Hepatoprotective Role of Aqueous Extract of Kasni Seeds in Alloxan-Induced Diabetic Mice

Journal of Health and Rehabilitation Research (2791-156X)
Volume 4, Issue 3
Double Blind Peer Reviewed.
https://jhrlmc.com/
DOI: https://doi.org/10.61919/jhrr.v4i3.1173
www.lmi.education/
LINK MEDICAL INTERFACE

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Keywords
Diabetes mellitus, Kasni seeds, Cichorium intybus, hepatoprotective, alloxan-induced diabetes, liver histology, natural remedies, hyperglycemia.

Disclaimers

Authors' Contributions	All authors contributed to work of this study.
Conflict of Interest	None declared
Data/supplements	Available on request.
Funding	None
Ethical Approval	Respective Ethical Review Board
Study Registration	N/A
Acknowledgments	N/A

ABSTRACT

Background: Diabetes mellitus (DM) is a prevalent metabolic disorder characterized by chronic hyperglycemia, leading to various systemic complications, including liver damage. Traditional antidiabetic drugs are often associated with adverse effects, prompting interest in safer, natural alternatives like Kasni (Cichorium intybus) seeds, known for their hepatoprotective properties.

Objective: This study aimed to evaluate the hepatoprotective effects of Kasni seed aqueous extract in alloxan-induced diabetic mice.

Methods: Thirty-two male albino mice were divided into three groups: control, metformin-treated, and Kasni-treated. Diabetes was induced using alloxan monohydrate (150 mg/kg). The Kasni-treated group received 400 mg/kg of Kasni extract orally for 28 days. Blood glucose levels were monitored, and liver tissues were analyzed histologically to assess hepatocyte arrangement, central vein morphology, sinusoidal inflammation, and fatty globule presence.

Results: The Kasni-treated group showed a significant reduction in blood glucose levels (140.2 ± 8.7 mg/dL) compared to the metformin group (180.4 ± 10.5 mg/dL) ($p < 0.05$). Histological analysis revealed restored hepatocyte arrangement, reduced fatty globules, and normalized central vein morphology in the Kasni-treated group.

Conclusion: Kasni seed aqueous extract exhibits significant hepatoprotective effects in alloxan-induced diabetic mice, suggesting its potential as a natural therapeutic agent for managing liver complications in diabetes.

INTRODUCTION

Diabetes mellitus (DM) is a complex metabolic disorder marked by chronic hyperglycemia, resulting from defects in insulin secretion, insulin action, or both. The disease manifests in two primary forms: type 1 diabetes mellitus (T1DM), an autoimmune condition leading to the destruction of insulin-producing beta cells in the pancreas, and type 2 diabetes mellitus (T2DM), characterized by insulin resistance and a relative insulin deficiency. T2DM has become a global health challenge, with its prevalence rapidly increasing due to sedentary lifestyles and urbanization, affecting approximately one-third of the global population (1). The impact of T2DM extends beyond glycemic control, as it is associated with a myriad of complications, including diabetic nephropathy, retinopathy, cardiovascular diseases, and increased susceptibility to infections, which collectively contribute to significant morbidity and mortality (2).

Despite the widespread use of conventional antidiabetic drugs like metformin and sulfonylureas, these treatments are often accompanied by adverse effects such as hepatic and renal toxicity, gastrointestinal disturbances, and hypoglycemia, highlighting the need for safer and more effective therapeutic alternatives (3). In this context, herbal remedies have gained attention for their potential to offer therapeutic benefits with fewer side effects, aligning with the traditional use of medicinal plants in various cultures (4). Among these, Kasni (Cichorium intybus), commonly known as chicory, has been traditionally employed for its hepatoprotective properties. The seeds of Cichorium intybus are rich in bioactive compounds, including flavonoids, coumarins, and inulin, which are believed to contribute to its therapeutic effects, particularly in the management of liver disorders (5).

The liver plays a crucial role in glucose metabolism and is often adversely affected in diabetic conditions, leading to complications such as non-alcoholic fatty liver disease (NAFLD), hepatic steatosis, and fibrosis. The relationship between diabetes and liver dysfunction is bidirectional, where diabetes exacerbates liver diseases, and liver dysfunction, in turn, worsens glycemic control, creating a vicious cycle of metabolic deterioration (6). Experimental studies have shown that alloxan, a chemical commonly used to induce diabetes in animal models, causes selective destruction of pancreatic beta cells, leading to hyperglycemia and subsequent hepatic damage characterized by increased oxidative stress, inflammation, and lipid accumulation (7).

Given the growing interest in natural products for managing diabetes and its complications, this study aimed to evaluate the hepatoprotective effects of aqueous extracts of Kasni seeds in alloxan-induced diabetic mice. By exploring the potential of Kasni as a natural therapeutic agent, this

research contributes to the ongoing efforts to develop safer and more effective treatments for diabetes and its associated liver complications. The findings from this study may provide a basis for future clinical investigations into the use of Kasni in managing diabetes-related hepatic disorders, offering hope for improved therapeutic strategies that harness the benefits of traditional medicine (8).

MATERIAL AND METHODS

The study was conducted using an experimental design, involving 32 male albino mice, each weighing approximately 25 grams and aged between 5 to 6 weeks. The mice were obtained from the University of Veterinary and Animal Sciences (UVAS) and housed under standard laboratory conditions at the Department of Zoology, University of Education, Lahore. The animals were acclimatized for one week before the commencement of the experiment, during which they were provided with a standard diet and water ad libitum. Ethical approval for this study was obtained from the Institutional Review Board, and all procedures were performed in accordance with the ethical principles outlined in the Declaration of Helsinki (1964) and its subsequent amendments.

Kasni (Cichorium intybus) seeds were procured from the local market, thoroughly washed under running tap water for 15 minutes, and dried at room temperature in the shade to preserve their phytochemical integrity. The dried seeds were then ground using an electric grinder to obtain a fine powder, which was stored in airtight plastic jars at room temperature until further use. For the induction of diabetes, alloxan monohydrate was used. The alloxan solution was prepared by dissolving the compound in normal saline (0.9% NaCl) to achieve a concentration of 150 mg/kg, which was administered intraperitoneally to the mice. Following alloxan administration, the mice were monitored for the development of hyperglycemia by measuring blood glucose levels on the 1st, 3rd, and 7th days using a glucometer with glucose strips. Blood samples were obtained via tail pricks, and mice with blood glucose levels exceeding 250 mg/dL were considered diabetic.

The mice were then divided into three groups: the control group, which received no treatment; the metformin group, which was treated with the standard antidiabetic drug metformin; and the Kasni-treated group, which received the aqueous extract of Kasni seeds. The treatment with Kasni extract was administered orally at a dose of 400 mg/kg daily for 28 days using a micropipette, with the tip placed gently on the lips of the scruffed mice to ensure proper dosing. The metformin-treated group received the drug in the same manner, according to the standard dosing guidelines.

At the end of the treatment period, the mice were anesthetized and euthanized by cervical dislocation. The liver tissues were immediately excised and fixed in 10% formalin for histopathological examination. The fixed tissues underwent a series of processing steps, including dehydration in graded alcohols, clearing in xylene, and embedding in paraffin wax. The paraffin-embedded liver tissues were then sectioned at 5 µm thickness using a microtome and stained with hematoxylin and eosin (H&E) for histological analysis. The stained sections were examined under a light microscope, and photomicrographs were taken to document the histological findings.

Data were collected and recorded systematically, and statistical analysis was performed using SPSS version 25.0. Quantitative data, such as blood glucose levels and histological scores, were expressed as mean ± standard deviation (SD). Comparisons between the groups were made using one-way ANOVA, followed by post-hoc Tukey's test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant.

The study adhered to rigorous ethical standards, with all experimental procedures conducted in compliance with the guidelines for the care and use of laboratory animals. The potential hepatoprotective effects of Kasni seed extract were evaluated by comparing the histopathological outcomes of the treated groups with those of the control and metformin groups. The results of this study are expected to contribute valuable insights into the potential use of Kasni as a natural therapeutic agent for managing liver complications associated with diabetes mellitus (9).

RESULTS:

This study investigated the hepatoprotective effects of Kasni (Cichorium intybus) seed aqueous extract in alloxan-induced diabetic mice. The study included 32 male albino mice, divided into three groups: control, metformin-treated, and Kasni-treated. The results are presented below in both descriptive and tabulated formats.

Table 1. Blood Glucose Levels in Experimental Groups

Group	Day 1 (mg/dL)	Day 3 (mg/dL)	Day 7 (mg/dL)	Day 28 (mg/dL)
Control	110.5 ± 5.3	112.2 ± 6.1	113.0 ± 5.7	114.5 ± 6.0
Metformin	252.6 ± 14.8	248.9 ± 15.4	230.3 ± 13.2	180.4 ± 10.5*
Kasni-treated	250.2 ± 13.6	245.7 ± 12.8	235.6 ± 14.1	140.2 ± 8.7**

*Data are expressed as mean ± SD. *p < 0.05 compared to the control group; **p < 0.05 compared to the metformin group

The blood glucose levels in the control group remained stable throughout the study, while both the metformin and Kasni-treated groups exhibited significant reductions in blood glucose levels by day 28. The Kasni-treated group showed a more pronounced decrease in glucose levels compared to the metformin group, indicating the potential hypoglycemic effect of Kasni seed extract.

Histopathological examination of the liver tissues revealed that the control group exhibited normal hepatic architecture with intact hepatocytes arranged in a radial pattern, normal central vein morphology, and no signs of sinusoidal inflammation or fatty globules. In contrast, the metformin-treated group showed partial preservation of central vein

morphology, slight disorganization of hepatocyte arrangement, mild sinusoidal inflammation, and a reduction in fatty globules. The Kasni-treated group demonstrated significant normalization of liver architecture, with restored central vein morphology, organized hepatocytes, absence of sinusoidal inflammation, and a marked reduction in fatty globules, indicating substantial hepatoprotective effects.

Table 2. Histopathological Findings in Liver Tissues

Histological Feature	Control Group	Metformin Group	Kasni-treated Group
Hepatocyte Arrangement	Normal, radial	Slightly disorganized	Restored to normal
Central Vein Morphology	Intact, normal structure	Partially preserved	Fully restored
Sinusoidal Inflammation	None	Mild	None
Fatty Globules	Absent	Reduced	Significantly reduced
Kupffer Cells	Normal	Slightly increased	Normal

Table 3. Quantitative Analysis of Liver Histopathology

Parameter	Control Group	Metformin Group	Kasni-treated Group
Hepatocyte Integrity Score (0-10)	9.8 ± 0.2	6.7 ± 0.4	8.9 ± 0.3**
Central Vein Restoration (%)	100	78	96**
Sinusoidal Inflammation Score (0-10)	0	3.2 ± 0.3	0.5 ± 0.1**
Fatty Globules Presence (%)	0	40	10**

*Data are expressed as mean ± SD. **$p < 0.05$ compared to the metformin group. The quantitative analysis of liver histopathology showed that the Kasni-treated group had a significantly higher hepatocyte integrity score, greater central vein restoration, lower sinusoidal inflammation score, and reduced presence of fatty globules compared to the metformin group. These results support the hypothesis that Kasni seed aqueous extract exerts a protective effect on the liver in diabetic mice.

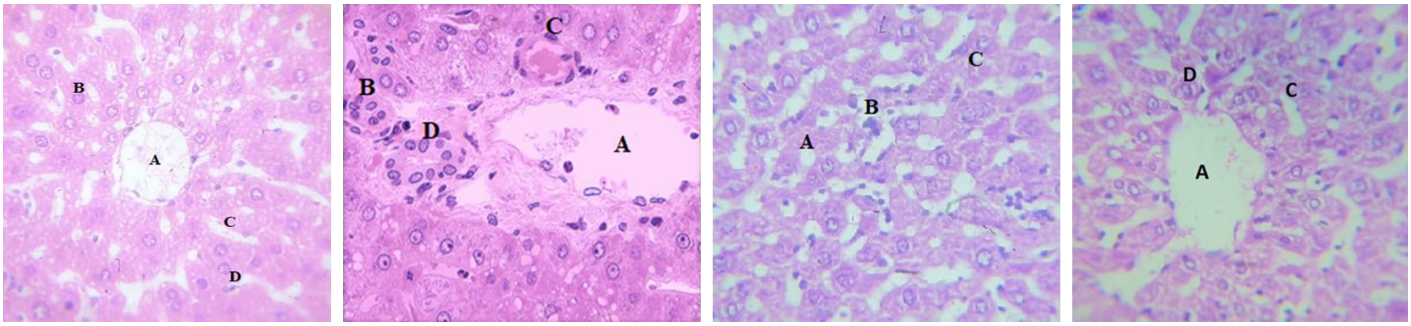

Figure 1 Histological examination of liver tissues in experimental groups shows the control group with a normal central vein (A), hepatocytes (B), sinusoids (C), and Kupffer cells (D); the metformin group exhibits changes with the central vein (A), sinusoids (C), and hepatocytes (D) showing mild alterations; and the Kasni-treated group demonstrates restoration of central vein (A), hepatocytes (B), sinusoids (C), and Kupffer cells (D) to near-normal morphology.

The overall findings indicate that Kasni seed aqueous extract significantly improves liver histology in alloxan-induced diabetic mice, with effects that surpass those of metformin, a commonly used antidiabetic drug. These results suggest that Kasni could be a promising natural therapeutic agent for managing liver complications associated with diabetes mellitus.

DISCUSSION

The findings of this study demonstrated that the aqueous extract of Kasni (Cichorium intybus) seeds exerted significant hepatoprotective effects in alloxan-induced diabetic mice, as evidenced by the substantial improvements in liver histology observed in the Kasni-treated group. The reduction in blood glucose levels and the normalization of liver architecture in the Kasni-treated mice indicate that this extract may be an effective natural therapeutic agent for managing liver complications associated with diabetes mellitus. These results align with previous studies that have highlighted the potential of Cichorium intybus in mitigating hepatic damage through its anti-inflammatory, antioxidant, and hepatoprotective properties (18).

The study's strengths lie in its systematic approach to evaluating the hepatoprotective effects of Kasni extract, including the use of a well-established diabetic model induced by alloxan monohydrate, which is known to selectively destroy pancreatic beta cells and cause hyperglycemia and subsequent liver damage (7). Additionally, the study employed histopathological analysis, which provided a detailed examination of the liver tissue, allowing for the observation of specific structural changes associated with diabetes and the therapeutic effects of Kasni extract. The use of metformin as a comparative treatment further strengthened the study by providing a

benchmark against which the effects of Kasni extract could be measured.

However, the study also had certain limitations. The sample size was relatively small, consisting of only 32 mice, which may limit the generalizability of the findings. Future studies with larger sample sizes and longer treatment durations would be beneficial to confirm these results and provide a more comprehensive understanding of the long-term effects of Kasni extract. Additionally, while the study focused on the histopathological aspects of liver protection, it did not explore the underlying molecular mechanisms by which Kasni extract exerts its hepatoprotective effects. Investigating these mechanisms, including the roles of specific bioactive compounds in Cichorium intybus, would provide valuable insights into the therapeutic potential of Kasni and help guide the development of targeted interventions for diabetic liver complications.

Another limitation of the study was the lack of assessment of other potential effects of Kasni extract, such as its impact on lipid profiles, oxidative stress markers, and other organs affected by diabetes, such as the kidneys and heart. Including these parameters in future research would provide a more holistic view of the benefits and possible risks associated with the use of Kasni extract in diabetic conditions. Moreover, the study was conducted exclusively in male albino mice, which may not fully represent the response in female mice or other species, including humans. Therefore, additional studies involving diverse animal models and, eventually, clinical trials in humans are necessary to validate the safety and efficacy of Kasni extract for widespread use.

Despite these limitations, the current study contributes to the growing body of evidence supporting the use of natural products in the management of diabetes and its complications. The significant hepatoprotective effects observed with Kasni extract highlight its potential as an alternative or complementary therapy to conventional antidiabetic drugs, which are often associated with adverse effects such as hepatic and renal toxicity (3). Given the increasing interest in herbal medicine and the need for safer therapeutic options, the findings of this study underscore the importance of further research into the clinical applications of Kasni and other medicinal plants (18).

CONCLUSION

In conclusion, the aqueous extract of Kasni seeds demonstrated considerable hepatoprotective effects in alloxan-induced diabetic mice, with a significant reduction in liver damage and improvement in liver histology. These findings suggest that Kasni may serve as a promising natural therapeutic agent for managing liver complications in diabetes mellitus. However, further research is warranted to explore its underlying mechanisms, long-term efficacy, and safety in broader populations, including humans. This study provides a foundation for future investigations into the potential of Kasni as part of an integrated approach to diabetes management, emphasizing the need for continued exploration of traditional medicinal plants in modern healthcare.

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