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ARTIKEL PENELITIAN

Evaluasi Efek Hepatoprotektif Barley terhadap Kerusakan Hati yang Diinduksi Aloksan pada Tikus Wistar Jantan Penderita Diabetes

Evaluating the Hepatoprotective Effects of Barley on Alloxan-Induced Liver Damage in Diabetic Male Wistar Rats

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Abstract

Type 2 diabetes mellitus often leads to non-alcoholic fatty liver disease, affecting 69-74% of patients. Barley (Hordeum vulgare) offers a potential therapeutic alternative with safer interventions. This study aimed to explore the effects of barley on liver histopathology in alloxan-induced male Wistar rats and compare it with metformin. The research was conducted using a laboratory experimental design with 36 male Wistar rats divided into six groups: normal control, negative control (alloxan induction without treatment), positive control (metformin), and three treatment groups with variations in barley administration. Treatment group 1 received barley before and after alloxan induction, treatment group 2 received barley and standard feed after alloxan induction, and treatment group 3 received standard feed mixed with barley after alloxan induction. Barley was administered for 28 days, and histopathological analysis was performed at the end of the study. Data were analyzed using the Kruskal-Wallis and Mann-Whitney tests. The results showed that barley was able to improve liver cell damage, although not as effectively as metformin. The positive control group with metformin showed an average liver damage score of 148,33 with 53,03% normal cells and 15,28% necrotic cells. In contrast, the barley treatment groups showed average liver damage scores of 169,67 and 159,67. Thus, barley can improve liver histopathology in alloxan-induced rats, highlighting its potential as a safe herbal therapy for preventing and managing liver damage caused by oxidative stress in diabetes mellitus.

Keywords: *barley, hepatoprotective, histopathology liver, hordeum vulgare, hyperglycemia*

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Abstrak

Diabetes mellitus tipe 2 seringkali menyebabkan penyakit hati berlemak non-alkohol (non-alcoholic fatty liver disease) yang mempengaruhi 69-74% pasien. Barley (Hordeum vulgare) menawarkan alternatif terapeutik yang potensial dengan intervensi yang lebih aman. Penelitian ini bertujuan untuk mengeksplorasi efek barley pada histopatologi hati tikus Wistar jantan yang diinduksi alloxan dan membandingkannya dengan metformin. Penelitian ini menggunakan desain eksperimental laboratorium dengan 36 tikus Wistar jantan yang dibagi menjadi enam kelompok: kontrol normal, kontrol negatif (induksi alloxan tanpa pengobatan), kontrol positif (metformin), dan tiga kelompok perlakuan dengan variasi pemberian barley. Kelompok perlakuan 1 diberikan barley sebelum dan sesudah induksi alloxan, kelompok perlakuan 2 diberikan barley dan pakan standar setelah induksi alloxan, serta kelompok perlakuan 3 diberikan pakan standar yang dicampur dengan barley setelah induksi alloxan. Barley diberikan selama 28 hari, dan analisis histopatologi dilakukan pada akhir penelitian. Data dianalisis menggunakan uji Kruskal-Wallis dan Mann-Whitney. Hasil penelitian menunjukkan bahwa barley mampu memperbaiki kerusakan sel hati, meskipun tidak seefektif metformin. Kelompok kontrol positif dengan metformin menunjukkan skor kerusakan hati rata-rata sebesar 148,33 dengan 53,03% sel normal dan 15,28% sel nekrosis. Sebaliknya, kelompok perlakuan dengan barley menunjukkan skor kerusakan hati rata-rata sebesar 169,67 dan 159,67. Dengan demikian barley mampu memperbaiki histopatologi hati pada tikus yang diinduksi alloxan, menyoroti potensinya sebagai terapi herbal yang aman untuk mencegah dan mengelola kerusakan hati yang disebabkan oleh stres oksidatif pada diabetes mellitus.

Kata Kunci: barley, hepatoprotektif, histopatologi hati, hordeum vulgare, hiperglikemia

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INTRODUCTION

Globally, including in Indonesia, non-communicable diseases (NCDs) are increasingly becoming a major concern, especially diabetes mellitus (DM), with type 2 DM being one of the most prevalent NCDs (Sormin dan Tenrilemba, 2019; Munira *et al.*, 2020; Pratiwi *et al.*, 2024). Diabetes mellitus is a chronic metabolic disease caused by impaired insulin synthesis or function, leading to hyperglycemia, an elevated blood sugar level (Rachdaoui, 2020; Alam *et al.*, 2021; Choudhury dan Rajeswari, 2021). This disease not only affects multiple organs but also increases the risk of major complications, such as heart disease, nerve damage, and eye damage (Sarkar *et al.*, 2019; Dwivedi dan Pandey, 2020; Jwad dan Al Fatlawi, 2022). It poses a significant burden on national health systems as well as financial strain on individuals and families. Saeedi *et al.* (2019) estimate that the global population living with type 2 DM will increase by 51% by 2045, affecting 783 million people (IDF, 2024). This projection highlights the importance of developing therapies that can reduce the incidence of DM while also mitigating its complications, including non-alcoholic fatty liver disease (NAFLD), which has also become a global health issue.

The increasing prevalence of type 2 DM is often linked to unhealthy lifestyles, such as the consumption of high-calorie, low-fiber foods, and a lack of physical activity (Saboo *et al.*, 2022). Obesity is also a significant risk factor that exacerbates DM. Indonesia has one of the highest incidences of DM in Southeast Asia (Herningtyas and

Ng, 2019; Priyadi *et al.*, 2019; Alfaqeeh *et al.*, 2024), leading to a rise in liver diseases like NAFLD (Murag *et al.*, 2021; Wong *et al.,* 2023). NAFLD is characterized by fat accumulation in the liver without excessive alcohol consumption (Caturano *et al.*, 2021; Rinaldi *et al.*, 2021), and it is often found in individuals with comorbidities such as obesity, dyslipidemia, and type 2 DM. Research indicates that approximately 50% of diabetes patients also suffer from NAFLD (Bril, 2020; Lee *et al.*, 2019). This underscores the need for more effective prevention and treatment strategies for managing DM complications, particularly in Indonesia (Andoko *et al.*, 2021; Darmawan *et al.*, 2024). One increasingly popular approach to managing diabetes complications, including NAFLD, is the use of herbal products like barley (*Hordeum vulgare*). Barley is rich in vitamins, fiber, and bioactive compounds, offering potential health benefits. These include β-glucan, a type of soluble fiber that slows glucose diffusion in the bloodstream (Mărginean *et al.*, 2021; Raj *et al.*, 2023), which can reduce the risk of hyperglycemia and heart disease (Singh dan Bhardwaj, 2023; Kabisch *et al.*, 2024). Additionally, phenolic acid in barley acts as an antioxidant that may lower the risk of type 2 DM and NAFLD (Raj *et al.,* 2023; Zeng *et al.*, 2020). Previous studies have also shown that β-glucan and phenolic acid in barley exhibit antidiabetic, antioxidant, and neuroprotective effects, potentially reducing oxidative stress and liver damage in diabetic patients (Kaur *et al.,* 2024; Zeng *et al.,* 2020). However, further research is needed in Indonesia to explore the benefits of barley as a natural intervention for preventing or reducing the effects of DM on liver health.

Several studies have emphasized the importance of dietary management and the use of natural products rich in fiber and antioxidants in managing DM. β-glucan in barley has been shown to slow down carbohydrate absorption in the small intestine, thus reducing blood glucose levels (Gotteland *et al.*, 2023; Haba, 2022). Phenolic acid also has anti-inflammatory and anticancer properties and can mitigate the harmful effects of free radicals, which increase due to hyperglycemia (Almeida *et al.*, 2022; Black, 2024). This is crucial, as free radicals can cause tissue damage, including liver damage (Chaudhary *et al.*, 2023; Gao *et al.*, 2019). Therefore, barley has the potential to serve as a safe alternative treatment for managing DM and its related complications, such as NAFLD (Ofosu *et al.*, 2021; Vaou *et al.*, 2022). This study aims to investigate the effects of barley (*Hordeum vulgare*) on improving liver histopathology in male Wistar rats induced with alloxan, a compound that causes hyperglycemia and mimics insulin-dependent diabetes mellitus in humans. Unlike previous studies that mainly used streptozotocin or a high-fat diet to induce pathological conditions and focused on using barley grass juice or sprouted barley, this study emphasizes the effects of whole barley as a food ingredient. Whole barley is expected not only to lower glucose levels but also to improve the structure and function of hepatocytes damaged by oxidative stress (Deng *et al.*, 2020). Thus, this study offers novelty by exploring the potential of barley as a preventive and therapeutic intervention for reducing liver damage caused by hyperglycemia. The findings are expected to pave the way for the development of safer and more effective herbal therapies for managing diabetes and its related complications, particularly in Indonesia, where similar research remains limited.

The proposed research hypotheses are as follows: The Null Hypothesis (Ho) states that the administration of barley (*Hordeum vulgare*) has no significant effect on the liver histopathology of male Wistar rats induced with alloxan. Conversely, the Alternative Hypothesis (Ha) states that the administration of barley (*Hordeum vulgare*) has a significant effect on improving the liver histopathology of male Wistar rats induced with alloxan. These hypotheses are interrelated and form the basis for analyzing

barley's impact on liver damage due to hyperglycemia, aiming to test the effectiveness of barley as a potential therapeutic agent.

METHODS

This study employed a laboratory-based experimental approach using a true experimental method with a post-test-only control group design. The experiment was conducted at the Faculty of Pharmacy Laboratory, Universitas Sumatera Utara, from March to May 2024. Ethical approval was obtained (Approval No. 057/KEPK/UNPRI/I1/2024), and strict protocols ensured compliance with animal welfare standards. Healthy male Wistar rats, aged 8–12 weeks and weighing 150–250 grams, were used as the research sample. The sample size was determined using Federer's formula, resulting in six rats per group and a total of 36 rats. Criteria for inclusion were male rats within the specified age and weight range, in good health (active, clear eyes, thick fur, no structural anomalies), and with fasting blood glucose levels between 50 and 135 mg/dL. Exclusion criteria included female rats, those outside the age and weight range, rats in poor health, those with fasting blood glucose above 135 mg/dL, and those that died during the study. The independent variable was barley (*Hordeum vulgare*), one of the most extensively cultivated cereal grains globally, measured in milligrams on a ratio scale. The dependent variable was liver histological characteristics in male Wistar rats, assessed using light microscopy to identify histopathological changes. These changes were evaluated with the Manja Roenigk score, comprising four categories: 1 = normal with no pathological changes, 2 = hemorrhage or parenchymal degeneration, 3 = fatty or hydropic degeneration, and 4 = necrosis, evaluated on a ratio scale (Astitu *et al*., 2023). Barley was administered to examine its effects on histopathological alterations induced by alloxan, a compound used to mimic diabetes in experimental models. All procedures, including alloxan induction and sample collection, were performed under anesthesia to minimize animal pain and stress. This methodology ensured rigorous ethical standards and reliable data collection for investigating the hepatoprotective potential of barley in mitigating liver damage associated with diabetes.

This study utilized various tools and materials, including a pot, ladle, blender, 1 cc syringe, digital scale, glucometer, surgical tray, dissecting set, and refrigerator, as well as equipment for histopathology specimen preparation such as tissue cassettes, tissue paper, an oven, cotton, spiritus, a rotary microtome, a disposable knife, an incubator, and glass slides. Microscopic examination was performed using a light microscope, object glass, cover glass, and immersion oil to observe the histopathological features of the liver in male Wistar rats. Materials included Aceh barley, filter paper, gloves, male Wistar rats, standard feed, drinking water, alloxan type A7413, cotton, alcohol, glucose strips, NaCl 0.9%, sample pots, and chloroform. Histopathology specimen preparation utilized a 10% neutral buffered formalin solution for fixation, alcohol, xylol, and hematoxylin-eosin (HE) staining. The research procedure began with sterilizing the tools using an autoclave at 121°C for 15 minutes, while Aceh barley was washed under running water, weighed (100 grams), and cooked to the desired consistency. Male Wistar rats (n = 36) were acclimatized for seven days before being induced with alloxan via intraperitoneal injection in fasted rats, with their blood glucose levels measured to confirm successful induction. The induced rats were then treated according to their respective groups for 28 days. After the treatment period, the rats were euthanized, and liver organs were collected for histopathology specimen preparation using the paraffin method and HE staining. The specimens were analyzed under a microscope at 400x

magnification, with liver cell damage assessed based on the Manja Roenigk classification. Damage scores were calculated by observing 20 random cells in five fields of view per specimen. The variable barley (*Hordeum vulgare*) was defined as one of the four main cereal grains globally, with the indicator being the mass of barley measured using a digital scale (ordinal scale). The variable liver histopathological features were measured through liver tissue preparations observed under a light microscope, with indicators including changes in histopathology assessed using the Manja Roenigk score: 1 = Normal, 2 = Hemorrhage/Parenchymal degeneration, 3 = Fatty/Hydropic degeneration, 4 = Necrosis (Astitu *et al.*, 2023). Data analysis was conducted using SPSS version 27, starting with a normality test (Shapiro-Wilk) and homogeneity test (Levene). If the data were normally distributed and homogeneous, parametric one-way ANOVA was used, followed by the Post-Hoc Tukey test for significant differences. If the data were not normally distributed or homogeneous, the non-parametric Kruskal-Wallis test was applied, followed by the Mann-Whitney test for significant differences among groups.

RESULTS AND DISCUSSIONS

Body weight measurement results

The body weight of the rats in this study was measured six times using a digital scale. The first measurement was conducted before the rats were induced with alloxan, and the second measurement was taken three days after alloxan induction. Once the alloxan induction was successful, each group began treatment for 28 days, and body weight was measured weekly on days 17, 24, 31, and 37. The results of the rats' body weight obtained from the study are presented in Table 1 below:

Table 1. Mean body weight of rats

Test Animal Groups	Average Body Weight ± SD (g)					
	Before Treatment	After Alloxan Induction	1 Week After Treatment	2 Weeks After Treatment	3 Weeks After Treatment	4 Weeks After Treatment
Normal	160,50 ± 2,66	173,16 ± 4,26	183,50 ± 5,68	195,17 ± 6,14	208,00 ± 6,29	218,83 ± 6,40
Negative Control	194,00 ± 8,57	179,20 ± 11,50	176,40 ± 9,96	176,00 ± 11,06	173,00 ± 12,88	173,00 ± 16,03
Positive Control	200,50 ± 12,58	186,00 ± 12,19	181,00 ± 14,44	184,00 ± 10,86	188,50 ± 9,95	193,25 ± 9,22
Treatment 1	160,17 ± 3,19	168,00 ± 2,68	173.,83 ± 2,93	180,00 ± 2,00	186,67 ± 1,21	190,83 ± 4,35
Treatment 2	193,17 ± 10,87	178,00 ± 10,52	179,50 ± 9,95	182,33 ± 10,05	184,83 ± 13,27	188,17 ± 13,31
Treatment 3	214,50 ± 21,06	197,50 ± 17,02	193,00 ± 20,07	195,00 ± 17,98	200.25 ± 17,89	204,25 ± 30

Note: Primary data, 2024

Table 1 shows the changes in body weight of the rats across different groups during the 28-day study. The normal group, which was not induced with alloxan, exhibited a significant increase in body weight, starting from 160,50 g before treatment to 218,83 g after four weeks of treatment. In contrast, the negative control group, which

was induced with alloxan, experienced a decrease in body weight from 194,00 g before treatment to 173,00 g after four weeks. The positive control group also experienced a reduction in body weight after alloxan induction, but it showed a gradual increase from 181,00 g in the first week to 193,25 g in the fourth week. The treatment groups 1, 2, and 3, which were given barley, showed varying degrees of weight gain, with treatment group 3 exhibiting the largest increase from 193,00 g in the first week to 204.25 g in the fourth week compared to Treatment group 2. Treatment group 1, which was not induced with alloxan, showed a steady increase from 173,83 g in the first week to 190,83 g in the fourth week. A larger increase in body weight was observed in the normal group, which was given standard feed, compared to the treatment groups that were given barley.

Blood glucose level measurements

The study measured blood glucose levels using the fasting blood glucose (FBG) parameter across all experimental groups. Measurements were recorded following a 12-hour fasting period of the rats. Blood samples were obtained from the rat's tail and analyzed using an Autocheck glucometer. The study involved six measurements of fasting blood glucose levels. The initial measurement (day 7) was taken following a 7-day period of acclimation without any intervention. The second measurement (day 11) was conducted three days after the induction of alloxan. Measurements were taken from the rats on four different occasions: days 17, 24, 31, and 37, after they received a 28-day treatment. The measurements are presented in Table 2, displaying the mean and standard deviation.

Table 2. Mean fasting blood glucose levels

Test Animal Groups	Average Fasting Blood Glucose ± SD (mg/dL)					
	Before Treatment	After Alloxan Induction	1 Week After Treatment	2 Week After Treatment	3 Week After Treatment	4 Week After Treatment
Normal	93,33 ± 9,54		78,17 ± 8,33	89,50 ± 8,67	105,83 ± 6,67	108,67 ± 12,97
Negative Control	85,20 ± 12,19	338,20 ± 156,13	323,50 ± 53,10	318,25 ± 54,67	347,33 ± 51,20	360,00 ± 103,06
Positive Control	95,25 ± 8,10	306,33 ± 72,40	289,50 ± 115,35	233,00 ± 86,57	147,00 ± 10,58	111,33 ± 11,50
Treatment 1	87,83 ± 14,65		99,67 ± 9,42	88,17 ± 11,69	93,67 ± 15,94	103,33 ± 8,85
Treatment 2	90,00 ± 11,56	377,50 ± 108,55	407,40 ± 156,36	371,60 ± 148,62	290,25 ± 107,40	240,25 ± 59,22
Treatment 3	81,50 ± 11,39	358,00 ± 51,23	341,75 ± 45,24	315,25 ± 59,24	289,25 ± 62,66	267,75 ± 73,07

Note: Primary data, 2024

The findings indicate that the initial fasting blood glucose levels in all rat groups fell within the normal range of 50-135 mg/dL. The average fasting blood glucose levels in the normal, negative control, positive control, Treatment 1, Treatment 2, and Treatment 3 groups were 93,33 mg/dL, 85,2 mg/dL, 95,25 mg/dL, 87,83 mg/dL, 90 mg/dL, and 81,5 mg/dL, respectively. Following alloxan induction in the negative control, positive control, Treatment 2, and Treatment 3 groups, blood glucose levels

rose above 250 mg/dL, indicating successful induction of diabetes. Throughout the 28-day treatment period, the group receiving barley, referred to as Treatment 1, consistently maintained blood glucose levels within the normal range. In contrast, the groups receiving Treatment 2 and 3 observed a reduction in blood glucose levels. Nevertheless, their levels stayed above 135 mg/dL. The blood glucose levels in the negative control group remained elevated, suggesting the presence of ongoing diabetes. On the other hand, the group that received metformin as a positive control demonstrated a noteworthy decrease in blood glucose levels. By week 4, their levels had returned to normal, measuring at 111,33 mg/dL.

Histopathological observations

The objective of this study was to assess the impact of barley (*Hordeum vulgare*) on the histopathological alterations in the liver of male Wistar rats. The assessment of liver cell damage was conducted using a modified Manja Roenigk scoring method. Scoring was conducted by examining one liver slide across five different fields of view, with each field evaluating 20 cells, resulting in a total of 100 liver cells per slide. Scores were assigned based on cell condition: normal cells received a score of 1, cells with parenchymatous degeneration were scored 2, cells with hydropic degeneration received a score of 3, and necrotic cells were scored 4. The scores from the five fields of view were summed, and the average was calculated, which provided the value for one replication in each treatment group. Liver cell damage was determined by examining liver slides to identify normal and damaged cells. Table 3 shows the results of liver damage scoring.

Table 3. Liver cell damage scoring percentage

Test Animal Groups	Percentage of Liver Cells Damage (%)				Average ± Standard Deviation of Total Score
	Normal	PD	HD	Necrosis	
Normal	82,89	0,59	14,16	2,36	113,00 ± 4,36
Negative Control	24,10	13,44	32,95	29,51	203,33 ± 2,08
Positive Control	53,03	00,00	31,68	15,28	148,33 ± 10,41
Treatment 1	57,44	1,42	29,79	11,35	141,00 ± 3,46
Treatment 2	38,31	10,61	30,65	20,43	169,67 ± 52,44
Treatment 3	41,96	14,2	31,31	12,53	159,67 ± 3,06

Note: Primary data, 2024

Observations of liver histopathology in male Wistar rats revealed variations in liver cell damage among the different treatment groups. In the normal group, the percentage of normal cells was found to be 82,89%. Minimal damage was observed in parenchymatous degeneration, hydropic degeneration, and necrosis. On the other hand, the negative control group, which received alloxan without any treatment, showed the most significant cell damage. The average score was 203,33, and necrosis reached 29,51%. The group that received metformin as a treatment, known as the positive control group, demonstrated a significant improvement with 53,03% of normal cells and less damage when compared to the negative control group. In the groups treated with barley, liver damage showed improvement compared to the negative control. Treatment 3 exhibited a higher percentage of normal cells (41,96%) compared to Treatment 2 (38,31%). On the other hand, Treatment 1, in which healthy rats received barley,

showed a higher incidence of liver damage compared to the normal group. The percentage of normal liver cells in Treatment 1 was only 57,44%, suggesting that the administration of barley in healthy rats could potentially elevate the risk of liver cell damage. Here are the histopathological microscopic figures of the liver for each group.

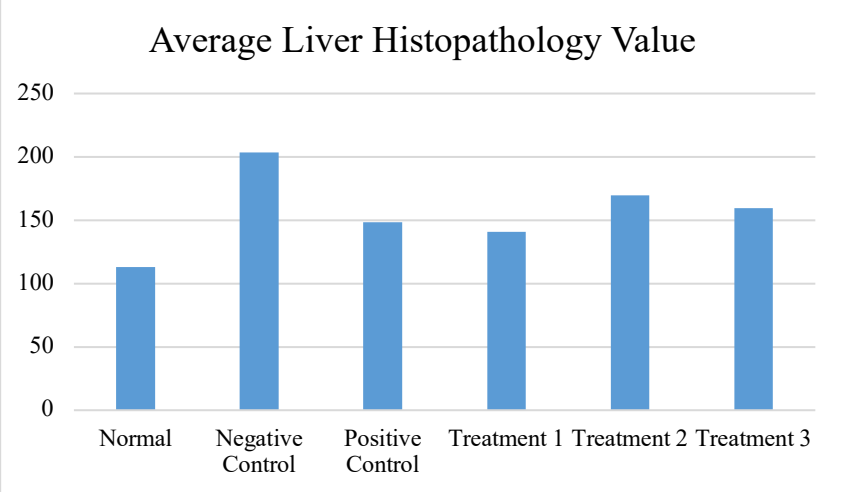


Figure 1. Average liver histopathology value

Table 4. Results of the microscopic histopathological examination of the liver

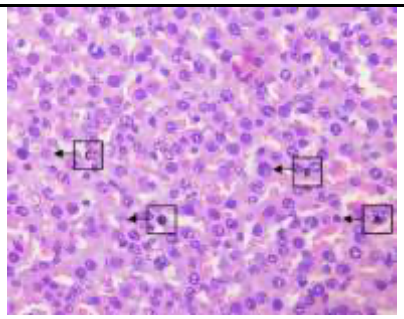
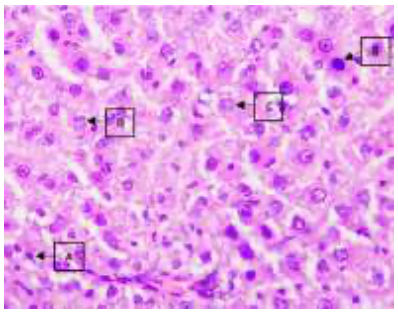
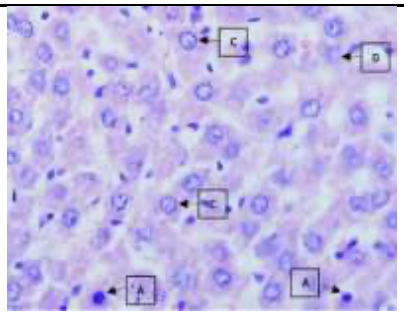
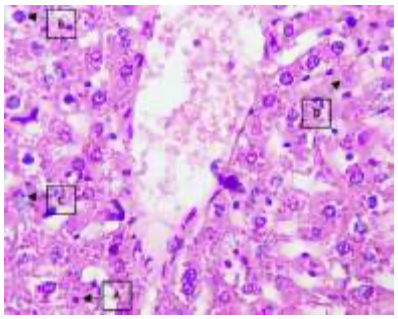
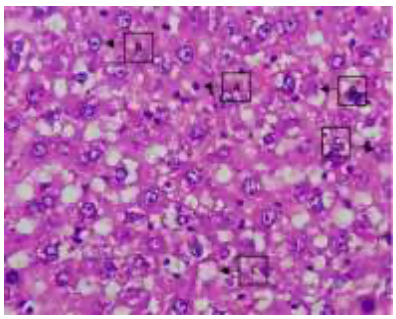
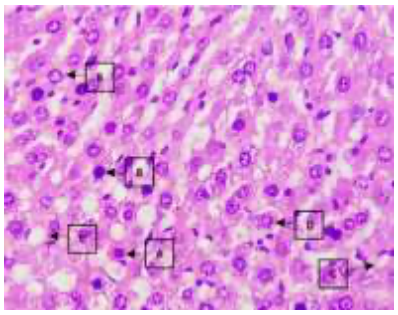
Figure	Description
	Histopathological Overview of the Normal Group (Magnification 10x40) Description: normal cells (A), parenchymatous degeneration cells (B), hydropic degeneration cells (C), necrotic cells (D)
	Histopathological Overview of the Negative Control Group (Magnification 10x40) Description: normal cells (A), parenchymatous degeneration cells (B), hydropic degeneration cells (C), necrotic cells (D)

Figure	Description
	Histopathological Overview of the Positive Control Group (Magnification 10x40) Description: normal cells (A), parenchymatous degeneration cells (B), hydropic degeneration cells (C), necrotic cells (D)
	Histopathological Overview of Treatment Group 1 (Magnification 10x40) Description: normal cells (A), parenchymatous degeneration cells (B), hydropic degeneration cells (C), necrotic cells (D)
	Histopathological Overview of Treatment Group 2 (Magnification 10x40) Description: normal cells (A), parenchymatous degeneration cells (B), hydropic degeneration cells (C), necrotic cells (D)
	Histopathological Overview of Treatment Group 3 (Magnification 10x40) Description: normal cells (A), parenchymatous degeneration cells (B), hydropic degeneration cells (C), necrotic cells (D)

Statistical test on the histopathological damage scores of liver cells

The data analysis process starts by conducting a normality test. The selection of the normality test relies on the size of the sample utilized. For sample sizes that are small (≤ 50), the Shapiro-Wilk test is typically selected. On the other hand, when the sample size exceeds 50, the Kolmogorov-Smirnov test is employed. If the significance value (p) is greater than 0,05 ($p > 0,05$), the data is considered normally distributed.

Conversely, if the significance value is less than or equal to 0,05 (p≤0,05), the data is not normally distributed. The analysis results showed that the significance values for the normal, negative control, positive control, treatment 2, and treatment three groups were all above 0,05. This suggests that the data in these groups were normally distributed. However, the treatment 1 group had a significance value below 0,05, indicating that the data in this group were not normally distributed. Subsequently, a homogeneity test was performed to assess the uniformity of the data. When the data is homogeneous, the analysis can be carried out using the Kruskal-Wallis test. The table displays the results of the homogeneity test, indicating that the significance value was 0,000, indicating a p-value less than 0,05 (p<0,05). These findings suggest that the data exhibited heterogeneity or unequal variances, which satisfies the requirements for conducting the Kruskal-Wallis test.

Kruskal-wallis test

This test is performed when the necessary conditions are fulfilled, namely when the data is not inherently uniform and follows a non-normal distribution. Table 5 below displays the results of the Kruskal-Wallis test.

Table 5. Kruskal-wallis test on histopathological damage scores of liver cells

Test	F	df	Sig.
Kruskal-Wallis	12,025	5	0,034

Mann-whitney test

The Mann-Whitney test is used to detect disparities between two randomly selected samples. The objective of this non parametric test for independent samples is analogous to that of the t-test in standard statistical analysis. A test result with a p-value less than or equal to 0,05 (p≤0,05) defines a statistically significant difference between the two groups.

Table 6. Mann-whitney test on histopathological damage scores of liver cells

	Test Animal Groups	Sig.
Normal	Negative Control	0,050
	Positive Control	0,050
	Treatment 1	0,046
	Treatment 2	0,050
	Treatment 3	0,050
Negative Control	Positive Control	0,050
	Treatment 1	0,046
	Treatment 2	0,513
	Treatment 3	0,050
Positive Control	Treatment 1	0,178
	Treatment 2	0,827
	Treatment 3	0,275
Treatment 1	Treatment 2	0,825

	Test Animal Groups	Sig.
	Treatment 3	0,046
Treatment 2	Treatment 3	0,513

Explanation:

a. Normal Group: Healthy rats were given standard feed and drinking water ad libitum.
b. Negative Control Group: Rats induced with alloxan at 125 mg/kg BW intraperitoneally, given once, and fed standard feed with drinking water ad libitum.
c. Positive Control Group: Rats induced with alloxan at 125 mg/kg BW, fed standard feed, given drinking water ad libitum, and administered metformin at 4.5 mg/100 g BW orally for seven days.
d. Treatment 1 Group: Healthy rats were given barley and drinking water ad libitum for 28 days.
e. Treatment 2 Group: Rats induced with alloxan at 125 mg/kg BW intraperitoneally, given once, and fed barley with drinking water ad libitum for 28 days.
f. Treatment 3 Group: Rats induced with alloxan at 125 mg/kg BW intraperitoneally, given once, and fed standard feed mixed with barley and drinking water ad libitum for 28 days.

The Mann-Whitney test findings for each group in Table 6 indicate that all experimental groups exhibit statistically significant differences compared to normal groups. The significance values for the negative control, positive control, treatment 2, and treatment 3 groups are all 0,05. The study group receiving treatment 1 has a significance level of 0,046. These findings suggest that there are variations in liver cell injury across all populations.

In comparison to the positive control, treatment 1, and treatment 3 groups, the negative control group exhibits statistically significant differences with significance values of 0,05, 0,046, and 0,05, respectively. Nevertheless, the negative control group did not exhibit a statistically significant distinction compared to the treatment 2 group, as shown by a significance value of 0,513. Thus, the histological damage scores of liver cells in the negative control group exhibit a significant difference compared to the positive control, treatment 1, and treatment three groups, but do not vary from the treatment 2 group. Hence, it can be inferred that the administration of barley to rats induced with alloxan does not efficiently decrease liver cell damage. However, feeding a combination of standard feed and barley is beneficial in decreasing liver cell damage in alloxan-induced rats, with a significance level of 0,05.

The analysis of the positive control group and treatment groups 1, 2, and 3 reveals that these groups exhibit significance values over 0,05, thereby suggesting the absence of statistically significant differences. Therefore, it may be inferred that the administration of barley to healthy rats and rats induced with alloxan does not lead to noteworthy variations in liver cell damage when compared to rats treated with metformin. This observation implies that barley has equivalent ability in the repairing of liver cell injury caused by alloxan. The average damage scores for the positive control, treatment 2, and treatment 3 groups, as shown in Table 3, are 148,33, 169,67, and

159,67, respectively. These findings suggest that barley is not superior to metformin therapy in healing liver cell damage.

The comparison of liver cell damage scores between treatment groups 1 and 2 shows no significant difference, with a significance value of 0,825. In contrast, the comparison between treatment groups 1 and 3 shows a significant difference in liver cell damage scores, with a significance value of 0,046. This means that administering barley to alloxan induced rats does not result in significantly different liver cell damage compared to healthy rats given barley. However, alloxan-induced rats fed standard feed mixed with barley have significantly different damage scores.

The significance value between treatment groups 2 and 3 is 0,513, indicating no significant difference. Therefore, to identify differences between these two groups, the mean liver cell damage scores in Table 3 should be examined. The treatment 2 group has a mean score of 169,67, while the treatment 3 group has a mean score of 159,67. This suggests that feeding standard feed mixed with barley is more effective in repairing liver cell damage induced by alloxan.

This study demonstrates that the body weight of the rats varied across different treatment groups, which aligns with previous findings regarding the effects of alloxan induction and metabolic treatment in rats. The normal group, which was not induced with alloxan, experienced a significant increase in body weight, reflecting stable metabolic conditions, from 160,50 g before treatment to 218.83 g after four weeks. In contrast, the negative control group, which was induced with alloxan, showed a decrease in body weight from 194,00 g to 173,00 g after four weeks, indicating the diabetogenic effects of alloxan observed in previous studies. The positive control group given metformin, as reported in the study by Davies *et al.* (2022) and Mirabelli *et al.* (2021), showed a gradual increase in body weight, reflecting the effectiveness of metformin in controlling hyperglycemia and maintaining body weight. The groups treated with barley, specifically treatment groups 1, 2, and 3, also showed an increase in body weight, with Treatment group 3 exhibited the most significant increase, rising from 193,00 g to 204,25 g, compared to Treatment group 2.

This supports the hypothesis that the bioactive compounds in barley might impact glucose and lipid metabolism. Blood glucose measurements revealed that the negative control group induced with alloxan had the highest fasting blood glucose levels, reaching 360 mg/dL in the fourth week, consistent with the findings of Al Chalabi *et al.* (2020) and Woldekidan *et al.* (2021), who reported alloxan's significant effect on increasing blood glucose levels. Meanwhile, the positive control group treated with metformin experienced a significant decrease in blood glucose levels, reaching a normal value of 111,33 mg/dL, in line with previous findings demonstrating metformin's effectiveness in reducing blood glucose levels in diabetic rats. The treatment groups given barley showed a reduction in blood glucose levels, but glucose levels remained above the normal range in treatment groups 2 and 3, indicating that barley has a moderate hypoglycemic effect. Histopathological observations revealed varying degrees of liver cell damage, with the negative control group showing the most severe damage, while the groups treated with barley showed improvement in liver damage, although not completely returning to normal.

The administration of barley (Hordeum vulgare) in male Wistar rats induced with alloxan showed varying effects on the histopathological picture of liver cells, consistent with Samtiya *et al.* (2021) research on barley's potential in modulating liver health through its bioactive components. The treatment groups given barley showed

improvement in liver cell damage compared to the negative control group that received only alloxan, with a higher percentage of normal cells in treatment group 2 (38,31%) and treatment group 3 (41,96%). These findings are consistent with the study by Kumar dan Goel (2019), which reported the antioxidant and hepatoprotective properties of phenolic compounds in barley. However, the improvement was not as significant as in the positive control group treated with metformin, where the percentage of normal cells reached 53,03%, supporting metformin's superior effectiveness in reducing alloxan-induced liver cell damage. Statistical tests showed that treatment group 3, given a standard feed mixed with barley, had a significant difference compared to the negative control group, indicating that barley has an effect in improving alloxan-induced liver cell damage. However, it is less effective than metformin. In this study, the administration of barley in healthy rats (treatment 1) actually increased liver cell damage, with an average damage score of 141, higher than the normal group that did not receive barley (113), indicating that barley consumption under optimal health conditions may pose a risk of liver cell damage. Nevertheless, the administration of barley in alloxan-induced rats resulted in better outcomes in improving liver cell damage compared to the untreated group. However, this treatment was still less effective than metformin treatment, suggesting that nutritional interventions such as barley need to be improved in effectiveness to achieve comparable results to pharmacological therapy in managing diabetic liver cell damage.

Barley (Hordeum vulgare) is known to contain β-glucan and phenolic acids, which act as powerful antioxidants. β-glucan is a soluble fiber that helps lower blood glucose levels by reducing glucose absorption and increasing insulin sensitivity (Zeng *et al.*, 2020). This mechanism helps reduce oxidative stress by decreasing reactive oxygen species (ROS) production, a major factor in hepatocyte damage under hyperglycemic conditions. The phenolic acids in barley, such as ferulic acid and p-coumaric acid, are known for their significant antioxidant effects, helping inhibit lipid peroxidation in hepatocyte cell membranes. This mechanism reduces cellular inflammation, protects the hepatocyte DNA structure, and prevents hydropic degeneration and necrosis in liver cells induced by oxidative stress. Recent studies also show that barley can enhance endogenous antioxidant enzyme activity, such as superoxide dismutase (SOD) and catalase, which play roles in ROS detoxification in the liver (Islam *et al.*, 2022). Additional research suggests that regular barley consumption can modulate the NF-κB and mTOR pathways, which are involved in inflammatory responses and hepatocyte apoptosis (Zhao *et al.*, 2021). Thus, the hepatoprotective effects of barley not only encompass lowering hyperglycemia but also include protecting cellular and molecular structures in the liver through antioxidant and anti-inflammatory mechanisms. Therefore, barley could be a safe and potential therapeutic alternative for reducing liver damage. However, this treatment is still less effective compared to metformin, indicating that nutritional interventions like barley need to be enhanced to achieve results comparable to pharmacological therapy in managing liver cell damage due to diabetes.

This study has several advantages over previous studies on barley's effects on liver health and metabolism. First, it emphasizes detailed histopathological observations of liver cells, providing a more comprehensive picture of liver cell damage and repair compared to the studies by Naseri *et al.* (2022) and Thatiparthi *et al.* (2019), which focused more on general organ function evaluation. Second, this study not only investigates barley's effects under pathological conditions, such as in the study by Al-

Shali dan Ramadan (2020) on steatohepatitis, but also explores the effects of barley in both normal diabetic conditions and those induced by alloxan, providing a broader perspective on barley's risks and benefits across different health conditions. Furthermore, this study evaluates the effects of barley across various treatment groups compared to metformin, offering valuable information about the efficacy of barley compared to commonly used pharmacological therapies. The findings of this study support previous research, particularly regarding barley's hepatoprotective properties and its potential to improve alloxan-induced liver damage. The reduction in liver cell damage in barley-treated groups aligns with the report by Kumar dan Goel (2019) on barley's antioxidant and hepatoprotective properties. This supports research findings demonstrating barley's role in liver health modulation, as seen in studies by Thatiparthi *et al*. (2019) and Al-Shali dan Ramadan (2020). However, it should be noted that while barley shows positive effects, this study also reveals that barley's ability to improve liver cell damage is not as effective as metformin, highlighting the need to enhance the efficacy of nutritional interventions like barley to achieve results comparable to pharmacological therapy in managing diabetes-related liver cell damage. Nonetheless, one of the advantages of whole barley studies is the retained dietary fiber content, as it does not undergo separation or extraction processes like processed barley. Whole barley offers additional benefits, such as more consistent antioxidant activity and improved glucose metabolism, which may be more effective in minimizing liver damage caused by hyperglycemia (Deng *et al.*, 2020).

The implications of this study suggest that barley (Hordeum vulgare) has the potential as a hepatoprotective agent that can help improve liver cell damage caused by alloxan. However, its effectiveness differs from metformin. These findings provide a basis for developing barley-based nutritional interventions in managing liver damage under diabetic conditions and open opportunities for further research to explore combinations of barley with existing pharmacological therapies, such as metformin, to enhance its therapeutic effects. Additionally, this study highlights the importance of determining the appropriate dosage and duration of barley use to avoid potential risks of liver cell damage in optimal health conditions, necessitating further research to ensure the safety and effectiveness of barley as part of nutritional therapy in the context of diabetes-related liver disease.

CONCLUSIONS

This study demonstrates that administering barley (Hordeum vulgare) to male Wistar rats induced with alloxan improved liver cell damage compared to the negative control group, which did not receive treatment. However, the effectiveness of barley has not yet equaled that of metformin, which resulted in a higher percentage of normal liver cells. The study's limitations include barley's inability to fully restore the liver's histopathological condition and the potential for increased liver cell damage in healthy rats given barley. Additionally, the study faced challenges in maintaining sample homogeneity and determining the optimal dosage and duration of barley administration to achieve significant results without side effects. Future research is recommended to focus on identifying the optimal dosage and duration of barley administration to maximize its hepatoprotective effects and minimize the risk of liver cell damage, particularly under optimal health conditions. Further studies should also explore combining barley with pharmacological therapies, such as metformin, to assess potential

synergies in repairing liver cell damage caused by alloxan. Moreover, larger and more diverse animal populations are required to ensure consistent and generalizable results. Long-term clinical trials in humans are also necessary to confirm these findings and evaluate the safety and efficacy of barley as part of a nutritional intervention in the management of diabetes-related liver diseases. This should include optimizing dosage and comparing it with other natural and pharmacological treatments to establish barley as a safe and effective alternative in managing liver diseases associated with diabetes.

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CONFLICT OF INTEREST

The authors state that they have no personal interests that could have influenced their research

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