**UTTAR PRADESH JOURNAL OF ZOOLOGY**

43(16): 7-14, 2022
*ISSN: 0256-971X (P)*

# HYPOGLYCEMIC AND ANTIOXIDANT POTENTIAL OF 1-DEOXYNOJIRIMYCIN IN HIGH GLUCOSE-INDUCED EXPERIMENTAL DIABETIC TILAPIA (*Oreochromis niloticus*)

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**AUTHORS' CONTRIBUTIONS**

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

***Article Information***

DOI: 10.56557/UPJOZ/2022/v43i163135

*Editor(s):*
(1) Dr. Pınar Oguzhan Yildiz**,** Ataturk University, Turkey.
(2) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
*Reviewers:*
(1) Ademolu, Lagos University Teaching Hospital, Nigeria.
(2) Workiyie Worie Assefa, Bahir Dar University, Ethiopia.

***Received: 13 July 2022***
***Accepted: 01 September 2022***
***Published: 02 September 2022***

***Original Research Article***

## ABSTRACT

The objective of the study was to assess 1-deoxynojirimycin's effects on high glucose levels and the tilapia *Oreochromis niloticus* (*O. niloticus*) propensities for hypoglycemia and antioxidant activity. *O. niloticus'* hypoglycemia was induced by adding glucose to the water of the fish pond. Glucose-given fishes were given either glibenclamide or the DJN. It was observed that DJN dissolves at doses of 10, 20 mg/kg b.w or glibenclamide (0.6 mg/kg b.w) tested in the fish's water. In comparison to the control group, the hypoglycemic-induced tilapia had greater blood sugar levels. After the induction of hypoglycemia, the blood glucose levels in tilapia were greater than in the control group for 90 minutes. The serum glucose level of the hypoglycemic tilapia was reduced by DJN or glibenclamide in a dose-dependent manner until it was comparable to that of the control group. According to the findings, DJN possessed anti-hypoglycemic action. This is the first study that we are aware of on the use of fish as a model for diabetes to test DJN or glibenclamide.

**Keywords:** Hypoglycemia; antioxidant potential; *Oreochromis niloticus*; DJN; glibenclamide; diabetes mellitus.

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## 1. INTRODUCTION

There are currently over 346 million diabetics worldwide, this ratio is anticipated to rise by 50% or more due to increases in sedentary behavior, the consumption of energy-dense meals, and obesity [1]. Due to financial limitations, providing modern medical treatment throughout the world is still a reserved objective. Therefore, we must continue to search for new, and ideally, more effective, medications, and phytotherapy may be an excellent target [2]. Worldwide, diabetes mellitus (DM) is today a serious health issue. The most prevalent chronic condition, diabetes mellitus (DM), is characterized by high blood sugar levels [3]. Reactive oxygen species are produced when high blood glucose levels are exposed over an extended period. One of the primary factors for the development of diabetes is oxidative stress, which actively causes cellular damage before the onset of many diabetic problems [4]. Additionally, hypoglycemia contributes to the emergence of microvascular and macrovascular problems, the main contributors to diabetes-related morbidity and mortality [5]. In the modern world, diabetes mellitus, which is characterized by hyperglycemia brought on by insulin resistance, is one of the leading causes of illness and mortality. Currently, dietary intervention has emerged as a desirable diabetes management and prevention technique [6]. It has been noted that many protein hydrolysates have anti-diabetic properties. In type 2 diabetic individuals, casein hydrolysate was found to increase insulin levels and lower glucose levels [7].

"The chronic metabolic condition known as diabetes mellitus (DM) is characterized by hypoinsulinemia, hyperglycemia, and poor metabolism of carbs, proteins, and lipids" [8,9]. "By 2045, the International Diabetes Federation predicts that the number of individuals with diabetes will have increased to 700 million, making it the seventh biggest cause of death worldwide" [10]. "Type-1 diabetes mellitus (T1DM) and type-2 diabetes mellitus were the two subtypes of DM that the American Diabetes Association identified (T2DM). Insulin-dependent diabetes mellitus (IDDM) is an immune-mediated condition marked by a lack of insulin secretion, or the loss of pancreatic beta cells, which results in a complete lack of insulin" [11]. "In non-insulin-dependent DM (NIDDM), also known as type-2 diabetes (T2DM), which is more common in adult age, high blood glucose levels and other biochemical abnormalities lead to either insufficient insulin secretion by pancreatic cells of the islet of Langerhans or insensitivity of target organs to insulin" [12].

Numerous oral medications are being developed by researchers to treat diabetes. But these medications have serious adverse effects, like weight gain and gastrointestinal discomfort. Therefore, it's important to discover new natural remedies that could stop DM [13]. "Several synthetic hypoglycemic medications, including sulfonylureas, biguanides, meglitinides, thiazolidinediones, glucosidase inhibitors, and peptide analogs, are currently used to treat diabetes either alone or in combination. However, some of these medications, such as biguanides and sulfonylureas, have serious side effects that include hypoglycemia, and gastrointestinal discomfort" [14]. The antioxidant potential and hypoglycemic effects of 1-deoxynojirimycin were examined in the current work on experimental diabetic (*Oreochromis niloticus*) tilapia.

## 2. MATERIALS AND METHODS

### 2.1 Fish

Healthy and dead tilapia fish (*O. niloticus*) samples were chosen at random from farms in Namakkal district- Tamil Nadu. Juvenile fish (55.6g ± 4.8g) of both sexes were acclimated in the test chamber for at least 14 days. In 5-L thermostated (28 ± 2°C) tanks with constant chemical, biological and mechanical water filtration and aeration (7.20 mg $O_2$/L), fishes were housed in groups of ten in each group. Fishes were fed with commercial flakes containing 48% protein, 8% fat, and 2% fibre, three times a day while being kept in a 14 h/10 h day/night photoperiod cycle [15]. The fish used in these tests were all randomly selected from various clusters.

### 2.2 Induction of Hyperglycemia in Tilapia

Ten fishes were taken into every six groups. The fish were kept in the water of the fish pond for 14 days after the addition of 50g/lit glucose. Transdermal induction of diabetes was based on the earlier method of Capiotti et al. [16] since the survival rate had to be the highest, and the blood glucose profile had to match the data that had been published. The fishes were placed in each solution, and stress indicators such as difficulty swimming or excessive gill movement were observed [17]. After that, blood samples were taken before the fishes were placed in clean freshwater.

### 2.3 Chemicals

These analytical-grade chemicals were utilized in the study: Glucose, potassium oxalate, sodium fluoride, ethidium bromide, anthrone, hematoxylin, and eosin. The analytical grade of all other chemicals and solvents was purchased from local vendors (India).

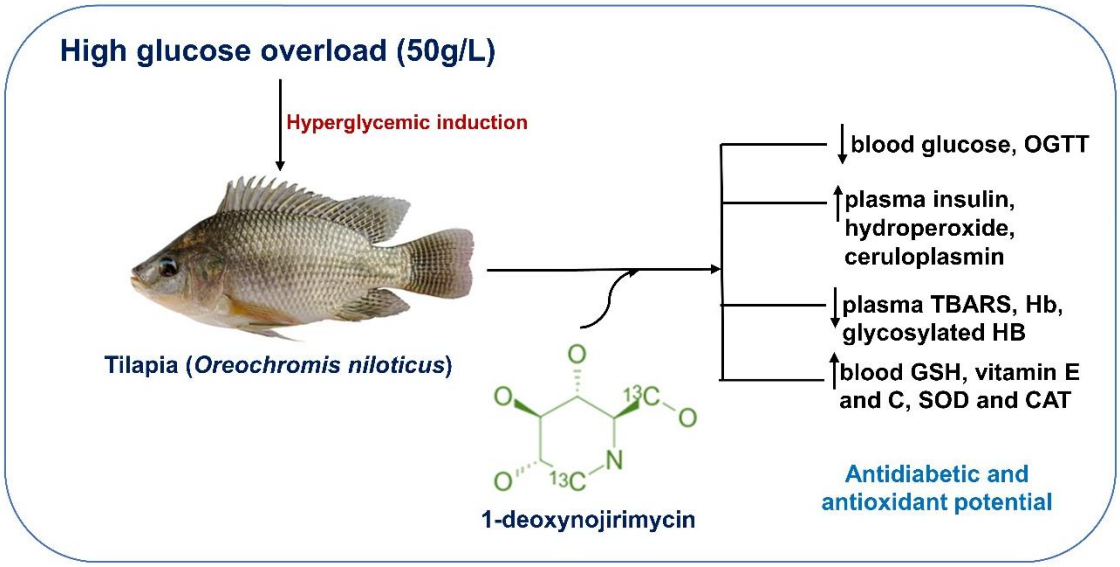


**Fig. 1. Overall protocol**

## 2.4 Experimental Design

The fishes were grouped into six groups (n = 10); a total of 60 fishes (40 diabetic surviving fishes, 20 control fishes) were used. Treatment with DJN and glibenclamide by transdermally in diabetic fishes were started after 14 days of treatment of high glucose induction. Different doses of DJN and glibenclamide were dissolved in water and transdermally administered for 7 days. After 7 days, blood samples were taken from all fish. 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) and Group VII: diabetic + glibenclamide (0.6 mg/kg b.w)

The fishes were sacrificed at the end of the study. Blood samples containing 150 to 200 µl were taken from the caudal vein of each fish and maintained in test tubes containing a mixture of potassium oxalate and sodium fluoride (3:1). Serum was then produced by centrifuging the test tubes at 3000 rpm for five minutes. Muscles, the pancreas, and the liver were promptly removed, removed the blood with ice-cold saline, and then preserved at -80$^{o}$ C for future use.

## 2.5 Treatment with DJN and Glibenclamide Drugs

Fishes that were given glucose, were given either glibenclamide or the DJN. In accordance with the equivalent concentrations of 10, 20, or glibenclamide (0.6 mg/kg b.w.) was dissolved DJN in the fish's water. Following DJN or glibenclamide administration for the OGTT, blood samples were taken at various time intervals.

## 2.6 Biochemical Parameters

### 2.6.1 Determination of glucose and insulin

Estimating glucose and insulin levels is crucial for diabetes diagnosis. Glucose levels were determined using commercially available glucose kits based on the glucose oxidase method (Quimefa, Cuba) for the assessment of biochemical parameters. An enzyme-linked immunosorbent test was used to measure plasma insulin using a commercial kit from Merck Millipore (Darmstadt, Germany). The cyanmethemoglobin technique was used to measure the haemoglobin (Hb) levels [18]. Measuring glycated haemoglobin is crucial for illness diagnosis since it serves as a marker for plasma glucose levels. The Nayak and Pattabiraman [19] method for calculating glycated haemoglobin (HbA1C) was modified in light of Bannon's [20] research.

### 2.6.2 Determination of oral glucose tolerance test

The oral glucose tolerance test is the finest tool for assessing glucose intolerance, an important phenomenon associated with diabetes (OGTT). OGTT was carried out using the Du Vigneaud and Karr technique (1925).

### 2.6.3 Determination of antioxidant enzymes

Antioxidants *in vivo* measurement, the method of Nichans and Samuelson [22], hydroperoxide [23], and

ceruloplasmin were used to measure TBARS in tissues [24]. GSH was calculated using the Ellman technique [25]. The methods of Kakkar et al. [26] and Sinha [27], respectively, were used to measure SOD and CAT.

### 2.7 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 used to determine the statistical significance in SPSS Version 22 (SPSS, Cary, NC, USA), and Duncan's multiple range test was used to determine individual comparisons (DMRT). When $p < 0.05$, values are deemed statistically significant.

## 3. RESULTS AND DISCUSSION

The chronic illness diabetes mellitus is quite dangerous. Although oral anti-hyperglycemic medications and insulin are frequently effective in treating diabetes [28], they have noticeable side effects and do nothing to change the course of diabetic complications [29,30]. Different animal models have been created to study diabetes. Most models involve mice, rats, small animals, or primates that have had diabetes chemically caused by being injected with streptozotocin or alloxan [31]. The chemicals used to generate insulin-dependent diabetes have adverse effects such as liver and kidney necrosis (alloxan) or kidney, lung, and kidney tumors (streptozotocin), demonstrating the lack of specificity of these medications [32]. In the current study, tilapia had hyperglycemia brought on by the addition of glucose to the water of the fish pond. This is consistent with the adult zebrafish hyperglycemia model that was suggested for the investigation of diabetic retinopathy [33].

The blood glucose levels were calculated for Basal, Day 1, Day 2, and Day 3. DJN was evaluated for level on glucose tolerance were Group I (62.4 ± 6.5 mg/dl, 142.4 ± 10.5 mg/dl, 132.8 ± 11.5 mg/dl and 112.3 ± 9.4 mg/dl), Group II (63.8 ±7.4 mg/dl, 138.8 ± 9.4 mg/dl, 121.6 ±10.5 mg/dl and 95.4 ±10.1 mg/dl), Group III (68.9 ± 7.7 mg/dl, 135.9 ± 9.7 mg/dl, 119.8 ± 9.3 mg/dl and 87.2 ± 8.1 mg/dl) and Group IV (68.5 ± 6.4 mg/dl, 128.5 ± 9.4 mg/dl, 112.5 ± 8.7 mg/dl and 85.2 ± 7.8 mg/dl).

The different treatment groups were analyzed on plasma insulin level (Plasma TBARS and Plasma insulin) and biochemical parameters such as Hydrogen-peroxide, ceruloplasmin, haemoglobin, and glycosylated Hb. Table 2 shows the different treatment groups (Normal control, DJN (20 mg/kg), Diabetic control, Diabetic + DJN (10 mg/kg), Diabetic + DJN (20 mg/kg), Diabetic + glibenclamide (0.6 mg/kg). The level of Plasma TBARS were 3.8 ± 0.8 nmol/ml, 3.6 ± 0.2 nmol/ml, 4.5 ± 0.3 nmol/ml, 4.2 ± 0.9 nmol/ml, 3.8 ± 0.7 nmol/ml and 3.6 ± 0.2 nmol/ml. The Plasma insulin were found to be 13 ± 3 µunit/ml, 16 ± 3 µunit/ml, 4 ± 1 µunit/ml, 7 ± 4 µunit/ml, 9 ± 3 µunit/ml and 9 ± 1 µunit/ml. Hydroperoxide were found to be 2.4 ± 0.8 nmol/ml, 2.4 ± 0.9 nmol/ml, 3.1 ± 1.4 nmol/ml, 3.0 ± 1.1 nmol/ml, 2.5 ± 0.8 nmol/ml and 2.4 ± 0.9 nmol/ml.

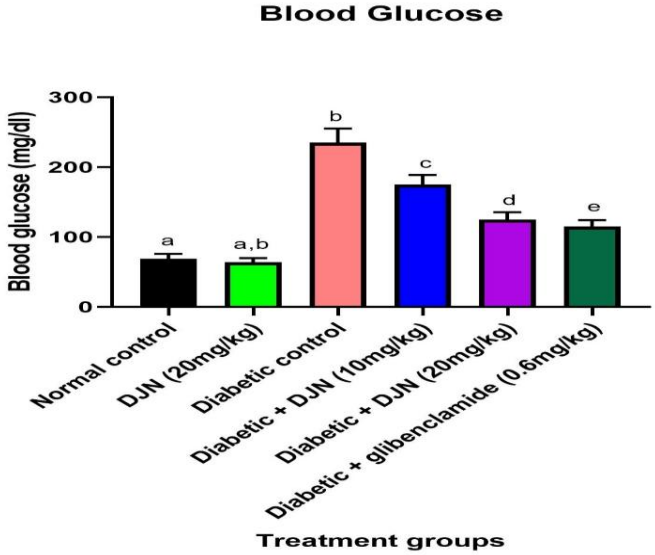


**Fig. 2. Effect of DJN on blood glucose of normal and experimental diabetic tilapia fish**

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

**Table 1. Effect of DJN on plasma insulin, and biochemical parameters in normal and experimental diabetic tilapia fish**

| Treatment groups | Plasma TBARS (nmol/ml) | Plasma insulin (µunit/ml) | Hydroperoxide (nmol/ml) | Ceruloplasmin (mg/dl) | Haemoglobin (g/dl) | Glycosylated Hb (mg/g Hb) |
|---|---|---|---|---|---|---|
| Normal control | $3.8 \pm 0.8^{a}$ | $13 \pm 3^{a}$ | $2.4 \pm 0.8^{a}$ | $38.3 \pm 3.9^{a}$ | $11.5 \pm 1.5^{a}$ | $4.4 \pm 0.5^{a}$ |
| DJN (20 mg/kg) | $3.6 \pm 0.2^{a}$ | $16 \pm 3^{b}$ | $2.4 \pm 0.9^{a}$ | $41.8 \pm 4.8^{a}$ | $12.6 \pm 1.4^{a}$ | $3.5 \pm 0.4^{a}$ |
| Diabetic control | $4.5 \pm 0.3^{b}$ | $4 \pm 1^{c}$ | $3.1 \pm 1.4^{b}$ | $53.5 \pm 6.4^{b}$ | $13.2 \pm 1.7^{b}$ | $13.3 \pm 2.5^{b}$ |
| Diabetic + DJN (10 mg/kg ) | $4.2 \pm 0.9^{bc}$ | $7 \pm 4^{d}$ | $3.0 \pm 1.1^{b}$ | $45.5 \pm 5.9^{c}$ | $12.5 \pm 1.5^{c}$ | $7.9 \pm 2.2^{c}$ |
| Diabetic + DJN (20 mg/kg) | $3.8 \pm 0.7^{ac}$ | $9 \pm 3^{e}$ | $2.5 \pm 0.8^{a}$ | $42.7 \pm 5.7^{c}$ | $11.4 \pm 1.9^{d}$ | $6.9 \pm 1.0^{d}$ |
| Diabetic + libenclamide (0.6 mg/kg) | $3.6 \pm 0.2^{a}$ | $9 \pm 1^{e}$ | $2.4 \pm 0.9^{a}$ | $41.9 \pm 4.8^{ac}$ | $10.9 \pm 1.4^{d}$ | $7.3 \pm 1.5^{d}$ |

*Values are given as mean ± S.D. for groups of ten fishes each. Values not sharing a common superscript (a–e) differ significantly at $p<0.05$, Duncan's Multiple Range Test (DMRT)*

**Table 2. Effect of DJN on blood GSH, vitamin E, vitamin C, SOD, and CAT in normal and experimental diabetic tilapia fish**

| Treatment groups | Blood GSH (mg/dl) | Vitamin E (mg/dl) | Vitamin C (mg/dl) | SOD (U/mg protein) | CAT ($H_2O_2$ decomposed/ min/mg protein) |
|---|---|---|---|---|---|
| Normal control | $38.4 \pm 4.5^{a}$ | $2.6 \pm 0.85^{a}$ | $2.4 \pm 0.27^{a}$ | $9.84 \pm 2.82^{a}$ | $72.6 \pm 2.5^{a}$ |
| DJN (20 mg/kg) | $38.1 \pm 4.7^{a}$ | $2.5 \pm 0.19^{a}$ | $2.3 \pm 0.29^{a}$ | $10.58 \pm 2.58^{a}$ | $81.65 \pm 8.91^{b}$ |
| Diabetic control | $29.1 \pm 4.8^{b}$ | $1.9 \pm 0.37^{b}$ | $1.6 \pm 0.24^{b}$ | $5.56 \pm 1.04^{b}$ | $39.87 \pm 2.42^{c}$ |
| Diabetic + DJN (10 mg/kg) | $33.2 \pm 4.4^{c}$ | $2.3 \pm 0.85^{b}$ | $2.0 \pm 0.36^{ab}$ | $7.22 \pm 1.97^{c}$ | $49.56 \pm 5.47^{d}$ |
| Diabetic + DJN (20 mg/kg) | $36.2 \pm 4.3^{a}$ | $2.5 \pm 0.15^{a}$ | $2.2 \pm 0.38^{a}$ | $8.26 \pm 1.98^{d}$ | $58.47 \pm 6.98^{e}$ |
| Diabetic + glibenclamide (0.6 mg/kg) | $38.1 \pm 4.7^{a}$ | $2.5 \pm 0.19^{a}$ | $2.3 \pm 0.29^{a}$ | $9.11 \pm 2.12^{a}$ | $64.59 \pm 7.89^{f}$ |

*Values are given as mean ± S.D. for groups of ten fishes each. Values not sharing a common superscript (a–f) differ significantly at $p<0.05$, Duncan's Multiple Range Test (DMRT)*

Ceruloplasmin were found to be 38.3 ± 3.9 mg/dl, 41.8 ± 4.8 mg/dl, 53.5 ± 6.4 mg/dl, 45.5 ± 5.9 mg/dl and 42.7 ± 5.7 mg/dl. Haemoglobin level was found to be 11.5 ± 1.5 g/dl, 12.6 ± 1.4 g/dl, 13.2 ± 1.7 g/dl, 12.5 ± 1.5 g/dl, 11.4 ± 1.9 g/dl and 10.9 ± 1.4. Glycosylated Hb was found to be 4.4 ± 0.5 mg/g Hb, 3.5 ± 0.4 mg/g Hb, 13.3 ± 2.5 mg/g Hb, 7.9 ± 2.2 mg/g Hb, 6.9 ± 1.0 mg/g Hb and 7.3 ± 1.5 mg/g Hb. Administration of DJN (20 and 10 mg/kg) or glibenclamide induced a significant reduction in plasma, hydroperoxide, and ceruloplasmin content as compared to diabetic control.

Hemoglobin that has been glycosylated is a common biochemical sign used to diagnose diabetes. A distinct type of haemoglobin called glycated haemoglobin is typically utilized to measure the average plasma glucose concentration over extended periods. It is created through a non-enzymatic process when haemoglobin is consistently exposed to high plasma levels of glucose [34]. Glycated haemoglobin levels were greater in the diabetic group, a sign of poor glycemic control. The muscles and other tissues of diabetic animals exhibit a negative nitrogen balance due to proteolysis, which is accompanied by decreased protein synthesis [35].

"Additionally, increased protein catabolism speeds up the production of urea, resulting in hyperuricemia. Uncontrolled diabetes causes rapid proteolysis because of aberrant glucagon-mediated regulation of cyclic AMP synthesis in the presence of insulin shortage" [36]. The observed decrease in the total protein level in diabetes mellitus is easily explained by this.

The enzymatic (SOD, CAT) and non-enzymatic antioxidant activity (Blood GSH, Vitamin E, Vitamin C) were analyzed for DJN on experimental diabetic tilapia fish. The reactive oxygen metabolites that are formed as a result of chronic hyperglycemia are scavenged by enzymatic antioxidants like SOD, catalase, glutathione peroxidase, and glutathione S-transferase [37]. "The harmful effects caused by superoxide radicals and other free radicals formed from secondary reactions are reduced as a result of the enzyme SOD's involvement in the dismutation of superoxide anion into hydrogen peroxide" [38]. "Catalase and glutathione peroxidase function may be inhibited by diabetes' increased levels of superoxide radical" [39]. Even at lower hydrogen peroxide concentrations, glutathione peroxidase is quite sensitive [40].

## 4. CONCLUSION

The use of DJN for the treatment of diabetes mellitus is supported by the findings of the current investigation. In conclusion, DJN (1-deoxynojirimycin) has strong biochemical, antioxidant, and anti-diabetic activities. Similar to the anti-diabetic medication gliclazide, the DJN showed anti-hyperglycemic efficacy. The results of the current investigation suggest that 1-deoxynojirimycin has antioxidant properties in experimental diabetic tilapia that have been exposed to high glucose levels (*Oreochromis niloticus*). The current study also calls for additional research to identify and describe the active ingredient responsible for its pharmacological effects.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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