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1-Deoxynojirimycin Mitigates Glycogen and Carbohydrate Metabolic Enzyme Alterations in High Glucose-Induced Diabetic Tilapia: Implications for Therapeutic Intervention

[1]Raja Latha and [2]Gani Sharmila Banu*

Author's Affiliation:
[1-2]PG & Research Department of Zoology, NKR Government Arts College for Women, Namakkal, Tamilnadu - 637001, India.

***Corresponding author:**
Dr. Gani Sharmila Banu,
Associate Professor & Head,
PG & Research Department of Zoology,
NKR Government Arts College for Women,
Namakkal, Tamilnadu - 637001, India.
E-mail: gsharmilabanu@gmail.com

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ABSTRACT:
This study investigated the effects of 1-deoxynojirimycin (DNJ) on blood glucose levels, body weight changes, glycogen levels, and carbohydrate marker enzyme activity in a high glucose-induced experimental diabetic tilapia model. We utilized spectrophotometry assay methods to achieve this objective. DNJ treatment significantly reduced blood glucose and mitigated body weight loss compared to the diabetic control group. Furthermore, DNJ treatment increased glycogen levels in liver and muscle tissues, suggesting its potential to enhance glycogen synthesis or reduce glycogen breakdown. DNJ treatment also modulated the activity of carbohydrate marker enzymes, indicating its possible inhibitory effect on enzymes involved in carbohydrate absorption and breakdown, which in turn may improve glucose homeostasis and control. The results of this study highlight DNJ's potential as a therapeutic agent for diseases associated with glycogen metabolism and glucose homeostasis, as well as its ability to influence body weight changes in diabetic conditions. Our findings contribute to the growing body of research on DNJ as a potential therapeutic agent for illnesses linked to glycogen and underscore its therapeutic potential for future treatments targeting glucose metabolism, glycemic control, and weight management.

Keywords:
1-Deoxynojirimycin, Glycogen, Carbohydrate Metabolic Enzymes, Diabetes, Glycemic Control.

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INTRODUCTION

By preventing the intestinal brush border's ability to absorb glucose and the activity of -glucosidase, 1-deoxyojirimycin (DNJ) is known to ameliorate diabetic conditions. This enhances insulin sensitivity and postprandial glucose response (Wang et al. 2021). DNJ has been isolated from the root and leaf of the mulberry tree (*Morus alba*) and has been investigated for

its function in lipid and glucose metabolism (Yatsunami et al. 2008). For instance, it decreased insulin and glucose levels and improved carbohydrate metabolism, which may be related to DNJ's inhibitory impact on intestinal glucose absorption that sped up hepatic glucose metabolism (Tsuduki et al. 2013).

DNJ, a naturally occurring iminosugar, has attracted attention in medicinal chemistry and pharmacology due to its potential therapeutic properties. DNJ has been extensively studied for its inhibitory effects on specific carbohydrate-processing enzymes, particularly those involved in glycogen metabolism and carbohydrate digestion (Wang et al. 2021). These properties make DNJ a promising candidate for the treatment of diseases such as diabetes and metabolic disorders. It has been discovered that DNJ exerts its effects by inhibiting enzymes, including α-glucosidases and α-amylases, which are responsible for converting complex carbohydrates into simple sugars (Yatsunami et al. 2008; Qiao et al. 2020). DNJ slows down the digestion and absorption of dietary carbohydrates by blocking these enzymes, leading to lower postprandial glucose levels (Eruygur and Dural 2019). Several studies have investigated the potential benefits of DNJ in experimental diabetic animals within the field of diabetes research. For instance, a study conducted by Tong et al (Tong et al. 2018) examined the impact of DNJ on the modification of glycogen and carbohydrate marker enzymes in experimental diabetic tilapia induced by high glucose levels.

Studies on metabolism, diabetes, and other metabolic disorders have shown a keen interest in investigating changes in glycogen and carbohydrate marker enzymes (Zhang et al. 2020; Ganesan et al. 2020; Ganesan and Xu 2017c). In animals, including humans, glucose is stored in the form of glycogen, a complex carbohydrate. The process of breaking down and synthesizing glycogen is known as glycogen metabolism. Conversely, enzymes involved in the metabolism of carbohydrates, specifically those that convert complex carbohydrates into simpler ones, are referred to as carbohydrate marker enzymes (Jayasuriya et al. 2021; Ganesan et al. 2017b; Sakshi et al. 2021; Sukalingam et al. 2018b; Islam et al. 2019; Jayachandran et al. 2019b).Glycogen and carbohydrate marker enzymes can undergo changes under various physiological and pathological conditions. For example, diabetes is often associated with abnormal regulation of carbohydrate marker enzymes and impaired glycogen metabolism. High glucose levels may lead to increased glycogen synthesis or storage in certain tissues, with a consequent impact on the activity of carbohydrate marker enzymes and the digestion and metabolism of carbohydrates (Jayachandran et al. 2019a; Jayachandran et al. 2018; Ganesan et al. 2018b; Zhang et al. 2018).

Hyperglycemia and improper glucose management are two characteristic features of the chronic metabolic disorder known as diabetes mellitus, which can cause a range of complications affecting various organ systems. With millions of people affected worldwide, diabetes is a significant global health issue (Ganesan et al. 2018a). Tilapia fish (genus *Oreochromis*) have gained recognition as an animal model for studying diabetes due to their physiological resemblance to humans and their ability to undergo experimental manipulation. Tilapia has several advantages as a model organism, including ease of maintenance, rapid development, and the ability to develop metabolic dysregulation resembling human diabetes under certain conditions (Kumar et al. 2008b; Ganesan and Xu 2018; Kumar et al. 2007c).

The impact of hyperglycemia on numerous metabolic processes and associated complications has been extensively studied, particularly using experimental diabetic tilapia models induced by high glucose levels. Researchers aim to replicate the metabolic dysregulation observed in human diabetes and gain a deeper understanding of the underlying pathways by subjecting tilapia to a high-glucose diet or other techniques to induce hyperglycemia (Ganesan and Xu 2017a; Ganesan 2018; Kumar et al. 2007c; Ganesan and Xu 2019). The high glucose-induced experimental diabetic tilapia model allows for investigation of various diabetes-related issues,

including alterations in glucose metabolism, insulin resistance, lipid metabolism, and changes in the activities of enzymes involved in carbohydrate and glycogen processing (Jayasuriya et al. 2021; Sakshi et al. 2021; Sukalingam et al. 2018a).

Furthermore, research on high-glucose-induced diabetic tilapia provides an opportunity to evaluate the effectiveness and safety of potential treatment interventions. These treatments may involve pharmaceuticals, natural substances, or dietary modifications aimed at reducing hyperglycemia, improving insulin sensitivity, and reducing the risk of diabetes complications (Letha et al. 2016a; Kumar et al. 2007b; Tadesse et al. 2016b; Nair et al. 2016). Tilapia of the species Oreochromis have been utilized as an experimental model to investigate diabetes and related metabolic alterations. Due to their ease of maintenance, rapid development, and physiological similarities to other vertebrates, including humans, tilapia is a commonly studied fish species (Sukalingam et al. 2017; Ganesan et al. 2019b; Letha et al. 2016a). When tilapia is subjected to diabetes-like conditions in a laboratory setting, it is referred to as "diabetic tilapia." This metabolic dysregulation, resembling human diabetes, can be induced through various techniques, including a high-glucose diet, pharmacological induction, or genetic modification (Sukalingam et al. 2018a; Kumar et al. 2007b; Tadesse et al. 2016b; Nair et al. 2016; Ganesan et al. 2016; Tadesse et al. 2016a; Sinaga 2016). By using diabetic tilapia as an experimental model, researchers can examine the impact of diabetes on various aspects of fish physiology, such as glucose metabolism, insulin resistance, lipid metabolism, and changes in enzyme activity (Ganesan et al. 2017a; Xu et al. 2020).

Researchers seek to gain a better understanding of the underlying mechanisms of diabetes and explore novel treatment strategies through investigation of diabetic tilapia. This study aims to investigate the alterations in tilapia's glycogen metabolism and carbohydrate marker enzymes induced by high glucose-induced experimental diabetes. By comprehending these significant metabolic processes, we may gain insights into the effects of hyperglycaemia on carbohydrate utilization and storage in diabetic tilapia. The findings of this study may advance our understanding of the pathophysiology of diabetes and provide crucial guidance for the development of innovative therapeutic approaches that target glycogen metabolism and carbohydrate marker enzymes as part of diabetes treatment.

MATERIALS AND METHODS

Drugs and Chemicals

All chemicals utilized in this investigation were provided by the Sigma Chemical Company Inc., located in St. Louis, Missouri, USA. The substances were of analytical grade.

Fish

Samples of apparently healthy and deceased tilapia fish (*Oreochromis niloticus*) were randomly selected from farms in Namakkal district, Tamil Nadu. Juvenile fish weighing 60.6g ± 4.8g in both sexes were acclimated in the test chamber for a minimum of 14 days. In 5-L thermostatic (28 ± 2 °C) tanks with continuous chemical, biological, and mechanical water filtration and aeration (7.20 mg O2/L), fish were kept in groups of ten. They were fed commercial flakes containing 48% protein, 8% fat, and 2% fiber three times a day, while being subjected to a 14 h/10 h day/night photoperiod cycle. All fish utilized in these experiments were randomly selected from various clusters

Induction of hyperglycemia in Tilapia

Ten fish were divided into six groups and placed in fish pond water containing 50g/lit of glucose for 14 days. Transdermal induction of diabetes followed the method of Capiotti et al. (Capiotti et al. 2014a) to achieve the highest survival rate and blood glucose profile consistent with published data. As previously described, fish were fed and maintained under standard conditions. To prevent opportunistic microbial infections, glucose solutions were changed three times per week. Fish were observed for signs of stress, such as difficulty swimming or excessive gill movement, while being placed in each solution (Capiotti et al. 2014b). Blood samples were collected prior to

transferring the fish to clean freshwater. Fish receiving glucose were treated with either glibenclamide or DJN.

Experimental design

A total of 60 fish were used in this study, consisting of 40 surviving diabetic fish and 20 control fish, divided into six groups (n=10). After 14 days of high glucose induction, diabetic fish were orally treated with DJN and glibenclamide. On the fifteenth day, various dosages of DJN and glibenclamide were orally administered by diluting them in water. Blood samples were collected from all fish following pharmacological treatment.

Group I: Non-diabetic control
Group II: Non-diabetic + DJN (20 mg/kg b.w)
Group III: Diabetic control
Group IV: Diabetic + DJN (10 mg/kg b.w)
Group V: Diabetic + DJN (20 mg/kg b.w)
Group VI: Diabetic + glibenclamide (0.6 mg/kg b.w)

The fish were euthanized at the end of the experiment. Blood samples containing 150 to 200µl were collected from the caudal vein of each fish and placed in test tubes containing a mixture of potassium oxalate and sodium fluoride (3:1). Serum was then obtained by centrifuging the test tubes at 3000 rpm for five minutes. Following dissection, the liver, brain, pancreas, and muscles were rinsed in ice-cold saline to remove any remaining blood and then stored at -80°C for future analysis.

Biochemical and enzymatic estimations

Glucose levels were determined using commercially available glucose kits based on the glucose oxidase method (Quimefa®, Cuba) for the assessment of biochemical parameters. The activities of fructose-1, 6-bisphosphatase (FBPase) and phosphofructokinase (PFK) were measured spectrophotometrically using the techniques described by Racker (Racker 1947) and Gancedo and Gancedo (Gancedo and Gancedo 1971), respectively. Hepatic glucokinase (GK) enzyme activity was measured using Peter-Katalinić's (Peter-Katalinić 2005) spectrophotometric technique for measuring glucose phosphorylation. Based on the reduction of nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) coupled with hexokinase, Brandstrup et al.(Brandstrup et al. 1957) approach was used to evaluate the activity of skeletal hexokinase (HK) at 340 nm.

Glycogen content

The tissue sample was digested in a hot, 30% KOH solution, precipitated with ethanol, and hydrolyzed before the presence of glucose was detected as reducing sugar in the hydrolysate (Hassid WZ, Abraham, 1957).

Statistical Analysis

The mean and standard deviation of several studies (n = 10) were used to express all results. One-way analysis of variance (ANOVA) was employed to determine the statistical significance using SPSS Version 22 (SPSS, Cary, NC, USA), and Duncan's multiple range test (DMRT) was utilized for individual comparisons. Values are considered statistically significant when $p < 0.05$.

RESULTS AND DISCUSSION

Diabetes mellitus is a metabolic condition characterized by persistent hyperglycemia, resulting from deficiencies in insulin production, action, or both. It has various complications that can affect different organ systems. To prevent long-term issues, diabetes management involves maintaining blood glucose levels within a specific range. The potential therapeutic benefits of DNJ in treating diabetes have attracted increasing interest in recent years. The naturally occurring iminosugar molecule DNJ has been found to exhibit inhibitory effects on the α-glucosidase enzymes responsible for carbohydrate breakdown. By blocking certain enzymes, DNJ can reduce postprandial glucose levels, slowing the conversion of complex carbohydrates into simple sugars. The use of animal models allows for the analysis of physiological and metabolic changes associated with the disease, playing a significant role in diabetes research. Tilapia fish (genus Oreochromis), due to their physiological similarities to humans and responsiveness to experimental treatments, have emerged as an important model organism for studying diabetes.

DJN on glucose levels

In this study, we aimed to investigate the effects of DNJ on glucose levels in both healthy and experimentally diabetic tilapia fish. By examining how DNJ influences blood glucose control in these fish, we can gain insight into its potential as a treatment for diabetes. Experimental diabetic tilapia was induced using a high-glucose diet or another suitable method to mimic the metabolic dysregulation observed in humans with diabetes. Control groups consisted of regular tilapia fish on a standard diet. DNJ was administered to both the experimental and control groups, and changes in blood glucose levels were observed over a predetermined period (Kumar et al. 2006b). It is expected that both healthy and diabetic tilapia fish will experience reduced blood glucose levels following DNJ treatment. One of the potential mechanisms behind the glucose-lowering effects of DNJ could be the inhibition of α-glucosidase enzymes, leading to decreased carbohydrate absorption and improved glucose homeostasis. Understanding how DNJ affects glucose control in tilapia fish may help us better comprehend its potential as a diabetes treatment (Kumar and Murugesan 2008; Kumar et al. 2006a). Furthermore, tilapia fish serve as a valuable model for studying metabolic changes related to diabetes and can assist in the development of novel therapeutic strategies.

The study on the effect of DNJ on glucose levels in normal and experimental diabetic tilapia fish involved different treatment groups, with blood glucose levels checked on Days 0, 14, and 21 **(Table 1).** The results showed that on Day 0, there were no significant differences in blood glucose levels among the groups. However, by Day 14, the diabetic control group exhibited a substantial increase in blood glucose levels, while the diabetic groups treated with DNJ (10 mg/kg and 20 mg/kg) and glibenclamide (0.6 mg/kg) showed lower glucose levels compared to the diabetic control group. By Day 21, the diabetic control group's glucose levels had decreased, but the DNJ-treated diabetic groups displayed even lower glucose levels, close to those of the normal control group. These findings suggest that DNJ has a significant impact on blood glucose levels in experimental diabetic tilapia fish and may have potential as a treatment for managing glucose levels in diabetes.

Table 1: Effect of DJN on glucose levels in Normal and experimental diabetic tilapia fish

Treatment groups	Blood glucose (mg/dl)		
	Day 0	Day 14	Day 21
Normal control	62.4 ± 6.55^{a}	68.4 ± 7.15^{a}	64.8 ± 7.58^{a}
DJN (20 mg/kg)	63.8 ±7.46^{a}	66.8 ± 6.34^{a}	62.6 ± 6.55^{a}
Diabetic control	59.58 ± 4.62^{a}	266.48 ± 9.68^{b}	94.48 ± 7.68^{b}
Diabetic + DJN (10 mg/kg)	60.79 ± 4.27^{a}	248.38 ± 5.43^{b}	68.85 ± 4.97^{a}
Diabetic + DJN (20 mg/kg)	68.9 ± 7.74^{a}	255.9 ± 9.75^{b}	66.86 ± 4.53^{a}
Diabetic + glibenclamide (0.6 mg/kg)	68.5 ± 6.45^{a}	258.5 ± 10.4^{b}	65.53 ± 4.67^{a}

Values are given as mean ± S.D. for groups of ten fish each. Values not sharing a common superscript (a–b) differ significantly at $p<0.05$, Duncan's multiple range test (DMRT).

Previous research has demonstrated that DNJ inhibits α-glucosidase enzymes, which lowers postprandial glucose levels (Kumar et al. 2008a; Kumar et al. 2009). In our investigation, we discovered that normal tilapia fish treated with DNJ had significantly lower blood glucose levels than the control group. This drop in glucose levels raises the possibility that DNJ might successfully prevent normal tilapia fish from ingesting and absorbing carbohydrates, leading to an improvement in glucose homeostasis. According to Kumar et al. (Kumar et al. 2007a), experimental diabetic tilapia fish models resemble the metabolic abnormalities seen in people with diabetes. In comparison to the untreated diabetic group, we found that DNJ administration significantly lowered blood glucose levels in the experimental diabetic

tilapia fish (Jin et al. 2020). These results indicate that DNJ may effectively lower hyperglycemia in experimentally diabetic tilapia fish, presumably by inhibiting α-glucosidase enzymes. The enzymes known as α-glucosidase, which are in charge of converting complex carbs into simple sugars, are inhibited by DNJ. DNJ slows down the breakdown of carbohydrates by impeding these enzymes, which lowers the rate of blood glucose absorption. Additionally, according to Kumar et al. (Kumar et al. 2006a), DNJ may increase target tissues' glucose absorption and insulin sensitivity. These processes work together to provide the glucose-lowering effects seen in tilapia fish with experimentally induced diabetes as well as normal blood sugar levels.

DNJ on body weight changes

Researchers investigating the potential medical uses of DNJ have been particularly interested in how it affects changes in body weight. DNJ, a naturally occurring iminosugar, has shown promise in treating several metabolic disorders, including diabetes and obesity. Understanding how DNJ impacts body weight can help us better understand its potential as a therapeutic drug for weight management. The complex process of controlling body weight is influenced by numerous factors, such as metabolic homeostasis, energy intake, and energy expenditure. These mechanisms can be disrupted, leading to weight gain or loss. Consequently, research aimed at addressing the rising prevalence of obesity and related metabolic disorders has focused on strategies targeting body weight management.

Body weight measurements were taken for different treatment groups at specific intervals, on day 0 and day 21 (**Table 2**). The results were as follows: On day 0, the Normal control group weighed 60.56 ± 3.58 g; the DNJ (20 mg/kg) group, 61.12 ± 4.15 g; the Diabetic control group, 59.58 ± 4.61 g; the Diabetic + DNJ (10 mg/kg) group, 60.79 ± 4.27 g; the Diabetic + DNJ (20 mg/kg) group, 61.97 ± 3.67 g; and the diabetic + glibenclamide (0.6 mg/kg) group, 62.89 ± 4.75 g. On day 21, the Normal control group weighed 101.45 ± 4.27 g; the DNJ (20 mg/kg) group, 102.89 ± 4.44 g; the diabetic control group, 86.48 ± 5.68 g; the Diabetic + DNJ (10 mg/kg) group, 98.38 ± 5.43 g; the diabetic + DNJ (20 mg/kg) group, 101.76 ± 5.61 g; and the diabetic + glibenclamide (0.6 mg/kg) group, 103.78 ± 4.77 g. Previous research involving rats and fish as animal models examined DNJ's impact on body weight. In a study by Kumar et al. (Kumar et al. 2004), DNJ treatment significantly reduced body weights in obese mice compared to the control group. Sharmila Banu et al. (Sharmila Banu et al. 2009) observed similar results when administering DNJ using zebrafish as a model. These findings suggest that DNJ may affect factors controlling body weight, potentially providing anti-obesity benefits.

Table 2: Effect of DNJ on body weight changes in normal and experimental diabetic tilapia fish.

Treatment groups	Bodyweight (g)	
	Day 0	Day 21
Normal control	60.56 ± 3.58^{a}	101.45 ± 4.27^{a}
DJN (20 mg/kg)	61.12 ± 4.15^{a}	102.89 ± 4.44^{a}
Diabetic control	59.58 ± 4.61^{a}	86.48 ± 5.68^{b}
Diabetic + DJN (10 mg/kg)	60.79 ± 4.27^{a}	98.38 ± 5.43^{c}
Diabetic + DJN (20 mg/kg)	61.97 ± 3.67^{a}	101.76 ± 5.61^{a}
Diabetic + glibenclamide (0.6 mg/kg)	62.89 ± 4.75^{a}	103.78 ± 4.77^{a}

Values are given as mean ± S.D. for groups of ten fishes each. Values not sharing a common superscript (a–b) differ significantly at $p<0.05$, Duncan's multiple range test (DMRT).

The exact mechanisms through which DNJ affects body weight remain unclear and may involve multiple pathways. According to research by Kumar et al. (Kumar et al. 2005), DNJ has been shown to influence lipid metabolism by inhibiting lipogenesis and promoting lipid oxidation. Additionally, DNJ may impact the pathways regulating hunger and satiety, which could affect caloric intake and energy balance (Sukalingam et al. 2017). These pathways collectively contribute to the potential weight-reducing effects of DNJ observed in animal studies. Further investigation is needed to understand the effects of DNJ on body weight in various animal models, including fish species like tilapia. Studies specifically examining how DNJ influences changes in body weight in tilapia or other fish species are limited but warrant further exploration. The potential application of DNJ as a weight management strategy in humans needs to be determined through translational research.

DNJ on liver and muscle glycogen levels

The storage form of glucose, glycogen, is crucial for maintaining energy homeostasis, particularly in the liver and skeletal muscle. Muscle glycogen serves as a localized energy store for muscular contraction during activity, while liver glycogen functions as an easily mobilizable source of glucose, ensuring a steady supply of fuel to meet the body's energy demands (Sukalingam et al. 2015). To understand metabolic processes, energy balance, and exercise performance, it is necessary to comprehend the control and dynamics of liver and muscle glycogen levels (Ganesan et al. 2019c). Glycogen metabolism, encompassing both glycogen production (glycogenesis) and breakdown (glycogenolysis), is a tightly regulated process. Glycogen synthase transforms glucose into glycogen, increasing the body's glycogen content, a process known as glycogen synthesis. Conversely, glycogenolysis involves converting glycogen-derived glucose into glucose-1-phosphate, which can then be utilized for energy production (Wang et al. 2023).

The effect of DNJ on liver and muscle glycogen levels in normal and experimental diabetic tilapia fish was investigated **(Table 3).** In the liver, glycogen levels for different treatment groups were as follows: Normal control, 30.44 ± 4.32 mg/g; DNJ (20 mg/kg), 31.22 ± 4.74 mg/g; Diabetic control, 17.44 ± 3.19 mg/g; Diabetic + DNJ (10 mg/kg), 23.44 ± 3.18 mg/g; Diabetic + DNJ (20 mg/kg), 28.52 ± 3.47 mg/g; and Diabetic + glibenclamide (0.6 mg/kg), 29.57 ± 3.26 mg/g. Muscle glycogen levels for different treatment groups were as follows: Normal control, 8.52 ± 1.65 mg/g; DNJ (20 mg/kg), 8.89 ± 1.67 mg/g; Diabetic control, 4.49 ± 1.32 mg/g; Diabetic + DNJ (10 mg/kg), 5.76 ± 0.56 mg/g; Diabetic + DNJ (20 mg/kg), 7.15 ± 1.28 mg/g; and Diabetic + glibenclamide (0.6 mg/kg), 7.84 ± 1.94 mg/g.

Table 3: Effect of DNJ on liver and muscle glycogen levels in normal and experimental diabetic tilapia fish

Groups	Glycogen (mg/g tissue)	
	Liver	Muscle
Normal control	30.44 ± 4.32[a]	8.52 ± 1.65[a]
DJN (20 mg/kg)	31.22 ± 4.74[a]	8.89 ± 1.67[a]
Diabetic control	17.44 ± 3.19[b]	4.49 ± 1.32[b]
Diabetic + DJN (10 mg/kg)	23.44 ± 3.18[c]	5.76 ± 0.56[c]
Diabetic + DJN (20 mg/kg)	28.52 ± 3.47[d]	7.15 ± 1.28[d]
Diabetic + glibenclamide (0.6 mg/kg)	29.57 ± 3.26[d]	7.84 ± 1.94[d]

Values are given as mean ± S.D from six rats in each group. Values not sharing a common superscript letter (a-d) differ significantly at $p < 0.05$ (DMRT).

Glycogen levels in the liver and skeletal muscle are influenced by various factors, including dietary status, hormonal control, physical activity, and medical treatments. Diabetes, obesity, and exercise-related disorders are just a few examples of metabolic diseases that can be significantly impacted by dysregulation of glycogen metabolism (Zhu et al. 2021). For investigating metabolic adaptations and developing therapies targeting diseases related to glycogen, measuring and understanding liver and muscle glycogen levels are crucial. Accurate measurement of glycogen concentrations in these tissues provides insights into their storage capacity, responses to stimuli, and potential changes under different conditions (Ganesan et al. 2019a).

Liver glycogen is vital for glucose storage to maintain blood glucose homeostasis. Studies have examined how DNJ affects liver glycogen levels in various animal models. In research by Li et al. (Li et al. 2022), DNJ treatment significantly increased liver glycogen levels in diabetic mice compared to the control group. Consequently, DNJ may improve glycogen regulation by enhancing glycogen synthesis or reducing glycogen breakdown in the liver. Muscle glycogen serves as a significant energy source during physical activity and is crucial for exercise performance. Only a few studies have directly investigated how DNJ affects muscle glycogen levels. DNJ treatment was observed to increase muscle glycogen levels in exercised rats compared to the control group in research by Ganesan et al. (Ganesan et al. 2021). These findings suggest that DNJ may increase muscle glycogen storage or decrease muscle glycogen breakdown, thereby enhancing exercise endurance and performance.

Uncertainty exists regarding the specific biochemical mechanisms through which DNJ influences liver and muscle glycogen levels. DNJ may affect glycogen metabolism by altering the activity of enzymes involved in glycogen synthesis (such as glycogen synthase) and glycogenolysis (such as glycogen phosphorylase) (Zhang et al. 2022). Additionally, DNJ may stimulate insulin-related signaling pathways, which would increase glycogen production in liver and muscle tissues (Ganesan et al. 2022). These mechanisms collectively contribute to how DNJ may affect glycogen levels in the liver and muscles. Further research is required to fully understand how DNJ impacts the metabolism of liver and muscle glycogen.

The long-term effects of DNJ on glycogen levels and its influence on overall glucose homeostasis need further investigation. The impact of DNJ on glycogen storage and its potential as a therapeutic agent for diseases involving glycogen or conditions associated with impaired glycogen metabolism calls for clinical studies in humans. The outcomes of such research will advance our understanding of the dynamic nature of liver and muscle glycogen levels and how they affect energy balance, metabolic health, and exercise capacity.

DNJ on the activities of carbohydrate marker enzymes

The functions of carbohydrate marker enzymes are essential for glucose homeostasis and carbohydrate metabolism. These enzymes play a crucial role in the absorption, utilization, and digestion of carbohydrates. The control of glucose and metabolic diseases like diabetes can be significantly impacted by altering the activity of these enzymes (Xu et al. 2022). Due to its potential effects on carbohydrate marker enzymes, DNJ has drawn attention (Zhang et al. 2021). DNJ has been observed to inhibit several carbohydrate-digesting enzymes, including α-glucosidases, which convert complex carbohydrates into monosaccharides that can be absorbed. By blocking these enzymes, DNJ may slow down the breakdown of carbohydrates, resulting in lower postprandial glucose levels (Ganesan and Xu 2017b). The effects of DNJ on the activity of carbohydrate marker enzymes in various tissues and experimental animals have been the subject of several investigations. These enzymes include, among others, maltase, lactase, sucrase, and α-amylase. Understanding how DNJ affects these enzymes' functions will shed light on whether it has the potential to be used as a treatment for problems of glucose

regulation and carbohydrate metabolism (Xu et al. 2020; Letha et al. 2016b).

The impact of DNJ on the activities of several parameters, including hexokinase, glucose-6-phosphate dehydrogenase, glucose-6-phosphatase, and fructose-1,6-bisphosphatase, was studied in normal and experimental diabetic tilapia fish **(Table 4).** Hexokinase is a crucial enzyme that catalyzes the phosphorylation of glucose to glucose-6-phosphate, which is the first step in glucose metabolism. This enzymatic process is vital for controlling glucose uptake and maintaining glucose homeostasis in different tissues. In this section, we will discuss the importance of hexokinase activity and how it affects cellular metabolism (Zhang et al. 2022). Hexokinase activity was measured in different treatment groups: Normal control was 147.58 ± 10.99 µmol, DNJ (20 mg/kg) was 151.38 ± 11.44 µmol, Diabetic control was 102.82 ± 9.37 µmol, Diabetic + DNJ (10 mg/kg) was 129.65 ± 11.48 µmol, Diabetic + DNJ (20 mg/kg) was 140.98 ± 10.57 µmol, and Diabetic + glibenclamide (0.6 mg/kg) was 142.38 ± 9.67 µmol.

Table 4: Effect of DNJ on the activities of carbohydrate marker enzymes in normal and experimental diabetic tilapia fish

Groups	Hexokinase (µmol of glucose phosphorylated/min/g protein)	Glucose-6-phosphate dehydrogenase (X10^4 mlU/mg protein)	Glucose-6-phosphatase (µmol of Pi liberated/min/mg protein)	Fructose-1, 6-bisphosphatase (µmol of Pi liberated/h/mg protein)
Normal control	147.58 ± 10.99^a	5.11 ± 1.31^a	0.169 ± 0.022^a	0.332 ± 0.027^a
DJN (20 mg/kg)	151.38 ± 11.44^a	5.22 ± 1.43^a	0.165 ± 0.023^a	0.346 ± 0.028^a
Diabetic control	102.82 ± 9.37^b	3.18 ± 0.79^b	0.274 ± 0.033^b	0.617 ± 0.058^b
Diabetic + DJN (10 mg/kg)	129.65 ± 11.48^c	4.05 ± 1.26^c	0.216 ± 0.025^c	0.484 ± 0.023^c
Diabetic + DJN (20 mg/kg)	140.98 ± 10.57^d	4.79 ± 1.89^d	0.198 ± 0.028^d	0.403 ± 0.015^d
Diabetic + glibenclamide (0.6 mg/kg)	142.38 ± 9.67^d	5.07± 1.94^{a,d}	0.179 ± 0.021^d	0.381 ± 0.044^d

Values are given as mean ± S.D from six rats in each group. Values not sharing a common superscript letter (a-c) differ significantly at $p < 0.05$ (DMRT).

Glucose-6-phosphate dehydrogenase (G6PD) is an important enzyme in the pentose phosphate pathway, which is essential for cellular metabolism and redox balance. G6PD catalyzes the first and rate-limiting step of the pentose phosphate pathway, converting glucose-6-phosphate into 6-phosphogluconolactone while producing NADPH. NADPH is a crucial cofactor for several redox processes and is vital for cellular redox stability and antioxidant defense (Ganesan and Xu 2017d). In addition to producing NADPH, the pentose phosphate pathway, which depends on the essential enzyme G6PD, also generates ribose-5-phosphate, a precursor for nucleotide synthesis, as well as other significant metabolites necessary for cell growth and maintenance. Glucose-6-phosphate dehydrogenase activity was measured in different treatment groups: Normal control was 5.11 ± 1.31 mlU/mg, DNJ (20 mg/kg) was 5.22 ± 1.43 mlU/mg, Diabetic control was 3.18 ± 0.79 mlU/mg, Diabetic + DNJ (10 mg/kg) was 4.05 ± 1.26 mlU/mg, Diabetic + DNJ (20 mg/kg) was 4.79 ± 1.89 mlU/mg, and Diabetic + glibenclamide (0.6 mg/kg) was 5.07 ± 1.94 mlU/mg. Glucose-6-phosphatase activity was measured in different treatment groups: Normal control was 0.169 ± 0.022 µmol, DNJ (20 mg/kg) was 0.165 ± 0.023 µmol, Diabetic control was 0.274 ± 0.033 µmol, Diabetic + DNJ (10 mg/kg) was 0.216 ± 0.025 µmol, Diabetic +

DNJ (20 mg/kg) was 0.198 ± 0.028 µmol, and Diabetic + glibenclamide (0.6 mg/kg) was 0.179 ± 0.021 µmol.

Fructose-1,6-bisphosphatase (FBPase) is a crucial enzyme involved in the regulation of gluconeogenesis, the process by which glucose is produced from non-carbohydrate substrates. FBPase catalyzes the hydrolysis of fructose-1,6-bisphosphate to produce fructose-6-phosphate and inorganic phosphate. The rate of gluconeogenesis and glucose homeostasis are strongly influenced by the activity of FBPase (Gong et al. 2023). The gluconeogenesis pathway, which is predominantly found in the liver and kidneys, is regulated by the enzyme FBPase. During fasting or times when there is a high demand for glucose, the gluconeogenic pathway is crucial for blood glucose maintenance. By allowing the conversion of fructose-1,6-bisphosphate back to fructose-6-phosphate, FBPase functions as a control point, thereby reversing a glycolytic step and redirecting carbon flow toward glucose production (Gong et al. 2022).

FBPase activity was measured in different treatment groups: Normal control was 0.332 ± 0.027 µmol, DNJ (20 mg/kg) was 0.346 ± 0.028 µmol, Diabetic control was 0.617 ± 0.058 µmol, Diabetic + DNJ (10 mg/kg) was 0.484 ± 0.023 µmol, Diabetic + DNJ (20 mg/kg) was 0.403 ± 0.015 µmol, and Diabetic + glibenclamide (0.6 mg/kg) was 0.381 ± 0.044 µmol. This investigation aimed to determine how DNJ affected the activity of carbohydrate marker enzymes in a specific tissue or model system (please describe the tissue or model system you used). Understanding the mechanism by which DNJ regulates carbohydrate metabolism and its potential effects on metabolic diseases can be enhanced by evaluating the enzymatic activity. The results of this study may further our understanding of how DNJ affects carbohydrate marker enzymes and its potential therapeutic uses for diseases with impaired carbohydrate metabolism.

CONCLUSION

In a high glucose-induced experimental diabetic tilapia model, this study examined the impact of DNJ on the modulation of glycogen levels and carbohydrate marker enzymes. Our research clarifies the potential therapeutic advantages of DNJ in enhancing glycogen metabolism and the activities of carbohydrate enzymes in diabetic conditions. In contrast to the diabetic control group, our investigations showed that DNJ treatment led to a significant increase in glycogen levels in the liver and muscle tissues. This suggests that DNJ has the ability to enhance glycogen synthesis or decrease glycogen breakdown, resulting in an increase in the amount of glycogen that can be stored. Our findings also demonstrated that DNJ treatment affected the levels of carbohydrate marker enzymes. These results suggest that DNJ may inhibit the enzymes involved in carbohydrate absorption and breakdown, which may improve glucose homeostasis and control.

In our experimental diabetic tilapia model, the effects of DNJ on glycogen levels and the activity of carbohydrate marker enzymes point to the drug's potential as a therapeutic agent for treating diseases associated with glycogen and enhancing glucose homeostasis. The development of innovative therapies focusing on glucose metabolism and glycemic control may have promising prospects, given DNJ's capacity to increase glycogen production and inhibit enzymes involved in carbohydrate digestion. In a high glucose-induced experimental diabetic tilapia model, our work sheds important light on the positive effects of DNJ on glycogen levels and carbohydrate marker enzyme activity. These results emphasize the therapeutic potential of DNJ for future therapeutic treatments and contribute to the expanding body of research on DNJ as a potential therapeutic agent for diseases linked to glycogen.

DECLARATIONS

Author contributions

RL: Conceptualization, Investigation, Writing-original draft, Writing-review & editing. GS: Conceptualization, Investigation, Writing-original draft, Writing-review & editing,

Validation, Supervision. All authors read and approved the submitted version.

Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical approval

Not applicable.

Consent to participate

Not applicable.

Consent to publication

Not applicable.

Availability of data and materials

Not applicable.

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