

Research Article



Biochemical Evaluation of Antidiabetic Activity of *Cocos nucifera* Flowers in STZ Induced Diabetic Rats

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ABSTRACT

Diabetes mellitus is a chronic metabolic disorder of multiple aetiologies resulting in drastic elevation of blood glucose under both fasting and postprandial conditions, which leads to multiple complications. Currently available drugs are unsuccessful for the treatment of diabetes due to their undesirable side effects. Hence, search for novel drugs, especially from plant origin continues. *Cocos nucifera* have been used in traditional medicine around the world to treat numerous ailments. The present study is aimed to evaluate the antidiabetic and antioxidant nature of *Cocos nucifera* flowers extract in STZ induced diabetic rats. Phytochemical analysis of the fruit extract revealed the presence of phytoingredients such as alkaloids, flavonoids, saponins, tannins, diterpenes, triterpenoids, glycosides and phenols. Oral administration of *Cocos nucifera* flower extract (300 mg/kg b.w/day) to diabetic rats for 30 days significantly reduced the levels of blood glucose, glycosylated hemoglobin, urea, uric acid and creatinine. The altered levels of serum amino transferases and alkaline phosphatase were normalized upon treatment with the fruit extract. The observed decrease in the levels of plasma protein in the diabetic rats was elevated to near normal by the extract treatment. The level of glycogen content, the altered activities of glycogen synthase and glycogen phosphorylase was improved upon treatment with the extract. The antioxidant competence was improved upon extract treatment. The results of the present study indicate that the flower extract is nontoxic and possess antidiabetic and antioxidant potential.

Keywords: Antidiabetic, Antioxidant, *Cocos nucifera* flower, Diabetes mellitus, STZ.

INTRODUCTION

Diabetes mellitus (DM) is a multi factorial, multi systemic endocrine disorder in which the body does not produce (type 1) and/or properly respond (type 2) to insulin, a hormone essential for the entry of glucose from plasma to cells for energy production.¹ Despite considerable progress in the treatment of diabetes by oral hypoglycaemic agents search for newer drugs continues because the existing synthetic drugs have several limitations. Plants play an important role as alternate medicine due to less side effects and low cost. However, the majority of traditional antidiabetic plants await proper scientific and medical evaluation for their ability to improve glucose homeostasis.

Medicinal values of many plants still remain unexplored for its enumerable activity of compounds responsible for later. Yet, plant materials remain important resources to combat serious diseases of the world. Pharmacological interventions of plants are carried out to find novel drugs or templates for the development of new therapeutic agents.^{2,3} India is a home to a variety of traditional medicine system that relies largely on native plant species for their raw drug material.⁴ Therefore, now there is a need to look back towards the traditional medicines, which can serve as novel therapeutic agents. One such medicinal plant that lacks scientific scrutiny for its antidiabetic activity is *Cocos nucifera*.

The coconut palm is botanically referred to as '*Cocos nucifera*'. It is a member of the *Arecaceae* or palm family. The coconut tree is tall palm that exhibits a height of approximately 30m. The coconut palm tree is a versatile and prolific plant, playing a vital part in the medical treatment of soldiers in World War II, sheltering residents of island nations, and providing ancient civilizations with spears for hunting. One of the most useful and interesting trees used commercially, the coconut tree yields hundreds of products from its various parts. While many plants are used commercially for the fruit they bear or the wood they are made of, the coconut palm is used for many purposes that rely on nearly every part of the species.⁵ The coconut, *Cocos nucifera* L., has been described as the "tree of life" or "tree of heaven" and nature's greatest gift to man. Each part of the coconut tree can be used to produce items of values for the community.

Cocos nucifera L., is a dominant type tree.⁶ Grown in 90 countries in the world, the coconut palm is one of the most important perennial tropical plantation crops. Coconut trees are growing to a height of 20-30m and flowering 5-7 years after planting with an average economic life of 60-70 years. The interval between the initiations of two successive leaves is influenced by the genotype, soil fertility and seasonal conditions but under normal conditions a palm produces on average 12-15 leaves annually. Plants are aurally unbranched and the



crown encloses a single, pleoanthic SAM, with indeterminate flowering.

The coconut is essentially a monoecious plant, that is, stamens and pistils are produced in the same inflorescence. Male flowers generally dry up or fall off while the pollinated (and fertilized) female flowers start growing as fruits (coconuts). The development pattern of the coconut inflorescence has been described.⁷ The inflorescence of the coconut palm is a spadix that develops in the axil of each leaf. Thus, the number of leaves produced annually also determines the number of spadices developed. Before opening, the spadix has a spear like shape, with a length of about one meter. When the inflorescence develops within a sheath, the latter finally splits open over its entire length, the inflorescence emerging and unfolding. The spadix consists of a main axis with 40-70 spikes, bearing flowers. The spadices are ivory in colour and turn brownish orange at mature stages. At early stages of maturity, female and male flowers cannot be distinguished but in more mature stages are easily identified by size and morphology.⁸ Female flowers are located at the base of the spikes the rest of the spike being fully covered by male flowers. Each spike may have one or more female flowers and generally about two to three hundred male flowers. The total number of female flowers per inflorescence usually varies between twenty to forty. First spadices usually have fewer female flowers per inflorescence than spadices in mature palm.⁹

Generally the male flowers opens immediately after splitting of the sheath starting with the male flowers and those at the sides of the female flowers and those at the tips of the upper spikes. Opening of the male flowers continues from the tips of the spikes towards the base. After shedding their pollen, the male flowers wither and fall off, usually within two days following their opening. However, the period between the opening of the first male flower and the shedding of the last takes about three weeks.¹⁰ When the female flower becomes receptive, it opens at the apex and the three sessile stigmas protrude from it like a three pointed star. Nectar is produced at their bases and at the three pores on the pericarp towards the top of the ovary. The stigmas turn from ivory to pinkish brown after the receptivity of the female flowers.

Immature or undifferentiated flowers have been used in traditional medicine around the world to treat numerous ailments, ranging from sore throat, colds, and earaches to tuberculosis, tumors, and ulcers. It is believed to be antibleorrhagic, antibronchitis, febrifugal, and antigitigivitic.¹¹ Recent medical studies have found that coconut flowers can have antibacterial, antifungal, antihelminthic, and antiviral properties, among other health benefits. Coconut oil was once avoided because it is composed of saturated fats, which were thought to raise cholesterol. However, recent research suggests that because it has medium- rather than long-chain fatty acids,

coconut oil does not raise cholesterol, but may actually protect against heart disease.

The flowers of *Cocos nucifera* used for the present study is depicted in Plate 1.



Plate 1: *Cocos nucifera* flowers

In the absence of systemic studies in the literature, the present study has been aimed to explore the antidiabetic and antioxidant potentials of *Cocos nucifera* flowers extract in STZ induced diabetic rats.

MATERIALS AND METHODS

Experimental Animals

Male albino Wistar rats (150-180 g) were purchased from TANUVAS, Madavaram, Chennai. The rats were housed in polypropylene cages lined with husk and maintained in Central Animal house Facility of University of Madras. The husk was renewed every 24 hours. The rats were fed with commercial pelleted rats chow (VRK Nutritional Solutions, Maharashtra, India) and had free access to water. The experimental rats were maintained in a controlled environment (12:12 hours light/dark cycle) and temperature ($30 \pm 2^\circ\text{C}$). The experiments were designed and conducted in accordance with the ethical norms approved by Ministry of Social Justices and Empowerment, Government of India and Institutional Animal Ethics Committee Guidelines for the investigation of experimental pain in conscious rats. The rats were acclimatized for one week before starting the experiments (IAEC NO. 17/01/2012)

Plant Material

The flowers of *Cocos nucifera* were collected from a mature tree in Chengalpattu, Kanchipuram district. The flowers were identified and authenticated and a voucher specimen was deposited at the Department of Botany, University of Madras, Chennai. The undifferentiated male and female flowers from the spadix were collected manually.

Preparation of Plant Extract

The flowers of *Cocos nucifera* were dried at room temperature and powdered in an electrical grinder, which was then stored in an airtight container at 5°C until further use. The powdered flowers were delipidated with petroleum ether (60 - 80° C) for overnight. It was then filtered and soxhlation was performed with 95% Ethanol. Ethanol was evaporated in a rotary evaporator at 40 – 50° C under reduced pressure.

Preliminary Phytochemical Screening

The ethanolic extract of *Cocos nucifera* flowers were subjected to preliminary phytochemical screening of various plant constituents.^{12,13}

Induction of Diabetes Mellitus

Diabetes was induced in overnight fasted rats by single intraperitoneal injection of streptozotocin (45 mg/kg b.w) dissolved in freshly prepared 0.1M of cold citrate buffer (pH 4.5).¹⁴ Since, STZ is capable of inducing fatal hypoglycaemia due to massive pancreatic insulin release, the rats were provided with 10% glucose solution after 6 h of STZ administration for the next 24 h to overcome drug induced hypoglycaemia.¹⁵ Neither death nor any other adverse effect was observed. After a week time, for the development and aggravation of diabetes, rats with moderate diabetes (i.e. fasting blood glucose concentration, >250 mg/dl) that exhibited hyperglycemias and glycosuria were selected for further experimentation.

Experimental Design

The rats were grouped into 4 groups, comprising of 6 rats in each group as follows:

- Group I** : Control rats
- Group II** : STZ induced diabetic Rats.
- Group III** : Diabetic Rats treated with *Cocos nucifera flowers* extract (300 mg/Kg Body weight/day) in aqueous solution orally for 30 days.
- Group IV** : Diabetic Rats treated with gliclazide (5mg/Kg body weight/day) in aqueous solution orally for 30 days.

During the experimental period, body weight and blood glucose levels of all the rats were determined at regular intervals. At the end of the experimental period, the rats were fasted over night, anaesthetized, and sacrificed by cervical decapitation. The blood was collected with or without anticoagulant for plasma or serum separation respectively.

The liver and pancreatic tissues were dissected out and washed in ice-cold saline, which is then used for further experimental studies.

Oral Glucose Tolerance Test (OGTT)

At the end of the experimental period, fasting blood samples were taken from all the groups of rats to perform

oral glucose tolerance test. Four more blood samples were collected at 30, 60, 90 and 120 min intervals after an oral administration of glucose solution at a dosage of 2g kg⁻¹ body weight. All the blood samples were collected with EDTA for the estimation of glucose.

Biochemical parameters

Blood glucose level was estimated by the method of glucose oxidase/peroxidase method¹⁶ using a commercial kit (Span Diagnostic Chemicals, India) and urea.¹⁷ Glycosylated hemoglobin was estimated.¹⁸ Plasma was used for protein assay. Urine sugar was detected using urine strips. Serum was used for the determination of creatinine¹⁹ and uric acid.²⁰ Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatase (ALP) were assayed by the method of King (1965(a, b)).^{21, 22}

Preparation of Tissue Homogenate

The liver and pancreatic tissues were excised, rinsed in ice- cold saline. Known amount of the tissues were homogenized in Tris-HCl buffer (100 mM, pH 7.4) at 4°C, in a Potter-Elvehjem homogenizer with a Teflon pestle at 600 rpm for 3 min. The homogenate was then centrifuged at 12,000-xg for 30 min at 4°C. The supernatant was collected as tissue homogenate, which was used to assay various parameters. The protein content in the tissue homogenate was estimated by the method of Lowry et al., (1951).²³ A portion of wet liver tissue was used for the estimation of glycogen content²⁴ Glycogen synthase²⁵ and glycogen phosphorylase²⁶ were assayed in liver tissues.

Assay of antioxidant status

The levels of lipid peroxides were determined in plasma and tissue homogenate.^{27,28} The activities of enzymatic antioxidants such as SOD, catalase and GPx were assayed in the tissue homogenate of control and experimental groups of rats.²⁹⁻³¹ The levels of nonenzymatic antioxidants such as vitamin C, vitamin E, and GSH were also determined.³²⁻³⁴

Statistical Analysis

The values were expressed as mean ± S.D for six rats in each group. All data were analyzed with SPSS/16.0 student software. Hypothesis testing method included one way analysis of variance (ANOVA) followed by post hoc testing performed with least significant difference (LSD) test. A Value of P < 0.05 was considered as significant.

RESULTS

From the preliminary phytochemical evaluation, it was found that the *Cocos nucifera* flowers extract found to contain alkaloids, flavonoids, Saponins, tannins, diterpenes, triterpenoids, glycosides and phenols.

Figure 1 shows the initial and final body weight changes in control and experimental group of animals. The body weight of control rats was gradually increased whereas



there was a significant decrease in the body weight of STZ induced diabetic rats. Diabetic rats treated with *Cocos nucifera* flowers extract and Gliclazide for 30 days showed a significant improvement in body weight indicating the beneficial effect of the flowers extract in controlling muscle wasting.

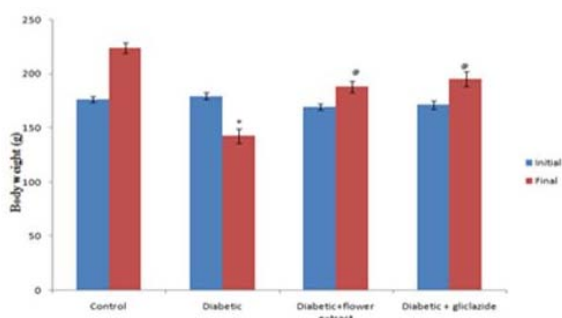


Figure 1: Effect of *Cocos nucifera* flowers extract on changes in body weight of experimental groups of rats after 30 days treatment

Values are given as mean \pm SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [®] compared with diabetic rats.

Figure 2 depicts the levels of blood glucose in certain durations after the oral administration of glucose (2g/Kg body weight) in normal and experimental group of rats. In control rats, the blood glucose level reached the maximum peak at 60 min after an oral glucose load and then it was gradually reverted back to near normal levels after 120 min. In STZ-induced diabetic rats the peak increase in blood glucose concentration was observed after 60 min and remained high over the next 60 min. Oral administration of the extract on STZ-induced diabetic rats showed significant decrease in blood glucose concentration at 60 and 120 min when compared with diabetic control suggesting improved glucose homeostasis.

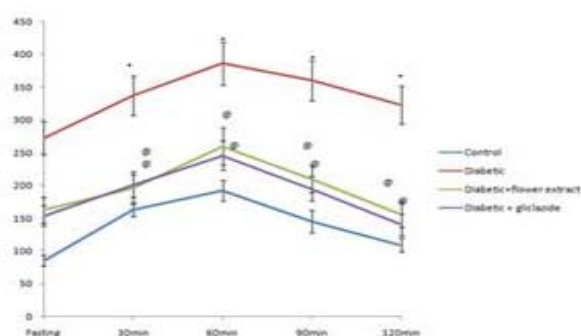


Figure 2: Effect of *Cocos nucifera* on the blood glucose level in the experimental groups of rats after receiving an oral glucose load

Units: mg/dl; Values are given as mean \pm SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [®] compared with diabetic rats.

Table 1 depicts the effect of fruit extract on the levels of biochemical parameters. STZ induced diabetic rats showed a significant elevation in the levels of blood glucose, presence of urine sugar and a simultaneous increase in Glycosylated haemoglobin. Oral administration of ethanolic extract of *Cocos nucifera* flowers to the diabetic group of rats significantly reduced the levels of blood glucose and Glycosylated haemoglobin. Urine sugar which was present in the diabetic group of rats was found to be absent in rats treated with the flowers extract. In STZ induced diabetic rats, there was a significant decrease in the total protein and increase in the levels of urea, uric acid and creatinine when compared with the control group of rats. Administration of an ethanolic extract of *Cocos nucifera* flowers as well as the standard drug, gliclazide to the diabetic group of rats significantly decreased the levels of blood urea, uric acid, serum creatinine and increased the levels of total protein.

Table 2 depicts the levels the levels of glycogen content and activities of glycogen synthase and glycogen phosphorylase in liver tissues control and experimental groups of rats. A significant decline in the glycogen level as well as in the glycogen synthase activity and a concomitant increase in the activity of glycogen phosphorylase were noted in the liver of diabetic group of rats. Oral administration of *Cocos nucifera* flowers extract as well Gliclazide to diabetic rats restored the level of glycogen and the activities of glycogen synthase, glycogen phosphorylase to near normalcy when compared to control group of rats.

Table 3 depicts the levels of aspartate transaminase, alanine transaminase and alkaline phosphatase in the control as well as experimental group of rats. Diabetic rats showed a significant elevation in the levels of aspartate transaminase, alanine transaminase and alkaline phosphatase when compared with the control group of rats. Administration of *Cocos nucifera* flowers extract and Gliclazide to the diabetic rats resulted in a significant decrease in the levels of these marker enzymes. The levels of TBARS in the plasma and pancreas of control and experimental group of rats are presented in Table 4. STZ induced diabetic rats showed marked increase in the levels of TBARS when compared to control rats. Treatment of *Cocos nucifera* flowers extract to diabetic rats showed a significant decrease in the levels of TBARS.

Table 5 and 6 illustrates the activities of enzymatic and non enzymatic antioxidants in pancreas and plasma of control and experimental group of rats. In STZ induced diabetic rats, there was a significant reduction in the activities of enzymatic and non enzymatic antioxidants in pancreas and plasma respectively Treatment of *Cocos nucifera* flowers extract to the diabetic rats showed an improvement in the activities of both enzymatic and non enzymatic antioxidants.

Table 1: Effect of *Cocos nucifera* flowers extract on the levels of biochemical parameters in the experimental groups of rats.

Groups	Glucose (mg/dl)	Glycosylated hemoglobin (%)	Protein (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)	Urine sugar
Control	92.55 ± 10.15	6.78 ± 1.58	8.15 ± 1.96	30.28 ± 4.10	0.98 ± 0.12	2.64 ± 0.84	Nil
Diabetic	286.69 ± 25.94*	12.95 ± 2.93*	5.72 ± 0.98*	51.45 ± 6.73*	2.26 ± 0.51*	5.19 ± 1.47*	+++
Diabetic + <i>Cocos nucifera</i> extract	149.96 ± 19.81 [®]	8.91 ± 1.97 [®]	6.99 ± 1.12 [®]	38.16 ± 5.94 [®]	1.40 ± 0.26 [®]	3.42 ± 1.04 [®]	Nil
Diabetic + gliclazide	156.10 ± 16.95 [®]	7.95 ± 2.14 [®]	7.18 ± 1.30 [®]	35.84 ± 4.97 [®]	1.51 ± 0.31 [®]	3.56 ± 1.27 [®]	Nil

Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [®] compared with diabetic rats.

Table 2: Effect of *Cocos nucifera* flowers extract on the levels of liver glycogen content and activities of glycogen metabolizing enzymes in the experimental groups of rats.

Groups	Glycogen	Glycogen synthase	Glycogen phosphorylase
Control	45.82 ± 4.78	825.67 ± 59.77	598.43 ± 42.10
Diabetic	20.98 ± 2.51*	568.91 ± 32.79*	855.90 ± 56.14*
Diabetic + <i>Cocos nucifera</i> extract	36.42 ± 4.95 [®]	725.36 ± 40.17 [®]	692.37 ± 38.90 [®]
Diabetic + gliclazide	31.63 ± 3.57 [®]	750.68 ± 48.66 [®]	668.30 ± 35.83 [®]

Units are expressed as: mg/g wet tissue for glycogen, μ moles of UDP formed/h/mg protein for glycogen synthase and μ moles of Pi liberated/h/mg protein for glycogen phosphorylase. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [®] compared with diabetic rats.

Table 3: Effect of *Cocos nucifera* flowers extract on the activity of AST, ALT and ALP in the serum of experimental groups of rats.

Groups	AST	ALT	ALP
Control	62.59 ± 9.86	23.47 ± 3.96	92.46 ± 12.08
Diabetic	130.97 ± 19.75*	52.39 ± 9.85*	175.28 ± 21.47*
Diabetic + <i>Cocos nucifera</i> extract	86.72 ± 12.64 [®]	32.95 ± 5.77 [®]	109.48 ± 19.94 [®]
Diabetic + gliclazide	94.50 ± 15.37 [®]	35.42 ± 4.82 [®]	104.56 ± 18.75 [®]

The enzyme activities are expressed as: AST and ALT μ moles of pyruvate liberated /h/mg of protein; ALP μ moles of phenol liberated/min/mg of protein. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [®] compared with diabetic rats.

DISCUSSION

STZ induced diabetes is similar to humans in many features. Streptozotocin is a cytotoxin specific to the pancreatic beta cells in mammals. STZ injection leads to the degeneration of the beta cells and is irreversible when compared to alloxan. The mechanism behind is that

STZ is preferentially up taken by pancreatic beta cells via GLUT2 transporter and causes DNA alkylation followed by the activation of poly ADP ribosylation leading to depletion of cytosolic concentration of NAD⁺ and ATP. Enhanced ATP dephosphorylation after STZ treatment supplies substrate for xanthine oxidase resulting in the formation of superoxide radicals and also NO moiety is liberated from STZ leading to the destruction of B cells by necrosis.³⁵ In the present study, STZ successfully induced experimental diabetes in all the rats.

Table 4: Effect of *Cocos nucifera* flowers extract on the level of TBARS in plasma and pancreas of experimental groups of rats.

Groups	TBARS	
	Plasma	Pancreas
Control	3.51 ± 0.95	38.39 ± 6.07
Diabetic	8.12 ± 2.26*	79.46 ± 16.71*
Diabetic + <i>Cocos nucifera</i> extract	5.11 ± 1.95 [®]	50.34 ± 12.76 [®]
Diabetic + gliclazide	5.38 ± 1.77 [®]	47.91 ± 15.20 [®]

Units: mM/100 g in tissues; nM/ml in plasma. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [®] compared with diabetic rats.

Table 5: Effect of *Cocos nucifera* flowers extract on the activity of SOD, Catalase and GPx in pancreas of experimental groups of rats.

Groups	SOD	Catalase	GPx
Control	4.73 ± 1.56	16.82 ± 3.99	6.42 ± 1.76
Diabetic	1.86 ± 0.91*	6.72 ± 2.05*	3.79 ± 0.93*
Diabetic + <i>Cocos nucifera</i> extract	3.54 ± 1.35 [®]	12.37 ± 2.16 [®]	4.75 ± 1.09 [®]
Diabetic + gliclazide	3.18 ± 1.05 [®]	12.95 ± 2.44 [®]	4.96 ± 1.28 [®]

Activity is expressed as: 50% of inhibition of epinephrine autooxidation/min/mg of protein for SOD; μ moles of hydrogen peroxide decomposed/min/mg of protein for catalase; μ moles of glutathione oxidized/min/mg of protein for GPx; mg/100 g tissue for GSH. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [®] compared with diabetic rats.



Table 6: Effect of *Cocos nucifera* flowers extract on the levels of vitamin C, vitamin E, ceruloplasmin and GSH in plasma of experimental groups of rats.

Groups	Vitamin C	Vitamin E	Ceruloplasmin	GSH
Control	1.64 ± 0.35	0.68 ± 0.11	12.85 ± 2.46	24.92 ± 4.67
Diabetic	0.59 ± 0.16*	0.35 ± 0.09*	5.12 ± 1.41*	14.08 ± 2.55*
Diabetic + <i>Cocos nucifera</i> extract	1.26 ± 0.19 [®]	0.52 ± 0.10 [®]	7.26 ± 1.95 [®]	19.64 ± 3.14 [®]
Diabetic + gliclazide	1.18 ± 0.15 [®]	0.55 ± 0.11 [®]	8.14 ± 2.26 [®]	20.12 ± 3.27 [®]

Units: mg/dl. Values are given as mean ± SD for groups of six rats in each. Values are statistically significant at $p < 0.05$. Statistical significance was compared within the groups as follows: *compared with control, [®] compared with diabetic rats.

Most plants with antidiabetic properties have been found to contain secondary metabolites such as glycosides, alkaloids and flavonoids.³⁶ It has been shown that many plants exhibit efficient antioxidant properties owing to their phenolic constituents. Flavonoids are the polyphenolic compounds that act as primary antioxidants or free radical scavengers.³⁷ Plant alkaloids have the tendency to release insulin from pancreatic beta cells and also have the potential to protect islets from hyperglycemia mediated oxidative stress.

Diabetic rats showed a clear muscle atrophy involving a decrease in both skeletal muscle mass and protein content. This was accompanied by marked loss of total carcass nitrogen. These changes are related to important alterations in the protein turnover in skeletal muscle.³⁸ Diabetic rats treated with *Cocos nucifera* flowers extract and gliclazide for 30 days showed a significant improvement in body weight indicating the beneficial effect of the flowers extract in controlling muscle wasting.

The oral glucose tolerance test is a more sensitive measure of early abnormalities in glucose regulation than fasting plasma glucose or glycosylated haemoglobin.³⁹ Impaired glucose tolerance reflects hepatic gluconeogenesis and reduced uptake of glucose from blood into skeletal muscles and adipose tissue following a meal.⁴⁰ Impaired glucose tolerance serves as a marker for the state of insulin resistance and predicts both large and small-vessel vascular complications.⁴¹ In STZ induced diabetic rats, the blood glucose level increased to peak at 60 min and remained high over the next 60 min. *Cocos nucifera* flowers extract treated diabetic rats showed an increase at 60 min and then a reduction in peak was observed at 120 min which finally exhibited the near normal range of control rats. The results obtained from GTT indicate the improved glycemic control in diabetic rats treated with the flowers extract.

Hyperglycaemia is a significant factor in the development and progression of the complications of diabetes mellitus.⁴² Experimentally induced diabetes leads to pancreatic β cell necrosis that is caused due to diabetic oxidative stress. As a result of pancreatic beta cell necrosis, insulin deficiency predominates resulting in repression of glycolytic enzymes and expression of gluconeogenic enzymes which promotes gluconeogenesis in liver, and decreased utilization of glucose by the peripheral tissues contributes to hyperglycemia.⁴³ The

elevated blood glucose level observed in the diabetic rats was significantly decreased in extract treated rats suggesting the antihyperglycemic nature of the extract.

In the persistent chronic hyperglycaemic state, the excess blood glucose non-enzymatically reacts with haemoglobin to form Glycosylated haemoglobin, which is a standard reliable marker for the diagnosis of ambient glycaemia for a period of 90 days.⁴⁴ The observed increase in the level of Glycosylated haemoglobin in the experimental diabetic rats implies the oxidation of sugars, extensive damage to both sugars and proteins in the circulation, vascular wall and lens proteins, continuing and reinforcing the cycle of oxidative stress and damage.⁴⁵ Oral administration of *Cocos nucifera* flowers extract to diabetic group of rats reduced the formation of Glycosylated haemoglobin by virtue of its normoglycemic activity.

Hyperglycaemia and glycosuria are the most critical abnormalities in diabetes. Therefore, the hypoglycaemic effect and consequent decrease in urine sugar excretion have been treated as one of the essential characteristics of anti-diabetic agents.⁴⁶ Oral administration of *Cocos nucifera* flowers extract to diabetic rats showed the absence of urine sugar due to the normalization of blood glucose levels.

Experimentally induced diabetic model indicates several alterations of amino acid metabolism, which may be attributed to increased muscle proteolysis, reduced protein synthesis, an energy-dependent process in the liver, and stimulated hepatic gluconeogenesis utilizing gluconeogenic amino acids.⁴⁷ This readily accounts for observed decrease in the total protein content in diabetic rats. Administration of *Cocos nucifera* flowers extract to diabetic rats significantly inhibits proteolysis caused by insulin deficiency and improves total protein level.

Creatinine is a byproduct of the breakdown of creatine and phosphocreatine, which are considered as an ability of the kidney to excrete creatinine. An elevation in creatinine usually occurs simultaneously with an increase in blood urea nitrogen. In the present study, the oral treatment with the flowers extract for 30 days significantly reduced the serum creatinine level indicating an improved renal function.

Urea is the main end product of protein catabolism in the body. Accumulation of urea nitrogen in experimental diabetes may due to the enhanced breakdown of both

liver and plasma proteins.⁴⁸ Alterations in nitrogen homeostasis may lead to increased hepatic elimination of urea nitrogen and increased peripheral release of nitrogenous substances. Thus, the observed negative nitrogen balance may partly be because of changes occurring within the hepatocytes. Oral administration of the flowers extract to diabetic rats significantly decreased the altered levels of blood urea. The protein glycation during diabetes is associated with muscle wasting and thereby, an increased release of purines. The elevated levels of purine nucleotides are the main source of uric acid, a water soluble antioxidant, by the increased activity of xanthine oxidase.⁴⁹ Oral administration of *Cocos nucifera* flowers extract decreased the levels of blood urea, serum creatinine and uric acid in STZ-induced diabetic group of rats, thereby eliciting the tissue protective nature.

Liver plays an important role in buffering postprandial hyperglycaemia and is involved in the synthesis of glycogen. Diabetes mellitus is known to impair the normal functioning of the liver to synthesize glycogen.⁵⁰ Glycogen is the primary intracellular storable form of glucose and its level in various tissues are a direct indication of insulin activity as insulin promotes the deposition of intracellular glycogen by stimulating glycogen synthase and inhibiting glycogen phosphorylase activities. Thus, assessment of glycogen level serves as a marker for the evaluation of antidiabetic activity. Moreover, glycogen synthase and glycogen phosphorylase are the two key regulatory enzymes that catalyze glycogenesis and glycogenolysis, respectively. Further, the decreased glycogen content in diabetic condition is due to the increased activity of glycogen phosphorylase and decreased activity of glycogen synthase.⁵¹ Oral administration of flowers to diabetic rats restored the glycogen content and the activities of glycogen metabolizing enzymes demonstrating the possible role of the flowers extract in the regulation of glycogen metabolism.

The pathophysiological enzymes such as ALT and AST are the intracellular enzymes that have escaped into the blood stream and serve as a clinical index of tissue injury chiefly hepatocyte as well as renal injury. ALP acts as a marker of biliary function and cholestasis. It is assumed that elevation in the levels of serum ALT, AST and ALP are considered as predictors of diabetes.⁵² Further, the elevation in the levels of these gluconeogenic enzymes such as ALT and AST, whose gene transcription is suppressed by insulin, could indicate impairment in insulin signalling rather than purely tissue injury. Other potential explanations for elevated aminotransferase in insulin-resistant states include oxidative stress from reactive lipid peroxidation, peroxisomal β -oxidation and recruited inflammatory cells. The increased activities of ALT, AST and ALP in the serum of diabetic rats may be primarily due to the leakage of these enzymes from liver as well as kidney into blood stream.⁵³ Oral administration of flowers extract to diabetic group of rats showed a notable decline in the activity of these enzymes to their

basal levels, signifying its non toxic as well as tissue protective nature.

The determination of malondialdehyde (MDA) affords a convenient index of lipid peroxidation.⁵⁴ and quantitatively determines the damage that the free radicals cause to cells. Lipid peroxidation may result in several sequelae, including structural and functional membrane modifications, protein oxidation and generation of oxidation products. Lipid peroxidation products are unstable, cytotoxic and highly reactive, leading to free radical damage to proteins and DNA and finally cause various diabetes-mediated complications. The degree of tissue damage persuaded by free radicals depends on the balance between free radical generation and the endogenous antioxidant defence mechanism.⁵⁵ In the present investigation, oral administration of the flowers extract to experimental diabetic rats significantly reinstates the elevated lipid peroxidation to near normalcy. Thus these results suggest that *Cocos nucifera* flower extract improves oxidative damage moderately by scavenging free radicals.

Persistent hyperglycaemia causes increased oxidative stress, which contributes to the development and progression of most of the diabetes-associated complications. Oxidative stress is an imbalance between the generation of reactive oxygen species (ROS) and antioxidant defence capacity of the body. The reduced antioxidant capacity potentially makes pancreatic β -cells sensitive to ROS mediated signal transduction and cellular response. Thus, maintenance of β -cell oxidant status and their protection against oxidative damage might delay the onset of diabetes as well as the progression of its complications.

The activities of enzymatic antioxidants and the levels of non-enzymatic antioxidants in diabetic group of rats were found to be decreased. Enzymatic antioxidants are involved in detoxification of free radicals and peroxides formed during oxidative stress, including diabetes. Enzymatic antioxidants such as SOD, CAT and GPx, are crucial cellular components of antioxidant defense system in the body, thus playing a crucial role in the maintenance of a balanced redox status.⁵⁶ The enzymatic antioxidants were found to be decreased in diabetic rats due to hyperglycaemia mediated oxidative stress.⁵⁷

The non-enzymatic antioxidants such as Vitamin C, E, ceruloplasmin and reduced glutathione are found to be decreased in diabetic state due to their free-radical scavenging property.⁵⁸ The declined levels of the non enzymatic antioxidants due to the increased production of free radicals during hyperglycaemia mediated oxidative stress.⁵⁹ Oral administration of *Cocos nucifera* flowers extract to diabetic group of rats showed improved status of non-enzymatic antioxidants suggesting the free radical scavenging potential of *Cocos nucifera* flowers extract. Naskar et al., (2011)⁶⁰ have reported the antioxidant properties of *Cocos nucifera*.

CONCLUSION

The results of the present study indicate that *Cocos nucifera* flowers extract possess antidiabetic as well as antioxidant properties. Phytochemical screening indicated the presence of pharmacologically active ingredients in the flowers. The results of OGTT clearly indicate the improved glycemic status upon treatment with flowers extract. The change in body weight gain indicates the beneficial effect of the flowers in controlling muscle wasting. The results of the biochemical analysis illustrated that the flowers extract normalizes the biochemical alterations. The activities of pathophysiological enzymes were normalized upon flowers extract treatment indicating its non toxic nature. The study on antioxidant status indicates the antioxidant property of the flowers extract. In conclusion, the observed antidiabetic as well as antioxidant property of *Cocos nucifera* flowers extract provides a scientific rationale for the use of flowers in the traditional medicine for the treatment of various ailments. The isolation and identification of active phytochemicals responsible for the observed pharmacological activities will throw more light on the possibility of considering the *Cocos nucifera* flowers as a potent source of novel lead molecules.

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