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COMPARATIVE EFFICACY OF NEEM (*AZADIRACHTA INDICA*) AND METFORMIN HYDROCHLORIDE (COMET®) IN STREPTOZOTOCIN INDUCED DIABETES MELITUS IN RATS

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ABSTRACT

The present study was investigated the comparative efficacy of extract of Neem (*Azadirachta indica*) and Metformin hydrochloride (Comet®) in respect of their hypoglycemic activity in Streptozotocin (STZ)-induced diabetic rats. Extract of Neem was administered @ 500 mg/kg body-weight (bwt) and 250 mg/kg bwt. Metformin hydrochloride @ 500 mg/kg bwt and 250 mg/kg daily orally for 21 days. Changes in the blood glucose level and body weight were measured and the results were compared statistically using Anova test. The extract of Neem leaves and Metformin hydrochloride showed significant ($p<0.01$) hypoglycemic effect and neem extracts at 250 mg/kg bwt showed more potent effects. The Neem extracts significantly ($p<0.01$) increased body weight @ 250 mg/kg. From this experiment it was observed that extracts of Neem leaves had hypoglycemic and body weight gain effect. For definite conclusion, details study is needed.

Key words: Diabetes mellitus hypoglycemia, body weight, blood glucose, *Azadirachta Indica*, metforminhydrochloride and Streptozotocin

INTRODUCTION

Bangladesh is a developing country and so it has lower per capita health care spending. Many people in the rural areas and also in the urban are affected with diabetes but they don't know. The most common forms of diabetes are (type 1) insulin dependent diabetes mellitus-IDDM (5%), which is an autoimmune disorder, and (type 2) non-insulin dependent diabetes mellitus-NIDDM (95%). There is a gradual increase in the number of diabetic patients in Bangladesh. It has been estimated that of the children born in the year 2000, 1 of 3 will suffer from diabetes at some point in their lifetime (Narayan *et al.* 2003). Streptozotocin selectively destroys insulin producing pancreatic β-cell and thus induces experimental diabetes mellitus. This diabetic rat model is used to determine the efficacy of various antidiabetic agents (Yamamoto *et al.,* 1981, Morgan *et al.,* 1994). Hypoglycemic reactions may occur in any diabetic patient treated with insulin. The patients who are taking insulin for long period insulin resistance may also occur (Larner, 2001). On the other hand, we have a great source of medicinal plants in the nature. Among the antidiabetic plants, neem is one of the most efficacious plants. It belongs to the family Meliaceae, order: Meliales. It is considered to be the "village pharmacy" and has played a key role in Ayurvedic medicine and agriculture since time immemorial (Chopra *et al.* 1956). Other plants which are also effective as antidiabetic agent are: Garlic oil (*Allium sativum*), Black berry seed extract (*Eugenia jambalana*), onion (*Allium cepa*) etc. (Zaman *et al.,* 1981; Bajaj and Srinivasan, 1999; Jain and Vyas, 1974). This study has been performed to investigate the comparative hypoglycemic activity and body weight changes of aqueous extract of neem leaves and Metformin in streptozotocin induced diabetic rats.

MATERIALS AND METHODS

This research work was conducted at animal Laboratory in the Department of Pharmacology, Bangladesh Agricultural University (BAU), Mymensingh from January/2009 to May/2009. Sixty long Evan's strain (Ratus norvegicus), aged of 6 weeks and weighing between 180 to 190 gms were collected from International Center of Diarrhea Disease Research, Bangladesh (ICDDR,B). The rats were allocated into six groups, each containing 10 individuals. Each group of rats was housed at screen bottomed wire cages arranged in rows and kept in animal house. The animals were fed in pellet form at a recommended dose of 100gm/kg as advised by and purchased from (ICDDRB). Drinking water was supplied *ad libitum*. All the groups of rats were kept for 18 days for acclimatization. The rats were given water *ad libitum,* their body weights and blood glucose levels were measured and recorded after acclimatization at the time when that of other groups were measured. Group A was kept as normal control, Group B, as diabetic control group and groups C, D, E and F were diabetic treatment

groups. After 18 hours of starvation, body weights and blood glucose level were measured after acclimatization of rats. Then streptozotocin injection was given at a dose rate of 65 mg/kg body weight in intraperitoneal route to each rat to induce diabetes in groups B, C, D, and F. The rats were fed normal diet and given water *ad libitum* from Day 1-15, on 15th day blood glucose level and the body weights were again measured to ensure diabetic condition. Then all the rats of this group were kept for 21 days for treatment. During that period on Day 0, 7, 14, and 21st the body weight and blood glucose level were measured. Aqueous extract of garlic were fed at a dose of 500mg/kg and 250mg/kg body weight daily for 21 days in groups C and D and aqueous solution of Metformin HCL were fed at a dose of 500mg and 250mg/kg body weight daily for 21 days in groups E and F.

Following parameters were studied

1.Working Instruments and Chemicals: Citric acid-l-hydrate, Tri-sodium citrate dihydrate, Streptozotocin (sigma), Accu CHEK$^{(R)}$ active monitor, Accu-chek test strip and other relevant instruments.

2. Preparation & Administration of Streptozotocin Solution: Streptozotocin was dissolved in 0.1 M citrate buffer having pH 4.5 which was injected intraperitonealy to rat and maintained fasting condition for 18 hours to induce diabetic condition in rat at a dose of 65 mg STZ per kg of body weight.

3. Collection, preparation, preservation & administration of aqueous extract of neem leaves and Metformin hydrochloride (Comet®) tablet

A) Aqueous solution of Comet®: The oral antidiabetic tablet, Comet® (Metformin hydrochloride) were bought from local market and was ground, dissolved in distilled water to make the concentration 500 mg/10ml.

B) Preparation of aqueous extract of neem leaves: Fresh neem leaves were collected, grinded, 10 gm neem was weighted and mixed with 100 ml distilled water and kept it in hot air oven over 24 hours to become 10 ml which was administered orally with the help of micropipette to the experimental rats at various doses.

Administration of drugs: Aqueous extract of neem leaves and Comet® tablet were fed orally to the experimental rats with the help of a micropipette.

4. Determination of blood glucose by **Accu CHEK(R) Active blood glucose system (strip method):** A drop of blood was collected from the tail vein. At the same time the Accu CHEK$^{(R)}$ Active monitor was started with a single soft press. After the monitor showed the code number the strip was inserted into the monitor. A drop of the blood was poured on the test zone of the strip. The values were expressed in m mol/L.

5. Determination of body weight by balance. Statistical analysis was made by using Anova test.

RESULTS AND DISCUSSION

Induction of diabetes: To induce diabetes mellitus, Streptozotocin injection was given through intraperitoneal route and blood glucose level was increased significantly ($p<0.001$) and also reduce body weight. Single dose of Streptozotocin administered intraperitoneal @ 55 mg/kg b.wt. (Anderson *et al.*, 1974). A number of workers reported similar observations (Amanullah, 2007 and Islam, 2008).In this experiment, polyuria, polydipsia and polyphogia after 24 hours of Streptozotocin injection were observed which is similar to Jound *et al.*, 1969. Thus, it is evident that the findings of this study were in well agreement with the findings of other workers.

1 Effects of Different Doses of Extract of Neem (*Azadirachta indica*) Leaves and Metformin Hydrochloride on Blood Glucose level in Streptozotocin Induced Diabetic Rats

In normal control rats, group A (n=10), the blood glucose concentration from day 0 to 21, ranged from 6.00±0.55 m mol/L to 6.18±1.27 m mol/L and upto 3.00% changes (Table 1 and Fig. 1) were found in comparison to day 0. In the diabetic control rats, group B (n=10), the blood glucose concentration ranged from 28.01±0.71 m mol/L to 31.00±1.71 m mol/L from beginning to the end and up to 10.64% changes (table 1and fig1) were found. Among the treated diabetic rats, group C (n=10), treated with aqueous extract of Neem (*Azadirachta indica*) leaves @ 500mg/kg bwt, the blood glucose concentrations were 28.00±0.71 m mol/L, 27.00±0.71 m mol/L, 26.00±0.18 m mol/L and 23.00±0.32 m mol/L on day 0, day 7, day 14 and day 21 respectively and 17.89% changes were found on day 21 in comparison with day 0. On the other hand, the blood glucose concentrations were 28.00±0.71 m mol/L, 27.00±0.75 m mol/L 26.00±0.32 m mol/L and 25.16±0.71 m mol/L and 10.14% changes were found on day 21 in comparison with day 0 in group D (n=10), was treated with aqueous extract of Neem (*Azadirachta indica*) leaves @ 250mg/kg bwt on day 0, day 7, day 14 and day 21 respectively from Table 1 and Fig. 1. The blood glucose concentrations were 28.01±0.71 m mol/L, 22.00±0.19 m

mol/L, 20.00±0.71 and 19.00±1.56 m mol/L on day 0, day 7, day 14 and day 21 respectively and 32.10% changes were found on day 21 in comparison with day 0 in group E (n=10), treated with Metformin hydrochloride (comet®tab) @ 500mg/kg and 28.59±0.37 m mol/L, 26.00±0.49 m mol/L, 25.00±1.35 m mol/L and 23.72±1.71 m mol/L, 17.01% changes were found in group F (n=10), treated with Metformin hydrochloride (comet® tab)@250mg/kg, on day 0, day 7, day 14 and day 21 respectively from Table 1 and Fig. 1. In this experiment, it was found that continuous treatment with aqueous extract of Neem caused a significant decrease ($p<0.01$) in blood glucose level in rats. More potent effects were found at 250 mg/kg bwt Chang *et al.,* 2005; Jeppesen *et al.*, 2003 also reported reduction in blood glucose level following administration of extract of Neem leaves. After treatment with Metformin hydrochloride, the blood glucose level was reduced significantly ($p<0.01$). Investigation carried out by Kemmer *et al.* (1977) found that Metformin hydrochloride lowers blood glucose levels by stimulating glucose uptake of skeletal muscle. Klepser and Kelly (1997) found that metformin hydrochloride is a antihyperglycemic agent; it lowers the blood glucose concentration without causing hypoglycemia.

Table 1. Changes in blood glucose concentration (Mean±SE m mol/L) in groups of normal and Streptozotocin (STZ) induced diabetic rats (n=10)

Groups	Drug, dose and route	Pre-treatment	Post-treatment			
		Day 0	Day 7	Day 14	Day 21	
					Mean± SE	%
A	Normal Control	6.00±0.55	5.00±0.68d	6.00±0.91c	6.18±1.27e	+3.00
B	Diabetic Control	28.01±0.71	29.00±0.71a	30.00±1.71a	31.00±1.71a	+10.64
C	Neem@500mg/kg	28.00±0.71	27.00±0.71b	26.00±0.18b	23.00±0.32c	-17.89
D	Neem@250mg/kg	28.00±0.71	27.00±0.75b	26.00±0.32b	25.16±0.71b	-10.14
E	Metformin HCL (Comet®) 500mg/kg	28.01±0.71	22.00±0.19c	20.00±0.71c	19.00±1.56d	-32.10
F	Metformin HCL Comet®)@250mg/kg	28.59±0.37	26.00±0.49bc	25.00±1.35bc	23.72±1.71c	-17.01
Levels of significant		NS	**	**	**	

Figures followed by different letter (s) in the same column differ significantly ($p<0.01$)

** = Significant increase /decrease for treated groups ($p<0.01$)

NS = Non Significant increase/decrease. # = Significant change in comparison to diabetic control group

2. Effects of Aqueous extract of Neem (*Azadirachta indica*) Leaves and Metformin Hydrochloride (Comet®) on Body Weight in Streptozotocin Induced Diabetes Rats

The percent increased in body weight gain over 21 days in normal control rats, Group A (n=10), was 0.45 %. On the contrary, in diabetic control rats, Group B (n=10), the percentage of body weight loss was 7.77 %, in Group C (n=10), 4.97 % body weight gain, following administration of aqueous extract of Neem leaves @ 500 mg/kg bwt, in Group D (n=10), body weight gain was 3.62 %, aqueous extract of Neem leaves @ 250 mg/kg bwt for 21 days. Similarly in Group E (n=10), body weight gain was 8.69%, treated with Metformin hydrochloride (Comet® tablet) @ 500 mg/kg bwt and body weight gain was 6.38% in group F (n=10) treated with Metformin hydrochloride (Comet® tablet) @ 250 mg/kg bwt for 21 days from Table 2 and Fig. 2. Aqueous extract of Neem (*Azadirachta indica*) leaves increased the body weight significantly @ 250 mg/kg. Among the doses of neem used in this experiment, 250 mg/kg bwt had more body increasing effect than 500 mg/kg. Rashed (2008) reported that aqueous extract of Neem @ 3 ml/kg reduces body weigh 1.44% from 0 day to day 7.

In this research work, the continuous treatment with aqueous extract of Neem (*Azadirachta indica*) and Metforminhydrochloride produced a significant reduction ($p<0.01$) of the blood glucose level and body weight also significantly increased ($p<0.01$) in STZ-induced diabetic rats. Among two doses of aqueous extract of Neem, 250 mg/kg bwt was more potent. To establish Neem leaves and metformin hydrochloride (Comet®) as an antidiabetic agent scientifically, the underlying mechanism of hypoglycemic activity of Neem leaves and metformin hydrochloride (Comet®) need to be discovered. We did the work in short term basis as modern equipments were also not available. It is required to trial for longterm on other biochemicals and histopathological studies.

Table 2. Changes of body weight (Mean±SE gm) in groups of normal and Streptozotocin (STZ) induced diabetic rats (n=10)

Groups	Drug, dose and route	Pre-treatment	Post-treatment			
		Day 0	Day 7	Day 14	Day 21	
					Mean± SE	%
A	Normal Control	220.10±1.19	221.00±1.76b	220.00±3.74b	221.00±1.57b	+0.45
B	Diabetic Control	180.00±1.57	170.00±1.13f	168.00±1.71e	166.00±1.71e	-7.77
C	Neem@500mg/kg	181.10±1.35	185.00±1.51e	188.00±1.16d	190.00±0.79d	+4.97
D	Neem@250mg/kg	193.00±1.14	195.00±1.17c	198.00±0.79c	200.00±1.82c	+3.62
E	Metformin HCL (Comet®)@500mg/kg	184.00±1.41	192.00±1.64d	196.00±1.37cd	200.00±1.37c	+8.69
F	Metformin HCL (Comet®) @250mg/kg	235.00±1.94	240.00±1.74a	242.00±1.24a	250.00±1.78a	+6.38
Levels of significant		NS	**	**	**	

Figures followed by different letter (s) in the same column differ significantly ($p<0.01$)

SE = Standard Error; SE = Standard Error, **= Significant increase /decrease ($p<0.01$).
* = Significant increase/decrease ($p<0.05$).
NS = Non Significant increase/decrease for treated groups. ($p<0.05$)
# = Significant change in comparison to diabetic control group

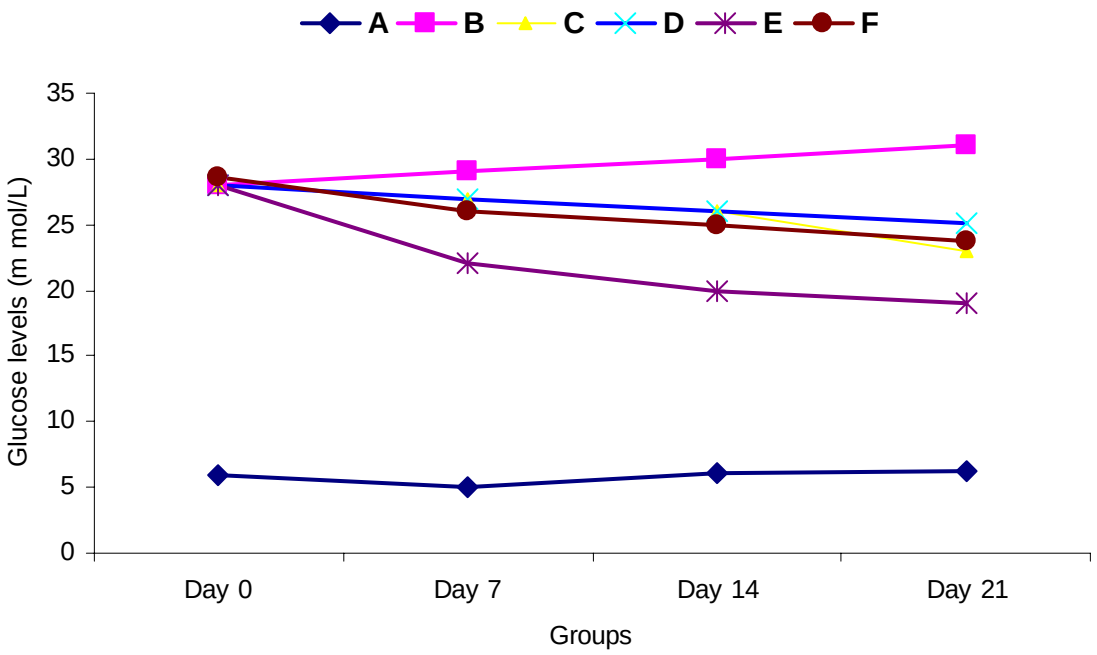

Figure 1. Changes on blood glucose concentration (Mean±SE m mol/L) in groups of normal and Streptozotocin (STZ) induced diabetic rats (n=10)

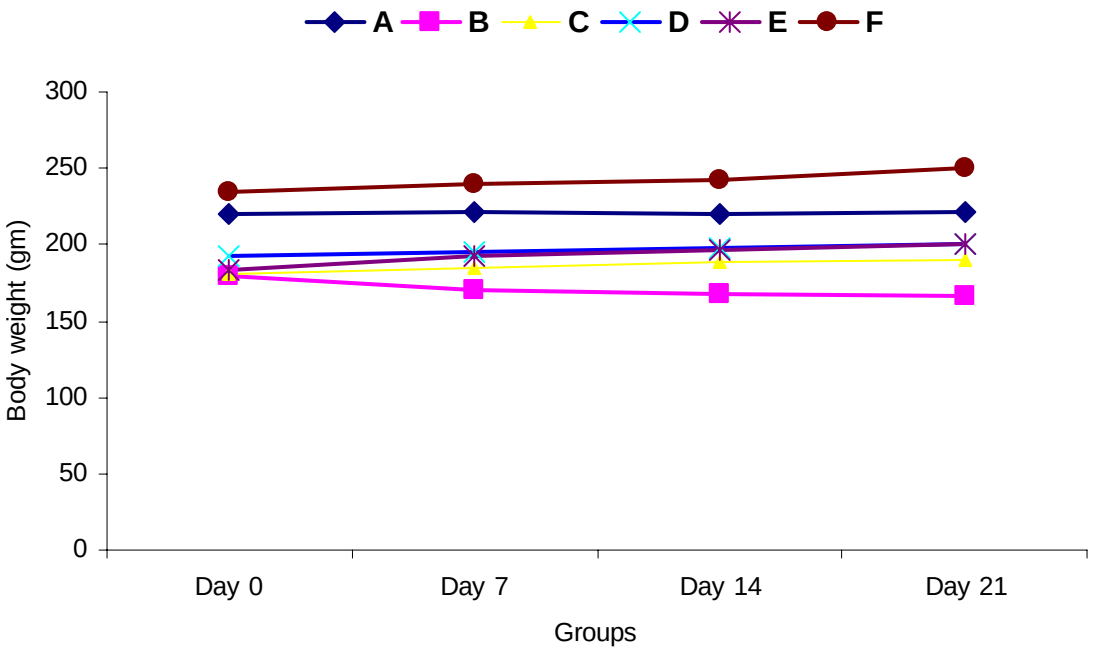

Figure 2. Changes of body weight (Mean±SE gm) in groups of normal and Streptozotocin (STZ) induced diabetic rats (n=10)

REFERENCES

1. Amanullah M (2007). Comparative Efficacy of Telakucha (*Coccinia indica*) and Amaryl® Tablet (Glimepiride) in induced Diabetes mellitus in Rat. *M.S. Thesis, Submitted to the Department of Pharmacology, Bangladesh Agricultural University*.
2. Anderson T, Schein PS, McMennamin MG and Cooney DA (1974). Streptozotocin diabetes, correlation with extent of depression of pancreatic islet nicotinamide adenine dinucleotide. *J. Clin. Invest.* 54:672-677.
3. Bajaj S and Srinivasan BP (1999). Investigation into the anti-diabetic activity of *Azadirachta indica*. *J. of Pharmacol.* 31: 138-141.
4. Chang JC, Wu MC, Liu IM and Cheng JT (2005). Increase of insulin sensitivity by stevioside in fructose-rich chow-fed rats. *Horm Metab Res.* 37(10): 610-6.
5. Chopra RN, Nayer SL and Chopra IC (1956). *Glossary of Indian Medicinal Plants*, CSIR, New Delhi.
6. Islam MS and Choi H (2008). Comparative effects of dietary ginger (Zingiber officinale) and garlic (Allium sativum) investigated in a type 2 diabetes model of rats. *J. Med. Food.* 11(1):152-9.
7. Jain RC and Vyas CR (1974). Hypoglycaemia action of onion on rabbits. *Brit. Medi. J.* 730.
8. Jeppesen PB, Gregersen S, Rolfsen SE, Jepsen M, Colombo M, Agger A, Xiao J, Kruhøffer M, Orntoft T and Hermansen K (2003). Antihyperglycemic and blood pressure-reducing effects of stevioside in the diabetic Goto-Kakizaki rat. *Metab.* 52(3): 372-8.
9. Jound A, Lamberd AE, Stauffacher W, Renold AE (1969). Diabetogenic actiono of streptozotocin: Relationship of dose to metabolic response. *J. Clim. Invest.* 48: 2129-2137.
10. Kemmer FW, Berger M, Herberg L and Gries FA (1977). Effects of metformin on glucose metabolism of isolated perfused rat skeletal muscle. *U.S. National Library of Medicine and the National Institutes of Health.* 27(8): 1573-6.
11. Klepser TB and Kelly MW (1997). Metformin hydrochloride: an antihyperglycemic agent. *Am. J. Hlth Syst. Pharm.* 54(8): 893-903.

12. 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.), *The MacMillan Publishing Co*., New York. ss61(2).
13. Morgan NG, Cable HC, Newcobe NR and Williams GT (1994). Treatment of cultured pancreatic β-cells with streptoxotocin induces cell death by apoptosis. *Biosci. Rep.* 14:243-250.
14. Narayan KM, Boyle JP and Thompson TJ (2003). Sorensen SW, Williamson DF. Lifetime risk for diabetes mellitus in the United States. *JAMA* 290(14):1884–1890.
15. Rashed MB, Mostofa MA, Hosssain MA and Yasmin F (2008). Efficacy of aqueous extract of *Stevia rebaudiana* bertoni leaves in rats with streptozotocin induced diabetes mellitus. *J. Bang. Agril. Univ.* 6(1): 73-77.
16. Yamanmoto H, Uchigata Y and Okamota H (1981). Streptozotocin and alloxan induce DNA strand breaks and poly and alloxan induce DNA strand breaks and poly (ADP-ribose) synthetose in pancreatic islets. *Nature.* 294: 284-286.
17. Zaman QAM, Banoo H, Chowdhoury S, Chowdhury SAR and Khaleque A (1981). Effect of garlic oil on serum cholesterol and blood sugar level in adult human volunteers in Bangldesh. *Bang. Medi. J.* 10(1):6-10.