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TO EVALUATE THE HYPOGLYCEMIC EFFECT OF SOLANUM SPIRALE ROXB.

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ABSTRACT

Diabetes mellitus is complex metabolic disorder resulting from either insulin insufficiency or insulin dysfunction a hormone that is required to convert sugar, starches and other food into energy. It is becoming the third "killer" of the health of mankind along with cancer, cardiovascular and cerebrovascular disease. The present study was conducted to evaluate the hypoglycemic effect of *Solanum Spirale Roxb*. Leaves. The air dried leaves were soaked in 500 ml of 95% ethanol in a round flask for 24 hours the process of extraction was done by reflux condensation method using soxhlet apparatus at 60-80 degree Celsius for 9 hours. The extract was concentrated by distillation apparatus. The hypoglycemic effect study was carried out by inducing diabetes in rats by injecting streptozotocin. The diabetic rats were treated with plant extract and standard drug orally once for 14 days. The blood glucose concentration was measured and noted. The serum collected was analyzed for total cholesterol, triglycerides, LDL, HDL, Sr. AST, Sr. ALT, Sr. creatinine. The result of the present study revealed that the *ethanolic* extract of *Solanum spirale Roxb*. Leaves possess anti diabetic effect.

KEYWORDS: *Diabetes, Hypoglycemic, Solanum spirale ethanol, streptozotocin.*

INTRODUCTION

Diabetes Mellitus is a complex metabolic disorder resulting from either insulin insufficiency or insulin dysfunction a hormone that is required to convert sugar, starches and other food into energy. Diabetes is caused by the abnormality of carbohydrate metabolism which is linked to low blood insulin level or insensitivity of target organs to insulin. It is characterized by constant high levels of blood glucose.

It is becoming the Third "killer" of the health of mankind along with cancer, cardiovascular and cerebrovascular diseases .Uncontrolled Diabetes mellitus over the time leads to serious damage to heart, blood vessels, eyes, kidneys and nerves which are the major causes of morbidity and mortality in diabetic subject.

The majority of people with Diabetes suffer from Type -2 diabetes. It usually occurs in obese individuals and is associated with Hypertension and Dyslipidemia.

Diabetes mellitus is described in AYURVEDA as "MADHUMEHA"- which literally means excessive urine with sweet taste like honey. Madhumeha is included among the "Ashtamaharoga" (eight major disorders) in CHARAK SAMHITA which indicates the graveness of the diseases. The understanding of Diabetes mellitus in Ayurvedic Medicine dates back to 600-700 BC where in a complete pathophysiology of the disease has been described by Vagbhat .The role of diet, physical activity and genetic in the etiopathogenesis and management of diabetes were described by Charak and Sushruta then and stand out remarkably well in the present era of scientific observations of the disease. Improper food habits, lack of exercise, stress, strain and obesity are the important causative factors that make an individual more prone to develop diabetes. Based on the current trends 425 million individuals all over the world are suffering from Diabetes Mellitus and 629 million individuals all over the world will have Diabetes Mellitus by the year 2045.

Plants have always been a good source of drugs .The beneficial uses of medicinal plants in traditional system of medicine of many cultures are extensively documented.

Several plants have been used as dietary adjuvant and in treating the number of diseases even without any knowledge on their proper functions and constituents. Although numerous synthetic drugs were developed for the treatment of Diabetes Mellitus but the safe and effective treatment paradigm is yet to be achieved.

WHO has recommended the evaluation of traditional plants for the treatment of Diabetes Mellitus as they are effective, non toxic with less or no side effects and are considered to be excellent aspirants for oral therapy.

From the reports on their potential effectiveness against Diabetes mellitus it is assumed that the phytochemicals have a major role in the management of the Diabetes mellitus which needs further exploration for the necessary development of drugs and nutraceuticals from natural resources.

One of the etiologic factors implicated in the development of Diabetes mellitus and its complication is damage induced by free radicals and hence an Anti-diabetic compound with Antioxidant property would be more beneficial.

Therefore, this present work will be concentrated on the Hypoglycemic effect (Experimental) and Antioxidant property of Solanum spirale Roxb.

AIM AND OBJECTIVE

- To evaluate the Hypoglycemic effect of *Solanum spirale Roxburgh* leaves
- To study the Antioxidant property
- To evaluate toxicity if any in leaves
- To find out an effective, low cost and safe and safe remedy to combat the disease.

MATERIALS AND METHODS

a) CHEMICAL AND KITS

All the chemicals used in this study were of analytical grate and purchased from Sigma Aldrich, USA. Biochemical kits were procured from Accurex, India. Solvents used for the extraction were obtained from Merck, Germany.

b) COLLECTION OF PLANT MATERIAL

The leaves of *Solanum spirale Roxb*. were collected on the month of August 2019 from Gune village, East siang district of Arunachal pradesh. The collected plant leaves were dried under shade.

C) PREPARATION OF PLANT EXTRACT

The air dried leaves of *SolanumspiraleRoxb*. weighing 100gm, were soaked in 500 ml of 95% ethanol in a round flask for 24 hrs. The process of extraction was done by reflux

condensation method using soxhlet apparatus at 60-80 degree Celsius for 9 hours at the state Drug testing laboratory (AYUSH), Jalukbari, Guwahati-781014. The extract was concentrated by distillation apparatus till a syrupy consistency was obtained. Finally, the extract was put in a china dish and evaporated at 40-60 degree Celsius temperature in a water bath untill 13.5 gm of dark green colour semisolid extract was obtained.

PLAN OF STUDY

The present study was conducted in two parts;

a) In vitro and

b) In vivo

In vitro antioxidant study

DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging assay

DPPH is a free radical compounds and has been commonly used to estimate the scavenging capacity of antioxidants (Huanh et.al. 2011). Ascorbic acid was used as a standard for the DPPH radical scavenging Assay. The DPPH scavenging activity of *Solanum spirale Roxb*. Ethanolic extract at different concentrations were taken and recorded. The plant extract showed the highest scavenging activity. The ethanolic extract of *Solanum spirale Roxb.* Leaves showed higher radical scavenging activity than the standard Ascorbic acid. The absorbance was recorded of 517 nm. The ethanolicextracts of *Solanum spirale Roxb*. Leaves showed strong scavenging activity and its power increased in accordance with the concentration.

PMD

Phosphomolybdate Assay. This method is used to analyse the cumulative antioxidant capacity based on the reduction of phosphate. MO (VI) to phosphate MO (V) by the sample and subsequent formation of bluish green coloured phosphate MO (V) complex at acid PH. Plant extract was taken as 100 µg/ml. different concentrations of the extracts viz. 10,25,50,75 and 100 µg/ml were taken. Then 250 µl of PMD reagent was added followed by vortex and incubated for 90 mins at 90^{0C} and cooled at room temperature. Absorbance increased measured at 695 nm.

% Inhibition = {(T-C) / T} x 100

T = Sample absorbance C = Control absorbance

ACUTE TOXICITY TEST

This study was performed to select optimum doses to evaluate the Anti diabeticeffect of *Solanum spirale Roxb*. leaves extract.

Wistar albino rats weighing 150 -250 gms of either sex (6animals) were randomly selected for this study and divided into two groups, group A and group B consisting of 3 animals in each group. They were fasted overnight with free access to water but not food before administration of the extract. And the next morning single dose of *Solanum spiral eRoxb*. ethanolic extracts at 2000mg/kg bodyweight and 4000mg/kg body weight were orally administered to the 3animals of each group i.e group A and group B respectively as per OECD guidelines. The animals were observed continuously for first 2 hours and then occasionally for 4 hours for any gross change in behavioural locomotors activity or any other symptoms of toxicity and finally for overnight mortality. If mortatility was observed even in one single animal, experiment was to be repeated again with same dose to confirm the toxic doseif mortality was observed then again experiment was to be carried out with lower doses(300,50 and 5 mg /kg body weight).

IN VIVO STUDYEVALUATION OF ANTI DIABETIC PROPERTY *OF SOLANUM SPIRALE ROXBURGH*

Adult male wistar albino rats weighing 150-250 gms were procured from animal house of Institute of Advanced Study in Science and Technology (IASST), Guwahati, Assam, India. All the animals were acclimatized for the laboratory environment before conducting the experiments and the animals were fed with standard pellet diet (provimi, India) and supplied with sufficient water. All the experiments were approved by Instiutional animal Ethical Committee(IAEC) of IASST (IASST/IAEC/2018-19/20) and performed in accordance to CPCSEA guidelines. Diabetes was induced to overnight fasted rats by single intraperitoneal (IP) injection of 55mg/kg bodyweight Streptozotocin (STZ) in citrate buffer (pH 4.35). After 3 days of STZ injection diabetes was confirmed by measuring the fasting blood glucose (FBG) levels. Animals having FBG more than 250mg/dl was confirmed as diabetic and included in the study. Normal control animals were given standard diet and water

ANIMALS GROUPING AND DRUG TREATMENT

Animals were randomly divided into 5 groups containing six rats in each group. The drug treatments were given as following:

- Group 1= Normal Control animals

- Group 2= Diabetic animals
- Group 3= Diabetic +standard drug Glibenclamide(10 mg / kg body weight)
- Group4= Diabetic + *SolanumspiraleRoxb*. ethanolic extract(200mg/kg bodyweight)
- Group 5= Diabetic + *SolanumspiraleRoxb*. ethanolic extract(400mg/kg bodyweight)

After the induction of Diabetes mellitus with streptozotocin, the diabetic rats were treated with plant extract and standard drug orally once for 14 days. The tails of the wistar albino rats was nipped gently with sterilized blade and one drop of fresh venous blood was squeezed out and placed on the sample cell of glucometer strip, FBS reading was taken at morning 8am on 0^{th} day, 8^{th} day and 15^{th} day of the study. The blood glucose concentration was measured by using glucometer and noted. After completion of 14 days of treatment, the animals were sacrificed on 15^{th} day, the animals were anesthetized by chloroform and diethyl ether inhalation, blood was collected by cardiac puncture and serum was separated by using centrifugation at 400rpm for 10 minutes for bio chemical estimation

BIOCHEMICAL ESTIMATION

All biochemical estimations were done by using fully automated analyzer, Sr.ALT, Sr.AST, Sr.Creatinine, Sr.LDL, Sr.HDL, Triglycerides, total Cholesterol.

STATISTICAL ANALYSIS

The data was statistically analysis by one way ANOVA test. The obtained information was analysed statistically in terms of mean score (x), standard deviation (SD), standard error (SE). Paired „t" test and unpaired „t" test was carried out at the level of 0.02, 0.05, 0.001 of p levels. The results were interpreted as-

$P<0.05$, <0.02, <0.01 significant $P<0.001$ highly significant $P>0.05$ insignificant difference

OBSERVATION AND RESULT

In vitro Anti- oxidant activity of *Solanum spirale Roxb.*ethanolic extract. DPPH (2,2-diphenyl-1-picryhydrazyl) radical scanenging assay:

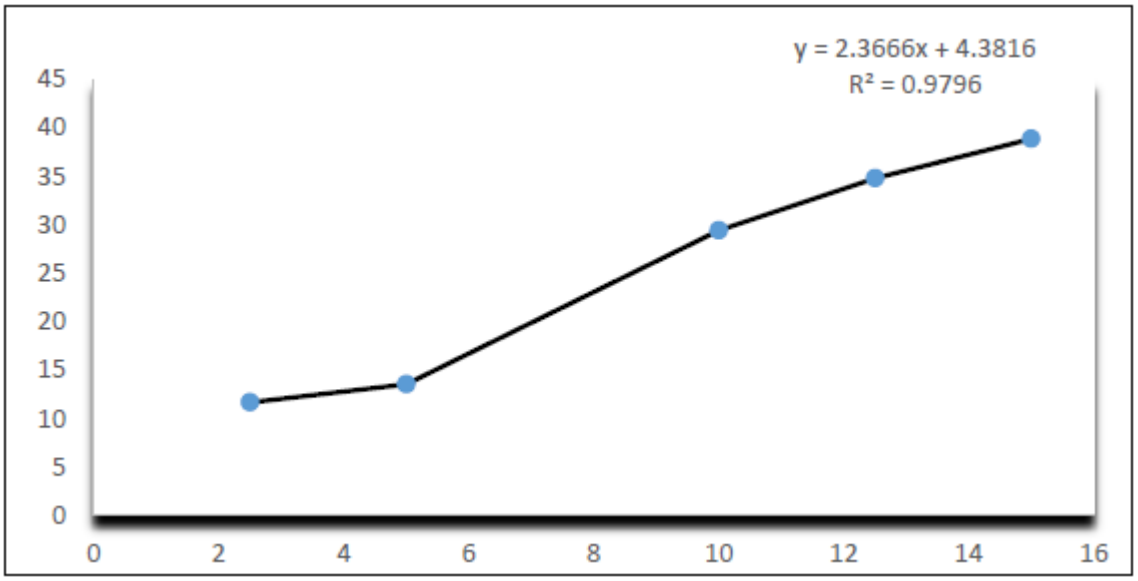
DPPH is a free radical compound and has been commonly used to estimate the scavenging capacity of antioxidants (Hung et.al. 2011). Ascorbic acid was used as standard for the DPPH radical scavenging assay. The DPPH scavenging activity of *solanum spirale Roxb*. Ethanolic extracts at different concentrations were taken and recorded as shown in fig.(1)below. The plant extract showed the highest scavenging activity. The ethanolic extract of *Solanum spirale Roxb.* leaves showed higher radical scavenging activity than the standard ascorbic

acid.

Percentage inhibition in various concentration of standard ascorbic acid. The absorbance was recorded at 517 nm.

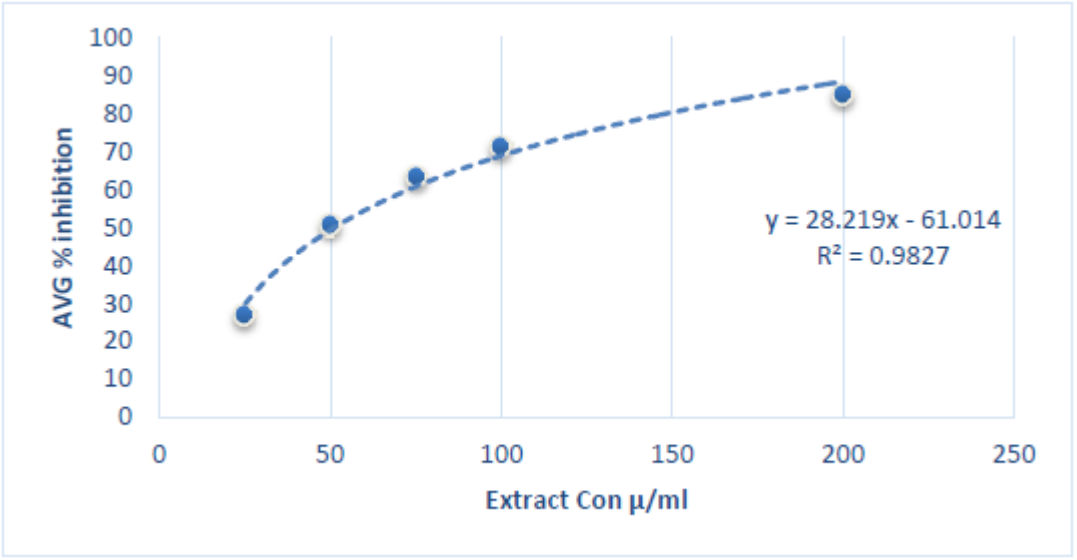
Slandered curve of Ascorbic Acid for DPPH assay

Slandered curve of ascorbic acid for DPPH assay.



Y= 2.366 x + 4.3816

IC_{50} of standard ascorbic acid = 19.275 □g/ml Plant extract for DPPH assay

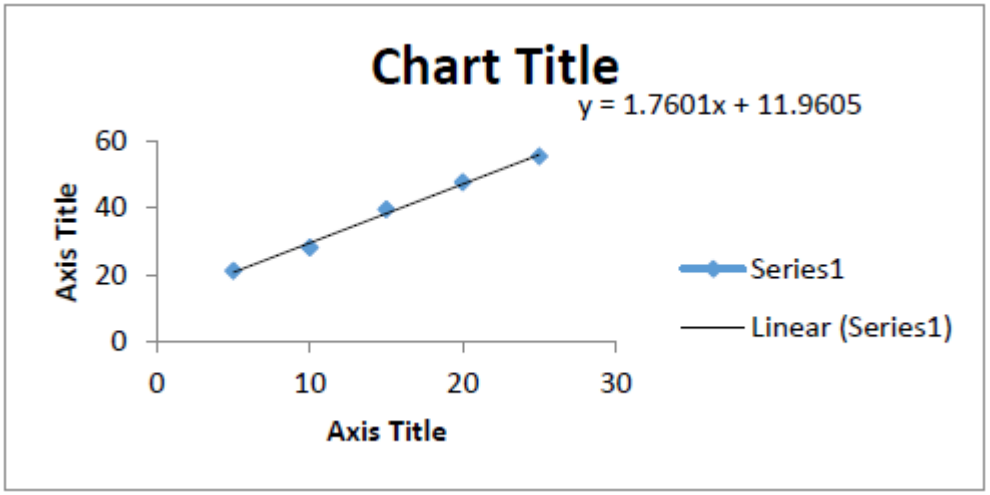


Y= 28.219x – 61.014

IC_{50} of plant extract = 3.934 □g/ml

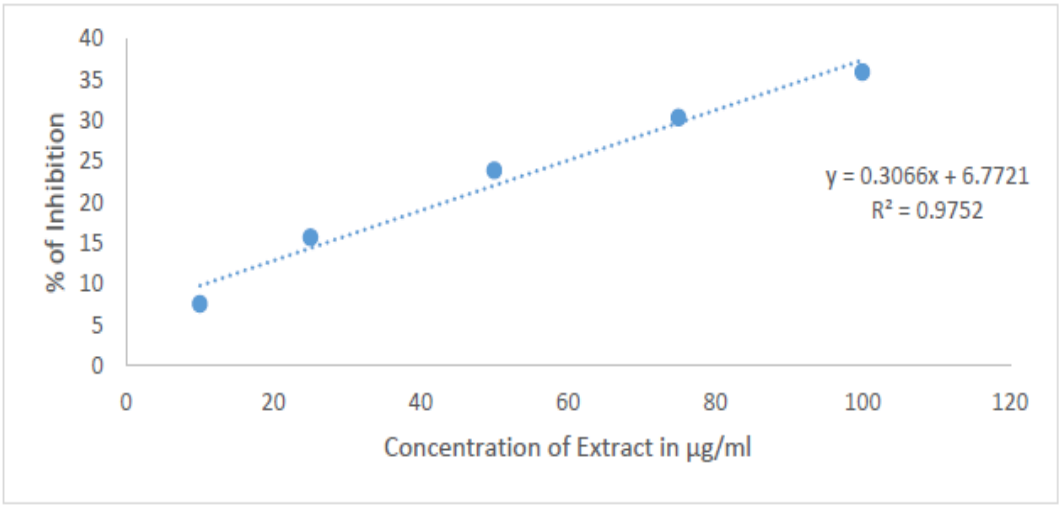
PMD: Phosphomolybdate assay, for this assay plant extract of various concentration were taken and recorded as shows in table below Ascorbic acid was taken as standard and the absorbance was recorded at 695nm.

Activity percentage in various concentration of ascorbic acid.



y = 1.7601x + 11.9605

IC_{50} of ascorbic acid = 21.612124 □g/ml PMDA assay of Plant extract



y = 0.306x + 6.7721

IC_{50} of plant extract = 141.267 □g/ml

ACUTE TOXICITY TEST

This study was performed to select optimum doses to evaluate the anti- diabetic properties of *Solanum spirale Roxb*. leaves extract. In this study wistar albino rats of either sex (6 animals) were randomly selected and divided in two groups, group A and group B. 3 animals in each group. The animals were fasted overnight with free access to water before administration of

the extract. Next morning animals were orally administered aqueous ethanolic extract of *Solanum spirale Roxb*. leaves in doses of 4000 mg/kg body weight and 2000 mg/kg body weight as per OECD guidelines. The tested dose was well tolerated. It produced no signs of toxicity, no any abnormality was detected on the behavior of the animals. No mortality was found even after overnight observation. Since the ethanolic extract of *Solanum spirale Roxb*. leaves was safe at the highest dose, no further test with lower doses 200 mg/ kg body weight and 400 mg/kg body weight were selected for further in vivo experiment.

Anti diabetic effect of ethanolic extract of *Solanum spirale Roxb*.

a) On serum glucose: Streptozocin injection (55 mg/kg body weight) caused significant increase in FBG levels in all the animals (group 2 to group 5) on the 3rd day as compared to animals in normal control group (group 1). Further animals treated with ethanolic extract of *Solanum spirale Roxb*. at dose of 200 mg/ kg body weight, 400 mg/kg body weight and Glibenclamide (10 mg/kg bodyweight) showed very significant ($p<0.01$) decline in FBG in comparison to diabetic control animals (group 2). The plant extract at a higher dose was more effective when compared to lower dose. The order of anti-diabetic activity of the test substances are Glibenclamide (10 mg/kg B/W)>*Solanum spirale Roxb*.ethanolic extract (400 mg/kg B/W)>*Solanum spirale Roxb*.ethanolic extract (200mg/kg B/W) extract

PAIRED T TEST

Table 1: Paired t test of group 4 (*Solanum spirale Roxb.* extract (200 mg) BT (0th day), AT (8th day)

$\bar{BT}$	$\bar{AT}$	SD_{BT}	SD_{AT}	SE	t_5
224.67	174.33	38.51	44.87	11.667	4.3143

P<0.01, R- very statistically significant t5 = 4.3143, p<0.01, result is very statistically significant. It implies that the trial drug has reduced the blood sugar after one week of administration.

Table 2: Paired t-test of group 4 *(Solanum spirale Roxb.* extract (200 mg) BT (0th day), AT (15th day)

$\bar{BT}$	$\bar{AT}$	SD_{BT}	SD_{AT}	SE	t_5
224.67	119.67	38.51	17.81	15.659	6.7055

P<0.01, R- very statistically significant, t_5= 6.7055, p<0.01, result is very statistically significant. It implies that the trial drug has reduced the blood sugar after two weeks of administration.

Table 3: Paired t-test of group 5 *(Solanum spirale Roxb*. extract (400 mg) BT (0th day), AT (8th day)

$\bar{BT}$	$\bar{AT}$	SD_{BT}	SD_{AT}	SE	t_5
303	234.l17	136.41	113.32	14.015	4.9113

P<0.01, R – very statistically significant, t_5 = 4.9113, p<0.01, result is very statistically significant. It implies that the trial drug has reduced the blood sugar after one week of administration.

Table 4: Paired t-test of group 5 (*Solanum spirale Roxb*. extract (400 mg) BT (0th day), AT (15th day)

$\bar{BT}$	$\bar{AT}$	SD_{BT}	SD_{AT}	SE	t_5
303	180.5	136.41	51.86	37.18	3.2948

P<0.05, R- statistically significant, t_5 = 3.2948, p<0.05, result is statistically significant. It implies that the trial drug has reduced the blood sugar after two weeks of administration.

Table 5: Paired t test of group 4 *Solanum spirale Roxb*. (200 mg extract in 0th, 8th and 15th day).

	$\bar{}$	SD	SE	T	p	Remarks
BT	224.67	38.51	11.667	4.3143	<0.01	Highly significant
8th Day	174.33	44.87				
15th day	119.67	17.81	15.659	6.7055	<0.01	Highly significant

Between before treatment and 8th day of treatment t_5= 4.3143, p<0.01 and between before treatment and 15th day of treatment t_5 = 6.7055, p<0.01 hence result are highly significant, it signifies that the trial drug of *Solanum spirale Roxb*. extract (200 mg/kg body weight) on FBS after 7 days of treatment is effective.

Table 6: Paired t-test of group 5 (*Solanum spirale Roxb*. 400 mg extract in 0th, 8th, and 15th day).

	$\bar{}$	SD	SE	t	p	Remarks
BT	303	136.41	14.015	4.9113	<0.01	Highly significant
8th Day	234.17	113.32				
15th day	180.5	51.86	37.18	3.2948	<0.05	Significant

Between before treatment and 8th day of treatment t_5= 4.9113, p<0.01 and between, before treatment and 15th day of treatment t_5= 3.2948 p<0.05 hence result are significant. It signifies that the trial drug of *Solanum spirale Roxb*.extract (400 mg/kg body wt.) on FBS after 7 days of treatment is effective.

UNPAIRED T-TEST

Unpaired t-test of group 4 with group 1, group 2, group 3, group 5

Unpaired t-test were done between group 4, group 1, group 2, group 3 and group 5. In between group 4 and group 1, t_{10}= 6.6419 $p<0.001$ which is highly significant which means that group 4 (200 mg/kg B/W plant extract) is more effective in controlling the blood sugar level than group 1 (normal control group).

In between group 4 (200 mg/kg B/W plant extract) and group 2 (diabetic group) t_{10}= 4.8936, $p<0.001$, which is highly significant. It implies that group 4 is more effective in controlling the blood sugar level than group 2.

In between group 4 (200 mg/kg B/W plant extract) and group 3 (standard drug glibenclamide 10mg/kg B/W), t_{10}= 0.6945, $p>0.05$ which means the difference is not statistically significant which implies that both the groups have equal effect in control of blood sugar level.

In between group 4 (200 mg /kg B/W plant extract) and group 5 (400 mg/kg B/Wplant exract), t_{10}= 0.4338, $p>0.05$ which means the difference is not significant. It implies that both groups have equal effect in control of blood sugar level.

Table 15: Unpaired t-test of group 5 with group 1,2,3 and 4

Unpaired t-test were done between group 5, group 1, group 2, group 3 and group 4. In between group 5 and group 1 t_{10}= 3.2715, $p<0.01$ which means result is highly significant. It implies that group 5 is more effective in controlling blood sugar than group 1.

In between group 5 and group 2 t_{10}= 2.7861 $p<0.05$, hence the result is significant. It means group 5 is more effective in controlling blood sugar level than group 2.

In between group 5 and group 3 t_{10}= 0.0634 $p>0.05$ which means result is not significant. It implies that both groups have equal effect in control of blood sugar level and the difference is not statistically significant.

In between group 5 and group 4 t_{10}= 0.4338 $p>0.05$ which means the result is not significant. It implies that both groups have equal effect in control of blood sugar level and the difference is not statistically significant.

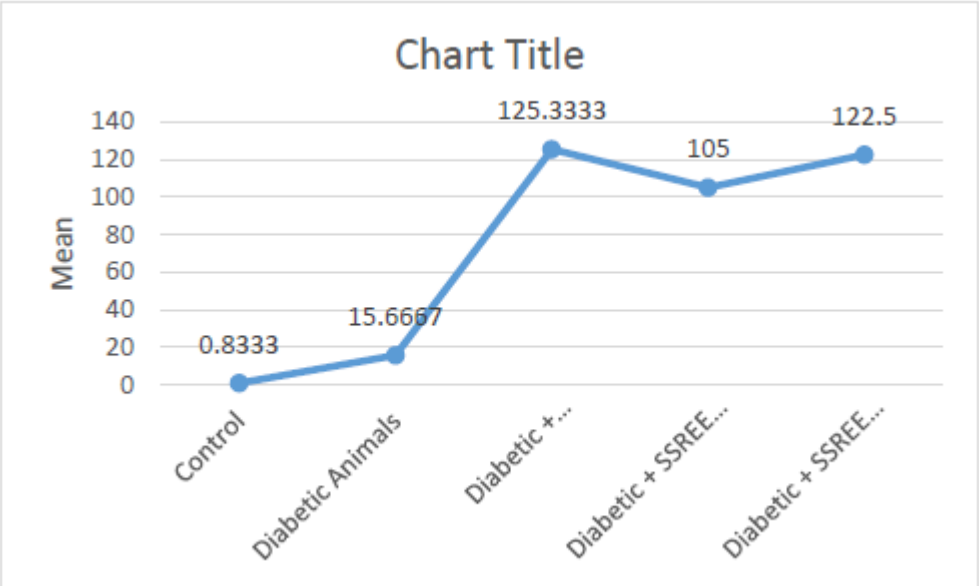
Table 16: One way ANOVA test Summary of Data.

	1	2	3	4	5	Total
N	6	6	6	6	6	30
$\sum$	5	94	752	630	735	2216
Mean	0.8333	15.6667	125.3333	105	122.5	73.867
$\sum^2$	27	4114	112608	73506	131507	321762
Std. Deviation	2.137	60.5926	22.9841	38.3562	91.0709	73.8296

Result Details

SOURCE	SS	df	MS	
Between the groups	88226.4667	4	22056.6167	F= 7.89462
Within the groups	69847	25	2793.88	
Total	158073.4667	29		

The f-ratio value is 7.89462. The p-value is .000294. It implies that out of all the groups. Group 3 has shown the highest efficacy in controlling the blood sugar level (FBS). Further animals treated with *Solonum spirale Roxb*. extract at a dose of (200mg/kg body weight) and (400 mg/kg body weight) and Glibenclamide (10 mg/kg body weight) showed highly significant (P<0.01) decline in fasting blood sugar compared to diabetic control animals (group 2). The plant extract at a higher dose of (400 mg/kg BW) was more effective when compared to lower dose (200 mg/kg body weight). The order of anti diabetic activity of the test substances are Glibenclamide (10mg/kg body weight) >*Solanum spirale Roxb*.ethanolic extract 400mg/kg body wt.) >*Solanum spirale Roxb*. ethanolic Ext. 200mg/kg body wt.



Effect *of Solanum spirale Roxb.* ethanolic extract on Serum Biomarkers

Serum creatinine, Sr. AST, Sr. ALT level estimation were done to evaluate the adverse effect of the test drug if any. As per results shown in fig. 5 (a) all values are within normal range and remained comparable to that of the normal control group. From the observations, no unpleasant effects on the experimental animals at the given dosages of 200 mg/kg body weight and 400 mg/kg body weight were seen.

It implies that the test drug has no toxicity and there is no any unfavourable outcome of the experimental trial drug on the general metabolism of the experimental rats after fourteen days of treatment with test drug. A slight increase in the serum levels were found in STZ induced diabetic animals in comparison to the normal control groups and diabetic treated groups.

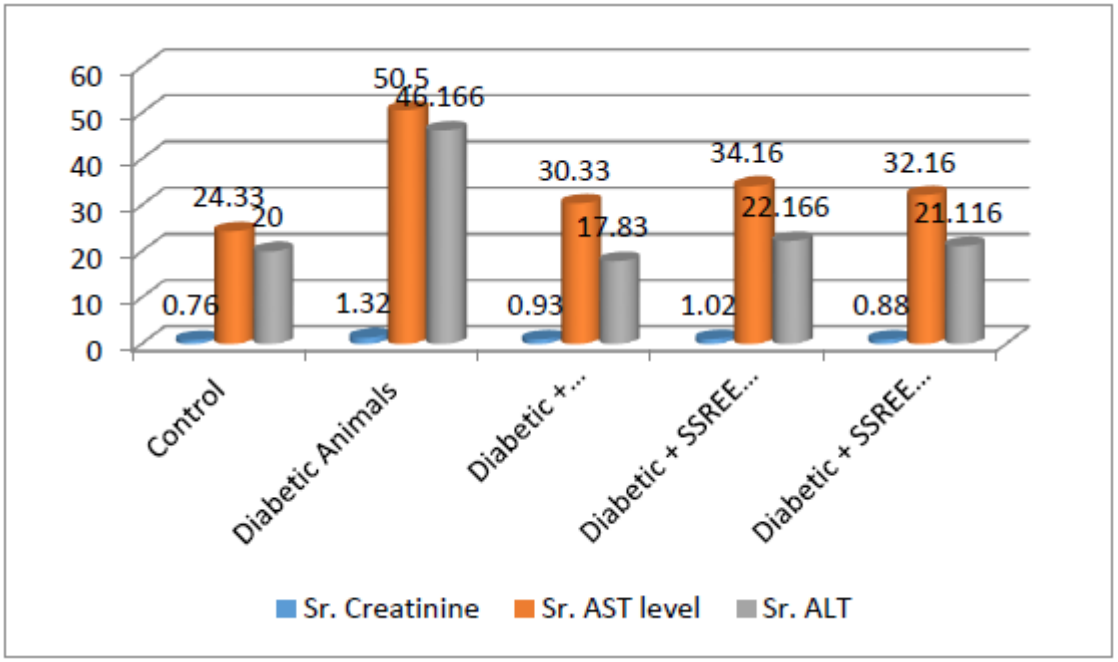


Fig. 5 (a)

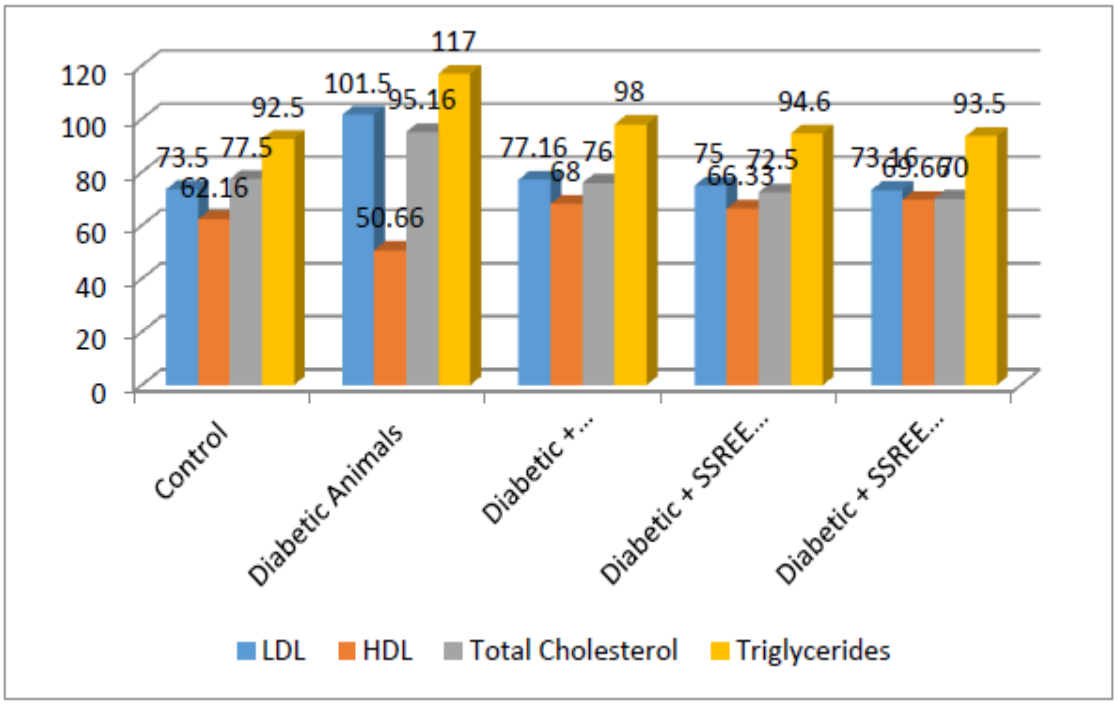


Fig. 5 (b)

LDL, HDL, total cholesterol, triglycerides level estimation were done to evaluate the adverse effect of the trial drug if any as per result in fig. 5 (b) all the values are within normal range. After 14 days of treatment and remained comparable to that of the normal control group. It implies that the test drug has no any adverse effect on the lipids. From the observations, no unpleasant effects or abnormalities were seen in the experimental animals at the given dosages of 200 mg/kg body weight and 400 mg/kg body weight.

CONCLUSION

The conclusions drawn from the whole study is presented below –

- In vitro study: antioxidant activity of the ethanolic extract of *Solanum spirale Roxb.* leaves was evaluated by DPPH Assay and PMD Assay with Ascorbic acid taken as standard, the plant extract showed higher radical scavenging activity than the standard Ascorbic acid which indicates that the plant extract is a good source of natural antioxidants.
- Toxicity test: Oral toxicity test of the plant extract was carried out in 6 wistar albino rats. No any abnormal behaviour and mortality were observed in the period of the study.
- In vivo study: The plant extract at both the experimenting doses group 4 (200 mg/kg body weight) and group 5 (400 mg/kg body weight) have the power to reduce blood sugar level. The plant extract at higher dose (400 mg/kg body weight) showed better results

compared to group 4 (200 mg/kg body weight) and group 1 (normal control animals). It might be due to the presence of phytoconstituents like flavonoids glycosides, alkaloids, saponins, tannins in the plant extract.

Recommendation for future studies

1. The present experimental study was done on animals only, clinical studies should be done on human beings too for better understanding of the clinical properties of *Solanum spirale Roxb*. leaves.
2. As the present study was conducted within a limited time period, further additional studies may be conducted on this formulation for longer duration and with larger sample size.
3. Identification and isolation of active phytochemical constituents of *Solanum spirale Roxb*. leaves and their possible mechanism of action for lowering blood glucose may be useful in developing new drug for treatment of diabetes and its complications in the coming future.

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RESEARCH PAPERS

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