Hypoglycemic Effect of *Terminalia Chebula* Retz. Fruit on Alloxan-Induced Diabetic Rats

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ABSTRACT

Objective: To evaluate the hypoglycemic effect of ethanolic extract of *terminalia chebula* Retz. fruits on alloxan-induced diabetic rats compared with standard oral hypoglycemic drug, metformin.

Methods: A randomized controlled experimental animal study was done. 30 Wistar albino rats were induced diabetes by alloxan (100 mg/kg). 80%-ethanolic extraction of fruits of *terminalia chebula* Retz. was performed by using Soxhlet extraction method. Group 1 was normal control. Group 2 was alloxan-induced diabetic control, group 3 was metformin 100 mg/kg standard group, groups 4, 5 and 6 were extracts 100, 200 and 400 mg/kg orally administered fruit extract for 28-days, respectively. Fasting blood glucose (FBG) levels were measured at the end of the first, second, third and fourth weeks by using standardized glucometer.

Results: At the end of first, second, third and fourth weeks, the FBG levels of diabetic control were 325.7±28.2, 308.5±69.8, 322.7±65.8 and 369.2±57.4 mg/dL, those of metformin (100 mg/kg) were 76.2±9.5, 92.5±14.9, 94.5±17.9 and 90.8±9.9 mg/dL, those of the *terminalia chebula* Retz. extract 100 mg/kg were 232.5±78.6, 122.8±41.4, 109.2±33.6 and 132.3±41.1 mg/dL, extract 200 mg/kg were 82.7±8.2, 82.7±8.2, 89.7±9.8 and 89±15.2 mg/dL, and extract 400 mg/kg were 80.2±9, 83.5±7.1, 91±11.5 and 82.7±5.9 mg/dL, respectively. When all treatment groups were compared with diabetic control, the FBG levels were significantly reduced ($p<0.001$). There was no significant difference in FBG levels between standard group and extract (200 and 400 mg/kg) groups.

Conclusion: The 80%-ethanolic extract of *terminalia chebula* Retz. has significant hypoglycemic effect on alloxan-induced diabetic rats and it was comparable with standard drug, metformin. The effective dose was 200 to 400 mg/kg.

Keywords: *Terminalia chebula* Retz.; hypoglycemic effect; alloxan-induced diabetic rats (Siriraj Med J 2017;69: 80-84)

INTRODUCTION

Diabetes mellitus is the most common endocrine disorder and constitutes a major health problem in non-communicable disease. The WHO estimated that in Myanmar, the total number of people with diabetes is projected to rise from 922, 000 in 2010 to 1, 755,000 in 2030.[1]

Increased urbanization, westernization and economic development in developing countries as well as changing lifestyles and eating habits have already contributed to a substantial rise in diabetes. The greatest increase in prevalence is expected to occur in Asia and Africa by 2030.[2]

In 2003-2004, a diabetes project was undertaken on prevalence of diabetes mellitus in rural and urban areas of Yangon Division. The overall prevalence within Yangon Division was 11.8 percent and the urban prevalence of diabetes was nearly two times higher than rural prevalence. Although the results of the study might not represent the whole nation, extrapolation could be made to estimate the prevalence of diabetes in Myanmar.[3,4]

The control of diabetes can be achieved by diet, exercise and insulin replacement therapy and/or different oral hypoglycemic drugs. In modern medical system, managing diabetes without side effects is still a challenge because the treatment with many oral hypoglycemic

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agents and insulin are usually associated with hypoglycemia and increased risk of cardiovascular and renal complications.[5] New agents are needed for the prevention and treatment of diabetes because of untoward effects of drugs. Among them, herbal remedies are one of the existing alternative therapies. A majority of the world's populations in developing countries still rely on herbal medicines to meet their health needs because they are readily available resources for primary health care.[6]

Terminalia chebula Retz., is a member of family Combretaceae. It is a native plant in India and South-east Asia.[7] *Terminalia chebula* Retz., has been reported to exhibit a variety of biological activities such as antidiabetic, antibacterial, anticancer, antioxidant, cardioprotective, hepatoprotective and antiulerogenic activities.[7-13] However, no scientific data is available regarding the effect of *terminalia chebula* Retz., fruits on blood glucose level in Myanmar. Therefore in this study, ethanolic extract of *terminalia chebula* fruits was investigated scientifically to evaluate the hypoglycemic activity in alloxan-induced diabetic albino rats.

MATERIALS AND METHODS

Materials

The fruits of *terminalia chebula* Retz., were collected from Moe Kaung monastery, Magway Division, Upper Myanmar and properly identified at the Botany Department, Yangon University. Alloxan monohydrate (Titan Biotech Ltd., Bhiwadi 301019 Rajasthan, India) and metformin tablet British pharmaceutical (BP) 500 mg (Denk Pharma GmbH and Co. KG, Germany) were included.

Preparation of *terminalia chebula* Retz. extract

The dried fruits of *terminalia chebula* Retz., were coarsely powdered in a blender. For extraction, 200 g of fruits (without seed) dry powdered were put into a 5 L conical flask together with 750 mL of 80% ethanol (ethanol/water = 8:2) and left in 60°C water bath for eight hours. It was then filtered and the filtrate was discarded. The residue was extracted by Soxhlet extraction. The yield of extract was 30 g/100g.

Experimental animals

Wistar albino rats of either sex weighing 200-250 g were locally bred in Animal Services Division, Department of Medical Research, Yangon. For feeding, conventional rodent laboratory diets were used with unlimited supply of drinking water. They were kept in clean and dry cages. This study was approved by Ethical Research Committee of University of Medicine 2.

Experimental design

The hypoglycemic effect of ethanolic extract of *terminalia chebula* fruits was investigated by using alloxan-induced diabetic rats. Randomization procedure was done by envelope method. Six Wistar albino rats were selected randomly and placed into each rat cage (block). Each block contained six Wistar albino rats and drugs were administered orally for 4 weeks as follows; group 1 (normal control group) was given distilled water 5 mL/kg/day; group 2 (diabetic group) was given distilled water 5 mL/kg/day; group 3 (standard group) was given metformin 100 mg/kg/day; group 4 (E 100 group) was given extract 100 mg/kg/day; group 5 (E 200 group) was given extract 200 mg/kg/day and group 6 (E 400 group) was given extract 400 mg/kg/day. All groups were given for 28 days. Fasting blood glucose levels were estimated by using standardized glucometer at the end of 1st, 2nd, 3rd and 4th weeks.

Induction of experimental diabetes

The animals were fasted overnight and induction of diabetes (groups 2-6) was performed by a single intraperitoneal injection of alloxan monohydrate at a dose of 100 mg/kg body weight. FBG levels were determined after 24-48 hours of alloxan administration. Wistar albino rats having BGL above 200 mg/dL after 24-48 hours of alloxan administration were selected for this study.

Method of collection of blood sample

Rats were put into a mechanical restraint device and blood sample was taken by cutting 1 mm at tip of tail. One drop of blood was collected on the test strip and blood glucose level was read by the standardized glucometer (mg/dL).[14]

Statistical analysis

Standard statistical methods were used in the calculation of arithmetic mean (X), and standard deviation (SD) by using SPSS 20 software. Comparison of data was done by one way analysis of variance "ANOVA" and general linear model (repeated measure) test. p value less than 0.05 was considered as statistically significant.

RESULTS

Results of hypoglycemic effect are summarized in Table 1 and Fig1 showing mean blood glucose level in different groups. When the metformin group was compared with diabetic group, the FBG levels were significantly reduced (p <0.001) as shown in Table 1. When the extracts (100, 200 and 400 mg/kg) groups were compared with

TABLE 1. Comparison of fasting blood glucose level between diabetic group and all other groups.

Group	Fasting blood glucose level (mg/dL) (Mean ± S.D)			
n = 6	First week	Second week	Third week	Fourth week
1	90.3 ± 10.3	84.7 ± 5.9	79.5 ± 4.2	89.2 ± 4.8
2	325.7 ± 28.2	308.5 ± 69.8	322.7 ± 65.8	369.2 ± 57.0
3	76.2 ± 9.5 ***	92.5 ± 14.9 ***	94.5 ± 17.9 ***	90.8 ± 9.9 ***
4	232.5 ± 78.6 ***	122.8 ± 41.4 ***	109.2 ± 33.6 ***	132.3 ± 41.1 ***
5	82.7 ± 8.2 ***	82.7 ± 8.2 ***	89.7 ± 9.8 ***	89.0 ± 15.2 ***
6	80.2 ± 8.9 ***	83.5 ± 7.1 ***	91.0 ± 11.5 ***	82.7 ± 5.9 ***

***p<0.001 between diabetic group and all treatment groups
Group 1 = Normal control group, Group 2 = Diabetic control group, Group 3 = Standard group with metformin 100 mg/kg, Group 4 = Extract 100 mg/kg, Group 5 = Extract 200 mg/kg, Group 6 = Extract 400 mg/kg

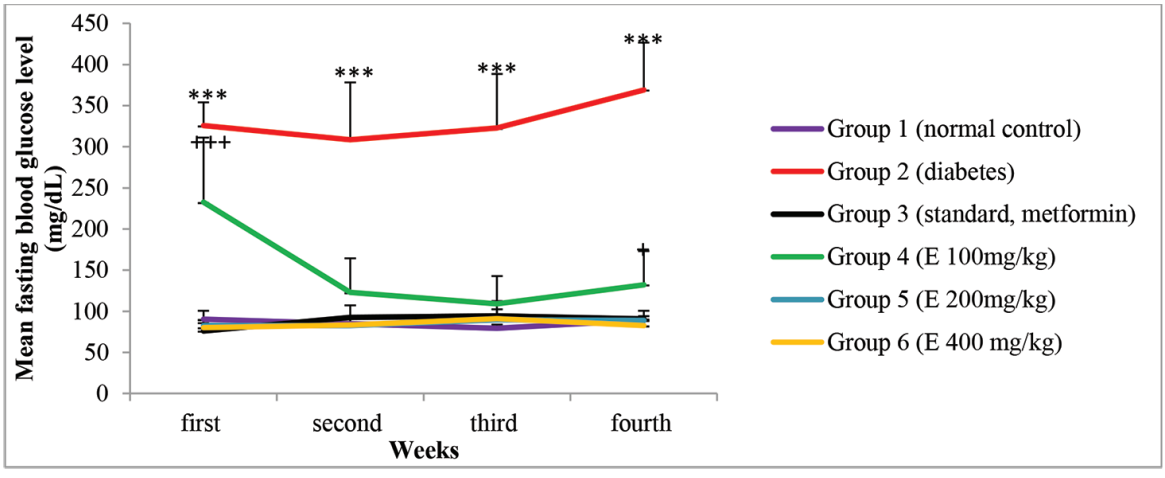

Fig1. Comparison of fasting blood glucose level between standard group and all other groups.
***p <0.001 between standard group and diabetic group, +++p <0.001, +p <0.05 between standard group and extract 100 mg/kg group

diabetic group, the FBG levels were also significantly reduced (p <0.001) as shown in Table 1. It showed that the extracts had hypoglycemic action. There was no significant difference when metformin group was compared with extract (200 and 400 mg/kg) groups as shown in Fig 1. The hypoglycemic action of the extract 200 mg/kg and 400 mg/kg is similar to the metformin. However, there was significant difference between metformin group and extract 100 mg/kg at first and fourth weeks as shown in Fig 1. It showed that the extract 100 mg/kg does not give the effective hypoglycemic action.

DISCUSSION

Terminalia chebula Retz., denotes "a fruit having dark greenish yellow color, which drives away diseases".[15] This fruit is easily available and edible because it can be cultivated in Upper Myanmar. It possesses hypoglycemic effect, but there is no scientific data in Myanmar. It has been used in India and China.[7,19] Therefore the present study was carried out to explore the hypoglycemic effect of ethanolic extract of fruits of *terminalia chebula* Retz., in alloxan-induced diabetic Wistar albino rats. The fruits were collected from the same season and same area to avoid difference of weather and soil which affects chemical constituents of the fruit.

Extraction is the crucial first step in the analysis of medicinal plants because it is necessary to extract the desired chemical components from the plant materials for further separation and characterization. There are several solvents which can be used in extraction such as aqueous, ethanol, methanol, and chloroform. The anti-diabetic effect of *terminalia chebula* Retz., had been reported by various extracts such as ethanol extract,[7] aqueous extract,[16] methanol extract,[17] and chloroform

extract.[18] Ethanolic extract of plant bioactives has displayed a higher yield compared with the aqueous extract.[19] Therefore, ethanolic extract of fruits of *terminalia chebula* Retz., was used in this study.

Experimental diabetic mellitus has been induced in laboratory animals by several methods that include chemical, surgical and genetic (immunological) manipulation. The diabetogenic drugs which are used, includealloxan monohydrate, streptozotocin with or without nicotinamide, ferric nitrilotriacetate, ditizona and antiinsulin serum. Streptozotocin is the most commonly used drug for induction of diabetes in rats, but there are some disadvantages to its use in chronic experiments, especially spontaneous recovery from high glucose levels by the development of functioning insulinoma and high incidence of kidney and liver tumours. Alloxan is the next most commonly used chemical for induction of diabetes mellitus because it causes pancreatic β cells destruction. In adrenaline-induced method, there is transient hyperglycemia. The surgical and genetic methods of diabetes induction are associated with a high percentage of animal morbidity and mortality. The induction of alloxan appeared to be the easiest, reliable and the most practicable method of inducing diabetes mellitus in rodents. Therefore, alloxan was used to induce diabetes in this study.[20,21]

Metformin is clinically used as the first line therapy in treatment of diabetes mellitus. Metformin reduces glucose level by decreasing hepatic glucose production and by increasing insulin action in muscle and fat. At a molecular level, these actions are mediated at least in part by activation of the cellular kinase AMP activated proteinkinase. It does not cause insulin release from the pancreas. Therefore, metformin was chosen as a standard drug for alloxan-induced diabetic rats because alloxan destroys the β cells and does not produce insulin.[22]

In alloxan-induced diabetic Wistar albino rats, the extract dose 200 mg/kg and 400 mg/kg significantly reduced the fasting blood glucose level in comparison with the diabetic group. The extract dose 200 mg/kg and 400 mg/kg had hypoglycemic effect. However, the dose 100 mg/kg did not have hypoglycemic efficacy because it FBG was significantly increased at first and fourth weeks in comparison with metformin group.

Kannan *et al.*, showed that the ethanolic extract of *terminalia chebula* Retz., 200 mg/kg had significant hypoglycemic activity against alloxan-induced diabetes rats.[23] Kumar *et al.*, mentioned that the ethanolic extract of fruit of *terminalia chebula* Retz., 200 mg/kg had hypoglycemic activity on streptozotocin-induced diabetes rats.[24] This study showed that the extract dose 200 and 400 mg/kg had significant hypoglycemic effect. Therefore, the result of this present study agreed with those two studies.

Borgohain *et al.*, stated that the ethanolic extract of *terminalia chebula* Retz., 100 mg/kg showed significant anti hyperglycemic effect in alloxan-induced diabetic model.[7] It also showed reduction in blood glucose level on adrenaline-induced hyperglycemic rats. The present study observed that the extract dose 200 and 400 mg/kg were found to be the optimum hypoglycemic doses. The extract dose 100 mg/kg had no significant hypoglycemic effect. Compared with Borgohain *et al.*, they had shown hypoglycemic effect with 100 mg/kg. It might be due to difference in nature, cultivation and climate.

The ethanolic extract of fruits of *terminalia chebula* Retz., has significant hypoglycemic effect and its action is comparable to standard hypoglycemic drug, metformin.

CONCLUSION

Ethanolic extract of *terminalia chebula* Retz., showed hypoglycemic effect evidenced by significant reduction in blood glucose concentration of alloxan-induced diabetes model in Wistar albino rats. Hence, the study suggested that the plant might help in control of diabetic mellitus or may be useful as a good adjunct to the anti-diabetic agents for effective treatment of diabetes mellitus.

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