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

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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*.

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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) [1,2]. 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[3]. 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[5].

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 Ethanobotanists, 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, Bilvaparni/vilvaparni 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 acitivity 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 Tirumala 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 68ºC-70ºC. The filtrates obtained were distilled and concentrated under reduced pressure at low temperature (40ºC to 45ºC) in the Buchi rotavapor R-200 and finally freeze dried. All the extracts were stored at 0ºC 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 antihyperglycemic 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.

Group1: Untreated normal rats

Group2: Untreated diabetic rats

Group3: Diabetic rats treated with 500mg 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 1ml 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:

Group1: Untreated normal rats

Group2: Untreated diabetic rats

Group3: Diabetic rats treated with 500mg NC hexane extract/kg bwt.

Group4: Diabetic rats treated with 500mg NC ethylacetate extract/kg bwt.

Group5: Diabetic rats treated with 500mg NC methanolic extract/kg bwt.

Group6: Diabetic rats treated with 500mg NC aqueous extract/kg bwt.

After an overnight fast different groups of diabetic rats received the hexane extract (dissolved in 1ml 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.

Experimental design for the dose fixation studies:

Seven groups of rats were used for the evaluation of antihyperglycemic effect of different doses of the selected solvents (methanol and aqueous) extracts of NC leaves.

Group1: Untreated normal rats

Group2: Untreated diabetic rats

Group3: Diabetic rats treated with 500mg methanol extract/kg bwt.

Group4: Diabetic rats treated with 500mg aqueous extract/kg bwt.

Group5: Diabetic rats treated with 750mg methanol extract/kg bwt.

Group6: Diabetic rats treated with 750mg aqueous extract/kg bwt.

Group7: Diabetic rats treated with 0.02g glibenclamide/kg bwt.

After an overnight fast the normal treated and diabetic treated rat groups in the respective batches received the methanol or aqueous extracts(dissolved in 1 ml of distilled water) by gastric intubation using a force feeding needle. Normal untreated and diabetic untreated rats were fed with 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 plant extract and the fasting 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.

Group1: Diabetic untreated rats

Group2: Diabetic rats treated with 750mg NCAE/kg.bwt.

The oral glucose tolerance test was performed in overnight fasted diabetic rats (Bonnarweir., 1998).Glucose(2g/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/kgbwt showed significant antihyperglycemic activity (Table 1). Hence the leaf powder of *Narengi crenulata* was used for preparing different solvent extracts and evaluation of antihyperglycemic activites of each extract. By screening of each extract in STZ induced diabetic rats, the aqueous extract at a dosage of 500mg/kgbwt. 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.

Groups	Blood glucose at different hours after the treatment(mg/dl)						
	0hr	1hr	2hr	3hr	4hr	5hr	6hr
NC	99±5.03	92±17.2	88±13.7	89±11.1	99±9.4	110±8.5	118±6.7
DC	328±35.2*	348±14.7	340±7.7**	359±12.5	373±11.7	382±8.7	395±16.3
DT 500mg crude suspension of NCL	418±11.8*	411±18.4**	391±17.9**	355±14.3**	311±19.5**	248±22.4*	157±17.4* (62.7%)

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<0.001 compared with initial level of blood glucose (0hr) in the respective group

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

	Blood glucose at different hours after the treatment(mg/dl)						
	0hr	1hr	2hr	3hr	4hr	5hr	6hr
NC	87±3.0	80.6±6.9	83.6±4.4	84.8±3.7	88.3±5.6	88.1±6.5	88.1±7.9
DC	310±35.2*	348±14.7	348±7.7	359±12.5	382±11.7	382±8.7	395±16.3
DT(Hexane extract) 500mg/Kg Bwt	348±34.3*	405±68.3	409±31.1	411±38.1	425±60.1	372±6.0	394±7.2
DT(Ethyl acetate extract)500mg/KgBwt.	314±19.8*	375±9.8	342±10.7	312±7.6	286±10.3	261±7.1	245±7.3
DT(Methanol extract) 500mg/Kg Bwt.	397±15.3*	385±5.8	343±9.2	312±6.2	275±5.9	245±5.9	216±8.4 (55%)
DT(Aqueous extract) 500mg/Kg. Bw	348±17.8*	335±6.6**	315±7.9**	295±10.6**	239±6.7**	213±4.5**	168±2.4** (63%)

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 (oh) in the respective group.

*P<0.001 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.

Groups	Blood glucose (mg/dl) at different hours after the treatment						
	0hr	1hr	2hr	3hr	4hr	5hr	6hr
NC	91.8±3.7	92±17.2	90.5±3.4	88±3.8	90.8±4.07	89.6±3.8	110±6.7
DC	347±35.2	368±45.4	348±7.8	403±68.9	405±36.5	337±38.3	395±15.3
DT(500mg methanol/KgBwt)	397±15.3	385±5.8	343±9.2	312±6.2	275±5.9	245±5.9	216±8.4 (55%)
DT(500mg aqueous /Kg Bwt)	348±17.8	335±6.6	315±7.9	295±10.6	239±6.7	213±4.5	168±2.4 (63%)
DT(750mg methanol/Kg Bwt)	472±5.5	395±4.4	372±17.8**	353±5.5**	323±9.8**	306±9.1**	203±9.1** (56.7%)
DT(750 mg aqueous /Kg Bwt)	415±8.8*	391±5.7	363±6.7	332±6.2	262±6.9	222±10.2**	148±6.8** (65%)
DG(0.02g)	322±34*	293.8±10.7**	265±24	243±24	216±21.4	208±14.9	233±23.5(27%)

Table 3. Effect of different doses of methanolic and aqueous extract 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

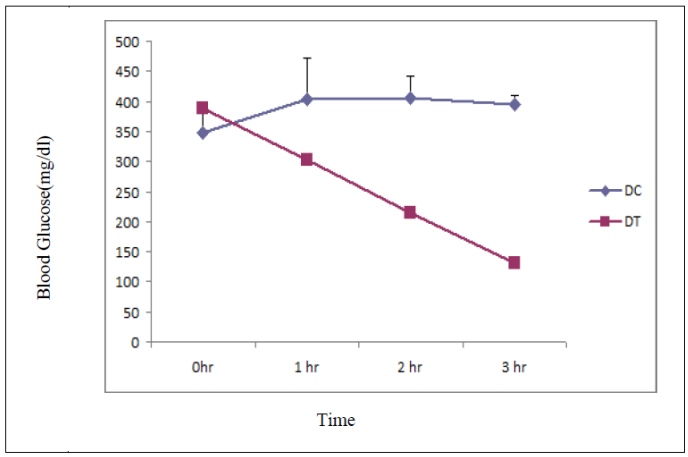
Values not sharing a common superscript letter differ significantly at p<0.01(DMRT)

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

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

Groups	Blood glucose at different hours after treatment(mg/dl)			
	0hr	1hr	2hr	3hr
DC	347±35.2	403±68.9	405±36.5	395±15.3
DT 750mg/kg. bwt	388±6.4	303±6.9	215±6.3	131±7.2**(51%)

Table 4: Effect of NCAE on oral glucose tolerance in STZ induced diabetic rats (OGTT)
**p<0.0001 compared with the initial level of blood glucose (0hr) in the respective group.
Numbers in parenthesis indicate the percentage of fall in 0hr blood glucose.



Among the different doses of NCAE and NCME, 750mg NCAE/Kg.b.wt. has produced higher antihyperglycemic activity than those with 500mg NCAE/Kg.b.wt., 500mg NCME and 750 mg NCME/Kg.b.wt.

The administration of NCAE at 750mg/Kg.b.wt. along with 2g/Kg.b.wt. glucose load has significantly improved the glucose tolerance in the diabetic rats. In the diabetic untreated rats the glucose levels remained higher without much change even at 3hr after glucose load. Where as in NCAE administered diabetic rats the glucose levels started falling from the 1st hour after glucose load and there was a consistent decrease in the blood glucose by the end of 3rd hour after glucose load. The phytochemical analysis of NCL methanolic and aqueous extracts showed the presence of alkaloids, flavonoids, phenols and saponins.

DISCUSSION:

Diabetes Mellitus is possibly the world's largest growing metabolic disease, and as the knowledge on the heterogeneity of this disorder is advanced, the need for more appropriate therapy increases[17]. In spite of the introduction of hypoglycemic agents, diabetes and its related complications continue to be a major medical problem. Since time immemorial patients with non-insulin dependent diabetes mellitus have been treated orally by folklore with a variety of plants extracts in the indigenous Indian system of medicine (Ayurveda). An attempt was made on good number of plants for the cure of diabetes or madhumeha and some of them have been experimentally evaluated and the active principles were isolated in India[18]. Such plants and remedies mentioned and used for the treatment of diabetes mellitus date back to the ancient authorities like Bhrigu, Charaka, Sushruta and Vagbatta, the last three called vriddha trayi of Ayurveda [19] .

In the present study 750mg/kg.b.wt. of aqueous extract of *Narengi crenulata* leaves has produced a maximum fall in blood glucose levels by about 65% in STZ induced diabetic rats, which is significantly higher than the hypoglycemic effect of 20mg/kg.b.wt. of glibenclamide in the diabetic treated rats. Hence, the aqueous extract may be considered to have good antihyperglycemic active principles without causing any hypoglycemic effect unlike insulin and other synthetic drugs. Drugs that normalize function, without causing hypoglycemia, would make attractive targets for diabetes[20]. The phytochemical analysis of NC aqueous extract showed the presence of flavonoids, phenols and alkaloids etc. The antidiabetic effect of NC aqueous extract may be due to the presence of more than one antihyperglycemic principle and their synergistic properties. Several phytomolecules including flavonoids, alkaloids, glycosides, saponins, glycolipids, dietary fibres, polysaccharides, peptidoglycans, carbohydrates, amino acids and others obtained form various plant sources have been reported as potent hypoglycemic agents[21]. Thus, 750mg/kg.bwt. of aqueous extract was found to be the effective dose of *Narengi crenulata* on FBG of normal as well as diabetic animals. Further studies are under progress to identify the active antihyperglycemic compound(s) present in the aqueous extract. Hence the results of our study strongly suggest that *Narengi crenulata* may be used for the treatment of diabetes mellitus.

Conclusion:

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.

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