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Evaluation of antidiabetic and antioxidant effect of *Schrebera swietenioides* fruit ethenolic extract

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ABSTRACT

Herbal medicines provide rational means for the treatment of many diseases that are obstinate and incurable in other systems of medicine. The aim of the study is the systematic screening of Schrebera swietenioides fruit ethenolic extract with the purpose of discovering new bioactive compounds for diabetes mellitus and to establish the scientific basis for the therapeutic actions of traditional plant medicines. In present study Schrebera swietenioides fruit ethenolic extract was studied for the acute and sub acute effects on alloxan induced diabetic rats. This extract was investigated for the antihyperglycemic action by using glucose hyperglycemic rats. Blood glucose levels and Serum lipid profiles were measured. The whole pancreas from each animal was removed after sacrificing the animal and subjected for histological examination. This extract's DPPH radical scavenging potential was also studied. In OGTT, reduction of fasting blood glucose levels took place from 60 min of extracts administration. In acute study, blood glucose lowering potential percentage of Schrebera swietenioides fruit ethenolic extract was 14 % at 6 hr after extract administration and in sub acute study, after 15 days of treatment it was 42 % when compared with diabetic rats. The ethenolic fruit extract of Schrebera swietenioides Linn. showed better activity in quenching DPPH radical with an IC_{50} value of 423 µg /ml when compared with Standard BHT(Butylated hydroxytolune) IC_{50} value of 107µg /ml.

Keywords: *Schrebera swietenioides*; Antidiabetic; Antioxidant; Alloxan induced diabetic rats; Hypoglycemic.

INTRODUCTION

Diabetes mellitus is a disease characterized by glycosuria, hyperglycemia and a disturbance in carbohydrate, fat and protein metabolism and water and electrolyte balance. The World Health

Organization predicts that the number of cases world wide for diabetes is now 150 million and will be doubled in coming years (1).

Free radicals have been implicated in the causation of several diseases such as liver cirrhosis, atherosclerosis, cancer, diabetes etc. and compounds that can scavenge free radicals have great potential in ameliorating these disease processes. Antioxidants thus play an important role to protect the human body against damage by reactive oxygen species. Increased oxidative stress has been postulated in the diabetic state (2).Antioxidants have been shown to reduce the risk of diabetes onset, improve glucose disposal and improve some of the associated complications (3).

Many plant species have been utilized as traditional medicines but it is necessary to establish the scientific basis for the therapeutic actions of traditional plant medicines as these may serve as the source for the development of more effective drugs.

Schrebera swietenioides Roxb (Oleaceae) is a moderate sized deciduous tree up to 20 m in height with thick grey bark; leaves imparipinnate, leaflets opposite, 3-4 pairs, flowers yellowish brown, fruits pendulous, pear shaped, 2-valved capsules; seeds 8, ending in long wings (4).The root, bark and leaves are bitter, acrid, appetizing, digestive, constipating and anthelmintic. They are useful in flatulence, skin diseases, leprosy, diarrhea, anemia and rectal disorders. The fruit is digestive, purgative and stomachic, and is useful in flatulence, anorexia, colic and diabetes (5).

The literature screened in the process of the proposed work indicates that the selected plant contain classes of chemical constituents which have shown antidiabetic and antioxidant activities. Literature survey revealed that *Schrebera swietenioides* fruit ethenolic extract has no scientific claims for anti-diabetic and antioxidant activity. Phytochemical and pharmacological investigations of this plant may yield useful information and material for better management for preventing the production of the free radicals and diabetes.

MATERIALS AND METHODS

Animals

Healthy adult male wistar albino rats weighing between 170-200 gm were used for the antidiabetic studies, whereas wistar albino rats of either sex were used for determination of acute toxicity study. The animals were housed in groups of 5 per cage with free access to commercial rat pallet diet (Lipton India ltd., Mumbai, India) and water *ad libitum*. The animal room was maintained at 25°c ± 2°c with timed lighting on from 6 am to 6 pm and relative air humidity of 30 to 60% . The Institutional Animal Ethics Committee (CPCSEA/1/15/2007) approved the study.

Chemicals

All chemicals and solvents used were of analytical grade from Merck Ltd., Mumbai, India and Sigma Aldrich Co., USA. Glibenclamide was obtained from Hoechst India as in form of Daonil tablet.

Collection of Plant material

The fruits of *Schrebera swietenioides* Linn were collected from local areas of Kolhapur (Maharashtra) & Belgaum (Karnataka) India. The specimen was authentificated from Dr.S.R.Yadav, Prof., Dept. of Botany, Shivaji University, Kolhapur (Maharashtra) India. The voucher specimen (KLEU/Pharm/ 07/15) was retained in the Herbarium of Department of Pharmacognosy, K.L.E.University, College of Pharmacy, Belgaum (Karnataka) India.

Preparation of plant extract

The collected plant material was washed thoroughly in water, chopped, shade dried at room temperature, reduced to a coarse powder in a mechanical grinder and passed through a 40 # sieve for desired particle size. The powder obtained was subjected for the extraction, with 95% ethanol in a soxhlet apparatus. The extract was concentrated under reduced pressure and dried. The yield of *Schrebera swietenioides* fruit ethenolic extract was 4.1 % (w/w). The obtained extract was stored in a refrigerator at 2-8°c until usage.

Preliminary phytochemical investigations

Preliminary phytochemical investigation revealed the presence of alkaloid, steroid, saponin and glycosides (root) (6) in the *Schrebera swietenioides* plant

Experimental design

Screening of *Schrebera swietenioides* fruit ethenolic extract for anti-diabetic action was done in rats by conducting glucose tolerance test (GTT) study and evaluating their effects (Single dose and Multidose treatment study) on blood glucose level and serum lipid profiles in alloxan diabetic rats.

1. Acute toxicity study

Determination of LD_{50} for extracts is done by OECD guidelines for fixing the dose for biological evaluation. The animals were fasted overnight prior to the experiment and maintained under standard conditions. The LD_{50} of the extract as per OECD guidelines 2001, falls under 5mg, 50 mg, 300 mg and 2000 mg/kg bw with no signs of acute toxicity at respective doses. The biological evaluation of extract is carried out at 1/10 doses of LD_{50} (7).

2. Oral glucose tolerance test (OGTT) (8)

Fasting blood glucose level of each rat was determined at zero time after overnight fasing with free access to water. Rats were divided into three groups containing six rats each. The first group of animals were received 1 ml of 1% gum acacia suspension orally (Control animals).Remaining groups received Glibenclamide (2.5 mg/kg - standard) and *Schrebera swietenioides* fruit ethenolic extract (200 mg/kg), by oral route using an orogastric tube respectively. Glucose (2 gm/kg) was orally administered 30 min. after the administration of extracts or Glibenclamide or gum acacia suspension. Blood samples were collected from the tail vein under ether anaesthesia just prior to and 30, 60, 120 and 240 min after glucose loading. Glucose levels were estimated using glucose-oxidase-peroxidase reactive strips and a glucometer (Sugar-check, Wockhardt Ltd, Mumbai, India).

3. Effect of Schrebera swietenioides fruit ethenolic extract on blood glucose levels in alloxan induced diabetic rats [Single dose (Acute) treatment] (9)

A single intraperitoneal injection of 120 mg/kg of alloxan monohydrate was employed to induce diabetes in overnight fasted male wistar albino rats weighing 170-200gm. After 72 hr, animals with blood glucose levels higher than 250 mg/dl were considered diabetic and were included in the study. Animals were divided into four groups including six rats each. Group I: Normal control rats administered 1 ml of 1% gum acacia suspension; Group II: Diabetic control rats administered 1 ml of 1% gum acacia suspension; Group III: Diabetic rats administered Glibenclamide (2.5 mg/kg) and Group IV: Diabetic rats administered *Schrebera swietenioides* fruit ethenolic extract (200 mg/kg) orally. Blood samples were collected from the tail vein prior to and at 30 min, 60 min, 2, 4, and 6 h intervals after the administration of the extract and blood glucose levels were estimated using glucometer.

4. Effect of Schrebera swietenioides fruit ethenolic extract on blood glucose levels and serum lipid profiles in alloxan induced diabetic rats [Multi dose (sub acute) treatment] (9)

Diabetes was induced in overnight fasted adult male wistar albino rats weighing 170-200gm by a single intraperitoneal injection of 120 mg/kg of alloxan monohydrate. After 72 hr, animals with blood glucose levels higher than 250 mg/dl were considered diabetic and were included in the study. Animals were divided into four groups including six rats each. Group I: Normal control rats administered 1 ml of 1% gum acacia suspension; Group II: Diabetic control rats administered 1 ml of 1% gum acacia suspension; Group III: Diabetic rats administered Glibenclamide (2.5 mg/kg) and Group IV: Diabetic rats administered *Schrebera swietenioides* fruit ethenolic extract (200 mg/kg) orally. These rats were given the same doses of the extract once daily for 15 days in this study. Blood samples were collected from the tail vein of nonfasted rats on days 0, 5, 10 and 15 of extract administration and blood glucose levels were estimated using glucometer. Serum lipid profiles on day 15 were measured by an autoanalyzer. The whole pancreas from each animal was removed after sacrificing the animal and subjected for histological examination.

5. In-Vitro Antioxidant –DPPH free radical scavenging activity (10)

The free radical scavenging activity of *Schrebera swietenioides* fruit ethenolic extract was measured by 1, 1-diphenyl-2-picryl-hydrazyl (DPPH). For DPPH assay, the method of Blois was adopted. The capacity of *Schrebera swietenioides* fruit ethenolic solvent extract to scavenge the lipid-soluble DPPH radical was monitored at an absorbance of 517 nm. Ethenolic fruit extract (1 ml) of *Bauhinia variegata*, at different concentration was allowed to react with DPPH. Thirty minutes later, the absorbance was measured at 517 nm. The percentage inhibition of absorbance was calculated for each concentration relative to a blank absorbance using the spectrophotometer. The DPPH scavenging capacity of the extracts is compared with that of BHT (Butylated hydroxytolune). Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. All determinations are carried out at least three times, and in triplicate. IC_{50} value in the tested compound is, the concentration required to scavenge 50% DPPH free radical. Percentage inhibition was calculated as DPPH radical scavenging activity.

DPPH radical Scavenging effect (%) = (Abs control – Abs sample) / (Abs control) × 100

Where, Abs control is the absorbance of initial conc. of DPPH radical;
Abs sample is the absorbance of DPPH radical + sample Extract / standard

Statistical analysis

Values are presented as mean ± S.E.M. Statistical difference between treatments and the controls were tested by one-way analysis of variance (ANOVA), followed by Dunnett's multiple comparison test using the "Stat" statistics computer program. A difference in the mean values of $P<0.05$ was considered to be statistically significant.

RESULTS

Acute toxicity study

Acute toxicity study revealed no mortality or any toxic reactions with oral administration of ethenolic extract of fruit of *Schrebera swietenioides* even at the highest dose (2000mg/kg). The biological evaluation of extract is carried out at 1/10 doses of LD_{50} (7).

Oral glucose tolerance test (OGTT)

Ethenolic extract of fruit of *Schrebera swietenioides* significantly (P<0.01) improved the glucose tolerance test up to 4 hrs (Table 1 and figure 1). Ethenolic extract of *Schrebera swietenioides* showing approximately 22 and 14 % reduction in blood glucose level from control values at the 2 hr and 4 hr respectively. The Glibenclamide also improved the glucose tolerance test up to 4 hrs.

Table 1 Effect of *Schrebera swietenioides* fruit ethenolic extract on the blood glucose levels in glucose loaded rats

Exp. Group	Treatment	Blood glucose concentration (mg/dl) (mean ± S.E.M.)				
(n= 6)		In fasting	30 min	60 min	120 min	240 min
I	Normal control (1% gum acacia)	93.5 ± 3.4	162.2 ± 4.5	163.3 ± 2.2	160.5 ± 3.9	131.3 ± 3.8
II	Glibenclamide (2.5 mg/kg)	95.0 ± 2.8	142.0 ± 1.7*	145.5 ± 1.1*	138.0 ± 2.8*	99.5 ± 1.6*
III	*Schrebera swietenioides* fruit ethenolic extract (200 mg/kg)	98.33 ± 14.2	131.0 ± 2.7*	132.7± 3.5*	125.7±3.2*	114.0 ± 1.4 *

Significantly different from control: *P < 0.01; n= no of animals in each group

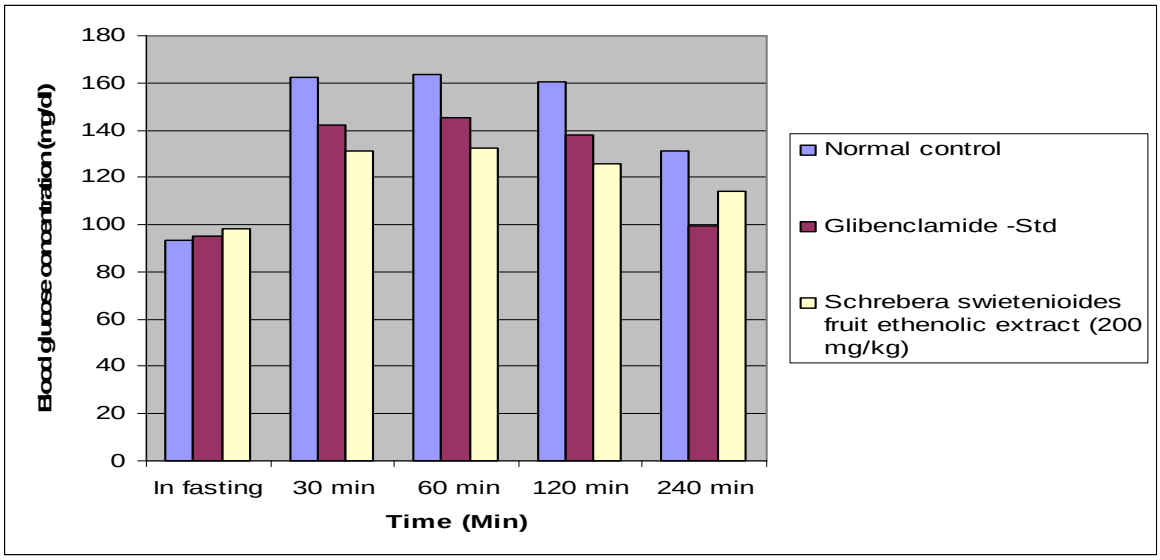

Figure-1 Effect of *Schrebera swietenioides* fruit ethenolic extract on the blood glucose concentration in glucose loaded rats

Effect of Schrebera swietenioides fruit ethenolic extract on blood glucose levels in alloxan induced diabetic rats [Single dose (Acute) treatment]

An administration of *Schrebera swietenioides* fruit ethenolic extract was found to reduce blood glucose level in alloxan induced diabetic rats in single dose study. *Schrebera swietenioides* fruit ethenolic extract exhibited significant (P<0.05) antihyperglycemic efficacy from 1 hr after its oral administration, the effect lasted up to 6 hrs when compared with normal rats and diabetic control rats. Blood glucose lowering potential percentage of *Schrebera swietenioides* was 13 % at 6 hr after administration, while the standard drug Glibenclamide (2.5mg/kg) caused 20 %

reduction of blood glucose at the same time interval when compared with diabetic control rats (Table 2).

Table 2 Effect of *Schrebera swietenioides* fruit ethenolic extract on the blood glucose levels in alloxan-diabetic rats (Single dose treatment /acute study)

Exp. Group (n= 6)	Treatment	Blood glucose concentration (mg/dl) (mean ± S.E.M.)					
		0 hour	30 min	60 min	120 min	240 min	360 min
I	Normal control (1% gum acacia)	85.25±2.6	86.25±1.8$^{\#\#}$	87.50±2.0$^{\#\#}$	89.75±1.8$^{\#\#}$	92.25±2.4$^{\#\#}$	93.50±3.5$^{\#\#}$
II	Diabetic control	287.5±5.	294.8±5.1*	294.5±5.2*	290.5±4.1*	292.0±3.5*	293.3±4.1*
III	Glibenclamide (2.5 mg/kg)	297.3±7.2	287.0±8.0*	274.0±9.7*	258.8±12.9* $^{\#}$	252.0±13.1* $^{\#\#}$	235.8±12.6* $^{\#}$ $^{\#}$
IV	*Schrebera swietenioides* fruit ethenolic extract (200 mg/kg)	279.5±6.3	271.0±6.5* $^{\#}$	265.8±8.1* $^{\#}$	262.8±9.9*	258.8±9.8* $^{\#}$	255.0±10.8* $^{\#}$

*P < 0.01 Significant, compared to normal, $^{\#}P$ < 0.05 & $^{\#\#}P$ < 0.01 Significant, compared to diabetic control.

n= no of animals in each group

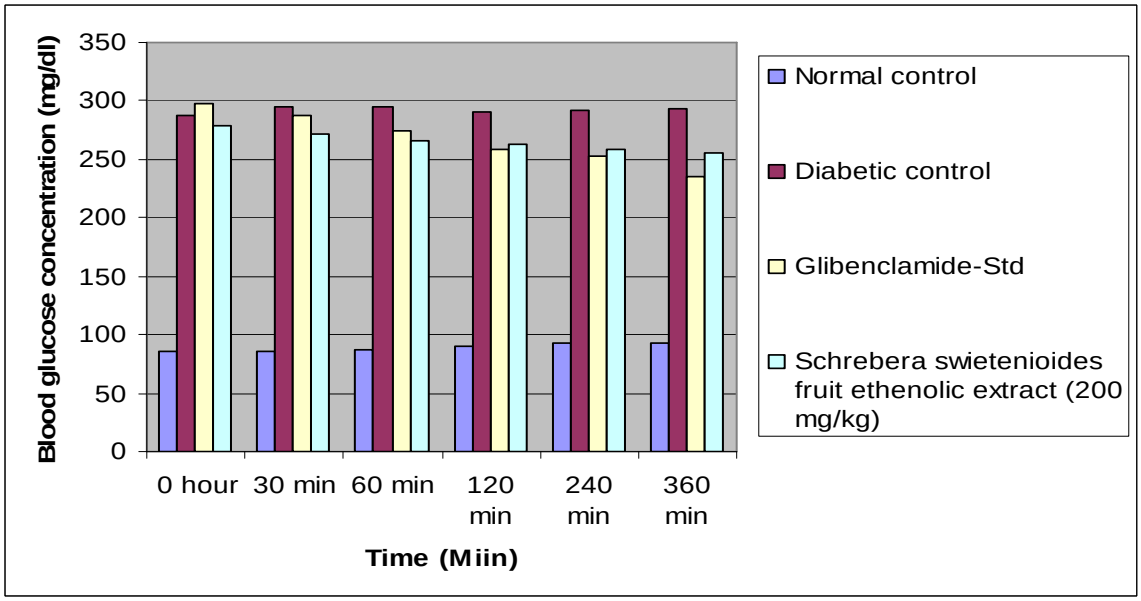

Figure-2 Effect of *Schrebera swietenioides* fruit ethenolic extract on the blood glucose concentration in alloxan-diabetic rats (Single dose treatment / acute study)

Effects of Schrebera swietenioides fruit ethenolic extract on blood glucose levels and serum lipid profiles in alloxan induced diabetic rats [Multi dose (sub acute) treatment]

In order to determine the sub acute effects, *Schrebera swietenioides* fruit ethenolic extract was administered throughout 15 days consecutively. The blood glucose level of each animal was monitored on 0th, 5th, 10th and 15th days after the administration of the test samples. As shown in the Table 3 and figure 3 initial antidiabetic activity was observed on 5th day and continued to increase in all groups during the experimental period.

Table 3: Effect of *Schrebera swietenioides* fruit ethenolic extract on the blood glucose levels in alloxan-diabetic rats (Multidose treatment /sub acute study)

Exp.Group (n= 6)	Treatment	Fasting blood glucose concentration (mg/dl) (mean ± S.E.M.)			
		0th Day	5th Day	10th Day	15th Day
I	Normal control (1% gum acacia)	85.25±2.6	85.75±1.8 ##	85.25±1.3 # #	87.25±1.1 ##
II	Diabetic control	287.5±5.2	274.3±7.1**	264.3±5.3**	255.8±5.1**
III	Glibenclamide (2.5 mg/kg)	299.3±6.9	217.3±14.3** # #	188.3±13.8** # #	158.3±15.3** # #
IV	*Schrebera swietenioides* fruit ethenolic extract (200 mg/kg)	277.3±6.7	223.3±12.5* # #	200.3±6.5* #	160.3±6.4* #

*P < 0.01 & **P < 0.05 Significant, compared to normal, #P < 0.01 & ##P < 0.05 Significant, compared to diabetic control.; n= no of animals in each group

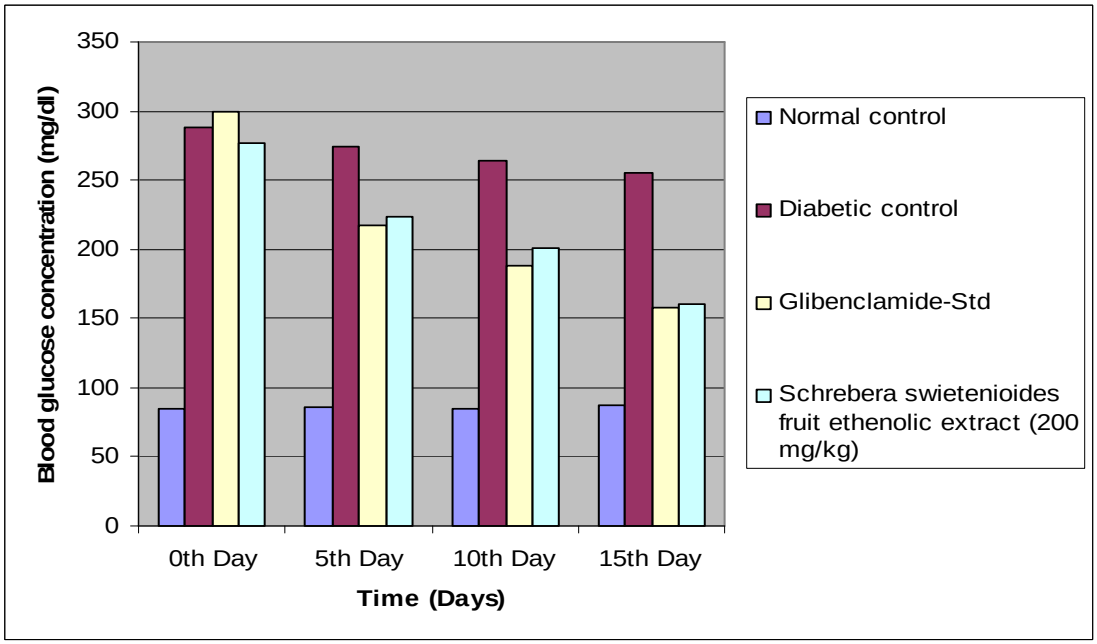

Figure-3 Effect of *Schrebera swietenioides* fruit ethenolic extract on the blood glucose levels in alloxan-diabetic rats (Multi dose treatment /sub acute study)

During the multidose treatment period, administration of ethenolic extract of fruit of *Schrebera swietenioides* (200 mg/kg/day) caused a significant decrease of 19%, 24% and 37% in blood glucose levels on 5th, 10th and 15th day intervals, respectively, when compared with diabetic control group.

In serum lipid profiles study on day 15, diabetes which is induced by alloxan lead to a significant change in levels of Serum cholesterol, triglyceride, LDL, VLDL, and HDL (Table 4 and figure 4). Serum cholesterol, serum triglyceride, serum LDL and serum VLDL levels were decreased and serum HDL level was increased significantly by Glibenclamide and ethenolic extract of fruit of *Schrebera swietenioides* (200mg /kg/day). Concurrent histopathological examination (Figure 5) of pancreas of these animals showed comparable regeneration of islets of Langerhans and β

cells by ethenolic extracts of *Schrebera swietenioides* fruit and Glibenclamide, which were earlier, necroses by alloxan.

Table 4 Effect of *Schrebera swietenioides* fruit ethenolic extract on the Serum profile in Alloxan-diabetic rats after 15 days of treatment

Exp. Group (n= 6)	Treatment	TGL mg/dl	HDL mg/dl	VLDL mg/dl	LDL mg/dl	TOTAL CHOLESTEROL mg/dl
I	Normal control (1% gum acacia)	85.25±1.5**	37.00±1.5*	19.00±0.73*	16.25±0.4*	55.50±1.6**
II	Diabetic control	123.5±2.4	30.00±1.4	27.75±1.3	34.42±3.7	83.00±2.0
III	Glibenclamide (2.5 mg/kg)	92.25±8.0**	51.50±1.9**	21.00±2.6*	19.00±1.9*	58.50±2.7**
IV	*Schrebera swietenioides* fruit ethenolic extract (200 mg/kg)	104.0±3.0	35.23±2.1*	23.00±0.73*	24.00±5.3*	68.00±5.1*

*P < 0.05 & **P < 0.01 Significant, compared with diabetic control.n= no of animals in each group

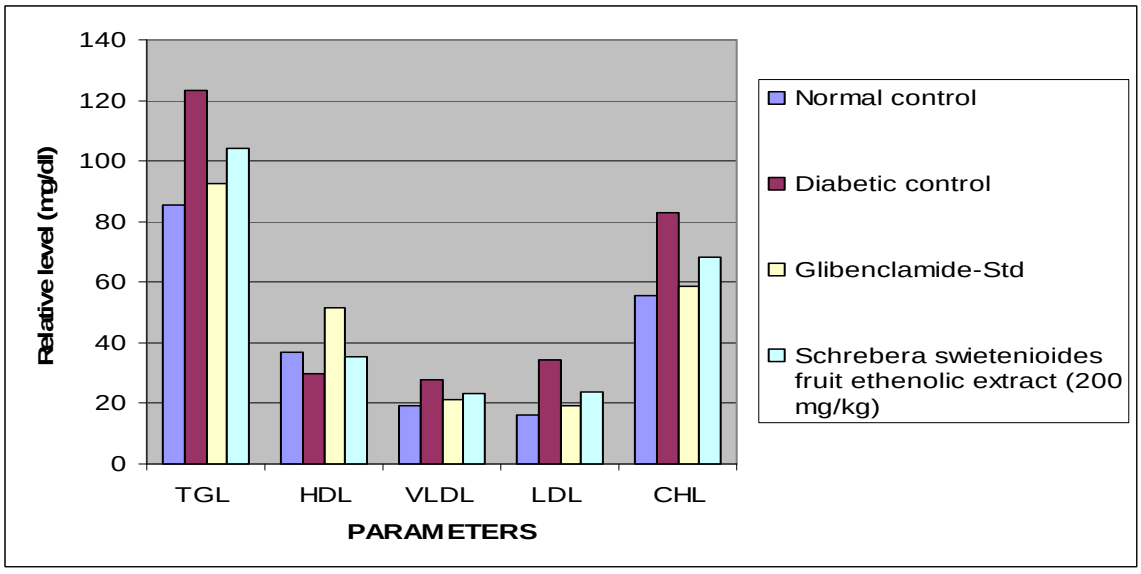

Figure-4 Effect of *Schrebera swietenioides* fruit ethenolic extract on serum lipid profiles in alloxan-diabetic rats (Multi dose treatment /sub acute study)

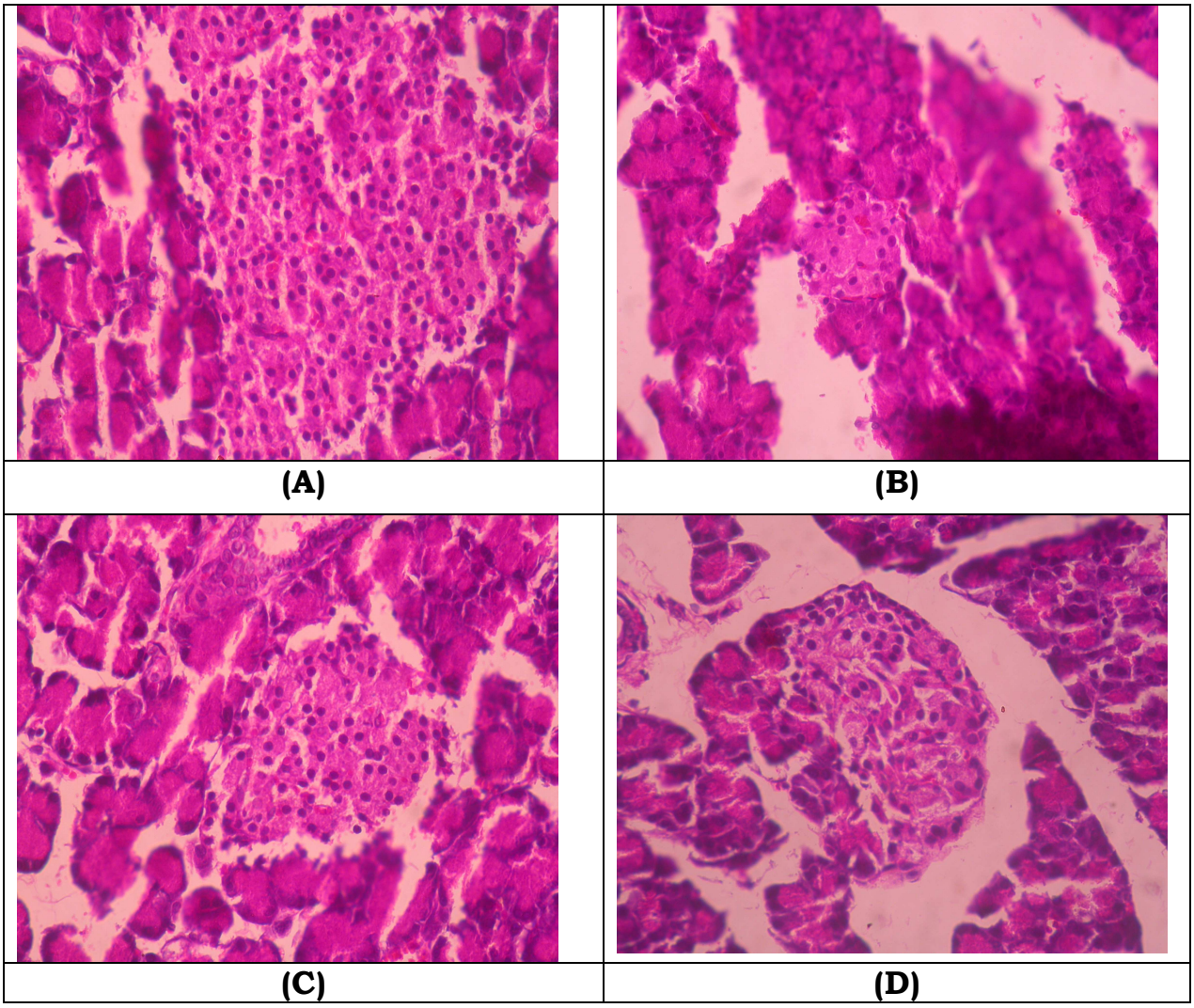

Fig-5. *Histological morphology of rat pancreas after 15 days of treatment with ethenolic extract of Schrebera swietenioides fruit. (A) Normal control rats showed normal pancreatic tissue, while (B) Alloxan-induced diabetic rats had severe decrease in number of islets of Langerhans cells and β cells. Pancreatic tissue of diabetic rats treated with (C) Glibenclamide (2.5 mg/kg) and (D) ethenolic extract of Schrebera swietenioides fruit (200 mg/kg) exhibited mild congestion with normal number of islets of Langerhans cells and β cells population.*

In-Vitro Antioxidant –DPPH free radical scavenging activity

Several concentrations ranging from 10-1000 µg /ml of the ethenolic extract of fruit of *Schrebera swietenioides* tested for their antioxidant activity by DPPH model. It has been observed that free radicals were scavenged by the *Schrebera swietenioides* fruit ethenolic extract in a concentration dependent manner in this DPPH assay (Table 5).The ethenolic extract of fruit of *Schrebera swietenioides* showed DPPH radical scavenging activity with an IC_{50} value of 423 µg /ml when compared with Standard BHT (Butylated hydroxytolune) IC_{50} value of 107 µg /ml.

DISCUSSION AND CONCLUSION

For a diabetic patient, blood sugar control is highly important over time. Usually as the patient ages, the oral hypoglycemic drugs are not as effective in keeping blood sugars in normal ranges, thus creating many complications. Medicinal plants are used in several countries to manage DM

and are thought to be less toxic than allopathic hypoglycemic drugs like the biguanides, sulphonylureas or insulin therapy.

Herbal medicines provide rational means for the treatment of many diseases that are obstinate and incurable in other systems of medicine. These are gaining popularity because of several advantages such as often fewer side effects, better patient tolerance, relatively less expensive and acceptance due to long history of use. Medicinal effects of plants tend to normalize physiological function and correct the underlying cause of the disorder. Plants are often less prone to the emergence of drug resistance.

Oxidative stress has been suggested as a contributory factor in the pathogenesis of diabetes. Diabetes, by itself, increases the production of tissue damaging reactive oxygen species (ROS) by glucose autoxidation and / or no enzymatic protein glycosylation (11). Plants provide a rich source of antioxidants, which include tochopherols, Vit.C, phenolic compounds, carotenoids (12), flavonoids, terpenoids, anthraquinones, steroids, strychnine and eugenol alkaloids (13). As this plant contains alkaloid, steroid, saponin and glycoside constituent, this study was therefore undertaken to assess antioxidant and antihyperglycemic properties of *Schrebera swietenioides* fruit, which have been reported in Ayurveda to be useful in diabetes mellitus.

The 1, 1-diphenyl -2-picryl hydrazyl (DPPH) radical was widely used as the model system to investigate the scavenging activities of several natural compounds. DPPH radical is scavenged by antioxidants through the donation of proton, forming the reduced DPPH. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517nm. Radical scavenging activity is increased with increasing percentage of the free radical inhibition**.** From the present results, it may be postulated that *Schrebera swietenioides* fruit ethenolic extract reduces the radical to corresponding hydrazine when it reacts with hydrogen donors in antioxidant principals. From the antioxidant results it can be concluded that the *Schrebera swietenioides* fruit ethenolic extract has potent in vitro antioxidant potential which may attributed to the presence of alkaloid, steroid, saponins, glycoside and other constituents present therein.

Overall results show that ethenolic extract of *Schrebera swietenioides* fruit possesses marked antihyperglycemic activity by improvement of glucose tolerance test and by lowering the blood glucose levels in alloxan-induced diabetic rats in single dose (acute) and multi dose (*sub acute*) treatment study. The ethenolic extract of fruit of *Schrebera swietenioides* and Glibenclamide exhibited remarkable blood glucose lowering effect in glucose tolerance test. This indicates that the extract has sound capacity to block glucose absorption.

Since diabetes is a chronic disorder requiring long-term therapy, there is a need to assess the effect of putative hypoglycemic/anti-hyperglycemic agents for a longer duration also. In addition, if plant extracts have a late onset of activity, their effect is likely to be missed in such screening studies. The present study was therefore planned to assess the effect of test drugs for a period of 6 hrs (single dose treatment study) and 15 days (multi dose treatment study).In this study the difference observed between the initial and final blood glucose levels under investigation reveals a significant elevation in blood glucose in diabetic control group at the end of 6 hrs (single dose treatment study) (Figure 2) or at the end of 15 days (multi dose treatment study) experimental period. Administration of extract to alloxan induced diabetic rats showed a significant decrease in the blood glucose level in both single dose and multi dose treatment study. The hypoglycemic effect comparable to glibenclamide suggested that the active fractions may act by regenerating the β cells in alloxan-induced diabetes (14). Alloxan causes diabetes

through its ability to destroy the insulin producing β cells of the pancreas. In *vitro* studies have shown that alloxan is selectively toxic to the pancreatic β cells, causing cell necrosis. The cytotoxic action of alloxan is mediated by reactive oxygen species, with a simultaneous massive increase in cytosolic calcium concentration, leading to a rapid destruction of β cells (15).

The components responsible and the mechanism by which this plant exert these antihyperglycemic and antioxidant activity is not completely understood.

From these results it may be concluded that the *Schrebera swietenioides* fruit ethenolic extract shown to have hypoglycemic and antioxidant action. It is conceivable that antioxidant/ free radical scavenging activity of *Schrebera swietenioides* fruit ethenolic extract is unlikely to be the only mechanism associated with antidiabetic effect. These results seem to confirm the alleged antidiabetic activity by the traditional medicine. However longer duration studies on chronic models are necessary to elucidate the mechanism of action so as to develop this plant as a potent antidiabetic drugs.

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