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

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ABSTRACT

Now-a-days medicinal plants are becoming popular for the treatment of various diseases all over the world and some of them are being used traditionally for the treatment of diabetes in human. The present study investigated the effects of Stevia (*Stevia rebaudiana* Bertoni) leaf, methi (*Trigonella foenum-graecum*) seed in terms of their hypoglycemic activity compared to Glimepiride (Amaryl®). Diabetes was induced experimentally in rats with Streptozotocin (STZ; 55 mg/kg, intraperitoneal). Stevia, methi, combination of stevia-methi extracts and Amaryl ® (glimepiride) tablet were orally administered daily at the rate of 100mg/kg, 500mg/kg, 500mg/kg and 800µg/kg respectively after 2 weeks of STZ injection for a period of 60 days. The combined stevia-methi extract showed potent hypoglycemic effect than stevia and methi alone, were assessed by oral glucose tolerance test and blood glucose measurement. STZ-induced body weight loss was improved in all groups except stevia treatment alone. In conclusion, Stevia and Methi extracts have hypoglycemic and body weight loss improving effects in STZ induced diabetic rats. Furthermore, combination therapy showed better effects. These results suggesting the possible use of the combination of Stevia leaves and Methi seeds aqueous extracts as antidiabetics in herbal medicine.

Keywords:- 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 and Rewwers, 1991). An estimation 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 (Journal of Diabetes 1999, BIRDEM). This disease alone ranks among the top ten causes of death in western world (Cotran *et al*., 1989). Regarding its treatment; a suitable drug is yet not to be available which can permanently cure this disease. Since the discovery of insulin in 1922, it has been used successfully in insulin-dependent diabetes mellitus (IDMM). But it cannot 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 insulin. Again insulin resistance, a state of relative tissue insensitivity to the action of insulin, is another drawback for patients taking insulin for a long period (Larner, 2001). On the other hand, oral hypoglycemic agents such as glimepiride, glibenclamide etc also have some adverse effects such as

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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 in 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 *S. rebaudiana* leaves. *Trigonella foenum-graecum* commonly known as methi which effect on blood glucose was evaluated in diabetic patients. Whole seeds have been shown to be hypoglycemic in normal and mildly diabetic animals but not in those with severe diabetic condition (Bailey and Day, 1989). Defatted seeds lowered blood glucose as well as glucagon in dogs both normal and diabetic (Lawrence Review, 1996). However, the combination use of the aqueous extract of stevia leaf and methi seeds in type-1 diabetes is unclear. Amaryl® Tablet (Glimepiride) was emerged from sulfonylurea group. Sulfonylurea 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 at a dose rate of 3 mg/kg lowered the plasma glucose levels in diabetic rats within three hours of administration. Considering the above fact, present study objective was to evaluate the effect of aqueous extract of the Stevia (*Stevia rebaudiana* Bertoni), Methi (*Trigonella foenum-graecum*) leaves and their combination therapy on fasting blood glucose and body weight compare to glimepiride (Amaryl®) in type-1 diabetic rats.

MATERIALS AND METHODS

The proposed experiment was conducted in the Department of Pharmacology, Bangladesh Agricultural University (BAU), mymensingh.

Animals

The experiment was carried out on male Long Evans (*Ratus norvegicus*) rats having 5 weeks age matching and average weighing is 132±3.1g. The rats were collected from International Center of Diarrhea Disease Research (ICDDRB), 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 use.

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 local Market. The plants were authenticated with the help of a taxonomist in the Department of Botany, BAU, Mymensingh, Bangladesh. And the Voucher samples were stored in the department of Pharmacology, Bangladesh Agricultural University.

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 10ml distilled water and were allowed to stay for whole night. Everyday fresh extract were prepared by using 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 in Central Laboratory, BAU. Finally the herbal drug was collected as powder form by Freeze drying in Central Laboratory, BAU.

Induction of diabetes

Streptozotocin was dissolved in 0.1 M citrate buffer having pH 4.5. To induce diabetic condition in rat a dose of 55 mg STZ per kg body weight were injected 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 (Rafiq *et al*., 2009). Blood samples were collected for blood glucose measurements at alternate weeks.

Experimental design

Animals were divided into 6 groups (n=6 in each group) and treated as follows: group-A as control (Con, without STZ). After 2 weeks of STZ injection diabetic rats were divided in to five groups (groups B, C, D, E, F). Group-B; diabetic control (STZ). Group-C; STZ + aqueous extract of stevia leaves at a dose rate of 100 mg/kg, Group-D; STZ + aqueous extract of methi leaves at a dose rate of 500 mg/kg, Group-E; STZ + combination of aqueous extract of stevia and methi leaves at a dose

rate of 500 mg/kg, and group-F, Tablet Amaryl® at a dose rate of 800 µg/kg. The drugs were administered orally once a day.

Oral glucose tolerance test (OGTT)

After the 60 days treatment with leaf aqueous extracts, oral glucose tolerance test was performed. Rats were fasted for 12-14 hrs before glucose was administered orally by gavage (2.0 g/kg); blood samples were collected from the tail by needle puncture before and at 30, 60, 90, 120 min after oral glucose load as previously described in our laboratory (Rafiq *et al*., 2009). Blood glucose level was determined by a commercial glucose kit based on the glucose oxidase method (Sigma Diagnostics, St. Louis, MO).

Statistical analysis

Data was expressed as Mean ± Standard Error of Means (Mean±SE). Statistical analysis was made by using Student's unpaired t-test. *P* values below 0.05 were considered statistically significant.

RESULTS

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

From beginning to end the fasting blood glucose concentration of normal control rats was 89.01±0.10 to 92.18±0.16 mg/dl. On the other hand in the diabetic control rats after Streptozotocin injection the blood glucose concentration on day 0 was 278.01 ±0.03 and on day 60 was 355.02±0.07 mg/dl (P<0.01). Interestingly, stevia leaves and methi seeds extract treatment significantly reduced fasting blood glucose levels. The blood glucose concentration of stevia leaves extract treated group on day 0 was 281.08±0.14 mg/dl and on 60 day was 229.05±0.15 mg/dl (P<0.05), and methi seeds extract treated group on day 0 was 275.02±0.24 mg/dl and on day 60 was 180.10±0.06 mg/dl (P<0.01). Moreover, the combination of stevia leaves and methi seeds aqueous extract treatment showed greater hypoglycemic effects. The blood glucose concentration of combination of stevia and methi leaves extract treatment on day 0 was 280.05±0.04 mg/dl and on day 60 was 139.72±0.11 mg/dl (P<0.01). The blood glucose concentration of Glimepiride (Amaryl®) treated group on day 0 was 277.63±0.35 mg/dl and on day 60 was 124.02±0.09 mg/dl (P<0.01) (Table - 1).

Effect of aqueous extracts of combination of stevia and methi seeds on glucose intolerance in STZ-induced diabetic rats

Blood glucose levels and its area under curves (AUC) during OGTT were shown in Figure 1. After oral glucose load STZ induced rats showed markedly increased blood glucose levels and its AUC compared to control rats (P<0.01). Interestingly, combination of stevia and methi seeds extract treatment showed markedly lowered blood glucose levels and it's AUC during OGTT which is more or less similar fashion like Amaryl® treatment group.

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

In this present study, at day 0 and day 60 of treatment, average body weight of control and STZ groups were 136.20±0.24g, 175.92±0.22g and 134.00±0.31g, 121.57±0.26g respectively. On the other hand, treatment with stevia leaf extracts showed 129.15±0.16g, 123.10±0.14g at day 0 and day 60 respectively. At day 0 and day 60 of treatment, methi seed extract treatment showed 129.80±0.26g, 128.25±0.08g body weight. Combined stevia and methi extracts showed 131.40±0.08g and 130.40±0.17g body weight at day 0 and day 60 respectively. At day 0 and day 60, tablet Amaryl (800µg/kg) treatment group showed 127.65±0.13g and 132.85±0.16g body weight respectively (Table-2).

Figure 1. Fresh stevia (*Stevia rebaudiana* Bertoni) leaf (left), methi (*Trigonella foenum-graecum*) seeds in petridish.

Figure 2. STZ-induced diabetic rats showed increased glucose intolerance which was improved by combination therapy. Oral glucose tolerance test were performed in rats at 60 day during treatment. After overnight fasting, rats were gavaged with glucose (2 g/kg) and blood samples were collected at 0, 30, 60, 90, 120 min, and blood glucose level (A) and its area under curve (AUC) (B). All data are expressed as means±SEM of n=5 per group. *****P*< 0.01 vs. control, ##*P*< 0.01 vs. STZ.

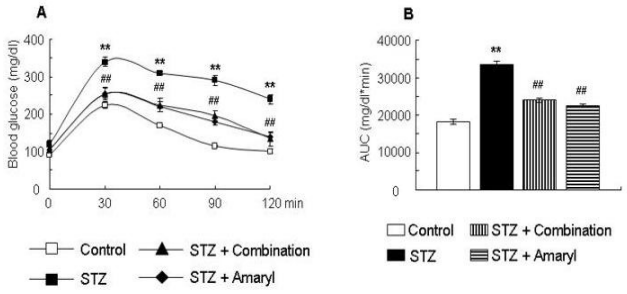

Table 1. Changes on blood glucose concentration (mean ± SE, mg/dl) in groups of normal and Streptozotocin (STZ) induced diabetic Rats (n=6)

Groups	Pre-treatment	Post-treatment			
	Day 0	Day 14	Day 28	Day 42	Day60
Normal control	89.01±0.10	92.89±0.34	88.50±0.23	95.89±0.32	92.18±0.16
Diabetic control	278.01 ±0.03	289.06±0.14	310.00±0.02	325.09±0.05	355.02±0.07
Stevia leaves extracts @ 100mg/kg, orally	281.08±0.14	237.06±0.04	230.05±0.10	236.09±0.05	229.05±0.15
Methi leaves extract @ 500 mg/kg, orally	275.02±0.24	228.09±0.21	210.08±0.31	202.06±0.12	180.10±0.06
Combined stevia and methi extract @ 500mg/kg, orally	280.05±0.04	169.01±0.12	150.29±0.12	142.65±0.16	139.72±0.11
Amaryl @ 800µg/kg, orally	277.63±0.35	148.05±0.12	122.04±0.13	128.06±0.04	124.02±0.09

Table 2. Changes on body weights (mean ± SE, g) in groups of normal and (Streptozotocin) STZ induced diabetic rats (n=6)

Groups	Pre-treatment	Post-treatment				
	Day 0	Day 14	Day 28	Day 42	Day60	Day 90
Normal control	136.20±0.24	145.36±0.33	151.61±0.06	159.70±0.02	165.70±0.14	175.92±0.22
Diabetic control	134.00±0.31	123.06±0.34	123.07±0.23	122.14±0.26	122.02±0.07	121.57±0.26
Stevia leaves extracts @ 100mg/kg orally	129.15±0.16	124.14±0.34	124.06±0.07	123.13±0.04	123.12±0.04	123.10±0.14
Methi leaves extract @ 500 mg/kg, orally	129.80±0.26	125.06±0.24	125.23±0.13	126.11±0.01	127.16±0.03	128.25±0.08
Combined stevia and methi extract @ 500mg/kg, orally	131.40±0.08	125.56±0.07	126.50±0.02	127.20±0.08	129.23±0.01	130.40±0.17
Amaryl @ 800µg/kg orally	127.65±0.13	125.95±0.08	127.45±0.10	129.65±0.03	131.75±0.13	132.85±0.16

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 (Kamalakkanan *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 preliminarily based on these effects.

In the present study, Streptozotocin injection in rats showed significant (P<0.01) increase in blood glucose level and reduction of body weight. Rafiq *et al*., (2009) shown that a single dose of Streptozotocin in rats exhibited characteristic signs of diabetic. Following treatment with Glimepiride (Amaryl®), blood glucose level was reduced significantly (P<0.01). Amanullah *et al*. (2007) also reported significant reduction in blood glucose level with Glimepiride @ 800µg/kg. Previous study 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. Chowdhury *et al*., (2005) reported the reduction of blood glucose following administration of methi seed extract. Previous studies from our laboratory and other researchers also support the present study findings such as Devi *et al*., (2003), Vats *et al*., (2002), Raju *et al*., (2001), Mostofa *et al*., (2007), Sumon *et al*., (2008), Rashed *et al*., (2008). However, in this study we observed that combination of stevia and methi showed better hypoglycemic effects than that of single therapy in STZ-induced rats.

Therefore, we did OGTT to evaluate the glucose intolerance effects of combination of stevia and methi extract treated groups. The combination of stevia and methi seeds aquous extract treated group showed reduced glucose intolerances like Glimepiride treated groups. These result suggesting that the combination treatment not only has hypoglycemic effects but also have glucose

intolerance lowering effects. Therefore, these findings lend pharmacological support to the suggested folkloric and ethnomedical user of these plants in managing and /or controlling of diabetes mellitus in rural communities of Bangladesh. Further pharmacological and biochemical investigations are needed to elucidate the exact mechanism of hypoglycemic effects of combination of stevia leaf and methi seeds aqueous extracts.

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