ACS OMEGA

Open Access

http://pubs.acs.org/journal/acsodf

Article

Biochemical Investigation of Therapeutic Efficacy of Berberine-Enriched Extract in Streptozotocin-Induced Metabolic Impairment

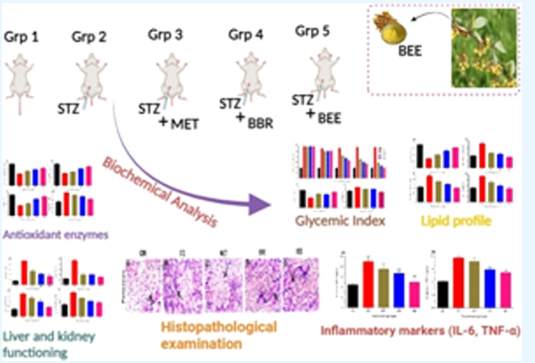
Muhammad Sajid Hamid Akash,* Sahrish Yaqoob, Kanwal Rehman,* Amjad Hussain, Zunera Chauhdary, Ahmed Nadeem, Asif Shahzad, Muhammad Ajmal Shah, and Pharkphoom Panichayupakaranant

Cite This: *ACS Omega* 2024, 9, 15677−15688

Read Online

ACCESS | Metrics & More | Article Recommendations

ABSTRACT: Metabolic disorders pose significant global health challenges, necessitating innovative therapeutic approaches. This study focused on the multifaceted therapeutic potential of berberine-enriched extract (BEE) in mitigating metabolic impairment induced by streptozotocin (STZ) in a rat model and compared the effects of BEE with berberine (BBR) and metformin (MET) to comprehensively evaluate their impact on various biochemical parameters. Our investigation reveals that BEE surpasses the effects of BBR and MET in ameliorating metabolic impairment, making it a promising candidate for managing metabolic disorders. For this, 30 male Wistar rats were divided into five groups (n = 6): control (CN), STZ, STZ + MET, STZ + BBR, and STZ + BEE. The treatment duration was extended over 4 weeks, during which various biochemical parameters were monitored, including fasting blood glucose (FBG), lipid profiles, inflammation, liver and kidney function biomarkers, and gene expressions of various metabolizing enzymes. The induction of metabolic impairment by STZ was evident through an elevated FBG level and disrupted lipid profiles. The enriched extract effectively regulated glucose homeostasis, as evidenced by the restoration of FBG levels, superior to both BBR and MET. Furthermore, BEE demonstrated potent effects on insulin sensitivity, upregulating the key genes involved in carbohydrate metabolism: GCK, IGF-1, and GLUT2. This highlights its potential in enhancing glucose utilization and insulin responsiveness. Dyslipidemia, a common occurrence in metabolic disorders, was effectively managed by BEE. The extract exhibited superior efficacy in regulating lipid profiles. Additionally, BEE exhibited significant anti-inflammatory properties, surpassing the effects of BBR and MET in lowering the levels of inflammatory biomarkers (IL-6 and TNF-α), thereby ameliorating insulin resistance and systemic inflammation. The extract's superior hepatoprotective and nephroprotective effects, indicated by the restoration of liver and kidney function biomarkers, further highlight its potential in maintaining organ health. Moreover, BEE demonstrated potent antioxidant properties, reducing oxidative stress and lipid peroxidation in liver tissue homogenates. Histopathological examination of the pancreas underscored the protective effects of BEE, preserving and recovering pancreatic β-cells damaged by STZ. This collective evidence positions BEE as a promising therapeutic candidate for managing metabolic disorders and offers potential benefits beyond current treatments. In conclusion, our findings emphasize the remarkable therapeutic efficacy of BEE and provide a foundation for further research into its mechanisms, long-term safety, and clinical translation.



1. INTRODUCTION

Plant-based secondary metabolites, notably alkaloids, play a significant role in the treatment of metabolic disorders due to their diverse pharmacological properties.[1] These bioactive compounds, found in various botanical sources, have shown promising effects in managing metabolic disorders, such as obesity, diabetes mellitus (DM), and dyslipidemia. Alkaloids such as berberine, present in *Berberis* species, exhibit antiobesity effects by modulating adipogenesis and lipid metabolism. This is achieved through the activation of AMP-activated protein kinase (AMPK) and the inhibition of peroxisome proliferator-activated receptor γ (PPARγ).[2] Alkaloids have shown potential in improving insulin sensitivity and glucose uptake in diabetic patients, partly by enhancing glucose transporter type 4 (GLUT4) translocation.[3] The use of alkaloid-rich extracts has been linked to improved lipid profiles, reduced hepatic steatosis, and enhanced insulin signaling, making them promising

Received: January 21, 2024
Revised: March 9, 2024
Accepted: March 12, 2024
Published: March 22, 2024

candidates for managing dyslipidemia and DM.[4] These studies highlight the therapeutic potential of plant-based alkaloids as natural remedies for metabolic disorders, paving the way for further research and development in this field.

Berberine is a quaternary protoberberine alkaloid with a vibrant yellow color having intensely bitter taste, commonly isolated from plant matrices using various extraction techniques.[5] Berberine-rich plants, such as *Berberis lycium* from the *Berberidaceae* family, are widely distributed in Pakistan and other countries, making them valuable traditional sources of this alkaloid. It possesses potent anti-inflammatory and antimicrobial properties, making it effective against various diseases and inflammation-related conditions.[5] Additionally, berberine has gained attention for its role in managing DM and associated metabolic disorders.[6] It exhibits significant antihyperglycemic and hypo-lipidemic activities, contributing to improved glycemic control and lipid profile.[7] Apart from these effects, berberine has shown its potential in the treatment of complications associated with DM, such as diabetic nephropathy, diabetic neuropathy, and diabetic cardiomyopathy.[8] Furthermore, recent studies indicate that berberine plays a protective role in preserving the β-cells of the pancreatic islets, which are responsible for insulin production.[9] The wide-ranging medicinal properties of berberine make it a fascinating natural compound with immense potential for therapeutic applications in the management of metabolic disorders and associated complications.[10]

Berberis extract and pure berberine demonstrate comparable efficacy, as they both exhibit similar effects. However, the use of the *berberis* extract is more cost-effective compared to the pure compound. *Berberis* extract is derived from the whole plant or specific parts, containing not only berberine but also a combination of other bioactive compounds, which may contribute to its effectiveness. This synergy of multiple compounds in the extract can enhance the overall therapeutic benefits, leading to comparable outcomes to pure berberine.[11] Moreover, the presence of other phytochemicals in the *berberis* extract may provide additional health benefits beyond what pure berberine can offer alone. These coexisting compounds might act synergistically to enhance the absorption, bioavailability, and cellular uptake of berberine, potentially improving its overall pharmacological actions. Additionally, using the extract reduces the need for extensive purification steps involved in obtaining pure berberine, thereby reducing the production costs.

The existing body of knowledge on plant-based alkaloids, particularly berberine, has witnessed significant expansion in recent years. Recent studies have delved into the diverse pharmacological properties of berberine, shedding light on its potential therapeutic applications.[6,12] However, a critical examination of the literature reveals notable gaps in comprehensively exploring the therapeutic effects of the berberine-enriched extract (BEE) against metabolic impairment, particularly in experimental animal models. Recent studies have emphasized the need for more detailed investigations into the biochemical profiling of BEE and its distinct impact on metabolic disorders.[8b13] Our study seeks to address this gap by undertaking a thorough examination of the biochemical effects of BEE in the context of metabolic impairment utilizing a well-established experimental model. By delving into the specific biochemical mechanisms and outcomes associated with BEE administration, we aim to provide a more comprehensive understanding of its therapeutic potential, thereby contributing to the current knowledge base and offering insights that can guide future research and potential clinical applications. This study aims to bridge the existing gap by elucidating the unique biochemical profile of BEE and its implications for mitigating metabolic disorders, enriching the scientific discourse on plant-based alkaloids.

In this study, our objective was to assess the therapeutic potential of BEE in mitigating streptozotocin (STZ)-induced metabolic disorder in rats, utilizing it as an experimental animal model. We aimed to compare the therapeutic effects of BEE with those of Berberine (BBR), a known compound with potential metabolic benefits. We hypothesized that BEE could effectively ameliorate the STZ-induced metabolic disorder by modulating and restoring normal levels of glycemic index, lipid profile, liver and kidney function markers, and inflammatory mediators more efficiently when compared to BBR. Furthermore, in addition to its overall effectiveness, we explored how BEE influenced the mRNA expressions of carbohydrate metabolizing enzymes and insulin sensitizing hormones. We anticipated that BEE would exert a more profound impact on the regulation of these targets compared to BBR. The findings may pave the way for future applications of BEE as a promising therapeutic agent in the management of metabolic imbalances and associated complications.

2. MATERIALS AND METHODS

2.1. Drugs and Chemicals. Metformin (MET) was sourced from Merck Limited. Streptozotocin (STZ), ketamine, agarose, ethidium bromide, chloroform, and isopropanol were procured from Sigma, USA. The cDNA kit (WizScript), cyber green master (WizPure), and primers were obtained from Thermo Fisher Scientific-US. Various enzyme-linked immunosorbent assay (ELISA) kits, including the insulin ELISA kit (catalogue number: IS130D, Calbiotech), and HbA1c ELISA kit (catalogue number: SG 10984, Elabsciences), HDL ELISA kit (catalogue number: E-BC-K222-S, Elabsciences), Cholesterol ELISA kit (catalogue number: BD090618, Human diagnostics), Triglyceride ELISA kit (catalogue number: BD090618, bioactive), Catalase ELISA kit (catalogue number: E-BC-K106, Elabsciences), superoxide dismutase ELISA kit (catalogue number: E-BC-K020, Elabsciences), and glutathione peroxidase ELISA kit (catalogue number: E-EL-R2491, Elabsciences), Malondialdehyde ELISA kit (catalogue number: E-EL-0060, Elabsciences), and IL-6 ELISA kit (catalogue number: E-EL-R0015, Elabsciences), and TNF-α ELISA kit (catalogue number: E-EL-R0019, Elabsciences), were employed in the study.

2.2. Berberine-Enriched Extract. The green method of extraction plays a vital role in ensuring the production of enriched extracts that are not only effective and safe but also environmentally friendly.[14] As consumers show a growing interest in natural products with sustainable origins, the green extraction approach becomes even more relevant and advantageous in the development of high-quality enriched extracts for various applications, including pharmaceuticals, functional foods, and nutraceuticals.[14a15] For this study, BBR and BEE were provided by Pharkphoom Panichayupakranant at the Phytomedicines and Pharmaceutical Biotechnology Excellence Centre, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Thailand. The green extraction method was employed for the preparation of BEE, as previously outlined.[16]

2.3. Experimental Design. In this experimental investigation, around 30 Wistar albino rats weighing between 200 and 350 g were employed. The rats were distributed into 5 groups, each comprising 6 rats, and were individually housed in cages at

https://doi.org/10.1021/acsomega.4c00696
ACS Omega 2024, 9, 15677−15688

Table 1. Primer Sequences for RT-PCR Analysis

gene	gene sequence (5′ to 3′) forward	reverse	basic product size
GCK	TGGTTCCTGTCCACCATTAGTT	CCAGGTCAGTGCCTTAGTGC	120 bp
IGF-1	GCTCCAAAGCAGACAAAAATACCC	GGTCTGGGCACAAAGATGGA	110 bp
GLUT-2	GCAGCCTTGGTTAAGAAGGTCA	CTTCTGACATGTTGCGTGCG	125 bp

the animal facility of Government College University, Faisalabad, Pakistan. The animal facility maintained standard environmental parameters, including an ambient temperature ranging from 20 to 24 °C, a relative humidity of 65 ± 5%, and a 12 h light−dark cycle, ensuring consistent and controlled conditions. Prior to the initiation of the experiments, the rats underwent a one-week acclimatization phase, during which they received a commercially available rat chow diet and had unrestricted access to water *ad libitum*.

The groups were classified as follows:

Group 1: Control group (CN): normal animals treated with normal saline.

Group 2: Diabetic control group—Animals treated with STZ at the dose of 40 mg/kg intraperitoneally to induce metabolic impairment.

Group 3: Positive control or standard group—Animals treated with STZ at the dose of 40 mg/kg intraperitoneally and MET at the dose of 500 mg/kg per orally, which served as the standard reference treatment for comparison.

Group 4: Animals received oral administration of BBR at a dose of 50 mg/kg and intraperitoneal injection of STZ (40 mg/kg).

Group 5: Animals received oral administration of BEE (50 mg/kg) and intraperitoneal injection of STZ (40 mg/kg).

These groups were constituted to assess and compare the therapeutic efficacy of BEE and BBR in managing STZ-induced metabolic disorders in an experimental animal model. The selected doses of BBR and BEE were administered orally, while STZ was administered intraperitoneally to induce the targeted metabolic condition.

2.4. Induction of Metabolic Impairment. Insulin resistance was induced in the experimental animals by subjecting them to a high-fat diet (per 100 g of diet contained 521 kcal with 60% of energy derived from fat, 20% from protein, and 20% from carbohydrates) for a duration of 4 weeks. After a 12 h fasting period, a low dose of STZ (40 mg/kg) was administered *via* intraperitoneal injection. The rationale for administering STZ after the 4-week period of the high-fat diet was to induce insulin resistance in the experimental animals. The high-fat diet serves to create a metabolic environment conducive to insulin resistance, and the subsequent administration of STZ exacerbates this condition. This sequential approach aims to model a more comprehensive representation of metabolic impairment, simulating a scenario closer to the multifactorial nature of insulin resistance observed in metabolic disorders. To prevent hypoglycemic shock in the diabetic groups, a 5% glucose solution was administered. Conversely, the nondiabetic groups received citrate buffer alone at a dose of 1 mL/kg i.p., with a pH of 4.5. Rat blood samples were obtained by tail vein puncture at 72 h after STZ administration. Blood glucose levels were determined using the On-Call glucometer and glucose strip method. Rats with plasma glucose levels ≥200 mg/dL were classified as diabetic animals and were incorporated into the study, following a method previously outlined with some modifications.[17] This method facilitated the identification and inclusion of diabetic rats, serving as subjects in the study to assess the impacts of induced insulin resistance and the therapeutic interventions under investigation.

2.5. Biochemical Analysis. The fasting blood glucose level was determined using the glucose strip method, with blood samples collected from the tail vein of experimental rats at predefined time points, including the 0-, 7-, 14-, 21-, and 28-day intervals. A glucometer (On-Call Extra) was utilized for these measurements, adhering to the manufacturer's instructions. After the 4-week period, blood samples were obtained through cardiac puncture under ketamine (24 mg/kg) anesthesia. Blood samples were allowed to coagulate at room temperature for 20 min, followed by centrifugation at 3000*g* for 15 min. The resulting serum was then carefully stored at −20 °C until further analysis. The subsequent analysis of these blood samples included parameters such as blood glucose, insulin, HbA1c, high-density lipoprotein (HDL), cholesterol, triglycerides (TGs), high-density lipoprotein (LDL), TNF-α, IL-6, aspartate aminotransferase (AST), alanine aminotransferase (ALT), blood urea nitrogen (BUN), and creatinine. Evaluation of these biochemical parameters was performed using their respective assay kits on a biochemical analyzer (Microlab-300, ELITech Group) and a microplate ELISA reader (BioTek 800 TS absorbance reader, Agilent Technologies). Strict adherence to the manufacturer's instructions for these assay kits ensured accuracy during the analysis process.

2.6. Preparation of Tissue Homogenates for Estimation of Oxidative Stress and Lipid Peroxidation. After the treatment period, liver tissues were promptly collected and stored in an icebox to maintain a low temperature and prevent cellular component alterations. Muscle samples, prepared for tissue homogenates, were homogenized by using a tissue homogenizer with a 0.01 M phosphate buffer (1:9 ratio of tissue to buffer) to achieve a homogeneous cellular suspension. The homogenates underwent a 10 min incubation on ice for stabilization, inhibiting enzymatic reactions. Centrifugation at 14,000*g* for 10 min effectively separated cellular debris, settling at the tube bottom, from clear supernatants kept for analysis. This process, driven by high centrifugal forces, facilitated the extraction of uncontaminated, clear supernatants. These meticulously collected supernatants were utilized for ELISA tests, ensuring a precise evaluation of specific components and biomarkers.

In the meticulous evaluation of oxidative stress biomarkers within the liver tissue homogenates, a comprehensive analysis was undertaken focusing on catalase (CAT), glutathione (GSH), superoxide dismutase (SOD), and malondialdehyde (MDA). The ELISA kit assay method was employed to analyze these biomarkers, and absorbance readings were precisely recorded at 450 nm by using a microplate ELISA reader.

2.7. Estimation of mRNA Expression in the Transcriptional Regulation of Glucose Metabolism and Insulin Secretion. To extract RNA from tissue homogenates, TRIzol reagent (Biobasic BS410A-MA18DR0J) was utilized following the TRIzol method, ensuring efficient total RNA extraction

https://doi.org/10.1021/acsomega.4c00696
ACS Omega 2024, 9, 15677−15688

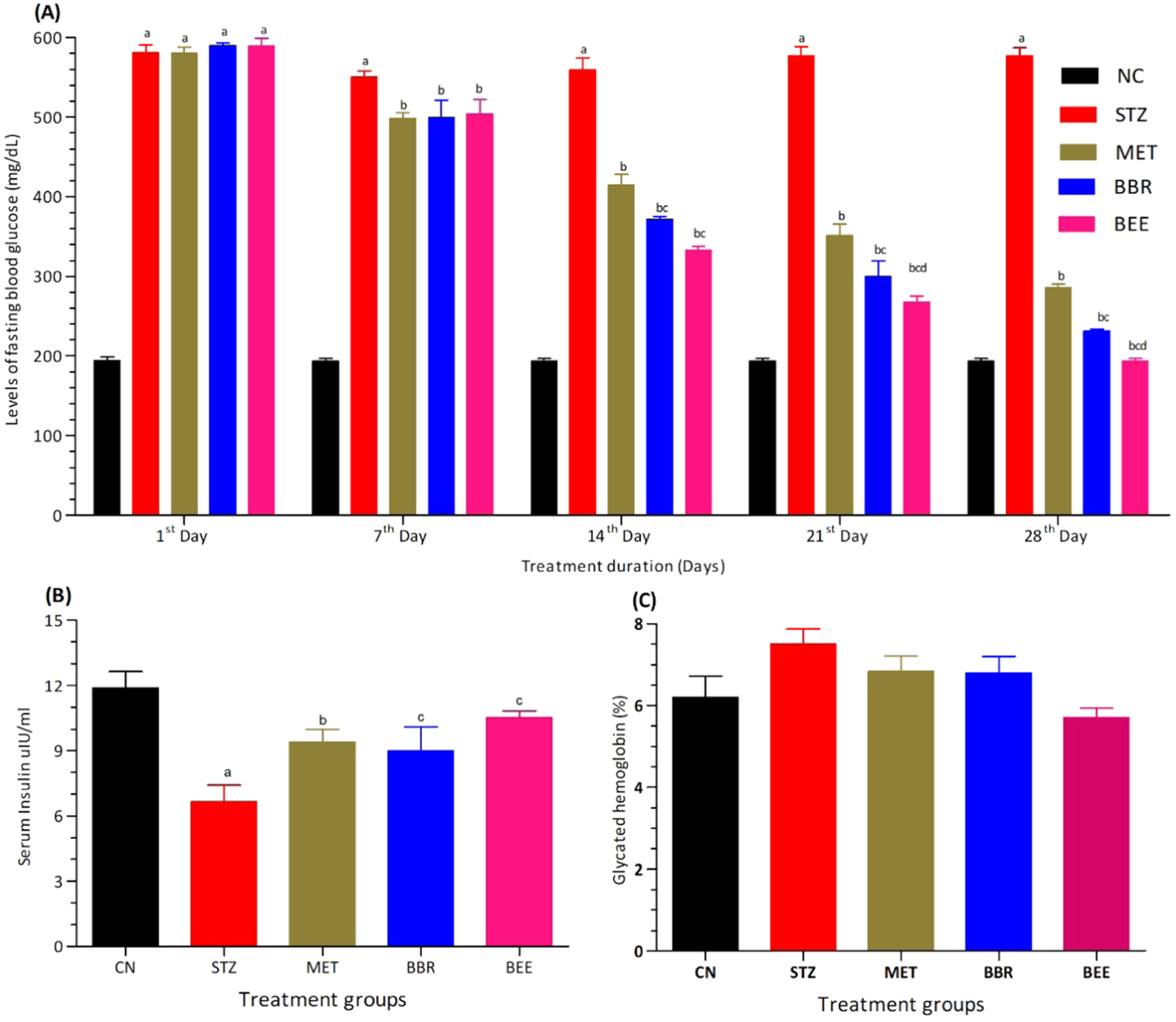


Figure 1. Fasting blood glucose (A), insulin (B), and glycated hemoglobin (C) analyses were conducted by using different statistical methods. For fasting blood glucose, a two-way analysis of variance (ANOVA) was employed. For insulin and glycated hemoglobin, one-way ANOVA was used. Post hoc analysis was performed using Bonferroni's multiple comparison test to compare all pairs of columns. [a] When compared with control group. [b] When compared with STZ group. [c] When compared with MET group. [d] When compared with MET and BRE groups. Abbreviations: | CN: control, STZ: Streptozotocin, MET: Metformin, BBR: Berberine, BEE: Berberine-enriched extract.

while maintaining its integrity. Subsequently, 2% agarose gel electrophoresis was used to evaluate the RNA quality. Extracted total RNA (2 μg) underwent reverse transcription into cDNA using the Revert Aid cDNA synthesis kit (Thermo Scientific). For gene expression quantification, quantitative polymerase chain reaction (qPCR) was performed with specific thermal cycling conditions: initial denaturation at 95 °C for 5 min, followed by 40 cycles of denaturation at 95 °C for 15 s, annealing at 60 °C for 20 s, and extension at 72 °C for 20 s. Table 1 presents sequence and size details of expected PCR products for reference and target genes (GCK, IGF-1, and GLUT2). Genes were selected based on their relevance to pancreatic function and metabolism. PCR initiation at 94 °C for 5 min, with subsequent cycling for 35−40 cycles, involved denaturation at 94 °C for 30 s, annealing at 52−55 °C for 30 s, and extension at 72 °C for 1 min. Gel electrophoresis with 1.5% agarose and staining with ethidium bromide facilitated the visualization of PCR products by using an image analyzer system.

2.8. Histopathological Analysis. Following the fourth week of the experimental period, animals were euthanized under anesthesia, and the pancreas was isolated for histopathological examination. Tissues were fixed in a 10% formalin solution, dehydrated, embedded in paraffin wax, and sectioned at a thickness of 5 μm using a microtome. The sections were then stained with hematoxylin and eosin (H&E), deparaffinized, hydrated, and observed under a microscope.

3. RESULTS

3.1. Biochemical Analysis of Glycemic Index Biomarkers. The results indicated that rats treated with STZ had the highest FBG levels, which were significantly different from each other. Conversely, rats treated with BEE showed the lowest FBG levels. Among the different time intervals, the maximum

https://doi.org/10.1021/acsomega.4c00696
ACS Omega 2024, 9, 15677−15688

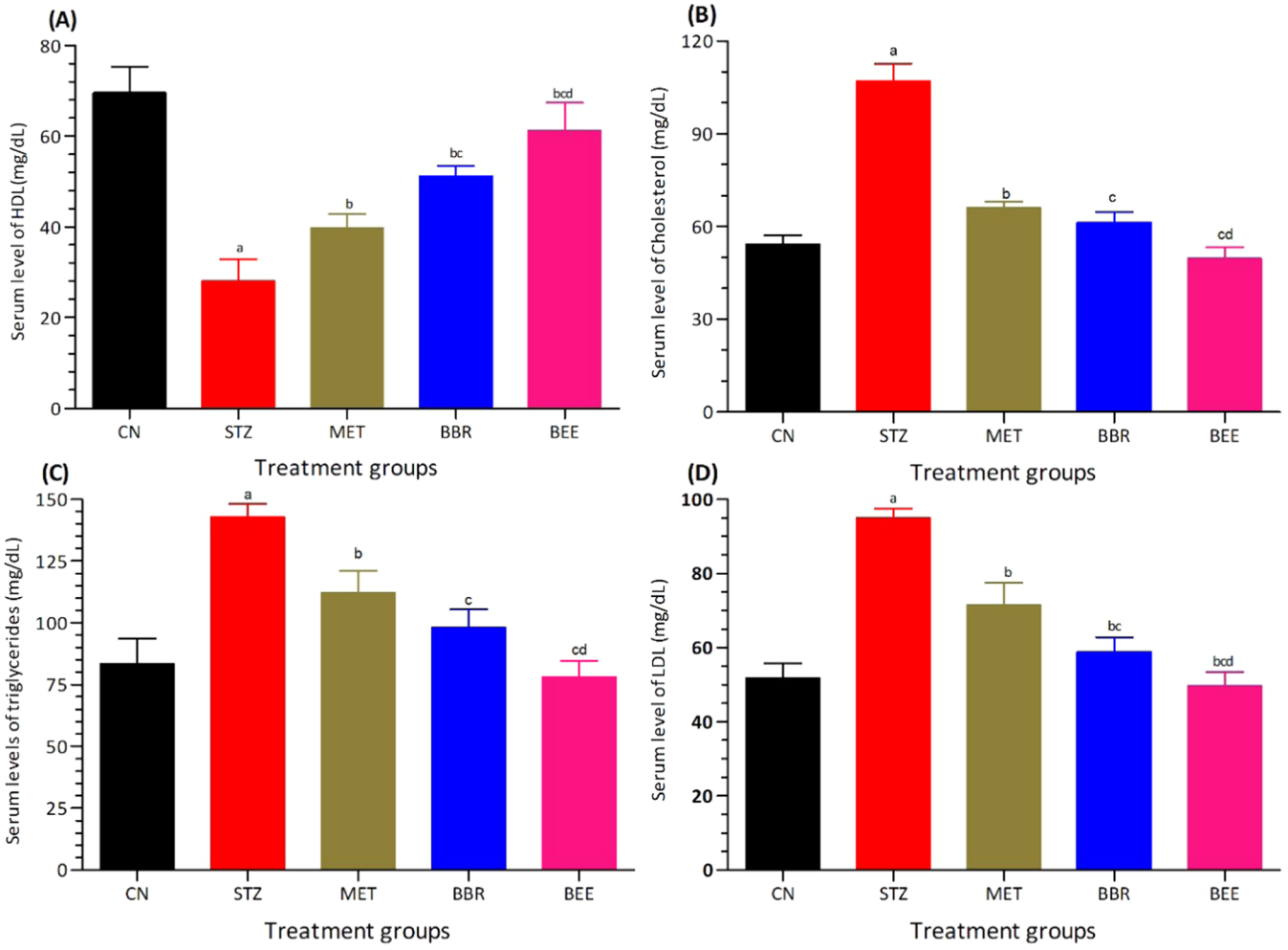


Figure 2. Serum levels of HDL (A), cholesterol (B), triglycerides (C), and LDL (D) were analyzed using one-way ANOVA. Post hoc analysis was conducted using Bonferroni's multiple comparison test to compare all pairs of columns. [a] When compared with control group. [b] When compared with STZ group. [c] When compared with MET group. [d] When compared with MET and BRE groups. Abbreviations: | HDL: high-density lipoprotein, LDL: low-density lipoprotein, CN: control, STZ: Streptozotocin, MET: Metformin, BBR: Berberine, BEE: Berberine-enriched extract.

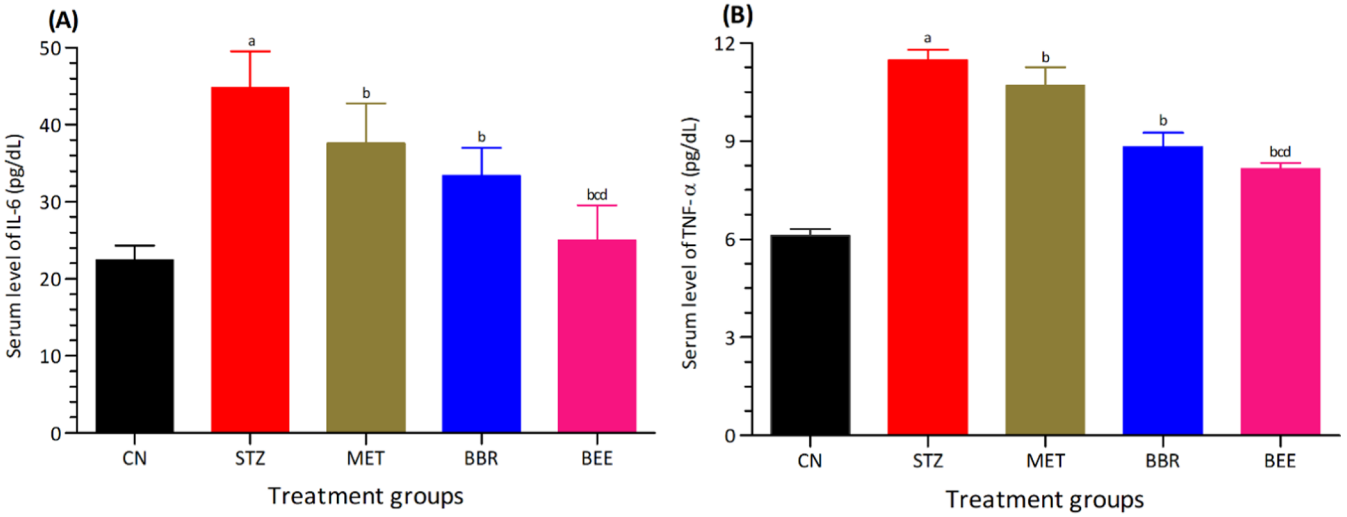


Figure 3. Serum levels of IL-6 (A) and TNF-α (B) analyzed using one-way ANOVA. Post hoc analysis was performed using Bonferroni's multiple comparison test to compare all pairs of columns. [a] When compared with control group. [b] When compared with STZ group. [c] When compared with MET group. [d] When compared with MET and BRE groups. Abbreviations: | IL-6: Interleukin 6, TNF-α: tumor necrosis factor α, CN: control, STZ: Streptozotocin, MET: Metformin, BBR: Berberine, BEE: Berberine-enriched extract.

https://doi.org/10.1021/acsomega.4c00696
ACS Omega 2024, 9, 15677−15688

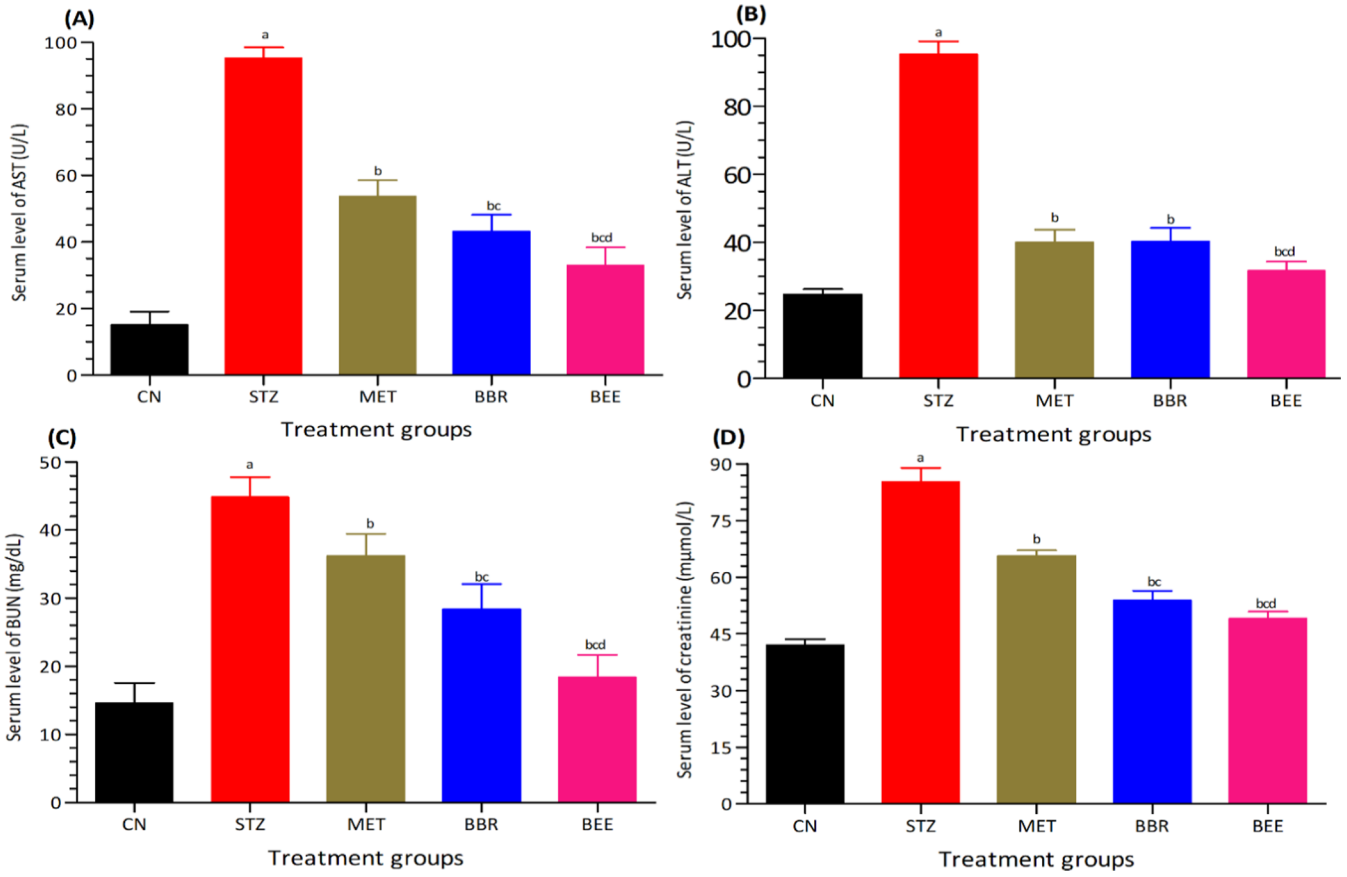


Figure 4. Serum levels of AST (A), ALT (B), BUN (C), and creatinine (D) were analyzed using one-way ANOVA. Post hoc analysis was performed using Bonferroni's multiple comparison test to compare all pairs of columns. [a] When compared with control group. [b] When compared with STZ group. [c] When compared with MET group. [d] When compared with MET and BRE groups. Abbreviations: | AST: aspartate aminotransferase, ALT: alanine aminotransferase, BUN: blood urea nitrogen, CN: control, STZ: Streptozotocin, MET: Metformin, BBR: Berberine, BEE: Berberine-enriched extract.

FBG was recorded on the 28th day (Figure 1A). Furthermore, the interaction effect analysis showed that the control group exhibited the maximum FBG on the 28th day, while the BEE-treated group had the lowest FBG levels on the same day. At the end of the treatment period, MET, BBR, and BEE significantly reduced FBG levels compared to those of the STZ group. However, the most pronounced effects were observed in the BEE-treated group, with a significant difference in FBG levels compared with the BBR-treated group. Additionally, both BEE and BBR treatment improved insulin sensitivity in the experimental animals (Figure 1B), with enhanced serum insulin levels in these groups. Among the treatments, BEE exhibited a more significant effect on serum insulin levels compared to both MET and BBR. Furthermore, BEE and BBR treatments demonstrated a more efficient reduction in the percent content of HbA1c in experimental animals compared to MET (Figure 1C). Notably, BEE exhibited better efficacy in reducing HbA1c levels compared to BBR.

3.2. Biochemical Analysis of Lipid Profile. In this study, we investigated the therapeutic effects of BBR and BEE on various lipid profile biomarkers, including HDL, cholesterol, TGs, and LDL (Figure 2). The results showed that STZ significantly increased the level of cholesterol, TGs, and LDL and decreased HDL level in comparison to the control group. This study revealed that both BEE and BBR demonstrated significant ($P < 0.001$) therapeutic effects by effectively regulating the levels of HDL, cholesterol, TGs, and LDL in STZ intoxicated rats in comparison to the MET group. These findings suggest that BEE and BBR could be beneficial in managing dyslipidemia and promoting a healthier lipid profile. Interestingly, when we compared the therapeutic efficiency of BEE with that of BBR, we observed that BEE exhibited greater efficacy in improving the serum levels of HDL (Figure 2A) and reducing the serum levels of cholesterol (Figure 2B), TGs (Figure 2C), and LDL (Figure 2D) in comparison to BBR. These findings suggest that BEE may be a particularly promising agent for enhancing HDL levels, known for its protective effects against cardiovascular diseases. Furthermore, BEE's ability to effectively reduce cholesterol, TGs, and LDL levels implies its potential in combating dyslipidemia and related metabolic disorders.

3.3. Biochemical Analysis of Inflammatory Mediators. In the development and pathogenesis of diverse metabolic disorders, inflammation plays a pivotal role. As depicted in Figure 3, the administration of STZ markedly elevated the levels of inflammatory markers IL-6 (Figure 3A) and TNF-α (Figure 3B) in comparison to the control group. These observations underscored the pro-inflammatory impact of STZ and its role in instigating an inflammatory response. However, in the groups subjected to treatment with MET, BBR, and BEE, a noteworthy decrease in the serum levels of IL-6 and TNF-α was evident when compared with the STZ-treated group. These outcomes indicate the effective attenuation of the inflammatory response induced by STZ through all three treatments, offering potential benefits in alleviating the adverse effects of inflammation on metabolic health. Notably, BEE demonstrated a more profound

https://doi.org/10.1021/acsomega.4c00696
ACS Omega 2024, 9, 15677−15688

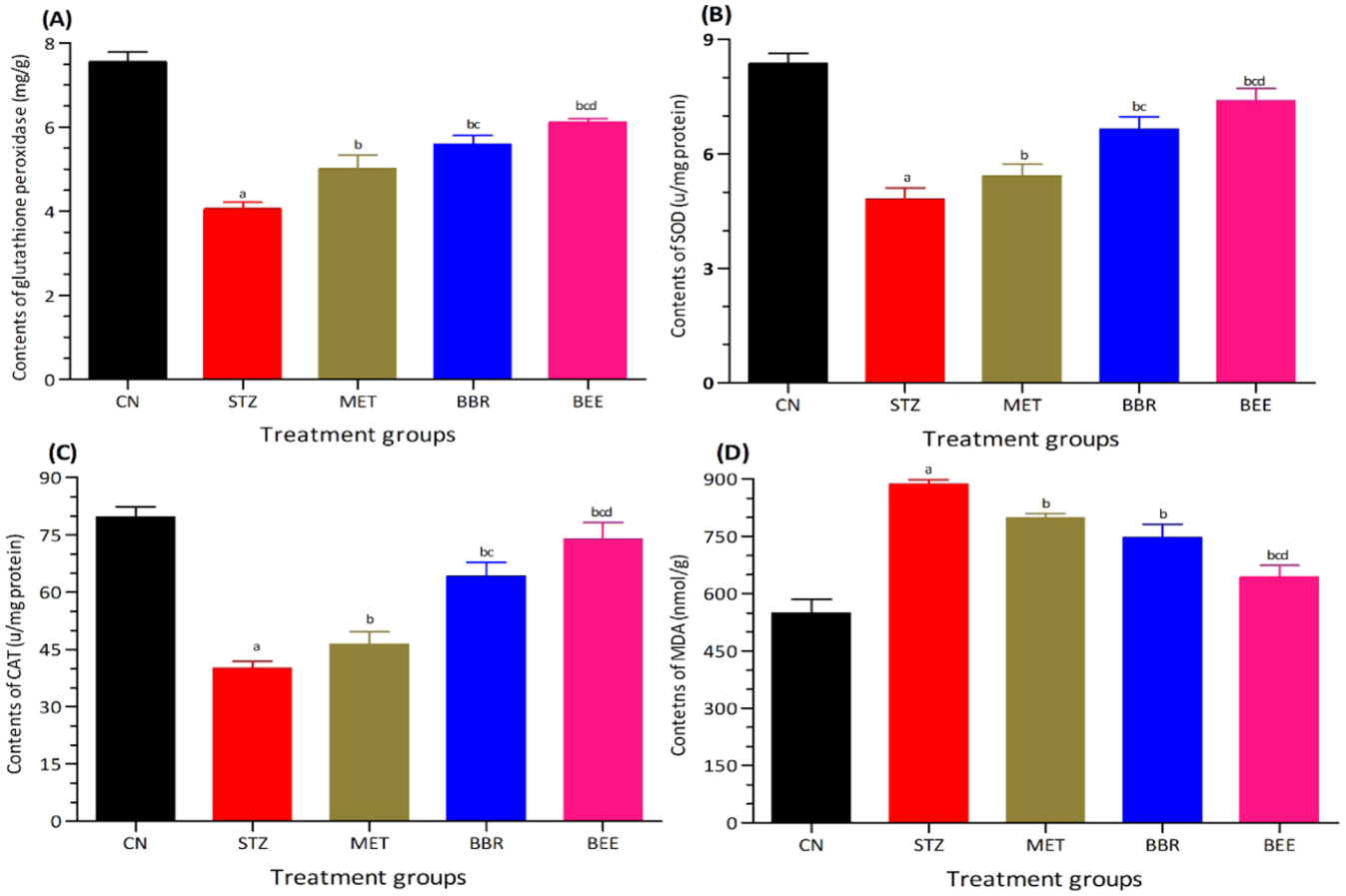


Figure 5. Contents of glutathione (A), SOD (B), CAT (C), and MDA (D) in liver tissue homogenate were analyzed using one-way ANOVA. Post hoc analysis was performed using Bonferroni's multiple comparison test to compare all pairs of columns. [a] When compared with control group. [b] When compared with STZ group. [c] When compared with MET group. [d] When compared with MET and BRE groups. Abbreviations: | SOD: superoxide dismutase, CAT: catalase, MDA: malondialdehyde, CN: control, STZ: Streptozotocin, MET: Metformin, BBR: Berberine, BEE: Berberine-enriched extract.

therapeutic effect, exhibiting superior efficacy in reducing the serum levels of IL-6 and TNF-α compared with BBR. This suggests that BEE may possess enhanced anti-inflammatory properties, positioning it as a promising candidate for the management of inflammation-associated metabolic disorders.

3.4. Biochemical Analysis of Liver Function Biomarkers. In the context of metabolic impairment, the measurement of serum AST and ALT levels is pivotal as they function as indicators of normal liver function. As illustrated in Figure 4A,B, the group treated with STZ displayed significantly heightened levels of serum AST and ALT in comparison to the control group. These observations signify that exposure to STZ resulted in liver dysfunction, as evidenced by the elevated levels of these liver enzymes. However, the administration of MET, BBR, and BEE in STZ-exposed rats led to a noteworthy restoration in the serum levels of AST and ALT, indicating the mitigating effect of these treatments on STZ-induced liver dysfunction. These results demonstrate the potential of MET, BBR, and BEE in restoring liver function and mitigating the adverse effects of STZ on hepatic health. Remarkably, both BBR and BEE showed more profound effects in improving liver function by effectively restoring the normal levels of AST and ALT when compared to MET. Moreover, BEE exhibited even better and more efficient therapeutic effects compared to BBR, indicating its potential as a potent liver protective agent.

3.5. Biochemical Analysis of Kidney Function Biomarkers. The kidneys play a critical role in filtering waste and maintaining the fluid and electrolyte balance in the body. In metabolic impairment, the measurement of serum BUN and creatinine is essential, as they serve as indicators of normal kidney function. As depicted in Figure 4C,D, the STZ-treated group exhibited significantly elevated levels of serum BUN and creatinine compared to the control group. However, the administration of MET, BBR, and BEE in STZ-exposed rats resulted in a remarkable recovery in the serum levels of BUN and creatinine, suggesting the ameliorating effect of these treatments on STZ-induced kidney dysfunction. Remarkably, both BBR and BEE showed more profound effects in improving kidney function by effectively restoring the normal levels of BUN and creatinine when compared with MET. This suggests that BBR and BEE may have superior therapeutic efficacy in combating STZ-induced kidney impairment. Moreover, BEE exhibited even better and more efficient therapeutic effects compared to BBR, indicating its potential as a potent renal protective agent.

3.6. Biochemical Analysis of Endogenous Antioxidant Enzyme Capacity and Lipid Peroxidation. Oxidative stress and lipid peroxidation play crucial roles in the development of metabolic disorders. In this study, we observed that STZ significantly decreased the levels of important antioxidant enzymes, including GSH (Figure 4A), SOD (Figure 5B), and CAT (Figure 5C), while increasing the content of MDA (Figure

https://doi.org/10.1021/acsomega.4c00696
ACS Omega 2024, 9, 15677−15688

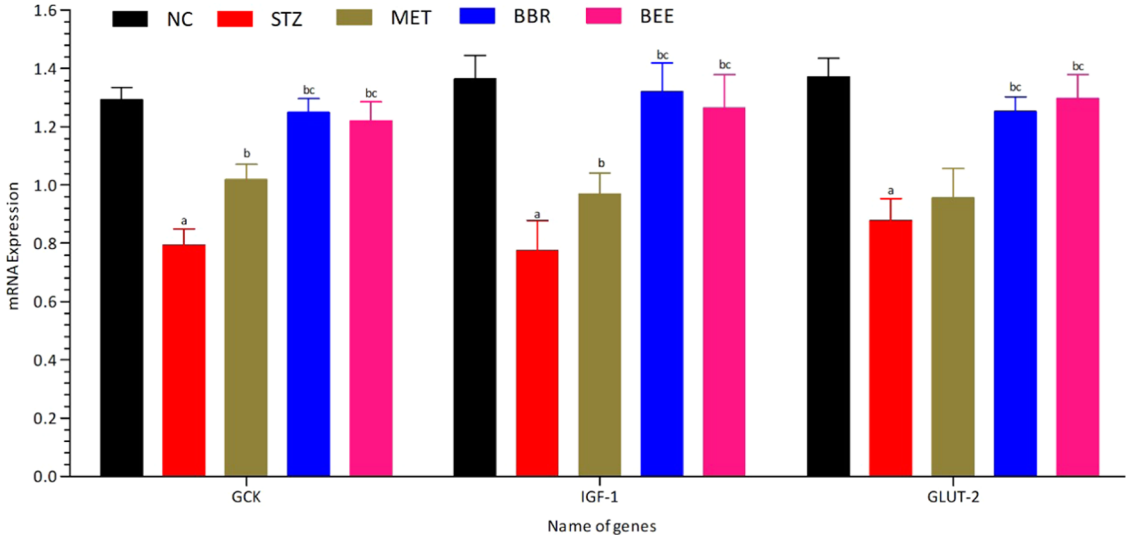


Figure 6. mRNA expression of GCK, IGF-1, and GLUT-2 was analyzed using two-way ANOVA. Post hoc analysis was performed using Bonferroni's multiple comparison test to compare all pairs of columns. [a] When compared with control group. [b] When compared with STZ group. [c] When compared with MET group. [d] When compared with MET and BRE groups. Abbreviations: | GCK: Glucokinase, IGF-1: Insulin-like Growth Factor 1, GLUT-2: Glucose Transporter 2, CN: Control, STZ: Streptozotocin, MET: Metformin, BBR: Berberine, BEE: Berberine-enriched extract.

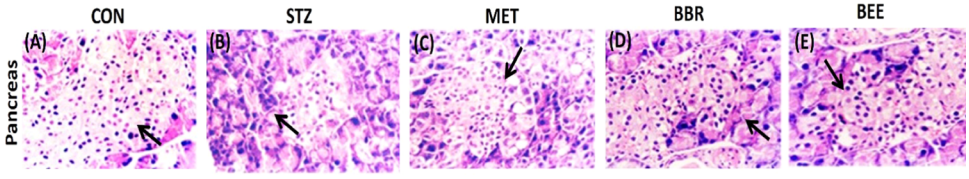


Figure 7. Histopathological examination of pancreas. (A) Normal rat pancreatic tissue. (B) STZ showing a severely injured pancreatic view in which β-cells of pancreatic islets have been shrunk. (C) Effect of MET in which β-cells of pancreatic islets have been recovered to some extent. (D, E) Recovery of β-cells of pancreatic islets after administration with BBR and BEE, respectively.

4D) in liver tissue homogenates compared to the control group. These findings suggest an imbalance between oxidative stress and antioxidant defense mechanisms induced by STZ, which contributes to liver tissue damage. However, treatment with MET significantly elevated the levels of antioxidant enzymes and reduced the levels of MDA when compared to the STZ-treated group. This indicates the potential of MET in mitigating oxidative stress and decreasing lipid peroxidation, leading to improved antioxidant capacity and cellular protection against oxidative damage. Remarkably, both BBR and BEE demonstrated more effective restoration of the normal levels of antioxidant enzymes and MDA as compared to MET. This suggests that BBR and BEE possess stronger antioxidant properties, which could contribute to their therapeutic effects in countering oxidative stress and lipid peroxidation induced by STZ. Moreover, BEE exhibited even better therapeutic effects compared to BBR, indicating its potential as a potent antioxidant agent.

3.7. Biochemical Analysis of mRNA Expression and Quantification of Carbohydrate Metabolizing and Insulin Sensitizing Enzymes. We meticulously examined the mRNA expression levels of pivotal genes, including GCK, IGF-1, and GLUT2, which play essential roles in the intricate regulation of carbohydrate metabolism and insulin stimulation. As illustrated in Figure 6, the group subjected to STZ treatment displayed a significant downregulation ($P < 0.05$) in the mRNA expression levels of GCK, IGF-1, and GLUT2 compared to the control group. This notable decrease in the expression of these critical genes, intricately involved in carbohydrate metabolism and insulin signaling, provides evidence of compromised cellular glucose utilization and a reduction in insulin sensitivity, thereby reflecting the detrimental impact of STZ on carbohydrate metabolism. Remarkably, the simultaneous administration of MET, BBR, and BEE with STZ led to a substantial increase ($P < 0.05$) in the expression of GCK, IGF-1, and GLUT2 compared to the STZ-treated group. This observed upregulation suggests the promising potential of these treatments in restoring the disrupted carbohydrate metabolism induced by STZ exposure. By augmenting the expression of GCK, IGF-1, and GLUT2, MET, BBR, and BEE may contribute significantly to the improvement of glucose metabolism and heightened insulin sensitivity—vital factors in maintaining a state of healthy carbohydrate homeostasis. Noteworthy is the comparative analysis among the treatments, where BEE demonstrated more pronounced effects in increasing the mRNA expressions of GCK, IGF-1, and GLUT2 compared to BBR. This heightened expression induced by BEE suggests its potential to enhance glucose utilization and insulin responsiveness, presenting a potential avenue for mitigating the adverse effects of STZ on carbohydrate metabolism.

3.8. Histopathological Analysis of Pancreas. Histopathology of the pancreas was conducted to assess the effect of BBR and BEE on the pancreatic tissue of rats (Figure 7). In the STZ-induced metabolic impairment model, the destruction of β-cells within the pancreatic islets was evident, as observed by the shrinkage of cells compared to that in the control group. STZ, known for its selective toxicity toward pancreatic β-cells, induced significant damage to these insulin-secreting cells,

https://doi.org/10.1021/acsomega.4c00696
ACS Omega 2024, 9, 15677−15688

contributing to impaired glucose homeostasis. Remarkably, treatment with both BBR and BEE resulted in the recovery of β-cells in the pancreatic islets. This observation suggests that BBR and BEE have beneficial effects on preserving the structural integrity and functionality of β-cells, which play a crucial role in regulating blood glucose levels. The presence of intact and recovered β-cells in the pancreatic islets of the BBR- and BEE-treated groups implies their potential to protect against STZ-induced damage and promote the restoration of insulin-secreting capacity. Notably, BEE demonstrated a more pronounced therapeutic effect in this regard compared with BBR. The presence of well-preserved β-cells in the BEE-treated group suggests its superior ability to mitigate the destructive effects of STZ on pancreatic tissue and β-cell function.

4. DISCUSSION

Metabolic disorders represent a significant public health concern worldwide. The present comprehensive preclinical investigation aimed to assess the therapeutic potential of BEE in STZ-induced metabolic disorder using a rat model and compare its effects with those of BBR and MET. The results of this study revealed the remarkable efficacy of BEE in mitigating metabolic impairment by regulating glucose homeostasis, improving lipid profiles, reducing inflammation, and preserving pancreatic and hepatic health. These findings hold significant implications for the development of natural and effective therapeutic interventions for metabolic disorders.

The assessment of FBG levels at different intervals throughout the experimental period served as a critical indicator of the treatment's effects on glucose metabolism. STZ-induced metabolic disorder resulted in elevated FBG levels, indicative of impaired glucose homeostasis. However, treatment with BEE, BBR, and MET effectively restored FBG levels, demonstrating their antidiabetic properties (Figure 1). Notably, BEE exhibited superior therapeutic effects in comparison to BBR and MET, indicating its potential as a potent intervention for managing hyperglycemia in metabolic disorders. Our findings align with previously published literature.[18] BEE has demonstrated efficacy comparable to metformin in lowering blood glucose and managing type 2 diabetes, and notably, no specific side effects have been reported. Therefore, it could serve as an effective and safe complementary therapy for diabetic patients.[19]

Dyslipidemia is a common co-occurring condition in metabolic disorders, contributing to cardiovascular complications. The assessment of lipid profiles revealed the beneficial effects of BEE on lipid regulation. BEE exhibited superior therapeutic efficacy compared with BBR and MET in regulating the levels of HDL, cholesterol, TGs, and LDL (Figure 2). This indicates its potential in reducing the risk of atherosclerosis and cardiovascular events in metabolic disorder management. The regulation of lipid profiles by BEE may be attributed to its impact on key enzymatic and signaling pathways involved in lipid metabolism. The results of our study showed similar results to previously reported literature.[20] Hyperlipidemia represents a clinical risk condition that heightens the susceptibility to various severe diseases, including atherosclerosis and coronary heart disease, consequently escalating costs for the public health system. In recent years, numerous natural products have been identified for their high effectiveness in alleviating hyperlipidemia. One such natural compound, BBR, exhibits a potent lipid-lowering effect by reducing elevated levels of plasma cholesterol.[21] In the pathogenesis of metabolic disorders, inflammation plays a pivotal role in contributing to insulin resistance and tissue damage.

The downregulation of inflammatory biomarkers such as IL-6 and TNF-α, holds significant therapeutic implications for metabolic disorders. These biomarkers are key contributors to insulin resistance and tissue damage, common features in conditions such as DM and dyslipidemia. By attenuating the levels of IL-6 and TNF-α, our study suggests that BEE exhibits potent anti-inflammatory properties. This aligns with previous findings that highlight the role of inflammation in metabolic dysfunction.[22] The ability of BEE to surpass the effects of BBR and MET in reducing these inflammatory markers suggests an enhanced anti-inflammatory potential. These outcomes are consistent with studies indicating the anti-inflammatory properties of berberine.[23] The collective evidence underscores the therapeutic promise of BEE in alleviating inflammation associated with metabolic disorders, providing a foundation for further investigations into its clinical applications. The results of our study demonstrated that BEE effectively lowered the levels of inflammatory markers, IL-6, and TNF-α, surpassing the effects of BBR and MET. The potent anti-inflammatory properties of BEE indicate its potential in ameliorating insulin resistance and reducing systemic inflammation, which are critical factors in managing metabolic disorders and their complications. Our results are supported by the previously published papers.[16]

Furthermore, BEE exhibited significant hepatoprotective and nephroprotective effects, as indicated by the restoration of liver and kidney function biomarkers. The superior efficacy of BEE in improving AST, ALT, BUN, and creatinine levels suggests its potential for preserving liver and kidney health (Figure 3). These findings are particularly relevant, as metabolic disorders can lead to hepatic and renal dysfunction, adding to the burden of the disease. The previous research also reported that berberine has potential to ameliorate the hepatic toxicity induced by STZ.[24] The researchers also reported that berberine has significant potential to reduce renal toxicity induced STZ.[25]

Oxidative stress occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the body's ability to neutralize them with antioxidants. This imbalance can lead to damage to cellular components, including lipids, proteins, and DNA, contributing to various pathological conditions. The measurement of oxidative stress markers provides insights into the extent of this imbalance and its implications for cellular health. The significant antioxidant properties of BEE, as demonstrated by elevated levels of antioxidant enzymes (GSH, SOD, and CAT) and reduced levels of MDA (Figure 4), further underscore its potential in combating oxidative-stress-induced damage, a common feature in metabolic disorders. The ability of BEE to scavenge free radicals and mitigate oxidative stress contributes to its therapeutic efficacy in preserving cellular health and function. Berberine has potential to increase the content of antioxidant enzymes and ultimately reduce oxidative stress which is major cause of metabolic disorders.[26] The study revealed significant downregulation by berberine in both mRNA and protein expression of inflammatory biomarkers. In the experimental model, treatment with BRE and berberine resulted in a remarkable recovery of antioxidant enzyme levels. Additionally, BEE demonstrated a mitigating effect on the levels of the antioxidant enzymes. These findings collectively suggest the potential use of berberine as an economical phytomedicine for the treatment of inflammatory disorders.[16]

https://doi.org/10.1021/acsomega.4c00696
ACS Omega 2024, 9, 15677−15688

In addition to improving FBG levels, BEE exhibited favorable effects on insulin sensitivity. The enriched extract demonstrated the capacity to upregulate key genes involved in carbohydrate metabolism, namely, GCK, IGF-1, and GLUT2 (Figure 5). The restoration of insulin sensitivity is vital in managing metabolic disorders, as it facilitates efficient glucose uptake and utilization in peripheral tissues. The pronounced effects of BEE on gene expression indicate its ability to enhance insulin signaling pathways and improve insulin sensitivity, making it a promising agent for addressing insulin resistance. The study of our result was affirmed by the previously published papers.[27] The upregulation of genes, particularly GCK, IGF-1, and GLUT2, is pivotal in understanding the molecular mechanisms underlying the therapeutic effects of BEE in our study. The increased expression of these genes suggests a positive impact on carbohydrate metabolism and insulin sensitivity. Significantly, the dose-dependent effects of BEE on gene upregulation provide valuable insights into its potential for tailored therapeutic interventions. Our study acknowledges the importance of exploring optimal dosages to maximize beneficial outcomes, aligning with previous research highlighting the dose-dependent nature of berberine's effects.[28] This nuanced approach adds depth to the discussion, emphasizing the need for further investigation to optimize the BEE dosage for enhanced therapeutic efficacy.

The histopathological assessment of the pancreas (Figure 6) further corroborated the therapeutic effects of BEE. In our study, the observed recovery of pancreatic β-cells in the BEE-treated group, as revealed by H&E staining, holds paramount significance for metabolic health. The preservation and recovery of β-cells in the pancreatic islets indicate the potential of BEE to protect against streptozotocin-induced damage and maintain insulin-secreting capacity. Since pancreatic β-cells play a crucial role in regulating blood glucose levels, the observed structural improvements further support the therapeutic efficacy of BEE in ameliorating metabolic impairment and sustaining pancreatic health. This finding aligns with the improvement in glucose metabolism observed with BEE treatment and supports its role in preserving pancreatic health. Berberine plays a significant role in the amelioration of pancreatic tissues in STZ-treated rats for the treatment of diabetes.[29] Berberine demonstrates robust anti-inflammatory, antioxidant, antidiabetic, and anticancer properties across various tissues and organs. Treatment with berberine effectively alleviated histopathological indications of inflammation and necrosis in the pancreas. Furthermore, the administration of BEE resulted in a reduction in oxidative stress, as evidenced by decreased levels of pancreatic MDA. When administered intraperitoneally, BEE demonstrated the capability to prevent and reverse pancreatic tissue damage in animal models. These effects correlated with a decrease in inflammatory markers and a reduction in pro-inflammatory signaling. Given that insulin resistance and pancreatitis pose a risk for malignant transformation leading to metabolic disorders, the ability of berberine to prevent and reverse insulin sensitivity in experimental animals with metabolic impairment is particularly noteworthy.[30] Overall, the comprehensive preclinical investigation highlights the multifaceted therapeutic potential of BEE for managing metabolic disorders. BEE exhibited superior effects compared to BBR and MET in regulating glucose homeostasis, improving lipid profiles, reducing inflammation, and preserving pancreatic and hepatic health. The collective results suggest that BEE holds promise as a potent and natural alternative for managing metabolic disorders, offering potential benefits beyond traditional treatments. Future research should focus on elucidating the underlying mechanisms through which BEE exerts its therapeutic effects as well as its long-term safety and efficacy. Clinical trials in human subjects are warranted to validate their efficacy and safety for clinical use. Furthermore, exploring combination therapies and optimizing BEE formulations to enhance their bioavailability and therapeutic potential could open new avenues for metabolic disorder management. The identification of specific biomarkers associated with BEE's therapeutic effects may aid in patient stratification and personalized treatment approaches.

5. CONCLUSIONS

In this comprehensive preclinical investigation, we explored the therapeutic potential of BEE in STZ-induced metabolic disorder using a rat model and compared its effects with BBR and MET. The results demonstrated that BEE exhibited remarkable therapeutic efficacy, surpassing the effects of BBR and MET, in ameliorating metabolic impairment, improving glucose homeostasis, regulating lipid profiles, reducing inflammation, and preserving pancreatic and hepatic health. The significant upregulation of key genes involved in carbohydrate metabolism, including GCK, IGF-1, and GLUT2, by BEE highlighted its ability to enhance glucose utilization and insulin sensitivity. This was supported by reduced oxidative stress, lipid peroxidation, and improved antioxidant enzyme activity in liver tissue homogenates, further affirming the extract's potent antioxidant properties. Histopathological examination of the pancreas revealed that BEE effectively preserved and recovered pancreatic β-cells, indicating its potential in mitigating STZ-induced damage and promoting insulin-secreting capacity. The beneficial effects of BEE extended to liver and kidney functions, as evidenced by the restoration of normal levels of AST, ALT, BUN, and creatinine, indicating its potential in maintaining organ health. In conclusion, this study establishes BEE as a promising therapeutic candidate for managing metabolic disorders. Overall, BEE exhibited superior therapeutic efficacy compared with BBR and MET in addressing STZ-induced metabolic disorders in rats. BEE effectively improved glucose homeostasis, regulated lipid profiles, reduced inflammation, preserved pancreatic and hepatic health, and upregulated key genes involved in carbohydrate metabolism. However, further research is essential to unravel its mechanisms, determine long-term safety, and pave the way for clinical translation, ultimately offering new hope for individuals affected by metabolic disorders.

■ ASSOCIATED CONTENT

Data Availability Statement

All data is available within the manuscript.

■ AUTHOR INFORMATION

Corresponding Authors

Muhammad Sajid Hamid Akash − *Department of Pharmaceutical Chemistry, Government College University, Faisalabad 38000 Punjab, Pakistan;* orcid.org/0000-0002-9446-5233; Email: sajidakash@gcuf.edu.pk

Kanwal Rehman − *Department of Pharmacy, The Women University, Multan 6000, Pakistan;* orcid.org/0000-0001-7873-6681; Email: kanwalrehman@wum.edu.pk

https://doi.org/10.1021/acsomega.4c00696
ACS Omega 2024, 9, 15677−15688

Authors

Sahrish Yaqoob − *Department of Pharmaceutical Chemistry, Government College University, Faisalabad 38000 Punjab, Pakistan*

Amjad Hussain − *Institute of Chemistry, University of Okara, Okara 56300 Punjab, Pakistan;* orcid.org/0000-0003-1697-7580

Zunera Chauhdary − *Department of Pharmaceutical Chemistry, Government College University, Faisalabad 38000 Punjab, Pakistan;* orcid.org/0000-0003-4191-8697

Ahmed Nadeem − *Department of Pharmacology and Toxicology, College of Pharmacy, King Saud University, Riyadh 11451, Saudi Arabia*

Asif Shahzad − *Department of Biochemistry and Molecular Biology, Kunming Medical University, Yunnan 650031, China*

Muhammad Ajmal Shah − *Department of Pharmacy, Hazara University, Mansehra 21300, Pakistan;* orcid.org/0000-0003-3471-184X

Pharkphoom Panichayupakaranant − *Department of Pharmacognosy and Pharmaceutical Botany, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai 90112, Thailand*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.4c00696

Author Contributions

Conceptualization, M.S.H.A. and K.R.; methodology, M.S.H.A. and S.Y.; validation, Z.C. and K.R.; formal analysis, S.Y., M.S.H.A., and Z.C.; investigation, K.R. and M.S.H.A.; resources, M.S.H.A.; data curation, S.Y.; writing—original draft preparation, M.S.H.A. and A.H.; writing—review and editing, K.R., A.H., and A.S.; visualization, Z.C.; supervision, K.R.; project administration, M.S.H.A.; funding acquisition, A.N. All authors have read and agreed to the published version of the manuscript.

Notes

The authors declare no competing financial interest.

Institutional Review Board Statement This study was approved by the Ethical Review Committee, Government College University, Faisalabad, Pakistan (ref no. GCUF/ERC/2195).

■ ACKNOWLEDGMENTS

The authors acknowledge and extend their appreciation to the Researchers Supporting Project Number (RSP2024R124), King Saud University, Riyadh, Saudi Arabia.

■ REFERENCES

(1) (a) Akbari, M.; Rasouli, H.; Bahdor, T. Physiological and pharmaceutical effect of fenugreek: a review. *IOSR J. Pharm.* **2012**, *2* (4), 49−53. (b) Loolaie, M.; Moasefi, N.; Rasouli, H.; Adibi, H. Peppermint and its functionality: A review. *Arch. Clin. Microbiol.* **2017**, *8* (4), No. 54, DOI: 10.4172/1989-8436.100053. (c) Rasouli, H.; Farzaei, M. H.; Mansouri, K.; Mohammadzadeh, S.; Khodarahmi, R. Plant cell cancer: may natural phenolic compounds prevent onset and development of plant cell malignancy? A literature review. *Molecules* **2016**, *21* (9), No. 1104, DOI: 10.3390/molecules21091104. (d) Rasouli, H.; Farzaei, M. H.; Khodarahmi, R. Polyphenols and their benefits: A review. *Int. J. Food Prop.* **2017**, *20* (Suppl. 2), 1700−1741. (e) Yarani, R.; Mansouri, K.; Mohammadi-Motlagh, H. R.; Mahnam, A.; Emami Aleagha, M. S. In vitro inhibition of angiogenesis by hydroalcoholic extract of oak (Quercus infectoria) acorn shell via suppressing VEGF, MMP-2, and MMP-9 secretion. *Pharm. Biol.* **2013**, *51* (3), 361−368.

(2) Cheng, Z.; Pang, T.; Gu, M.; Gao, A. H.; Xie, C. M.; Li, J. Y.; Nan, F. J.; Li, J. Berberine-stimulated glucose uptake in L6 myotubes involves both AMPK and p38 MAPK. *Biochim. Biophys. Acta* **2006**, *1760* (11), 1682−1689.

(3) Chang, W.; Chen, L.; Hatch, G. M. Berberine as a therapy for type 2 diabetes and its complications: From mechanism of action to clinical studies. *Biochem. Cell Biol.* **2015**, *93* (5), 479−486.

(4) Kumar, S.; Narwal, S.; Kumar, V.; Prakash, O. α-glucosidase inhibitors from plants: A natural approach to treat diabetes. *Pharmacogn. Rev.* **2011**, *5* (9), 19−29.

(5) Neag, M. A.; Mocan, A.; Echeverría, J.; Pop, R. M.; Bocsan, C. I.; Crişan, G.; Buzoianu, A. D. Berberine: Botanical occurrence, traditional uses, extraction methods, and relevance in cardiovascular, metabolic, hepatic, and renal disorders. *Front. Pharmacol.* **2018**, *9*, No. 557, DOI: 10.3389/fphar.2018.00557.

(6) Ai, X.; Yu, P.; Peng, L.; Luo, L.; Liu, J.; Li, S.; Lai, X.; Luan, F.; Meng, X. Berberine: A Review of its Pharmacokinetics Properties and Therapeutic Potentials in Diverse Vascular Diseases. *Front. Pharmacol.* **2021**, *12*, No. 762654.

(7) (a) Pang, B.; Zhao, L.-H.; Zhou, Q.; Zhao, T.-Y.; Wang, H.; Gu, C.-J.; Tong, X.-L. Application of berberine on treating type 2 diabetes mellitus. *Int. J. Endocrinol.* **2015**, *2015*, No. 905749. (b) Collaboration, E. R. F. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet* **2010**, *375* (9733), 2215−2222.

(8) (a) Zhou, J.; Zhou, S.; Tang, J.; Zhang, K.; Guang, L.; Huang, Y.; Xu, Y.; Ying, Y.; Zhang, L.; Li, D. Protective effect of berberine on beta cells in streptozotocin-and high-carbohydrate/high-fat diet-induced diabetic rats. *Eur. J. Pharmacol.* **2009**, *606* (1−3), 262−268. (b) Askari, V. R.; Khosravi, K.; Baradaran Rahimi, V.; Garzoli, S. A Mechanistic Review on How Berberine Use Combats Diabetes and Related Complications: Molecular, Cellular, and Metabolic Effects. *Pharmaceuticals* **2024**, *17* (1), No. 7, DOI: 10.3390/ph17010007.

(9) (a) Lv, X.; Zhao, Y.; Yang, X.; Han, H.; Ge, Y.; Zhang, M.; Zhang, H.; Zhang, M.; Chen, L. Berberine Potentiates Insulin Secretion and Prevents β-cell Dysfunction Through the miR-204/SIRT1 Signaling Pathway. *Front. Pharmacol.* **2021**, *12*, No. 720866. (b) Wu, W.; Xia, Q.; Guo, Y.; Wang, H.; Dong, H.; Lu, F.; Yuan, F. Berberine enhances the function of db/db mice islet β cell through GLP-1/GLP-1R/PKA signaling pathway in intestinal L cell and islet α cell. *Front. Pharmacol.* **2023**, *14*, No. 1228722.

(10) Och, A.; Och, M.; Nowak, R.; Podgórska, D.; Podgórski, R. Berberine, a Herbal Metabolite in the Metabolic Syndrome: The Risk Factors, Course, and Consequences of the Disease. *Molecules* **2022**, *27* (4), No. 1351, DOI: 10.3390/molecules27041351.

(11) (a) Gulfraz, M.; Mehmood, S.; Ahmad, A.; Fatima, N.; Praveen, Z.; Williamson, E. Comparison of the antidiabetic activity of Berberis lyceum root extract and berberine in alloxan-induced diabetic rats. *Phytother. Res.* **2008**, *22* (9), 1208−1212. (b) Akash, M. S. H.; Akbar, M.; Rehman, K.; Shah, M. A.; Panichayupakaranant, P.; Imran, M.; Assiri, M. A. Biochemical profiling of berberine-enriched extract in aluminum chloride induced oxidative damage and neuroinflammation. *Environ. Sci. Pollut. Res. Int.* **2023**, *30*, 85263−85275, DOI: 10.1007/s11356-023-28392-1.

(12) (a) Nadalin, P.; Kim, Y. G.; Park, S. U. Recent studies on berberine and its biological and pharmacological activities. *EXCLI J.* **2023**, *22*, 315−328. (b) Tian, E.; Sharma, G.; Dai, C. Neuroprotective Properties of Berberine: Molecular Mechanisms and Clinical Implications. *Antioxidants* **2023**, *12* (10), No. 1883, DOI: 10.3390/antiox12101883.

(13) Cai, Y.; Yang, Q.; Yu, Y.; Yang, F.; Bai, R.; Fan, X. Efficacy and underlying mechanisms of berberine against lipid metabolic diseases: a review. *Front. Pharmacol.* **2023**, *14*, No. 1283784.

(14) (a) Chemat, F.; Vian, M. A.; Cravotto, G. Green extraction of natural products: concept and principles. *Int. J. Mol. Sci.* **2012**, *13* (7), 8615−8627. (b) Usman, M.; Nakagawa, M.; Cheng, S. Emerging Trends in Green Extraction Techniques for Bioactive Natural Products. *Processes* **2023**, *11* (12), No. 3444, DOI: 10.3390/pr11123444.

(15) (a) Pojić, M.; Mišan, A.; Tiwari, B. Eco-innovative technologies for extraction of proteins for human consumption from renewable protein sources of plant origin. *Trends Food Sci. Technol.* **2018**, *75*, 93−104. (b) Chemat, F.; Abert-Vian, M.; Fabiano-Tixier, A. S.; Strube, J.; Uhlenbrock, L.; Gunjevic, V.; Cravotto, G. Green extraction of natural products. Origins, current status, and future challenges. *TrAC, Trends Anal. Chem.* **2019**, *118*, 248−263.
(16) Naz, I.; Masoud, M. S.; Chauhdary, Z.; Shah, M. A.; Panichayupakaranant, P. Anti-inflammatory potential of berberine-rich extract via modulation of inflammation biomarkers. *J. Food Biochem.* **2022**, *46* (12), No. e14389.
(17) Salah-Eldin, A.-E.; Ibrahim, M. A.; Soliman, M. M. RT-PCR analysis of genes expression to evaluate the biomedical importance of medical-herbal extracts in diabetes treatment. *Eur. Sci. J.* **2015**, *11* (30), 189−210.
(18) Yin, J.; Xing, H.; Ye, J. Efficacy of berberine in patients with type 2 diabetes mellitus. *Metab., Clin. Exp.* **2008**, *57* (5), 712−717.
(19) Sanjari, M.; Shamsinejad, B.; Khazaeli, P.; Safi, Z.; Mirrashidi, F.; Naghibzadeh-Tahami, A. Safety and efficacy of Berberis integerrima root extract in patients with type 2 diabetes. A parallel intervention based triple blind clinical trial. *J. Diabetes Metab. Disord.* **2020**, *19*, 71−80.
(20) Derosa, G.; D'Angelo, A.; Bonaventura, A.; Bianchi, L.; Romano, D.; Maffioli, P. Effects of berberine on lipid profile in subjects with low cardiovascular risk. *Expert Opin. Biol. Ther.* **2013**, *13* (4), 475−482.
(21) Yang, Y.-N.; Wang, Q.-C.; Xu, W.; Yu, J.; Zhang, H.; Wu, C. The berberine-enriched gut commensal Blautia producta ameliorates high-fat diet (HFD)-induced hyperlipidemia and stimulates liver LDLR expression. *Biomed. Pharmacother.* **2022**, *155*, No. 113749.
(22) (a) Akash, M. S. H.; Rehman, K.; Chen, S. Role of inflammatory mechanisms in pathogenesis of type 2 diabetes mellitus. *J. Cell. Biochem.* **2013**, *114* (3), 525−531. (b) Rehman, K.; Akash, M. S. Mechanisms of inflammatory responses and development of insulin resistance: how are they interlinked? *J. Biomed. Sci.* **2016**, *23* (1), No. 87, DOI: 10.1186/s12929-016-0303-y.
(23) (a) Li, Z.; Geng, Y. N.; Jiang, J. D.; Kong, W. J. Antioxidant and anti-inflammatory activities of berberine in the treatment of diabetes mellitus. *J. Evidence-Based Complementary Altern. Med.* **2014**, *2014*, No. 289264. (b) Zhang, Y.; Li, X.; Zou, D.; Liu, W.; Yang, J.; Zhu, N.; Huo, L.; Wang, M.; Hong, J.; Wu, P.; Ren, G.; Ning, G. Treatment of type 2 diabetes and dyslipidemia with the natural plant alkaloid berberine. *J. Clin. Endocrinol. Metab.* **2008**, *93* (7), 2559−2565.
(24) Chandirasegaran, G.; Elanchezhiyan, C.; Ghosh, K. Effects of Berberine chloride on the liver of streptozotocin-induced diabetes in albino Wistar rats. *Biomed. Pharmacother.* **2018**, *99*, 227−236.
(25) Zhang, X.; He, H.; Liang, D.; Jiang, Y.; Liang, W.; Chi, Z. H.; Ma, J. Protective Effects of Berberine on Renal Injury in Streptozotocin (STZ)-Induced Diabetic Mice. *Int. J. Mol. Sci.* **2016**, *17* (8), No. 1327, DOI: 10.3390/ijms17081327.
(26) Albasher, G.; Alkahtani, S.; Alarifi, S. Berberine mitigates oxidative damage associated with testicular impairment following mercury chloride intoxication. *J. Food Biochem.* **2020**, *44* (9), No. e13385.
(27) Zhang, M.; Yang, H.; Yang, E.; Li, J.; Dong, L. Berberine Decreases Intestinal GLUT2 Translocation and Reduces Intestinal Glucose Absorption in Mice. *Int. J. Mol. Sci.* **2022**, *23* (1), No. 327, DOI: 10.3390/ijms23010327.
(28) Habtemariam, S. The therapeutic potential of Berberis darwinii stem-bark: quantification of berberine and in vitro evidence for Alzheimer's disease therapy. *Nat. Prod. Commun.* **2011**, *6* (8), 1089−1090.
(29) Zhou, J.; Zhou, S.; Tang, J.; Zhang, K.; Guang, L.; Huang, Y.; Xu, Y.; Ying, Y.; Zhang, L.; Li, D. Protective effect of berberine on beta cells in streptozotocin- and high-carbohydrate/high-fat diet-induced diabetic rats. *Eur. J. Pharmacol.* **2009**, *606*, 262−268.
(30) Vlavcheski, F.; O'Neill, E. J.; Gagacev, F.; Tsiani, E. Effects of berberine against pancreatitis and pancreatic cancer. *Molecules* **2022**, *27* (23), No. 8630, DOI: 10.3390/molecules27238630.

https://doi.org/10.1021/acsomega.4c00696
ACS Omega 2024, 9, 15677−15688