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EFFECT OF HEXANE EXTRACT OF *NARINGI CRENULATA* LEAFS ON EXPERIMENTALLY INDUCED DIABETES MELLITUS IN RATS

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ABSRACTS

Diabetes mellitus is a heterogeneous group of metabolic syndromes characterized by an elevation of blood glucose caused by a relative or absolute deficiency of insulin. The incidence of diabetes is growing rapidly worldwide. Drug treatment for diabetes mellitus is expensive and carries risks for many adverse effects. India is one among rich sources of medicinal plants useful in the treatment of diabetes. The study was performed to investigate the blood glucose lowering effect of *Naringi crenulata* leaves in experimentally induced diabetic rats. Administration of Hexane extract of *N. crenulata* leaves produced significant change in the blood glucose level as compared to control. Histological examination of pancreas showed destruction of beta cells in Islets of pancreas in group-C whereas retaining of islets and few degranulations of beta cells of pancreas found in group-D. These observations and results provide information that hexane extract of *N. crenulata* leaves has hypoglycaemic effect in experimentally induced diabetic rats which requires further investigation.

Key words: Diabetes mellitus, *Naringi crenulata,* albino wistar rats, Alloxan, Blood glucose.

INTRODUCTION

Diabetes mellitus is a metabolic disorder resulting in raised blood glucose (hyperglycaemia) from defects in insulin secretion; insulin action or both that arise from genetic as well as environmental factors. The chronic hyperglycaemia is associated with long-term damage, dysfunction and failure of various organs, especially the eyes, kidneys, liver, heart and blood vessels (Mahtab *et al*., 2007). The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (Wild *et al*., 2004).

Patients with DM often suffer from motility disorders in the gastrointestinal tract, which are manifest in clinical symptoms as nausea, vomiting, heart burn, constipation, diarrhea and fecal incontinence. Such disorders occur in both type I (insulin dependent-IDDM) and type II (noninsulin dependant-NIDDM) diabetes mellitus. These disorders are believed to be caused by autonomic neuropathy, although these disorders are only weakly correlated with that neuropathy (Maxton and Whorwell, 1991, Clark and Nowak, 1994 and Folwaczny *et al*., 1995).

Animal models in diabetes research are very common where rats are the first choice of use, comprising over 85 percent of these models. It may have been because of the pathogenesis of diabetes in animal models is most likely similar to the pathogenesis in humans. Alloxan has been extensively used to induce diabetes for various diabetes studies in laboratory animals. Alloxan has been reported to be capable of generating reactive oxygen species resulting in oxidative stress and cell death. Also, alloxan has been found to be a better chemical inducer for diabetes than alloxan (Szkudelski, 2001).

The present study was designed to demonstrate the hypoglycemic activity in alloxan induced diabetes in a rat model.

MATERIALS AND METHODS

Sample Collection

The plant sample *Naringi crenulata* was collected from local of Chidambaram town, Cuddalore, Tamil Nadu and was identified by a taxonomist Dr. R. Selvaraj, Professor of Botany, Annamalai University. The collected plant was immediately transported to the lab and a voucher specimen is submitted to our lab.

Preparation of hexane extracts of *N. crenulata*

The hexane extract of *N. crenulata* leaves was prepared by Yajninik (2003) method. Approximately 500 g of fresh plant material was shade dried and then powdered using a blender. It was soaked in 1500 ml of 95 % hexane at room temperature over night. The above soaked contents were filtered through Whatman No. 1 filter paper. The residue was again resuspended with equal volume of 95 % hexane and incubated at room temperature for 48 hours, and filtered again. The filtrates were pooled and evaporated at 40-50°C and the residue weighed. The extract was made into a semisolid by mixing with 80 % hexane and stored below 10°C until use. The extract was dark brown in color.

Experimental Animals

Adult albino rats (Wistar strain) were collected from Central Animal House, Rajah Muthiah Medical College, Annamalai University, Annamalainagar, Chidambaram (approved by the Institutional Animal Ethical Committee of Rajah Muthiah Medical College (160/1999/CPCSEA, Proposal No. 711). Wistar albino rats, the experimental animals were acclimatized to lab condition. The toxicity analysis of plant extract was done. Diabetes was induced by injecting alloxan.

Animals are divided into five groups and treated as follows: A total number of 30 animals were divided into 2 lots with 12 and 18 animals respectively. The first lot of animals served as control and such divided into two groups of each. One group did not receive any treatment and the other one received just plant extract for (100 mg/kg bw) 30 days. The second lot were induced with alloxan monohydrate (100 mg / kg bw) after overnight fast for 12 hours, sub divided into 3 groups of each. Group I did not received anything (other than alloxan), group II received plant extract (100 mg/kg bw) and group III received standard anti-diabetic drug, tolbutamide (100 mg/kg bw). At the end of the study, the animals were euthanized between 0900-1100 h to minimize diurnal variation.

Glucose Estimation

Fasting blood glucose level was estimated by glucose oxidase - peroxidase method.

Insulin Estimation

The assay of insulin in the plasma of normal and diabetic rats was performed by enzyme-linked immunosorbent assay (ELISA) method.

Glycosylated Hemoglobin

Glycosylated hemoglobin in the blood was estimated.

Serum Biochemical Estimation

Serum glutamic oxaloacetic transaminase (SGOT), serum ALT, ALP and was determined.

Statistical Analysis

All the data were expressed as Mean ± SEM. Statistical analysis was carried using Student's t-test to analyze the significance between the groups. A value of $P<0.05$ was considered to be significant.

RESULTS & DISCUSSION

Table 1 shows the study of blood glucose, body weight; plasma insulin and total haemoglobin in normal diabetic and *N. crenulata* extract treated animals in different groups. In the present study, the initial (0 day) and final body weight (45^{th} day) of each group were observed. In the control group, the initial and final bodyweight tremendously altered respectively. The average growth recorded in control group is 44g. The growth was slightly increased in control treated with *N. crenulata*. The initial and final growth between control and treated with *N. crenulata* extract was (24.53g) and in the diabetic control group, the growth rate was negative. The status of plasma glucose is shown in Table 1. Blood glucose level in diabetic control group was observed to be very high (280.50±15.90 mg/dl) than that of normal control which was (82.55±7.90), lowering in the blood glucose of diabetic rats was observed when treated with *N. crenulata* extract and glibenclamide as (121.18±7.97 mg/dl), which is relatively lesser than that of diabetic induced.

The plasma insulin level was increased in diabetic treated with *N. crenulata* extract (12.15±0.94 µU/ml) and glibenclamide (14.1±1.51 µU/ml). There was a promising increase in the level of plasma insulin in normal rats when treated with *N. crenulata* extract (12.15±0.94 µU/ml), which is near normal standard glibenclamide. Table 2 also shows the level of total haemoglobin which was totally decreasing in the diabetic rats (7.60±0.9 g/dl) than control group (13.40±1.51 g/dl). There was an astonishing increase in diabetic rats when treated with the *N. crenulata* extract (15.1±1.26 g/dl).

Glycosylated haemoglobin (HbA1C) level in diabetic was (7.50±0.48HbA1c %) which seems very high as compared to that of normal control which was (3.95±0.25 HbA1c %) and it was found that *N. crenulata* extract is vigorously lowering the level of glycosylated haemoglobin and was found (4.10±0.55 HbA1c %) (Table.2). Table.3 shows the level of liner glycogen in diabetic animals which is relatively less (25.10 ± 2.12 mg /g) compared to that of its control which was observed (41.10 ± 3.24 mg /g).

On the contrary, *N. crenulata* extract was found an efficient to increase lower glycongen in diabetic up to promising level of (33. 54±3.14 mg /g) which was near to standard glibenclamide treated (35.45±3.15 mg /g). Urea level was totally found more in diabetic (32.54±3.15 mg/dl) and the same was found recovering in *N. crenulata* extract treated (26.20±2.52 mg/dl) which is near to standard treated (Table 2). Similarly, creatinine level was found more in case of diabetic induced (1.30±0.28 mg/dl) which is totally against to that of normal control level of (0.45±0.14 mg/dl) and the *N. crenulata* extract was found significant enough in lowering the level of creatinine up to level of (0.77±0.08 mg/dl) (Table 3). The evaluation of creatinine was found decreased when treated with *N. crenulata* extract and there was also a slight decrease in normal control when treated with *N. crenulata* extract.

Table 1. Effect of *Naringi crenulata* on blood glucose, plasma insulin, haemoglobin, and changes in body weight of normal and experimental animals.

Groups	Body weight (g)		Net weight gain (g)	Plasma glucose (mg/dl)	Plasma Insulin (µU/ml)	Total Hb (g/dl)
	Initial	Final				
Normal	166.95±11.1	200.78±6.52	44±6.7^{a}	82.55±7.9^{a}	13.09±1.12^{b}	13.40±1.51^{a}
Normal+ NC	165.42±11.41	189.95±4.65	24.53±4.15^{a}	79.10±6.6^{a}	12.15±0.94^{a}	15.1±1.26^{a}
Diabetic control	190.16±10.15	148.90±11.12	42.26±12.17^{c}	280.5±15.9^{e}	6.49±0.3^{f}	7.60±0.9^{d}
Diabetic + NC	154.99±12.17	177±13.28	22.01±13.28^{b}	199.79±10.4^{d}	10.34±1.31^{e}	10.12±0.86^{c}
Diabetic+GLCD	160±11.06	187.16±11.43	27.16±11.43^{b}	121.18±7.97^{b}	14.1±1.51^{c}	13.79±1.11^{a}

Values are given as mean ± SD (n=6 rats).
Values that are not sharing a common superscript letter in the same column differ significantly at p<0.05 (DMRT).

Table 2. Glycosylated Hb, liver glycogen and urine sugar and creatinine of normal and experimental animals.

Groups	Glycosylated Hb (HbA1c %)	Liver glycogen (mg /g)	Urea (mg/dl)	Creatinine (mg/dl)
Normal	3.95±0.25^{a}	41.10±3.24^{a}	19.49±2.17^{a}	0.45±0.14^{a}
Normal+ NC	3.67±0.28^{a}	41.47±2.74^{a}	17.84±1.51^{a}	0.43±0.02^{a}
Diabetic control	7.50±0.48^{e}	25.10±2.12^{d}	32.54±3.15^{d}	1.30±0.28^{d}
Diabetic + NC	4.10±0.55^{d}	34.54±3.14^{c}	26.20±2.52^{c}	0.77±0.08^{c}
Diabetic+GLCD	5±0.4^{b}	35.45±3.15^{c}	21.19±2.12^{b}	0.7±0.06^{b}

Values are given as mean ± SD (n=6 rats).
Values that are not sharing a common superscript letter in the same column differ significantly at p<0.05 (DMRT).

Table 3. Effect of *Naringi crenulata* on serum total proteins, ALP, ALT, AST and Billirubin of normal and diabetic rats.

Groups	Total protein (mg/dl)	ALP (IU/L)	AST (IU/L)	ALT (IU/L)	Bilirubin (mg/dl)
Normal	7.20±0.42^{a}	75.45±4.85^{a}	49.09±3.13^{a}	20.10±2.02^{a}	0.65±0.14^{a}
Normal+ NC	6.56±0.55^{a}	71.51±4.83^{a}	47±3.28^{a}	18.26±1.92^{a}	0.62±0.19^{a}
Diabetic control	5.2±0.55^{b}	132.13±6.52^{d}	78.23±3.6^{d}	33.65±2.52^{e}	1.18±0.09^{c}
Diabetic + NC	5.63±0.4^{b}	85.43±1.75^{c}	66.09±5.15^{c}	25.06±1.12^{c}	0.82±0.16^{b}
Diabetic+GLCD	6.41±0.41^{a}	80.08±5.08^{b}	52.09±5.55^{b}	22.89±2.12^{b}	0.74±0.15^{a}

Values are given as mean ± SD (n=6 rats).
Values that are not sharing a common superscript letter in the same column differ significantly at p<0.05 (DMRT).

Conclusion

From the analysis of the results, it is evident that there was a progressive fall of blood sugar level and significant increase in body weight and insulin level after the intake of *N. crenulata* extract. These results were on par with the standard drug used. The phytochemical screening of *N. crenulata* leaves reveals the presence of phytoconstituents like phenols, tannin, flavonoids, saponins, quinine, protein and lipids. These results expose that the plant has quite a number of chemical constituents, which may be responsible for the many pharmacological actions and will be useful in finding out the quality of the drug. Hence *Naringi crenulata* can be used as an antidiabetic herbal formulation.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this article.

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