Evaluation of Anti-Hyperglycemic Activity of *Narengi crenulata* leaf in STZ induced Diabetic Rats

N.Ramya, K.Peddanna, Y.K.Prabhakar, Ch.Apparao*
Department of Biochemistry, Sri Venkateswara University, Tirupati-517502, Andhra Pradesh, India.

Abstract

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. Though there are various synthetic drugs available for the treatment of Diabetes Mellitus, they are associated with one or more side effects. Hence herbal drugs are gaining popularity to treat diabetes. The present study was aimed to evaluate the antihyperglycemic effects of *Narengi crenulata* leaf extracts i.e. *Narengi crenulata* methanol extract (NCME) and aqueous extract (NCAE) in streptozotocin (STZ) induced diabetic rats. Animals were divided into 6 groups (n=6). Group I (normal control), Group II (diabetic control), Group III (diabetic +NCME 500mg/Kg bw), Group IV (diabetic + NCAE 500mg/kg BW), Group V (Diabetic NCME+750mg/kg bw), and Group VI (diabetic NCAE+750mg/kg,bw). Among these extracts, the aqueous extract NCAE (Group VI) at a dose of 750 mg/kgbw. has produced significant antihyperglycemic effect when compared to the 500mg/kg bwt. dose of NCAE and the two doses of NCME. Blood glucose levels were measured after administration of the extracts at every one hour interval upto six hours in all the groups of rats.

Keywords: Diabetes, antihyperglycemic activity, herbal drugs, aqueous extract, *Narengi crenulata*.
INTRODUCTION

Diabetes Mellitus is a group of disorders of multiple etiologies characterized by chronic hyperglycemia with disturbances of carbohydrate, fat and protein metabolism resulting from the defects in insulin secretion, insulin action or both. Four clinical classes of Diabetes Mellitus were identified by WHO (World Health Organization) and ADA (American Diabetes Association)\(^1\). The prevalence of diabetes for all age groups worldwide was estimated to be 2.8% in 2000 and expected to become 4.4% in 2030. Existence of diabetes was increasing rapidly at an alarming rate\(^2\). The number of people suffering with diabetes mellitus was expected to rise from 171 million in the year 2000 to 366 million in 2030 \(^4\). Though there are various drugs available for the treatment of Diabetes Mellitus, they are associated with one or more side effects. So in order to minimize them, herbal drugs are widely used with their biologically active compounds\(^3\).

The existing modern medicines are accompanied by serious side effects such as hepatotoxicity, abdominal pain, flatulence, diarrhea and hypoglycemia etc. Drug resistance to these medicines is also reported after prolonged treatment\(^6\). Hence, investigations on hypoglycemic agents derived from medicinal plants have gained popularity in recent years. For instance, a medicinal plant, Galega officinalis, led to the discovery and synthesis of Metformin\(^7\). Therefore, plants play a major role in the discovery of new therapeutic agents which includes antioxidant, hypoglycemic and hypolipidemic agents\(^8\). Various laboratories are conducting research on these medicinal plants in a scientific manner for the development of alternative drugs and strategies for better management of diabetes. India has a recorded list of 45,000 plant species and about 7500 species of medicinal importance\(^9\). According to the ethanobotanical information reports, 800 plants were found to possess antidiabetic potential\(^10\).

Tirumala hills, are known for their rich heritage of plants and scientifically unexplored\(^11\). This area is inhabited by a number of tribes which includes The Chenchus, Nakkalas, Sugalis, Yanadis and Yerukalas. In a survey conducted by Ethnobotanists, over 35 species were found to have antidiabetic activity in the effective treatment of diabetes\(^12\). One such plant is *Narengi crenulata*. *Narengi crenulata* belongs to the family of Rutaceae commonly known as Kukkavelaga in Telugu, Bīlvaparnī/vīlvaparnī in sanskrit, Nagavilvam in tamil. It has been used as folk medicine in curing vomiting, dysentery, colic disorder etc\(^16\). There are no studies on the antidiabetic activity of *Narengi crenulata*. Hence an attempt has been made to investigate the antihyperglycemic activity of the leaves of *Narengi crenulata*.

In the present study, STZ was used to induce diabetes mellitus in rats. At low dose, STZ (50mg/kgbwt) partially destructs the β-cells, which secreted insufficient insulin causing type 2 diabetes\(^13\). It is widely accepted animal model and reported to resemble human hyperglycemic nonketotic diabetes mellitus\(^14\), is associated with renal damage, hepatotoxicity, oxidative stress and hypercholesterolemia\(^15\).

MATERIALS AND METHODS:

Leaves of *Narengi crenulata* were collected from Tirumal hills, and identified by the Taxonomist of the Herbarium, Department of Botany, S.V.University, Tirupati. A voucher specimen was deposited in the herbarium of the Department of Botany, S.V.University, Tirupati (Voucher No.2017). The powder was stored in the airtight containers and was used for the extraction of the bioactive compounds in different solvents.

Preparation of extracts:
The leaf powder of *Narengi crenulata* was extracted into the following solvents in the increasing order of polarity: Hexane, ethylacetate, 95% methanol and water. Hexane, ethylacetate and methanolic extracts were prepared by successive solvent extraction of *Narengi crenulata* leaf powder in soxhlet apparatus at 68°C-70°C. The filtrates obtained were distilled and concentrated under reduced pressure at low temperature (40°C to 45°C) in the Buchi rotavapor R-200 and finally freeze dried. All the extracts were stored at 0°C in airtight containers until needed for further studies.

Aqueous extract:
To prepare aqueous extract the *Narengi crenulata* leaf powder was soaked in distilled water (3 volumes) in a glass jar for two days at room temperature and the solvent was filtered. This was repeated 3 to 4 times until the filtrate gave no coloration. The filtrate was concentrated under reduced pressure in the Buchi rotavapor R-200 and finally freeze dried.

Induction of diabetes:
Diabetes was induced in healthy male Wistar Albino rats aged 3-4 months, with body weights 180-200g, by single intraperitoneal injection of freshly prepared Streptozotocin(50mg/kgbwt) dissolved in ice cold 0.1M, citrate buffer pH 4.5 after overnight fasting for 12 hours 17. Since STZ is capable of producing fatal hypoglycemia as a result of massive pancreatic insulin release due to the destruction of β-cells, 6 hours after STZ administration, the rats were kept for next 24 hours on 15% glucose solution to prevent hypoglycemia. Diabetes was assessed by determining the fasting blood glucose after 48 hours of injection of STZ. The blood glucose levels in STZ induced diabetic rats were increased to markedly higher levels than...
normal. After a week rats with marked hyperglycemia (FBG ≥ 250 mg/dl) were selected and used for the study. All the animals were allowed free access to tap water and pellet diet and maintained at room temperature in plastic cages, as per the guidelines of Institute Animal Ethics Committee. (Resolution No: 43/2012-2013/(i)/a/CPCSEA/IAEC/SVU/CAR-NRY dt. 08-07-2012).

**Experimental design:**

**Experimental design for the evaluation of anti-hyperglycemic activity of aqueous crude suspension of leaf powder of Narengi crenulata in STZ induced diabetic rats:** The animals were divided into three groups and each group consisted of six rats.

**Group 1:** Untreated normal rats

**Group 2:** Untreated diabetic rats

**Group 3:** Diabetic rats treated with 500 mg of aqueous crude suspension of NC leaf powder/kg bwt.

After an overnight fast the diabetic treated rat group received the crude suspension of leaf of Narengi crenulata (in 1 ml of distilled water) by gastric intubation using a force feeding needle, while the normal and untreated diabetic rats were fed distilled water alone. Blood samples were collected for the measurement of blood glucose from the tail vein at 0, 1, 2, 3, 4, 5 and 6 hr after the administration of crude suspension and blood glucose levels were determined by using dextrostix (Glucose oxidase peroxidase method) with Basic One Touch Accuchec Glucometer.

**Evaluation of anti-hyperglycemic effect of different solvent extracts of leaves of NC in STZ induced diabetic rats:** Rats were divided into 6 groups and each group consisted of six rats:

**Group 1:** Untreated normal rats

**Group 2:** Untreated diabetic rats

**Group 3:** Diabetic rats treated with 500 mg of aqueous crude suspension of NC leaf powder/kg bwt.

**Group 4:** Diabetic rats treated with 500 mg NC hexane extract/kg bwt.

**Group 5:** Diabetic rats treated with 500 mg NC ethylacetate extract/kg bwt.

**Group 6:** Diabetic rats treated with 500 mg NC aqueous extract/kg bwt.

After an overnight fast different groups of diabetic rats received the hexane extract (dissolved in 1 ml of 5% Tween 80) or ethyl acetate or methanol or aqueous extracts (dissolved in 1 ml of distilled water) by gastric intubation using a force feeding needle while the untreated normal and diabetic rats were fed distilled water alone. Blood samples were collected for the measurement of blood glucose from the tail vein at 0, 1, 2, 3, 4, 5 and 6 hr after the administration of NC extracts and blood glucose levels were determined by using dextrostix (Glucose oxidase peroxidase method) with Basic One Touch Accuchec Glucometer.

**Effect of NCAE on oral glucose tolerance in STZ induced diabetic rats (OGTT):**

Two groups of diabetic rats each group containing six rats were used for the present study.

**Group 1:** Diabetic untreated rats

**Group 2:** Diabetic rats treated with 750 mg NCAE/kg.bwt.

The oral glucose tolerance test was performed in overnight fasted diabetic rats (Bonarweir., 1998). Glucose (2 g/kg bwt.) was administered orally to both groups of rats using a force feeding needle. NCAE was orally administered to group 2 rats immediately after glucose administration. Blood samples were collected from the tail veins of all the animals from 0 min (before glucose administration) to 120 min of glucose administration for estimation of blood glucose using dextrostix with Basic One Touch Accuchec Glucometer (Glucose oxidase peroxidase method).

**Phytochemical analysis:** Phytochemical analysis of the NCAE and NCME were carried out by the different methods (Harbone, 2005).

**Statistical Analysis:**

All values are expressed as Mean ± S.D. The data was statistically analyzed by student's t-test.
RESULTS:
The aqueous crude suspension of leaves of *Narengi crenulata* at a dosage of 500mg/kg bwt showed significant antihyperglycemic activity (Table 1). Hence the leaf powder of *Narengi crenulata* was used for preparing different solvent extracts and evaluation of antihyperglycemic activities of each extract. By screening of each extract in STZ induced diabetic rats, the aqueous extract at a dosage of 500mg/kg bwt showed marked antihyperglycemic activity (63%) by decreasing the fasting blood glucose level in STZ induced diabetic rats. The methanolic extract also produced but less (55%) antihyperglycemic activity when compared to the aqueous extract and it did not produce any hypoglycemic activity in normal rats. The hexane and ethylacetate extracts did not show any antihyperglycemic activity.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose at different hours after the treatment (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0hr</td>
</tr>
<tr>
<td>NC</td>
<td>99±5.03</td>
</tr>
<tr>
<td>DC</td>
<td>328±35.2*</td>
</tr>
<tr>
<td>DT 500mg crude suspension of NCL</td>
<td>418±11.8*</td>
</tr>
</tbody>
</table>

### Table 1. Effect of crude suspension of NCL on fasting blood glucose levels of normal and diabetic treated rats

Values are calculated as mean±S.D from six rats in each group.

Values not sharing a common superscript letter differ significantly at $p<0.01$ (DMRT).

**$P<0.0001$ compared with the initial level of blood glucose (0h) in the respective group.

Numbers in parenthesis indicate the percentage of fall in 0h blood glucose.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose at different hours after the treatment (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0hr</td>
</tr>
<tr>
<td>NC</td>
<td>87±3.0</td>
</tr>
<tr>
<td>DC</td>
<td>310±35.2*</td>
</tr>
<tr>
<td>DT (Hexane extract) 500mg/Kg Bwt</td>
<td>348±34.3*</td>
</tr>
<tr>
<td>DT (Ethyl acetate extract) 500mg/Kg Bwt</td>
<td>314±19.8*</td>
</tr>
<tr>
<td>DT (Methanol extract) 500mg/Kg Bwt</td>
<td>397±15.3*</td>
</tr>
<tr>
<td>DT (Aqueous extract) 500mg/Kg Bw.</td>
<td>348±17.8*</td>
</tr>
</tbody>
</table>

### Table 2. Effect of different extracts at fixed dose NCLE on fasting blood glucose of normal and diabetic rats.

+$P<0.0001$ compared with the initial level of blood glucose (0h) of normal rats.

**$P<0.0001$ compared with the initial level of blood glucose (0h) in the respective group.

Numbers in parenthesis indicate the percentage of fall in 0h blood glucose.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Blood glucose (mg/dl) at different hours after the treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0hr</td>
</tr>
<tr>
<td>NC</td>
<td>91.8±3.7</td>
</tr>
<tr>
<td>DC</td>
<td>347±35.2</td>
</tr>
<tr>
<td>DT (500mg methanol/Kg Bwt)</td>
<td>397±15.3</td>
</tr>
<tr>
<td>DT (500mg aqueous/Kg Bwt)</td>
<td>348±17.8</td>
</tr>
<tr>
<td>DT (750mg methanol/Kg Bwt)</td>
<td>472±5.5</td>
</tr>
<tr>
<td>DT (750mg aqueous/Kg Bwt)</td>
<td>415±8.8*</td>
</tr>
<tr>
<td>DG (0.02g)</td>
<td>322±34*</td>
</tr>
</tbody>
</table>

### Table 3. Effect of different doses of methanolic and aqueous extract of NCL on fasting treated rats

Values are calculated as mean±S.D from six rats in each group.

Values not sharing a common superscript letter differ significantly at $p<0.01$ (DMRT).

+$P<0.0001$ compared with the initial level of blood glucose (0h) of normal rats.

**$P<0.0001$ compared with the initial level of blood glucose (0h) in the respective group.

Numbers in parenthesis indicate the percentage of fall in 0h blood glucose.
The present investigations clearly indicated significant antidiabetic activity of *Narengi crenulata* in STZ induced diabetic rats. The leaves seems to have a promising value for the development of potent phytomedicine for diabetes.

**ACKNOWLEDGEMENTS:**

The author is grateful to parents for their moral and financial support and Prof. Ch. Apparao for supervising the research work. Author also thanks Prof. O. V. S. Reddy, each and everyone whoever supported throughout the work.

**REFERENCES:**


