Journal of Pharmaceutical Research International

22(5): 1-11, 2018; Article no.JPRI.41878
ISSN: 2456-9119
(Past name: British Journal of Pharmaceutical Research, Past ISSN: 2231-2919, NLM ID: 101631759)

Antihyperglycemic Activity of *Swertia chirata* on nSTZ-T2DM Rats: A Chronic Study

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Authors' contributions

This work was carried out in collaboration between all authors. Author AB designed the proposal and protocol, performed the experiments, collection and assembly of data and analysis and wrote the first draft of manuscript. Author MM gave financial and logistic supports. Authors MM, BR and YK designed the protocol. Author BR corrected the protocol, managed the experiments and revised the manuscript. All authors approved the manuscript.

Article Information

DOI: 10.9734/JPRI/2018/41878
Editor(s):
(1) Salvatore Chirumbolo, Clinical Biochemist, Department of Medicine, University of Verona, Italy.
Reviewers:
(1) Mohamed Ahmed Mohamed Nagy Mohamed, Egypt.
(2) Dennis Amaechi, Veritas University, Nigeria.
Complete Peer review History: http://www.sciencedomain.org/review-history/25088

Original Research Article

Received 4th March 2018
Accepted 16th May 2018
Published 11th June 2018

ABSTRACT

Aim: The present study aimed to investigate antidiabetic effects and to explore the underlying mechanism of *S. chirata* on neonatal-streptozotocin induced type 2 diabetic model (nSTZ-T2DM) rats.

Methodology: Stem-barks were collected from local market, identified from Bangladesh National Herbarium; 96% ethanol extract and overnight soaked water solution were prepared. T2DM was induced by a single *ip* injection of STZ to 48 hours Long Evans neonatal pups. After 3 months, T2DM adult rats (confirmed by OGTT) were divided into 4 groups: i) water control; ii) Glibenclamide (5 mg/kg bw); iii) soaked water (SCWS, 0.25 g/10 ml/kg bw) and iv) ethanol extract (SCE, 0.25 g/kg bw)treated groups respectively. Blood was collected by cutting tail tip on 0 and 21 day; by cardiac

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puncture on 28 day for measuring biochemical parameters. The data were analyzed using univariate & multivariate tools.
Results: SCE (p=0.003) and SCWS (p<0.001) treated group significantly improved OGTT of T2DM rats after three weeks administration. A significant decrease of fasting glucose level (p=0.01) and HOMA IR (p=0.01) were noticed in SCWS treated group after 28th day in comparison to initial day value respectively. Cholesterol was significantly decreased in SCWS on 28th day (p=0.02 and p=0.03) in comparison to water control and initial day respectively. Triglycerides was decreased in both treated groups and LDL level decreased by 42% in SCWS group respectively. Hepatic glycogen content of SCE was significantly increased (p=0.05) compared to water control. Serum ALT and creatinine level almost remained unchanged. A gradual fall of glucose absorption in SCE-krebs-glucose solution through GIT after 30 minutes was shown in Gut Perfusion Technique. **Conclusion:** *S. chirata* stem-bark possesses significant antihyperglycemic activity in T2DM rats which may be improved glucose tolerance, increased glycogenesis, decreased insulin resistance and total cholesterol level that may provide a rationale for using it in diabetic treatment.

Keywords: S. chirata; antihyperglycemic; STZ; GI Tract; T2DM.

ABBREVIATIONS

BIRDEM= Bangladesh Institute of Research and Rehabilitation in Diabetes; Endocrine and Metabolic Disorders; DM =Diabetes Mellitus; FSG= Fasting serum glucose; GIT= Gastrointestinal tract; GOD-PAP = Glucose Oxidase; GSH = Reduced gluthathion; HDL= High density lipoprotein; HOMA B%= B cell secretion; HOMA IR= Insulin Resistance index; HOMA S%= insulin sensitivity; i.p = intraperitonial; LDL= Low density lipoprotein; MDA = Malondialdehyde; nSTZ = neonatal-streptozotocin; OGTT = Oral Glucose Tolerance Test; TBARS = Thiobarbituric acid reactive substances; TG = Triglycerides.

1. INTRODUCTION

Diabetes Mellitus is a clinical syndrome characterized by hyperglycemia caused by a relative or absolute deficiency of insulin at the cellular level. It is the most common endocrine disorder affecting mankind all over the world, prevalence of which is increasing day by day [1]. Traditional preparations from plant sources are widely used almost everywhere in the world to treat this disease. Therefore, plant materials are considered to be the alternative sources for finding out new leads for hypoglycemic agents. A total of more than 400 species were reported to display hypoglycemic effects, but few of them have been investigated scientifically [2]. The plant product undertaken in this study for anti-diabetic effect was *S. chirata* known as Chirota available in South Asian countries and much in Indian subcontinent. S. *chirata* belongs to the family Gentianaceae [3,4]. It can be traced through the medicinal history as a nontoxic and safe ethnomedicinal herb utilized for its bitter bioactive compounds [5]. The chemical constituents of *S. chirata* include secoiridoid bitters, alkaloids, xanthones and triterpenoids [6, 7,8]. Amarogentin, amaroswerin, gentiopicroside and swertiamarin are the reported bitter secoiridoid glycosides of the plant [9-12]. A xanthone rich extract of this plant has shown significant anti-inflammatory, anti-platelet, anti-cancer, CNS stimulant, anti-fungal and antimalarial effects [13]. Swerchirin, a xanthone from S. *chirata* is a potent hypoglycaemic agent [14-17]. Mukherjee et al. reported significant blood sugar lowering effects of the 95% ethanolic extract of *S chirata* in fed, fasted and glucose-loaded albino rats [18]. The hypoglycaemic activity of tolbutamide was increased in healthy albino rats by giving S *chirata* extract orally [19]. Antidiabetic activity of swerchirin isolated from hexane fraction of *S. chirata*has been reported by Bajpai et al. [20]. Except in rats with severe pancreatic damage, swerchinin showed better glucose lowering effect compared to tolbutamide [20-22].

Traditional healers in Bangladesh use most commonly Stem-bark of *S. chirata* for antidiabetic activity [23,24]. Although a huge number of publications have been done with *S. chirata,* still the probable mechanisms of its antidiabetic activity remained unclear. For this reason, we have tried to investigate some probable mechanisms of antidiabetic activity of *S. chirata*. This study evaluated the glycemic, insulinemic, lipidemic and antioxidant properties of *S. chirata* stem-bark in nSTZ-diabetic rats. Gut perfusion

technique was also performed to study the upper intestinal glucose absorption by *S. chirata* stem-bark.

2. MATERIALS AND METHODS

2.1 Place of Study

The study was conducted in the Department of Pharmacology in Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) and Bangladesh University of Health Sciences (BUHS).

2.2 Plant Material

In this study, *S. chirata* stem-barkwas used. The stems were collected from the commercially available sources and identified by the Bangladesh National Herbarium, Dhaka (DACB Accession no 37789).

2.3 Preparation of *S. chirata* Extracts

After collection, *S. chirata* stem-barks were washed by fresh water thoroughly and sun dried in laboratory. The stem-barks were prepared for two types of extracts: 1) ethanolic extract and 2) water soaked solution. For preparation of ethanolic extract, stem-barks were grinded to make fine powder by a grinding machine. The grinded powder was extracted by dissolving overnight absolute (96%) ethanolic solvent at -8°C. Following the completion of extraction, extract prepared from stem-barks was concentrated under reduced pressure using a rotary evaporator (BUCHI R-114, Switzerland) maintained at 55°C. The semi-dried ethanolic extract was further dried in a freeze drier (HETOSICC, Heto Lab Equipment, Denmark) at -55°C and stored in a reagent bottle at -8°C in a refrigerator for future use. The soaked water solution of *S. chirata* was prepared dissolving by overnightdistilled water.

2.4 Animals

The Long Evans rats bred at Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders(BIRDEM) animal house, were used in this study. The animals were maintained at a constant room temperature of 23°C with humidity of 40-70% and the natural 12 hours day-night cycle. The rats were fed on a standard laboratory pellet diet and water supplied *ad libitum*. The experiments were conducted according to the ethical guidelines approved by Bangladesh Association for Laboratory Animal Science.

2.5 Preparation of Type 2 Diabetes Model Rats

Diabetes was induced by a single *intraperitoneal* injection of streptozotocin (STZ) at a dose of 90 mg/kg body weight to the neonate rats (48 hours old) as described by Bonner-Weir et al. [25]. Following 3 months of STZ injection, rats were examined for their blood glucose level by oral glucose tolerance test (OGTT, Glucose 2.5 g/kg bw). Diabetic model rats in body weight 150-200 gm with blood glucose level >7.00 mmol/l, at fasting condition was selected for studying the effects of the extracts in chronic studies.

2.6 Experimental Groups of T2DM Rats

The chronic experiment was carried out for duration of 28 days on 48 rats. Type 2 rats were divided into four different groups. Those were followed as:

1) **Water Control group** (n = 7): Treated with deionized water at a dose of 10 ml/kg body weight (bw).
2) **Glibenclamide (positive) control group** (n = 7): Treated with glibenclamide at a dose of 5 mg//kg bw [26].
3) **Soaked water treated group SCWS** (n = 8)**:** Treated with 0.25 g stems overnight soaked within distill water at a dose of 0.25 mg/ 10 ml/ kg bw.
4) **Extracttreated group SCE**(n = 8)**:** Fed with ethanol extract at a dose of 0.25 mg /kg bw.

Water, Glibenclamide, soaked water solution and ethanol extract were administered intragastrically through metallic tubes to the corresponding group of rats after 12 hrs fast.

2.7 Collection of Blood Sample for Biochemical Analysis

Blood samples were collected from rats kept under fasting conditions (12 hours) by amputation of the tail tip under diethyl ether anesthesia on the 0 day and 21st day. On the 28th day, blood was collected from the rats by cardiac puncture (diethyl ether anesthesia). The collected blood samples were centrifuged at 2,500 rpm for 15 minutes and finally the serums were separated into another eppendorf tubes

for biochemical analysis. Two (2) mL of blood was collected in heparinized tubes and then packed red cells were used for estimation of Malondialdehyde (MDA) and reduced Glutathione (GSH).

2.8 Effects on Intestinal Glucose Absorption

An intestinal perfusion technique [27] was used to study the effects of extract on intestinal absorption of glucose in type 2 diabetic rats fasted for 36 hours and anesthetized with sodium pentobarbital (50 mg/kg). The plant extract was added to a kreb's solution (g/L 1.02 $CaCl_2$, 7.37 NaCl, 0.20 KCl, 0.065 $NaH_2PO4.6H_2O$, 0.6 $NaHCO_3$, pH 7.4), supplemented with glucose (54.0 g/L) and perfused at a perfusion rate of 0.5 mL/min for 30 min through the duodenum. The perfusate was collected from a catheter set at 40 cm. Both Extracts were added to Kreb's solution to final conc. of 25 mg/mL so that the amount of extract in the perfused intestine is equivalent to the dose of 1.25 g/kg. The control group was perfused only with Kreb's buffer supplemented with glucose. The results were expressed as percentage of absorbed glucose, calculated from the amount of glucose in solution before and after the perfusion.

2.9 Biochemical Analysis

Serum glucose was measured by Glucose Oxidase (GOD-PAP) method using micro-plate reader (Bio-Tec, ELISA); total cholesterol and Triglyceride (TG) by enzymatic colorimetric method (Randox Laboratories Ltd., UK), using autoanalyzer. LDL-cholesterol was calculated by Friedewald equation [28]. Serum insulin was estimated by ELISA (Crystal Chem Inc., USA); and HOMA B% (Beta-cell function) and HOMA S% (Insulin sensitivity) were calculation by HOMA SIGMA Software [29]. HOMA IR (Insulin Resistance Index) was calculated by International Formula: fasting Glucose (mmol/L) × fasting Insulin (mU/L)/22.5. Serum Creatinine and Amino Alanine Transferase (ALT) by Auto-analyzer. Hepatic glycogen was measured by Anthrone-sulphuric acid method. Reduced Glutathione (GSH) and plasma Malondialdehyde (MDA) estimated by using Ellman's [30] and Thiobarbituric Acid Reactive Substances (TBARS) method respectively [31].

2.10 Statistical Analysis

Data from the experiments were analyzed using the Statistical Package for Social Science (SPSS) software for windows version 16 (SPSS Inc., Chicago, Illinois, USA).All the data were expressed as Mean ± SD or as Median (Range) as appropriate. Statistical analysis of the results was performed by using the student's t-test (paired and unpaired), ANOVA (analysis of variance) followed by Bonferroni post hoc test and Mann Whitney (u) test. The limit of significance was set at $p<0.05$.

3. RESULTS

3.1 Effect on the Body Weight of T2DM Rats

Body weight of each rat was taken at seven days interval. As shown in Fig. 1 the gradual increase ofbody weight was observed after 28 days in all of the groups i.e. water control, glibenclamide and both treated groups.

3.2 Effects on Glucose Homeostasis

Effect of oral administration of *S. chirata* extracts on type 2 model rats on 30 min before with glucose load were observed on the 0 day and 21st day at four consecutive times (60, 90 and 120 min) respectively.On initial day, no significant change was found in any of the treated groups.

On 21st day, glucose level was lowered in SCWS treated group when it compared among groups (p=ns) at 60 min (Fig. 2). At 90 min after glucose load, almost similar rise in serum glucose level was found in glibenclamide and SCE treated groups, and a significantly reduced glucose was found in SCWS group in comparison to water control ($p=0.002$). Moreover, at 120 min the glucose level decreased in all treated group compared to 90 minutes value; and a significant decreased were found in SCWS ($p<0.001$) and SCE ($p=0.003$) treated groups when compared with water control respectively. The positive control glibenclamide showed a significant ($p=0.03$) decreased of glucose level at 120 min in comparison to water control, as expected.

Fasting serum glucose (FSG) levels of type 2 diabetic models rats for experimental groups were almost similar on 0 day (Table 1). After oral administration of respective treatment to T2DM rat of different groups for 28 days of experimental period, it was found that FSG level of all the groups of rats decreased on final day except water control. However, type 2 rats treated with

SCWS (p=0.01) showed a significant decreased when compared within groups. As expected, glibenclamide also ameliorated the diabetic condition on 28th day. There was 17% deceased level was found in SCE group on final day in comparison to baseline value.

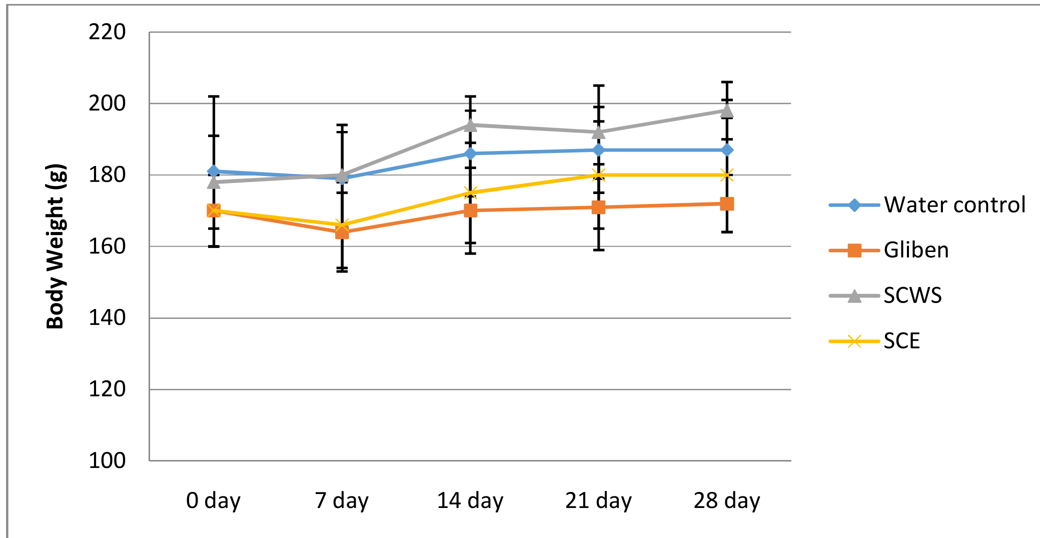

Fig. 1. Effect of *S. chirata* extract on the body weight of type 2 diabetic model rats

Results are expressed as Mean ±SD. Statistical analysis between group comparison was done by using one way ANOVA with post hoc Bonferroni test and Within groups, comparison was done using paired t test. WC = Water Control; Gliben = Glibenclamide treated group; SCWS = S. chirata water soaked group; SCE =S. chirata ethanol extract.

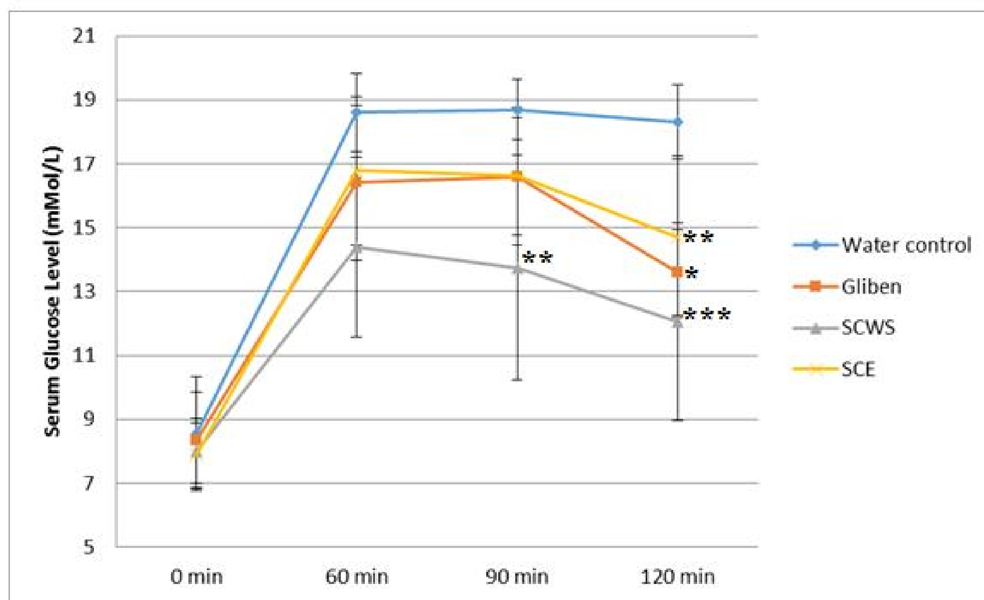

Fig. 2. Effect of *S. chirata* extracts on the blood glucose levels of T2DM rats in 30 min before with glucose load on 21st day

*Results are expressed as Mean ±SD. Statistical analysis between group comparison was done by using one way ANOVA with post hoc Bonferroni test and Within groups, comparison was done using paired t test. . WC = Water Control; Gliben = Glibenclamide treated group; SCWS = S. chirata water soaked group; SCE = S. chirata ethanol extract. *= p<0.05; **= p<0.005 ***= p<0.001*

Table 1. Chronic effects of *S. chirata* extracts on fasting serumglucose (FSG) level of STZ-inducedT2DM rats

Groups	Glucose (mmol/l)	
	0 day	28th day
WC (n=7)	8.28±1.62 (100%)	8.27±1.91 (100%)
Gliben (n=7)	9.21±1.85 (100%)	8.46±1.62 (92%)
SCWS (n=8)	9.24±0.92 (100%)	7.11±1.11* (76%)
SCE (n=8)	9.52±1.76 (100%)	7.93±0.94 (83%)

*Results are expressed as Mean±SD (percentage). Between groups, comparison was done using one-way ANOVA with post hoc Bonferroni test and Within groups, comparison was done using paired t test. *p=0.01, WC = Water Control; Gliben = Glibenclamide treated group; SCWS = S. chirata water soaked group; SCE =S. chirata ethanol extract.*

3.3 Chronic effect of *S. chirata* extracts on β-cell function (HOMA B%), insulin sensitivity (HOMA S%) and insulin resistance HOMA IR

There were no significant changed of HOMA S% and HOMA B% in SCWS and SCE treated group after 28 days in comparison to baseline value. Insulin resistance index HOMA IR decreased by 30%, 72%, and 77% in glibenclamide, SCWS and SCE treated groups on 28th day in comparison to 0 day (mean of baseline) value respectively. But the decreased was significant (p=0.01) only in SCWS group on 28th day when it compared with baseline values. But the decreased was significant (p=0.01) only for SCWS group.

3.4 Effect of *S. chirata* on the Serum Lipid Profiles of T2DM Rats

Chronic effects of *S. chirata* on serum lipid profiles were presented in Table 3. Treatment of diabetic model rats for 28 days with *SCWS* (p=0.02) and *SC*E (p=0.01) showed a significant decrease in serum cholesterol level when it compared with water control value respectively. In comparison to initial day, total cholesterol level was also decreased in SCWS group (p=0.03) on 28th day significantly. The triglyceride levels were decreased by 13% in *SC*E group and LDL level deceased by 42% in *SCWS* treated rats on final day in comparison to baseline value (p=ns) respectively. Besides these all groups do not have any changes in the serum HDL- and LDL-cholesterol levels on final day.

3.5 Effect on Liver and Kidney Function

As shown Table 4, the serum ALT level was decreased by 18% and 8% in SCWS and SCE treated group respectively on final day in comparison to initial day, which was not statistically significant and serum creatinine level almost remained the same for all groups on final day.

Table 2. Effect of *S. chirata* extracts on HOMA B%, HOMA S% and HOMA IR of STZ-induced T2DM rats

Groups	HOMA B%		HOMA S%		HOMA IR	
	0 day	28 day	0 day	28 day	0 day	28 day
WC (n=7)	48.02±21.22 (100%)	49.26±19.10 (102%)	121.09±99.72 (100%)	128.27±118.00 (105%)	4.7±3.5 (100%)	10.9±13.9 (231%)
Gliben (n=7)	39.98±14.73 (100%)	42.23±22.69 (105%)	92.41±85.59 (100%)	106.05±79.11 (111%)	5.5±3.4 (100%)	3.9±2.8 (70%)
***SCWS* (n=8)**	56.94±22.68 (100%)	43.00±26.07 (76%)	42.50±18.71 (100%)	162.63±80.92 (387%)	8.2±4.0 (100%)	2.3±2.8 (28%) **p=0.01**
***SCE* (n=8)**	40.24±13.00 (100%)	30.97±13.56 (77%)	78.40±65.91 (100%)	164.95±69.41 (211%)	6.4±4.8 (100%)	2.1±1.8 (33%) p=0.08

Results are expressed as Mean±SD (percentage). Between groups, comparison was done using one way ANOVA with post hoc Bonferroni p value and Within groups, comparison was done using paired t test. WC = Water Control; Gliben = Glibenclamide treated group; SCWS = S. chirata water soaked group; SCE =S. chirata ethanol extract.

Table 3. Effect of *S. chirata* extracts on lipid profiles of STZ-induced T2DM rats

Groups	Chol(mg/dL)		TG (mg/dL)		HDL (mg/dL)		LDL (mg/dL)	
	0 day	28 day	0 day	28 day	0 day	28 day	0 day	28 day
WC (n=7)	67±7 (100%)	72±5 (107%)	83±11 (100%)	89±14 (107%)	43±2 (100%)	46±5 (106%)	7±6 (100%)	8±7 (114%)
Gliben (n=7)	61±5 (100%)	61±5 (100%)	72±16 (100%)	71±13 (98%)	39±6 (100%)	40±6 (102%)	7±7 (100%)	10±5 (142%)
***SCWS* (n=8)**	68±9 (100%)	61±5 (89%) p =**0.02a** **p= 0.03c**	68±11 (100%)	66±18 (97%)	41±5 (100%)	41±6 (100%)	12±7 (100%)	7±9 **(58%)**
***SCE* (n=8)**	62±6 (100%)	59±6 (95%) p= **0.01b**	86±21 (100%)	75±17 (87%)	39±4 (100%)	38±5 (97%)	5±4 (100%)	9±8 (180%)

Results are expressed as Mean±SD (percentage). Between groups, comparison was done using one way ANOVA with post hoc Bonferroni p value and Within groups, comparison was done using paired t test. WC = Water Control; Gliben = Glibenclamide treated group; SCWS = S. chirata water soaked group; SCE =S. chirata ethanol extract. a= WC Vs SCWS, b = WC Vs SCE, c= 0 day vs 28 day

Table 4. Effect on liver and Kidney function of STZ-induced T2DM rats

Groups	ALT (U/L)		S Creatinine (mg/dL)	
	0 day	28 day	0 day	28 day
WC (n=7)	64±37 (100%)	91±51 (142%)	0.77±0.11	0.79±0.09
Gliben (n=7)	62±22 (100%)	93±26 (147%)	0.73±0.14	0.83±0.18
SCWS (n=8)	74±29 (100%)	61±13 **(82%)**	0.71±0.04	0.75±0.08
***SCE* (n=8)**	76±25 (100%)	70±21 **(92%)**	0.73±0.07	0.71±0.08

Results are expressed as Mean±SD (percentage). Between groups, comparison was done using one-way ANOVA with post hoc Bonferroni test. Within groups, comparison was done using paired t test. WC = Water Control; Gliben = Glibenclamide treated group; SCWS = S. chirata water soaked group; SCE =S. chirata ethanol extract.

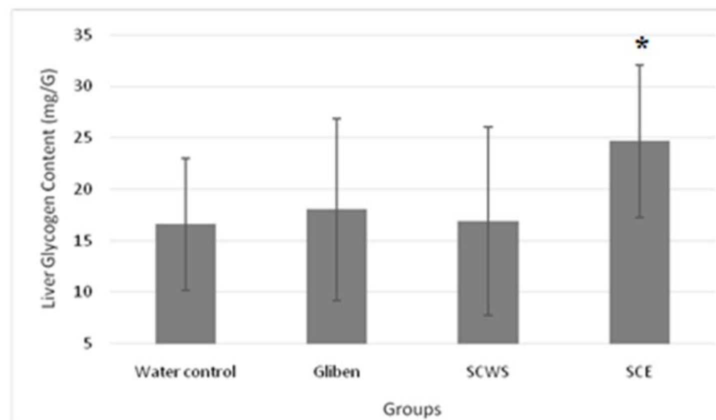

Fig. 3. Effect of *S. chirata* extracts on hepatic glycogen content of STZ-induced T2DM rats

Results are expressed as Mean ± SD. Statistical analysis between group comparison was done by using Independent Sample T Test. WC = Water Control; Gliben = Glibenclamide treated group; SCWS = S. chirata water soaked group; SCE =S. chirata ethanol extract

3.6 Effect on Hepatic Glycogen Content of T2DM Rats

The Fig. 3 showed that there were no significant changed in hepatic glycogen content on glibenclamide and SCWS treated group; but 48% increased was shown in SCE treated group after 28th day of chronic oral administration when it compared to water control, which was statistically significant (p=0.05).

3.7 Investigation of Antioxidant Activities

Table 5shows the concentration of erythrocyte lipid peroxidation products i.e. malondialdehyde (MDA) and reduced Glutathione (GSH) in different groups of rats after 28 days of the study period. The levels of erythrocyte MDA was lower by 10% in SCWS treated groups in comparison to water control group and GSH level did not showed any change in treated group respectively.

Table 5. Effects of *S. chirata* extracts on serum malondialdehyde (MDA) and reduced glutathione (GSH) of STZ-induced T2DM rats

Groups	MDA (μmol/ml)	GSH (mg/g Hb)
WC (n=7)	1.74±0.10 (100%)	16.37±4.32 (100%)
Gliben (n=7)	1.78±0.37 (102%)	16.52±6.40 (100%)
***SCWS* (n=8)**	1.57±0.81 (90%)	14.75±8.39 (90%)
***SCE* (n=8)**	1.87±0.95 (107%)	15.11±8.77 (92%)

Results are expressed as Mean ±SD (percentage). Statistical analysis between group comparison was done by using Independent Sample T Test. WC = Water Control; Gliben = Glibenclamide treated group; SCWS = S. chirata water soaked group; SCE =S. chirata ethanol extract.

3.8 Effect of *S. chirata* on Upper Intestinal Glucose Absorption

As shown in Fig. 4, the upper intestinal glucose absorption was almost constant during 30 minutes of perfusion with glucose. When the *S. chirata* extract and soaked water solution were supplemented with the glucose solution, type 2 model rats showed decrease in intestinal glucose absorption almost constantly during 30 min of perfusion.

SCWS and SCE strongly affected the amount of absorbed glucose throughout the notable period of experiment in type 2 rats. Fig. 4 depicts the gradual fall (p=ns) in glucose absorption during the whole perfusion period in type 2 rats compared to Krebs solution only as control. Therefore, the obtained results suggest that both extract delays glucose absorption in the upper part of the gastrointestinal tract.

4. DISCUSSION

The present study was undertaken to assess the antidiabetic effect after chronic administration of *S. chirata* extract on T2DM rats and explore the mode of antidiabetic action. At the beginning (0 day) extracts of *S. chirata* non-significantly opposed the rise of postprandial serum glucose level when fed 30 minutes before glucose load. When OGTT was examined again at the 21st day after chronic feeding of SCE and SCWS,produced a significant antihyperglycemic effect in T2DM rats. Observed glucose lowering effect in T2DM rats after oral administration of extract of *S. chirata* with a simultaneous glucose load, indicated that *S. chirata* may interfere with the intestinal glucose absorption in the gut. It may also act by modifying the peripheral glucose uptake and probably increasing insulin sensitivity [32]. Our 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 extracts 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 28 days of consecutive feeding, when the rats were sacrificed, a significant reduction in the fasting glucose level was observed in SCWS (p=0.01) fed group compared with baseline day and SCE group was decreased by 17% respectively. In this experiment, glibenclamide treated group (positive control) also decreased fasting blood glucose level after chronic feeding. This obtained result is supported by the finding of other investigators [14-16].

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 control and treated (extract and glibenclamide) groups. The tendencies were of similar proportion in the control and treated groups; and thus, they do not responsible for the hypoglycemic effect found in the extract group. The findings also suggest that

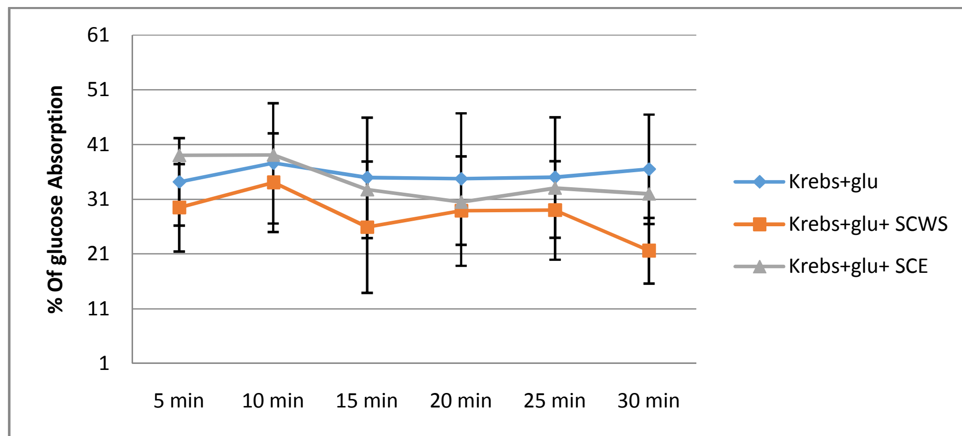

Fig. 4. Effects of *S. chirata* soaked water solution and extract on upper intestinal glucose absorption on STZ-induced T2DM rats

Results are presented as mean± SD (n=6). Between groups, comparison was done using one-way ANOVA with post hoc Bonferroni test. Within groups, comparison was done using paired t test. Rats were fasted for 36 hours and intestine was perfused with glucose solution (54 g/l) with or without SCWS/SCE (0.25mg/ml); K-g=Kreb's solution +glucose; K-g-SCWS= Kreb's solution + glucose + water shocked solution of S. chirata, K-g-SCE= Kreb's solution + glucose + extract solution of S. chirata.

S. chirata extract does not alter normal metabolic parameters like food and water intake.

A significant decrease of insulin resistance index (HOMA IR) was shown in SCWS (p=0.01) after 28th day in comparison to initial day and SCE group was decreased (p=ns) by 77% respectively. The result indicates that the extracts affect the β-cell of pancreas directly for secretion of insulin by decreasing insulin resistance. Again, in our experiment, *S. chirata* extract showed a significantly higher (p=0.05) glycogen content in the liver (Fig. 3). Synthesis of liver glycogen may be due to increased glycogen sythase activity. The levels of erythrocyte MDA was lowered by 10% in SCWS treated groups in comparison with water control (Table 5), which indicates that SCWS has antioxidant property. The extract may also improve insulin sensitivity by reducing glucotoxicity which is one of the causes of insulin resistance in type2 rats [33].

Apart from the blood sugar lowering effect, beneficial changes in lipid profile was also observed by *S. chirata* extract. It has been demonstrated that postprandial hyperglycemia is an important cardiovascular risk factors in Type 2 diabetic patients [34]. It has been claimed that hypercholesterolemia and hypertriglyceridaemia occurred in STZ induced diabetic rats [35]. Abnormalities in lipid profiles are one of the most common complications in diabetes mellitus. 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. In this study, *SCWS* (p=0.02) and SCE (p=0.01) groups significantly decreased serum total cholesterol level. Regarding triglyceride level, it was decreased by 13% in SCE group and LDL level was decreased by 42% in SCWS group on final day in comparison to initial day. After chronic administration there was no significant change in serum SGPT and creatinine level which indicates that there was no toxic effect in the liver or in the kidney by the extracts of *S. chirata.*

In gut perfusion *in situ* experiment where both extract of *S. chirata* showed an inhibition of glucose absorption. The inhibition of intestinal glucose absorption may contribute to the reduction of postprandial glucose level which was observed in this study.

5. CONCLUSION

S. chirata extracts has potential antihyperglycemic effect on T2D model rats. The underlying mechanism may be at least partly due to improved glucose tolerance, increased glycogenesis, decreased insulin resistance and improvement in lipid profile. Therefore, treatment with *S. chirata* provides a rationale for its use in diabetic treatment.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard, written approval of Ethics committee has been collected and preserved by the authors.

ACKNOWLEDGEMENTS

We gratefully acknowledge the financial support of International Program for the Chemical Sciences (IPICS), Uppsala, Sweden; and Asian Network of Research of Antidiabetic plants (ANRAP) in conducting this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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http://www.sciencedomain.org/review-history/25088