EVALUATION OF ANTIDIABETIC POTENTIAL OF ETHANOLIC AND AQUEOUS EXTRACT OF COCOS NUCIFERA ENDOCARP

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ABSTRACT

The present study was aimed to investigate the effect of ethanolic and aqueous extract of Cocus nucifera endocarp on blood glucose level. The extracts were prepared and analysed separately for the present phytoconstituents. Total Phenolics contents were estimated and were found 17.2 mg (CNAE) and 21.4 mg (CNEE) gallic acid equivalent in ethanolic and aqueous extract of Cocos nucifera respectively, while the estimated total present flavonoid contents were 23.71 mg (CNAE) and 37.57 mg (CNEE) rutin equivalent in respective extract. The effect on blood glucose level was studied using streptozotocin induced diabetes. Ethanolic extract of cocus nucifera posses highest antidiabetic potential than aqueous extract of cocus nucifera.

KEYWORDS: Cocus nucifera, Antidiabetic,

INTRODUCTION

Cocos nucifera L. (Family Arecaceae) commonly known as coconut, is considered as an important fruit crop in the tropical countries. The coconut, Cocos nucifera L., has been described as "the tree of life" or the tree of plenty and nature’s greatest gift to man. Cocos nucifera is a large palm, growing up to 30 meters (98 ft) tall, with pinnate leaves 4–6 meters (13–20 ft) long and pinnate 60–90 cm long; old leaves break away cleanly, leaving the trunk smooth (Grimwood, 1975). The fruit is a fibrous drupe. It consists of a thin hard skin (exocarp), a thicker layer of fibrous mesocarp (husk), the hard endocarp (shell), the white endosperm (kernel) and a large cavity filled with liquid (water). The seed comprises the dark brown shell and kernel. The surrounding husk, which is brown and dry at maturity, always remains intact (Edward Chan & Craig R. E., 2006). Unlike some other plants, the palm tree has neither tap root nor root hairs; but has a fibrous root system. Leaves
pinnate, feather shaped, 4-7m long and 1-1.5 m wide at the broadest part. Leaf stalks 1-2 cm in length and thorn less (Coble L.S & Steele W.M, 1976). Leaves are among the largest of any plant (up to 20 ft), pinnately compound with 200 or more leaflets, and borne in a spiral arrangement at the apex of the trunk (Orwa et al, 2009).

The coconut plants have a number of uses. It is being used since longer time as a source of timber, food, fermented and unfermented drink, alcohol, vinegar, thatching materials, splints, strips and fibers for making baskets, mats, rope, hats, brushes, brooms and other articles like utensils for household use (such as cups, bowls, spoons). At the same time it is also used for cooking, illumination, for making soap, substitutes for butter and lard, ointments and oil cake for feeding domestic animals and for fertilizers. The palm is ornamental and is frequently planted for decorative (Sivakumar et al, 2011). Along with these it has several beneficial health effects like antitumor, antihelmintic, antidotal, antiseptic, aphrodisiac, astringent, bactericidal, depurative, diuretic, refrigerant, stomachic, styptic, suppurative, vermifuge, antioxidant, antihypertensive and vasorelaxant (Singla et al, 2011).

Diabetes mellitus refers to the group of diseases that lead to high blood glucose levels due to defect in either insulin secretion or in its action (Guyton and Hall, 2002). Without enough insulin, the cells of body cannot absorb sufficient glucose from the blood and hence blood glucose level raises i.e. hyperglycemia. If the glucose level in the blood remains high over for a longer period, this can result in long term damage to organs, such as the kidney, liver, eyes, nerves, heart and blood vessels. Complication of some of these organs can lead to death also (Pari and Saravanan, 2004).

MATERIAL AND METHODS

Plant Material

_Cocos nucifera_ fruits were collected from local market of Rohtak (Haryana, India) and endocarp was separated manually and was authentified from Department of Botany, University of Rajasthan, Rajasthan viz. RUBL 21099. the voucher specimen plant material was deposited in concern department. The endocarp of _Cocos nucifera_ was dried under shade at room temperature (25± 2°C). The endocarp was powdered manually to protect thermolabile material and was passed through sieve no. 40 and were subjected to ethanolic extraction and aqueous extraction in soxhlet assembly for 72 h at 60°C. The excess of solvent was removed. The collected mass from extraction of ethanolic extraction of _Cocos nucifera_ (yield: 2.5%) and aqueous extraction of _Cocos nucifera_ (yield: 4%) was respectively termed as extract.
Chemical and reagents

All the chemicals used for the research work were of analytical grade and the reagents used for phytochemical screening were freshly prepared. Streptozotocin (Hi-Media), Ethanol (Merck Chemicals) Rutin (Sigma Aldrich), Folin-Ciocalteu’s phenol reagent (Fisher scientific) and Gallic acid (Hi-Media) were incorporated in study.

Physico-Chemical Evaluation

Preliminary Phytochemical screening

Preliminary phytochemical screening of extracts were performed separately to detect the phytoconstituents and the result data revealed the presence of carbohydrates, phenols and flavonoids in both the extracts.

Estimation of Total Phenolic Contents

The Total Phenols in all three extracts were measured at 765 nm by Folin-ciocalteu’s reagent method (Singleton & Rossi, 1965).

Estimation of Total Flavonoid

The total flavonoid content was determined with aluminium chloride (AlCl3) using rutin as a standard at 510 nm (Zhishen et al, 1999).

Pharmacological study

Animals

Preliminary phytochemical Wistar rats of either sex (150–200 g) were used for the experimental study. The animals were maintained under standard husbandry conditions of temperature (25 ± 2) °C, 12 h light/dark cycle in polypropylene cages and provided with standard pellet diet and water ad libitum. Animals were fasted overnight prior to the experiment.

Effect of Cocos nucifera endocarp extracts (aqueous & ethanolic) in normal rats (Siddaiah et al, 2011)

The study was carried out to test the effect of Cocos nucifera endocarp extracts i.e. aqueous (CNAE) & ethanolic (CNEE) on the blood sugar levels in normal rats. The rats weighing 150–250 g were divided into six groups of four animals each. The animals were fasted overnight before the experiment but were allowed free access to water. Group I was treated with normal saline and served as normal control. Groups II & III were treated with CNAE
orally at doses of 200 and 400 mg/kg body weight, respectively. Group IV & V were treated with CNEE orally at doses of 200 and 400 mg/kg body weight, respectively. Group VI was administered with glibenclamide (5 mg/kg). Blood glucose levels (BGLs) were determined using One Touch Glucometer (Mankind Pvt. Limited) at different time intervals, viz. 0 (before drug administration) and 1, 2 and 4 hrs after drug administration.

**Effect of Cocos nucifera endocarp extracts (aqueous & ethanolic) on blood glucose level against streptozotocin induced diabetic rats**

**Induction of diabetes in animals**
Streptozotocin was freshly prepared in 10mmol/citrate buffers, pH 4.5. Male wistar rats weighing 150–220 g fed with standard diet were injected with 60 mg/kg streptozotocin i.p. Initially, blood glucose level was increased, reaching values of 150–200 mg after 3 h. Six–eight h after streptozotocin, the serum insulin values were increased up to 4 times, resulting in a hypoglycemic phase which was followed by persistent hyperglycemia. After 72 hrs of streptozotocin administration, the blood glucose levels were measured and the rats showing blood glucose level > 250 mg/dl were considered to be diabetic and were used in the study.

**Effect of Cocos nucifera endocarp extracts (aqueous & ethanolic) in streptozotocin induced diabetic rats (Naskar et al, 2011)**

The study was carried out to test the effect of *Cocos nucifera* endocarp extracts i.e aqueous (CNAE) & ethanolic (CNEE) on the blood glucose levels in streptozotocin induced diabetic rats. 28 healthy wistar rats (weighing 150–250 g) were divided into seven groups of four animals each. The animals were fasted overnight before the experiment and were allowed free access to water. Nondiabetic rats (n = 4) treated with normal saline (0.5 ml/kg) were considered as normal control; Group I. Diabetic rats (n = 24) were randomly divided into six groups of four rats each. Group II was treated with normal saline (0.5 ml/kg) and served as the diabetic control. Group III & IV animals were treated with CNAE at dose levels of 200 and 400 mg/kg orally, respectively. Group V & VI animals were treated with CNEE at dose levels of 200 and 400 mg/kg orally, respectively. Group VII was treated with glibenclamide (5 mg/kg). All the animals were treated for 21 days. Glucose levels were measured on day 0, i.e. just prior to the initiation of any treatment, on day 7th, 14th day & 21th day using One Touch Glucometer (Mankind Pvt. Limited).

**STATISTICAL ANALYSIS**
Results are expressed as mean ± SEM. Statistical significance was determined using one way
ANOVA followed by Dunnett’s multiple comparison test. p < 0.5 and p < 0.001 was considered significant and highly significant respectively Limited).

RESULT AND DISCUSSION

PHYSICO-CHEMICAL STUDY RESULTS

Total Phenol Estimation
The standard curve equation was; \( y = 0.0085x + 0.0038; \) \( R^2 = 0.998 \). The total phenolic content of aqueous and ethanolic extract of *Cocos nucifera* endocarp were contained 17.2 mg (CNAE) and 21.4 mg (CNEE) gallic acid equivalent, respectively.

Total Flavonoid Estimation
The standard curve equation was found; \( y = 0.0062x + 0.0012; \) \( R^2 = 0.9986 \). The total flavonoid contents (Rutin equivalents, mg/g) aqueous and ethanolic extract of *Cocos nucifera* endocarp were contained 23.71 mg (CNAE) and 37.57 mg (CNEE) .

PHARMACOLOGICAL SCREENING

EFFECT OF *COCOS NUCIFERA* ENDOCARP ON NORMAL BLOOD GLUCOSE LEVEL

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose (mg/dl)</th>
<th>0 hr</th>
<th>1 hr</th>
<th>2 hrs</th>
<th>4 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Normal control</td>
<td>Normal saline</td>
<td>84.5±1.9</td>
<td>82±2.04</td>
<td>81.25±1.7</td>
<td>82.5±2.06</td>
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</tr>
<tr>
<td>II</td>
<td>Aqueous extract</td>
<td>200 mg/kg</td>
<td>85.75±1.1</td>
<td>77.5±1.1</td>
<td>73.5±1.5</td>
<td>76±1.4**</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>Aqueous extract</td>
<td>400 mg/kg</td>
<td>83.25±1.5</td>
<td>74.5±2.1</td>
<td>70±1.3**</td>
<td>72.75±1.6***</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Ethanolic extract</td>
<td>200 mg/kg</td>
<td>85.5±1.7</td>
<td>74±1.9**</td>
<td>68.75±2.6</td>
<td>70.25±1.5**</td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Ethanolic extract</td>
<td>400 mg/kg</td>
<td>85.5±2.2</td>
<td>71±2.6***</td>
<td>65±2.6***</td>
<td>67.25±2.5***</td>
<td></td>
</tr>
<tr>
<td>VI</td>
<td>Glibenclamide</td>
<td>5 mg/kg</td>
<td>86.75±1.7</td>
<td>69±1.25***</td>
<td>60.25±1.75***</td>
<td>63.5±1.85***</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; n=4; **P<0.01, ***P<0.001 vs. control group at the respective hour; one way ANOVA followed by Dunnett’s multiple comparisons test; CNAE, aqueous extract of *Cocos nucifera* endocarp; CNEE, ethanolic extract of *Cocos nucifera* endocarp.
Fig 5: Effect of aqueous and ethanolic extract of Cocos nucifera endocarp on blood glucose levels in normal rats.

The effect of CNAE and CNEE extracts on blood glucose level in normal rats was measured on 0, 1, 2 and 4hr and compared with normal control groups. Both the extracts showed significant (P<0.01, P<0.001) decline in the blood glucose level of normal rats. In normal rats, administration of CNAE (200 mg/kg) showed 9.62%, 14.29%, 11.37% and CNAE (400 mg/kg) showed 10.51%, 15.91%, 12.61% decline in blood glucose levels on 1, 2 & 4hr respectively. CNEE (200 mg/kg) administration showed 13.45%, 19.59%, 17.83% and CNEE (400 mg/kg) showed 16.96%, 23.97%, 21.34% decline in blood glucose levels on 1, 2 & 4hr respectively.

EFFECT OF COCOS NUCIFERA ENDOCARP ON BLOOD GLUCOSE LEVEL OF STREPTOZOTOCIN INDUCED DIABETIC RATS

Table 8: Effect of aqueous and ethanolic extract of Cocos nucifera endocarp on blood glucose levels in streptozotocin induced diabetes rats.

<table>
<thead>
<tr>
<th>S.No</th>
<th>Group</th>
<th>Treatment</th>
<th>Blood glucose (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0 day</td>
</tr>
<tr>
<td>I</td>
<td>Normal control</td>
<td>Normal saline</td>
<td>87.5 ±1.6</td>
</tr>
<tr>
<td>II</td>
<td>Diabetic control</td>
<td>Normal saline</td>
<td>262 ±5.3</td>
</tr>
<tr>
<td>III</td>
<td>Aqueous extract</td>
<td>200 mg/kg</td>
<td>278.75 ±5.4</td>
</tr>
<tr>
<td>IV</td>
<td>Aqueous extract</td>
<td>400 mg/kg</td>
<td>280.75 ±2.9</td>
</tr>
<tr>
<td>V</td>
<td>Ethanol extract</td>
<td>200 mg/kg</td>
<td>282.25 ±3.8</td>
</tr>
<tr>
<td>VI</td>
<td>Ethanol extract</td>
<td>400 mg/kg</td>
<td>276.5 ±7.1</td>
</tr>
<tr>
<td>VII</td>
<td>Glibenclamide</td>
<td>5 mg/kg</td>
<td>272.5 ±6.4</td>
</tr>
</tbody>
</table>
Values are expressed as mean ± SEM; n=4; group II was compared with group I & group III-VII were compared with group II; *P<0.05, **P<0.01; one way ANOVA followed by Dunnett’s multiple comparisons test; CNAE, aqueous extract of Cocos nucifera endocarp; CNEE, ethanolic extract of Cocos nucifera endocarp.

The effect of CNAE and CNEE extracts on fasting blood glucose level was measured on 0th, 7th, 14th and 21th day of post induction and compared with normal and diabetic control groups. Streptozotocin induced diabetic rats showed a significant increase (P<0.01) in fasting blood glucose level as compared to normal rats. We observed a significant decrease (P<0.01, P<0.05) in blood glucose in CNAE & CNEE treated diabetic rats, when compared with diabetic control rats. However, CNEE showed much significant result than the CNAE. Streptozotocin induced diabetic rats administered with CNAE showed 33.09% & 41.22% decline in the blood glucose level at 200 mg/kg & 400 mg/kg respectively on 21th day where as CNEE showed 44.02% & 53.35% decline at 200 mg/kg & 400 mg/kg respectively. Standard drug (Glibenclamide, 5 mg/kg) showed 60.64% decline in the blood glucose level on 21th day. The possible mechanism of the extracts on hyperglycemic effect; may be through potentiation of pancreatic secretion of insulin from β-cell of islets and/or due to enhanced transport of blood glucose to the peripheral tissue or by other mechanism such as stimulation of glucose uptake by peripheral tissue, inhibition of endogenous glucose production or activation of gluconeogenesis in liver and muscles (Burcelain et al, 1995).
The antidiabetic activity of *Cocos nucifera* endocarp may be due to the presence of flavonoids. It was already being reported that flavonoids constitute the active biological principle of most medicinal plants with hypoglycaemic and anti-diabetic properties.

**REFERENCES**
