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Effects of *Swertia chirata* on some blood biochemical parameters in streptozotocin diabetic rats

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Abstract

The antidiabetic effect of aqueous and hexane extracts of *Swertia chirata* was studied in streptozotocin-induced diabetic rats. Type II diabetes was induced by injecting streptozotocin (STZ) subcutaneously to adult (ten to twelve weeks old) mixed albino male (Long Evan's Strain) rats. Aqueous extracts of *Swertia chirata* at 75 and 125 mg/kg body wt. ($AqSC_{75}$ and $AqSC_{125}$), hexane extracts of *Swertia chirata* at 50 and 100 mg/kg body weight ($HeSC_{50}$ and $HeSC_{100}$) and glibenclamide at 600 µg/kg body wt. (an oral antidiabetic drug) were given orally for 16 days to STZ-induced diabetic rats. In diabetic rats, all the different extracts as well as glibenclamide produced a significant ($P<0.01$) antihyperglycemic effect, the markedly higher being in the groups treated with $HeSC_{50}$ and $HeSC_{100}$ followed by $AqSC_{125}$ and $AqSC_{75}$. Both aqueous and hexane extracts of *Swertia chirata* exhibited anti-hypercholesterolemic effect in STZ diabetic rats. But glibenclamide had virtually no cholesterol lowering effect. All forms of extracts at different doses decreased serum TAG levels significantly ($P<0.01$) in diabetic rats; the highest decrease (60%) in TAG level was observed in animals of the group treated with $HeSC_{100}$. In STZ-rats, a fall in SGPT levels was observed in all the groups treated with different extracts and glibenclamide; the most affected was the group treated with $HeSC_{50}$. On the other hand, an increase in SGOT level, another liver enzyme, was observed in groups treated with glibenclamide. But aqueous and hexane extracts of *Swertia chirata* decreased SGOT levels compared with diabetic control group. Thus the above observations suggest that extracts from *Swertia chirata* possess antidiabetic principle and can presumably be used for the treatment of diabetes mellitus.

Keywords: Type II diabetes, Antidiabetic principles, *Swertia chirata* and Glibenclamide

Introduction

Diabetes mellitus is a life long disease, the prevalence of which is increasing day by day throughout the world. More and more world's population are becoming new victims of this disease. According to an estimate, 336 million of the world's population will be diabetic by 2030 as against 171 million in 2000. While most of the 40 countries studied will have their diabetic population more than twice, the diabetic population in Bangladesh will be increased to 3.6 times during the period (Manning, 2004). Type II diabetes, i.e.; non-insulin dependent diabetes mellitus (NIDDM) occurs when the body does not produce enough insulin, or the insulin that is produced become less effective and 80-90% diabetes belong to this type (Johnson, 1998). Diabetes mellitus is a group of incurable diseases, but if properly managed through diet, drug and discipline, a diabetic can live almost a normal life. Natural remedies from medicinal plants have been widely used in the treatment of diabetes mellitus for centuries. Plant sources of hypoglycemic agents are, in fact, easily available, cost-effective and presumably devoid of side effects. Among the indigenous plants that are commonly used for hypoglycemic and antihyperglycemic activity are neem (Bajaj and Srinivasan, 1999), methi (Ali *et al.*, 1995 and Khosla *et al.*, 1995), chirata (Saxena *et al.,* 1993), karalla (Srivastava *et al.*, 1988) etc. Besides there are a number of reports (Nammi *et al.*, 2003; Rathi *et al.*, 2002; Zhang and Tan, 2000 and Ponnachan *et al.*, 1993) of different plant extracts having antihyperglycemic effect on induced diabetic rats or rabbits.

Chirata (*Swertia chirata*) is an erect annual herb, which belongs to the family of Gentianaceae. It is distributed in the mountainous region of Asia, Europe, America and Africa. In Bangladesh it is used for its medicinal value (CSIR, 1976). But information is still lacking with regard to the precise action of *Swertia chirata* on some important metabolic activities in animals. In view of economic and medicinal importance of *Swertia chirata*, the present study aims at investigating the efficacy of aqueous and hexane extracts of *Swertia chirata* in comparison with glibenclamide as antihyperglycemic agent in streptozotocin (STZ)-induced diabetic rats.

Materials and Methods

Dried whole plant of chirata (*Swertia chirata*) was collected from the local market. Ten to twelve week old mixed albino male rats (*Long Evan's Strain*) weighing 180-230 g and the feed pellets were collected from International Center for Diarrhoeal Disease Research, Bangladesh (ICDDR, B).

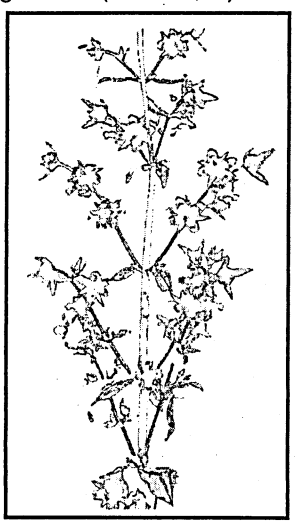

Fig. Chirata plant

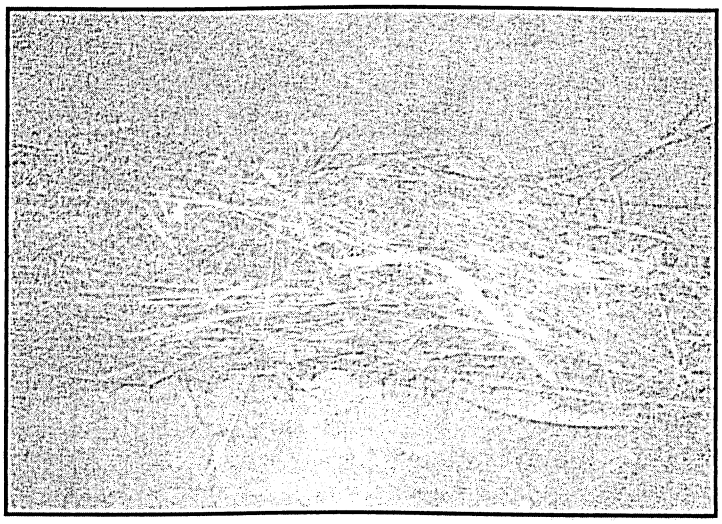

Fig. Whole dried chirata plant

The aqueous extract was prepared according to method described by Mukherjee *et al.* (1997) with slight modification. The whole plant (*Swertia chirata*) was dried, cut into small pieces with secateur and grinded into powder. Each 100 g portions of powdered *Swertia chirata* sample was soaked with 200 mL distilled water and kept for two days with occasional stirring and then filtered. Three portions of filtrates were mixed together and evaporated to slurry in a rotary vacuum evaporator at temperature around 45°C. The slurry mass was then freeze-dried and stored in refrigerator till its application.

The hexane extract of *Swertia chirata* was prepared according to the method described by Chandrasekar *et at.* (1993) with slight modification. Four hundred grams of dried, powdered whole plant of *Swertia chirata* were divided into four 100g portions. Each 100 g portion was percolated with hexane thrice at room temperature by Soxhlet apparatus. The combined percolates were concentrated under reduced pressure below 45°C. Finally the percolate was kept under air at room temperature for complete removal of solvent leaving 3 g of residue (A). Then the defatted plant material was air-dried and each 100 g portion was soaked overnight

with 200 mL redistilled ethanol. After filtration the residue was re-extracted twice with 100 ml of ethanol each time. Three filtrate portions were mixed together and evaporated in a rotary vacuum evaporator at temperature around 45°C. The concentrated slurry was then freeze dried. After freeze drying the crude concentrate was extracted thrice with hexane at room temperature and the combined hexane extracts were kept in air at room temperature for removal of all solvent leaving 500 mg residue (B).

Daonil®, manufactured by Aventis Hoechst Marion Roussel Ltd. containing 5 mg glibenclamide per tablet, was purchased from the local market and dissolved in distilled water to obtain the desired concentration. Glibenclamide served as an antihyperglycemic reference standard drug.

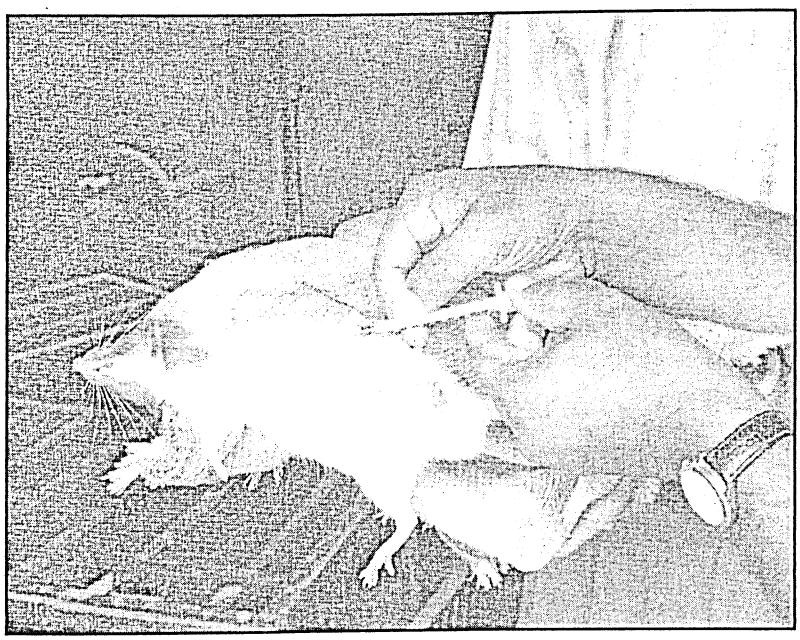

Fig. Subcutaneous injection of streptozotocin

Streptozotocin (STZ) was dissolved in 0.1 M citrate buffer having pH 4.5. A freshly prepared solution of STZ was injected subcutaneously to rats maintained fasting condition for 18 hours in a volume of one ml/kg. To induce diabetes in rat a dose of 50 mg STZ per kg of body weight was chosen following the recommendation of works done previously (Rossini *et al.*, 1977 and Szkudelski, 1998).

Seven groups of rats, aged ten to twelve weeks having five individuals in each group were acclimatized for 14 days and then starved for 18 hrs prior to treatments as follows.

Group A: Normal control
Group B: Diabetic control
Group C: STZ rats treated with aqueous extract of chirata at 75 mg/kg body wt. for 16 days
Group D: STZ rats treated with aqueous extract of chirata at 125 mg/kg body wt. for 16 days
Group E: STZ rats treated with hexane extract of chirata at 50 mg/kg body wt. for 16 days
Group F: STZ rats treated with hexane extract of chirata at 100 mg/kg body wt. for 16 days
Group G: STZ rats treated with glibenclamide at 600 µg/kg body wt. for 16 days

The normal control group was maintained on commercial pellet feed and water *ad libitum* without any treatment till the end of the experiment. The other six groups received STZ injection at 50 mg/kg body weight (Sigma Chemical Co., USA), following the fasting period to develop into diabetic. The diabetic control group did not receive any further treatment. They were fed with normal feed and water *ad libitum*. At 14 days of STZ injection, the other five groups were orally fed the extracts and glibenclamide with the help of a micropipette to ensure the administration of requisite quantity. Aqueous extracts of *Swertia chirata* at 75 and 125 mg/kg body wt. ($AqSC_{75}$ and $AqSC_{125}$), hexane extracts of *Swertia chirata* at 50 and 100 mg/kg body weight ($HeSC_{50}$ and $HeSC_{100}$) and glibenclamide at 600 µg/kg body wt. (an oral antidiabetic drug) were given orally for 16 days to STZ-induced diabetic rats. Doses of *Swertia chirata* and glibenclamide were chosen according to the works done by Saxena *et al* (1993) and Mukherjee *et al* (1997) with slight modification.

On the 16^{th} day of treatment after 2 hrs of meal, 3 to5 mL blood was directly collected from the heart of the rats under mild ether anesthesia, with the help of disposable syringe and needle (size 23). For separation of plasma, 20 mg sodium fluoride was added to and gently mixed with 2 ml of blood. This blood mixture was then centrifuged for 10 min at 2500 rpm and the supernatant carefully collected by a micropipette to an Eppendorf vial. Another 2 ml of blood was kept at normal temperature in a test tube in an inclined position. After 20 min the serum was collected with a micropipette, centrifuged for 7 min at 2500 rpm and the clear supernatant serum was collected and preserved in a freeze at 2-8°C for 2days before use.

The kits of Biosystems, Spain, were purchased to assay the concentration of the biochemical blood parameters. The kits exploited the underlying principle of determination outlined by Trinder, 1969 for glucose, by Allian *et al.*, 1974 for total cholesterol (TCh), by Fossati and Prencipe, 1982 for triacylglycerol (TAG) and by Gella *et al.* 1985 for serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) activity.

The data were expressed as Mean ± SD and subjected to complete randomized design under one way of analysis of variance (ANOVA), followed by students t-test to evaluate the significant difference.

Results and Discussion

The blood levels of glucose, TCh, TAG and SGPT and SGOT activities in normal, diabetic control and treated animals are shown in Table 1 and the per cent variation in these parameters due to different treatments in Table 2.

The normal control group had the plasma glucose level 104.04 mg/dL. All the STZ-treated groups showed significantly ($P<0.01$) higher contents of plasma glucose. The diabetic control group had a mean of 250.84 mg/dL plasma glucose. Two different doses were used for both aqueous and hexane extracts of *Swertia chirata.* $AqSC_{75}$, $AqSC_{125}$, $HeSC_{50}$ and $HeSC_{100}$ showed plasma glucose levels 205.36, 171.70, 128.56 and 147.88 mg/dL respectively. The glucose level of glibenclamide treated group ($GLB_{600\mu g}$) was 134.54 mg/dL. The aqueous extracts of chirata were found less effective compared to hexane extract with regard to its hypoglycemic effect. All the treated groups significantly ($P<0.01$) decreased plasma glucose level compared with diabetic control.

Table 1. Effects of aqueous and alcohol extracts of *Swertia chirata* and glibenclamide on some blood biochemical parameters in STZ diabetic rats

Groups	Treatment with dose	Blood parameters after 16 days of treatment				
		Plasma glucose mg/dL mean±SD	Serum total cholesterol mg/dL mean±SD	Serum triacylglycerol mg/dL mean±SD	SGPT U/L mean±SD	SGOT U/L mean±SD
A	Normal control (No treatment)	104.04±5.32f	75.48±3.35b	64.86±4.05b	9.58±2.69de	48.44±3.01c
B	Diabetic control (STZ induction at 50 mg/kg body weight)	250.84±4.80a	95.64±3.21a	80.78±3.92a	30.38±3.39a	50.30±3.51c
C	STZ rat + aqueous extract of chirata at 75 mg/kg b.w. for 16 days	205.36±3.61b	77.32±3.77b	42.36±3.55e	12.34±2.47e	34.48±2.41d
D	STZ rat + aqueous extract of chirata at 125 mg/kg b.w. for 16 days	171.70±4.50c	75.78±2.97b	47.74±3.05d	13.38±2.57cd	37.24±1.70d
E	STZ rat + hexane extract of chirata at 50 mg/kg b.w. for 16 days	128.56±4.00e	68.74±4.16c	40.45±3.50e	6.02±0.77e	24.38±3.57e
F	STZ rat + hexane extract of chirata at 100 mg/kg b.w. for 16 days	147.88±4.60d	75.82±4.07b	32.44±3.65f	17.30±2.57bc	36.76±2.27d
G	STZ rat + glibenclamide (600μg/kg b.w for 16 days)	134.54±5.11e	90.22±3.82a	53.50±2.65c	20.42±3.46b	60.30±3.47b

Values after 2 hrs of meal are given as mean ± SD for five rats in each group
Means having different superscript letters differ significantly at $P<0.01$.

Table 2. Per cent decrease in the blood biochemical parameters in STZ diabetic rats

Groups	Treatment with dose	Blood biochemical parameters				
		Plasma glucose	Serum total cholesterol	Serum triacylglycerol	SGPT	SGOT
A	Normal control (No treatment)					
B	Diabetic control (STZ induction at 50 mg/kg body weight)					
C	STZ rat + aqueous extract of chirata at 75 mg/kg b.w. for 16 days	18.13%	19.16%	47.56%	59.38%	31.45%
D	STZ rat + aqueous extract of chirata at 125 mg/kg b.w. for 16 days	31.55%	20.77%	40.90%	55.96%	25.96%
E	STZ rat + hexane extract of chirata at 50 mg/kg b.w. for 16 days	48.74%	28.17%	49.93%	80.18%	51.53%
F	STZ rat + hexane extract of chirata at 100 mg/kg b.w. for 16 days	41.05%	20.72%	59.84%	43.05%	26.92%
G	STZ rat + glibenclamide (600μg/kg b.w for 16 days)	46.36%	5.67%	33.77%	32.78%	*19.88%

* The only value which was increased

The TCh of normal control and diabetic control group was 75.48 and 95.64 mg/dL respectively. Aqueous extracts of chirata at 75 and 125 mg/kg body wt. ($AqSC_{75}$ and $AqSC_{125}$), hexane extracts of chirata at 50 and 100 mg/kg body wt. ($HeSC_{50}$ and $HeSC_{100}$) showed considerable cholesterol lowering effect and the effects were 27.94, 19.16, 20.77, 28.17 and 20.72% lower respectively in comparison with that in the diabetic control. Glibenclamide ($GLB_{600\mu g}$) had virtually no cholesterol reducing effect.

There was a significant ($p<0.01$) rise in the serum TAG in the diabetic control group (80.78 ±3.92 mg/dL) compared with that in the normal control group (64.86 ± 4.05 mg/dL). Hypertriglyceridemia is one of the risk factors in coronary heart disease (CHD). Glibenclamide ($GLB_{600\mu g}$) as well as aqueous and hexane extracts of *Swertia chirata* ($AqSC_{75}$, $AqSC_{125}$, $HeSC_{50}$ and $HeSC_{100}$) decreased serum TAG levels significantly ($P<0.01$) in comparison with that in the diabetic control and may presumably prevent the progression of CHD. Hexane extract of *Swertia chirata* at 100 mg/kg body wt. ($HeSC_{100}$) showed highest reducing effect in serum TAG levels.

In this study the diabetic control group (30.38 ± 3.39 U/L) showed significantly ($p<0.01$) much higher SGPT activity compared with the normal control group (9.58 ± 2.69 U/L). In STZ-rats, a fall in SGPT levels of 59.38, 55.96, 80.18, 43.05 and 32.78% was observed in groups treated with aqueous extracts of *Swertia chirata* at 75 and 125 mg/kg body wt. ($AqSC_{75}$ and $AqSC_{125}$), hexane extracts of *Swertia chirata* at 50 and 100 mg/kg body wt. ($HeSC_{50}$ and $HeSC_{100}$) and glibenclamide ($GLB_{600\mu g}$) respectively compared with diabetic control group. Hexane extract of *Swertia chirata* at 50 mg/kg body wt. ($HeSC_{50}$) treated groups reduced the SGPT level compared to diabetic as well as normal control groups. SGOT activities in normal control and in diabetic control were 48.44 ± 3.01 and 50.30 ± 3.51 U/L respectively. Aqueous extracts of *Swertia chirata* at 75 and 125 mg/kg body wt. ($AqSC_{75}$ and $AqSC_{125}$) and hexane extracts of *Swertia chirata* at 50 and 100 mg/kg body wt. ($HeSC_{50}$ and $HeSC_{100}$) decreased SGOT levels by 44.70, 59.38, 55.96, 80.18% respectively compared with diabetic control group. The result shows that glibenclamide ($GLB_{600\mu g}$) increased SGOT levels by 19.88% compared with diabetic control group.

Chirata (*Swertia chirata*) contains swerchirin, which is a potent antihyperglycemic compound (Bajpai *et al.* 1991). It is probable that hexane fraction of chirata contains more active compounds which showed hypoglycemic effect. Hexane fraction of chirata induced a significant fall in blood glucose in STZ-rats compared to that of aqueous extracts of chirata. Hexane fraction of chirata at the rate of 50 mg/kg body wt. was found better dose. Similar result was reported by Saxena *et al.* (1993).

Although different extracts of *Swertia chirata* apparently reduced STZ-induced hyperglycemia compared with diabetic control, the levels were found far above the normal control. This reflects that STZ induced hyperglycemia could not be fully restored by the plant extracts ($AqSC_{75}$, $AqSC_{125}$, $HeSC_{50}$, and $HeSC_{100}$) and glibenclamide, an antidiabetic drug.

It was possible that in STZ rats a large amount of fat was burnt to supply energy in absence of glucose utilization. The amount of acetyl-CoA, thus formed from the oxidation of fatty acids, might have exceeded the capacity of Krebs cycle to oxidize it. The excess acetyl-CoA was likely to be converted to ketone bodies and cholesterol. This is possibly why diabetogenic STZ produced more cholesterol than that in the control (Abrams *et al* 1982). This condition of apparent hypercholesterolemia was relieved by different extracts of *Swertia chirata* but glibenclamide could not reduce the elevated cholesterol levels appreciably in diabetic rats.

Recently it is reported that elevated TAG is associated with type II diabetes, which is also evidenced from the present study showing higher serum TAG among the rats of diabetic control. The biochemical reason for TAG elevation in diabetic mellitus due to increased circulating free fatty acids which are the main source of TAG because lipogenesis from acetyl-CoA is depressed under this condition (Murray *et al*, 2000). It is worthy to note here that different extracts of *Swertia chirata* showed efficacy as that of glibenclamide in lowering TAG in the sera of diabetic rats.

Assay of serum glutamate pyruvate transaminase (SGPT) and serum glutamate oxaloacetate transaminase (SGOT) can provide the physician with valuable diagnostic and prognostic clinical evidence of acute hepatic and cardiac disease (Martin *et al.*1985 and Ohlson *et al.*1988). The much higher SGPT activity in the diabetic control compared with the normal control observed in this study complies with that of Ohlson *et al.* 1988, who found a close association between SGPT activity and type II diabetes. The higher SGOT activity due to glibenclamide itself is difficult to explain because these treatments efficiently lowered blood glucose level in the diabetic rats. The significantly much higher SGPT activity without any effect on SGOT activity in the diabetic control in relation to normal control group suggests that rat liver and not the heart might have been affected by STZ induction. However, higher SGOT activity is reported to be associated with later development of diabetes (Perry *et al.*, 1998).

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