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## Herbal Products: A Novel Approach for Diabetic Patients

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**Abstract:** A research work of herbal medicine viz. Neem leaf extract (*Azadirachta indica*), nayantara leaf extract (*Catharanthus roseus*) and bitter melon fruit (*Momordica charantia*) juice with the patent drug gliclazide (Compid®, Square Pharmaceuticals Bangladesh Ltd.) were studied on blood glucose level, hematological parameters and on body weight in rats. Twenty-five apparently healthy adult rats were randomly divided into 5 equal groups namely A, B, C, D and E. One group (group A) was kept as control. The rest four groups (B, C, D and E) of rats were treated with gliclazide (Compid®) @ 4.5 mg/kg bd. wt./day, neem leaf extract (NLE) @ 500 mg/kg bd. wt./day, nayantara leave extract (NtLE) @ 500mg/kg bd. wt./day and bitter melon fruit juice @ 500 mg/kg b. wt./day respectively for 14 consecutive days. Blood glucose levels were significantly ( $P<0.01$ ) reduced in all treated four groups of rats (39.78-44.31%) in comparison to their pre-treatment values. Total erythrocyte count (TEC), total leukocyte count (TLC) and differential leukocyte count (DLC) were not changed significantly in any treated group (B, C, D & E). Eosinophil and monocytes and hemoglobin contents were decreased significantly ( $P<0.01$ ) in all groups. Among the herbal drugs used in the study bitter melon fruit juice was more effective (7.45%) in increasing the body weight in comparison with other herbal preparations i.e. NtLE (7.4%) and NLE (4.86%). From the present study, it may be further revealed that although the patent drug gliclazide was found to be highly effective, as blood glucose lowering agent, but the efficacy of three different combined form of herbal preparations was also seemed to be encouraging.

**Key words:** Herbal medicine, neem leaf, nayantara leaf, diabetic patients

### Introduction

The use of herbal medicines is widespread. Bangladesh is full of medicinal plants, which are used by the people for the treatment of various diseases even at this modern era. There are various medicinal plants in the world, which are the potential sources of the drugs. Traditionally various plants are being used to treat diabetic patients. It is believed that herbal medicine has little side effects as well as it requires no cost in few cases. So, the herbal medicine can solve the economic problem for the poor. Now days, scientists and researchers are very much busy on research of natural plant products all over the world and a large number of evidence have shown the immense potential of medicinal plants used traditionally. Diabetes mellitus is a major endocrine disorder affecting nearly 10% of the population all over the world (Burke *et al.*, 2003). Diabetes is a deadly disease that affects an estimated 135 million people worldwide (Roberts, 2001) and the numbers are increasing in rural and poor populations throughout the world. In Bangladesh about 5 million people are affected with diabetes. Shapiro and Gong (2002) listed that some plants which are being used to control blood glucose level, such as neem, , agrimony, aloe, bilberry, cinnamon, fenugreek, American ginseng, Asian ginseng, garlic, vincarosea, bitter melon, onion etc. Diabetes is one of the leading causes of death in human and animals. In animals it occurs most

frequently in the dog, with an incidence of approximately 0.2%. Since time immemorial, patients with non-insulin dependent diabetes mellitus have been treated orally by folklore with a variety of plant extracts. In the indigenous Indian system of medicine, good number of plants were mentioned for the cure of diabetes and some of them have been experimentally evaluated and the active principles were isolated (Grover *et al.*, 2002). However search for new anti-diabetic drugs continues. In Bangladesh and India, neem is widely used as a medicinal plant for thousand of years. Different parts neem (*Azadirachta indica*) like seed and neem leaf extract (NLE) have been shown to possess hypoglycemic effect. Bitter melon (*Momordica charantia*) popularly known as karela which is widely used as vegetable throughout the world including Bangladesh, Brazil, Africa, China, India, The Far East, The Caribbeans and is cultivated as a tropical vegetables in Asia, Africa, and South America where it has been used as a traditional therapy for diabetes. The peptides and terpenoids from bitter melon perhaps the most widely used traditional antidiabetic remedy, are believed to be responsible for the plant's hypoglycemic properties. Considering the economic resource constraints and cheapness of these plant products (neem, noyantara and bitter melon) the present study was conducted to investigate the efficacy of these herbal products on blood glucose level, hematological parameters and body weight gain, if any.

Table 1: Comparative efficacy of three different herbal preparations with patent drug, gliclazide on blood glucose (mg/dl) in rat

Group No. of rat	Drug with dose	Pre-treatment	Treatment period		
		Day 0	Day 1	7th Day	14th Day
A (n=5)	Normal rats (Control)	7.2±1.10	6.82±1.01	6.38±0.81	6.6±1.02
B (n=5)	gliclazide (Compid®) 4.5 mg/kg bd. wt./day	8.26±0.34	4.56±0.59** (-44.79%)	5.36±0.35** (-35.11%)	4.6±0.21** (-44.31%)
C (n=5)	Nyantara leaf extract (NtLE) @500mg/kg bd. / wt.	7.34±0.8	6.18±0.58** (-15.80%)	5.18±0.44** (-29.43%)	4.42±0.33** (-39.78%)
D (n=5)	Neem leaf extract (NLE) @ 500 mg/kg bd. /day	7.54±0.89	6.2±0.56** (-17.77%)	5.02±0.58** (-33.42%)	4.54±0.6** (-39.79%)
E (n=5)	Bitter melon fruit juice @ 500mg/ kg bd. wt./day	8.34±0.73	6.34±0.29** (-23.98%)	5.34±0.27** (-35.97%)	4.78±0.28** (-42.69%)

Values expressed are mean ± SE of 5 rats, \*\* Significant at (P<0.01)

Table 2: Comparative efficacy of three herbal preparations with patent drug, gliclazide on body weight (gm) in rat

Group No. of rat	Drug with dose	Pre-treatment	Treatment period	
		Day 0	7th Day	14th Day
A (n=5)	Normal rats (Control)	105.80±39.92	113.60±30.03 (+7.37%)	119.20±28.62 (+ 12.66%)
B (n=5)	Gliclazide (Compid®) 4.5 mg/kg bd. wt./day	166.80±34.87	171.00±33.55 (+ 2.52%)	174.40±33.69 (+ 4.56%)
C (n=5)	Nyantara leaf extract (NtLE) @500mg/kg bd. /day	158.40±38.68	166.00±38.07 (+4.80%)	159.80±28.61 (+ 4.86%)
D (n=5)	Neem leaf extract (NLE) @ 500 mg/kg bd. wt /day	152.40±38.29	156.60±30.93 (+2.76%)	170.20±37.59 (+ 7.45%)
E (n=5)	Bitter melon fruit juice @ 500mg/ kg bd. wt/day	154.00±39.08	160.20±36.13 (+4.03%)	165.40±34.66 (+ 7.40%)

Values expressed are mean ± SE of 5 rats, + = Increase

Table 3: Comparative efficacy of three herbal preparations with patent drug, gliclazide on total erythrocyte count (TEC) (million/cu.mm) in rat

Group No. of rat	Drug with dose	Pre-treatment	Treatment period
		Day 0	Day 14th
A (n=5)	Normal rats (Control)	7.72±0.16	7.72±0.16
B (n=5)	Gliclazide (Compid®) 4.5 mg/kg bd. wt./day	7.72±0.16	7.78±0.03 (+0.78%)
C (n=5)	Nyantara leaf extract (NtLE) @ 500mg/kg bd. wt./day	7.72±0.16	8.02±0.05 (+3.89%)
D (n=5)	Neem leaf extract (NLE) @ 500 mg/kg bd. /day	7.72±0.16	7.74±0.05 (+0.26%)
E (n=5)	Bitter melon fruit @ 500mg/ kg bd. wt./day	7.72±0.16	7.71±0.02 (-0.13%)

Values expressed are mean ± SE of 5 rats, + = Increase, - = Decrease

### Materials and Methods

The experiment was performed in the Department of Pharmacology, Bangladesh Agricultural University, Mymensingh during the period of October to November, 2003. Twenty-five healthy adult rats were collected from the International Centre for Diarrhoeal Diseases Research, Bangladesh (ICDDR, B), Mohakhali, Dhaka, Bangladesh. Prior to the commencement of the experiment, all the rats were acclimatized to the new environmental condition for a period of one week. During the experimental period, the rats were kept in a well-ventilated animal house at room temperature of 25°C

and were supplied with standard ration and fresh drinking water *ad libitum*. The rats were randomly divided into five groups, each group consists of five rats. Rats were placed in separate cage according to group. Each cage was labeled for identification of different groups. Food and water were provided *ad libitum* during the experimental period.

Blood glucose and hematological parameters (TEC, DLC, TLC and Hb) were estimated in all groups of rats before and during treatment.

### Collection and Preservation of Compid® (gliclazide):

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Table 4: Comparative efficacy of three different herbal preparations with patent drug, gliclazide on total leukocyte count (TLC) (thousand/cu.mm) in rat

Group No. of rat	Drug with dose	Pre-treatment	Treatment period
		Day 0	Day 14th
A (n=5)	Normal rats (Control)	9.66±0.11	9.66±0.11
B (n=5)	Gliclazide (Compid®) 4.5 mg/kg bd. wt./day	9.66±0.11	10.68±0.15(+10.56%)
C (n=5)	Nayantara leaf extract (NtLE) @ 500mg/kg bd. /day	9.66±0.11	9.65±0.19 (-0.10%)
D (n=5)	Neem leaf extract (NLE) @ 500 mg/kg bd. wt /day	9.66±0.11	9.64±0.15 (-0.21%)
E (n=5)	Bitter melon fruit juice @ 500mg/ kg bd. wt/day	9.66±0.11	9.80±0.23 (+1.45%)

Values expressed are mean ± SE of 5 rats, - = Decrease, + = Increase

Table 5: Comparative efficacy of three different herbal preparations with patent drug, gliclazide on differential leukocyte count (DLC) in rat

Group No. of rat	Drug with dose	Pre-treatment				
		Neutrophil (%)	Eosinophil (%)	Basophil (%)	Lymphocyte (%)	Monocyte (%)
A (n=5)	Normal rats (Control)	24.40±2.20	3.00±0.00	0.0±0.00	68.40±2.41	4.0±0.00
B (n=5)	Gliclazide (Compid®) @ 4.5 mg/kg bd. wt./day	24.40±2.20	3.00±0.00	0.0±0.00	68.40±2.41	4.0±0.00
C (n=5)	Nayantara leaf extract (NtLE) @500mg/kg bd. wt./day	24.40±2.20	3.00±0.00	0.0±0.00	68.40±2.41	4.0±0.00
D (n=5)	Neem leaf extract (NLE) @ 500 mg/kg bd. wt. /day	24.40±2.20	3.00±0.00	0.0±0.00	68.40±2.41	4.0±0.00
E (n=5)	Bitter melon fruit juice @ @500mg/kg bd. wt./day	24.40±2.20	3.00±0.00	0.0±0.00	68.40±2.41	4.0±0.00
Group No. of rat	Drug with dose	Treatment period				
		Neutrophil (%)	Eosinophil (%)	Basophil (%)	Lymphocyte (%)	Monocyte (%)
A (n=5)	Normal rats (Control)	24.40±2.20	3.00±0.00	0.0±0.00	68.40±2.41	4.0±0.00
B (n=5)	Gliclazide (Compid®) @ 4.5 mg/kg bd. wt./day	25.20±1.30 (+3.28%)	2.40±0.55 (-20%)	0.00±0.00	69.00±1.73 (+0.88%)	3.40±0.55 (-15%)
C (n=5)	Nayantara leaf extract (NtLE) @500mg/kg bd. wt./day	25.80±1.79 (+5.74%)	2.60±0.55 (-13.33%)	0.00±0.00	67.80±1.64 (-0.88%)	3.80±0.45 (-5.0%)
D (n=5)	Neem leaf extract (NLE) @ 500 mg/kg bd. wt. /day	23.40±1.14 (-4.1%)	3.0±0.71	0.00±0.00	69.20±0.84 (-1.17%)	3.40±0.55 (-15%)
E (n=5)	Bitter melon fruit juice @ @500mg/kg bd. wt./day	23.0±1.58 (-5.74%)	2.80±0.45 (-6.67%)	0.00±0.00	70.40±1.67 (+2.92%)	3.80±0.45 (-5.0%)

Compid® (gliclazide) manufactured by Square the local Pharmaceuticals Ltd., Bangladesh, was collected from market. (Compid®) & was preserved at room temperature and used during 14 days treatment period @ 4.5mg/kg bd. wt./day.

**Collection, preparation and preservation of neem leaf extract, nayantara leaf extract and Bitter melon fruit juice:**

**Procedure:** Fresh neem and nayantara leaves were collected from medicinal garden, Department of

Pharmacology, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh bitter melon was collected from local market. The leaves and fruits were measured by electronic balance @ 500mg /kg bd. wt./day and then ground with mortar and pestle. Finally only the leaf extract was mixed with 10 ml distilled water and stirred for homogenous mixture, kept 6 hours and then filtered with silk cloth. Bitter melon fruit juice was collected by grinding with pestle and mortar without adding any water. It was then kept in refrigerator (4°C). All the parameters i.e., blood glucose, hematological values (TEC, DLC, TLC and Hb) and body wt. were

Table 6: Comparative efficacy of three different herbal preparations with patent drug, gliclazide on hemoglobin (gm %) in rat

Group No. of rat	Drug with dose	Pre-treatment	Treatment period
		Day 0	Day 14th
A (n=5)	Normal rats (Control)	12.0±0.35	12.0±0.35
B (n=5)	Gliclazide (Compid®) 4.5 mg/kg bd. wt./day	12.0±0.35	11.10±0.22 (-7.50%)
C (n=5)	Nayantara leaf extract (NtLE) @ 500mg/kg bd. wt /day	12.0±0.35	11.80±0.45 (-1.67%)
D (n=5)	Neem leaf extract (NLE) @ 500 mg/kg bd. wt /day	12.0±0.35	11.20±0.27 (-6.67%)
E (n=5)	Bitter melon fruit juice @ 500mg/ kg bd. wt/day	12.0±0.35	11.40±0.42 (-5.00%)

Values expressed are mean ± SE of 5 rats, - = Decrease, + = Increase

estimated and recorded before and during treatment of different herbal preparations of & patent drug.

Blood samples were collected from retroferengeal vein on day 0 (pre-treatment), day 1, day 7 and day 14 for estimation of blood glucose by Glucotrend test kit and day 0 & day 14 for routine hematological parameters (cutting the tip of the tail) as per method cited by Coffin (1955)

**Statistical analysis:** The data were analyzed statistically between treatment and control values by well-known student's test ('t' test).

**Dosage schedule:**

- Group A: Control (normal rat without any treatment).
- Group B: Normal rats treated with Compid® (gliclazide) @ 4.5 mg/kg bd. wt./day orally for 14 days.
- Group C: Normal rats treated with NLE 500-mg/kg bd. wt./day orally for 14 days.
- Group D: Normal rats treated with nayantara @ 500 mg/kg bd. wt./day orally for 14 days.
- Group E: Normal rats treated with Bitter melon @ 500 mg/kg bd. wt./day orally for 14 days.

**Results and Discussion**

**Effects on blood glucose level:** The effects of gliclazide and three herbal preparations i.e. neem leaf extract (NLE), nayantara leave extract and bitter melon fruits juice significantly decreased blood glucose level (P<0.01) with a highest reduction by gliclazide (45%) . In accordance with the present finding other scientists also reported similar results (Ahmed, 2003; Khosla *et al.*, 2000; Chattopadhyay, 1999; Bajaj and Srinivasan, 1998; Bopanna *et al.*, 1997). The exact mechanism in reducing blood glucose level is not well understood. The mechanism of reducing blood glucose might be due to increased uptake of glucose peripherally and increased sensitivity of insulin receptor. The active constituents of these herbal products might be responsible for anti-hyperglycemic activity, which is not known clearly. Some laboratory studies have shown that extracts of bitter melon may block the absorption of sugar molecules in the intestine to improve the body's ability to use sugar which would help to reduce blood sugar levels (Meir and

Yaniv, 1985). Bitter melon extracts might enhance the secretion of insulin from pancreas (Chakravarthy *et al.*, 1982). Ahmed *et al.* (1998) found that the juice of bitter melon fruit might actually help to renew or recover partially destroyed insulin secreting cells in the pancreas.

In dichloromethane-methanol extract of leaves and twigs of nayantara (*Catharanthus roseus*) @ 500mg/kg b. wt./day given orally for 7 and 15 days showed 48.6% and 57.6% hypoglycemic activity respectively (Singh *et al.*, 2001). Enzymic activities of glycogen synthase, glucose-6-phosphate dehydrogenase, succinate dehydrogenase and malate dehydrogenase were decreased in liver of diabetic animals in comparison to normal and were significantly improved after treatment with extract @ 500mg/kg b. wt./day P.O. for seven days resulting increased metabolization of glucose in treated rats (Singh *et al.*, 2001).

**Effects on hematological parameters:** TEC and TLC were not significantly changed in any treated group (B, C, D & E). Variable findings on TEC and TLC have been reported by many scientists indicating that variation is not severe and harmful (Ahmed, 2003; , Lyons *et al.*, 1988; Bansal *et al.*, 1994 and Olefsky and Reaven, 1976). On the other hand, during the treatment period DLC values were slightly increased or decreased following patent drug (gliclazide) and three other herbal preparations but was not significant. Eosinophil, monocyte and hemoglobin contents were significantly (P<0.01) decreased in hypoglycemic treated groups B, C, D and E. The neem leaf extract (NLE), nayantara leaf extract (NtLE),bitter melon fruit juice (KFJ) and patent drug gliclazide decreased the hemoglobin content (6.67%), (5.0%), (1.67%) and (7.5%) respectively. It is revealed that all the herbal treated groups of rats could decrease the hemoglobin content. The present study is partially in agreement with findings of Bansal *et al.*, (1994). The decrease in hemoglobin may be due to decrease in TEC.

**Effects on body weight:** Among the herbal drugs used in the study bitter melon fruit juice was more effective (7.45%) in increasing the body weight in comparison

with other herbal preparations i.e. NtLE (7.4%) and NLE (4.86%). Results of the present study supports partially the findings of Ponnachan *et al.* (1993) and Bopanna *et al.* (1997) who also observed significant increase in body weight after treatment with herbal preparations in hyperglycemic animals.

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