

Protective Modulation of Hepatocyte Enlargement by Stevia Rebaudiana and Bempedoic Acid in Dexamethasone-Induced Hepatic Changes in Male Wistar Rats

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is the most prevalent liver disorder globally and represents as the primary cause of liver-related morbidity and mortality. In Pakistan, the prevalence of NAFLD has increased in recent years. Clinical studies involving human subjects in context of using NAFLD and NASH (Non-Alcoholic Steatohepatitis) are constrained by ethical limitations, mainly because liver biopsy, the most accurate way to diagnose these conditions is invasive. Furthermore, the manifestation of NASH in humans can take many years even many decades. Because of this, researchers rely on animal models to study the disease and test treatments. This study evaluates the effects of dexamethasone on liver in male Wistar rats and compares histological alterations among control, dexamethasone-exposed stevia, and bempedoic acid-treated groups. Dexamethasone, a synthetic glucocorticoid, is recognized for its capacity to produce hepatic steatosis. Bempedoic acid, an ATP citrate lyase inhibitor, is employed to regulate

hypercholesterolemia and demonstrates potential in treating NAFLD, while natural options such as Stevia rebaudiana are attracting interest for their possible hepatoprotective properties. This study evaluates the effectiveness of Stevia rebaudiana as a natural adjunct to bempedoic acid in treating dexamethasone-induced fatty liver in male rats.

This is an experimental study that used quantitative and qualitative criteria to evaluate the effects, adhering to a positivist paradigm and utilizing deductive reasoning, to objectively assess the hepatoprotective efficacy of Stevia rebaudiana in conjunction with bempedoic acid.

Male Wistar rats were categorized into control, dexamethasone-exposed, stevia-treated, bempedoic acid-treated, and combined stevia-bempedoic acid-treated groups. Dexamethasone was delivered to induce hepatic steatosis, followed by the implementation of therapeutic treatments. Histopathological analysis of hepatic tissues was performed to assess therapy outcomes.

Histopathological findings diameter of hepatocyte had a p-value 0.002 after one-way ANOVA among groups, which is statistically significant. Further studies are recommended in this aspect to find the benefits of SR and BA acting together.

Exhibiting a synergistic impact would aid in recognizing a cost-efficient, natural, and safer adjunct therapy for NAFLD. This study contributes to the expanding literature on plant-derived hepatoprotective drugs and provides practical insights for future translational research targeting human clinical trials.

Introduction

Background

NAFLD is presenting as the major part of chronic hepatic disease around the world.(Loomba et al., 2021) This affects around 1 in 4 individuals globally. (Cataldo et al., 2021) NAFLD is marked by excessive deposition of lipids in the liver, which leads to lipotoxicity.(Nassir, 2022) This condition may progress to more severe stages, including metabolic-associated steatohepatitis (NASH), liver fibrosis, and hepatocellular carcinoma.(Cataldo et al., 2021) The basic histologic criterion for diagnosing NAFLD is the presence of more than 5% macro vesicular fat accumulation in hepatocytes on liver biopsy samples.(Brunt et al., 2021)Hepatocytes are usually 1.5

-2 times larger than their usual size, located in pericentral region in NASH. (Cataldo et al., 2021)The manifestation of NASH in humans can take many years, even many decades. Because of this, researchers rely on use of animal models to examine the disease and test treatments. (Mustika et al., 2023)

Dexamethasone is a commonly used synthetic drug that helps reduce inflammation.(Elbadr et al., 2023) It also causes the liver to make more fat. It does this by increasing production of two important genes, FASN and CD36, which are involved in making and taking in of fatty acids. (Selim et al., 2025) Hence, two main pathways by which dexamethasone can cause fatty liver are decreased breakdown of fatty acids and increased formation of fatty acids. (Elbadr et al., 2023)It has been shown that giving dexamethasone to male Wistar rats can lead to the development of NAFLD. (Amer et al., 2024)

BA blocks ATP citrate lyase enzyme. It works as a prodrug, which means that it only becomes active in the body.(Biolo et al., 2022) In liver a long-chain enzyme called acyl-CoA-synthetase1 turns it into coenzyme A, which then stops the ATP citrate lyase (ACYL) from working.(Cicero et al., 2021)BA is a good option for people who cannot take statins because it does not cause the same muscle-related problems. This makes it a possible choice for patients who are not able to handle statin treatment. Studies encompassing phase II and phase III trials have indicated positive outcomes with BA.(Albosta et al., 2023)

Stevia rebaudiana, people call it Honey leaf or sweet leaf because its leaves taste incredibly sweet.(Peteliuk et al., 2021) Many studies have found that, besides sweetness, stevia rebaudiana has several health benefits. Studies have shown that Stevioside can help improve myocardial fibrosis and renal fibrosis in mice, possibly by lowering oxidative stress and inhibiting the NF- κ B/TGF- β 1/ Smad pathway. It has been found to inhibit liver fibrosis caused by thioacetamide.(Hao et al., 2023)

Rationale

Glucocorticoid-induced hepatic injury, particularly following prolonged dexamethasone exposure, is characterized by metabolic dysregulation, oxidative stress, lipid accumulation, and hepatocellular ballooning. Many conventional hepatoprotective agents primarily focus on symptomatic relief or isolated biochemical markers rather than addressing the underlying metabolic and inflammatory pathways that contribute to hepatocyte ballooning. The selection of Stevia rebaudiana and bempedoic acid in the present study is based on their complementary mechanisms of action. While Stevia rebaudiana primarily targets oxidative stress and inflammation through natural bioactive compounds, bempedoic acid modulates hepatic lipid metabolism at a molecular level. Investigating and comparing the modulatory effects of these agents on hepatocyte ballooning provides valuable insight into both natural and pharmacological approaches for managing glucocorticoid-induced hepatic injury. This comparative evaluation may help identify effective adjunctive strategies for preventing or reducing steroid-associated liver damage.

Research Gap

Lack of studies using Stevia Rebaudiana and Bempedoic acid as adjunct focusing on hepatocyte enlargement after dexamethasone insult

Aim and Objectives

To assess the modulatory effects of Stevia rebaudiana and bempedoic acid on dexamethasone-induced hepatocyte ballooning after dexamethasone exposure To compare histopathological changes among treatment groups To quantify hepatocyte ballooning severity

Materials and Methods

Study Design

Experimental, randomized, controlled animal study

Ethical Approval

Ethical approval was taken from the Ethical Review Board, Superior University

Experimental Animals

A total of thirty adult (8-10 weeks) male rats of Wistar strain, weighing 170 – 200 grams (Elbadr et al., 2024). were housed in the Experimental Research Laboratory for one week for acclimatization. They were individually kept in a climate-controlled conditions of temperature $22\pm0.5^{\circ}\text{C}$, humidity ($50\pm10\%$), and 12 hours light/dark cycles. (Elbadr et al., 2024) Animals were provided with standard rat food and water ad libitum.

Chemicals and Drugs

Dexamethasone The dosing regimen for Dexamethasone is 8mg /kg body weight. Injection Decadron 4mg/ml(5ml) was used in research, manufactured by OBS Pakistan (Pvt) Ltd. Based on this, the calculated volume for administration is 0.4ml per rat. It was administered intraperitoneally daily for 21 days

Stevia rebaudiana extract The dosing regimen for Stevia rebaudiana is 400mg/kg body weight. Stevia was used be form of sachet. Equal Stevia one sachet contains 1 gm stevia manufactured by The Searle company Ltd. It was administered orally, diluted in 10 ml of distilled water, and 0.8ml was given through oral gavage method to the rats daily for 21 days.(El Moshy et al., 2023)

Bempedoic acid The dosing regimen for Bempedoic Acid is 30mg/kg body weight. Tablet Bempex (Bempedoic acid)180mg was used in research, manufactured by Horizon Pharmaceuticals (Pvt) Ltd. It was administered orally after being diluted with 6ml of distilled water, and 0.2ml will be given through oral gavage method to the rats daily for 21 days.(Yaseen & Mohammad Ali, 2022)

Grouping and Treatment Protocol

Group A Control group Rats were given distilled water orally at the time of dose administration to rats of experimental groups for 21 consecutive days.

Group B Dexamethasone treated group

Group C Dexamethasone and Bempedoic acid treated group

Group D Dexamethasone and Stevia rebaudiana treated group

Group E Dexamethasone, Bempedoic acid and Stevia rebaudiana treated group

Tissue Collection and Processing

Rats were sacrificed, keeping in mind the international protocol for euthanasia.(UK IACUC Policies Guidlines, 2020) liver was dissected out.

Liver specimen was taken after dissection and thoroughly washed with tap water before being placed on the bloating paper to remove any debris. Weight of liver was recorded carefully on an electronic weighing scale (Sartorius Precision Balance, Germany).

Liver was fixed in formalin.(Dunk, 2007)

Histopathological Assessment

Briefly, after tissue collection and fixing, they were typically dehydrated and embedded in melted paraffin wax; the resulting block is mounted on a microtome and cut into thin slices. The slices were affixed to microscope slides at which point the wax is removed with a solvent and the tissue slices attached to the slides are rehydrated and are ready for staining. The liver tissue was prepared for histopathological assessment stained by a combination of hematoxylin and eosin (H&E) . (Dunk, 2007)

The hematoxylin-stained cell nuclei a purplish blue, and eosin stains the extracellular matrix and cytoplasm pink. Both dyes demonstrate the general histological architecture of the tissue.(Onica et al., 2025)

Size of hepatocytes were measured with a micrometer at 40X. Slides were observed for cell enlargement, pale or granular cytoplasm, and loss of normal polygonal shape with H & E staining, especially in centrilobular zone at 10X and 40X in 10 random non-overlapping fields per slide. (Cataldo et al., 2021)

Photographic magnification (photographs of slides)

(Nikon ECLIPSE Si Digital Sight 1000 Microscope) at 10X and 40X magnifications, with digital head camera-mounted microscope was used for taking the photographs of slides.

Statistical Analysis

The observations were recorded in MS Word® and Excel(R) data sheet and summarized in tabulated form . Data was entered and analyzed using SPSS (Statistical Package for Social Sciences). All observations were documented in a tabular format and analyzed using SPSS v28. Normality of data was checked with Shapiro-Wilk Test. Mean $\pm$ SD was calculated for quantitative variables like weight of liver and diameter hepatocyte. One way ANOVA (Analysis of Variance) was applied to determine the mean difference in quantitative variables among the groups which were normally distributed.

Results

The animals were examined carefully throughout the experiment to assess the state of their health. Rats remained healthy throughout the experimental period. There was no mortality or morbidity among the groups.

Weight of Liver

Weight of liver was observed at the end of the experiment. The descriptive analysis of liver weights among the five groups revealed a progressive increase from the control group to the treatment groups. Treatment with DEX (8 mg/kg IP daily for 21 days), alone or combined with either Bempedoic Acid (30mg/kg PO daily for 21 days) or Stevia Rebaudiana (400mg/kg PO daily for 21 days) or combination of both Stevia rebaudiana and Bempedoic Acid with Dexamethasone revealed a significant increased liver weight of the rats in comparison with the control group.

Table 1: Descriptive analysis of Liver Weight among groups

	Mean	Std. Deviation	Std. Error	Minimum	Maximum
A	7	0.5	0.2	6.1	7.2
B	7	1.0	0.4	6.3	8.4
C	8	1.5	0.6	7	10

D	8	1.2	0.5	7.2	10.2
E	9	1.7	0.7	6.4	10.5
Total	8	1.4	0.3	6.1	10.5

A one-way ANOVA was performed to ascertain if liver weight varied substantially among groups. The analysis demonstrated a statistically significant impact of the treatment group on liver weight, $p=0.032$.

Histological Findings

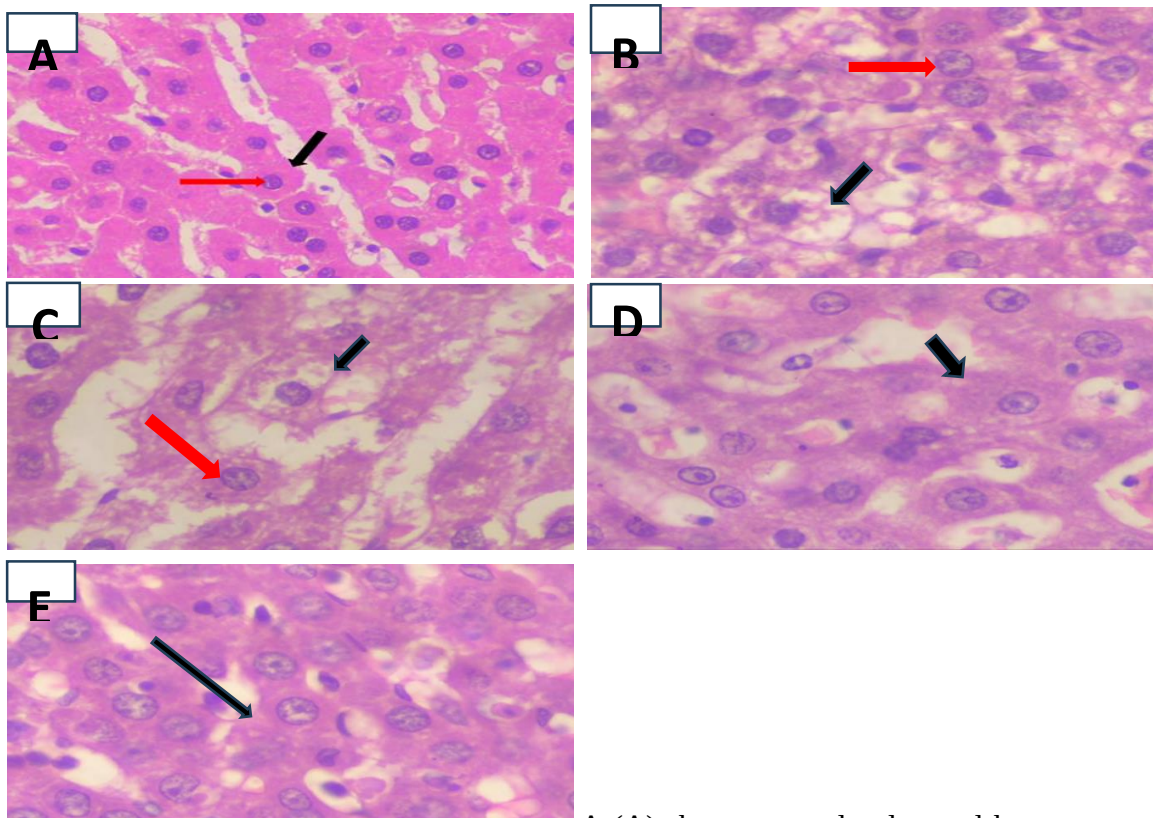
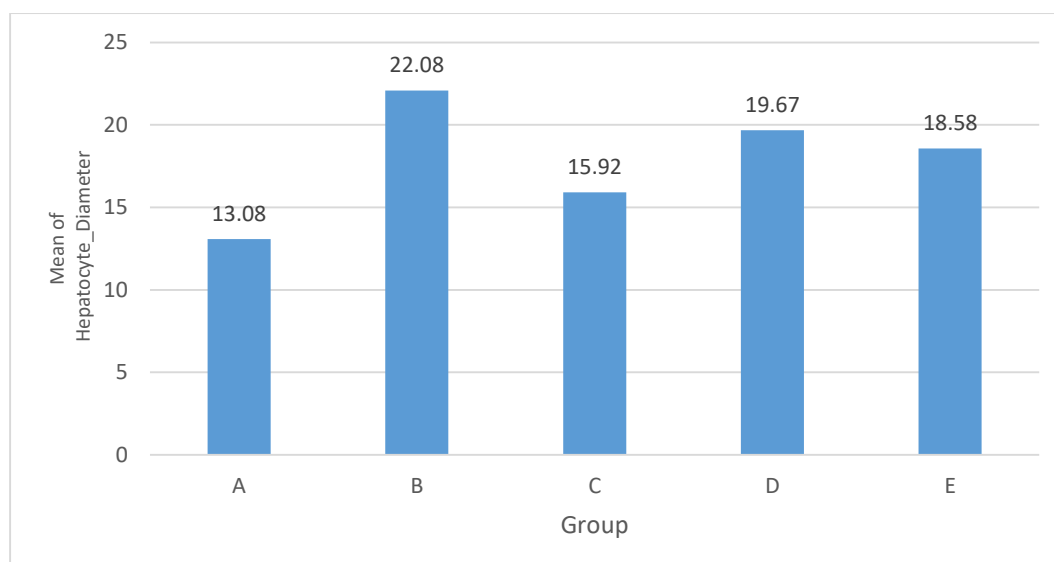


Figure 1: Photomicrograph of liver Group A (A) shows normal polygonal hepatocyte (black arrow) with normal round central nucleus. Group B(B)shows hepatocyte ballooning (black arrow) with large euchromatic nucleus (red arrow). Group C (C) shows hepatocyte ballooning (black arrow) normal hepatocyte(red arrow). Group D (D) shows mild hepatocyte ballooning (black arrow). Group E (E) shows normal hepatocyte (black arrow)H&E (40X)

Quantitative Analysis

Hepatocyte diameter	Between Groups	464.05	4	102.165	3.67	0.002
	Within Groups	783.261	27	31.425		
	Total	1247.311	34			

The diameter of the hepatocytes exhibited a highly significant variation among groups, with ANOVA revealing a substantial group effect, $p < .002$.



• Figure 2 :Bar chart showing comparison of Mean of Hepatocyte Diameter

Discussion

Dexamethasone is extensively utilized for many inflammatory and autoimmune disorders, such as asthma, TB, rheumatism, and acute allergic responses (24/Menkes/2022, 2022). Nonetheless, despite its therapeutic benefits, the administration of dexamethasone is extensively established to induce a wide array of metabolic abnormalities. (Selim et al., 2025) Wistar rats are the favored model in experimental settings because of their genetic consistency, placid temperament, predictable physiological responses, and ability to endure extended experimental treatments. (Ashmita Debnath, M Ayub Ali, Kekungwi Newmai, Palagiri Madhuri & Malsawmsangi, 2024)

The weight of organs, especially the liver, offered more understanding of steroid-induced hepatomegaly. In the present study, a progressive increase in mean liver weight was observed from the control to the dexamethasone-treated groups, with dexamethasone administration resulting in significant hepatomegaly. This finding is consistent with established evidence that chronic glucocorticoid exposure promotes hepatic enlargement through a combination of lipid accumulation, glycogen deposition, sinusoidal congestion, and hepatocellular hypertrophy (Jasim et al., 2022). A recent report from 2024 also recorded analogous increases in hepatic mass following prolonged glucocorticoid exposure, confirming that pathological response is consistent and reproducible. (Elbadr et al., 2024).

The present study found that BA (Bempedoic Acid) administration led to a significant increase in liver weight, a finding that appears paradoxical when interpreted alongside its known lipid-lowering and metabolic regulatory properties. However, evidence from murine models of steatosis, bempedoic acid has been shown to increase liver weight by up to 66%, potentially due to enhanced hepatocellular metabolic activity, peroxisomal proliferation, or temporary lipid redistribution during metabolic correction (A et al., 2022). These findings suggest that the effects of bempedoic acid on the liver are complex and cannot be fully understood by organ weight alone. This underscores the importance of evaluating changes in liver weight in conjunction with histomorphometric and functional assessments, rather than viewing them in isolation.

The administration of Stevia resulted in hepatic enlargement, in contrast with the finding of previous reports, indicating that Stevia, even at higher dosages, does not significantly alter organ weights or induce histological abnormalities (Uddin1 et al., 2022).

Histomorphometric analysis further reinforced these observations. The rats treated with dexamethasone exhibited significantly enlarged hepatocytes with ballooning degeneration, indicative of cellular swelling, cytoskeletal disruption, and impaired lipid handling. These alterations are typical of glucocorticoid-induced hepatic injury and are frequently reported in experimental models of steroid-associated NAFLD (Amer et al., 2024). Diameter of hepatocytes exhibited a clear, consistent trend, i.e., Dexamethasone group displayed markedly swollen hypertrophic hepatocytes whereas the treated groups showed a reduction in cell size with values approaching near to that of control group. The significant ANOVA results indicate that Dex-induced damage was considerable and that interventions resulted in notable structural recovery, providing strong evidence for the initial hypothesis, i.e., treatment with bempedoic acid and *Stevia rebaudiana* resulted in a marked decrease in hepatocyte diameter, with values approaching near normal. These improvements suggest attenuation of cellular stress, restoration of hepatocellular architecture, and improvement in metabolic functionality. Collectively, these results provide strong support for the study hypothesis that *Stevia rebaudiana* and bempedoic acid exert modulatory effects on dexamethasone-induced hepatocyte ballooning, likely through potential underlying distinct mechanisms. Despite the promising results, certain limitations must be recognized. The lack of biochemical assessment such as liver enzymes, lipid profiles, and oxidative stress parameters, constrains the functional correlation with histological changes. Moreover, the relatively short duration of the study and the use of an animal model may restrict direct translation to clinical settings. Further studies incorporating molecular markers of lipid metabolism, inflammation, and oxidative stress, along with extended treatment durations, would further clarify the mechanistic pathways underlying these effects.

Conclusion

The present study confirms that dexamethasone causes significant changes in hepatic architecture, consistent with findings of hepatomegaly and pronounced hepatocyte ballooning. Administration of *Stevia Rebaudiana* and Bempedoic Acid resulted in marked improvement in hepatocyte diameter with a consistent rise in hepatic weight, which can be studied further in prospects of congestion, inflammation, and other biochemical changes. Collectively, these findings suggest that both agents possess therapeutic potential in mitigating steroid induced hepatic injury. *Stevia rebaudiana* proves to be a promising natural adjunct to Bempedoic Acid. Future studies incorporating biochemical markers and molecular analyses are suggested to confirm the underlying mechanisms and support translational relevance.

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