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Research Article

ANTI-HYPERGLYCEMIC AND LIPID LOWERING EFFECT OF *TERMINALIA ARJUNA* BARK EXTRACT ON STREPTOZOTOCIN INDICED TYPE 2 DIABETIC MODEL RATS

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ABSTRACT

The present study was carried out to evaluate the antidiabetic and antilipidemic property of *Terminalia arjuna* in streptozotocin-induced type 2 diabetic model rats. 50% ethanol extract of stem bark of *Terminalia arjuna* were administered 1.25gkg-1 body weight for 21 consecutive days to type 2 diabetic male Long-Evans rats. Ethanol extract of *T. arjuna* significantly ($p<0.05$) improved oral glucose tolerance in type 2 rats in comparison to control group at the end of study period. It was also found that fasting serum glucose level decreased significantly ($p<0.05$) compared to water control after 21 days of feeding of *T. arjuna*. However, no change was observed in the liver glycogen content and serum insulin level at the end of the study period. In addition to hypoglycemic effect of *T. arjuna*, beneficial effect was also observed in lipid profile. Serum total cholesterol and triglyceride were decreased significantly by ($p<0.01$) and ($p<0.001$) at the end of the study period. Administration of Glibenclamide (5 mgkg-1) also produced significant reduction ($p<0.01$) in serum glucose concentration in type 2 diabetic rats. Thus, the results of the experimental study suggest that *T. arjuna* possesses hypoglycemic and hypolipidemic effects and can be served as a source of potent antidiabetic agent.

Keywords: Anti-hyperglycemic, Anti-atherosclerotic, *Terminalia arjuna* (Combretaceae), Streptozotocin, Type 2 diabetes.

INTRODUCTION

Diabetes mellitus is a disease due to abnormality of carbohydrate metabolism and it is mainly linked with low blood insulin level or insensitivity of target organs to insulin. It is the most prevalent chronic disease in the world affecting nearly 25% of the population1.

Hyperglycemia and hyperlipidemia are two important characters of diabetes mellitus, an endocrine disorder based disease. In modern medicine, no satisfactory effective therapy is still available to cure diabetes mellitus1. Though pharmaceutic drugs like sulfonylureas and biguanides are used for the treatment of diabetes but these are either too expensive or have undesirable side effects or contraindications2, 3. In recent years, there has been renewed interest in plant medicine4, 5, 6 for the treatment against different diseases as herbal drugs are generally out of toxic effect7, 8 reported from research work conducted on experimental model animal. Although in human, whether there is any toxic effect are not investigated, isolated studies screened various plants having "folk medicine reputation" by biochemical test for this for antidiabetogenic effect9.

Terminalia arjuna (family - Combretaceae), a large tree, is found throughout the South Asian region. It is one of the most versatile medicinal plants having a wide spectrum of biological activity10, 11. Traditional healers claim that the stem bark of the plant possess antidiabetic properties. Scientific reports also support the hypoglycemic activity of this plant12. However, no published report supports both the acute and chronic hypoglycemic effect of *T. arjuna* on streptozotocin induced Type 2 diabetic model rats. As the majority of the diabetic population suffers from Type 2 diabetes, we undertook the present study to evaluate the anti-diabetic effect on Type 2 diabetic model rats and to explore the possible hypoglycemic / anti-hyperglycemic and lipid lowering activity of the extract as well as to investigate the possible chemical constituents responsible for the activity and the target tissue(s) involved in this action.

MATERIALS AND METHODS

Plant materials and preparation of test sample

The barks of *Terminalia arjuna* were collected from Khamarpara, a village of Magura, Bangladesh. The plant was identified by the Bangladesh National Herbarium, Dhaka and the specimens were stored in there for the further reference (Voucher Specimen No. DACB-35235).

The stem barks of the *T. arjuna* were cut into small pieces and then water washed carefully. After washing, the fresh barks were air dried and then oven dried at 40°C temperature. The dried barks are then grinded to make powder, which were then screened to get fine powder. 1500g of barks were dried in oven and finally 500g of fine powder was obtained. 500g of dried bark powder were soaked in 50% ethanol. These suspensions were filtered with thin and clean cloth and then filtered by filter paper. The suspensions were evaporated by BUCHI Rota vapor R-114 [BUCHI, Germany], connected with BUCHI water bath B-480 at 500C. In this case, 175mbar (to remove ethanol), 72mbar (to remove water) pressure and 160rpm rotation speed were maintained constantly. Finally, small amount of liquid were evaporated from the semi-solid extracts by using a freeze-drier (HETOSICC, Heto Lab Equipment, Denmark) and 75 g of ethanol extracts were obtained.

Phytochemical Screening

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents. Phytochemical screening of the extract was performed using the following reagents and chemicals: Alkaloids with Molisch's reagent, flavonoids with the use of Mg and HCl; tannins with ferric chloride solutions and saponins with ability to produce stable foam and steroids with Libermann-Burchard reagent. Gum was tested using Molish reagent and concentrated sulphuric acid; reducing sugars with Benedict's reagent; terpenoids with chloroform and conc. sulphuric acid. These were identified by characteristic color changes using standard procedures13.

Experimental Animals

The study was conducted with adult male Long-Evans rats (weighing 160-210g). They were bred at the BIRDEM animal house maintained at a constant room temperature of 22±50C, 40-70% humidity conditions and the natural day-night cycle with an ad libitum access to food except the day of experimental procedure when animals were used after 12hrs fasting. The rats had no access to food during

the whole period of blood sampling. The influence of circadian rhythms was avoided by starting all experiments at 8.30 a.m.

Induction of type 2 diabetes to the rats

Diabetes stimulating Type 2 was induced by a single intra-peritoneal injection of streptozotocin (90mg/kg body weight, dissolved in 0.1 citrate buffer, pH 4.5) to 48hr old pulps of Long-Evans rats14. Experiments were carried out 3 months latter to STZ injection and those rats having blood glucose level 8-12 mmol/l at fasting condition, were considered to carry out the experiments. The animals were divided into 3 groups of 7-8 rats in each as Control group (fed with water), Positive control (fed with glibenclamide) 15, Treated group (fed with ethanol extract of *T. arjuna*). Rats of all groups were kept under similar environmental conditions, and were provided with enough food and water throughout the experiment. The body weight of each rat was measured in every weekend.

Acute study

The ethanol extracts (1.25 mg/10 ml/kg body weight) were fed simultaneously with glucose (2.5g / 10 ml / kg body weight) to the overnight fasting (12h) diabetic rats orally at 0 minute using a syringe (3ml) with a metallic tube that was smooth and curved at the end, which led the feed direct to the stomach and then blood samples were drawn at 0, 30, 75 minutes. Both positive control and water control rats were fed with glucose solution at a dose of 2.5g / 10 ml / kg body weight15. Blood samples were collected by amputation of the tail tip under mild diethyl ether anesthesia.

Chronic study

The ethanol extract (1.25 mg/10 ml/kg body weight), glibenclamide (5 mg/10 ml/kg body weight), and water (10 ml/kg body weight) were fed to the rats for 21 consecutive days. An oral glucose tolerance test (OGTT) was performed on the 1st, 8th, 15th, and 22nd day of the study. Blood samples were collected to measure serum glucose, total cholesterol, triglyceride and high density lipoprotein (HDL) levels. On 22nd day, all rats were sacrificed to collect liver to measure the glycogen level. Insulin level was measured by collecting the blood smples on the 1st and 22nd day of the experiment.

Biochemical analysis

Serum glucose was measured by glucose-oxidase method (Sera Pak, USA). The total cholesterol, HDL and triglyceride (TG) were measured by enzymatic-colorimetric method (Randox Laboratories Ltd., UK). Serum insulin by Rat Insulin enzyme linked immunosorbent assay (ELISA) method (Crystal Chem Inc., USA) and liver glycogen levels were estimated by Anthrone-sulphuric acid method. The absorbance was measured by microplate ELISA Reader (Bio-Tek EL-340, USA).

Statistical analysis

Data from the experiments were analyzed using the Statistical Package for Social Science(SPSS) software for windows version 12 (SPSS Inc., Chicago, Illinois, USA). All the data were expressed as Mean ± SD or as Median (Range) as appropriate. Statistical analysis of the results were performed by using the student's t-test (paired and unpaired) or ANOVA (analysis of variance) followed by Bonferroni post hoc test or Mann Whitney (u) test. The limit of significance was set at $p<0.05$.

RESULTS

Phytochemical screening

Phytochemical screening of the crude extract revealed the presence of tannins, flavonoids, saponins, gums, steroids, alkaloids, reducing sugar and terpenoids. The intensity of the component content was high in all of the tested groups except saponins and terpenoids (Table 1).

Acute effect on blood serum with simultaneous glucose load

The first oral glucose tolerance test (OGTT) was performed on the first day (termed as 0 day) of the experiment and the results showed that serum glucose level sharply raised following glucose load in the control rats. *T. arjuna* extracts tend to oppose the rise of serum glucose at both time point i.e, at 30 min and 75 min, although it was not significantly (Table 2). As expected glibenclamide, the positive control, opposed the rise in serum glucose to a greater extent in comparison with *T. arjuna*.

Table 1: Result of chemical group test of the ethanol extract of *Terminalia arjuna*

Plant Extract	Tanins	Flavonoids	Saponins	Gum & Carbohydrate	Steroids	Alkaloids	Reducing sugar	Terpenoids
50% ethanol	+++	+++	++	+++	+++	+++	+++	++

High = +++; Moderate = ++.

Table 2: Acute effects of 50% ethanol extract of *T. arjuna* on serum glucose level (M±SD) of type 2 Diabetic model rats when Fed simultaneously with glucose load at the initial and 21st day

	Group	Glu_0 Min (mmol/l)	Glu_30 Min (mmol/l)	Glu_75 Min (mmol/l)	iobv
Day_1	WC (n = 7)	8.88±1.73	18.45±2.88	16.56±4.14	17.25±9.58
	Glib (n = 8)	8.82±1.08	16.41±2.93	13.62±3.27	12.38±5.67
	Extract (n = 7)	8.29±1.09	15.75±1.38	15.03±3.29	14.20±5.91
	WC (n = 7)	7.83±1.11	16.59±2.79	17.79±2.71	18.73±3.45
Day_21	Glib (n = 8)	6.67±2.13	17.58±1.67	16.57±4.62	20.82±6.80
	Extract (n = 7)	7.58±1.28	13.75±1.53**	13.53±2.39	13.66±4.44

Results are expressed as Mean **±** SD; one way ANOVA with post hoc Bonferroni test was performed as the test of significance. **P<0.05.

Second oral glucose tolerance test (OGTT) was performed on 21st day and it was found that 21-day consecutive feeding of ethanol extract *T. arjuna* resulted in a significant fall in serum glucose level at 30 min ($p<0.05$). At 75 min, *T. arjuna* extract also tend to oppose the risen in glucose level although non-significantly (serum glucose mmol/l, M±SD, (13.53±2.39) *T. arjuna* vs. (17.79±2.71) water control group. Glibenclamide treated group showed lower fasting value in comparison with water control and *T. arjuna* treated groups (Table 2).

Effect on the body weight (BW)

It was found that body weight was increased among the all groups from the initial day to the end of the experiment. However, only glibenclamide treated rats showed significant rise in body weight at the end of study period (Table 3).

Chronic effect on fasting glucose level

At the initial day of the chronic experiment, fasting blood glucose levels were only slightly higher in type 2 model rats, indicating the presence of functioning β cells. But after 21 days (on 22nd day) of chronic feeding, *T. arjuna* extract had significant effect ($p<0.05$) on lowering of fasting glucose levels of type 2 diabetic rats (serum glucose levels, mmol/l, M±SD, 6.66±1.39 in the extract feed group compared with 8.00±1.18 in water control group). The standard drug glibenclamide, which served as positive control, also showed significant ($p<0.01$) hypoglycemic effect (serum glucose levels,

mmol/l, M±SD, 8.00±1.44). These data clearly showed that ethanol extract of *T. arjuna* tend to decrease serum glucose level gradually. Fasting blood glucose level was mmol/l (M±SD) 8.47±0.79; 7.73±1.22 and 6.66±1.39 at 8th, 15th, and 22nd day compared with the initial day. While comparing between groups, glibenclamide treated group significantly lowered serum glucose level on the 15th day (p=0.01) (Table 4).

Table 3: Chronic effect of 50% ethanol extract of *T. arjuna* on body weight (Bw) of type 2 diabetic model rats

Group	BW_ 1st day (gm)	BW_ 8th day (gm)	BW_ 15th day (gm)	BW_ 22ns day (gm)
Water control (n = 7)	183±17	184±14	190±15	193±15
Glibenclamide (n = 8)	169±15	173±12	176±13	183±16**
Extract (n = 8)	176±23	174±25	181±31	178±42

Data are presented as Mean ± SD and compared using paired't' test. **P<0.05.

Table 4: Chronic effect of *T. arjuna* ethanol extract on fasting glucose level of type 2 diabetic model rats

Group	Glu_1st day (mmol/l)	Glu_8th day (mmol/l)	Glu_15th day (mmol/l)	Glu_22nd day (mmol/l)	t/p value 1st day vs 8th day	t/p value 1st day vs 15th day	t/p value 1st day vs 22nd day
WC (n = 7)	8.88±1.73	8.29±1.95	8.49±1.44	8.00±1.18	0.47/0.66	0.33/0.75	1.91/0.11
Glib (n = 8)	9.64±1.38	8.62±1.18	6.64±1.42*	8.00±1.44*	1.78/0.12	3.93/0.01	3.61/0.01
Extract (n=8)	8.36±1.03	8.47±0.79	7.73±1.22	6.66±1.39**	-0.26/0.81	1.05/0.33	2.77/0.03

Results are expressed as Mean **±** SD. Between groups: comparison was done using one way ANOVA with post hoc Bonferroni test. Within groups: comparison was done using paired t test. *P<0.01; **P<0.05.

Effect on serum insulin level and hepatic glycogen content

There is no significant change in serum insulin and hepatic glycogen content among the test groups after 21 days of chronic oral administration of ethanol extract of *T. arjuna* (Table 5, 6).

Effects on lipidemic status

T. arjuna has some effect on atherogenic lipids. It is found that there is a decrease in the total cholesterol level among all the groups but significant changes (p<0.05) were found in case of extract and glibenclamide treated groups after 21 days of consecutive feeding. The mean total cholesterol levels were mg/dl (M±SD) 57±13 in extract fed group compared with 60±7 in glibenclamide treated group and 66±10 in the water control. This data clearly showed that total cholesterol level was decreased more effectively by the *T. arjuna* extract rather than glibenclamide and water treated control groups from the initial day.

Serum triglyceride level was also decreased among all the groups but significant levels were found in the both cases of extract (p<0.001) and glibenclamide (p<0.05) treated groups. The data from the table-7 clearly showed that the decreasing tendency of triglyceride was by 50%, 28% and 24% in respect of extract, glibenclamide and water treated groups after 21 days (on 22nd day).

Table 5: Chronic effect of *T. arjuna* ethanol extract on serum insulin level of type 2 diabetic model rats

Group	Insulin_ 1st day (ng/ml)	Insulin _Final day (ng/ml) Median (Range)
Water control (n = 7)	0.67 (0.52-1.84)	0.88 (0.48-1.20)
Glibenclamide (n = 8)	0.63 (0.32-1.36)	0.77 (0.60-1.31)
Extract (n = 5)	0.71 (0.32-1.84)	0.78 (0.48-1.31)

Results are expressed as Median (Range: Minimum-Maximum); Mann Whitney test was does as the test of significance.

Table 6: Chronic effect of *T. arjuna* ethanol extract on hepatic glycogen content of type 2 diabetic model rats

Group	Glycogen (mg/gm)
Water control (n = 7)	1.32±1.40
Glibenclamide (n = 8)	1.82±1.91
Extract (n = 8)	1.49±2.72

Data are presented as Mean ± SD and compared using paired't' test.

Table 7: Chronic effect of *T. arjuna* ethanol extract on lipidemic status (cholesterol & triglyceride) of type 2 diabetic rats

Group	CH_1 (mg/dl)	CH_22 (mg/dl)	TG_1 (mg/dl)	TG_22 (mg/dl)
Water (n=7)	78±16	66±10	83±19	63±11
Glibenclamide (n=8)	79±19	60±7	75±22	54±14**
Extract (n=8)	73±10	57±13**	117±15	59±10***

Data are presented as Mean ± SD and compared using paired't' test. **P<0.05; P<0.001.

Effects on HDL-cholesterol are presented in Fig-1. The figure illustrates that there were no significant changes in case of HDL-cholesterol level among all the test groups after 21 days of chronic experiment.

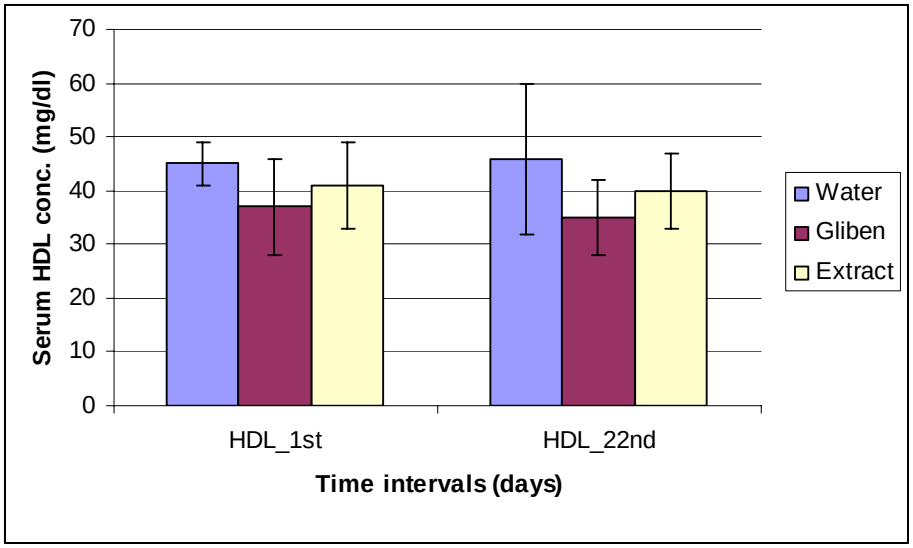

Fig. 1: Chronic effects of *T. arjuna* ethanol extract on lipidemic status (hdl-cholesterol) of type 2 diabetic rats

Data are compared using paired't' test.

DISCUSSION

Synthetic drugs such as Sulphonylureas and Biguanides are valuable in the treatment of DM, but their uses are restricted by their limited action, pharmacokinetic properties, secondary failure rates and accompanying side effects16. Moreover, these therapies only partially compensate for metabolic derangements seen in diabetes and do not necessarily correct the fundamental biochemical lesion17. As the incidence of diabetes is rising relatively around the world, there is an urgent need to expand the range of effective palliatives available to patients.

The present study was undertaken to assess the acute as well as chronic antihyperglycemic effect of *Terminalia arjuna* ethanol extract on Type 2 diabetic model rats. An attempt was also made to assess the mode of antidiabetic and anti-lipidemic action of *T. arjuna*.

It has been demonstrated that postprandial hyperglycemia is an important cardiovascular risk factors in Type 2 diabetic patients18. Studies have shown that the post-meal hyperglycemia doubled the rise of heart disease and fatal cardiovascular diseases19. In acute test, ethanol extract of *T. arjuna* opposed the rise of postprandial serum glucose level when was fed simultaneously with glucose load, although the effect was non significant. On the 21st day after chronic feeding when the 2nd oral glucose tolerance test was performed, ethanol extract of *T. arjuna* produced a significant antihyperglycemic effect in Type 2 model rats. Antihyperglycemic activity, when given with a simultaneous glucose load in Type 2 rats indicates that *T. arjuna* may interfere with the intestinal glucose absorption in the gut. It may also act by modifying the peripheral uptake of glucose and probably increasing the sensitivity of insulin20. The obtained results also indicate that in case of Type 2 diabetic rats both first as well as second phase of insulin response to glucose are impaired, whereas *T. arjuna* extract and glibenclamide treatment improved glucose tolerance. It was, may be, and due to restoration delayed insulin response.

In the chronic study, the most important finding was that, after 21 days of consecutive feeding, when the rats were sacrificed on the 22nd day, a significant reduction ($p<0.05$) in the fasting glucose level was observed in extract fed group compared with the water control group. In this experiment, glibenclamide treated group (positive control) also significantly decreased ($p<0.01$) fasting blood glucose level after chronic feeding. This obtained result is supported by the finding of other investigators12.

The possible mechanism of hypoglycemic effect might be explained by the findings of some other investigators as, the oral administration of the extract of *T. arjuna* increases the activities of glycolytic enzymes (hexokinase, phosphoglucoisomerase) and decreases aldolase enzyme activities in the liver and kidney. The extract also reduces the gluconeogenic enzymes (glucose-6-phosphatase and fructose-1, 6-biphosphatase) to a normal level whenever it becomes high12.

It was explored whether the blood glucose lowering effect was due to reduction of food intake. This was done by comparing the body weight between the control and treated groups. The result showed that there was an increasing tendency of body weight in both control and treated (extract and glibenclamide) groups. The tendencies were of similar proportion in the control and treated groups and thus they do not explain the hypoglycemic effect in the extract group. The findings also suggest that *T. arjuna* extract does not alter normal metabolic parameters like food and water intake.

No statistically significant change was found in serum insulin level after chronic feeding of *T. arjuna* although serum insulin level tend to rise in all groups. The result indicates that the extract does not affect the B-cell of pancreas directly for secretion of insulin. Again, in our experiment, *T. arjuna* did not show any effect on glycogen deposit in the liver. This effect may be due to absence of the effect on insulin secretion.

The present study was performed on Type 2 model rats, which were made diabetic by the single intraperitoneal injection of Streptozotocin (STZ). The STZ has been shown to induce free radical production and cause tissue injury21. The pancreas is very much susceptible to the action of STZ induced free radical damage. The ethanol extract of *T. arjuna* was evaluated recently for its potent antioxidant potential against OH•, O2•– and lipid peroxidation. It has been shown that, due to high degree of some derivatives of arjunic acid like arjunoglycoside (I, II, III and IV), arjungenin, arjunolone, arjunetin, tanins, ellagic acid and other content22, it significantly decreased free radical damage and hepatic lipid peroxidation10. Therefore, the antidiabetic effect of *T. arjuna* ethanol extract in our studies, which was found after 21 days of consecutive feeding may be due to increased insulin sensitivity. Insulin sensitivity can be increased by affecting these mechanisms. The extract may also improve insulin sensitivity by reducing glucotoxicity which is one of the causes of insulin resistance in type 2 rats20.

Apart from the blood sugar lowering effect, beneficial changes in lipid profile have also been observed by *T. arjuna* extract. Abnormalities in lipid profile are one of the most common complications in diabetes mellitus, which is found in about 40% of diabetics. Since dyslipidemia plays an important role in the pathogenesis of macro- and micro vascular complications of diabetes, hence, improvement in the lipid abnormalities must play beneficial role in inhibiting the complications of diabetes. It has been claimed that hypercholesterolemia and hypertriglyceridaemia occurred in STZ induced diabetic rats23. In this present study, ethanol extract of *T. arjuna* significantly decreased serum total cholesterol ($p<0.05$) and triglyceride ($p<0.001$).

Considering triglyceride level, it was found that ethanol extract of *T. arjuna* decreased TG level more significantly ($p<0.001$) than glibenclamide treated group ($p<0.05$). The observed results also comply with other investigators10, 12, suggesting that arjunic acid as well as its derivatives when undergo biotransformation by hepatic drug metabolism, produce common active metabolites, which probably responsible for lipid lowering activity. This demonstrates that *T. arjuna* ethanol extract have potential antihyperlipidemic effect in type 2 diabetic model rats.

From phytochemical screening, the presence of various chemical components was found at a higher intensity. Polyphenolic compounds, like flavonoids, tannins, alkaloids and steroids commonly found in plants have been reported to have multiple biological effects, including cardio protective, antioxidant and anti-cancer activity. Tannins present in the plant extract, as evident from phytochemical screening, may be responsible for the lipid lowering effect10, 24.

CONCLUSION

Thus it can be concluded that, *Terminalia arjuna* has got promising anti-hyperglycemic and hypolipidemic effects in respect to Type 2 diabetic model rats. In this experiment, specific component analysis has not been performed. So, further analysis for component isolation should be studied.

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REFERENCE

1. Ghosh S, Suryawanshi SA. Effect of Vinca rosea extracts in treatment of alloxan diabetes in male albino rats. Indian Journal of Experimental Biology 2001; 39:748-759.
2. Berger W. Incidence of severe side effects during therapy with sulphonylureas and biguanides. Hormones Metabolic Res 1985; 17:111-5.
3. Rang HP, Dale MM, Rittar JM. The endocrine system Pharmacology. In: Pharmacology. Longman Group Ltd., UK. 1991; 504-508.
4. Dubey GP, Dixit SP, Alok S. Alloxan-induced diabetes in rabbits and effect of herbal formulation D-400. Indian Journal of Pharmacology 1994; 26:225-226.
5. Prince PS, Menon VP, Pari L. Hypoglycemic activity of Syzigium cuminii seeds: effect on lipid peroxidation in alloxan diabetes rats. Journal of Ethnopharmacology 1998; 61:1-7.
6. Ladeji O, Omekarah I, Solomon M. Hypoglycemic properties of aqueous bark extract of Ceiba pentandra in streptozotocin-induced diabetic rats. Journal of Ethnopharmacology 2003; 84:139-142.
7. Geetha BS, Biju CM, Augusti KT. Hypoglycemic effect of leucodelphidin derivative isolated from Ficus bengalensis (Linn.).Indian Journal of Pharmacology 1994; 38:220-222.
8. Rao BK, Sudarshan PR, Rajasekhar MD, Nagaraju N, Rao CA. Antidiabetic activity of Terminalia pallida fruit in alloxan-induced diabetic rats. Journal of Ethnopharmacology 2003; 85:169-172.
9. Vats V, Grover JK, Rathi SS. Evaluation of antihyperglycemic effect of Trigonella foenum-graecum Linn, Occium sanctum Linn. and Pterocarpus marsupium Linn., in normal and alloxanised diabetic rats. Journal of Ethnopharmacology 2002; 79:95-100.
10. Ramesh Chander, Kavita Singh, A K Khanna, S M Kaul, Anju Puri, Rashmi Saxena, Gitika Bhatia, Farhan Rizvi, A K. Rastogi. Antidislipidemic and antioxident activities of defferent fractions of *T. arjuna* stem bark. Indian Journal of Clinical Biochemistry 2004; 19(2):141-148.
11. M. Alam Morshed, Azim Uddin, Akhlaqur Rahman, Tahrim Hasan, Saurov Roy, Abdullah Al Amin, Rajibul Ahsan, Rezuanul Islam. In vitro Antimicrobial and cytotoxicity screening of *Terminalia arjuna* ethanol extract. International Journal of Biosciences 2011; 1(2): 31-38.
12. Ragavan B, S Krishnakumari. Anti-diabetic effect of *T. arjuna* bark extract in alloxan induced diabetic rats. Indian Journal of Clinical Biochemistry 2006; 21(2):123-128.
13. Ghani A. Medicinal Plants of Bangladesh, 2nd Ed. The Asiatic Society of Bangladesh, Dhaka. p331-332, (2003).
14. Bonner-Weir S, Trent DF, Honey RN, Weir G.C. Responses of neonatal rat islets on srteptozotocin limited beta cell regeneration and hyperglycemia. Diabetes 1981; 30:64-69.
15. Ali L, Azad Khan AK, Mamun MIR, Mosihuzzaman M, Nahar N, Nur-E-Alam M, Rokeya B. Studies on Hypoglycemic Effects Fruit Pulp, seed and Whole plant of Momordica charantia on Normal and Diabetic Model Rats. Planta Medica 1993; 59:408-412.
16. Bailey CJ, Day C. Traditional Plant medicines as treatments for Diabetes. Diabetes Care 1989; 12(8):553-564.
17. Tailor R, Agius L. The Biochemistry of Diabetes. Biochemistry Journal 1988; 250: 650-740.
18. Paolisso G., MR Rizzo, M Barbieri, D Manzella, E Ragno, D Maugeri. Cardiovascular risk in type 2 diabetics and pharmacological regulation of mealtime glucose excursions. Diabetes & Metabolism 2003; 29(4):335-340.
19. Barrett-Connor E, Ferrara A. Isolated postchallenge hyperglycemia and the risk of fatal cardiovascular disease in older women and men. The Rancho Bernardo Study. Diabetes Care 1998; 21:1236–1239.
20. Nahar N, Rokeya B, Ali L, Hassan Z, Nur-e-Alam M, Choudhury NS, Khan AKA, Mosihuzzaman M. Effect of three medicinal plants on blood glucose levels in nondiabetic and diabetic model rats. Diabetic Research 2000; 35:41-49.
21. Bolzan AD, Bianchi MS. Genotoxicity of Streptozotocin. Mutat Res 2002; 512:121-134.
22. Row LR, Murti DS, Subba Rao GSR, Sastry CSP, Rao KVF. Chemical examination of Terminalia species: Part III- isolation and structure determination of arjunetin from *Terminalia arjuna*, species. Ind. J. Chem 1970; 8:772-775.
23. Sharma SR, Dwided SK, Swarup D. Hyperglycemia and hypolipidemic effects of Chinamomum tamala Nees leaves. Indian Journal of Experimental Biology 1996; 31:26-29.
24. Pankaj Nainwal, Karan Dhamija, Sacchidananmani Tripathi. Study of antihyperlipidemic effect on the juice of the fresh fruits of Lagenaria siceraria. International Journal of Pharmacy and Pharmaceutical Sciences 2011; 3(1): 88-90.