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COMPARATIVE EFFICACY OF STEVIA LEAF (*STEVIA REBAUDIANA BERTON*), METHI SEEDS (*TRIGONELLA FOENUM-GRAECUM*) AND GLIMEPIRIDE IN STREPTPZOTOCIN INDUCED DIABETEIC RATS

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ABSTRACT

Medicinal plants are becoming very popular for the treatment of different diseases all over the world. The present study was conducted during January 2009 to June 2009 and investigated the effects of Stevia (*Stevia rebaudiana* Bertoni) leaves, methi (*Trigonella foenum-graecum*) seeds in terms of their hypoglycemic activity compared to Glimepiride (Amaryl®, Aventies Pharmaceuticals). Diabetes was induced experimentally in the rats with Streptozotocin (STZ; 55 mg/kg, i.p.). Stevia, methi, combination of stevia-methi extracts and Amaryl ® tablet were orally administered daily at the rate of 100mg/kg, 500mg/kg, 500mg/kg and 800µg/kg respectively after 15 days of STZ injection for a period of 60 days. Changes in the blood glucose levels and body weights were measured and the results were compared statistically. The combined stevia-methi extract showed potent hypoglycemic effect than stevia and methi alone. STZ-induced body weight loss was improved in all groups except stevia treatment group.

Key words: Stevia, methi, glimepiride, STZ-induce diabetic rats

INTRODUCTION

The worldwide prevalence of diabetes is increasing at such a rapid that the World Health Organization (WHO) has identified diabetes as an epidemic condition (King *et al,* 1991). An estimate by WHO, there will be about 250 million cases of diabetes mellitus throughout the world by 2025 (Friedman, 2002). In Bangladesh about 5 million people are suffering from diabetes . This disease alone ranks among the top ten causes of death in western world (Cotran *et al,* 1989). Since the discovery of insulin in 1922, it has been used successfully in insulin-dependent diabetes mellitus (IDMM). But it can not be given orally, daily intake through injection is obviously troublesome and hypoglycemic reactions as an adverse effect may occur in any diabetic patient treated with it. Again insulin resistance, a state of relative tissue insensitivity to the action of insulin, is another drawback for patients taking it for a long period (Larner, 2001). On the other hand, oral hypoglycemic agents such as glimepiride, glibenclamide have some adverse effects such as vomiting, epigastric discomfort, jaundice, headache etc. Traditional medicinal plants are being used throughout the world for a range of anti-diabetic preparations and prior to the availability of insulin, dietary measures (Khajuria and Thomas, 1992), the traditional medicines derived from plants, were the major forms of treatment (Bailey and Day, 1989). Ethnobotanical studies of traditional herbal remedies used for diabetes around the world have identified more than 1,200 species of plants with hypoglycemic activity (Marles and Farnsworth, 1995). The study of such agents might offer a natural key to unlock a diabetologist's pharmacy for future.

Stevia (*Stevia Rebaudiana* Bertoni) is an herbaceous native South American perennial plant containing two major compounds, stevioside and rebaudioside. It is reported that Steviosides has insulinotropic effects in the beta-cell (in vitro), increase the insulin secretion and thereby decreasing blood glucose level. Oviedo *et al* (1979) found an antihyperglycemic effect in rats when supplementing the diet with dried *Stevia rebaudiana* leaves. *Trigonella foenum-graecum* commonly known as methi which effect on blood glucose was evaluated in diabetic patients. Seeds have been shown to be hypoglycemic in normal and mildly diabetic animals but not in those with severe disease (Bailey and Day, 1989). Defatted seeds lowered blood glucose as well as glucagon in dogs both normal and diabetic. Amaryl® Tablet (Glimepiride) was emerged from Sulfonylureas cause hypoglycemia by (a) stimulating insulin release from pancreatic β cells, (b) reduction of serum glucagon level and (c) increased binding of insulin to the target tissue or receptor. Cetto et al. (2000) reported that a single oral administration of glibenclamide @ 3 mg/kg lowered the plasma glucose levels in diabetic rats within three hours of administration. Considering the above, our objectives was to study the effect of aqueous extract of the Stevia (*Stevia rebaudiana* Bertoni), Methi (*Trigonella foenum-graecum*) leaves on fasting blood glucose and body weight compare to glimepiride (Amaryl®) in streptozotocin-induced diabetic rats.

MATERIALS AND METHODS

The proposed experiment on conducted in the Department of Pharmacology, Bangladesh Agricultural University (BAU) during the period from January 2009 to June 2009.

Experimental Rats

The experiment was carried out on 30 apparently healthy rats of Long Evans (*Ratus norvegicus*) rats having 5 weeks age matching and weighing between 100 to 150 g weights. The rats were collected from International Center of Diarrhea Disease Research (ICDDR'B), Dhaka, Bangladesh. During the experimental period, the rats were fed food in pellet form and tap water was supplied *ad libitum*. The rats were maintained in this condition for a period of one week to acclimatize them prior to experimental uses.

Plant materials

Young Stevia plants were collected from BRAC tissue culture nursery at Joydevpur, Gazipur and then were reared for about three months on the roof of the Building-2, Faculty of Veterinary Science, Bangladesh Agricultural University, Mymensingh. Fresh Stevia leaves were obtained from that garden. Fresh methi leaves were collected from the Botanical Garden of BAU Campus and tablet Amaryl® were collected from K.R Market of BAU. The plants were authenticated from the Department of Botany, BAU, Mymensingh, Bangladesh.

Fresh Stevia leaves that were collected from the garden were oven dried first and then dried leaves were grinded with Grinder machine. Then 1g dried leaves samples were mixed with 10 ml distilled water and were allowed to stay for whole night. Everyday new extract were prepared following these techniques. Water extract of methi was made from 100g fresh seed sample by grinding with Grinder machine, and mixed with 2000 ml distilled water. Then the water extract was lyophilized collected as powder form by Freeze drying in Central Laboratory, BAU.

Induction of diabetes

Streptozotocin (STZ) was dissolved in 0.1 M citrate buffer having pH 4.5. To induce diabetic condition in rats STZ injected 55 mg/kg body weight intraperitoneal as done previously. STZ injection rapidly produced the characteristic signs of diabetes, such as increased intake of food and water, frequent urination and increased blood glucose concentration. After one week of STZ injection rats having more than 250 mg/dl random blood glucose concentrations and showing above-mentioned characteristic signs of diabetes were selected for this experiment. Blood samples were collected for blood glucose measurements at alternate weeks.

Experimental design

Experimental 30 rats were divided into 6 groups (n=5) and treated intraperitoneal as follows: group-A as control (Con, without STZ). STZ-induced diabetic rats were divided into five groups (groups B, C, D, E, F) each consisting 5 rats. Group-B; diabetic control (STZ). Group-C; STZ + aqueous extract of stevia leaves @100 mg/kg, Group-D; STZ+ aqueous extract of methi leaves @ 500 mg/kg/ ,Group-E; STZ+ combination of aqueous extract of stevia and methi leaves @ 500 mg/kg, and group-F, Tablet Amaryl®(glimepiride, Aventis) @ 800 µg/kg.

Statistical analysis

Data was expressed as Mean ± Standard Deviation of Means.Statistical analysis was made by using Student's unpaired t-test.

RESULTS AND DISSCUSSION

Diabetes mellitus is a complex disorder or more properly described as a malfunction of the Pancreas (Adams, 1995). Hyperglycemia is the most critical problems in the diabetes with generally, decrease of body weight as progress of diabetes (Kamalakkannan *et al*., 2003). Therefore, the hypoglycemic (Mahomed and Ojewole, 2003) as well as body weight maintaining effects have been considered as the essential characteristics of an anti-diabetic agent, and the efficacy of this herbal extracts has been screened primarily based on these effects.

In this study, Streptozotocin injection in rats showed significant ($p<0.001$) increase in blood glucose level and reduction of body weight. Chowdhury *et al* (2005) shown that a single dose of Streptozotocin (50 mg/kg, intraperitonially) in rats exhibited characteristic signs of diabetic. Following treatment with Glimepiride (Amaryl®), blood glucose level was reduced significantly ($P<0.001$). Amanullah *et al* (2008) also reported significant reduction in blood glucose level with Glimepiride @ 800µg/kg. Some other authors also reported the

reduction of blood glucose level following administration of Stevia (*Stevia rebaudiana* Bertoni) leaves. (Chang *et al*., 2005, Raskovic *et al*., 2004, Jeppesen *et al*., 2003). In this study treatment with Methi (*Trigonella foenum-graecum*) and Talekucha (*Coccinia indica*) was significantly reduced (P< 0.01) blood glucose level. Choudhury et al (2005) reported the reduction of blood glucose following administration of methi seed extract, our previous study and other researcher also support present study data Devi *et al*(2003), Vats *et al* (2002),Raju J *et al* (2001), Mostofa *et al* (2007), M.H.Sumon *et al*(2008) . However, in this study we observed that combination of stevia and methi showed better hypoglycemic effects then that of single therapy in STZ-induced rats.

Antihyperglycemic effect of aqueous extracts of indigenous medicinal plants in STZ-induced diabetic rats

From beginning to end the blood glucose concentration of normal control rats (Group A) was 5.47±0.10 to 5.55±0.56 mmol/L and in the diabetic control rats (GroupB) after Streptozotocin injection the blood glucose concentration on day 0 was 21.01 ±0.83 and on day 60 was 24.02±78mmol/L. In Group C, the blood glucose concentration of stevia (100mg/kg) leaves extract treated group on day 0 was 23.08±0.47 mmol/L and on 60 day was 17.05±0.34mmol/L. In Group D, the blood glucose concentration of Methi seeds extract (500 mg/kg) treated group on day 0 was 22.02±0.24mmol/L and on day 60 16.10±0.56mmol/L. In Group E, the blood glucose concentration of combination of stevia and methi leaves extract treatment (500 mg/kg) on day 0 was 24.05±0.04 mmol/L and on day 60 was 16.02±0.11mmol/L. In Group F, the blood glucose concentration of Glimepiride (Amaryl® @ 800 µg/kg) treated group on day 0 was 23.63±0.35mmol/L and on day 60 was 10.02±0.54mmol/L (Table 1).

Table 1. Changes on blood glucose concentration (mean ± SD, m mol/L) in groups of normal and Streptozotocin (STZ) induced diabetic Rats

Groups (number of rats)	Drug, dose and route	Pre-treatment	Post-treatment				
		Day 0	Day 14	Day 28	Day 42	Day60	% Increase(+) decrease(-) in blood glucose level
A(n=5)	Normal control	5.47±0.10	5.49±0.34	5.50±0.23	5.53±0.32	5.55±0.56	+2.00
B(n=5)	Diabetic control	21.01 ±0.83	22.06±0.34	23.00±0.23	23.09±0.56	24.02±78	+13.00
C(n=5)	Stevia leaves extracts @ 100mg/kg orally	23.08±0.47	22.06±0.04	20.00±0.34	18.09±0.34	17.05±0.34	-26
D(n=5)	Methi leaves extract @ 500 mg/kg, orally	22.02±0.24	21.09±0.21	19.08±0.56	17.06±0.32	16.10±0.56	-28
E(n=5)	Combined stevia and methi extract@ 500mg/kg, orally	24.05±0.04	23.01±0.12	21.09±0.12	14.05±0.67	16.02±0.11	-34
F(n=5)	Amaryl@800µg/kg, orally	23.63±0.35	21.05±0.75	20.04±0.43	13.06±0.34	10.02±0.54	-57

Effect of aqueous extracts of indigenous medicinal plants on body weight gain in STZ-induced diabetic rats

In this present study, at day 0 and day 90 of treatment, average body weight of control and STZ groups were 136.20±5.24g, 124.00±7.11g and 136.92±5.22g, 121.57±7.26g respectively. On the other hand treatment with stevia leaf extracts lowered body weight gain 1.6%. At day 0 and day 90 of treatment, methi seed extract treatment showed 124.80±7.76g, 128.25±6.98g body weight. Combined stevia and methi extracts (500mg/kg) showed 125.40±8.08g and 130.40±8.17g body weight at day 0 and day 90 respectively. At day 0 and day 90 of treatment, tablet Amaryl (800µg/kg) treatment group showed 125.65±5.13g and 132.85±4.96g body weight respectively (Table 2)

Table 2. Changes on body weights (mean ± SD,g) in groups of normal and Streptozotocin (STZ) induced diabetic rats

Groups(number of rats)	Drug, dose and route	Pre-treatment	Post-treatment					% Increase(+) decrease(-) in body weight
		Day 0	Day 14	Day 28	Day 42	Day60	Day 90	
A(n=5)	Normal control	136.20± 5.24	136.36± 5.33	136.61± 5.06	136.70± 5.02	136.70± 5.14	136.92± 5.22	+0.53
B(n=5)	Diabetic control	124.00± 7.11	123.06± 0.34	123.07± 0.23	122.14± 0.56	122.02± 0.78	121.57± 7.26	-2.0
C(n=5)	Stevia leaves extracts @ 100mg/kg orally	125.15± 8.16	124.14± 0.34	124.06± 0.74	123.13± 0.64	123.12± 0.34	123.10± 7.94	-1.6
D(n=5)	Methi leaves extract @ 500 mg/kg, orally	124.80± 7.76	125.06± 0.24	125.23± 0.34	126.11± 0.34	127.16± 0.34	128.25± 6.98	+3.0
E(n=5)	Combined stevia and methi extract@ 500mg/kg, orally	125.40± 8.08	125.56± 8.07	126.50± 8.02	127.20± 7.08	129.23± 7.01	130.40± 8.17	+4.0
F(n=5)	Amaryl@800µg/kg orally	125.65± 5.13	125.95± 4.78	127.45± 4.13	129.65± 3.23	131.75± 5.13	132.85± 4.96	+5.6

Leaves of Stevia plant

Petridish containing methi seeds

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