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RESEARCH ARTICLE

COMPARATIVE EFFICACY OF METHANOLIC EXTRACT OF NAYANTARA, TELAKUCHA AND THEIR COMBINATIONS USED IN STREPTOZOTOCIN INDUCED DIABETIC RATS

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Abstract

Plants have been the major source of medicine throughout human history. Even today, this trend continues. The aim of the present study was to evaluate the antidiabetic activity of the methanolic extract of *Catharanthus roseus* (Nayantara), *Cephalandra indica* (Telakucha) and their combination compared to standard drug Glimpepride (Amaryl®). Diabetes was induced experimentally in the rats with Streptozotocin (STZ; 55 mg/kg, i.p.). The extract of *Catharanthus roseus* (Nayantara), *Cephalandra indica* (Telakucha) & their combination were administered orally at the rate of 500 mg/kg and Glimpepride at 800 µg/kg body weight daily. The herbal preparations of these plants significantly increased body weight and decreased blood glucose as compared with the patented drug Glimpepride (Amaryl®). The present study clearly indicated the significant antidiabetic activity of *Catharanthus roseus* (Nayantara), Telakucha (*Cephalandra indica*) combination of Nayantara & Telakucha. Telakucha was more effective which decreased blood glucose, thereby justifying the use of those plants in the Unani & Ayurvedic system of medicine.

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Introduction:-

Diabetes mellitus is a major challenge for health care systems around the world (González-Villalpando et al., 2008). Type I diabetes is caused by immune system mediated destruction of insulin-producing beta cells in the pancreatic islets of langerhans (Tsai et al., 2008). Clinically, diabetes mellitus is one of the most important risk factors for cardiovascular diseases (Tripathi et al., 2006). Currently, more than 150 million people worldwide are suffering from this disease and it is expected that the number of them reach 366 million in 2030 (Wild et al., 2004). Diabetes is characterized by hyperglycemia due to absolute or relatively deficient insulin levels (World Health Organization, 2014). Although insulin therapy is widely used for management of diabetes mellitus, its many side effects, such as, insulin resistance, anorexia nervosa, brain atrophy, and fatty liver after chronic treatment, makes it a risky

proposition (Rutter, 2001; Taylor & Agius, 1998). Prior to the discovery of insulin and other hypoglycaemic synthetic drugs, herbal medicine has been long used for the treatment of diabetic patients and they are currently accepted as an alternative therapy for diabetic treatment and control. Recent scientific investigation and clinical studies have confirmed the efficacy of many medicinal plants and herbal preparations in the improvement of normal glucose homeostasis.

There are several drugs in clinical practice for the treatment of diabetes mellitus. Many of these oral anti-diabetic agents have been reported to show serious adverse effects. It is apparent that due to the side effects of the currently used drugs, there is a need for a potent drug with minimal adverse effects, which can be taken for long durations. Plant materials which are being used as traditional medicine for the treatment of diabetes are considered one of the good sources for a new drug or a lead to make a new drug. Throughout the world, many traditional medicinal plant treatments for diabetes exist. However, few have received scientific or medical scrutiny and the WHO has recommended that traditional medicinal plant treatments for diabetes warrant further evaluation (Ansarullah et al., 2009; Daisy et al., 2009 & Wadkaret al., 2008).

Catharanthus roseus (Vinca rosea) a traditionally used medicinal plant, belongs to the family:-

Apocynaceae. The plant has been considered due to its wide range of pharmacological activity, like anti-inflammatory, antimalarial, antimutagenic, antihypertensive, antifertility, antihypercholesterolemic, antimutagenic, antidiuretic, antifungal, antispasmodic, antiviral, cardio tonic, CNS depressant, antitumor, cytotoxic, antispermatic, and anticancer activities. The study involved the whole plant of *Catharanthus roseus* for evaluating both antibacterial and antidiabetic activity (Huxley, 1992). It contains 150 alkaloids, including vincristine, vinblastine, ajmalicine, (Treas and Evans, 2002) etc. and also contains glycosides, carbohydrates, flavonoids, phenols, tannins, proteins, amino acids, fats, and oils (Khandelwal, 2007).

Cephalandra indica belongs to the family Cucurbitaceae. It is famous for its Antitussive (Chandrasekar, 1989), Antidiabetic (Ramakrishnan et al., 2011), Antioxidant (Venkateswaran & Pari, 2003), Anti-inflammatory & Analgesic (Niaz et al., 2009), Antihyperlipidemic (Singhet et al., 2007), Chemoprotective, Hepatoprotective (Sunil et al., 2009) activities. Aerial parts of *Cephalandra indica* contain heptacosane, cephalandrol, tritriacontane, β -sitosterol alkaloids cephalandrine α and β (Dhargalkaret et al., 1959 & Qudrat-i-Khuda et al., 1965). Fruit contains taraxerone, taraxerol, and (24R)-24-ethylcholest-5-en-3 β -ol glucoside, β -carotene, lycopene, cryptoxanthin, apo-6'-lycopenal and β -sitosterol (Kundu & Ray, 1987; Basu & Ghosh, 1972; Bhakuni et al., 1962). The leaves contain isoprenoid alcohol, C60-polyphenol ((Singhet et al., 2007).

Catharanthus roseus (Nayantara), *Cephalandra indica* (Telakucha) are indigenous medicinal plants of Bangladesh. Considering the above, our objective was to study the effect of methanolic extract of *Catharanthus roseus* (Nayantara), *Cephalandra indica* (Telakucha) and their combination on fasting blood glucose and body weight compared to Glimperide (Amaryl®) in streptozotocin-induced diabetic rats.

Materials and Method:-

Plant material collection:-

Catharanthus roseus (Nayantara), *Cephalandra indica* (Telakucha) are available in Mymensing, Chittagong, Cox's Bazar, Barguna, Rajshahi, Pabna, Jessore, Kustia, Faridpur, Dhaka, and all over in Bangladesh. For our study, the sample was collected from the Gazipur near Dhaka and identified by the experts of Bangladesh National Herbarium, Dhaka.

Preparation of methanolic extracts:-

Catharanthus roseus (Nayantara), *Cephalandra indica* (Telakucha) plants were carefully cut with the help of scissors and separated from other parts. About 500 gm of the *Catharanthus roseus* (Nayantara) and *Cephalandra indica* (Telakucha) were dried for 15 days without the direct contact of sunrays. When the moisture content of sample drug was found to be below 5% by loss on drying (LOD) at 100°C, the *Catharanthus roseus* (Nayantara), *Cephalandra indica* (Telakucha) were powdered and weighed (300gm) and then extracted with 95% of methanol in a Soxhlet's apparatus at an elevated temperature. The extract was concentrated by evaporation under reduced pressure at 40°C using a Buchi rotary evaporator to yield a gummy reddish black colored extract (yield App. 9.6%) (Nyeem et al., 2016).

Collection and preparation of Glimepiride (Amaryl®):-

The oral antidiabetic drug Glimepiride (Amaryl®) was bought from local market of Bangladesh Agricultural University Campus Mymensingh. The Glimepiride (Amaryl®) was dissolved in distilled water to make a concentration of 800µg/ml. The Glimepiride (Amaryl®) was given orally at the doses of 800µg/kg body weight daily for 14 days.

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 (ip) injection of STZ dissolved in 0.1M 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 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 further experiments.

Experimental design:-

In our experiment, we used a total of 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 Nayantara (500 mg/kg bwt), Group-D Diabetic rats treated with Telakucha (500mg/kg bwt). Group E Diabetic rats were treated with a combination of Nayantara and Telakucha (500mg/kg bwt) and Group F Diabetic rats were treated with Glimepiride (Amaryl®)(800µg/kg bwt) orally for 14 days. Body weight of all treated rats (4 groups), normal control and diabetic control groups were taken before and after, i.e. day 0, 7th and 14th days of post treatment by electronic balance. Blood glucose level of all treated rats (4 groups), normal control and diabetic control groups were taken before and after i.e. day 0, 7th and 14th days of after treatment by using a Glucotrend kit machine.

Drug:-

Glimepiride (Amaryl®)(Aventis, Bangladesh Limited)

Animals:-

Swiss albino rats 3 to 4 weeks old and weighing about 160-220g of either sex were obtained from the Animal house of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were housed under standard laboratory conditions (relative humidity 55-65%, r.t. 23.0 ± 2°C and 12 h light: dark cycle). The animals were fed with standard diet and water ad libitum. The animals were fasted 12 hours before the conduct of experiment and during the experiment they were withdrawn from food and water. The University Animal Research ethical Committee approved the experimental protocol.

Acute toxicity test:-

Acute oral toxicity (Ecobichon, 1997) study was performed as per OMCD-423 guidelines. Test animals (n = 5) of either sex selected by random sampling technique were for the study. The animals were kept fasting for overnight providing only water, after which the aqueous extracts were administered orally at the dose label of 5 mg/kg body weight by intragastric tube and observed for 14 days. If mortality was observed in 2-3 animals, then the dose administered was assigned as toxic dose. If mortality was not observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose, such as 50, 200, 500, 1000 and 2000 mg/kg body weight.

Results and Discussion:-**Effect on body weight in control and experimental rat:-**

The effects of methanolic extracts of Catharanthus roseus (Nayantara), Cephalandra indica (Telakucha), and their combination of body weight in normal control, diabetic control and diabetic treated rats were shown in Table 1. After 14 days of treatment with Catharanthus roseus (Nayantara), Cephalandra indica (Telakucha), combination of Nayantara&Telakucha, and standard drug Glimepiride (Amaryl®), body weight were increased significantly (p< 0.01) (Table 1) in comparison with before treatment period. In diabetic control group, body weight decreased (3.22%). Among the herbal drugs treated groups, higher body weight (5.63%) was recorded in Telakuchatreated groups, followed by Nayantara (2.52%) and combination of Nayantara andTelakucha(3.22%) treated groups.

Glimepiride (Amaryl®) also increased the body weight (7.35%). Results of the present study reported a significant increase in body weight after treatment with herbal preparations in hyperglycemic animals.

Table1. Effect of Nayantara, Telakucha, Combination of Nayantara &Telakucha, and standard drug Glimepride (Amaryl®),on body weight gain in normal and STZ treated diabetic rat.

Groups	Dose	Route of administration	Before Treatment	After Treatment			
			0 Day	7 th Day	(%)	14 th Day	(%)
A-Normal control received distilled water	10ml/kg body weight	p.o.	191.06±6.74	188.26±8.13 ^a	- 1.47	192.46±6.386 ^b	+0.73
B-Diabetic control	55mg/kg body weight	p.o.	190.60±7.53	188.46±7.52 ^a	- 1.12	184.46±7.17 ^a	-3.22
C-Nayantara	500mg/kg body weight	p.o.	199.78±4.77	202.28±4.07 ^a	+1.25	204.88±4.28 ^a	+2.52
D-Telakucha	500mg/kg body weight	p.o.	188.26±6.62	191.12±6.02 ^a	+1.52	198.86±4.71 ^a	+5.63
E-Combination of Nayantara&Telakucha	500mg/kg body weight	p.o.	183.34±5.13	193.76±5.46 ^c	+5.68	189.24±5.44 ^a	+3.22
F-Glimepride (Amaryl®)	800µg/kg body weight	p.o.	179.92±3.53	181.78±3.57 ^a	+ 1.03	193.14±3.41 ^b	+7.35

^ap<0.01, ^bp<0.05, ^cp<0.10 vs. control, Student's t-test; values are mean ± S.E (N=5).

Effects on blood glucose level in normal and experimental rats:-

STZ is a nitrosurea compound produced by *Streptomyces achromogenes*, which specifically induces DNA strand breakage in β -cells, causing diabetes mellitus. Therefore, the STZ-diabetic model has been widely employed to induce diabetes in experimental animals (Calabresi&Chabner, 1987). The effect of STZ by damaging the beta cells of islets of langerhans interfered with glucose metabolism, resulting in increasing blood glucose levels. The effect of different doses of methanolic extracts of *Catharanthus roseus* (Nayantara), *Cephalandra indica* (Telakucha), and their combination of Nayantara &Telakucha on blood glucose in normal control, diabetic control and diabetic treated rats was shown in Table 2.

Table 2. Effect of Nayantara, Telakucha, Combination of Nayantara &Telakucha, and standard drug Glimepride (Amaryl®), on blood glucose in normal and STZ treated diabetic rats.

Groups	Dose	Route of administration	Before Treatment	After Treatment			
			0 Day	7 th Day	(%)	14 th Day	(%)
A-Normal control received distilled water	10ml/kg body weight	p.o.	5.56±0.24	5.62±0.21 ^a	+1.08	5.66±0.20 ^a	+1.80
B-Diabetic control	55mg/kg body weight	p.o.	30.94±1.85	31.38±1.52 ^a	+1.42	32.16±1.2 ^a	+3.94
C-Nayantara	500mg/kg body weight	p.o.	31.26±1.91	29.56±1.55 ^a	-5.44	27.3±1.84 ^a	-12.67

D-Telakucha	500mg/kg body weight	p.o.	31.46±1.92	26.86±1.02 ^a	-14.62	22.68±1.01 ^b	-27.91
E-Combination of Nayantara &Telakucha	500mg/kg body weight	p.o.	31.66±1.76	27.3±0.82 ^a	-13.77	28.18±1.05 ^a	-10.99
F- Glimepride (Amaryl®)	800µg/kg body weight	p.o.	31.82±1.76	27.7±0.65 ^a	12.95	16.48±1.50 ^b	-48.21

^ap<0.01 ^bp<0.05 vs. control, Student's t-test; values are mean ± S.E (N=5).

After 14 days of treatment with *Catharanthus roseus* (Nayantara), *Cephalandra indica* (Telakucha), and their combination and standard drug Glimepride (Amaryl®), blood glucose decreased significantly (p<0.01) in comparison with pretreatment period. The plant materials in the study Telakucha was more effective, which decreased the blood glucose (27.91 %) in comparison with other plant materials in this study, i.e. the combination of Nayantara&Telakucha(10.99 %) and Nayantara (12.67 %). Glimepiride (Amaryl®)also decreased the blood glucose (48.21 %). After 14 days of treatment with Glimepiride (Amaryl®)the blood glucose level reduces significantly (p< 0.01).

Conclusions:-

The results of the present study indicate that the methanolic extract of Nayantara &Telakucha, and their combination possess significant antidiabetic activity and significantly increased body weight and decreased blood glucose as compared with the patented drug. The present data justify the traditional uses of this plant for the treatment of various diseases. However, further studies are required for isolation and purification of the active principles of the plant responsible for these effects and to better understand the mechanism of such actions.

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