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Hypoglycemic effect of Nyctanthes arbor-tristis leaf extract on Alloxan induced Diabetic rabbits

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ABSTRACT

Currently available drugs for diabetes mellitus pose considerable side effects. This necessitates the development of new products, particularly herbal preparations which are known to have lesser side effects. Several herbal products are used to treat diabetes; but their hypoglycemic effects are complex and few anti-diabetic plants have received proper scientific validation.

The present study was undertaken to validate the use of the leaves of Nyctanthes arbor-tristis plant as anti-diabetic agent. This study was conducted on thirty healthy albino rabbits of either sex; the effect of the Nyctanthes arbor-tristis leaf extract (NALE) at doses 200mg/kg body weight and 400mg/kg body weight was evaluated for a period of 14 days on alloxan induced diabetic rabbits. The hypoglycemic effect of the leaf extract was compared with the standard Glibenclamide at dose 2.5mg/kg body weight. Results revealed that the hypoglycemic effect of the Nyctanthes arbor-tristis leaf extract at 200mg/kg body weight was not significant whereas at 400mg/kg body weight it was highly significant and comparable to Glibenclamide. However, the result of the present study can only be confirmed after evaluating Nyctanthes arbor-tristis on larger number of animals as well as clinically.

Keywords: Nyctanthes arbor-tristis, hypoglycemic effects

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INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by chronic hyperglycemia, with disturbances of carbohydrate, lipid and protein metabolism resulting from defects in insulin secretion, insulin action or both, causing long term complications in many organs1. Globally, the prevalence of diabetes mellitus is increasing. By the year 2025, India shall have the maximum number of diabetics in the world, making it the 'Diabetic Capital of the world'[2]. In modern medicine, treatment of diabetes mellitus with sulfonylureas, biguanides and insulin poses undesirable side effects[3]. So, management of diabetes without side effects is a challenge to the medical system. There is an increasing demand to use the natural products with anti-diabetic activity. Plants are useful sources for the development of anti-diabetic drugs1. However, few traditional anti-diabetic plants have received proper scientific validation. Today, it is necessary to provide scientific proof about the justification of use of plant products as anti-diabetic agents which are also claimed to have low toxicity.

Nyctanthes arbor-tristis Linn.(Synonyms are Parajiatham or Harsinghar) of the family Oleaceae, is a perennial, terrestrial tree widely distributed in the Indo-Pak subcontinent and South East Asia, having remarkable folk medicinal use. The various parts of the plant are used as carminative, purgative[4], anti-oxidant[5], immune-stimulant[6, 7,] anti-bacterial[8] and significant anticancer [9]activity. Earlier studies have shown the root extract of this plant to possess acute hypoglycaemic effect at 500mg/kg[10]and hypolipidaemic effect at 200mg/kg11in rats. Traditional medicine practitioners have used various parts of the plant as anti-hyperglycaemic agent. The present study was undertaken to evaluate the hypoglycemic activity of Nyctanthes arbortristis leaf extract (NALE) at different dose levels in rabbits and to compare its efficacy with the standard drug Glibenclamide.

Preparation of *Nyctanthes Arbor-tristis* leaf extracts (NALE):

The mature leaves of *N. arbor-tristis* were collected from the trees during the months of March and April from the plants available locally. The leaves were then dried and powdered. The powdered material was taken and extracted by the process 'maceration' with 90% ethanol at room temperature. The ethanolic extract was filtered and the residue obtained was rejected. The filtrate was evaporated to dryness and *Nyctanthes arbor-tristis* leaf extract was thus obtained. The extract was suspended in Gum Tragacanth and freshly prepared solution was used for each experiment for *in vivo* studies, the NALE was administered orally.

Selection of animals:

The study was conducted on 30 healthy albino rabbits of either sex weighing between 1.5—2kgs. The animals were housed in separate cages under controlled conditions of temperature (22±20C) and humidity (50—60%). All the animals were fed with standard laboratory diet and water regularly. Animals were uniformly divided into five groups, each group consisting of six animals, group I was treated as normal control. Remaining animals were fasted overnight and diabetes was induced by a single intra-peritoneal injection of freshly prepared aqueous solution of alloxan in a dose of 150mg/kg12. The animals which did not develop hyperglycaemia i.e. fasting glucose levels more than 200mg/dl after 48 hours of alloxan administration were rejected and replaced with new animals. After confirmation of diabetes, animals were equally divided into four groups. Group II served as alloxan induced diabetic control. Group III and IV received NALE in doses of 200mg/kg and 400mg/kg body weight orally respectively. Group V received standard drug Glibenclamide in a dose of 2.5mg/kg orally. Drugs were administered orally consecutively for 14 days on once daily basis, with the help of a gastric catheter2. The experiment was conducted with the approval of the Institutional Animal Ethics Committee.

Sample collection and assessment of blood glucose:

3ml blood was collected aseptically from the marginal vein of pinna of the rabbits13 each time on day 0, day 7 and day 14 for the estimation of fasting blood sugar level (Day 0 was the day after 48 hours of injection of alloxan). After collecting the blood, it was taken in a fluorinated vial. The serum was separated by centrifugation at 3000rpm. Then the blood sugar level was analysed by automated analyser (ERBA EM200) by Glucose-oxidase peroxidase method (GOD-POD method).

Statistical analysis:

After collection of data regarding blood sugar level of day 0, day 7 and day 14 of all the five groups, the data was double entered in Microsoft Excel 2007 and cross checked. The checked sheet was copied into a fresh sheet of SPSS (version 16.0). The analysis of the data was done in SPSS. Mean and standard deviation of fasting blood sugar in all groups were analysed. The reduction of blood sugar with low and high dose of NALE as well as with standard drug Glibenclamide was analysed by comparing with diabetic control. The efficacy of low and high dose of NALE was also compared with the efficacy of Glibenclamide in reducing blood sugar. For all these purpose unpaired t test was used to find out whether the difference was statistically significant or not. Finally the results were tabulated.

RESULTS AND DISCUSSION

Table 1 shows the mean and standard deviations of fasting blood sugar levels in different groups. It indicates that with normal control, as well as in diabetic control there was negligible change in the blood sugar level from day 0 to day 7 and day 14. These groups received the vehicle Gum Tragacanth (1ml). So it indicates that Gum Tragacanth itself does not have any hypoglycemic effect. There was reduction in blood sugar level in group III (received NALE at 200mg/kg), group IV (received NALE 400mg/kg) as well as group V (received Glibenclamide 2.5mg/kg). From the table it is obvious that the reduction in blood sugar in group III is to a lesser extent as compared to group IV and group V.

Table 1: Hypoglycemic activity of NALE on alloxan induced diabetes in rabbits (n=6 for each group)

Groups	Mean± S.D. of blood glucose levels (mg/dl)		
	Day0	Day7	Day14
I (normal control)	70.67±10.33	72.17±7.08	71.33±8.38
II (diabetic control)	266.17±19.31	270.83±19.17	271.83±18.23
III (NALE 200mg/kg)	270.50±7.48	247.83±10.01	227.17±11.29
IV (NALE 400mg/kg)	264.33±8.34	129.83±7.55	99.67±7.31
V (Glibenclamide 2.5mg/kg)	267.00±10.02	119.00±3.23	93.67±2.88

To find out the significance of reduction of blood sugar by NALE in different doses and Glibenclamide, unpaired t tests were performed with comparison to diabetic control. Table 2 shows that there is no significant difference in reduction in blood sugar level with NALE (200mg/kg dose) on day 7 ($p=0.539$) as well as on day 14 ($p=0.168$). On the other hand the unpaired t test shows the reduction in blood sugar level with NALE (400mg/kg body weight) is statistically significant on day 7 ($p<0.001$) as well as on day 14 ($p<0.001$). The reduction of fasting blood sugar level by standard drug Glibenclamide was also found to be significant on day 7 ($p<0.001$) as well as on day 14 ($p<0.001$). Efficacy of NALE at high dose (400mg/kg) was compared with Glibenclamide (2.5mg/kg). The table shows that on day 7 the reduction in blood sugar by Glibenclamide is superior to the NALE at higher dose ($p=0.009$). But on long term (day 14) there is no significant difference in reduction in blood sugar by NALE at 400mg/kg and Glibenclamide at 2.5mg/kg. So, on long term the efficacy of NALE is catching the efficacy of Glibenclamide.

Herbal medicines have been used since long times for the treatment of diabetes and continue to be currently accepted as an alternative therapy. Considerably large numbers of anti-diabetic drugs are known through folklore, however, pharmacological evaluation by scientific methods is required to establish the anti-diabetic activity. The study of such medicine might offer natural key to unlock a diabetologist's pharmacy for the future14. In the present study, rabbits were

selected as experimental animals as they were docile, easy to handle because of the size, economical, larger volume of blood could be taken from them at any stage of the experiment as compared to rats, and the physiology of the rabbits being similar to human beings. Alloxan, a β-cytotoxin, induces diabetes by destroying β cells of the pancreas partially through production of reactive oxygen species15. Hence, result of the present study reveals that the intraperitoneal administration of alloxan effectively induced diabetes in rabbits. A rabbit was considered as being diabetic if the blood sugar level was 200mg/dl or greater12. The maximum values accepted as normal were blood sugar levels below 140mg/dl. Diabetic rabbits had much higher blood glucose levels than that of the normal control. The study showed that the vehicle Gum Tragacanth did not have any hypoglycaemic activity in normal and diabetic control rabbits. The results revealed that the Nyctanthes arbor-tristis leaf extract in a dose of 200mg/kg body weight showed less significant effect in diabetic rabbits and lowered the blood glucose to little extent while the leaf extract in a dose of 400mg/kg body weight showed highly significant effect on 7th and 14th day of NALE administration. Administration of Nyctanthes arbor-tristis leaf extract restored the blood sugar level in alloxan induced diabetic rabbits near to the normal level. Glibenclamide was used as a standard antidiabetic drug to compare the activity of Nyctanthes arbor-tristis in a dose of 2.5mg/kg body weight1. The main mechanism of action of Glibenclamide is stimulation of insulin release and inhibition of glucagon secretion. The comparable effect of NALE (400mg/kg) with Glibenclamide (2.5mg/kg) may suggest similar mode of action

Table 2: Unpaired t-tests for fasting blood sugar levels of different groups

Comparison groups	Day	t-value	d.f.	p-value	Mean difference	95% confidence interval of the difference	
						Lower	Upper
NALE 200mg/kg with Diabetic control	0	+0.513	10	0.619	+4.33	—14.50	+23.17
	7	—0.636	10	0.539	—5.67	—25.52	+14.19
	14	—1.485	10	0.168	—12.17	—30.42	+6.08
NALE 400mg/kg with Diabetic control	0	—0.213	10	0.835	—1.83	—20.97	+17.30
	7	—16.766	10	<0.001	—141	—156.50	—116.50
	14	—21.475	10	<0.001	—172.17	—187.68	—149.99
Glibenclamide 2.5mg/kg with Diabetic control	0	+0.094	10	0.928	+0.83	—18.96	+20.62
	7	—19.135	10	<0.001	—151.83	—166.34	—128.33
	14	—23.653	10	<0.001	—178.17	—192.66	—157.00
Glibenclamide 2.5mg/kg with NALE 400mg/kg	0	+0.504	10	0.627	+2.67	—9.19	+14.52
	7	—3.233	10	0.009	—10.83	—18.30	—3.37
	14	—1.871	10	0.091	—6	—13.15	+1.15

The leaves of the plant contain D-mannitol, β-sitosterole, Flavanol glycosides- astragaline, nicotiflorin, oleanolic acid, nyctanthic acid, tannic acid, ascorbic acid, methyl salicylate, an amorphous glycoside and resin, traces of volatile oil, carotene, friedeline, lupeol, mannitol, glucose and fructose, iridoid glycosides and benzoic acid[16]. Presence of flavonoids has been reported in *Nyctanthes arbor-tristis* and flavonoids are frequently implicated as having antidiabetic effects[17]. It has been described that Glibenclamide is effective in moderate diabetic state and ineffective in severe diabetic animals where pancreatic β cells are totally destroyed[18]. The hypoglycaemic effect of medicinal plant extract generally depends on the degree of β cell destruction[19]. A number of other plants have also been observed to exert hypoglycaemic activity through insulin release stimulatory effects[20]. The possible mechanism by which NALE brings about its hypoglycaemic action may be by potentiating the insulin effect by increasing the pancreatic secretion of insulin from β cells[21] through interaction with Sulfonylurea receptor in the plasma membrane of pancreatic β cells. NALE may promote insulin secretion by closure of K+ ATP channels, membrane depolarization and stimulation of Ca++ influx, an initial key in insulin secretion[22]. The findings also suggest that flavonoids in the plant extract may regenerate the damaged β cells and increased insulin sensitivity[23]. However, NALE (400mg/kg) was not able to restore the blood glucose level to the baseline value. This indicates that the extract should be used with alternatives for diabetes control like diet and hypoglycaemic agents.

CONCLUSION:

Nyctanthes arbor-tristis leaf extract in a dose of 400mg/kg produced an anti-diabetic effect comparable to that of Glibenclamide (2.5mg/kg), suggesting the mechanism similar to that of Glibenclamide. Nyctanthes arbor-tristis seems to have a promising value for the development of potent phytomedicines for diabetes, which may be due to the presence of flavonoids. Hence, further studies on larger number of animals and in human beings are required to evaluate potential side effects and to justify the use of the plant for treating diabetes.

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