Myanmar Health Sciences Research Journal, Vol. 30, No. 3, 2018

Hypoglycemic Effect of *Terminalia chebula* Retz. (Phan-kha-thee) on Diabetic Albino Rat Models

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This study was aimed to evaluate the hypoglycemic effect of ethanolic extract of *Terminalia chebula* Retz. fruits (Phan-kha-thee) on diabetic albino rats. In first part, a randomized controlled experimental animals study was done. The 80% - ethanolic extraction of fruits of *Terminalia chebula* Retz. was done by using Soxhlet apparatus. Diabetes mellitus was induced in wistar albino rats by intraperitoneal injection of alloxan (100 mg/kg).The extracts (100, 200 and 400 mg/kg) and metformin were orally administered to rats for 28 days. Fasting blood glucose (FBG) levels were measured weekly using glucometer. At the end of first, second, third and fourth weeks, the FBG levels of diabetic group were 325.7±28.2 mg/dl, 308.5±69.8 mg/dl, 322.7±65.8 mg/dl and 369.2±57.4 mg/dl, those of metformin (100 mg/kg) group were 76.2±9.5 mg/dl, 92.5±14.9 mg/dl, 94.5±17.9 mg/dl and 90.8±9.9 mg/dl, those of the *Terminalia chebula* Retz. extract 100 mg/kg group were 232.5±78.6 mg/dl, 122.8±41.4 mg/dl, 109.2±33.6 mg/dl and 132.3±41.1 mg/dl, respectively. When all treatment groups compared with diabetic group, the FBG levels were significantly reduced ($p<0.001$). There was no significant difference in FBG levels between metformin group and extract (200 and 400 mg/kg) groups. In second part, Quasi experimental study was done in maltose-induced models. Acarbose (18.5 mg/kg) and extract (100, 200 and 400 mg/kg) were orally administered first and maltose 2 g/kg was also administered after 10 minutes apart. Blood glucose concentrations were measured at 0, 30, 60, 90 and 120 minutes by glucometer. There was no significant difference between that acarbose and that of the extract 200 mg/kg. The ethanolic extract has significant hypoglycemic effect on alloxan-induced diabetic rats and its action is comparable to metformin.

Key words: Terminalia chebula Retz., Hypoglycemic effect, Alloxan-induced diabetic rats, Maltose-induced models

INTRODUCTION

Diabetes mellitus is the most common endocrine disorder and constitutes a major health problem in non-communicable disease. In 2003-2004, diabetes project had undertaken on prevalence of diabetes mellitus in rural and urban areas of Yangon Division. The overall prevalence within Yangon Division was 11.8% and the urban prevalence of diabetes was nearly two times higher than that of rural. Although the results of the study may not represent the whole nation, extrapolation can be made to estimate the prevalence of diabetes in Myanmar.[1, 2]

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 effect is still a challenge[3] because the treatments with many oral hypoglycemic agents are usually associated

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with hypoglycemic and increased risk of cardiovascular and renal adverse effects.

New agents which have less side effects and less costs are needed for the treatment of diabetes because of longterm use. Among them, herbal remedies are one of the existing alternative therapies. A majority of the world's population in developing countries still relies on herbal medicines to meet its health needs because they are readily available resources for primary health care.[4]

Terminalia chebula Retz. is a member of family Combretaceae. It is a native plant in India and Southeast Asia.[5] *Terminalia chebula* Retz. has been reported to exhibit a variety of biological activities such as antidiabetic, antibacterial, anticancer, anti-oxidant, cardioprotective, hepatoprotective and antiulerogenic activity.[5-10] 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 and maltose-induced hyperglycemic rats.

MATERIALS AND METHODS

The fruits of *Terminalia chebula* Retz. was collected from Moe Kaung Monastery, Magway Region, 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 used.

Preparation of Terminalia chebula Retz. extract

The dry fruits of *Terminalia chebula* Retz. were coarsely powdered in a blender. For extraction, 200 g dry powder of fruits (without seed) were put into a five-liter conical flask together with 750 ml of 80% ethanol (ethanol: water - 8:2) and incubated at 60^{o}C in water bath for eight hours. It was then filtered through filter paper and the filtrate was evaporated on a boiling water bath for a week.

Phytochemical analysis of Terminalia chebula Retz.

Phytochemical test for the dry powder fruits was investigated the presence or absence of organic compounds by color changes in test tube according to Harbone phytochemical methods.[11]

Procedure of acute toxicity study

According to limit test of OECD 425 Guide-line, one mouse was given single oral dose 2000 mg/kg of the extract. If the mouse was survived, the same done was given to another four mice. If all mice were survived, the test was terminated.

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, conven-tional rodent laboratory diets were used with unlimited supply of drinking water. They were kept in clean and dry cages.

Experimental design

In first part, 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 5ml/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 6 groups were given

daily for 28 days. Fasting blood glucose levels were estimated by using glucometer at the end of 1st, 2nd, 3rd and 4th weeks.

In second part of the maltose-induced hyperglycemic experiment, twenty-four Wistar albino rats were grouped into four blocks. By using above procedure, four envelopes were placed onto the block randomly. Group A was given acarbose 18.5 mg/kg, group B was given extract 100 mg/kg, group C was given extract 200 mg/kg and group D was given extract 400 mg/kg, respectively. Post prandial blood glucose levels were measured at time 0, 30, 60, 90 and 120 minutes.

Induction of experimental diabetes

The animals were fasted overnight and induction of diabetes (groups 2-6) were made by a single intraperitoneal injection of alloxan monohydrate at a dose of 100 mg/kg body weight. Fasting blood glucose (FBG) levels were determined after 24-48 hours of alloxan administration. Wistar albino rats having FBG levels 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.[12]

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.

Ethical consideration

This study was approved by Ethics Review Committee of University of Medicine 2, (Yangon).

RESULTS

It was observed that the yield of 80% ethanolic extract of *Terminalia chebula* Retz. fruits was 30 g/100 g. As first part of study, hypoglycemic effects of *Terminalia chebula* Retz. extract groups and metformin group are summarized in Table 1. As shown in Table 1, metformin group significantly reduced the FBG levels ($p<0.001$) when compared with diabetic group. The extracts (100, 200 and 400 mg/kg) groups also significantly reduced the FBG levels ($p<0.001$) compared with diabetic group.

Table 1. Comparison of fasting blood glucose levels between diabetic group and all other groups

Group	Fasting blood glucose level (mg/dl) (Mean±SD)			
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***

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

***p<0.001 between diabetic group and all treatment groups

The extract (200 and 400 mg/kg) groups showed no significant difference in blood glucose levels when compared with metformin group. But extract 100 mg/kg showed significant difference in blood glucose levels when compared with metformin group at first and fourth weeks. Therefore, the extract 200 and 400 mg/kg had similar hypoglycemic effect to metformin except the extract 100 mg/kg.

As second part of the study, post prandial blood glucose concentrations of acarbose group and all extract groups are summarized in Table 2.

Table 2. Comparison of mean post prandial blood glucose concentrations of acarbose group and all extract groups

Group n=6	Mean post prandial blood glucose concentrations after maltose taken (mg/dl) (Mean±SD)				
	0 min	30 min	60 min	90 min	120 min
A	82.5 ±5.6	133.0 ±24.3	117.5 ±7.1	113.3 ±6.9	107.0 ±7.8
B	79.83 ±4.7 (NS)	142.5 ±21.6 (NS)	138.3 ±17.8*	113.8 ±12.4 (NS)	99.8 ±12.9 (NS)
C	82.2 ±9.2 (NS)	129.3 ±12.6 (NS)	119.5 ±15.8 (NS)	107.5 ±8.0 (NS)	91.33 ±10.3 (NS)
D	89.2 ±4.8 (NS)	147.5 ±16.7 (NS)	137.7 ±16.1*	115.7 ±8.8 (NS)	102.2 ±9.0 (NS)

Group A = Acarbose 18.5 mg/kg
Group B = Extract 100 mg/kg
Group C = Extract 200 mg/kg
Group D = Extract 400 mg/kg

*$p<0.05$ between acarbose and groups B and D.
NS=Not significant between acarbose and groups B, C and D.

When acarbose 18.5 mg/kg group compared with extract 100 mg/kg group and extract 400 mg/kg group, the significant differences $p<0.05$ were found at 60 minutes. When acarbose group compared with extract 200 mg/kg group, there was no difference statistically. Therefore, the acarbose 18.5 mg/kg group and the extract 200 mg/kg group had similar hypoglycemic effect on post prandial blood glucose concentrations.

DISCUSSION

Terminalia chebula Retz. 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 claimed to exhibit a variety of biological activities in India and China such as antidiabetic, antibacterial, anticancer, antioxidant, cardioprotective, hepatoprotective and antiulerogenic activities.[5-11] The fruits were collected from the same area during the same season to avoid differences of soil and wealther that affect 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 water, ethanol, methanol, chloroform. The antidiabetic effect of *Terminalia chebula* Retz. had been reported by various extracts such as ethanol extract,[5] aqueous extract,[13] methanol extract,[14] and chloroform extract.[15] Ethanolic extract of plant bioactives has displayed a higher yield compared with the aqueous extract.[16] Therefore, ethanolic extract of fruits of *Terminalia chebula* Retz. was used in this study.

In this acute oral toxicity test, limit test of OECD 425 guideline was used and it was reported that LD_{50} was supposed to be more than 2000 mg/kg and was considered to be slight toxic.

Phytochemical analysis of this study revealed the presence of carbohydrate, reducing sugar, glycoside, flavonoid, tannis, amino acid, steroid, saponins and polyphenol in 80% ethanolic extract of fruits of *Terminlia chebula* Retz.

Experimental diabetic mellitus has been induced in laboratory animals by several methods that include chemical, surgical and genetic (immunological) manipulation. The diabetogenic drugs used are alloxan monohydrate, streptozotocin with or without nicotinamide, ferric nitrilotriacetate, dithizona and anti-insulin serum. Streptozotocin is the most commonly used drug for induction of diabetes in rats. 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 appears to be the easiest, reliable

and the most practicable method of inducing diabetes mellitus in rodents. So, alloxan is used to induce diabetes in this study.[17, 18]

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 molecular level, these actions are mediated at least in part by activation of the cellular kinase AMP activated protein-kinase. It does not cause insulin release from the pancreas. Therefore, metformin was chosen as a standard drug for alloxan-induced diabetic rats because alloxan destructs the β cells and does not produce insulin.[19]

The alpha-glucosidase inhibitor, acarbose, reduces intestinal absorption of starch, dextrin and disaccharides by inhibiting the action of alpha-glucosidase in the intestinal brush border. Inhibition of this enzyme slows the absorption of carbohydrates. The post prandial rise in plasma glucose is blunted in both normal and diabetic subjects. Huang, *et al.*[16] showed that the extract had similar hypoglycemic effect to the acarbose after maltose-induced hyperglycemic rats. In this study, acarbose was chosen as a standard drug for maltose-induced hyperglycemic rats.

In this study, all dose levels of the extract significantly reduced the fasting blood glucose levels in alloxan-induced diabetic Wistar albino rats when compared with the diabetic group. All extract groups had hypoglycemic effect. The extracts 200 mg/kg and 400 mg/kg showed similar efficacy to metformin although the extract 100 mg/kg did not have the comparable efficacy with metformin.

Kannan, *et al.*[20] showed that the ethanolic extract of *Terminalia chebula* Retz. 200 mg/kg had significant hypoglycemic activity against alloxan-induced diabetes rats. Kumar, *et al.*[21] mentioned that the ethanolic extract of fruit of *Terminalia chebula* Retz. 200 mg/kg had hypoglycemic activity on streptozotocin-induced diabetes rats. In this study, it was observed that the extract at the doses of 200 and 400 mg/kg had significant hypoglycemic effect. Therefore, the result of present study agreed with these two studies.

Borgohain, *et al.*[5] stated that the ethanolic extract of *Terminalia chebula* Retz. 100 mg/kg showed significant antihyperglycemic effect in alloxan-induced diabetic model. It also showed reduction in blood glucose level on adrenaline-induced hyperglycemic rats. In the present study, it was observed that the extract of *Terminalia chebula* Retz. fruits at the doses of 200 and 400 mg/kg were the optimum hypoglycemic doses. The extract (100 mg/kg) had no significant hypoglycemic effect. Borgohain, *et al.*[5] reported the hypoglycemic effect of 100 mg/kg of *Terminalia chebula* extract. It might be due to difference in nature, cultivation and climate.

Huang, *et al.*[16] stated that chebulagic acid (100 mg/kg) of *Terminalia chebula* Retz. significantly reduced post-prandial blood glucose level in maltose-loaded SD-rats. They also found that the hypoglycemic effect of the extract had more potent effect on maltose than on sucrose and glucose. In this study of maltose-induced hyperglycemic rats, there was a significant difference between ethanolic extract (100 mg/kg and 400 mg/kg) and acarbose at time 60 minutes. However, there was no significant difference between acarbose and the extract 200 mg/kg at time 30, 60, 90 and 120 minutes. The extract was most effective at the dose of 200 mg/kg and the extract had no dose-dependent hypoglycemic activity.

The ethanolic extract of fruits of *Terminalia chebula* Retz. has significant hypoglycemic effect and its action is comparable to standard hypoglycemic drug, metformin. The study showed similar efficacy with acarbose in post prandial glucose reduction.

Conclusion

Eighty percent ethanolic extract of *Terminalia chebula* Retz. fruits showed

hypoglycemic effect evidenced by significant reduction in blood glucose concentration of alloxan-induced diabetic albino rat models. Hence, the study suggested that the fruits of *Terminalia chebula* Retz. 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.

Competing interests

The authors declare that they have no competing interests.

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