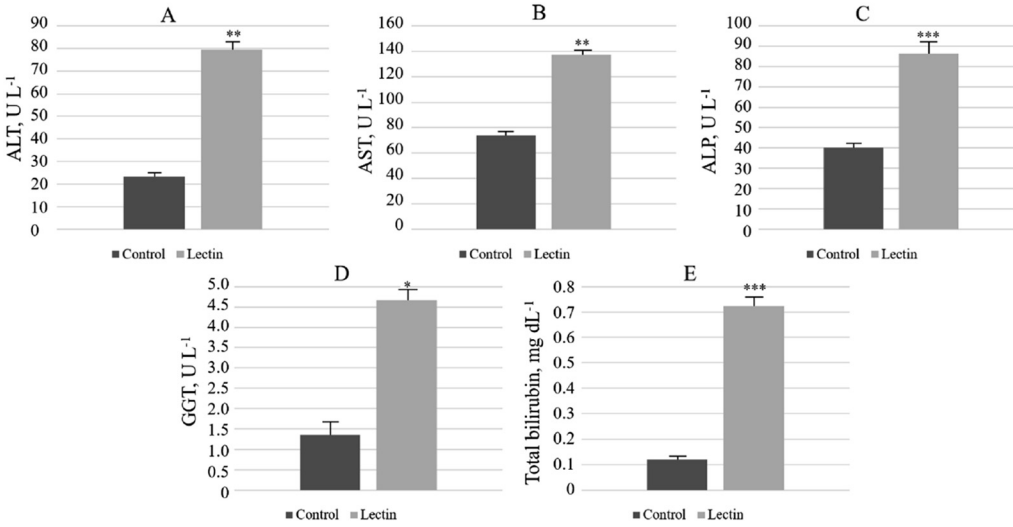

Fig. 1. Activity of liver enzymes (U L^{-1}): alanine aminotransferase (A); aspartate aminotransferase (B); alkaline phosphatase (C); gamma-glutamyl transferase (D), and concentration of total bilirubin (mg dL^{-1}) (E) in serum. Data represent mean ± SEM. Statistical analysis was performed using One-way ANOVA followed by Tukey's post hoc tests. Significance annotations: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ compared to control group (Group I)

toxic plant lectins, such as concanavalin A and BTKL, supporting the view that lectin exposure can compromise liver integrity through systemic bioactivity (Gantner et al., 1995; Fang et al., 2011).

3.2. Oxidative stress and antioxidant defence

Assessment of liver antioxidant enzymes revealed a clear shift toward oxidative imbalance (Fig. 2). SOD ($F(1,48) = 47.1$; $P < 0.001$) and CAT ($F(1,48) = 49.8$; $P < 0.001$) activities were markedly decreased, indicating diminished capacity to detoxify superoxide anions and hydrogen peroxide. In contrast, GPx ($F(1,48) = 4.9$; $P < 0.05$) and GR ($F(1,48) = 7.23$; $P < 0.05$) activities were significantly increased, likely reflecting a compensatory upregulation of the glutathione-dependent antioxidant system in response to accumulating peroxides. These enzymatic changes strongly suggest mitochondrial and cytosolic oxidative stress.

This functional impairment of antioxidant defences was accompanied by robust increases in oxidative stress markers (Fig. 3). BS-Gal treatment elevated H_2O_2 levels by ~35% ($F(1,48) = 166.9$; $P < 0.001$), NO levels by ~24% ($F(1,48) = 4.88$; $P < 0.05$), and MDA levels rose by approximately 35% ($F(1,48) = 8.0$; $P < 0.01$).

The rise in MDA indicates enhanced lipid peroxidation, while elevated NO and H_2O_2 point to concurrent oxidative and nitrosative stress. Together these data suggest that reactive oxygen species (ROS) accumulation is a central mechanism of BS-Gal–mediated hepatic damage.

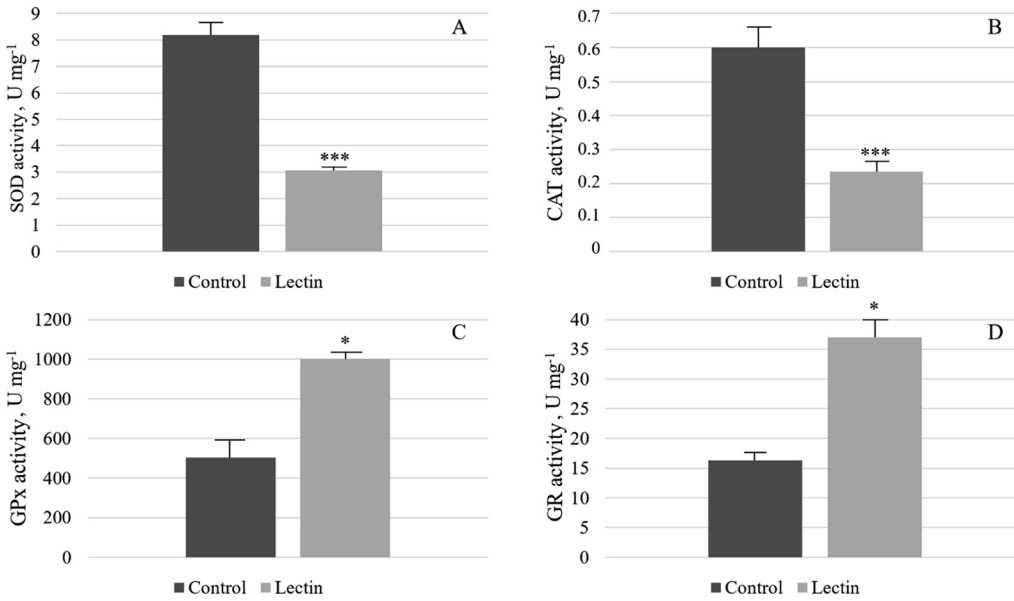

Fig. 2. Activity of antioxidant enzymes (U mg^{-1} protein): superoxide dismutase (A); catalase (B); glutathione peroxidase (C); and glutathione reductase (D) in liver tissue. Data represent mean ± SEM. Statistical analysis was performed using One-way ANOVA followed by Tukey's post hoc tests. Significance annotations: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ compared to control group (Group I)

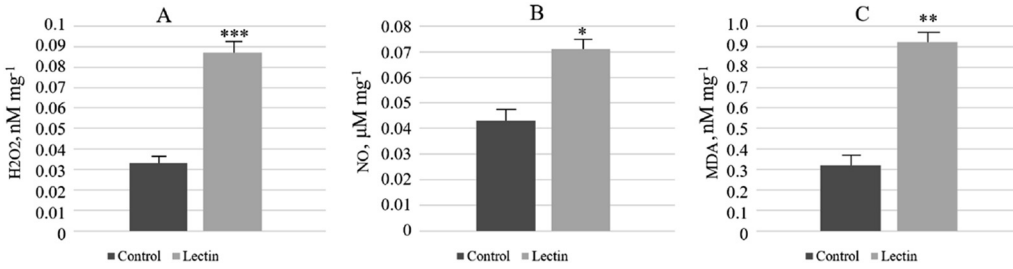

Fig. 3. Concentration of oxidative stress markers: H_2O_2 (nM mg^{-1}, A); NO (µM mg^{-1}, B); MDA (nM mg^{-1}, C) in liver tissue. Data represent mean ± SEM. Statistical analysis was performed using One-way ANOVA followed by Tukey's post hoc tests. Significance annotations: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ compared to control group (Group I)

3.3. Inflammatory responses

Given the established link between oxidative stress and inflammation, we next evaluated NF-κB p65 and proinflammatory cytokines. BS-Gal significantly increased NF-κB p65 levels ($F(1,48) = 10.54$; $P < 0.01$), accompanied by upregulated IL-6 ($F(1,48) = 12.21$; $P < 0.001$), IL-1β ($F(1,48) = 5.55$; $P < 0.05$), and TNF-α ($F(1,48) = 17.16$; $P < 0.001$) (Fig. 4). NF-κB activation is a canonical response to ROS, and its upregulation is known to amplify inflammatory cascades in the liver. The cytokine profile observed here mirrors inflammatory signatures reported for other bioactive dietary lectins, including wheat germ agglutinin and soybean agglutinin, which can activate NF-κB signalling in both intestinal and extraintestinal tissues (Sodhi and Kesherwani, 2007). Thus, BS-Gal–induced oxidative stress appears to drive robust hepatic inflammation, contributing to tissue injury.

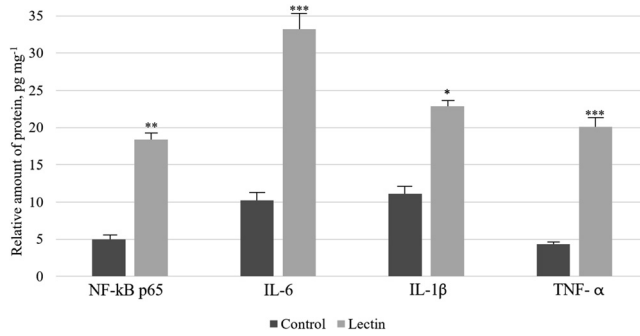

Fig. 4. Levels of proinflammatory factor NF-kB p65 and cytokines: IL-6, IL-1β, TNF-α (pg mg^{-1}) in liver tissue. Data represent mean ± SEM. Statistical analysis was performed using One-way ANOVA followed by Tukey's post hoc tests. Significance annotations: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ compared to control group (Group I)

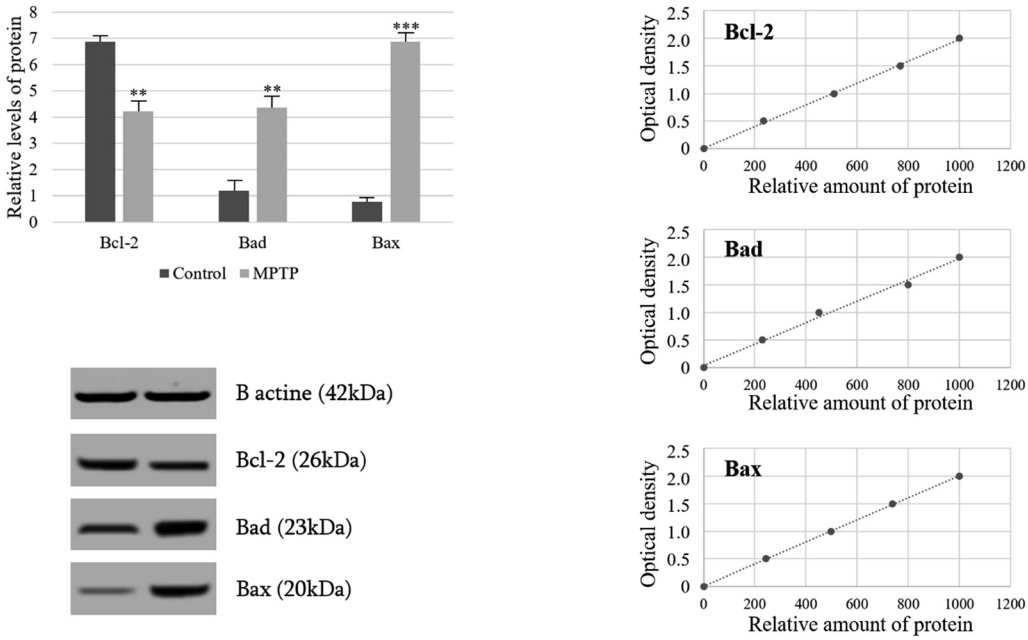

Fig. 5. Expression of pro- and antiapoptotic factors: Bcl-2, Bad, and Bax in liver tissue. Data represent mean ± SEM. Statistical analysis was performed using One-way ANOVA followed by Tukey's post hoc tests. Significance annotations: $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ compared to control group (Group I)

3.4. Apoptotic signalling

To determine whether hepatocellular injury progressed to apoptosis, we assessed the expression of mitochondrial apoptotic regulators (Fig. 5). BS-Gal induced significant upregulation of the pro-apoptotic proteins BAD ($F(1,48) = 11.02$; $P < 0.01$) and BAX ($F(1,48) = 17.57$; $P < 0.001$), alongside a decrease in the anti-apoptotic protein Bcl-2 ($F(1,48) = 6.10$; $P < 0.01$). This expression profile is characteristic of mitochondrial outer membrane permeabilisation, a key step in intrinsic apoptosis. Both oxidative stress and inflammatory cytokines such as TNF-α are known activators of this pathway. The observed imbalance in BAX/Bcl-2 suggests that hepatocytes exposed to BS-Gal shift toward a pro-apoptotic state, which is consistent with previously described mechanisms for cytotoxic lectins in liver tissues (Elmore, 2007; Gong et al., 2017)

4. CONCLUSIONS

Collectively, these results show that BS-Gal exerts hepatotoxic effects by elevating liver enzymes, oxidative stress, inflammatory mediators, and triggering mitochondrial apoptosis. Its resistance to gastrointestinal degradation suggests that intact BS-Gal reaches the liver, causing systemic toxicity. Compared with other dietary lectins, BS-Gal is particularly potent, raising concerns over the consumption of raw bean sprouts. Future studies should explore dose dependence,

reversibility of hepatotoxicity, and the role of gut integrity and microbiota in BS-Gal bioavailability to assess relevance to human diets.

Declaration: During the preparation of this work, the authors used ChatGPT to improve language and grammar. After using this tool/service, the authors reviewed and edited the content as needed and take full responsibility for the publication's content.

Conflict of interest: The authors declare no conflicts of interest regarding the publication of this paper.

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