ANTIDIABETIC AND ANTIHYPERLIPIDAEMIC EFFECTS OF S*OLANUM XANTHOCARPUM* TOTAL EXTRACT IN ALLOXAN INDUCED DIABETIC RATS.

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Summary

The antidiabetic and antihyperlipidaemic effects of aqueous-methanol (40:60) extract of *Solanum xanthocarpum* whole plant (Sx) were investigated in normoglycemic, glucose fed, and alloxan-induced diabetic rats. In normoglycemic rats, the powdered extract of Sx, administered at doses of 200 mg/kg and 400 mg/kg b.w x 1 p.o. resulted in reduction of blood glucose level by 10.55% and 12.83% respectively. In glucose fed diabetic rats, the reduction of blood glucose level was also achieved dose dependently and significantly compared to the vehicle control group. In another experiment, powdered Sx extract was administered daily at doses of 200 mg/kg and 400 mg/kg p.o., for 7 days, after alloxan administration (150 mg/kg i.p.). The treatment showed significant dose-dependent percentage blood glucose lowering in the diabetic rats. This effect of Sx was comparable to that of a reference standard drug, glibenclamide (10 mg/kg b.w p.o. x 7). Additionally, the Sx plant extract also had potent antihyperlipidaemic activity and was found to improve the lipid levels of alloxan induced diabetic rats at the end of the treatment period. Phytochemical screening of the *Solanum xanthocarpum* total plant extract showed the presence of spirosta-steroidal saponins, amino acids, phytosterols, flavonoids, glycoalkaloids, tannins and tarpenoids, many of which were previously unreported and now contributed to the bioactivity of Sx.

Keywords: *Solanum xanthocarpum,* antidiabetic, alloxan, antihyperlipidaemic, Glibenclamide.

Introduction

Diabetes mellitus (DM) is a metabolic disorder characterized by elevation of blood glucose level. DM is found worldwide and becoming a serious threat to mankind. It is third killer of human beings after cancer, cardiovascular and cerebrovascular diseases [1]. DM is of two types: type 1 and type 2. Both type 1 and type 2 diabetes are known to be multifactorial diseases caused by a combination of genetic (inheritance) and environmental (diet and lifestyle) factors [2, 3]. DM also leads to hyperglycemia, which is the landmark of this disease syndrome [4] and it has also been associated with an increased risk for premature arteriosclerosis due to increase in triglycerides and low density lipoprotein levels and ultimately results in Coronary Heart Disease (CHD). CHD morbidity is two to four times higher in patients with diabetes than nondiabetics, and the mortality from CHD is up to 100% higher in diabetic patients than in nondiabetics over a 6-year period. So, an ideal treatment for diabetes would be a drug that not only controls the glucose levels but also prevents the development of arteriosclerosis and other complications of diabetes [5]. For a long time, DM has been treated with several medicinal plants or their extractives. Before the discovery of insulin in 1922, the options for treatment of diabetes were based on traditional knowledge [4]. Ethnobotanical knowledge played an important role in historical diabetes therapies; with over 1200 medicinal plant species recognized throughout the World for their ability to treat diabetes. Although different types of hypoglycemic agents are available, along with insulin, for the treatment of DM, there has been an increased demand for natural products with antihyperglycemic activity [5] and also antihyperlipidaemic activity. However, there is a need for thoroughly controlled studies on the effectiveness and potential risks of treatment with natural products [6].

Solanum xanthocarpum, commonly known as the Indian nightshade or yellow berried night shade, is a prickly, diffusely bright-green, perennial shrub which grows abundantly in arid areas of India. It has been used in Ayurveda for a variety of therapeutic purposes. As natural remedies, its fruit juice is used in sore throats and rheumatism; decoction of the plant is used in gonorrhea; paste of leaves is applied to relieve pains; seeds act as expectorant in cough and asthma; roots are expectorant and diuretic, and are also useful in the treatment of fever, coughs, asthma and chest pain [7-9].

The antidiabetic potential of the fruit and leaves of Sx were studied previously in streptozotocin (STZ) induced diabetic rats [10-12]. However, the antidiabetic potential of Sx total plant extract in alloxan induced diabetic animals has not been reported before. This was considered necessary because of the basic differences in the mechanisms of actions of the two established diabetogens, streptozotocin and alloxan. STZ is a nitrosourea derivative and can produce both type-I and type-II DM. STZ is taken up by pancreatic beta cells via the glucose transporter GLUT-2. Its toxicity depends on the potent alkylating properties combined with the synergistic action of systemic nitric oxide and reactive oxygen species that cause DNA degeneration [13-16].

Alloxan (administered intraperitoneally) induces a specific necrosis of pancreatic islets. Toxic effects are manifested through multiple sequences,- rapid uptake of alloxan by the beta cells, via GLUT-2, reduction of alloxan to dialuric acid together with oxidation of SH groups, inhibition of glucokinase, generation of reactive oxygen species, and disturbances in intracellular calcium homeostasis [17-19] .

In an earlier study, (not reported before), Sx also showed antihyperlipidaemic activity, due to the presence of steroidal saponins. A detailed study of the antihyperglycemic and antihyperlipidaemic activity of *S. xanthocarpum* total extracts, on alloxan induced diabetic rats, has now been carried out. The findings constitute the subject of this paper.

Material and Methods

Test sample- Authenticated plant material of *S. xanthocarpum*, cultivated in the Western Himalayas, was obtained from Indian Herbs Ltd, Saharanpur (U.P.). A specimen has been preserved in our file as reference.

Extraction and isolation of chemical constituents of *S. xanthocarpum* (Sx):

Dried and powdered whole plant of Sx was continuously (Soxhlet) extracted (6h) with aqueous-methanol (40:60). The solvent was evaporated under reduced pressure. The total extractives were subjected to solvent-gradient separation followed by comprehensive column chromatographic and spectroscopic analyses, using markers, where possible. In a typical experiment, the major bioactive fraction(s), viz. the spirosta-steroidal saponins were processed as follows and identified by GC-MS analyses.
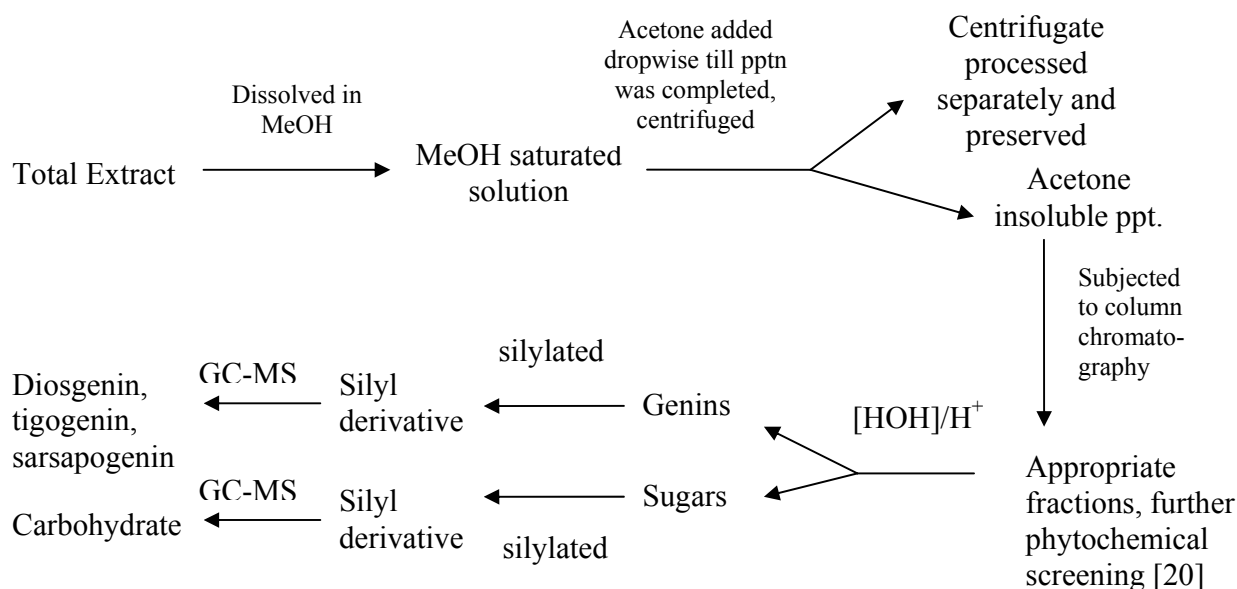
Scheme-1: A general method of isolation of spirosta-saponins and analyses of component moieties of Sx.

Pharmacology:

Animals- Albino rats (Sprague Dawley strain) of either sex, 3-4 months old and weighing around 180 to 240 gm, procured from Central Research Institute (Ayurveda), Govt. of India, Salt Lake City, Kolkata, were used. The animals were housed in an animal room with alternating light-dark cycle of 12 hr each. The animals were acclimatized for at least 7 days to the laboratory conditions before conducting experiments. Experiments were carried out between 0900 h and 1700 h. The study was conducted in accordance with Good Laboratory Practice (GLP) Regulations of WHO (WHO Document, 1998). The "Principles of laboratory animal care" (NIH Publication # 85-23, 1985) were also followed in the study. The 'Institutional Animal Ethics Committee' (IAEC) approved the experimental protocol.

Preparation of standard drug solution
Glibenclamide (Bal Pharma, Bangalore, India) was used as the reference drug for evaluating antihyperglycemic activity. Glibenclamide suspension was prepared in distilled water using Carboxymethyl cellulose (0.8%) as suspending agent.
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Induction of experimental diabetes
The animals were allowed to fast 24 h and were injected with alloxan monohydrate (Loba Chemie Pvt. Ltd. Mumbai, India) dissolved in sterile normal saline (0.9%) at a dose of 150 mg/kg body weight i.p. The general behavior and blood glucose level were checked for 5 days at constant intervals. After completion of specified days, a stabilized increased blood glucose levels were registered and animals having more than 250 mg/dl blood glucose were separated and opted for experimentation. During the period of induction, 25% death was registered.

Experimental protocol

Effect of Sx/total extract on blood glucose levels in normoglycemic rats:
Animal were divided into three groups of six rats in each group
Group-1: Normal control (Animals received vehicle only i.e. 0.8% CMC).
Group-2: Animals received Sx total extract 200mg/kg body wt. per orally.
Group-3: Animals received Sx total extract 400mg/kg body wt. per orally.
In this study the entire groups of animals were fasted over night and administered with respective drugs as per the above mentioned dosage schedule.
Blood glucose levels were determined at 0 (before drug challenge), 30, 60, 120 and 240 min, after drug administration.

Effect of Sx/total extract on Blood Glucose Level on Glucose Fed Hyperglycemic Rats (Oral Glucose Tolerance Test):
The animals were divided into four groups of six rats in each group
Group-1: Animals received glucose at a dose 2g/kg body wt. per orally.
Group-2: Animals received Glibenclamide 10mg/kg body wt. and glucose Solution at a dose 2g/kg body wt. per orally.

Group-3: Animals received Sx total extract 200mg/kg body wt. p.o and glucose Solution at a dose 2g/kg body wt. per orally.
Group-4: Animals received Sx total extract 400mg/kg body wt. p.o and glucose Solution at a dose 2g/kg body wt. per orally.
In this study, the entire group of animals were fasted and treated with above dosage schedule orally. The Sx total extract 200mg/kg, 400mg/kg and 10 mg/kg Glibenclamide were administered half an hour before administration of glucose solution. Blood glucose levels were determined at 0 (before glucose challenge) 30, 60, 90, 120 min after glucose administration.

Effect of Sx/total extract on Blood Glucose Level in Alloxan Induced Diabetic Rats.
In this experiment, total 30 surviving rats were used, having 6 rats in each group.
Group-1: Normal control animals received vehicle only i.e. 0.8% CMC.
Group-2: Alloxan (150mg/kg body wt. i.p.) induced diabetic animals received vehicle only.
Group-3: Alloxan (150mg/kg body wt. i.p.) induced diabetic animals received glibenclamide 10 mg/kg, body wt. per orally.
Group-4: Alloxan (150mg/kg body wt. i.p.) induced diabetic animals received Sx total extract 200mg/kg, body wt. per orally**.**
Group-5: Alloxan (150mg/kg body wt. i.p.) induced diabetic animals received Sx total extract 400mg/kg, body wt. per orally. .
In acute study, all the surviving diabetic animals and normal animals were fasted over night. Blood samples were collected from the fasted animals prior to the treatment with above schedule and after administration at each day up to 7days.

Determination of blood glucose level:
In this experiment, blood glucose level of the animals was estimated by Glucose Oxidase-Peroxidase Enzymatic Method (GOD-POD) [21]. Blood from the tail vein was collected. About 100 μl of blood was taken in Eppendorff's tube (1.5 ml capacity) to which anticoagulant (potassium EDTA + sodium fluoride, 2:1) was previously added. Blood was mixed properly and centrifuged at 3000 rpm for 10 min. Out of it 10 μl of plasma was further used with 1ml of enzyme solution. Thoroughly mixed plasma and enzyme were incubated at room temperature for 30 min. Then the absorbance of pink colored solution formed was noted at 505 nm. The glucose value was calculated using 100 mg/dl standard solution of glucose.
Glucose conc. (mg/dl) = Absorbance (sample) / Absorbance (standard) X 100

Determination of lipid parameters:
Total cholesterol (TC), Triglyceride (TG), High Density Lipoprotein in serum were determined using enzymatic kits (Span diagnostic Ltd.). Low Density Lipoprotein (LDL) was calculated using the following Friedewald's equation:
LDL = TC- TG/5 – HDL.
Very Low Density Lipoprotein (VLDL) was calculated using the following formula:
VLDL= TC – (HDL + LDL).

Statistical analysis- Statistical analysis was carried out using Prism software ver.4.0 (Graph pad Inc). All the results were expressed as Mean ± standard deviation of the mean (SD). Data were analyzed using one-way ANOVA followed by Tukey's test. In the entire test, the criterion for statistical significance was *p<0.05.*

Results

The different types of chemical constituents found to be present in Sx are reported in Table-1.

Table 1. Chemical constituents of Sx/total extract

Phytochemicals	Sx/total extract
Spirosta-steroidal saponins	+++
Amino acids	++
Phytosterols	+
Flavonoids	+
Steroidal glycoalkaloids	+
Tannins	+
Tarpenoids	+

+++ indicates ≥2%; ++ indicates 1-2%; + indicates ≤0.5% w/w of total plant.

Spirosta-steroidal saponins and amino acids of Sx:
Comprehensive GC-MS analyses of spirosta-steroidal saponins of Sx were performed. Some of the spirosta-steroidal saponins present in Sx are new. This was indicated by the occurrence of previously unreported carbohydrate and cyclitol moieties associated with spiro-stanols (Table 2).

Comprehensive GC-MS analyses of amino acids of Sx were performed. The results are given in Table 2. The amino acids also contributed to the bioactivities of the Sx extract (data not shown).

Table 2. Amino acids and spirosta-steroidal saponins of *S. xanthocarpum*

Amino acids	Spirosta-steroidal saponins	
	sapogenins	Sugars
Alanine, glycine, valine, L-proline, aspartic acid, glutamine, L-aspargine.	Diosgenin, tigogenin, sarsapogenin	L-altrose, talose, gluconic acid lactone, glucitol, β-D-glucopyranose

Effect of Sx/total extract on Blood Glucose in Normoglycemic Rats:
After administration at doses 200mg/kg and 400mg/kg body weight p.o of Sx/total extract, fasting blood sugar levels were assessed in normal rats at various time intervals. The results are shown in Table-3. The mean blood glucose level decreased from 79.34mg/dl to 70.97mg/dl at dose of 200mg/kg body weight p.o of Sx/total extract and from 80.06mg/dl to 68.42 mg/dl at dose of 400mg/kg body weight p.o in rats treated with Sx/total extract at 60 min, which was the optimum time of activity after the treatment.

Table 3. Effect of Sx/total extract on blood glucose levels in normoglycemic rats

Groups	Blood glucose level in mg/dl				
	Initial (0 min)	30 min	60 min	120 min	240 min
Gr-1 (Normal control)	76.93±2.64	76.96±3.85	79.08±4.38	76.05±2.44	77.96±2.75
Gr-2 (Sx 200mg/kg)	79.34±5.70	72.71±3.40	70.97±4.03	75.80±4.04	78.88±6.24
Gr-3 (Sx 400mg/kg)	80.06±5.62	69.79±7.45*	68.42±5.16**	78.82±4.70	80.05±6.08

Data represented as Mean ± SD; for 6 rats.
The blood glucose values of Group 2, 3 and 4 are compared with control animal's values
where, *$p<0.05$; ** $p<0.01$; *** $p<0.001$;

Effect of Sx/total extract on Blood Glucose Level of Glucose Fed Hyperglycemic Rats (Oral Glucose Tolerance Test):
At doses 200 mg/kg and 400 mg/kg body weight p.o of Sx/total extract on blood sugar levels of glucose fed rats were assessed. The results are incorporated in Table 4.

Table 4. Effect of Sx/total extract on blood glucose levels in glucose fed hyperglycemic rats.

Groups	Blood glucose level in mg/dl				
	Initial (0 min)	30 min	60 min	90 min	120 min
Gr-1 (glucose)	78.10±4.28	123.82±6.13	116.80±8.96	89.35±4.40	77.59±3.98
Gr-2 (glucose+ glibenclamide)	81.21±4.53	126.31±4.57	104.35±5.69**	80.88±6.58*	77.71±5.97
Gr-3 (glucose+ Sx 200mg/kg)	79.23±3.07	123.61±4.57	109.40±3.28	85.85±5.15	80.20±4.71
Gr-4 (glucose+ Sx 400mg/kg)	78.40±1.73	125.34±7.13	107.72±1.61*	81.90±2.39	77.38±1.67

Data represented as Mean ± SD; for 6 rats.
The blood glucose values of Group 2, 3 and 4 are compared with control animal's values
where, *$p<0.05$; ** $p<0.01$; *** $p<0.001$;

Effect of Sx/total extract on Blood Glucose Level in Alloxan Induced Diabetic Rats.

The antihyperglycemic effect of the Sx/total extract on the blood sugar level on diabetic rats was shown in figure-1. The blood glucose level of diabetic animals reduced from 288.71 to 189.98 at 200 mg/kg body wt. p.o and 285.65 mg/dl to 178.14 mg/dl at 400 mg/kg body wt. p.o of Sx/total extract. These results are comparable with 10 mg/kg of glibenclamide. The % blood glucose change in each day has also been calculated for 7 days and the results were incorporated in Table 5. The % of blood glucose level of diabetic animals significantly ($p<0.001$) reduced to 34.08% at 200mg/kg body wt. p.o of Sx/total extract and to 37.29% at 400mg/kg body wt. p.o of Sx/total extract. These results are comparable with that of 10 mg/kg oral dose of glibenclamide (41.63%).

Effect of Sx/total extract on Lipid Level in Alloxan Induced Diabetic Rats.

The antihyperlipidaemic effect of the extracts on the serum lipid level on diabetic rats was shown in Table-6. Due to treatment with alloxan, there were significant ($p<0.001$) elevation of TC, TG, LDL and VLDL with a concomitant decrease in HDL. Sx/total extracts in both the doses (200mg/kg b.w. and 400 mg/kg b.w.) showed significant improvement of all the lipid parameters.
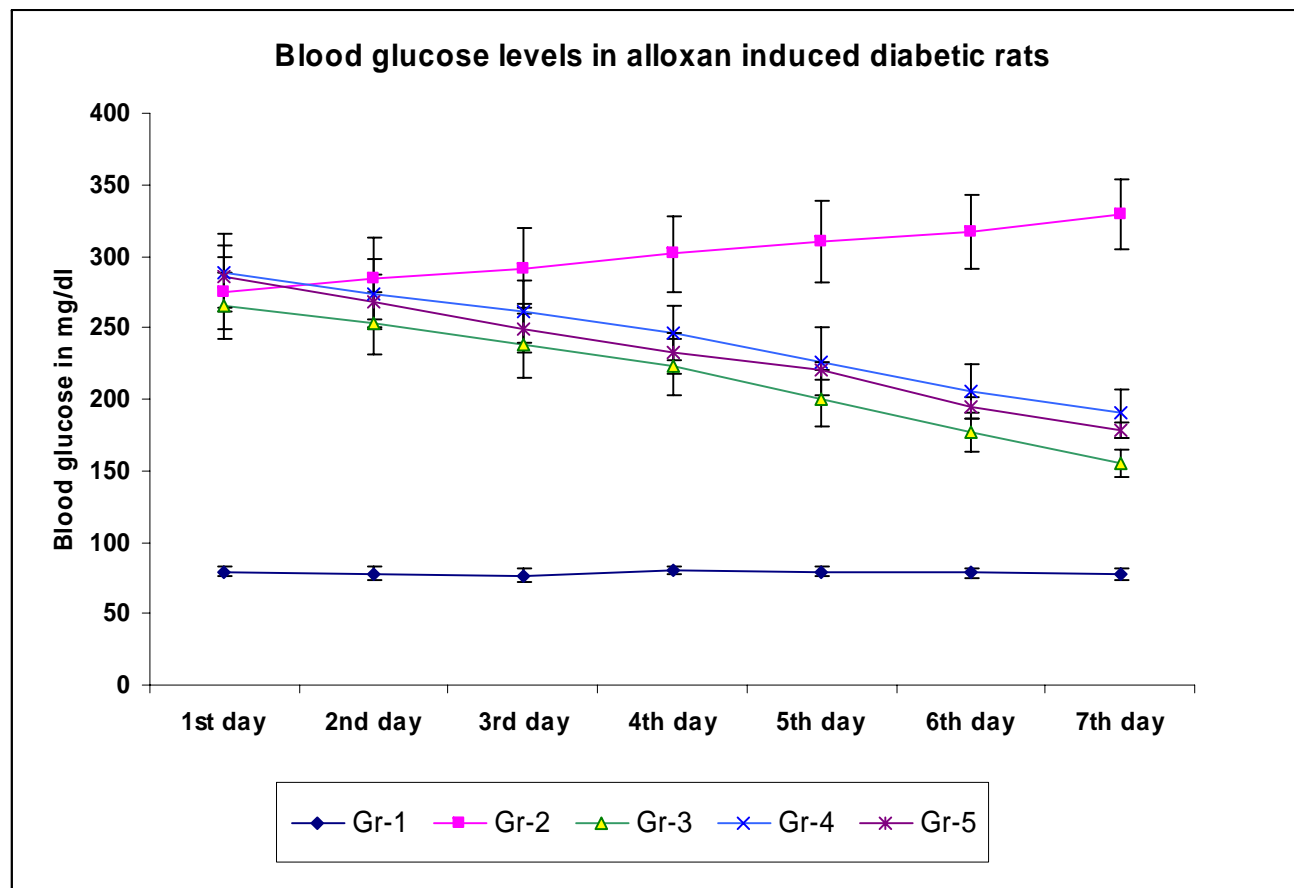
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Blood glucose levels in alloxan induced diabetic rats

Figure 1. Effect of Sx/total extract on Blood Glucose Level in Alloxan Induced Diabetic Rats

Table 5. % change in Blood Glucose Level by Sx/total extract in Alloxan Induced Diabetic Rats

Groups	% change in blood glucose level in mg/dl in each day						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Gr-1 (Normal control)	-	-1.03±2.83	-2.89±3.03	1.70±2.31	0.18±4.81	-0.67±4.79	-1.86±1.81
Gr-2 (Alloxan control)	-	3.49±2.03	6.41±2.33	10.02±1.94	13.17±2.02	15.94±2.06	20.40±4.87
Gr-3 (Alloxan+ Glibenclamide)	-	-4.59±2.74 [a]	-10.38±2.51 [a]	-16.16±2.62 [a]	-24.54±2.90 [a]	-33.14±2.33 [a]	-41.63±2.03 [a]
Gr-4 (Alloxan+ Sx 200 mg/kg)	-	-5.09±1.79 [a]	-9.41±1.81 [a]	-14.59±2.83 [a]	-21.59±3.46 [a]	-28.81±2.87 [a]	-34.08±3.67 [a]
Gr-5 (Alloxan+ Sx 400 mg/kg)	-	-5.94±1.04 [a]	-12.68±1.69 [a]	-18.63±3.36 [a]	-22.69±6.02 [a]	-31.71±6.49 [a]	-37.29±6.00 [a]

Data represented as Mean ± SD; for 6 rats.

[a] denotes *p <0.001;* in comparison to Group 2 (negative control) rats treated with the vehicle after alloxan induced hyperglycemia.

Table 6. Effect of Sx/total extract on Blood Lipid Level after 7 days in Alloxan Induced Diabetic Rats.

Groups	Blood lipid level in mg/dl				
	Total cholesterol	**Triglyceride**	**LDL**	**HDL**	**VLDL**
Gr-1 **(Normal control)**	99.58 ± 7.31	64.46 ± 3.82	41.06 ± 10.67	45.63 ± 4.54	12.89 ± 0.76
Gr-2 **(Alloxan control)**	$128.21 \pm 11.24^{\#\#\#}$	$137.36 \pm 8.07^{\#\#\#}$	$68.61 \pm 11.18^{\#\#\#}$	$32.12 \pm 3.34^{\#\#\#}$	$27.47 \pm 1.61^{\#\#\#}$
Gr-3 **(Alloxan+Glibenclamide)**	$113.66 \pm 7.90^*$	$119.95 \pm 6.65^{***}$	$51.54 \pm 5.78^*$	38.13 ± 3.70	$23.99 \pm 1.33^{***}$
Gr-4 **(Alloxan+ Sx 200 mg/kg)**	120.98 ± 7.45	128.72 ± 6.01	60.80 ± 7.17	34.44 ± 4.05	25.74 ± 1.20
Gr-5 **(Alloxan+ Sx 400 mg/kg)**	$113.03 \pm 8.09^*$	$117.90 \pm 7.42^{***}$	$50.28 \pm 5.45^{**}$	$39.17 \pm 4.04^*$	$23.58 \pm 1.48^{***}$

Data represented as Mean $\pm$ SD; for 6 rats.

$^{\#}p<0.05$; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$; in comparison to Group 1 (normal control) rats treated with vehicle.

$^*p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$; in comparison to Group 2 (negative control) rats treated with the vehicle after alloxan induced hyperglycemia.

Discussion

DM is possibly the World's largest metabolic disease, and with the advanced knowledge of the heterogeneity of this disease, there has been increasing need for more appropriate therapy increases [22]. Overproduction (excessive hepatic glycogenolysis and gluconeogenesis) and decreased utilization of glucose by the tissue is believed to be the fundamental mechanism underlying hyperglycemia [8]. The maintenance of blood glucose balance is a critical physiological function which involves a dynamic equilibrium requiring a series of cellular metabolic events [23]. Extensive atherosclerosis involving cardiovascular, renal and peripheral level remains the major complication of DM. Alteration in serum lipid profile is known to occur in DM which is likely to increase the risk of CVD. In the treatment of DM, lowering of glucose and improvement of lipid levels are two very important aspects for the long term prognosis of the patients suffering from DM [24]. Alloxan, used for induction of "chemical diabetes" is a potent diabetogen. It produces the partial destruction of pancreatic β cells of the islets of langerhans and induces hyperglycemia [25]. The standard drug Glibenclamide causes hypoglycemia by increasing insulin secretion from pancreas and by reducing hepatic clearance of the hormone [26].

In the present study, the antihyperglycemic and antihyperlipidaemic activity of the total plant extract of *Solanum xanthocarpum* has been evaluated. In the normoglycemic rats, Sx/total extract caused reduction in blood glucose level at both the doses with a peak at 60 minutes after oral administration. In case of glucose fed hyperglycemic rats, a dose dependent significant glucose lowering activity was observed. In case of alloxan induced diabetic rats, after 7 days of treatment, both the doses of Sx/total extract caused significant, dose dependent glucose lowering commensurate with the standard drug, glibenclamaide (10mg/kg b.w p.o.).

Sx/total extract also showed a significant improvement of blood lipid levels of the rats treated with alloxan. Alloxan treatment significantly increased the TC, TG, LDL and VLDL levels of animals with a concomitant decrease in HDL level. Treatment with different doses of Sx/total extract, dose dependent improvement of the lipid status occurred which was commensurate with that of glibenclamide treatment.

Phytochemical analyses of Sx revealed the presence of spirosta-steroidal saponins, amino acids, phytosterols, flavonoids, steroidal glycoalkaloids, tannins and tarpenoids in the decreasing order of abundance. Tannins and flavonoids are known to reduce glucose level in diabetic animals [27, 28]. Compounds like steroidal saponins are known for their hypolipidaemic activity [29]. The contribution of these constituents to the bioactivities of the total extract of Sx is noteworthy. The antihyperglycemic and antihyperlipidaemic activities of Sx/ total extract may be attributed to the collective contributions of the identified compounds. This study, in essence, provides an analysis of the total chemical constituents of Sx, in respect of its antidiabetic and antihyperlipidaemic activities in alloxan-induced diabetes in rats.

Acknowledgement

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