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Impact of *Carissa Carandas* Linn. Fruit Extract On Streptozotocin-Induced Diabetes Mellitus

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Carissa carandas is traditionally used as an herbal remedy in Asian countries for diabetes due to its multiple pharmacological activities. The potential activity of the ethanolic extract of C. carandas fruits as an anti-diabetic agent was investigated in this study. Rats with streptozotocin-induced (STZ) diabetes were used to evaluate the effects of the ethanolic extract of C. carandas fruit. Various parameters, including body weight, oral glucose tolerance test, blood glucose levels, biochemical and hematological parameters, antioxidant enzyme activities, DNA damage inhibition, and qRT-PCR, were analyzed. The LD50 of the ethanolic extract of C. carandas was determined to be 2000 mg/kg body weight. The results demonstrate that, compared to the diabetic control group, the ethanolic extract of C. carandas fruit significantly reduced high blood glucose levels ($p<0.05$) to 400 mg/dL. Additionally, the levels of peroxidase, superoxide dismutase (SOD), and catalase in the liver were significantly lower in the diabetic controls than in the groups treated with 400 mg/kg of the fruit extract. The STZ-induced diabetic controls showed higher levels of Glyceraldehyde-3-phosphate dehydrogenase (GPD) gene expression and lower levels of hexokinase gene expression compared to the other groups. These findings suggest that the ethanolic extract of C. carandas fruit has potential as an ethnic medicine for treating diabetes mellitus and holds medicinal value in drug discovery as a therapeutic agent.

Keywords: *Carissa carandas*; Streptozotocin; Blood Glucose; Biochemical Parameters; DNA Damage; qRT-PCR.

Diabetes mellitus (DM) is a prevalent and widespread chronic non-communicable disease worldwide, particularly in developing countries. It can lead to complications such as cardiovascular disease, micro angiopathy, retinopathy, nephropathy, and cognitive deficits, all of which can be managed by promoting retinal tissue regeneration[1]. Traditional treatments like laser therapy, antiangiogenesis, vitrectomy, and steroid hormones have limitations due to their

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potential negative impacts. Consequently, the search for new, more effective phytotherapeutic substances has gained significant attention[2]. Diabetes also has a serious long-term side effect known as diabetic retinopathy. Nanotechnology has enhanced the accuracy and efficacy of retinal tissue regeneration[3], with innovations such as nano stents and conductive hydrogels being used for this purpose. Another serious and costly long-term complication of diabetes is diabetic foot, which often leads to trauma-free amputations[4]. Stem cell transplants and nanomaterials have been utilized to promote angiogenesis and wound healing in diabetic foot ulcers[5]. The global population is approaching a diabetes pandemic, with the number of people living with diabetes expected to rise from 171 million in 2000 to 366 million by 2030[6, 7].

Approximately 10% of the global population is affected by diabetes mellitus (DM), posing a significant societal threat. This chronic metabolic disorder is characterized by hyperglycemia, which arises from inadequate insulin production, stress, obesity, gender, and lifestyle factors. Common phytoconstituents such as 13-docosenamide, quercetin, phytol, 1-hexadecanol, and 1-hexadecene exhibit antimicrobial, anti-inflammatory, and antioxidant properties, which are essential for wound healing in both acute and chronic conditions [8,9]. Conventional medications for obesity are often limited due to availability and potential side effects. Thus, developing safe, effective, and accessible plant-derived treatments is crucial for maintaining health and preventing diseases [10,11]. Recent years have seen a surge in research on plant biochemical and their potential pharmacological effects, with polyphenols being the primary bioactive compounds used to treat diabetes, cancer, and oxidative stress[12-14]. The American Diabetes Association (ADA) conducted a study in 1997 to examine the outcomes of long-standing hyperglycemia, categorizing it into Type I, Type II, and gestational diabetes mellitus (GDM). Diabetes can lead to complications in the kidneys, nerves, and heart [15,16]. The World Health Organization (WHO) has advocated the use of medicinal plants for diabetes management, with approximately 800 plants being utilized. Strategies to mitigate hyperglycemia include medications such as sulfonylureas, metformin, and glucosidase inhibitors [17-19]. Streptozotocin, a toxic xenobiotic, is often used to induce diabetes in experimental animals.

Karonda, scientifically known as *Carissa carandas* Linn., is a plant indigenous to India and Southeast Asia. Historically, the fruits of this plant have been utilized in various traditional remedies due to their believed health benefits. Recent scientific studies have explored the therapeutic properties of *C. carandas* fruit extract in the treatment of diabetes. This exotic, lesser-known fruit, *C. carandas* Linn. (Family: Apocynaceae), or Karanda, is widely recognized among Indian tribes for its medicinal uses. It thrives in sandy loam, laterite, alluvial sand, and calcareous soil and can grow to a height of 3-5 meters. Research indicates that the roots of this plant contain numerous essential compounds. The fruit is typically round or oval and ranges in color from pinkish-red to black. The pulp juice is sweet, resembling berry flavors, and is rich in vitamin C. Harvested during the rainy season, it is used both as a culinary ingredient and a medicinal remedy, exhibiting properties such as pain relief, anti-inflammatory, antimicrobial, antiviral, anthelmintic, antipyretic, appetite-stimulating, and lipase-inhibitory effects. The therapeutic potential of these herbs is attributed to their key bioactive components, including alkaloids, flavonoids, saponins, glycosides, triterpenoids, phenolic substances, and tannins. The antioxidant capacity of *C. carandas* fruit has been evaluated in several studies [20-22]. Investigations into the effects of *C. carandas* fruit extract in STZ-induced diabetic animal models have yielded promising results. The extract appears to exert antidiabetic effects by potentially preserving pancreatic beta-cell function, enhancing insulin secretion, and improving glucose metabolism. Moreover, its antioxidant properties may help mitigate oxidative stress, a critical factor in the pathogenesis of diabetes and its complications [23-25].

This study focused on the ethanolic extract of *C. carandas* fruit and its hypoglycemic effects. The objective was to investigate the changes in biochemical, hematological parameters, and antioxidant effects in streptozotocin-induced diabetic rats. Based on the information provided, the research aimed to assess the effectiveness of *C. carandas* fruit extract as a treatment for hyperglycemia induced by STZ.

MATERIALS AND METHODS

Collection and Preparation of Plant Materials

The fruits of *C. carandas* were collected from Jawadhi Hills (Tirupattur and Thiruvannamalai districts, latitude: 12°34'N, longitude: 78°49'E) in March 2019. All plant materials and fruits were identified and authenticated by Prof. P. Jayaraman, a botanist at the Herbarium of the Plant Anatomy Research Center. A voucher specimen of *C. carandas* (2022/4685) was deposited in the Plant Anatomy Research Center. The fruits were harvested, washed thoroughly under running tap water, and then oven-dried for one week at 40-60°C. The dried fruit pulp was ground uniformly using an electric grinder. The fruit powder (100 g) was then homogenized with 500 ml of ethanol, filtered through Whatman No. 1 paper, and the filtrate was evaporated to dryness using a rotary evaporator. The residues were stored at 4°C for further use.

Animals

The study involved male albino rats of the Wistar strain, weighing between 150-200 g, obtained from the Animal House of Saveetha Medical College, Chennai, Tamil Nadu, India. The temperature and humidity were controlled at 22 ± 5°C with a 12-hour light/dark cycle and 45–50% relative humidity. The animals were fed a standard pellet diet and provided water ad libitum. The protocols for animal experimentation were approved by the Institutional Animal Ethical Committee (BRULAC/SDCH/SIMATS/IACE/8-2021/073).

Acute Toxicity Study

An acute toxicity study of the ethanolic fruit extract of *C. carandas* was conducted in male Wistar albino rats weighing 150–200 g, following the guidelines of the Organization for Economic Co-operation and Development (OECD) 423[26]. A single administration of the ethanolic extract was given at a starting dose of 2000 mg/kg body weight to six rats. The rats were observed for 14 days for changes in body weight, behavior, and any other abnormalities. No signs of toxicity were observed before or after treatment.

Experimental Diabetes Induction

Type II diabetes was induced by injecting Streptozotocin (STZ) at a dose of 55 mg/kg, freshly prepared in 100 mM cold citrate buffer (pH 4.5). The onset of diabetes was confirmed by measuring the blood glucose levels after 72 hours. Diabetic rats were maintained under standard laboratory conditions for up to 14 days, with blood glucose levels monitored. Rats exhibiting blood glucose levels >200 mg/dL were selected to assess the antidiabetic activity of *C. carandas* fruit extract.

Preparation of Tissue Homogenate

The livers of both diabetic control and treated rats were collected, washed with ice-cold saline, homogenized, and used for enzymatic antioxidant assays.

Experimental Design

Rats were divided into five groups, each consisting of six male rats. Group I: control (0.9% sodium chloride); Group II: diabetic control (STZ 55 mg kg-1) Group III: diabetic rats with ethanolic fruit extract of *C. carandas* at 200 mg kg^{-1} Group IV: diabetic rats with ethanolic fruit extract of *C. carandas* at 400 mg kg^{-1}; and Group V: diabetic rats administered with a standard drug (metformin) at 75 mg kg^{-1}. All the treatments were administered by oral feeding for up to 28 days. After day 28 of oral administration, all the animals fasted overnight, and blood glucose and body weight were measured, the next day the rats were anesthetized with ketamine (100 mg kg^{-1}). The liver was removed from the animals, cleaned with ice -cold physiological saline, and refrigerator at "80'" C.

Oral Glucose Tolerance Test

On day 28, an oral glucose tolerance test was performed[27]. All groups of animals were fasted overnight, and 2 mg/kg of glucose was administered to each animal. Blood glucose levels were measured at 0, 30, 60, 90, and 120 minutes.

Biochemical Analysis

Creatinine, SGPT, and SGOT levels were determined using assay kits. The activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase were assayed according to the method described by Gancedo and Gancedo (1971)[28]. Enzymatic antioxidants, such as superoxide dismutase (SOD), were measured using the method described by Beauchamp and Fridovich (1971)[29], and catalase (CAT) activity was assayed according to the protocol of Koroliuk (1975)[30]. Peroxidase (POD) activity in the liver was measured following the method described by Gupta *et al* . (2023)[31]. Total cholesterol and phospholipids were determined as per the methods outlined by

Sharma *et al* . (2022)[32].

Determination of Hematological Parameters

Hematological parameters, including WBCs (x 10^3/ìL), lymphocytes (%), monocytes (%), RBCs (x 10v /ìL), hemoglobin concentration (g/dL), mean corpuscular hemoglobin (pg), mean corpuscular volume (fL), mean corpuscular hemoglobin concentration (g/dL), and packed cell volume (%), were analyzed using the Horiba ABX 80 Diagnostics system (ABX Central, Montpellier, France).

Determination of protein oxidation inhibition

The protein oxidation was determined with different concentrations of fruit extract (0.1 ml) incubated with 0.3 ml of 2mM bovine serum albumin (BSA), 0.2 ml of 2.5mM hydrogen peroxide, 0.2 ml of 1mM FeCl2, 0.1 ml of 1mM ascorbate, and 3 mM EDTA at 25 °C for 30 min incubation, adding 10% TCA and centrifuged at 5000×g for 10 min. The supernatant was decanted, and the pellets were dissolved in 1 ml of 0.05M potassium phosphate buffer at pH 7.5. 50 µL of Ellman's reagent (5,5dithiobis-(2 nitrobenzoic acid) DTNB (5mg in 2.5 ml of potassium phosphate buffer pH 7.5), 2 ml of phosphate buffer, and 50 µl of supernatant were mixed and kept at room temperature for 15 min. The absorbance of the solution was read at 412nm.

Protein oxidation inhibition = [(Abs control-Abs sample)/ (Abs control)] × 100.

DNA damage inhibition assay

The ethanolic fruit extract of C. carandas was used to prevent DNA damage by photolyzing the DNA of the pBR322 plasmid with UV radiation and H_2O_2[33]. Agarose gel electrophoresis was done with 1 ìl of pBR322 DNA (200 ìg/ml), and 50 ìg of the plant extracts were added to tube A. Tube B has a control that has been exposed to radiation and does not have any extracts. The tubes were filled with 4 ìl of 3% H_2O_2 and then exposed to 300 nm UV radiation for 15 minutes with a UV transilluminator (8000 ìW/cm2). A control tube without radiation has a 1 ì l sample of stock pBR322 plasmid DNA. The DNA samples were tested on 1% agarose gel and then analyzed using a Lourmat gel imaging system [34].

qRT-PCR analysis

The RNA was isolated from each rat liver tissue using TRIzol Reagent (Thermo Fisher Scientific, Waltham, MA). RNA(1 ìg) was denatured at 72°C for 5 min and then reverse transcribed using 100 units of Moloney Murine Leukemia virus reverse transcriptase (Gibco) at 37°C for 1hr. After heating at 94°C for five minutes, 2.5 units of Taq DNA polymerase (Perkin-Elmer, Foster City, CA, USA) were used for PCR amplification. In a 50 mL volume, both the forward and reverse primers of the gene were mixed with MgCl2 3 mM and OneTaqVR 2X Master Mix (NEB). For 35–40 cycles, the denaturation, annealing, and extension phases of the q RT-PCR thermal profile were set for 30 seconds at 94°C, 52–55°C, and 1 minute at 72°C, respectively. Melting analysis was carried out in the same reaction tube right away following PCR. The primer set that was employed was: Hexokinase
Forward: GTGTACAAGCTGCACCCGA
Reverse: CAGCATGCAAGCCTTCTTG, Glucose-6-phosphatase: Forward: GCTCCGTGCCTCTGATAAA Reverse: CCACGAAAGATAGCGAGAGTAG. GADPH normalized the expression level of the genes studied, and the band density was measured using Image J, which is plotted as a bar graph as illustrated[35]. Electrophoresis was done on a 1% agarose gel using the above PCR product and visualized under a UV transilluminator. Band identification was performed by using the product size and documented with the Gel Documentation System (Gel DocTMXR System, Bio-Rad), followed by digitalization of the prints. Densitometry was used to quantify the scanned images with assistance from the NIH image program (http://rsb.info.nih.gov/nih-image/).

Statistical Analysis

The results were expressed as mean ± SD. Statistical significance (p) was calculated using one-way ANOVA followed by Dunnett's test. ***P < 0.001, **P < 0.01, *P < 0.05.

RESULTS

The acute toxicity study demonstrated that the ethanolic fruit extract of *C. carandas* had low toxicity. A dosage of 2000 mg/kg body weight did not result in fatalities or behavioral abnormalities in the animals.As shown in Table 1, the body weight of the diabetic control group (induced

by STZ) was significantly lower than the other groups. In comparison, the ethanolic fruit extract of *C. carandas* (200 mg/kg and 400 mg/kg) groups showed a significant increase in body weight over the 28 days, compared to the diabetic control group. The body weight gain in the ethanolic fruit extract-treated rats was more pronounced than in the diabetic control group, which lost weight likely due to disturbances in protein and fat metabolism. Additionally, the group treated with metformin also showed a notable increase in body weight.

Table 2 shows that, in comparison to the normal control groups, the diabetic control group (STZ 55 mg/kg) had significantly higher fasting blood glucose levels from day 0 to day 28. A comparison was made between the diabetic control group (107.31 ± 1.13 mg/dL) and the ethanolic fruit extract (200, 400 mg/kg). Among these, the group treated with ethanolic fruit extract at 400 mg/kg had lower blood sugar levels (101.52 mg/dL) than the other groups treated with the same drug. When compared to the diabetic control group, the metformin-treated diabetic groups (75 mg/kg), with levels of 104.56 ± 0.15 mg/dL, had much lower glucose levels, but these were still higher than those of the groups treated with the fruit extract. In contrast, the diabetic animal groups given the ethanolic extract of *C. carandas* at 400 mg/kg exhibited significantly reduced blood glucose levels, whereas the diabetic control groups still showed elevated glucose levels.

Table 3 show the performance of the rats in the oral glucose tolerance test (OGTT). At 30 minutes, the diabetic control group exhibited significantly elevated blood glucose levels compared to the ethanolic fruit extract and metformin-treated groups. The groups treated with 200 mg/kg and 400 mg/kg ethanolic fruit

Table 1. Effects of ethanolic fruit extract of *C. carandas* L. on body weight in streptozotocin-induced diabetic rats

Group	0 Day (g)	7 Day (g)	14 Day (g)	21 Day (g)	28 Day (g)
Normal Control	114.83 ± 1.42	122.5 ± 1.31	130.33 ± 1.66	141 ± 1.52	148 ± 1.06
Diabetic Control (STZ)	133 ± 0.856	142.17 ± 1.27	132 ± 0.894	123.5 ± 0.95	120.333 ± 1.30
Diabetic + Ethanolic Extract of *C. carandas* (200 mg/kg)	130.33 ± 1.66	132 ± 0.894	122.33 ± 0.95	130.33 ± 1.28	136.16 ± 1.27
Diabetic + Ethanolic Extract of *C. carandas* (400 mg/kg)	141 ± 1.52	137.5 ± 1.31	131 ± 1.52	137.5 ± 1.31	142.67 ± 1.11
Diabetic + Metformin (75 mg/kg)	148 ± 1.06	120.333 ± 1.30	138 ± 2.12	144 ± 1.15	150.83 ± 1.04

The values are expressed as the mean ± S.D; Statistical significance (p) calculated by one way ANOVA

Table 2. Effect of ethanolic fruit extract of *C. carandas* on blood glucose level in Streptozotocin induced diabetic rats

Group	0 Day	7 Days	14 Days	21 Days	28 Days
Normal Control	97.07 ± 0.75	95.79 ± 0.82	95.03 ± 0.35	93.78 ± 0.80	95.89 ± 0.90
Diabetic Control (STZ)	295.35 ± 0.28	326.03 ± 3.20	313.09 ± 0.80	305.55 ± 0.78	297.51 ± 0.78
Diabetic + Ethanolic Extract of *C. carandas* (200 mg/kg)	293.07 ± 0.52	164.65 ± 0.50	140.80 ± 0.80	125.05 ± 0.92	107.31 ± 1.13
Diabetic + Ethanolic Extract of *C. carandas* (400 mg/kg)	295.35 ± 0.41	156.83 ± 0.76	135.81 ± 0.66	121.44 ± 0.56	101.52 ± 1.00
Diabetic + Metformin (75 mg/kg)	292.34 ± 0.29	195.09 ± 0.32	152.26 ± 1.34	125.69 ± 0.63	104.56 ± 1.15

Each value represents mean ± S.D, n=6. Significantly ($p \leq 0.05$)

extract showed a more efficient reduction in blood glucose levels over time, with the 400 mg/kg group achieving the lowest levels.

Biochemical Parameters

Table 4 depicts the effect of *C. carandas* fruit extract on the biochemical profiles in the plasma of the experimental animals. The cholesterol levels increased significantly ($P < 0.05$) in the diabetic control group compared to groups III and IV. When compared to the diabetic control group, there was a significant reduction in total cholesterol (TC) in the metformin (75 mg/kg)-treated groups (200, 400 mg/kg). The SGOT, SGPT, and ALT (serum alanine aminotransferase) levels were significantly elevated in the diabetic control group when compared to the normal control group. Additionally, there was a significant reduction in ALT levels in the metformin (75 mg/kg)-treated groups (200, 400 mg/kg) compared to the diabetic group.

Table 5 shows that glucose-6-phosphatase activity is significantly increased in the STZ-induced diabetic control group, reflecting the typical increase in gluconeogenesis observed in

Table 3. Effect of ethanolic fruit extract of *C. carandas* on oral glucose tolerance test in Streptozotocin induced diabetic rats

Group	0 min	30 min	60 min	90 min	120 min
Normal Control	95.06 ± 0.80	95.80 ± 0.46	96.22 ± 0.76	96.26 ± 1.08	96.99 ± 0.77
Diabetic Control (STZ 55 mg/kg)	275.50 ± 1.07	291.36 ± 0.84	303.47 ± 0.89	297.66 ± 1.27	296.74 ± 0.78
Diabetic + Ethanolic Extract of *C. carandas* (200 mg/kg)	185.12 ± 0.76	171.78 ± 1.03	166.45 ± 0.65	158.02 ± 1.57	148.04 ± 1.43
Diabetic + Ethanolic Extract of *C. carandas* (400 mg/kg)	186.11 ± 0.39	153.25 ± 1.03	148.77 ± 0.64	130.28 ± 0.57	114.57 ± 1.47
Diabetic + Metformin (75 mg/kg)	186.52 ± 0.76	161.24 ± 0.77	142.29 ± 0.90	131.55 ± 1.27	115.65 ± 0.79

Table 4. Effects of ethanolic fruit extract of *C. carandas* on biochemical parameter in Streptozotocin induced diabetic rats

Group	TC (mg/dL)	SGPT (U/L)	SGOT (U/L)	ALT (U/L)	Creatinine (mg/dL)
Normal Control	179.45 ± 1.07	48.38 ± 1.19	146.56 ± 2.03	143.43 ± 1.88	1.69 ± 1.01
Diabetic Control (STZ)	237.10 ± 1.87	84.69 ± 1.43	213.18 ± 1.98	170.92 ± 1.91	2.43 ± 1.09
Diabetic + Ethanolic Extract of *C. carandas* (200 mg/kg)	220.45 ± 1.02*	70.15 ± 1.38*	205.14 ± 1.54*	160.65 ± 1.45*	2.245 ± 1.25*
Diabetic + Ethanolic Extract of *C. carandas* (400 mg/kg)	172.16 ± 1.23*	49.33 ± 1.58*	150.32 ± 1.61*	145.22 ± 1.73*	1.781 ± 1.03*
Diabetic + Metformin (75 mg/kg)	195.45 ± 2.70*	51.26 ± 1.01*	165.26 ± 1.11*	149.39 ± 1.10*	1.454 ± 1.00*

Values are expressed as the mean ± S.D; Statistical significance (p), calculated by one way ANOVA followed by dunnett's $^{***}P< 0.001$, $^{**}P < 0.01$, $^{*}P < 0.05$

Table 5. Effects of ethanolic fruit extract of *C. carandas* on Carbohydrate Metabolic Enzymes in Streptozotocin-Induced Diabetic Rats

Groups	Glucose-6-phosphatase (unit/min/mg protein)	Fructose 1,6-bisphosphatase (unit/min/mg protein)	Glucokinase (unit/min/mg protein)
Normal control	0.119 ± 0.0089	0.503 ± 0.043	0.875 ± 0.032
Diabetic control (STZ 55 mg/kg)	0.242 ± 0.0201**	0.737 ± 0.12	0.866 ± 0.030 ns
Diabetic + Ethanolic fruit extract *C. carandas* (200 mg/kg)	0.154 ± 0.0106 ns	0.465 ± 0.061 ns	0.708 ± 0.02987 *
Diabetic + Ethanolic fruit extract *C. carandas* (400 mg/kg)	0.121 ± 0.0413 ns	0.426 ± 0.030 ns	0.467 ± 0.0607 ***
Diabetic + Metformin (75 mg/kg)	0.182 ± 0.0084 ns	0.555 ± 0.054 ns	0.793 ± 0.035 ns

*The values represent Mean ± SEM. One-way ANOVA followed by Dunnett comparison was performed. (***P < 0.001) control group was compared with the STZ group-II. (***P < 0.001, **P < 0.01, P < 0.05) treated groups III, IV, V, and VI were compared with group I.

Table 6. Effects of ethanolic fruit extract of *C. carandas* on Liver Glycogen in Streptozotocin-Induced Diabetic Rats

Groups	Glycogen (mg/dl)
Normal control	1.426 ± 0.113
Diabetic control (STZ 55 mg/kg)	2.231 ± 0.115 ***
Diabetic + Ethanolic fruit extract (200 mg/kg)	1.109 ± 0.117 ns
Diabetic + Ethanolic fruit extract (400 mg/kg)	1.044 ± 0.097 *
Diabetic + Metformin (75 mg/kg)	0.844 ± 0.038 **

*The values represent Mean ± SEM. One-way ANOVA followed by Dunnett comparison was performed. (***P < 0.001) control group was compared with only the STZ group-II. (***P < 0.001, **P < 0.01, P < 0.05) treated groups III, IV, and V were compared with group I.

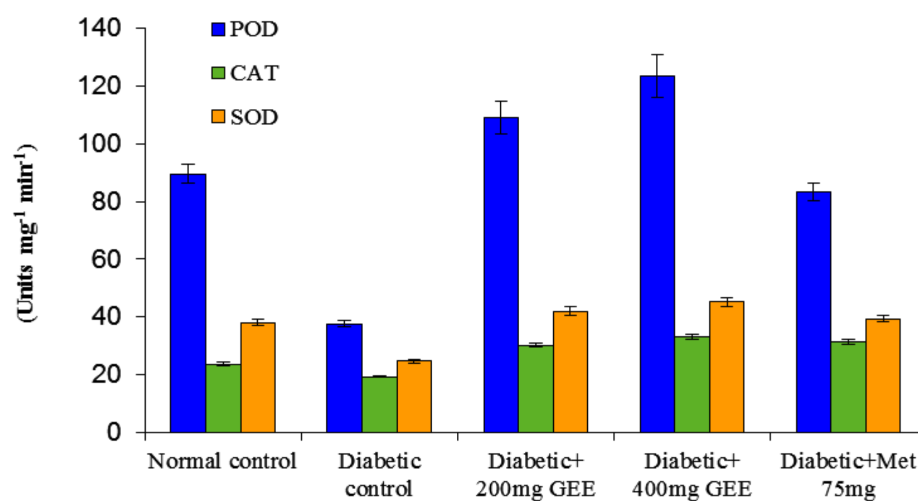

Fig. 1. Effect of ethanolic fruit extract of *C.carandas* on antioxidant enzyme activity in Streptozotocin-induced diabetic group and control group

Table 7. Effects of Ethanolic Fruit Extract of *C. carandas* on Hematological Parameters in STZ-Induced Diabetic Rats

Parameters	Normal Control	Diabetic Control (STZ 55 mg kg-1)	Diabetic + Metformin (75 mg kg-1)	Diabetic + Ethanolic Fruit Extract of *C. carandas* (200 mg kg-1)	Diabetic + Ethanolic Fruit Extract of *C. carandas* (400 mg kg-1)
RBC (X10^6/µL)	4.62 ± 0.2155	5.13 ± 0.203 (ns)	3.297 ± 0.05239 (***)	4.05 ± 0.1242 (ns)	3.217 ± 0.1985 (***)
WBC (X10^3/µL)	12.27 ± 0.5608	12.8 ± 0.681	13.4 ± 0.3215	13.07 ± 0.5207	11.47 ± 0.5364
Hemoglobin (Hb) (g/dL)	13.9 ± 0.649	15.1 ± 0.555 (ns)	9.9 ± 0.153 (**)	11.5 ± 0.681 (*)	9.63 ± 0.617 (**)
Leukocyte (X10^9/µL)	5.333 ± 1.202	9.333 ± 1.453 (ns)	5.67 ± 0.882 (ns)	6.33 ± 1.2 (ns)	7.667 ± 0.8819 (ns)

**The values are expressed as the mean ± S.D. Statistical significance (p) was calculated using one-way ANOVA followed by Dunnett's test. ns = not significant, *P<0.05, **P<0.01, *P<0.001.

diabetes. Both ethanolic fruit extract (at doses of 200 mg/kg and 400 mg/kg) and metformin showed no significant impact on glucose-6-phosphatase activity compared to the diabetic control group, suggesting that these treatments do not notably alter this enzyme's activity under the given conditions. Notably, there were no substantial changes in fructose 1,6-bisphosphatase activity across all groups, including normal controls and treatments in the diabetic group, implying that neither the ethanolic fruit extract nor metformin significantly influences this activity under diabetic conditions. Conversely, a decrease in glucokinase activity is evident in groups receiving ethanolic fruit extract of *C. carandas* at both 200 mg/kg and 400 mg/kg doses, indicating a potentially positive impact on glucose metabolism. In contrast, metformin did not significantly alter glucokinase activity compared to the diabetic control group.

Table 6 shows a significant increase in glycogen levels in the diabetic control group compared to the normal control group. This increase could be due to altered glycogen metabolism in response to diabetes induction (STZ). Glycogen levels were not significantly different from the diabetic control group when treated with the ethanolic fruit extract at the 200 mg/kg dose. However, the ethanolic fruit extract at the 400 mg/kg dose showed a significant decrease in glycogen levels compared to the diabetic control group, suggesting that the higher dose of the ethanolic fruit extract may reduce glycogen accumulation in diabetic conditions. Metformin treatment led to lower glycogen accumulation, indicating its role in altering glycogen metabolism in diabetes.

Antioxidant Enzymes

When comparing the normal control group and the STZ-induced diabetic group, peroxidase (POD) levels were elevated in the liver of diabetic rats. The high dose of ethanolic fruit extract of *C. carandas* reduced POD levels compared to the other treated groups. The standard drug, metformin (75 mg/kg), significantly decreased POD levels compared to the other treatments.When compared to the normal control group, the enzyme activity of catalase was significantly decreased in the liver of the diabetic group. However, catalase activity was observed to be comparable to the control group after 21 days of treatment. Among all groups, a remarkable increase in catalase activity

was observed in the liver of rats treated with the ethanolic fruit extract of *C. carandas* (400 mg/kg), compared to the other groups.

Diabetic rats had relatively lower liver superoxide dismutase (SOD) levels than the control group. When compared to the other groups, the rats treated with ethanolic fruit extract showed a considerable increase in SOD activity in their livers.

Fig. 1 reveals that the STZ-induced diabetic control group has lower levels of Superoxide Dismutase (SOD), Catalase (CAT), and Peroxidase (POD) compared to the normal control group. The administration of an ethanolic fruit extract of *C. carandas* for 28 days resulted in a significant increase in enzyme activity in the treated groups compared to the control groups. There was an increase in the activity of POD, SOD, and CAT in the diabetic groups treated with 400 mg/kg of extract when compared to the metformin-treated groups. These antioxidant enzymes play a vital role in scavenging free radicals. The STZ-induced diabetic control groups showed significantly lower enzyme activity in the liver, indicating oxidative damage to the organs.

Hematological Parameters

The hematological parameters are shown in Table 7. A significant decrease in WBC, RBC,

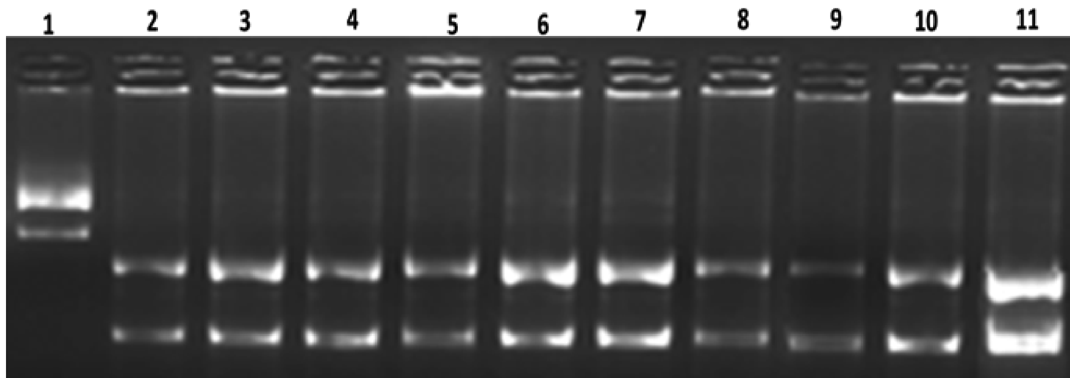

Fig. 2. Electrophoregram of plasmid DNA after ultraviolet photolysis of H_2O_2 (30%) in the presence or absence of the extract. Lane 1: UV + H_2O_2 + DNA, lane 2,3 and 4: 500 mg/ml ethanol extract + UV + H_2O_2 + DNA, lane 5,6 and 7: 500 mg/ml ethanol extract + UV + H_2O_2 + DNA, lane 8,9 and10: 500 mg/ml ethanol extract + UV + H_2O_2 + DNA, lane 11: untreated DNA

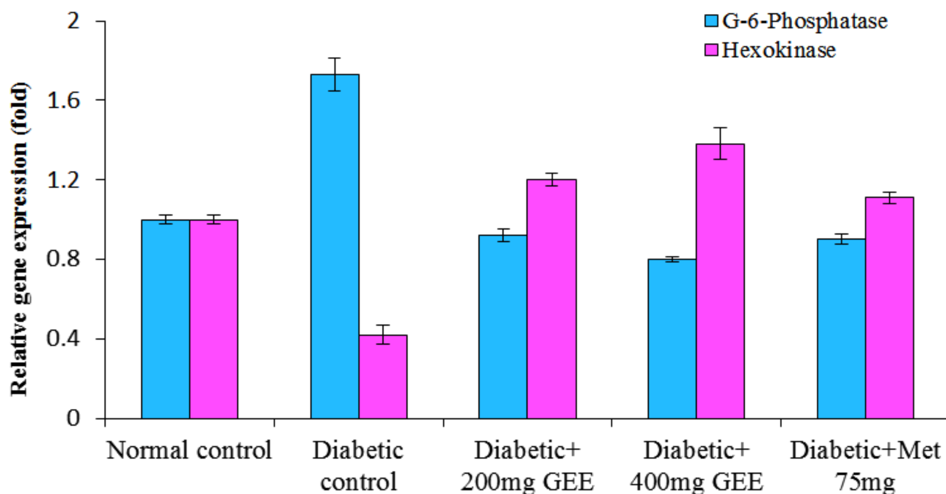

Fig. 2.1. Effect of ethanolic fruit extract of *C. carandas* on gene expression

Hb, and leukocyte levels was observed in diabetic animals, but these values dramatically increased to near-normal levels in the STZ-induced diabetic group following the administration of the extract, particularly at doses of 200 mg/kg and 400 mg/kg body weight. Hemoglobin (Hb) and red blood cell count (RBC) were substantially higher in normal, non-diabetic rats fed with *C. carandas* fruit ethanolic extract than in untreated rats ($p < 0.05$). The RBC count in the diabetic group was significantly lower than in the control group ($p < 0.05$). Compared with untreated rats, diabetic rats treated with *C. carandas* exhibited significantly higher RBC counts ($p < 0.05$).

Effect of Ethanolic Fruit Extract of *C. carandas* on Gene Expression

From the scanned images, which were quantified densitometrically using the NIH image program, the following observations were recorded: There was a significant decrease in the gene expression of hexokinase and an increase in glucose-6-phosphatase expression in the liver of STZ-induced diabetic rats compared to the other groups (Figs. 2 & 2.1).

A remarkable elevation in hexokinase gene expression and a reduction in glucose-6-phosphatase gene expression were observed in diabetic groups administered the ethanolic fruit extract of *C. carandas* and metformin. The ethanolic fruit extract of *C. carandas* stimulates the glycolysis pathway by activating hexokinase gene expression, while down-regulating the gluconeogenesis pathway via glucose-6-phosphatase gene expression. In rats treated with 400 mg/kg of the ethanolic fruit extract, hexokinase gene expression was significantly up-regulated, and glucose-6-phosphatase expression was significantly down-regulated, indicating an enhancement of glycolysis and inhibition of gluconeogenesis. The results of this study show a significant decrease in hexokinase and an increase in glucose-6-phosphatase gene expression in the livers of STZ-induced diabetic control groups, in comparison to the other groups.

DISCUSSION

The present study demonstrates that diabetic rats exhibit a significant decrease in body weight in the STZ-induced group, which can be attributed to protein and fat catabolism as well as dehydration. The body weight observed in the diabetic control group reflects the catabolic state commonly seen in poorly managed diabetes due to insulin deficiency. Administration of *C. carandas* fruit extract, particularly at the higher dose (400 mg/kg), appears to mitigate the decline in body weight associated with diabetes. This suggests a potential beneficial effect on overall health and metabolism in diabetic conditions. The efficacy of *C. carandas* extract, especially at higher doses, in stabilizing body weight is comparable to metformin, a standard antidiabetic medication known for its ability to improve metabolic parameters in diabetes.The administration of ethanolic *C. carandas* fruit extract at doses of 200 mg/kg and 400 mg/kg significantly reduced blood glucose levels and improved oral glucose tolerance in the STZ-induced diabetic rats. The diabetic rats in the STZ-induced group exhibited a marked decrease in body weight, likely due to altered protein and fat metabolism. Compared to the diabetic group, the melatonin-treated animals showed a better rate of body weight gain. Several studies have suggested that melatonin may enhance food consumption, likely through its influence on eating habits associated with melatonin peak secretion, which could account for the short-term increase in food intake [36].The present study also revealed the non-toxic nature of ethanolic fruit extract of *C. carandas*, supporting its potential as an antidiabetic agent in both normal and STZ-induced diabetic rats. The results showed that the ethanolic fruit extract of *C. carandas* (400 mg/kg) exhibited hypoglycemic activity by lowering blood glucose levels in STZ-induced diabetic rats. The induction of diabetes by STZ and subsequent treatment with the aqueous extract highlight the hypoglycemic effects of the extract[37].

Both doses (200 mg/kg and 400 mg/kg) of *C. carandas* fruit extract demonstrated significant antihyperglycemic effects, effectively reducing blood glucose levels in streptozotocin-induced diabetic rats. This suggests that the extract may improve insulin sensitivity, enhance glucose uptake, or protect pancreatic beta-cells. The efficacy of *C. carandas* extract, particularly at the higher dose (400 mg/kg), in reducing blood glucose levels was comparable to metformin, a standard antidiabetic medication. This indicates

that *C. carandas* fruit extract may be a promising therapy for managing diabetes mellitus.

Both doses of *C. carandas* fruit extract and metformin showed a time-dependent reduction in blood glucose levels, with maximum effects observed at 120 minutes post-administration. This suggests that continuous treatment with the extract may lead to sustained improvements in glucose regulation. The exact mechanisms by which *C. carandas* fruit extract exerts its antidiabetic effects require further investigation. Potential mechanisms could include modulation of insulin signaling pathways, enhancement of glucose uptake in peripheral tissues, or protection against oxidative stress associated with diabetes.

Hypercholesterolemia[38], often characterized by a decrease in HDL and an increase in TC, LDL, VLDL, and TG, is commonly associated with hyperglycemia in diabetes. Following *C. carandas* extract treatment, the altered serum lipid profile returned to normal. This suggests that the extract may also possess lipid-lowering effects, which could be related to the inhibition of cholesterol synthesis or increased lipolysis, potentially mediated by insulin regulation[39].*C. carandas* fruit extract, especially at both doses (200 mg kg^{-1} and 400 mg kg^{-1}), showed significant reductions in TC levels compared to the diabetic control group. Elevated TC is often associated with dyslipidemia in diabetes, and the extract's ability to lower TC suggests potential lipid-lowering effects. According to a study, administering melatonin to diabetic rats helped to prevent a rise in blood glucose levels by reversing the activity of glucose-6-phosphatase, transketolase, catalase, and pentose phosphate enzymes in the diabetic liver[40].

In the diabetic control group, elevated SGPT (ALT) and SGOT (AST) levels indicated liver impairment, a common complication associated with diabetes. However, treatment with *C. carandas* fruit extract at both doses (200 mg/kg and 400 mg/kg), as well as metformin, significantly reduced these liver enzyme markers, suggesting hepatoprotective effects. The reduction in ALT levels is particularly noteworthy because elevated ALT typically indicates liver dysfunction or damage. The normalization of these enzymes suggests that *C. carandas* extract may protect the liver in diabetic conditions[41-44].The ethanolic fruit extract of *C. carandas* indicates its high antioxidant potential, likely due to the presence of polyphenols and other phytochemical constituents. These compounds may play a role in combating oxidative stress and preventing disorders associated with diabetes mellitus. Creatinine levels were reduced by *C. carandas* fruit extract at 200 mg/kg but remained slightly elevated at 400 mg/kg compared to the normal control group. Metformin effectively lowered creatinine levels, indicating improved kidney function.

The rate of insulin-stimulated muscle glucose uptake in vivo is important in addressing insulin resistance, with hexokinase playing a key role in glucose phosphorylation. Studies suggest that normalization of plasma glucose concentration in diabetic rats leads to normalization of hepatic glucose-6-phosphatase mRNA expression. Further research is necessary to elucidate the exact mechanisms responsible for these effects and to explore their safety and efficacy in clinical settings. *C. carandas* fruit extract likely exerts its antidiabetic effects, at least in part, by enhancing glucokinase activity, rather than directly inhibiting gluconeogenesis enzymes like glucose-6-phosphatase and glucose-1,6-bisphosphatase. This mechanism promotes glucose utilization and could lower blood glucose levels in diabetic conditions. Since glucose-6-phosphate gene expression in the diabetic liver is â-independent of insulin, sustained hyperglycemia can lead to excess glucose production through elevated expression of this protein.

CONCLUSION

In conclusion, the ethanolic fruit extract of *C. carandas* demonstrates significant antidiabetic properties. It effectively reduces blood glucose levels and enhances insulin sensitivity by protecting pancreatic â-cells from STZ-induced damage. The extract's antioxidant properties may be attributed to its modulation of insulin signaling pathways and other biochemical mechanisms involved in glucose metabolism. A dose-dependent response was observed, with varying concentrations of the fruit extract (200 mg/kg and 400 mg/kg) producing different levels of efficacy. This plant shows promising medicinal value in the field of drug discovery and could be a potential therapeutic

agent for diabetes management. While the rat study shows encouraging results, human clinical trials are needed to assess the safety, efficacy, and appropriate dosing of this treatment. Comparative studies with metformin and long-term safety evaluations will be crucial. Further research will help determine whether this treatment can replace or complement metformin in managing metabolic diseases.

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Informed Consent Statement

This study did not involve human participants, and therefore, informed consent was not required.

Clinical Trial Registration

This research does not involve any clinical trials.

Author's Contributions

Sudha: Completed the research work plan, manuscript writing, and supervision; Gokulakrishnan: Handled the editing and review; Malarkodi: Completed the review of the literature collection; Every author has reviewed and approved the published version of the manuscript.

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