



Original Article

Asian Pacific Journal of Tropical Biomedicine



apjtb.org

doi: 10.4103/apjtb.apjtb_383_24

Asiaticoside ameliorates type 2 diabetes mellitus in rats by modulating carbohydrate metabolism and regulating insulin signaling

B. Prathap¹✉, V. Satyanarayanan², K. Duraipandian³, P. Subashree⁴¹Department of Pharmacology, Karpaga Vinayaga Institute of Medical Sciences and Research Center, Maduranthakam 603308, Tamil Nadu, India²Department of Pharmacology, SRM Medical College Hospital and Research Center, Kattankulathur 603211, Tamil Nadu, India³Department of Anatomy, Karpaga Vinayaga Institute of Medical Sciences and Research Center, Maduranthakam 603308, Tamil Nadu, India⁴Department of Ophthalmology, Karpaga Vinayaga Institute of Medical Sciences and Research Center, Maduranthakam 603308, Tamil Nadu, India

ABSTRACT

Objective: To evaluate the effect of asiaticoside on streptozotocin (STZ) and nicotinamide (NAD)-induced carbohydrate metabolism abnormalities and deregulated insulin signaling pathways in rats.

Methods: Asiaticoside (50 and 100 mg/kg body weight) was administered to STZ-NAD-induced diabetic rats for 45 days, and its effects on hyperglycaemic, carbohydrate metabolic, and insulin signaling pathway markers were examined.

Results: Asiaticoside increased insulin production, lowered blood glucose levels, and enhanced glycolysis by improving hexokinase activity and suppressing glucose-6-phosphatase and fructose-1,6-bisphosphatase activities. Abnormalities in glycogen metabolism were mitigated by increasing glycogen synthase activity and gluconeogenesis was decreased by decreasing glycogen phosphorylase activity. Furthermore, asiaticoside upregulated the mRNA expressions of *IRS-1*, *IRS-2*, and *GLUT4* in STZ-NAD-induced diabetic rats and restored the beta cell morphology to normal.

Conclusions: Asiaticoside has the potential to ameliorate type 2 diabetes by improving glycolysis, gluconeogenesis, and insulin signaling pathways.

KEYWORDS: Asiaticoside; Type 2 diabetes mellitus; Metabolic disorders; Carbohydrate metabolism; Insulin signaling

1. Introduction

Diabetes mellitus (DM) is a complex metabolic disorder with various etiologies affecting the metabolism of carbohydrates, fats, and proteins. It is characterized by chronic hyperglycemia

and disruptions in insulin secretion, insulin action, or both[1–4]. Abnormalities in insulin action lead to disturbances in carbohydrate metabolism due to altered enzyme activities governing glycolysis and gluconeogenesis in the liver and muscles, resulting in hyperglycemia[5]. The current oral hypoglycemic medications have their limitations, necessitating the exploration of novel therapeutic options. The maintenance of glucose homeostasis relies on the coordinated regulation of enzymes engaged in glucose utilization and production[6]. Insulin plays a pivotal role in modulating glucose utilization intracellularly by activating glycolytic enzymes and inhibiting gluconeogenic enzyme activity to regulate postprandial blood glucose levels effectively[7]. Insulin deficiency and insulin resistance (IR) in DM disrupt carbohydrate metabolism, leading to

Significance

Asiaticoside, a pentacyclic triterpenoid saponin from *Centella asiatica*, exhibits diverse pharmacological properties. However, there is still no scientific data that proves its antidiabetic potential. This study found that asiaticoside could alleviate hyperglycemia in rats by regulating glucose metabolism enzymes, promoting glycolysis, reducing gluconeogenesis, and modulating insulin signaling pathways.

✉To whom correspondence may be addressed. E-mail: prathap.thap@gmail.com

This is an open access journal, and articles are distributed under the terms of the Creative Commons Attribution-Non Commercial-ShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

For reprints contact: reprints@medknow.com

©2024 Asian Pacific Journal of Tropical Biomedicine Produced by Wolters Kluwer-Medknow.

How to cite this article: Prathap B, Satyanarayanan V, Duraipandian K, Subashree P. Asiaticoside ameliorates type 2 diabetes mellitus in rats by modulating carbohydrate metabolism and regulating insulin signaling. Asian Pac J Trop Biomed 2024; 14(9): 401-409.

Article history: Received 3 July 2024; Revision 14 August 2024; Accepted 18 September 2024; Available online 27 September 2024

impaired peripheral glucose utilization and increased hepatic glucose production[5].

In humans and other mammals, more or less 75% of insulin-stimulated glucose transport takes place in skeletal muscle, underscoring its importance in systemic glucose homeostasis[8]. The impaired responsiveness of skeletal muscles to insulin significantly disrupts overall glucose regulation. Increased expression and activity of essential elements in the insulin signal transduction pathway are closely related to improved insulin action in skeletal muscle. According to Swapna *et al.*[2], errors in this cascade are essential for the development of IR and impaired glucose utilization. Insulin-sensitive tissues such as skeletal muscle, liver, and adipose tissue are predominantly affected. Chronic elevation of blood glucose levels is a significant contributor to dysfunction in various tissues and organs, along with the onset of diabetic complications[9,10]. Studies have demonstrated that in animal models of type 2 DM (T2DM), treatment with plant-based compounds decreased blood glucose levels, improved glycemic control, reduced IR, and enhanced glucose uptake by skeletal muscle[2,5].

The global utilization of medicinal plants and their bioactive components for managing DM and its associated complications is mainly due to their safety, accessibility, and cost-effectiveness. The World Health Organization (WHO) has endorsed the use of traditional plants for treating chronic and life-threatening conditions, including DM[6,11–14]. Asiaticoside, a pentacyclic triterpenoid saponin derived from *Centella asiatica*, exhibits diverse pharmacological properties such as anticancer, neuroprotective, hepatoprotective, anti-inflammatory, and antidepressant activities[15]. Despite this, there is a scarcity of literature investigating the potential antidiabetic effects of asiaticoside and its impact on glycolysis, gluconeogenesis, and the insulin signaling pathway. The present investigation was conducted to assess the effects of asiaticoside on the functions of carbohydrate metabolic enzymes and insulin signaling pathway markers in streptozotocin (STZ) and nicotinamide (NAD)-induced diabetic rats.

2. Materials and methods

2.1. Chemicals

Asiaticoside, identified by CAS No: 16830-15-2, was procured from Sigma-Aldrich, India, while all other chemicals and solvents used were of analytical grade and obtained from Himedia, Bangalore, India.

2.2. Animals

For this research, male Wistar albino rats weighing 180–200 g

were employed and acquired from Mass Biotech, Chengelpet, Tamil Nadu, India. These animals underwent one week of acclimatization under standard laboratory conditions [temperature: $(22 \pm 2)^\circ\text{C}$; humidity: 40%–60% and a 12 h light/dark cycle]. Animals were fed with commercial pellet diet and given water *ad libitum*.

2.3. Experimental design

DM was induced in rats by a single dose of STZ (45 mg/kg body weight) and NAD (110 mg/kg body weight) intraperitoneally. After one week, blood glucose levels were monitored, and rats with values above 250 mg/dL were considered diabetic and included in the study. The diabetic rats were then randomly divided into different groups of 10 animals each. The study included five groups: (i) normal control rats, (ii) STZ-NAD-induced diabetic control, (iii) diabetic rats treated with asiaticoside (50 mg/kg body weight)[16], (iv) diabetic rats treated with asiaticoside (100 mg/kg body weight)[17] and (v) diabetic rats treated with gliclazide (5 mg/kg body weight)[18]. Groups 1 and 2 were administered placebo treatment with normal saline (2 mL/kg body weight), while groups 3 to 5 received the respective drugs orally once daily for 45 d. At the end of the study, blood samples were collected using retro-orbital sinus puncture for plasma and serum separation with or without anticoagulant (EDTA).

2.4. Estimation of body weight, blood glucose, insulin, and IR

Upon completion of the experimental procedures, the rats' body weights were recorded. The effect of asiaticoside on hyperglycemic indicators was assessed using specific kits for insulin (Bio-Merieux, RCS, Lyon, France), plasma glucose (Stan Bio Laboratory Kits, USA), hemoglobin, and glycated hemoglobin (Hitachi 912, Boehringer Mannheim, Germany). IR was determined through the homeostasis model assessment (HOMA) formula, calculated as $\text{HOMA} = \text{fasting serum insulin (mU/L)} \times \text{fasting plasma glucose (mM)} / 22.5$.

2.5. Oral glucose tolerance test (OGTT)

OGTT was conducted post-experimentation following an overnight fast, with glucose administered orogastrically at 2 g/kg body weight. Blood samples were collected from the supraorbital sinus at intervals of 0, 30, 60, 90, and 120 min for glucose level analysis.

2.6. Assessment of blood urea, creatinine, and uric acid

Blood urea, creatinine, and uric acid levels were determined utilizing commercially available diagnostic kits from Sigma Aldrich, USA.

2.7. Estimation of renal damage biomarkers

A Roche automated biochemical analyzer was used to evaluate *N*-acetyl- β -*D*-glucosaminidase (NAG) in urine. The urinary albumin to creatinine ratio (UACR), calculated as the ratio of urinary albumin (mg) to urine creatinine (g), was also determined. Concentrations of urinary albumin, neutrophil gelatinase-associated lipocalin (NGAL), and transforming growth factor (TGF)- β 1 were determined using the corresponding ELISA kits.

2.8. Determination of markers for liver carbohydrate metabolism

Liver samples from experimental rats were excised, rinsed in ice-cold saline, and stored at -20°C until needed. The samples were weighed and a 10% tissue homogenate was prepared using 0.025 M Tris-HCl buffer, pH 7.5. After centrifugation at 2000 rpm for 10 min, the clear supernatant was used for the analysis of hexokinase, glucose-6-phosphatase, fructose-1,6-bisphosphatase, glycogen content, glycogen synthase, and glycogen phosphorylase using appropriate methods[5].

2.9. Histopathological analysis of the pancreas

At the end of the experimental period, animals were anaesthetized by administering pentobarbital sodium (40 mg/kg/bodyweight) intraperitoneally and sacrificed by cervical decapitation. and the pancreas was removed, immediately washed with saline, and then fixed in 10% buffered formalin. The pancreatic tissues fixed in 10% buffered formalin were embedded in paraffin. Sections were cut at 5 μm and stained with hematoxylin and eosin (H&E). These sections were then examined under a light microscope (ZEISS Axiolab 5, Munich, Germany) for histoarchitectural changes.

2.10. RT-PCR analysis

Skeletal muscle tissue was collected from rats and frozen tissue was thawed and homogenized. Total RNA was isolated using Trizol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions and reverse transcribed to cDNA using a DNA synthesis kit (Applied Biosystems, Foster City, USA). Then 20 ng of cDNA was used for semiquantitative PCR. PCR amplification was performed for 38 cycles under the following cycling conditions: 30 s of denaturation at 94°C , 30 s of annealing at 59°C , and 1 min of extension at 72°C with the indicated primers (Table 1).

Table 1. Primer sequences used for RT-PCR analysis.

Primer	Sequence
<i>GLUT4</i>	F: 5'-GCAAGG ACAGTGGACGCTCTCTTT-3' R: 5'-CTGGGTAGGCAG GGTCTG GGG G-3'
<i>IRS-1</i>	F: 5'- GATTAAAGCACCTATGCCAG-3' R: 5'-GAATCG TGAAAGAGTTTCGAG-3'
<i>IRS-2</i>	F: 5'-GCGAGCTCTATGGGTATATG-3' R: 5'-AGTCCTCTTCCTCAGTCTC-3'
β -actin	F: 5'-CCTGCTTGCTGATCCACA-3' R: 5'-CTGACCGAGCGTGGCTAC-3'

F: forward; R: reverse.

2.11. Statistical analysis

Statistical analysis was performed using SPSS software (version 20.0). Data are presented as mean $\pm$ SD. One-way analysis of variance (ANOVA) and least significant difference (LSD) test were used to determine the significance of variances between groups. A *P* value less than 0.05 was considered statistically significant.

2.12. Ethical statement

All procedures were conducted following the guidelines of the Institutional Animal Ethics Committee (IAEC) at Karpaga Vinayaga Institute of Medical Science and Research Center, Maduranthakam, Tamil Nadu, India (IAEC Approval No: 027/IAEC/KIMS/2023).

3. Results

3.1. Asiaticoside attenuates STZ-NAD-induced hyperglycemia

Figure 1 illustrates the alterations in body weight in diabetic rats. Administration of STZ-NAD resulted in a noteworthy ($P<0.05$) reduction in body weight compared to the normal control group; however, supplementation of asiaticoside notably increased body weight ($P<0.05$) (Figure 1A). Moreover, there was a significant ($P<0.05$) elevation in plasma glucose (Figure 1B), IR (Figure 1C), and glycated hemoglobin (Figure 1D), and a decline in insulin (Figure 1E) and hemoglobin levels (Figure 1F). Oral administration of asiaticoside alleviated STZ-NAD-induced changes in blood glucose, IR, glycated hemoglobin, and plasma insulin levels ($P<0.05$).

3.2. Effect of asiaticoside on OGTT

Figure 2 depicts the impact of asiaticoside on OGTT in diabetic rats. In the normal control group, there was a significant elevation in blood glucose levels peaking at 60 min post-glucose administration, and then gradually decreasing to basal levels at 120 min. Conversely,

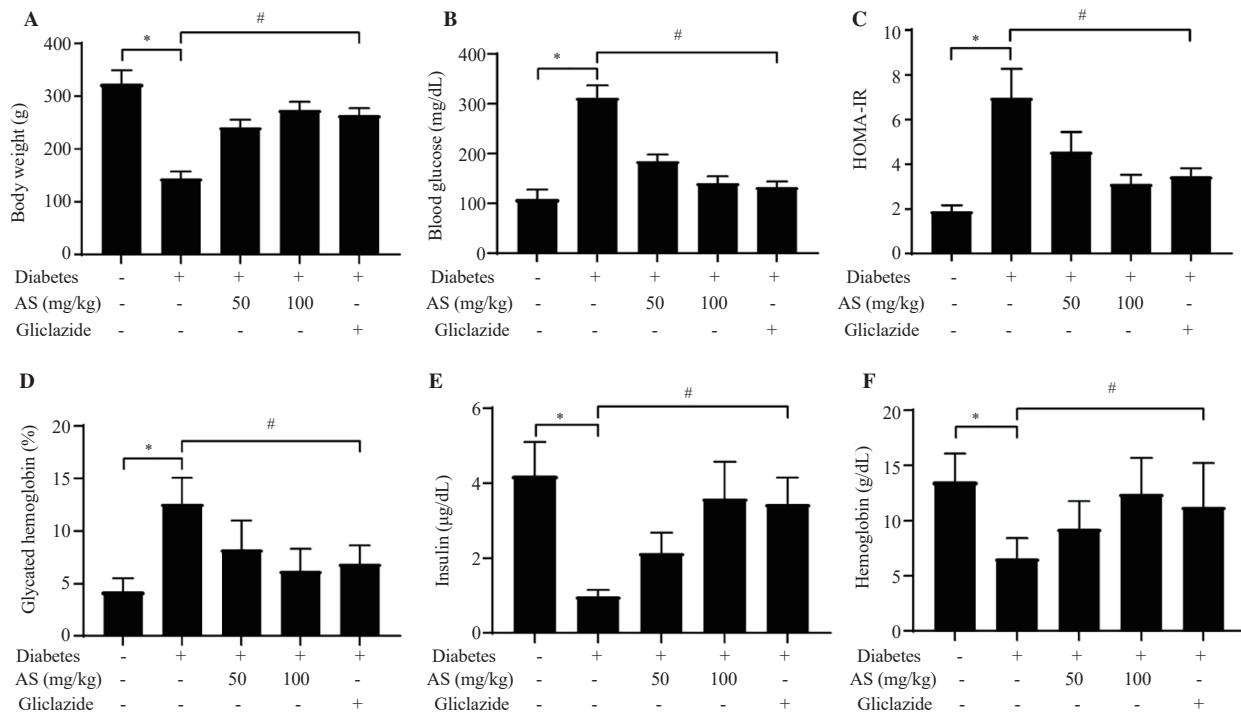


Figure 1. Effect of asiaticoside on (A) body weight, (B) plasma glucose, (C) insulin resistance, (D) glycated hemoglobin, (E) insulin, and (F) hemoglobin levels in rats with type 2 diabetes mellitus. The data are expressed as mean $\pm$ SD ($n=10$) and analyzed by one-way analysis of variance (ANOVA) and least significant difference (LSD) test. * $P<0.05$ vs. the normal control, # $P<0.05$ vs. the diabetes group. AS: asiaticoside.

in the diabetic group, the peak rise in blood glucose levels occurred at 60 min and remained elevated for the subsequent 90 min. Notably, oral administration of asiaticoside resulted in a substantial ($P<0.05$) reduction in blood glucose levels starting from 90 min onwards, reaching near-normal levels by 120 min compared to untreated diabetic rats.

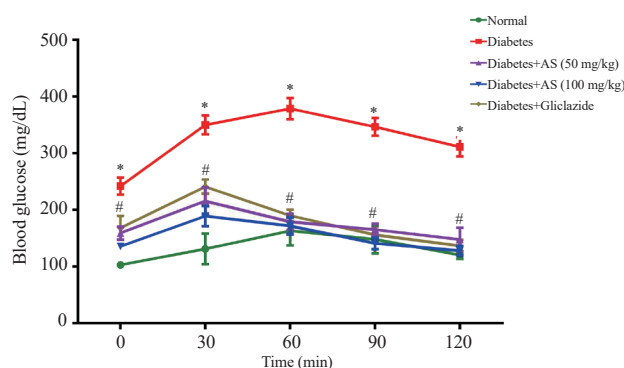


Figure 2. Effect of asiaticoside on oral glucose tolerance test in rats with type 2 diabetes mellitus. The data are expressed as mean $\pm$ SD ($n=10$) and analyzed by one-way ANOVA and LSD test. * $P<0.05$ vs. the normal control, # $P<0.05$ vs. the diabetes group.

3.3. Asiaticoside effectively mitigates albuminuria

Figure 3 demonstrates the levels of biochemical markers. A significant ($P<0.05$) elevation in blood urea, uric acid, and creatinine levels was observed in diabetic rats compared to the normal control

group. Interestingly, asiaticoside treatment led to a marked ($P<0.05$) reduction in urea (Figure 3A), uric acid (Figure 3B), and creatinine levels (Figure 3C) in diabetic rats.

Figure 3D&E shows the influence of asiaticoside on urinary albumin excretion and UACR in diabetic rats. A significant ($P<0.05$) increase in urinary albumin excretion (Figure 3D) and UACR (Figure 3E) was observed in STZ-NAD-induced diabetic rats compared to the normal control. After treatment with asiaticoside, these values were significantly ($P<0.05$) reduced to near-normal values compared to untreated diabetic rats. Figure 3F-H shows the effect of asiaticoside on NGAL, NAG, and TGF- β 1 in rats. A significant ($P<0.05$) increase in urinary levels of NGAL (Figure 3F), NAG (Figure 3G), and TGF- β 1 (Figure 3H) was observed in STZ-NAD-induced diabetic rats compared with normal rats. Treatment with asiaticoside notably decreased NGAL, NAG, and TGF- β 1 levels ($P<0.05$).

3.4. Asiaticoside restores glucose homeostasis by enhancing glycolysis and attenuating the gluconeogenesis

Figure 4 illustrates the levels of carbohydrate metabolism markers in the liver in both experimental and control rats. A significant ($P<0.05$) decrease in hexokinase levels (Figure 4A) along with a concomitant increase in glucose-6-phosphatase levels (Figure 4B) and fructose-1,6-bisphosphatase levels (Figure 4C) was observed in the livers of diabetic rats, which was ameliorated after 45 days of supplementation with asiaticoside.

Figure 4D-F shows the tissue glycogen levels and the activities of

glycogen phosphorylase and synthase in all experimental groups. Diabetic control rats showed significantly ($P<0.05$) lower liver glycogen levels (Figure 4D), decreased glycogen synthase activity (Figure 4E) and higher glycogen phosphorylase activity (Figure 4F) compared to the normal control. Treatment with asiaticoside effectively restored altered glycogen levels and activities of glycogen phosphorylase and synthase in diabetic rats, especially at a higher concentration.

3.5. Histopathological analysis of the pancreas

Histopathological analysis of the pancreas revealed that rats in the normal control group exhibited normal-sized and circular islets of Langerhans (Figure 5A). Conversely, the diabetic control displayed a significant decrease in the size of the islets, along with necrosis and increased hemorrhaging surrounding them (Figure 5B). In contrast, rats treated with asiaticoside (50 mg/kg body weight)

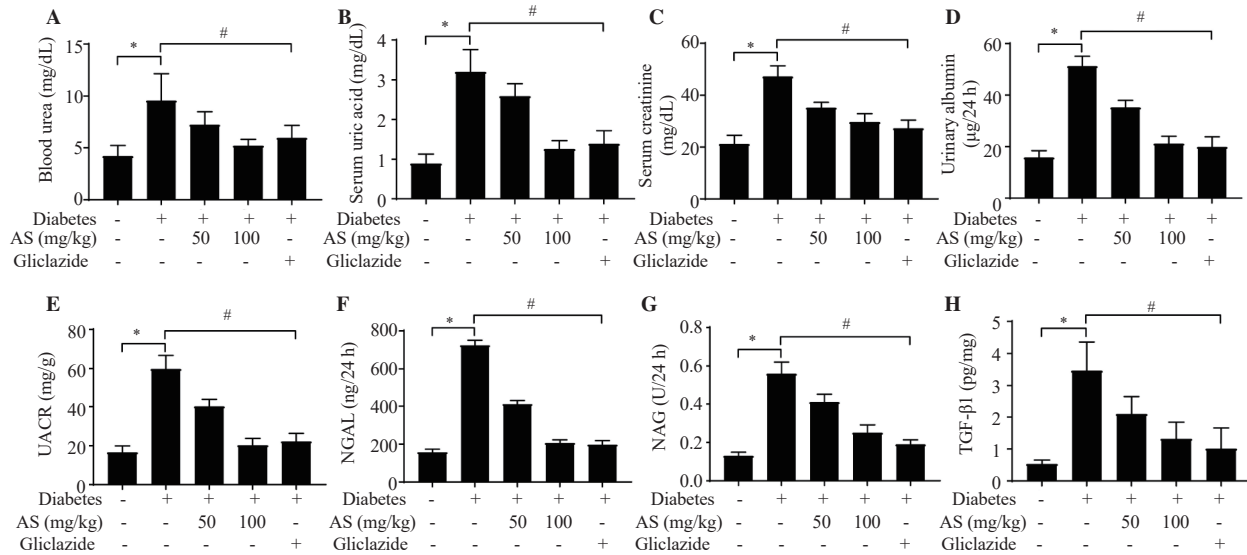


Figure 3. Effect of asiaticoside on (A) blood urea, (B) uric acid, (C) creatinine, (D) urinary albumin excretion, (E) urinary albumin to creatinine ratio (UACR), (F) neutrophil gelatinase-associated lipocalin (NGAL), (G) *N*-acetyl- β -D-glucosaminidase (NAG) and (H) transforming growth factor (TGF)- β 1 in rats with type 2 diabetes mellitus. The data are expressed as mean $\pm$ SD ($n=10$) and analyzed by one-way ANOVA and LSD test. * $P<0.05$ vs. the normal control, # $P<0.05$ vs. the diabetes group.

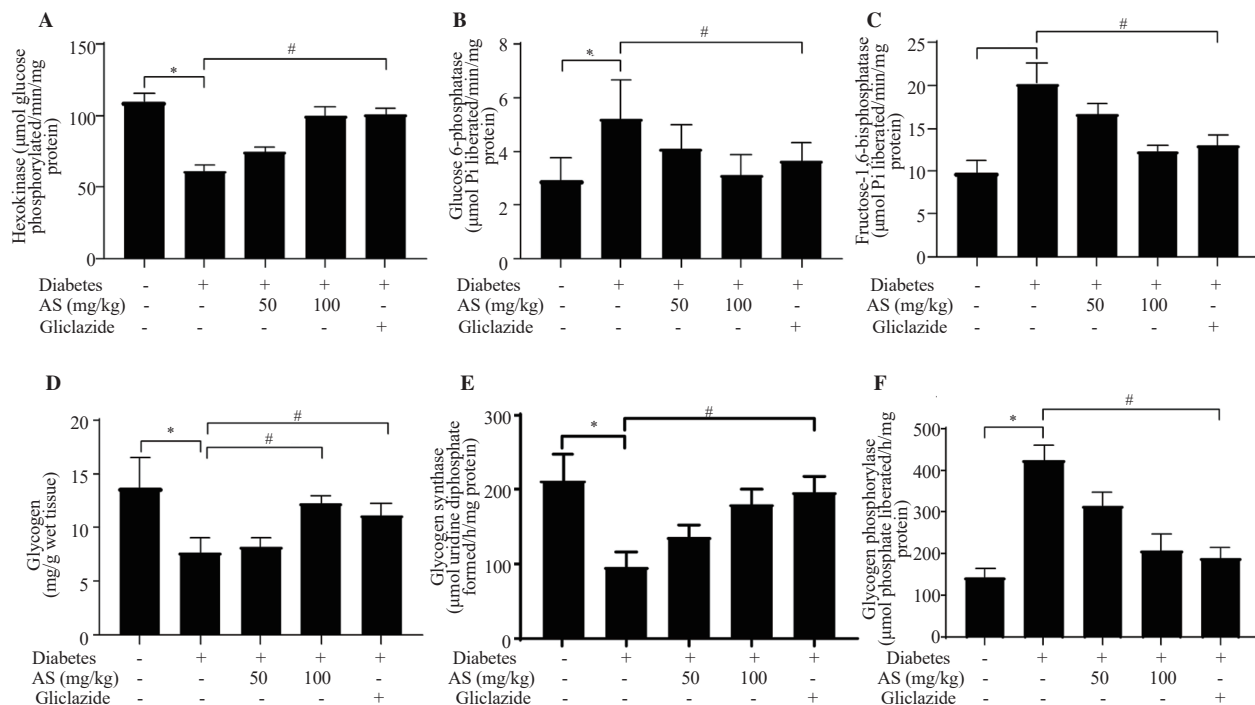


Figure 4. Effect of asiaticoside on glucose homeostasis in rats. (A) Hexokinase, (B) glucose 6-phosphatase, (C) fructose-1,6-bisphosphatase, (D) glycogen content, (E) glycogen synthase, and (F) glycogen phosphorylase. The data are expressed as mean $\pm$ SD ($n=10$) and analyzed by one-way ANOVA and LSD test. * $P<0.05$ vs. the normal control, # $P<0.05$ vs. the diabetes group.

showed a moderate level of necrosis in the islets while maintaining an adequate quantity of pancreatic α and β -cells (Figure 5C). Additionally, the administration of asiaticoside (100 mg/kg body weight) alleviated STZ-NAD-induced damages in diabetic rats, leading to an enlargement of the islets of Langerhans, an increase in pancreatic α and β -cells, and the presence of endocrine cells undergoing regeneration towards their original state (Figure 5D).

3.6. Asiaticoside regulates the insulin signaling pathway by upregulating the mRNA expressions of *IRS-1*, *IRS-2*, and *GLUT4*

As shown in Figure 6, *IRS-1*, *IRS-2*, and *GLUT4* expressions were significantly downregulated in diabetic rats ($P < 0.05$). Treatment with asiaticoside remarkably upregulated the expressions of *IRS-1* (Figure 6A), *IRS-2* (Figure 6B), and *GLUT4* (Figure 6C) in skeletal muscles of diabetic rats ($P < 0.05$).

4. Discussion

T2DM is perceived as a widespread epidemic disease and poses a significant global health concern. The presence of high blood glucose, known as hyperglycemia, serves as a primary indicator of DM arising from abnormal glucose metabolism[18,19]. Studies have identified gluconeogenesis as notably active in T2DM patients, leading to increased production of glucose within the body[20]. Therefore, the regulation of gluconeogenesis plays a crucial role in blood glucose management. Current research suggests that specific natural compounds can effectively lower blood glucose levels by controlling gluconeogenesis[5,19]. Pathologically, IR and subsequent pancreatic β cell damage-induced insulin deficiency are the main features of T2DM, with their varying combinations adding complexity to the condition and diversity in patient presentations[21]. Therapeutically, many glucose-lowering medications act through various signaling pathways. Consequently, extensive exploration has been conducted into herbal-based medicines to utilize their therapeutic properties[11,13,22]. While conventional Western medicine

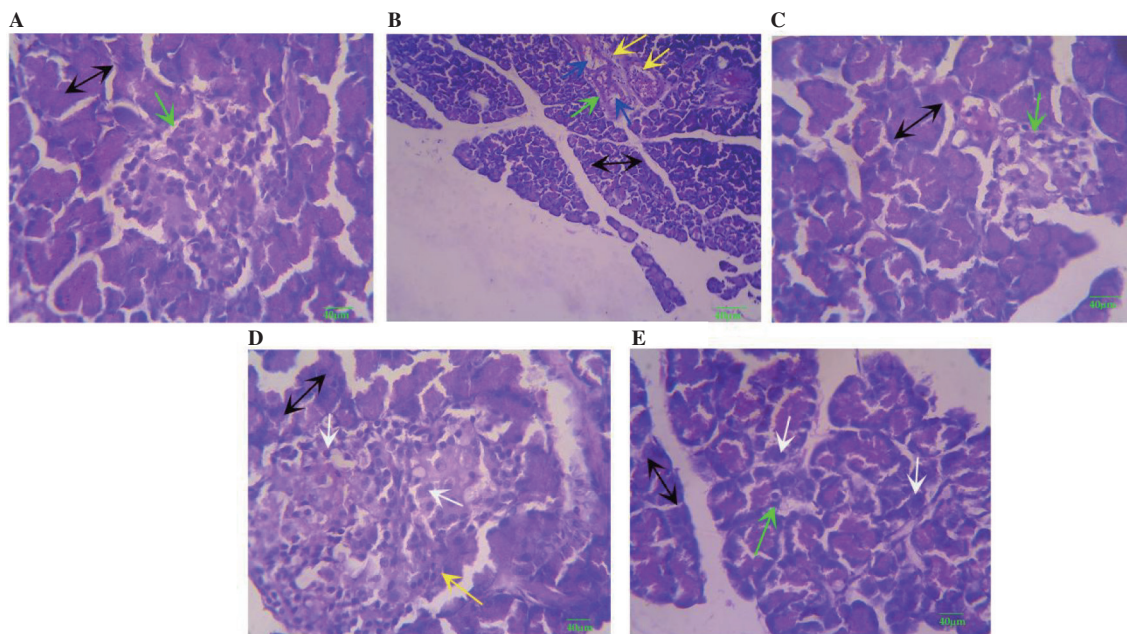


Figure 5. Photomicrographs of the pancreas of (A) normal control, (B) diabetes control, (C) diabetes + asiaticoside (50 mg/kg), (D) diabetes + asiaticoside (100 mg/kg) and (E) diabetes + gliclazide (5 mg/kg) (scale bar: 40 μ m, magnification: 40 $\times$). Green arrow: islets of Langerhans; yellow arrow: clear shrinkage in the islets of Langerhans with increased hemorrhaging around the islets; blue arrow: empty room due to necrosis; white arrow: an increase in the size of the islets of Langerhans and double-headed arrow: exocrine glands.

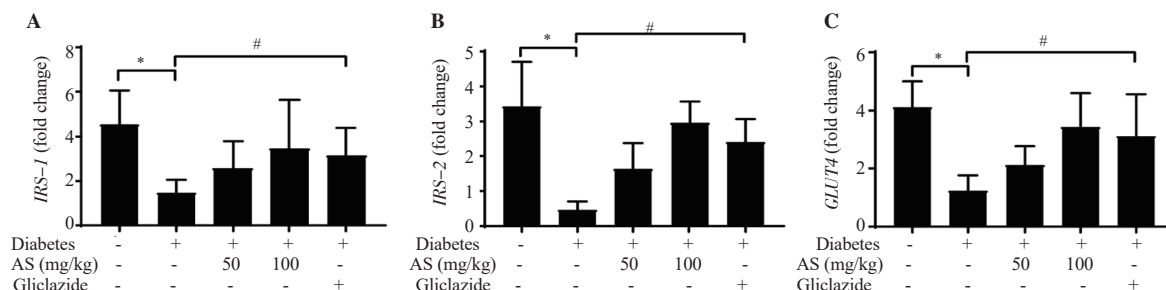


Figure 6. Effect of asiaticoside on the mRNA expressions of (A) *IRS-1*, (B) *IRS-2*, and (C) *GLUT4* in rats with type 2 diabetes mellitus. β -actin was used as internal control. The data are expressed as mean $\pm$ SD ($n=10$) and analyzed by one-way ANOVA and LSD test. * $P < 0.05$ vs. the normal control, # $P < 0.05$ vs. the diabetes group.

remains dominant, traditional alternative healthcare practices have been widely accepted and employed since ancient times[11]. Plant-derived natural compounds are increasingly vital in addressing issues related to carbohydrate metabolism and IR which are pivotal factors in T2DM[19,23]. The current research delves into the therapeutic potential of asiaticoside in combating STZ-NAD-induced T2DM, focusing on its impact on gluconeogenesis, glycolysis, and insulin signaling transduction for DM amelioration.

In individuals with T2DM and IR, disruptions in the control of hepatic glucose metabolism and output lead to issues, such as increased hepatic glucose production and hyperglycemia, due to the liver's diminished response to insulin[24]. T2DM arises when there is inadequate insulin production to sustain normal blood glucose levels, typically due to a combination of IR in peripheral tissues and beta-cell dysfunction, resulting in persistently elevated blood glucose levels or hyperglycemia. The chronic nature of DM is characterized by progressive beta-cell deterioration[25]. Similarly, our current investigation revealed that STZ-NAD administration destroyed pancreatic beta cells, as confirmed by histopathological analysis. Moreover, it showed reduced insulin levels alongside increased blood glucose, IR, and glycated hemoglobin, indicating successful induction of T2DM features by STZ-NAD. Conversely, treatment with asiaticoside demonstrated potential in reducing hyperglycemia by restoring pancreatic beta cell morphology and enhancing insulin production from these cells.

Hyperglycemia-induced perturbations in metabolism lead to the development of glucotoxicity, initiating pathological alterations in T2DM. Abnormalities in renal function, a critical complication of DM, manifest as heightened levels of serum creatinine, blood urea, and uric acid. These parameters serve as key clinical markers of renal impairment in the context of hyperglycemia-mediated renal dysfunction[9]. The persistent elevation in blood glucose levels compromises renal filtration efficacy, resulting in renal impairment and aberrant retention of substances within the body. Consequently, there is a noticeable surge in the concentrations of urea, uric acid, and creatinine in diabetic rats under scrutiny. Treatment with asiaticoside significantly enhances renal function in diabetic rats, suggesting a nephroprotective effect. Conversely, NAG is a widely recognized marker of tubular injury, consistently appearing in urine samples to indicate tubular damage; its high molecular weight precludes glomerular filtration, leading to its release in the tubular lumen following proximal tubular injury[26]. Investigations have demonstrated the utility of NGAL as a novel biomarker for early detection of renal injury and its strong association with diabetic nephropathy progression. Elevated levels of serum and urine NGAL are characteristics of early-stage diabetic renal injury[27]. TGF- β 1 plays a pivotal role in the pathogenesis of diabetic nephropathy, with DM exacerbating oxidative stress and upregulating TGF- β 1 expression. This cytokine promotes cellular hypertrophy and extracellular matrix deposition in the mesangium, resulting in reduced glomerular filtration rate and eventual progression to end-stage renal disease. Recent research has elucidated TGF- β 1's modulation of urinary albumin excretion through concurrent reductions in reabsorption and increases in glomerular permeability

within the proximal tubules[28]. The current investigation revealed tubular injury in diabetic rats following STZ-NAD administration, as evidenced by elevated levels of tubular injury biomarkers such as NAG, NGAL, and TGF- β 1; asiaticoside treatment effectively mitigated these elevations. These findings suggest that asiaticoside may hold promise in preventing further complications arising from hyperglycemia.

Glucose homeostasis entails the coordinated regulation of various metabolic pathways, such as gluconeogenesis and glycolysis, which is attributed to compromised carbohydrate utilization due to inadequate insulin secretion or function[29]. In individuals with T2DM, elevated hepatic glucose production, coupled with decreased hepatic glycogen synthesis and glycolysis, are the primary manifestations leading to hyperglycemia. Within the diabetic context, hepatic glucokinase serves as a highly sensitive marker of the glycolytic pathway, potentially enhancing the utilization of blood glucose for glycogen storage in the liver. Conversely, hexokinase, the primary regulatory enzyme in glycolysis, is reliant on insulin and is mostly inhibited in hyperglycemic rat livers lacking in insulin[30]. Devi and Ramesh[30] have highlighted a reduction in hepatic hexokinase activity among diabetic rats. Interestingly, treatment of diabetic rats with asiaticoside resulted in heightened liver hexokinase activity, indicating asiaticoside's potential in modulating the glycolytic pathway through the activation of hexokinase.

Conversely, hepatic gluconeogenic enzymes like glucose-6-phosphatase and fructose-1,6-bisphosphatase were found to be elevated in diabetic rats. The increased activity of glucose-6-phosphatase in diabetic rats generates hydrogen, which binds with NADP^+ to form NADPH, thereby promoting the conversion of carbohydrates into fats (lipogenesis) and ultimately contributing to elevated blood glucose levels. In DM, increased hepatic glucose production is associated with impaired control of fructose-1,6-bisphosphatase[19]. This study observed a significant increase in the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase in diabetic rats. Reductions in the activities of these gluconeogenic enzymes could potentially lower blood glucose levels through asiaticoside supplementation. Notably, hyperglycemia is correlated with a noticeable decrease in liver glycogen levels[5], which is attributed to decreased glycogen synthase activity and increased glycogen phosphorylase activity. Diabetic rats exhibited reduced glycogen synthase activity and increased glycogen phosphorylase activity, likely due to decreased circulating insulin levels. Insulin generally stimulates glycogen synthase activity and inhibits glycogen phosphorylase activity, so lower insulin levels would result in reduced glycogen synthesis and increased glycogen breakdown[30]. Treatment of diabetic rats with asiaticoside restored enzyme activities to nearly normal levels.

Skeletal muscle glucose clearance is crucial due to the translocation of the glucose transporter through the insulin signaling cascade. The effect of insulin on skeletal muscle metabolism is mainly to stimulate glucose transport and glycogen synthesis[31]. Physiologically, insulin binds to its receptor, which belongs to the superfamily of receptor tyrosine kinases, resulting in the phosphorylation of specific tyrosine residues. These residues bind to the phosphorylated tyrosine 960

residue of the Juxta membrane domain, a group of large docking proteins known as IRS. In humans, there are two IRS proteins, IRS-1 and IRS-2, which play critical roles in the insulin signaling pathway. Studies by Atmodjo *et al.*[32] and Zhao *et al.*[33] have suggested that IRS1 acts as a primary docking protein for phosphoinositide 3-kinase and leads to enhanced glucose uptake by cells. Therefore, assessment of insulin levels in cells can be done by measuring tyrosine phosphorylation of IRS-1, a downstream product of insulin binding to cellular receptors[32]. Furthermore, a reduction in IRS-1 levels has been observed in various IR animal models, suggesting its involvement in the onset or progression of T2DM[19].

Tissues that respond acutely to insulin by increasing glucose uptake have a unique feature: they predominantly express various transcription factors such as IRS-1, IRS-2, and GLUT4[19]. Notably, GLUT4 transporters represent the majority of glucose carriers present in adipocytes and skeletal muscles of rats[34]. This observation suggests that GLUT4 may contribute to the regulation of metabolic homeostasis by participating in the insulin-mediated glucose uptake mechanism[35]. This finding is consistent with the impaired insulin secretion and IR observed in diabetic rats. Due to reduced transcriptional activity, reduced GLUT4 protein and mRNA levels occur during DM and fasting. It is well established that IR in DM is related to decreased phosphorylation of IRS-1 and IRS-2. The process of glucose uptake is initiated by the binding of insulin to its cell surface receptors, followed by insulin-induced enhancement of glucose transport in muscle tissue *via* GLUT4[11]. Administration of asiaticoside to diabetic rats resulted in increased mRNA expression of IRS-1 and IRS-2 in skeletal muscle. Furthermore, this study provides insight into the increased GLUT-4 levels promoted by asiaticoside in diabetic rats, suggesting that the activation of the insulin signaling pathways is responsible for enhancing GLUT4 translocation and subsequent glucose influx into the liver and muscle could be responsible.

In conclusion, asiaticoside could mitigate hyperglycemia in rats by regulating glucose metabolic enzymes, promoting increased glycolysis, decreasing gluconeogenesis, and modulating the insulin signaling pathway, which suggests that asiaticoside may be considered a potential therapeutic option for managing T2DM. However, further clinical trials are necessary to validate these effects. On the other hand, while the study suggests that asiaticoside affects insulin levels and glucose metabolic pathways, the exact mechanisms and interactions involved may not be fully understood. Additional research is needed to elucidate these mechanisms.

Conflict of interest statement

The authors have no conflicts of interest.

Funding

The authors received no extramural funding for the study.

Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

BP wrote the manuscript; BP, VS, KD, and PS performed experiments and collected the data; BP, VS, KD, and PS analyzed the data; BP and VS designed the study; KD and PS reviewed the manuscript. All authors read and approved the final manuscript.

References

- [1] Sathibabu Uddand Rao VV, Brahmanaidu P, Ravindarnaik R, Suresh P, Vadivukkarasi S, Saravanan G. Restorative potentiality of S-allylcysteine against diabetic nephropathy through attenuation of oxidative stress and inflammation in streptozotocin-nicotinamide-induced diabetic rats. *Eur J Nutr* 2019; **58**(6): 2425-2437.
- [2] Swapna K, Sathibabu Uddand Rao VV, Parim B, Ravindarnaik R, Suresh P, Ponmurugan P, et al. Effects of asiatic acid, an active constituent in *Centella asiatica* (L.): Restorative perspectives of streptozotocin-nicotinamide induced changes on lipid profile and lipid metabolic enzymes in diabetic rats. *Comp Clin Pathol* 2019; **28**: 1321-1329.
- [3] Antony Rathinasamy JIR, Uddand Rao VVS, Raveendran N, Sasikumar V. Antiobesity effect of biochanin-A: Effect on trace element metabolism in high fat diet-induced obesity in rats. *Cardiovasc Hematol Agents Med Chem* 2020; **18**(1): 21-30.
- [4] Sethumathi PP, Sathibabu Uddand Rao VV, Chandrasekaran P, Sengottuvelu S, Tamilmani P, Ponmurugan P, et al. Biochanin-A attenuates high-fat diet and streptozotocin-induced hyperlipidemia and oxidative stress in rats by improving antioxidant status and lipid metabolic markers. *Asian Pac J Trop Biomed* 2023; **13**(11): 460-468.
- [5] Pudhupalayam SP, Uddand Rao VVS, Ponnusamy C, Sengottuvelu S, Tamilmani P, Ponmurugan P, et al. Biochanin A attenuates hyperglycemia in high-fat diet-streptozotocin-induced diabetic rats by modulating the activities of carbohydrate-metabolizing enzymes in vital organs. *Rev Bras Farmacogn* 2022; **32**: 608-617.
- [6] Sankaranarayanan C, Nishanthi R, Pugalendi P. Ameliorating effect of berbamine on hepatic key enzymes of carbohydrate metabolism in high-fat diet and streptozotocin induced type 2 diabetic rats. *Biomed Pharmacother* 2018; **103**: 539-545.
- [7] Rahman MS, Hossain KS, Das S, Kundu S, Adegoke EO, Rahman MA, et al. Role of insulin in health and disease: An update. *Int J Mol Sci* 2021; **22**(12). doi: 10.3390/ijms22126403.
- [8] Feraco A, Gorini S, Armani A, Camajani E, Rizzo M, Caprio M. Exploring the role of skeletal muscle in insulin resistance: Lessons from cultured cells to animal models. *Int J Mol Sci* 2021; **22**(17). doi: 10.3390/ijms22179327.
- [9] Uddand Rao VVS, Parim B, Singaravel S, Ponnusamy P, Ponnusamy C, Sasikumar V, et al. Polyherbal formulation ameliorates diabetic cardiomyopathy through attenuation of cardiac inflammation and

- oxidative stress *via* NF- κ B/Nrf-2/HO-1 pathway in diabetic rats. *J Cardiovasc Pharmacol* 2022; **79**(1): e75-e86.
- [10]Uddand Rao VVS, Parim B, Ramavat R, Pothani S, Vadivukkarasi S, Ponmurugan P, et al. Effect of S-allylcysteine against diabetic nephropathy *via* inhibition of MEK1/2-ERK1/2-RSK2 signalling pathway in streptozotocin-nicotinamide-induced diabetic rats. *Arch Physiol Biochem* 2023; **129**(1): 213-221.
- [11]Parim B, Sathibabu Uddand Rao VV, Saravanan G. Diabetic cardiomyopathy: Molecular mechanisms, detrimental effects of conventional treatment, and beneficial effects of natural therapy. *Heart Fail Rev* 2019; **24**(2): 279-299.
- [12]Kalaivani A, Uddand Rao VVS, Parim B, Ganapathy S, Nivetha PR, Kancharla SC, et al. Reversal of high fat diet-induced obesity through modulating lipid metabolic enzymes and inflammatory markers expressions in rats. *Arch Physiol Biochem* 2019; **125**(3): 228-234.
- [13]Kotturu SK, Uddand Rao VVS, Ghosh S, Parim B. Bioactive compounds in diabetic cardiomyopathy: Current approaches and potential diagnostic and therapeutic targets. *Cardiovasc Hematol Agents Med Chem* 2021; **19**(2): 118-130.
- [14]Raziani Y, Karami K, Mohammadi HR, Mahmoudvand H, Moradi MN, Yadegari JG. *Astragalus adscendens* extract shows antidiabetic effects through controlling oxidative stress, inflammation and apoptosis in streptozotocin-induced diabetic rats. *Asian Pac J Trop Biomed* 2023; **13**(6): 242-249.
- [15]Tan SC, Bhattamisra SK, Chellappan DK, Candasamy M. Actions and therapeutic potential of madecassoside and other major constituents of *Centella asiatica*: A review. *Appl Sci* 2021; **11**(18). doi: 10.3390/app11188475.
- [16]Luo J, Zhang T, Zhu C, Sun J, Zhu W, Ai W, et al. Asiaticoside might attenuate bleomycin-induced pulmonary fibrosis by activating cAMP and Rap1 signalling pathway assisted by A2AR. *J Cell Mol Med* 2020; **24**(14): 8248-8261.
- [17]Tosapol A, Mayuree HT, Tsutomu S, Yoshimichi S, Phisit K. Multiple oral dosing pharmacokinetics of standardized extract of *Centella asiatica* ECa 233 and its inductive effect on efflux transporters in rats. *Planta Med Int Open* 2017; **4**: e66-e73.
- [18]Naidu PB, Sathibabu Uddand Rao VV, Naik RR, Pothani S, Munipally PK, Meriga B, et al. Effects of S-allylcysteine on biomarkers of the polyol pathway in rats with type 2 diabetes. *Can J Diabetes* 2016; **40**(5): 442-448.
- [19]Kalidhindi S, Uddand Rao VVS, Sasikumar V, Raveendran N, Ganapathy S. Mitigating perspectives of asiatic acid in the renal derangements of streptozotocin-nicotinamide induced diabetic rats. *Cardiovasc Hematol Agents Med Chem* 2020; **18**(1): 37-44.
- [20]Hatting M, Tavares CDJ, Sharabi K, Rines AK, Puigserver P. Insulin regulation of gluconeogenesis. *Ann N Y Acad Sci* 2018; **1411**(1): 21-35.
- [21]Cao R, Tian H, Zhang Y, Liu G, Xu H, Rao G, et al. Signaling pathways and intervention for therapy of type 2 diabetes mellitus. *MedComm (2020)* 2023; **4**(3). doi: 10.1002/mco2.283.
- [22]Sathibabu Uddand Rao VV, Brahmanaidu P, Nivedha PR, Vadivukkarasi S, Saravanan G. Beneficial role of some natural products to attenuate the diabetic cardiomyopathy through Nrf2 pathway in cell culture and animal models. *Cardiovasc Toxicol* 2018; **18**(3): 199-205.
- [23]Brahmanaidu P, Uddand Rao VVS, Sasikumar V, Naik RR, Pothani S, Begum MS, et al. Reversal of endothelial dysfunction in aorta of streptozotocin-nicotinamide-induced type-2 diabetic rats by S-allylcysteine. *Mol Cell Biochem* 2017; **432**(1-2): 25-32.
- [24]Lewis GF, Carpentier AC, Pereira S, Hahn M, Giacca A. Direct and indirect control of hepatic glucose production by insulin. *Cell Metab* 2021; **33**(4): 709-720.
- [25]Rines AK, Sharabi K, Tavares CD, Puigserver P. Targeting hepatic glucose metabolism in the treatment of type 2 diabetes. *Nat Rev Drug Discov* 2016; **15**(11): 786-804.
- [26]Bouvet BR, Paparella CV, Arriaga SM, Monje AL, Amarilla AM, Almará AM. Evaluation of urinary *N*-acetyl-beta-*D*-glucosaminidase as a marker of early renal damage in patients with type 2 diabetes mellitus. *Arq Bras Endocrinol Metabol* 2014; **58**(8): 798-801.
- [27]Chou KM, Lee CC, Chen CH, Sun CY. Clinical value of NGAL, L-FABP and albuminuria in predicting GFR decline in type 2 diabetes mellitus patients. *PLoS One* 2013; **8**(1). doi: 10.1371/journal.pone.0054863.
- [28]Chang AS, Hathaway CK, Smithies O, Kakoki M. Transforming growth factor- β 1 and diabetic nephropathy. *Am J Physiol Renal Physiol* 2016; **310**(8): F689-F696.
- [29]Röder PV, Wu B, Liu Y, Han W. Pancreatic regulation of glucose homeostasis. *Exp Mol Med* 2016; **48**(3): e219. doi: 10.1038/emmm.2016.6.
- [30]Devi RC, Ramesh B. Amelioration of streptozotocin-induced diabetes by *Bougainvillea spectabilis* leaf extract through modulation of carbohydrate metabolic enzymes and oxidative stress in rats. *Biosc Biotech Res Comm* 2021; **14**(2): 842-852.
- [31]Sylov L, Tokarz VL, Richter EA, Klip A. The many actions of insulin in skeletal muscle, the paramount tissue determining glycemia. *Cell Metab* 2021; **33**(4): 758-780.
- [32]Atmodjo WL, Larasati YO, Jo J, Nufika R, Naomi S, Winoto I. Relationship between insulin-receptor substrate 1 and Langerhans' islet in a rat model of type 2 diabetes mellitus. *In Vivo* 2021; **35**(1): 291-297.
- [33]Zhao X, An X, Yang C, Sun W, Ji H, Lian F. The crucial role and mechanism of insulin resistance in metabolic disease. *Front Endocrinol (Lausanne)* 2023; **14**. doi: 10.3389/fendo.2023.1149239.
- [34]Chadt A, Al-Hasani H. Glucose transporters in adipose tissue, liver, and skeletal muscle in metabolic health and disease. *Pflugers Arch* 2020; **472**(9): 1273-1298.
- [35]Kandasamy N, Ashokkumar N. Protective effect of bioflavonoid myricetin enhances carbohydrate metabolic enzymes and insulin signaling molecules in streptozotocin-cadmium induced diabetic nephrotoxic rats. *Toxicol Appl Pharmacol* 2014; **279**(2): 173-185.

Publisher's note

The Publisher of the *Journal* remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.