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RESEARCH ARTICLE

VALIDATION OF ANTI-DIABETIC ACTIVITY OF *Naringi crenulata* IN CONTROL AND STREPTOZOTOCIN INDUCED DIABETIC RATS

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ABSTRACT

The effect of the leaf extract of *Naringi crenulata* in normal and streptozotocin (STZ) induced diabetic rats were studied. Blood glucose levels were determined after oral administration of graded doses of *Naringi crenulata* (300, 600mg/kg) in fasted normal and diabetic groups. In both groups, 600 mg/kg of the extract, significantly reduced blood glucose levels 8 h after administration, which was consistent and time-dependent. The higher doses of 300 mg/kg did not affect significantly the blood glucose levels. The plant extract (Acetone) is given to the diabetic rats as well as normal rats orally by intragastric tubes. The blood glucose levels were increased when diabetes is induced in all the experimental rats. The methanolic extract of *N. crenulata* action was significant in increasing the plasma insulin levels in diabetic rats when compare to acetone extract treated rats. Total Hb was also found increasing in extract administered rats. The normal rats blood glucose levels did not change much when treated with plant extract alone. The plant extract was capable of ameliorating at the glycosylated Hb levels and moderate increase in glycogen levels and significant decrease in urea and creatinine levels while administering deferent dosage of *N. crenulata* extracts.

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INTRODUCTION

Diabetes mellitus is a common endocrine disorder caused due to either deficiency in insulin production or due to ineffectiveness of the insulin. Such a deficiency results in impaired metabolism of glucose and other energy- yielding fuels like lipids and proteins (Gale and Anderson, 2002). The metabolic disturbance contributes massively to most neurological, cardiovascular, retinal and renal diabetic complications (Mohanty *et al*., 2000). Diabetes mellitus has become a growing problem in the contemporary world (Piyush *et al.,* 2006). India has today become the diabetic capital of the world with over 20 million diabetes and this number is likely to increase to 57 million by 2025 (Cook and Plotnick, 2008). This astronomic increase in the prevalence of diabetes has made diabetes a major public health challenge for India and is become important human ailment afflicting many from various walks of life in different countries and once again the whole world being looked upon Ayurvedic the oldest healing system of medicine for the treatment of diabetes (Joseph, 2011). The global population is in the midst of a diabetes epidemic with people in south East Asia and western pacific being mostly at risk. The number of cases for diabetes is predicted to reach 366 million by the end of 2030. Diabetes mellitus is an endocrine disease which is related to the disorders of carbohydrate metabolism brought about by deficiency in insulin production, insulin resistance or both (Raju *et al.,* 2006). The classic well-known symptoms of diabetes are polyuria, polydipsia, polyphagia and loss of body weight (Guthrie and Guthrie, 2003). Diabetes and its complications are among the leading causes for mortality and morbidity worldwide. Diabetes mellitus is characterized by hyperglycemia in the postprandial fasting state and its severe form leads to ketosis and protein wasting (Bell, 1991). Blood glucose elevation is a common phenomenon of diabetes mellitus and is primarily due to reduced glucose intake by the tissues and its production increased via gluconeogenesis and glycogenolysis. Diabetes mellitus is basically characterized by high levels of blood glucose caused by defective insulin production and action that are often responsible for severe health problems and early death (Leahy, 2005). One of the reasons for injury related to hyperglycemia is the formation of glycated proteins, glucose oxidation and increase free fatty acids (Devi and Falco, 2005). Chronic hyperglycemia of diabetes is associated with long term damage, dysfunction and eventually the failure of organs, especially the eyes, kidneys, nerves, heart and blood vessels (Hung *et al.,* 2005). It is a commonest endocrine disorder that affects more than 100 million people worldwide (about 6% of population) and in the next 10 years, it may affect about 5 times more people than it does now (WHO, 1992; ADA, 1997). According to WHO report, India has 19.4 million diabetes patients (King *et al.,* 1998). It is the fourth leading cause of death in the most developed countries and there is substantial evidence that it is epidemic in many developing and newly industrialized nations. Diabetes mellitus is a syndrome resulting from a variable interaction and environmental factors and is characterized by depleted insulin secretion, hyperglycemia and altered metabolism of lipid, carbohydrates and proteins, in addition to damaged β-cells of pancreas and increased risk of complications of vascular diseases (Davis and Granner., 1996). In the present study, it is aimed to focus the potential of *N.crenulata* plant extract that has been confirmed by scientific investigation, which appear to be most effective relatively nontoxic having substantial documentation of efficacy.

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MATERIALS AND METHODS

Plant material

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 extracts of *N. crenulata*

The methanol and acetone extracts of *N. crenulata* leaves were prepared by Yajninik *et al*. (2003). Approximately 500 g of fresh plant material was shade dried and then powdered using a blender. It was soaked in 1500 ml of 95 % acetone and methanol 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 % acetone and methanol 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 residue is stored at 10°C until use.

Experimental Animals

Adult (albino Wistar rats) 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 streptozotocin. Animals are divided into seven groups with six animals each.

Group 1: Served as control animals.
Group 2: STZ induced diabetic rats without any drug treatment
Group 3: Diabetic rats treated with 300mg/kg of methanolic *N.crenulata* extract
Group 4: Diabetic rats treated with 600mg/kg of methanolic *N.crenulata* extract
Group 5: Diabetic rats treated with 300mg/kg of acetone *N.crenulata* extract
Group 6: Diabetic rats treated with 600mg/kg of acetone *N.crenulata* extract
Group 7: Diabetic rats treated with 600µg/kg of Glibenclamide standard drug
At the end of the study, the animals were euthanized between 0900-1100 h to minimize diurnal variation.

Body weight

Body weight was determined by observing the initial (0 day) and final body weight (45th day) of each group were observed.

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.

Estimation of haemoglobin

Haemoglobin in the blood was estimated by the method of Drabkin and Austin (1932). The dilution of blood in an alkaline solution containing potassium cyanide and potassium ferricyanide form the basis of this method. Haemoglobin gets oxidized forming cyanmethaemoglobin whose absorbance was then measured at540 nm.

Estimation of glycosylated haemoglobin (HbA1C)

Glycosylated haemoglobin in the blood was estimated by the method of Sudhakar and Pattabiraman, (1981).

Estimation of glycogen

Liver glycogen was extracted and estimated by the method of Morales *et al.* (1973).

Estimation of urea

Urea in the plasma was estimated by using the diagnostic kit based on the method of Fawcett and Scott (1960). Urea is hydrolyzed in the presence of water and urease to produce ammonia and carbon dioxide. Under alkaline conditions, the ammonia so formed reacts with hypochlorite and sodium salicylate in the presence of sodium nitroprusside to form a green colored chromophore. The intensity of the colour produced is proportional to the concentration of urea in the sample.

Estimation of Creatinine

Creatinine in the plasma was estimated by using the diagnostic kit based on the method of Tietz, (1987) using Jaffe's (1886) colour reaction. The assay of creatinine has been based on the reaction of creatinine with alkaline picrate as described by Jaffe. Most of the contaminants reacting with the Jaffe reagent produce a colour at a lower rate of colour formation are proportional to the concentration of creatinine in the sample.

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

Table 1 shows the study of body weight, blood glucose, plasma insulin and total haemoglobin in normal diabetic and extract treated animals in different groups.

Body weight changes

In the present study, the initial (0 day) and final body weight (45th day) of each group were observed. In the control group, the initial and final bodyweight was (159.92±12.18g) and (192.98± 8.72g), respectively. The average growth recorded in control group is 36g. The growth was slightly increased in *N.crenulata* extract treated diabetic groups. The initial and final growth of methanolic *N.crenulata* extract treated group was (158.99±12.12g) and (199.93±4.65g), respectively. At the same time *N.crenulata* acetone extract treated group exhibited average weight gain of 177.21±13.28 which is lesser than the methanolic extract treated group. The effect of glibenclamide treated group exhibited the weight gain which is near equal to that of *N.crenulata* extract treated group.

Plasma glucose

Plasma glucose level has extremely shot up to a level of 320.5±15.9 mg/dl in diabetic induced rats, whereas it is normal in the control group of 110.52±8.9 mg/dl. *N.crenulata* extract treated diabetic rats has shown considerable plasma glucose reducing effect as in case of methanolic extract of 600 mg/kg dose the plasma glucose level has reduced to 115.10±6.6 mg/dl and in case of acetone extact treated group 139.8±9.2 mg/dl level of glucose reduction was observed (Table 1).

Plasma insulin

Plasma insulin of normal control was relatively higher as compared to that of diabetic control rats. Plasma insulin level in normal control and diabetic control were observed as (15.09±1.11 µU/ml) and (7.49±0.3 µU/ml). The plasma insulin level was increased in group treated with methanolic *N.crenulata* extract (13.16±1.02µU/ml) and glibenclamide (13.1±1.51 µU/ml). There was a promising increase in the level of plasma insulin in normal rats when treated with *N.crenulata* extract respectively more than that of standard glibenclamide (Table 1).

Total haemoglobin

Table 1. shows the level of total haemoglobin which was totally decreasing in the diabetic rats (7.69±0.9 g/dl) than control group

Table 1. The effect of *Naringi crenulata* on Blood glucose, Plasma insulin, Total 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 H_b (g/dl)
	Initial	Final				
Control	159.92±12.18	192.98±8.72	35.82±8.7^{a}	110.52±8.9^{a}	15.09±1.11^{b}	13.49±1.51^{a}
Diabetic	156.26±14.15	151.99±12.17	-4.27±12.17^{c}	320.5±15.9^{e}	7.49±0.3^{f}	7.69±0.9^{d}
N.crenulata (M)/300	160.1±13.28	180.66±11.43	26.66±11.43^{b}	205.5±11.5^{d}	9.95±0.4^{e}	9.52±0.88^{c}
N.crenulata (M)/600	158.99±12.12	199.93±4.65	32.66±4.65^{a}	115.10±6.6^{a}	13.16±1.02^{a}	12.49±1.26^{a}
N.crenulata (A)/300	154.99±12.17	177±13.28	22.01±13.28^{b}	184.79±10.4^{d}	10.34±1.31^{e}	10.12±0.86^{c}
N.crenulata (A)/600	157.63±6.87	180±10.44	22.37±10.44^{b}	139.8±9.2^{c}	12.51±0.94^{d}	12.2±1.23^{b}
Glibenclamide	154.21±11.06	187.16±11.43	27.16±11.43^{b}	121.18±7.97^{b}	13.1±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 H_b, 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)
Control	4.01±0.35^{a}	40.14±4.14^{a}	19.49±2.17^{a}	0.49±0.04^{a}
Diabetic	8.04±0.57^{e}	23.14±2.14^{d}	30.24±3.13^{d}	1.28±0.18^{d}
N.crenulata (M)/300	5.79±0.5^{c}	34.94±4.14^{c}	24.14±2.27^{c}	0.77±0.07^{b}
N.crenulata (M)/600	5.04±0.4^{b}	33.24±3.43^{c}	26.20±2.52^{c}	0.84±0.08^{c}
N.crenulata (A)/300	7.23±0.42^{d}	30.24±2.14^{b}	27.25±2.82^{c}	0.77±0.07^{b}
N.crenulata (A)/600	7.04±0.58^{d}	33.24±3.43^{c}	30.24±3.13^{d}	0.89±0.08^{c}
Glibenclamide	4.55±0.42^{d}	35.54±4.12^{c}	21.19±2.12^{b}	0.71±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)

(13.49±1.51 g/dl), and there was an steady increase in total haemoglobin of methanol treated group *N.crenulata* formulation (12.49±1.26g/dl) which is highly significant to all other treated group.

Glycosylated Hemoglobin (H_bA_1C)

Glycosylated haemoglobin (H_bA_1C) level in diabetic was (8.04±0.57%) which seems very high as compared to that of normal control which was (40.14±4.14). *N.crenulata* methanolic extract has vigorously lowered the level of glycosylated haemoglobin and was found to (5.04±0.5%) (Table 2).

Liver glycogen

Liver glycogen in diabetic rats which is relatively less (23.14±2.14mg/g) compared to that of control animals which was observed (40.1±4.14 mg/g). As a contrary, *N.crenulata* methanol extract treated group exhibited efficient level of glycogen level icrease (33.24± 3.43 mg/g) which is near to standard glibenclamide treated (35.54± 4.12 mg/g) (Table 2).

Urea level

Urea level was totally found more in diabetic group (30.24±3.13 mg/dl) and the reverting state in *N.crenulata* methanol extract treated (26.20±2.52 mg/dl) near to standard glibenclamide treated group (Table 2).

Creatinine level

Similarly, creatinine level was found more in case of diabetic induced group (1.28±0.18 mg/dl) which is totally reverse to that of normal control level of (0.49±0.04 mg/dl). *N.crenulata* methanol extract was found significant enough in lowering the level of creatinine up to level of (0.77±0.07 mg/dl) and (0.84±0.08^{c}) significantly (Table 2). The evaluation of creatinine was found decreased when treated with *N.crenulata* extract.

DISCUSSION

Medicinal plants have created the foundation of health care system throughout the world since the initial stage of humanity and still plant products are the major source drug/formulation in treatment of various diseases (Kamboj, 2000). The treatment of diabetes with medicines of plant origin that proved much safer than synthetic drugs is an integral part of many cultures throughout the world and has gained importance in recent years. India has a rich history of using various potent herbs and herbal components for treating various diseases including diabetes (Yeh *et al.,* 2003). Several phytomolecules including flavonoids, alkaloids, glycosides, saponins, glycolipids, dietary fibers, polysaccharides, peptidoglycans, carbohydrates, amino acids and others obtained from various plant sources have been reported as potent hypoglycemic agent (Khaled *et al.,* 2008; Baldi *et al.,* 2010). In the present study herbal drug and phytoconstituents resembles safely and efficacy, they produces no side effect when compare to synthetic drugs. In the present study, untreated diabetic rats showed severe body weight loss. This characteristic weight loss in diabetic rats could be due to degradation and catabolism of fats and proteins (Nurlan and Garlick, 1979). Thus, increased catabolic reactions leads to muscle wasting which may be the major cause for weight loss in diabetic rats (Rajkumar *et al.,* 1991). However, *N.crenulata* extract treated groups showed a sign of recovery in the body weight which suggest the protective effect of the extract by preventing it from muscle wastage and other macromolecular degradations. The present study confirms the antihyperglycemic and antihyperlipidemic effects of *N. crenulata* extracts in streptozotocin induced diabetic rats. Administration of *N. crenulata* extracts to diabetic rats reduced the blood glucose levels to near normal and the optimum activity of the extract was found at the dose of 600 mg/kg bw. Although, the exact mechanism of action of the extract is unknown, the reduction in blood glucose level could be due to increased pancreatic insulin secretion from existing β-cell of the pancreas (Ghosh and Suryawanshi, 2001). This antihyperglycemic activity of *N.crenulata* was associated with increase in plasma insulin levels. The extents of changes in insulin levels with plant phenolic extract are insufficient to account for the obvious improvement in the glucose profile (Sachdewa and Khemani, 1999). The etiology of the complications of diabetes involves oxidative stress perhaps as a result of hypoglycemia, because glucose itself and hyperglycemia-related increased protein glycosylation are important sources of free radicals. In physiological condition, antioxidant enzyme protects the cells against harmful free radicals. A number of plant derived products have been possessed hypoglycemic, hyperlipidemic as well as antioxidant properties (Vasu *et al*., 1975). Kidney maintains optimum chemical composition of body fluid by acidification of urine and removal of metabolic wastes such as urea, uric acid, creatinine and ions. During renal diseases, the concentration of these metabolites increases in blood (Jaspreet *et al.,* 2000). In the present study it was observed that, administration of *N.crenulata* extract at 600 mg/kg doses reduced elevated levels of urea and creatinine, which was comparable to the effect observed with glibenclamide. This indicates

the prevention of any significant kidney change, which may be possible in diabetic animals. The liver is an important organ that plays a vital role in glycolysis and gluconeogenesis pathways. Glucose-6-phosphatase is the key enzyme in homeostatic regulation of blood glucose level (Massillon *et al*., 1996). Hence, In the present study administration of extract of interest to the STZ diabetic rats enormously increase the liver glycogen to its normal level of existence hence in the diabetic rats the glucokinase activity was decreased in the liver of diabetic rats, which may be due to deficiency of insulin. Decrease in the enzymatic activity of hexokinase and increase in the level of glycogen phosphorylase observed in the present study are responsible for the depletion of liver glycogen. In addition fructose1, 6-bisphosphatase catalyzes on the irreversible step in gluconeogenesis and serves as a site in the regulation of the process (Gupta *et al.,* 1999). Glucose is transported out of the liver to increase in blood glucose concentration. Normally insulin inhibits the hepatic glucose production by glucose-6-phosphatase and fructose-1, 6-bisphosphatase activity. Administration of *N.crenulata* extract decreased both enzymes activities in diabetic rats in a dose dependent manner thereby decreasing gluconeogenesis.

Conclusion

Thus from the above findings, it is clear evident that, methanolic extract of *N. crenulata* exhibited well worsed anti-hyperglycemic activities mainly at the dose of 600 mg/kg without showing any adverse toxic effects. Further studies on the characterization and the active principles responsible for antidiabetic activity of *N. crenulata* are highly warranted.

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