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ANTIDIABETIC EFFECTS OF *CATHARANTHUS ROSEUS, AZADIRACHTA INDICA, ALLIUM SATIVUM* AND GLIMEPRIDE IN EXPERIMENTALLY DIABETIC INDUCED RAT

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ABSTRACT

Catharanthus roseus (Nyantara), *Azadirachta indica* (Neem), *Allium sativum* (Garlic) are medicinal plants, used in Ayurveda for treating various diseases, one of which is diabetes mellitus. In the present study of 12 months period from January to December 2007, aqueous extract of this plants were prepared and blood glucose lowering effect and improvement of body weight gain in Streptozotocin (50 mg/kg bwt i.p.) induced diabetic rats were measured and compared with that of a patent drug glimepride in the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh. Rats were administered *Catharanthus roseus, Azadirachta indica, Allium sativum* extracts at the dose rate of 1g/kg, 500 mg/kg and 1g/kg bwt orally for 14 days, respectively. Blood glucose level and body weight was measured by Glucotrend kit and Electronic balance and that compared with a patent drug Glimepride at a dose rate of 100 mg/kg bwt. The data were compared statistically by using student's unpaired *t*-test. The herbal preparations of these plants significantly increased body weight gain and decreased blood glucose as compared with the patent drug. The present study clearly indicated the significant antidiabetic activity of *Catharanthus Roseus, Azadirachta indica* and *Allium sativum* and supports the traditional usage of the herbal preparations by Ayurvedic physicians for the therapy of diabetics.

Key words: *Catharanthus roseus, Azadirachta indica, Allium sativum*, Glimepride, Streptozotocin, diabetic rat

INTRODUCTION

Diabetes mellitus is a major health problem not only in urban but also in the rural areas of Bangladesh. Diabetic patients of rural areas usually do not register themselves in diabetic clinics or hospitals because the medical facilities are mainly concentrated in the urban areas. So, with the ever-increasing incidence of diabetes, it's management is becoming costly not only for the individual and his/her family but also for the national health care sector. Now-a-days various medicinal plants are becoming very popular for the treatment of different diseases in our country as well as all over the world. There are a number of plants to control the blood glucose level such as Nayantara (*Catharanthus roseus),* Neem (*Azadirachta indica*), Garlic (*Allium sativum*), Karela (*Momordica indica*), Methi (*Trigonella foenum graecum*), etc, which are indigenous plants of Bangladesh. So, this study with such above-mentioned plants might offer a natural key to unlock a dialectologist's pharmacy in future. For considering all these constraints, in this experiment we wanted to establish indigenous system of medicine (herbal therapy) as anti-diabetic drugs instead of chemical drugs and rat was used as model of animal.

MATERIALS AND METHODS

The experiment was performed in the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh for a period of 12 months from January to December 2007 to evaluate the comparative efficacy of Neem, Nayantara, Garlic and Glimepride (Amaryl®) on streptozotocin induced diabetic rats.

Collection and acclimatization of rats

Thirty mixed albino rats, long Evan's strain (*Ratus norvegicus*) aged between 3 to 4 months and weighing between 200 to 300 g were collected from International Centre of Diarrhoeal Disease Research, Bangladesh (ICDDR, B). All the rats were grouped into six, each containing 5. Each group of rats was housed at serene bottomed wire cages arranged in rows and kept in the animal house of Department of Pharmacology, Bangladesh Agricultural University, Mymensingh. The animals were fed with pellet at a recommended dose of 100 g/kg as advised by ICDDR, B. Drinking water was supplied *ad libitum*. The rats were maintained in this condition for a period of three weeks to acclimatize them prior to experimental uses.

Induction of diabetes

Streptozotocin (STZ) induced hyperglycemia has been described as a useful experimental model to study the activity of hypoglycemic agents (Junod *et al.,* 1969; Ledoux *et al*., 1966). After overnight fasting (deprived of food for 16 hours had been allowed free access to water) diabetes was induced in rats by intraperitoneal injection of STZ (Sigma, St. Louis, Mo) dissolved in O.1M sodium citrate buffer pH 4.5, at a dose of 55mg/kg body weight (Chattopadhyay, 1999) in five groups of rats. The control rats received the same amount of 0.1 M sodium citrate buffer. The animals were allowed to drink 5% glucose solution overnight to overcome the drug-induced hypoglycemia. After a week time for the development of diabetes, the rats with diabetes having glycosuria and hyperglycemia (blood glucose range of above 250-300 mg /dl) were considered as diabetic rats and used for the further experiments.

Collection and preparation of plants extracts

Fresh nayantara, neem leaves were collected from Medicinal Plants Garden, Department of Pharmacology, BAU, Mymensingh and garlic seeds was purchased from the K.R. market, BAU campus. The plant materials were measured separately by electronic balance and grinded with mortar and pestle. Finally, the extracts were mixed with 50 ml distilled water separately and stirred to make homogenous mixture and then filtered through silk cloth and stored in refrigerator until used.

Collection, preparation and preservation of Glimepride (Amaryl®)

The oral antidiabetic drug Glimepride (Aventis, Bangladesh Limited) was bought from K.R. Market of Bangladesh Agricultural University Campus Mymensingh. The Glimpride was dissolved in distilled water to make a concentration of 100 mg/ml. The Glimepride was given orally @ 100mg/kg bwt daily for 14 days.

Experimental design

In our study, we used total 30 rats (five normal rats and twenty-five STZ induced diabetic rats). The diabetic rats were divided into five groups having five rats in each: Group A Normal control, Group B Diabetic control, Group C Diabetic rats treated with NyLEt (1g/kg bwt), Group D Diabetic rats treated with NLEt (500mg/kg bwt). Group E Diabetic rats treated with GSEt (1g/kg bwt) and Group F Diabetic rats treated with Amaryl® (100mg/kg bwt) orally for 14 days.

Body weight of all treated rats (4 groups), normal control and diabetic control groups were taken on pre and post i.e. day 0, 7^{th} and 14^{th} days of post treatment by electronic balance.

Blood glucose level of all treated rats (4 groups), normal control and diabetic control groups were taken pre and post i.e. day 0, 7^{th} and 14^{th} days of post treatment by Glucotrend kit machine (Boehringer Mannheim, U.K).

Statistical Analysis

Data was expressed as Mean ± SD of means. Statistical analysis was made by using unpaired *t*-test (Steel and Torre, 1960).

RESULTS AND DISCUSSION

Effect on body weight in control and experimental rat

The effects of different doses of aqueous extracts of nayantara (*Catharanthus roseus*) leaves, neem (*Azadirachta indica*) leaves, garlic (*Allium sativum*) the body weight in normal control, diabetic control and diabetic treated rats were shown in Table 1. After 14 days of treatment with nayantara leaves, Neem leaves, garlic and Amaryl® (Glimepride), body weight were increased significantly ($p < 0.01$) to the extent of 4.54 to 12.3 percent (Table 1) in comparison with pretreatment period. In diabetic control group body weight decreased (4.10%). Among the herbal drugs treated groups, higher body weight (7.58%) was recorded in neem treated groups followed by nayantara (4.54%) and garlic (1.37%) treated groups. Amaryl® also increased the body weight (14.03%). Results of the present study supports partially the findings of Devi *et al*. (2003), Pari and Saravanan (2000) and Bopannaet *et al*. (1997) who also reported significant increase in body weight after treatment with herbal preparations in hyperglycemic animals.

Table1. Effect of nayantara leaves, neem leaves, garlic and Amaryl® (Glimepride) tablet on body weight gain in normal and STZ treated diabetic rat

Groups	Treatment (mg/kg bwt) PO	Body weight (g) (n = 5)			
		Pre-treatment	Post-treatment		
		Day 0	Day 7	Day 14	
			Mean ± SD	Mean ± SD	%
A	Normal control	265.28±5.25	263.6±5.26*	266.36±5.17*	+ 0.41
B	Diabetic control	226.06±12.13	222.04±12.34[a]***	216.80±12.23[a]***	- 4.10
C	Nayantara leaves 1000	163.38±8.71	166.76±8.73[b]***	170.80±8.74[b]***	+ 4.54
D	Neem leaves 500	147.66±9.31	149.68±9.21[b]***	158.86±9.27[b]***	+ 7.58
E	Garlic 1000	152.80±9.23	173.12±10.41[b]**	154.90±10.29[b]***	+ 1.37
F	Amaryl® (Glimepride) tablet 100	176.18±5.72	179.90±5.99[b]***	200.90±5.88[b]***	+ 14.03

PO = Per os (Orally), n = Number of rats, *Significant at 5%, **Significant at 1%, ***Significant at 0.1%, Values expressed in parenthesis denotes maximum percentage of increase[b]/decrease[a].

Effects on blood glucose level in normal and experimental rat

The effect of streptozotocin by damaging the beta cells of pancreas interfered glucose metabolism resulting increasing blood glucose level. The effect of different doses of aqueous extracts of nayantara (*Catharanthus roseus*) leaves, neem (*Azadirachta indica*) leaves and garlic (*Allium sativum*) on blood glucose in normal control, diabetic control and diabetic treated rats was shown in the Table 2.

Table 2. Effect of nayantara leaves, neem leaves, garlic and Amaryl® (Glimepride) tablet on blood glucose in normal and STZ treated diabetic rat

Groups	Treatment (mg/kg bwt) PO	Blood glucose (m mol/L) (n = 5)				
		Pre-treatment	Post- treatment			
		Day 0	Day 7		Day 14	
			Mean ± SD	%	Mean ± SD	%
A	Normal control	5.52±0.15	5.65±0.19 NS	+2.36	5.65±0.16NS	+2.36
B	Diabetic control	32.70±0.84	32.99±0.89[b] NS	+0.89	33.64±0.91[b] NS	+2.87
C	Nayantara leaves 1000	30.12±0.63	28.20±0.34[a]**	-6.37	26.16±0.46[a] ***	-13.15
D	Neem leaves 500	31.24±0.61	26.14±0.43[a]***	-16.33	22.33±0.46[a] ***	-28.52
E	Garlic 1000	31.44±0.58	27.88±0.19[a]**	-11.32	28.44±0.18[a] ***	-9.54
F	Amaryl® (Glimepride) tablet 100	31.52±0.33	26.10±0.33[a]***	-17.20	14.26±0.21[a] ***	-54.75

PO = Per os (Orally), n = Number of blood samples, *Significant at 5%, **Significant at 1%, ***Significant at 0.1%, NS = Non-significant, Values expressed in parenthesis denotes maximum percentage of increase[b]/decrease[a].

After 14 days of treatment with nayantara (*Catharanthus roseus*) leaves, neem (*Azadirachta indica*) leaves, garlic (*Allium sativum*) and Amaryl® (Glimepride) blood glucose were decreased significantly ($p < 0.01$) to the extent of 15.14 to 54.75 percent in comparison with pretreatment period. The plant materials were in the study neem was more effective which decreased the blood glucose (28.52 %) in comparison with other plant materials used in this study i.e. nayantara (13.15%) and garlic (9.54 %). Amaryl® also decreased the blood glucose (54.75%). After 14 days of treatment with Amaryl® (Glimepride) the blood glucose level reduce significantly ($p < 0.01$) which agreed with that of Zhang and Tan (2000).

The exact mechanism in reducing blood glucose level is not well understood. But the probable cause of reduction of blood glucose might be due to increased uptake of glucose peripherally and increased sensitivity of insulin receptor in case of neem leaves. In accordance to the present finding some author also reported reduction of blood glucose following administration of insulin and neem such as Pari *et al*. (2001), Khosla *et al.* (2000). On the other hand, Chattopadhyay (1999) reported that *Azadirachta indica* leaf extract was found to have the most potential activity as blood sugar lowering agent followed by *Catharanthus roseus, Gymnemaq sylvestre* and *Ocimum sanctum.* In case of garlic the exact mechanism of action is still unclear. The antidiabetic effect of garlic is thought to be due to the formation of a colloidal type suspension in the stomach and intestines when the mucilaginous fiber of garlic is hydrated, therefore affecting gastro-intestinal transit and slowing glucose absorption. Shalini *et al.* (2004) also reported the reduction of blood glucose following administration of garlic extract. Devi *et al.* (2003) and Vats *et al.* (2002) also reported similar results.

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