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Antidiabetic effects of *Azadirachta indica* , *Trigonella foenum-graecum*, *Olea europea* and Glibenclamide in experimentally diabetic induced rat

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Abstract

Medicinal plants as a folk medicine are used for the treatment of diabetes all over the world. The present study investigated, the effects of some indigenous medicinal plants *viz Olea europea, Azadirachta indica* and *Trigonella foenum-graecum* in terms of their antidiabetic activity such as hypoglycemic effect and improvement of body weight gain in Streptozotocin induced diabetic rats and compared with that of a patent drug as Glibenclamide.The blood glucose lowering activity of these selected plants was studied in Streptozotocin (50mg/kg, i.p.) induced diabetic rat, following oral administration of *Olea europea, Azadirachta indica* and *Trigonella foenum-graecum* at the dose rate of 1gm/kg, 500mg/kg and 1gm/kg body weight respectively. Blood glucose level and body weight gain were measured by Glucotrend Kit and Electric balance respectively and that compared with a patent drug Glibenclamide at dose rate of 800µg/kg body weight. The data were compared statistically by using Student's unpaired t-test. The herbal preparations of these plants significantly reduce blood glucose level in Streptozotocin induced diabetic rats and comparable with that of the standard drug, Glibenclamide. The herbal preparation also significantly increased body weight gain as compared with the patent drug. The present study clearly indicated the significant antidiabetic activity of *Olea europea, Azadirachta indica* and *Trigonella foenum-graecum* and supports the traditional usage of the herbal preparations by Ayurvedic physicians for the therapy of diabetes.

Keywords: *Azadirachta indica, Trigonella foenum-graecum*, *Olea europea,* glibenclamide, blood glucose, body weight, streptozotocin, diabetic rat

Introduction

Diabetes mellitus is regarded as a syndrome, a collection of disorders that have hyperglycemia as the hallmark (Zimmet, 1997). It is a systemic illness. Now a days this disease is a matter of concern because, it is an important cause of morbidity and mortality throughout the world, including Bangladesh. It is one of the major causes of disability leading to loss of manpower, labour and decreased productivity in all spheres of life. Bangladesh is one of the poorest countries in the world and has lowest health care spending per capita. Statistical data in Bangladesh showed the gradual increase in the number of diabetic patients. It is estimated that about 5 million people have diabetes in Bangladesh (BIRDEM, 1999).

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. Although modern allopathic system of medicine is greatly accepted in the treatment of diabetes throughout the world, it has not been able to reach the remote rural areas for various reasons. In our country, a vast majority of the population can not afford the treatment of diabetes mellitus.

Insulin can not be given orally, and daily intake of injection is obviously troublesome. Hypoglycemic reactions as an adverse effect may occur in any diabetic patient treated with insulin. Insulin resistance is a state of relative tissue insensitivity to the action of insulin, which is another drawback for patients taking insulin for a long period (Larner, 2001). On the other hand, modern oral hypoglycemic agents such as glimepiride, glibenclamide etc are costly are to be continued lifelong. Moreover, they have some adverse effects and they are unavailable in rural area.

Because of the Local availability of certain herbs, treatment of diabetes mellitus according to the traditional system of medicine is often cheaper if effective remedy could be provided. Traditional medicinal plants are used throughout the world for a range of diabetic presentations. The study of such agents might offer a successful key to unlock a diabetologist's pharmacy for future.

Spontaneous diabetes is a common occurrence in human being which may be due to destruction of the pancreatic β- cells. Animal can rendered diabetic by a wide variety of experimental procedures and the diabetic animals may be regarded as models of the disease in man. There fore, although induction of the disease is impermissible in man, it thus may be studied in animals. As a corollary, the efficacy of new agents or drugs to treat or reverse the disease should be logically demonstrated in animals, advantages afforded by animal models allow them to be used as valuable research tools (Mordes and Rossini, 1981).

Neem, Methi and Olive plants are available in rural area of our country. For considering all these constraints, in this experiment we wanted to establish indigenous system of medicine (herbal therapy) as antidiabetic drugs instead of chemical drugs and rat is used as model of animal.

This pioneer work has been under taken with the following objectives:

1. To study the comparative antidiabetic activity of Neem (*Azadirachta indica*) capsule, Methi (*Trigonella foenum-graecum*) seed and Olive leaf (*Olea europea*) with Dibenol®(Glibenclamide).
2. To compare the hypoglycemic effects of Neem (*Azadirachta indica*) capsule, Methi (*Trigonella foenum graecum*) seed and Olive leaf (*Olea europea*) with Dibenol®(Glibenclamide).
3. To compare the effects of Neem (*Azadirachta indica*) capsule, Methi (*Trigonella foenum graecum*) seed and Olive leaf (*Olea europea*) with Dibenol®(Glibenclamide) on body weight.

Materials and Methods

The present study was undertaken to demonstrate the antidiabetic effects of Olive (*Olea europea*) leaves, Neem (*Azadirachta indica*) capsule and Methi (*Trigonella foenum-graecum*) seed. The hypoglycemic effects of these herbal preparations were compared with patent drug Dibenol®(Glibenclamide) in rat. Attempts were also made to study the effects of this drug Dibenol®(Glibenclamide) and herbal preparation of Olive (*Olea europea*) leaves, Neem (*Azadirachta indica*) capsule and Methi (*Trigonella foenum-graecum*) seed on body weight gain in mixed albino rats, long Evan's strain.

To perform this experiment, after acclimatization thirty rats were randomly divided into six equal groups and rats of Group A were kept as non-hyperglycemic control. Then hyperglycemia was induced in other four groups of rats (B, C, D, E and F) by administering Streptozotocin (STZ) at a dose of 50-gm/kg-body weight intraperitoneal. After fifteen days of Streptozotocin (STZ) injection, Group B were kept as hyperglycemic control, four groups of rats (C, D, E and F) were administered with Olive (*Olea europea*) leaves, Neem (*Azadirachta indica*) capsule, Methi (*Trigonella foenum-graecum*) seed and Dibenol® (Glibenclamide) as per schedule dose and all the control and treated rats were closely observed during 14 days of herbal treatment and all above mentioned parameters were investigated as per schedule.

Streptozotocin (STZ) induced hyperglycemia

In the present study, diabetes was induced by streptozotocin(STZ). The dose and route of administration of Streptozotocin (STZ) were applied according to the procedure described by Arison *et al.* (1967) and Anderson *et al.* (1974). In this study, intraperitoneal administration of single dose of Streptozotocin (STZ) at the dose rate of 50 mg/kg body weight in rats increased blood glucose level significantly.

Experimental layout

Thirty, mixed albino rats, Long Evans strain were used. Diabetic condition was induced by injecting Streptozotocin to rats in a dose rate of 50-mg/kg i.p. (Bonner *et al.* 1981) in 0.1M citrated buffer, P^H 4.5, Rats were kept fasted overnight but were given water *ad libitum.* Animals were devided into six groups. Group A (non-diabetic control) and Group B (diabetic control). Groups C, D, E and F were treated with Olive leaf extract (1 gm/kg), Neem capsule (500mg/kg), Methi seed extract (1gm/kg) and tablet Diabenol®(800 µg /kg) respectively. After 15 days of Streptozotocin injection, blood samples were taken from tail vein and blood glucose was estimated by strip method and body weight were taken by Electric balance and 1 week of herbal treatment blood glucose and body weight gain were estimated. Then the experiment was continued for 7 days and blood glucose level and body weight were estimated .The results of blood glucose were expressed in m mol/L. Statistical analysis was made by using Student's unpaired t-test.

Results and Discussion

Effects on blood glucose level

After 15 days of Streptozotocin (STZ) injection (0 day of experiment) blood glucose level was markedly increased in all the groups except the normal control (Group A). The hyperglycemic effect of Streptozotocin (STZ) may be due to damage of pancreatic β-cell which inturn might increase the blood glucose level.

After 15 days of treatment with glibenclamide the blood glucose level were reduced significantly ($P<0.01$) which agreed with that of Okyar *et al* (2001) and Zhang *et al.* (2000). The groups treated with Olive (*Olea europea*) leaves, Neem (*Azadirachta indica*) capsule and Methi seed (*Trigonella foenum-graecum*) reduced blood glucose level significantly ($P< 0.05$).

The exact mechanism of 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 incase of Neem capsule. In accordance to the present finding some author also reported reduction of blood glucose following administration of Neem extract 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 potent activity as blood sugar lowering agent followed by *Catharanthus roseus, Gymnemaq sylvestre and Ocimum sanctum.* Incase of methi seed the exact mechanism of action is still unclear. The antidiabetic effect of methi seed might be due to the formation of a colloidal-type suspension in the stomach and intestines when the mucilaginous fiber of the seeds is hydrated, therefore affecting gastrointestinal transit and slowing glucose absorption. (Linh T.H. Tran, 2003). Shalini *et al.* (2004) also reported the reduction of blood glucose following administration of methi seed extract. Devi *et al.* (2003), Vats *et al.* (2002) and Raju *et al.* (2001) also reported similar results. The hypoglycemic action of olive leaves agreed with the finding of Ray Sahelian (2003).

Table 1. Effects of oral administration of Olive leaves, Neem capsule, Methi seeds and Dibenol® tablet on blood glucose (m mol/L,mean±SE) in normal and STZ treated diabetic Rat (n=5)

Group		0 day	7day	14day
A	Normal control	5.50±0.15	5.62±0.19 NS	5.62±0.16 NS
B	Diabetic control	32.74±0.84	32.98±0.89 ^{b}NS	33.24±0.91 ^{b}NS
C	Olive leaves	30.72±0.63	28.50±0.34 ^{a} **	27.06±0.36 ^{a} ***
D	Neem capsule	31.38±0.61	25.14±0.43 ^{a} ***	22.38±0.46 ^{a} ***
E	Methi seed	31.34±0.58	27.28±0.19 ^{a} **	26.14±0.18 ^{a} ***
F	Dibenol® tablet	31.02±0.33	26.80±0.33 ^{a} ***	24.46±0.21 ^{a} ***

*** = Significant increase/decrease. (P<0.01)
** = Significant increase /decrease. (P<0.05)
* = Significant increase/decrease. (P<0.1)
NS = Non Significant increase/decrease. (P<0.5)
a = Decrease, b = Increase

Effects on body weight

After 15 days of treatment with Olive (*Olea europea*) leaves, Neem (*Azadirachta indica*) capsule, Methi (*Trigonella foenum-graecum*) seed and Dibenol® (Glibenclamide) the body weight were increased significantly (P< 0.05). Among the herbal drugs Methi seed was more effective (14.64%) in comparison with other herbal preparations i.e., neem (3.47%) and Olive leaves (3.46%). Dibenol® also increases the body weight. Simillar results were reported by Baynes *et al.* (1993), Devi *et al.* (2003) and Pari and Saravanan (2000) reported similar increase in body weight.

The present study supports the use of herbal drugs such as *Azadirachta indica , Trigonella foenum-graecum, Olea europea* for the treatment of diabetes. But further study is needed to find out the active ingredients, those responsible for their antidiabetic effect.

Table 2. Effects of oral administration of Olive leaves, Neem capsule, Methi seed and Dibenol® tablet on body weight gain in gm (mean ± SE) in normal and STZ treated diabetic Rat (n=5)

Groups		0 day	7day	14day	% increase in b.wt. over 14 days
A	Normal control	263.28 ± 5.25	263.4 ± 5.26 *	263.36±5.17 *	+ 0.03
B	Diabetic control	226.86±12.13	222.84±12.34[a] ***	219.0±12.23[a] ***	- 3.46
C	Olive leaves	162.38±8.71	166.06±8.73 [b] ***	170.50±8.74 [b] ***	+ 5
D	Neem capsule	147.06±9.31	149.88±9.21 [b] ***	152.16±9.27 [b] ***	+ 3.47
E	Methi seed	152.16±9.23	172.12±10.41[b] **	174.44±10.29 [b] ***	+ 14.64
F	Dibenol® tablet	176.18±5.72	179.70±5.99 [b] ***	183.48±5.88 [b] ***	+ 4.14

*** = Significant increase/decrease. (P<0.01)
** = Significant increase /decrease. (P<0.05)
* = Significant increase/decrease. (P<0.1)
NS = Non Significant increase/decrease. (P<0.5)
a = Decrease, b = Increase

References

Anderson, T., Schein, P.S., McMennamin, M.G. and Cooney, D.A. 1974. Streptozotocin diabetes, correlation with extent of depression of pancreatic islet nicotinamide adenine dinucleotide. *J. Clin. Invest.* 54: 672-677.

Arison, R.N., Giaccio, E.I., Glitzer, M.S., Cassaro,J.A. and Pruss, M.P. 1967. Light and electron microscopy of lesions in rats rendered diabetic with Streptozotocin. *Diabetes*, 16(1):51-56.

Baynes, C., Elkeles, R.S., Henderson, A.D., Richmond, W. and Johnst, D.G. 1993. The effects of glibenclamide on blood Glucose homeostasis and lipoprotein metabolism in poorly controlled type II diabetes. *Hormone and Metabolic Research.* 25(2): 96-101.

BIRDEM. 1999. Statistical Department.

Bonner weir, S., Trent, D.F., Honey, R.N. and Weir, G.C. 1981. Response of neonatal rats islets to Streptozotocin. *Diabetes.* 30:64-9.

Chattopadhyay, R.R. 1999. A comparative evaluation of some blood sugar lowering agents of plant origin. *J. of Ethnopharmiacology.* 67(3): 367-372.

Devi, B.A., Kamalakkannan, N., Prince, P.S.M. 2003. Supplementation of fenugreek leaves to diabetic rats. Effect on carbohydrate metabolic enzymes in diabetic liver and kidney. *Phytotherapy-Research.* 17(10): 1231-1233.

Khosla, P., Bhanwra, S., Singh, J., Seth, S. and Srivastava, R.K. 2000. A study of hypoglycemic effects of Azadirachta indica (Neem) in normal and alloxan diabetic rabbits. *Indian Journal of Physiology and Pharmacology.* 44(1):69-74.

Larner,J. 2001. Insulin and oral hypoglycemic drug, glucagon. In: Goodman and Gillman's the Pharmacological Basis of Therapeutics. Gilman A.G., Goodman L.S., Rail T.W. and Murad, F. (eds.), vol. 2, 10th ed. The MacMillan Publishing Co., New York, Chapter 61.

Linh, T.H. Tran, 2003. Reviewed Susan Paulsen Pharm. D.

Mordes, J.P. and Rossini, A.A. 1981. Animal models of diabetes. *American Journal of medicine.* 70: 353-360.

Okyar, A., Matsuda, K., Sato, M., Yamagata, S. and Sato, T. 2001. Effect of *Aloe vera* leaves on blood glucose level in type I and type II diabetic rat Models. *Phytotherapy Research. 15(2):157-161.*

Pari, L., Ramakrishnan, R. and Venkateswaran, S. 2001. Antihyperglycemic effect of Diamed, a herbal formulation, in experimental diabetes in rats. *Journal of Pharmacy and Parmacology.* 53 (8): 1139-1143.

Pari, L. and Saravanan, G. 2000. Antidiabetic effect of Cogent db, a herbal drug in alloxan-induced diabetes mellitus. *Comparative-Biochemistry-and-Physiology.-C,-Toxicology-and-Pharmacology.* 131: 1, 19-25; 40 ref.

Raju,J., Gupta, J., Rao,AR., Yadava, PK. and Baquer, NZ. 2001 *Trigonella foenum-gracum* seed powder improves glucose homeostasis in alloxan diabetic rat tissues by reversing the altered glycolytic, gluconeogenic and lipogenic enzymes. *Molecular Cellular Biochemistry.* 224(1-2): 45-51.

Ray Sahelian, M.D. 2004. Olive leaf benefits olive leaf research.

Shalini, T., Siddiqui, M.R. and Baquer, N.Z. 2004. *Trigonella foenum graecum* seed powder protects against histopathological abnormalities in tissues of diabetic rats. *Molecular-and-Cellular-Biochemistry.* 266(1-2): 151-159.

Vats,V.; Grover, J.K. and Rathi, S.S. 2002. Evaluation of anti-hyperglycemic and hypoglycemic effect of *Trigonella foenum-graecum* Linn, *Ocimum sanctum* Linn and *Pterocarpus marsupium* Linn in normal and alloxanized diabetic rats. *Journal-of-Ethnopharmacology.* 79: 1: 95-100.

Zhang, X.F. and Tan, B.K.M. 2000. Effects of an ethanolic extract of *Gynura procumbens* on serum glucose cholesterol and stretozotocin-induced diabetic rats. *Singapore Medical Journa.* 40 : 9-13

Zimmet, P.1997. Diabetes-definition and classification