POTENTIAL HYPOGLYCEMIC EFFECT OF *SWERTIA CHIRATA* - AN INDIAN SUBCONTINENT HERB WITH IMPORTANT MEDICINAL VALUE

Khondoker Dedarul Alam [1*], Mohammad Shawkat Ali [1],Sanjida Mahjabeen[2], Md. Rajib Hassan[1], Md. Fazlur Rahman[1], R.M.A.A. Chowdhury[3]

[1]Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Bangladesh
[2]Department of Pharmaceutical Technology, University of Dhaka, Bangladesh
[3]Department of Pharmaceutical Chemistry, University of Dhaka, Bangladesh

* Corresponding Author: Khondoker Dedarul Alam
Present Address: Department of Clinical Pharmacy and Pharmacology, University of Dhaka, Bangladesh
Phone: +8801714042246, E-mail: kh.dedarul@yahoo.com

Summary

The aim of the present study was to investigate hypoglycemic effect of ethanolic extract of leaf and its different fractions i.e. pet-ether, dichloromethane and methanol fraction of *Swertia chirata* (family-Gentianaceae) on *Swiss albino* mice at fasting condition. Tail tipping method was used as the mean of the investigation. Ethanolic extract of leaf and its pet-ether fraction showed significant hypoglycemic activity by about 32% and 47.2% reduction of blood glucose level respectively after 3 hours of test sample administration. Dichloromethane and methanol fractions show mild to moderate hypoglycemic effect which is about 14.1% and 15.9% reduction in blood glucose level respectively after 3 hours of drug administration. The test samples were administered at a dose of 250 mg/kg body weight of *Swiss albino* mice. Glibenclamide at a dose of 5 mg/kg body weight was used as standard in this study.

Key words: Hypoglycemic effect, *Swertia chirata*, Gentianaceae, *Swiss albino* mice, Pet-ether, Glibenclamide

Introduction

Diabetes mellitus is a chronic disease which has been described as a state of raised blood glucose level. As per International Diabetes Federation (IDF), diabetes currently affects 246 million people worldwide and is expected to affect 380 million by 2025. The prevalence of diabetes is more pronounce in third world countries like Bangladesh. In 2000, about 3.2 millions of people of Bangladesh were recognized to have diabetes and this ranked 10th position of global consideration. It is estimated that in 2030 about 11.1 millions of people of Bangladesh will be affected by diabetes which will rank the 7th position of global consideration[1].

At present, several drugs are available for the management of hyperglycemia but they are expensive and possess side effects also. Therefore, search for a suitable alternative is continued. For a developing country like Bangladesh herbal plants may be the most attractive target for their availability, low cost and better safety margin. Hence, the present study was undertaken to investigate the hypoglycemic activity of *Swertia chirata* (Family: Gentianaceae*)*. The plant has has an erect, about 2–3 ft long stem, the middle portion is round, while the upper is four-angled, with a prominent decurrent line at each angle. The stems are orange brown or purplish in colour. The root is simple, tapering and stout, short, almost 7 cm long and usually half an inch thick. Flowering in Swertia chirata is in the form of numerous small, axillary, opposite, lax cymes arranged as short branches and the whole inflorescence is 2 ft long. Flowers are small, stalked, green-yellow, tinged with purple colour. The flower-tube is twice as long as the sepal-cup and divided near the base into four ovate–lancelike segments. The upper surface of the petal has a pair of nectaries covered with oblong scales and ending as fringes. The plant is found at an altitude of 1200–3000 m, from Kashmir to Bhutan, and in the Khasi hills at 1200–1500 m. It can be grown in sub-temperate regions between 1500 and 2100 m altitudes[2].

The plant extract have been reported to possess anti-inflammatory[3] (Islam *et al.,* 1995), antipyretic[4] (Bhargava *et al.,* 2009), anti-viral[5] (Verma *et al.,* 2008), anthelmintic[6] (Iqbal *et al.,* 2006), anticarcinogenic[7] (Saha *et al.,* 2004), hepatoprotectative[8] (Mukherjee *et al.,* 1997) activities. Early studies documented the presence of flavonoids, xanthones, terpenoids, iridoid and secoiridoid glycosides in the *Swertia chirata* plant[9] (Pant *et al.,* 2002). In this study, ethanol extract of *Swertia chirata* leaf and its different fractions i.e. pet-ether, dichloromethane, methanol, were investigated to find out hypoglycemic activity on *Swiss albino* mice at fasting condition.

Materials and Methods

Plant material

The plant sample was collected from Chawk bazar, Dhaka in November 2009. The authentication of the plant samples have been confirmed by the taxonomist of the National Herbarium of Bangladesh, Mirpur, Dhaka, Bangladesh (accession number - 34333). The plant was dried in shade and then leaf part was ground in coarse powder using high capacity grinding machine. The powders were then preserved in air tight containers.

Extraction and fractionations

Coarsely powdered leaf of *Swertia chirata* was extracted with ethanol by cold extraction process. The crude extracts were then filtered and the vehicle were removed until solid/semisolid mass were produced. Then the crude leaf extract was dissolved in 10% water in methanol (100 ml) and partitioned between pet-ether, dichloromethane, and methanol fractions.

Experimental animals

The experiment of hypoglycemic effect was conducted on *Swiss albino* mice, aged 4-5 weeks, weighting about 20-25 g. The mice were procured from the Animal Resource Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR, B). Before initiating the experiment, the mice were acclimatized for few days under standard environmental conditions (12hours dark/12hours light cycle; temperature 20-22 ^{0}C; relative humidity 40-60%), and fed ICDDR,B formulated rodent food and water.

Chemicals and drugs

Glibenclamide (Daonil) from Sanofi-Aventis was used as standard drug. Tween 80 from BDH Chemicals Ltd and Dimethyl sulphoxide (DMSO) from Merck Chemicals Ltd (Germany) were used as suspending agent for extract fractions. Sterile normal saline solution (0.9% NaCl) from Beximco Infusion Ltd. (Bangladesh) is used as vehicle for standard and test samples.

Preparation of the test materials, standard and control
75 mg crude extract of leaf and its different fractions were measured and triturated unidirectional way by the addition of small amount of suspending agent (tween-80 and dimethyl sulphoxide). After proper mixing of sample and suspending agent, normal saline was slowly added to make the final volume of the suspension up to 3 ml. The suspensions were then stirred by vortex mixture to stabilize it. To prepare the standard, Glibenclamide 5 mg tablet was dissolved into 0.9% normal saline and made the volume up to 10 ml. For preparing control sample, tween-80 (1%) and dimethyl sulphoxide are mixed properly in the normal saline to make the volume up to 5 ml.

Designing of the experiment
The experimental animals were randomly divided into six groups consisting of 5 rats in each group. The groups were denoted as group-I, group-II, group-III, group- IV, group-V and group-VI. Each group of mice received a specific treatment. Test samples at a dose of 250 mg/kg body weight of mice were used to evaluate the hypoglycemic activity. Standard glibenclamide was used at a dose of 5 mg/kg body weight. Prior administering the drugs, each mouse was weighed properly and the doses were adjusted accordingly.

Experimental procedure
In the evaluation of the hypoglycemic effect, the blood glucose level of the experimental animals were measured at 0 hour by tail tipping method[10] (Durschlag *et al.,* 1996) using a glucometer (Bioland G-423 S). Then the control, standard, ethanolic extract of *Swertia chirata* and its different fractions were administered orally to the experimental animals with the help of feeding needle. After 1, 2 and 3 hours, the blood glucose level of the experimental animals were again measured to see the hypoglycemic effect of the test samples in relative to control and standard group.

Statistical Analysis

Data were expressed as mean±SEM. Statistical significance was determined via Student's *t*-test. $P<0.05$ was considered as statistically significant.

Results

The hypoglycemic activities of the ethanolic extract of leaf and its different fractions i.e. pet-ether, dichloromethane, and methanol fractions of *Swertia chirata* were investigated on *Swiss albino* mice at the dose of 250 mg-kg^{-1} body weight. The results are summarized in the **table 1 and table 2.**

After 3 hours of drug administration, the ethanolic extract of leaf showed 32% reduction in blood glucose level relative to 0 hour of the observation period. Among different fractions, pet-ether fraction showed notable hypoglycemic effect which is sustained throughout the study period. About 42.1%, 44.8%, 47.2% blood glucose reduction were observed at first, second and third hour of the study period respectively. Dichloromethane and methanol fractions show mild to moderate hypoglycemic effect which is about 14.1% and 15.9% reduction in blood glucose level respectively after 3 hours of drug administration.

Table 1: Effect of ethanolic leaf extract of *Swertia chirata* and its different fractions on blood glucose of *Swiss albino* mice

Groups	Sample	Blood Glucose Level (m mole/L)			
		0 hour	1 hour	2 hour	3 hour
Gr-I	Control	6.52±0.215	6.20±0.148 *	5.90±0.167 *	5.72±0.183 *
Gr-II	Standard	5.96±0.308	2.00±0.071 *	2.54±0.133 *	2.76±0.163 *
Gr-III	Leaf extract	7.06±0.172	5.16±0.112 *	4.88±0.116 *	4.80±0.110 *
Gr-IV	Pet-ether fraction	6.74±0.449	3.90±0.192 *	3.72±0.037 *	3.56±0.144 *
Gr-V	Dichloromethane fraction	5.68±0.242 *	4.68±0.269 *	4.90±0.245 *	4.82±0.297 *
Gr-VI	Methanol fraction	5.92±0.254	5.50±0.134 *	5.24±0.040 *	4.98±0.080 *

Values in the table are presented as mean ± SEM ($N = 5$). Values are statistically significant at $^{*=}P<0.05$

Table 2: Percentage of blood glucose reduction on *Swiss albino* mice by ethanolic leaf extract of *Swertia chirata* and its different fractions

Groups	Sample	% of blood glucose reduction		
		1 hour	2 hour	3 hour
Gr-I	Control	4.9	12.0	15.3
Gr-II	Standard	66.4	57.4	53.7
Gr-III	Leaf extract	26.9	30.9	32.0
Gr-IV	Pet-ether fraction	42.1	44.8	47.2
Gr-V	Dichloromethane fraction	17.6	13.7	14.1
Gr-VI	Methanol fraction	7.1	11.5	15.9

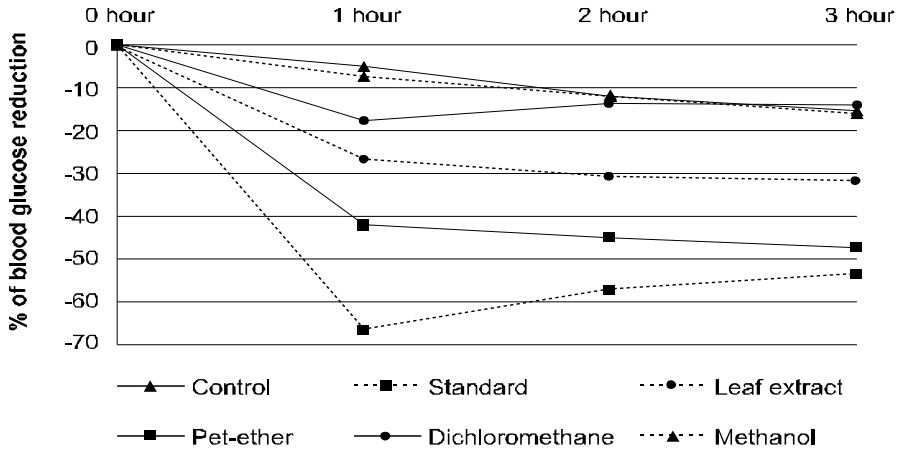

Figure-1: Hypoglycemic effect of ethanolic leaf extract of *Swertia chirata* and its different fraction in comparison of control and standard

Discussion

In recent time, interest with herbal medicine for hypoglycemic activity has been increased significantly. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicines are used throughout the world for a range of diabetic presentations[11] (Mukherjee *et al.,* 2006). The economic crisis, high cost of industrialized medicines, inefficient public access to medical and pharmaceutical care, in addition to the side effects caused by synthetic drugs are some of the factors contributing to the central role of medicinal plants in health care[12] (Johann *et al.,* 2007). *Swertia chirata* is one of such products which is extensively investigated in present time for its antidiabetic potential. In the present study pet-ether fraction of *Swertia chirata* also shows significant hypoglycemic activity on *Swiss albino* mice in relative to control and standard group. Glibenclaide which is used as standard in the study showed significant fall in blood glucose in the first hour of the observation and blood glucose tend to rise slightly in the next two hours (**Figure-1**). Where as, pet-ether fraction showed sustain hypoglycemic effect at the end the observation (**Figure-1**). Mild hypoglycemic effect of Dichloromethane and Methanol fractions was observed may be as a natural corollary due to elonged fasting condition of the mice.

As many of currently available hypoglycemic agent fails to provide sustain action, this observation is valuable to find out new therapeutic option for the patients who seek sustain hypoglycemic action. This observation confirms the use of *Swertia chirata* in ethnomedical application for diabetes management and this study demands further investigation to evaluate the clinical effectiveness of *Swertia chirata* to be used as hypoglycemic agent on commercial basis.

Acknowledgements

The authors gratefully acknowledge Professor Sitesh Chandra Bachar for technical assistance. The authors also show their gratitude to Animal Resource Branch of the International Centre for Diarrhoeal Diseases and Research, Bangladesh (ICDDR,B) to provide experimental animals and also the Department of Clinical Pharmacy and Pharmacology to give necessary support along with different instruments and platform to continue the research work.

References

1. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care 2004;27:1047-1053.
2. Chirayita. (2008, August 28). Indipedia: India's Wikipedia at OSDD. Available from http://crdd.osdd.net/indipedia/index.php?title=Chirayita&oldid=1117. Retrieved, July 18, 2011.
3. Islam CN, Bandyopadhyay SK, Banerjee MK, Das PC. Preliminary studies on the anti-inflammatory effects of *Swertia chirata* in albino rats. Indian J Pharmacol 1995;27:37–39.
4. Bhargava S, Rao PS, Bhargava P, Shukla S. Antipyretic Potential of Swertia chirata Buch Ham. Root Extract. Sci Pharm 2009,77:617–623.
5. Verma H, Patil PR, Kolhapure RM, Gopalkrishna V. Antiviral activity of the Indian medicinal plant extract *Swertia chirata* against herpes simplex viruses: a study by in-vitro and molecular approach. Indian J Med Microbiol 2008;26:322-326.
6. Iqbal Z, Lateef Z, Khan MN, Jabbar A, Akhtar MS. Anthelmintic activity of *Swertia chirata* against gastrointestinal nematodes of sheep. Fitoterapia 2006;77:463-465.

7. Saha P, Mandal S, Das A, Das PC, Das S. Evaluation of the anticarcinogenic activity of *Swertia chirata* Buch. Ham, an Indian medicinal plant, on DMBA-induced mouse skin carcinogenesis model. Phytother Res 2004,18:373-378.
8. Mukherjee S, Sur A, Maiti BR. Hepatoprotective effect of Swertia chirata on rat. Indian J Exp Biol 1997;35:384-388.
9. Pant N, Jain DC, Bhakuni RS. Some chemical constituents of *Swertia chirata*. Indian J Chem 2002;41B:1980-1986.
10. Durschlag M, Wurbel H, Stauffacher M, Von Holst D. Repeated Blood Collection in the Laboratory Mouse by Tail Incision—Modification of an Old Technique. Physiol Behav 1996;60: 1565–1568.
11. Mukherjee PK, Maiti K, Mukherjee K, Houghton PJ. Leads from Indian medicinal plants with hypoglycemic potentials. J Ethnopharmacol 2006;106:1–28.
12. Johann S, Pizzolatti MG, Donnici CL, Aparecida de Resende M. Antifungal properties of plants used in Brazilian traditional medicine against clinically relevant fungal pathogens. Braz J Microbiol 2007;38:632-637.