ANTI-HYPERGLYCEMIC EFFECT OF *GLIRICIDIA SEPIUM* LEAF EXTRACT ON ALLOXAN INDUCED DIABETIC RATS

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Abstract: The aim of this study is to investigate the Antidiabetic activity of leaves extract of *Gliricidia sepium* (EEGSL) in Alloxan-induced diabetic rats. The animals were grouped as control (distilled water and Glibenclamide) and experimental (250, 333, and 500 mg/kg body weight). Type 2 DM was induced by a single intraperitoneal injection of alloxan at 120 mg/kg body weight. The blood sugar levels were estimated by employing the glucose kit from Aspen diagnostics and measured in Semi auto analyzer. The lipid profile and histopathological studies of experimental animal were studied. The results of three dose levels and Standard treatment showed downstream levels from hyperglycemic condition on blood glucose level ($P<0.01$) and restored the normal functioning of the various organ functions and brought the lipid levels from hyperlipidemia due to diabetes. The extract has shown notable therapeutic effects which can be seen clearly in regeneration capacities histopathological changes of the pancreas in alloxan-induced diabetes.

Keywords: Alloxan, Antidiabetic, Flavonoids, Tannins, *Gliricidia sepium*, Glibenclamide

INTRODUCTION:

A study of reports indicates that diabetes in India was fairly known and well-conceived as an entity in India as the term "Madumeha". It' is a disease in which a patient experiences high blood sugar levels so that it can be excreted in urine and sweat also and then the patient is considered hyperglycemic. In daily life consuming the raw fruits and juice of plants as folklore medicines have shown a reduction in the level of blood glucose. The elevated blood sugar level was later termed diabetes mellitus (DM). It is one of the most common metabolic disorders in India. As per the existing survey data, 1.3% of the population living and suffering from this disease globally and it affects annually by 6%. It is estimated that the mortality is about 300,000 each year are attributed to diabetes. The management of diabetes is a major challenge in recent years in this regard the insulin and other oral hypoglycemic agents still play a major role as there are so many limitations are there for existing antidiabetic drugs, hence there is a scope for the development of more efficacious anti-diabetic drugs. [1] The incidence of type-2 DM is more than that of type-1 DM. There are lot many side effects related to the therapy by insulin and the existing medicines from the synthetic origin, research for efficacious and attentive antihyperglycemic plant drugs is going worldwide. [2] Many Scientific reports reveal that the use of medicinal plants to treat various ailments is common in developed and developing countries. As per World Health Organization reports, 80% of the world population believes mainly in herbal medicinal therapies which make use of plant extracts as bioactive substances. [3] The selected plant possessed many activities some of which include sedative effects, as an insecticide, rodenticide, etc. There are some studies already to determine the antibacterial property of Gliricidia root bark extracts. The antidiabetic activity of this particular part of the leaf has not been done or documented in any scientific reports. Hence, based on the literature and phytochemical data of the plant the present investigation has been planned to rule out the possibilities of the anti-diabetic property.[4] Diabetes mellitus is a metabolic disorder accompanied with the multifactorial etiology which is characterized by chronic hyperglycemia [5], abnormalities in lipoproteins[6], elevated basal metabolic rate[7], low antioxidant property and high oxidative stress[8] damage to pancreatic beta cells due to oxidative stress.[9] Given the above scientific data, the present project task was undertaken to evaluate the anti-diabetic property of Gliricidia sepium leaf extract on Alloxan-induced diabetic rats.

MATERIALS AND METHODS:

Plant

The Gliricidia Sepium leaves were collected in the midsummer season from the farm areas of Harapanahalli town. Professor K. Prabhu, Department of Pharmacognosy, S.C.S College of Pharmacy, Harapanahalli authenticated and certified the plant and leaves.

Preparation of extract

The plant leaves were collected and shade dried at room temperature. The dried plant leaves were coarsely powdered and then the coarse powder is packed in the column and subjected for successive extraction with various solvents based on polarity and also with distilled water. The products after soxhlet extraction were taken out and kept in a rota flash evaporator to remove the moisture content under pressure.

Experimental animals

Wistar rats (180-200 g) of both sexes, female swiss albino mice (20-25 g) were used in the experiments. The animals were supplied by Sri Venkateshwara enterprises Bangalore (CPCSEA Reg. no:-157/1999/CPCSEA). Soon after procurement, the animals were kept for 7 days for acclimatization. The standard conditions as per CPCSEA such as temperature (26 + 20C), relative humidity (45-55%), and 12 h dark/light cycle were maintained. All the animals were fed with a standard pellet diet and water was allowed ad libitum under strict hygienic conditions. Ethical clearance for this experiment was obtained before the initiation of the work from the Institutional Animal Ethics Committee (IAEC). SCSCP/583/4/2011-12 before the beginning of the project work.

Acute toxicity studies [10]

The acute toxicity for ethanolic extract of Gliricidia sepium was established in female Swiss albino mice. The mice were left for fasting allnight prior to the experiment, the method for fixed-dose was approved as per OECD Guideline No. 423 of CPCSEA. For each test dose group of three mice were taken.

The doses for extract were selected as per OECD guidelines No. 423 and the methods for fixed-dose are mentioned below.

70% Ethanolic extract 250 mg/kg (1/10th dose from cutoff value)
333 mg/kg (1/7.5th dose from cutoff value)
500 mg/kg (1/5th dose from cutoff value)

Experimental protocol [11-13]

Blood glucose levels were evaluated after depriving food for about 18h with free access to drinking water. Diabetes was induced or produced by a single i.p. injection of alloxan monohydrate at a dose of 120mg/kg and is obtained from (s.d. fine-chem. Ltd., Mumbai, India) which is prepared sterile saline. The rats with glucose levels> 250 mg/dl were separated after 5 days of injection and segregated into different groups of 6 rats in each for the anti-hyperglycemic study. The treatment (p.o.) was started from the same day of selection and separation of hyperglycaemic rats except for normal control and diabetic control groups for 10 days. Bodyweight and blood glucose levels are the two parameters that are taken into consideration and were estimated on three different time schedules i.e 5th, 10th, and 15th day of the treatment. On the last day of completion of the experiment i.e 15th day, the blood samples were collected by tail vein under light anesthetic conditions and serum is separated and biochemical parameters were estimated.

Histopathology [14]

The isolated pancreatic tissue from the animal was separated after sacrificing the animal thus the tissue was washed in ice-cold saline without any delay. The desired section of pancreatic tissue was subjected to a 10% neutral formalin fixative solution for histopathological examination. After the above procedure tissues were embedded in paraffin, sections were cut at the diameter of 5µm and were stained with hematoxylin and eosin.

A. Photomicrograph of normal rat pancreas showing normal acinar cells and normal Islet of Langerhans cell groups (White arrow) and varying sized capillaries (thin black arrow).
B. Sections studied showed predominantly acinar cells with occasional very small-sized clusters of Islet of Langerhans cells. Photomicrograph of this rat pancreas showing areas of necrosis (white arrow), degenerative changes in the form of karyolysis, karyorrhexis, and pyknosis (thin black arrow), and sparse capillaries.

C. Photomicrograph of pancreas showing a regenerative cluster of Islet of Langerhans cell groups (white arrow) and capillaries with normal surrounding acinar cell (thin black arrow).
D. Section studied shows intact acinar cells with their intralobular ducts. The number of islets in each lobule appears adequate. The selected part of islet cells consists of some degenerated beta-cells (Long-Arrow) and normal Beta-cells (Short-Arrow), while the region is with alpha-cells (Short-Arrow).
E. The selected section observations shows fibrovascular septa separating the pancreatic lobules. The center of islet cells consists of a few degenerated beta-cells (Long-Arrow) and normal beta-cells (Short-Arrow), while the periphery comprises Alpha-cells (Short-Arrow).
F. Section studied shows intact acinar cells with their intralobular ducts. The center of islet cells consists of a quantitative increase in Beta-cells (Long-Arrow), while the periphery comprises Alpha-cells (short Arrow). There are seen some congested blood vessels (Short-Arrow) between the islet cells.

Statistical analysis

The parameters selected like body weight, glucose levels, and biochemical markers were expressed as mean ± standard error of the mean (S.E.M.) and they are analyzed by applying the ANOVA methods followed by Dunnet's multiple comparison test.

RESULTS

Table1. Effect of 70% EEGSL on body weight

Treatment	Body weight of the animal (gm)			
	Initial	5th day	10th day	15th day
Normal	234.2±8.207	230.0±7.638	225.8±6.50	217.5±5.737
Alloxan (120 mg/kg)	240.0±6.191	179.2±5.833	185.0±5.000	153.3±4.014
Glibenclamide (10 mg/kg)	231.7±5.270	188.3±4.773	206.7±6.667	200.8±4.167***
Low dose(250 mg/kg) EEGSL	217.5±8.539	186.7±8.131	191.7±3.575	165.0±3.416
Median Dose(333 mg/kg) EEGSL	230.8±4.729	180.8±3.962	195.0±3.416	179.2±2.386**
High dose(500 mg/kg EEGSL)	222.5±7.042	205.0±7.188	201.7±3.073	185.0±3.651***

Values are Mean ± S.E.M. (n=6); Significance values are ***P< 0.001; **P < 0.01 and *P <0.05. positive Control group vs all groups.

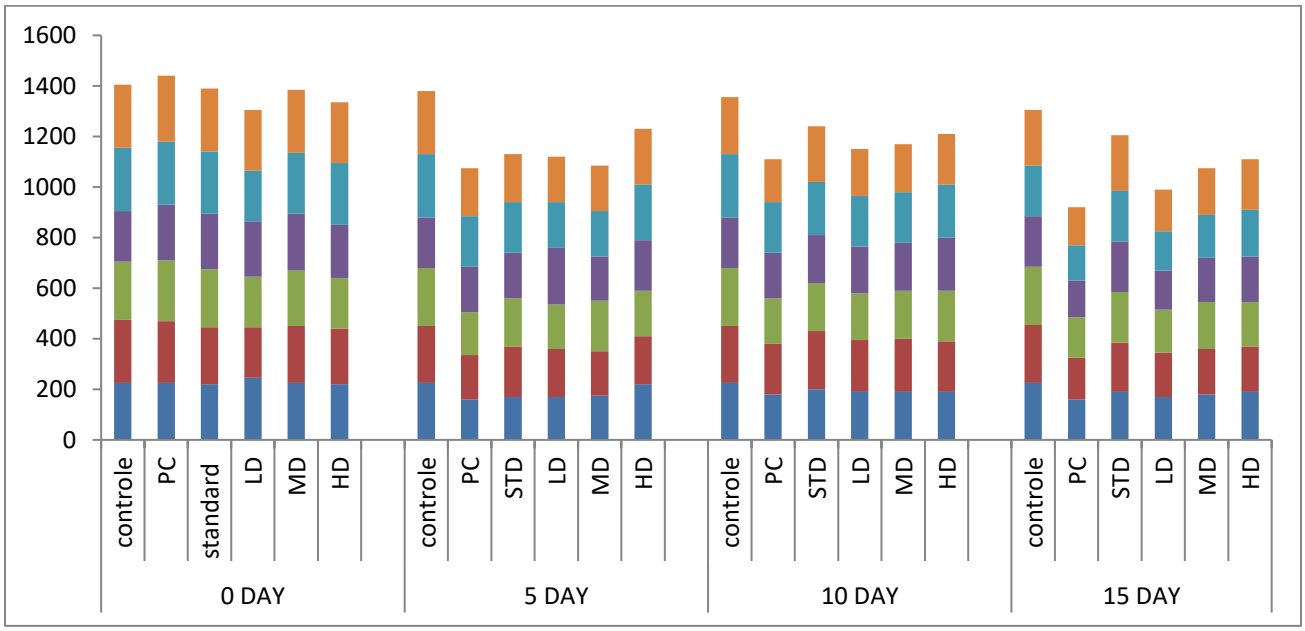


Figure 1. Influenceof 70% EEGSL on body weight

Table.2 Effect of 70% EEGSL on Fasting Serum Glucose (mg/dl) in alloxan induced diabetic rats

Treatment	**Fasting Serum Glucose (mg/dl) Mean ± S.E.M)**			
	Initial	**5th day**	**10th day**	**15th day**
Normal rats	164.7±7.375	384.1±9.555	167.4±4.845	93.35±8.796
Vehicle	252.8±24.17	241.9±41.61*	313.1±17.99	175.1±6.349
Glibenclamide (10 mg/kg)	258.7±33.50	221.2±37.42*	167.4±24.53***	78.54±9.077***
Low dose(250 mg/kg) EEGSL	214.4±25.48	204.7±18.53**	181.7±4.288**	111.1±3.381***
Median dose (333 mg/kg) EEGSL	198.7±18.63	180.8±3.962	203.6±20.15**	100.6±10.87***
High dose(500 mg/kg) EEGSL	348.3±48.94	205.0±7.188	240.1±31.11*	91.09±3.006***

Values are Mean ± S.E.M. (n=6); Significance values are ****P*< 0.001; **P<0.01 and *P < 0.05. Diabetic Control group vs all groups

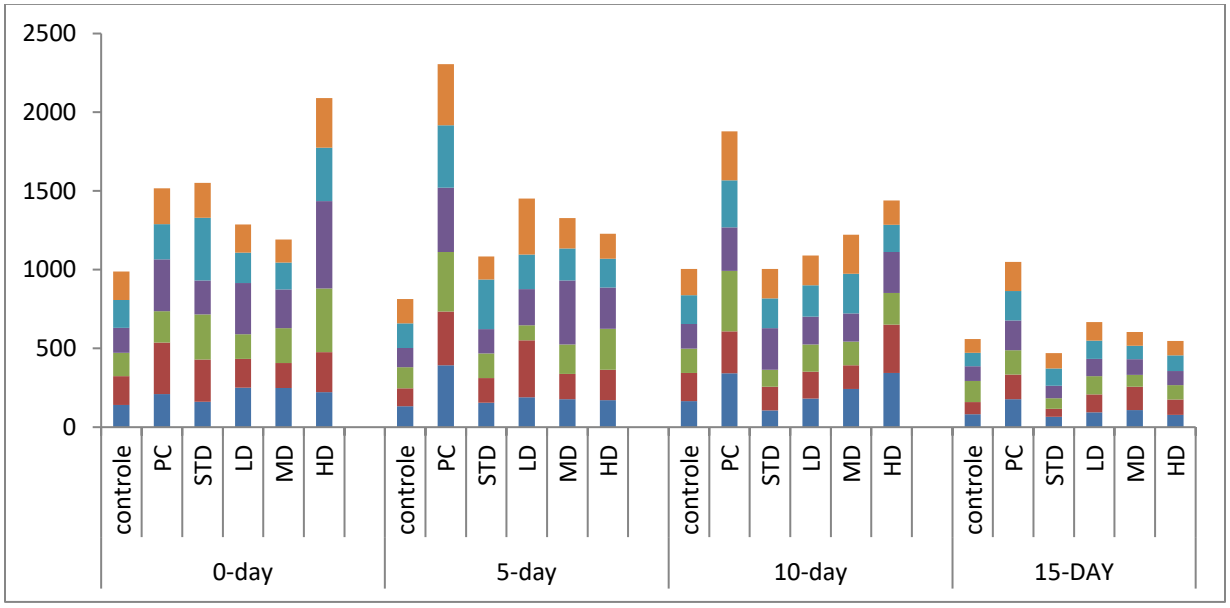
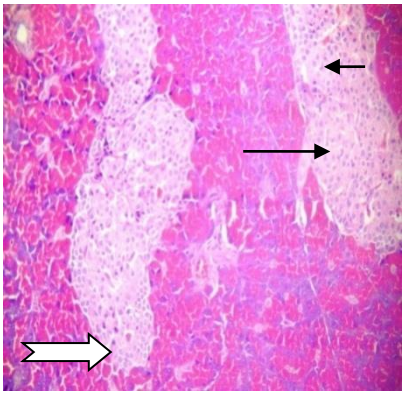


Figure 2. Influence of 70% EEGSL on Fasting Serum Glucose (mg/dl) in alloxan induced diabetic rats

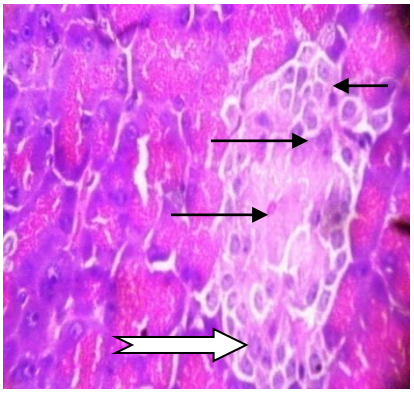
Table 3. Effect of 70% EEGSL on biochemical parameters in alloxan induced diabetic rats

Groups	Dose (mg/kg)	Serum protein (gm/dl)	Serum urea (mg/dl)	Serum creatinine (mg/dl)	Serum cholesterol (mg/dl)	Pancreas weight gm	Hepatic glycogen test (mg/dl)	Tri Glycerides (gm/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)
NC	Normal rats	9.337± 0.309	27.04± 8.559	0.3680± 0.023	36.23± 2.900	0.1982± 0.017	2.411± 0.405	0.1982± 0.017	2.411± 0.405	48.73± 1.940	0.0561± 0.004
PC	Allaxon (120mg/kg)	4.918± 0.155	164.0± 13.94	0.8155± 0.065	84.78± 3.090	0.7230± 0.057	7.450± 0.419	0.7230± 0.057	7.450± 0.419	52.62± 2.523	0.4107± 0.066
STD	Glibenclamide (10mg/kg)	12.18± 0.690***	54.92± 9.773***	0.3503± 0.027***	56.28± 3.736***	0.2757± 0.015***	2.867± 0.241***	0.2757± 0.015***	2.867± 0.241***	72.77± 5.774***	0.2422± 0.06***
LD	250mg/kg EEGSL	9.687± 0.387***	93.63± 2.238***	0.5402± 0.030*	66.81± 2.949**	0.4462± 0.042***	4.850± 0.201***	0.4462± 0.042***	4.850± 0.201***	39.50± 6.99	0.3219± 0.045*
MD	333mg/kg EEGSL	8.672± 0.162**	109.4± 7.609*	0.5183± 0.070*	63.34± 1.395***	0.4802± 0.031**	4.837± 0.084***	0.4802± 0.031**	4.837± 0.084***	50.74± 1.479**	0.3155± 0.032*
HD	500mg/kg EEGSL	3.730± 0.251	89.85± 6.321**	0.4852± 0.038**	62.17± 0.678***	0.5090± 0.019*	4.800± 0.364***	0.5090± 0.019*	4.800± 0.364***	50.93± 2.085***	0.3842± 0.057*

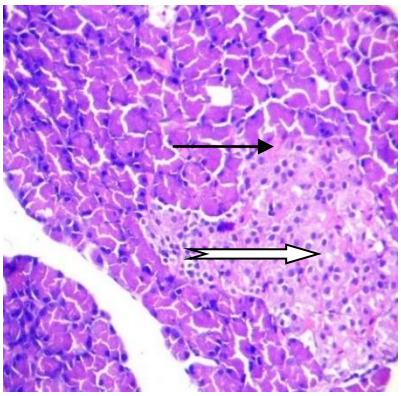
Values are Mean ± S.E.M. (n=6); Significance values are ****P*< 0.001; **P<0.01 and *P < 0.05. Diabetic Control group vs all groups



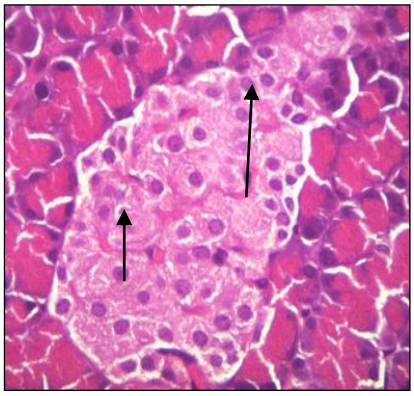
A. Normal Control



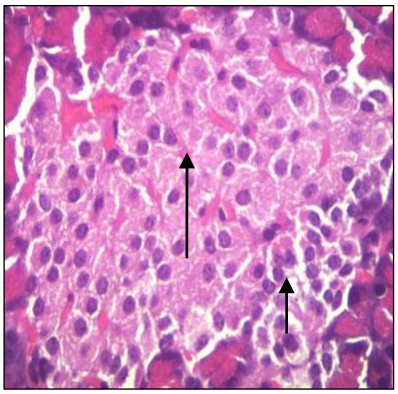
B. Positive control



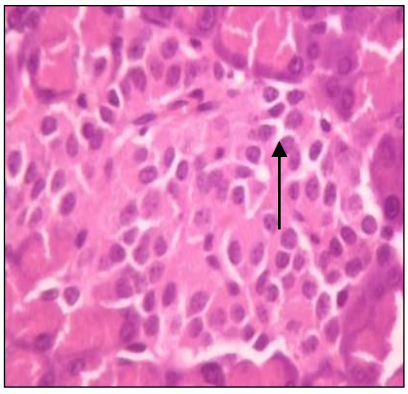
C. Standard



D. Lower Dose



E. Median Dose



F. High Dose

Figure 3. Histopathology Photographs

A. Photomicrograph of normal rat pancreas showing normal acinar cells and normal Islet of Langerhans cell groups (White arrow) and varying sized capillaries (Thin black arrow).
B. Sections studied showed predominantly acinar cells with occasional very small sized clusters of Islet of Langerhans cells. Photomicrograph of this rat pancreas showing areas of necrosis (White arrow), degenerative changes in the form of karyolysis, karyorrhexis, and pyknosis (Thin black arrow) and sparse capillaries
C. Photomicrograph of pancreas showing regenerative cluster of Islet of Langerhans cell groups (White arrow) and capillaries with normal surrounding acinar cells (Thin black arrow).
D. Section studied shows intact acinar cells with their intralobular ducts. The number of islets in each lobule appears adequate. The selected part of islet cells contain of some degenerated beta-cells (Long-Arrow) and normal Beta-cells (Short-Arrow), while the region is with alpha-cells (Short-Arrow).
E. The selected section observations show fibrovascular septa separating the pancreatic lobules. The center of islet cells consists of few degenerated beta-cells (Long-Arrow) and normal beta-cells (Short-Arrow), while the periphery comprises of Alpha-cells (Short-Arrow).
F. Section studied shows intact acinar cells with their intralobular ducts. The center of islet cells consists of quantitative increase in Beta-cells (Long-Arrow), while the periphery comprises of Alpha-cells (short Arrow). There are seen some congested blood vessels (Short-Arrow) between the islet cells.

DISCUSSION:

The scientific reports clearly explain how alloxan causes diabetes through its destructive capacity on insulin-producing pancreatic beta-cells [15-16]. The data generated through this study is preliminarydata and an initial assessment of the anti-diabetic property of the extract of Gliricidia sepium. The extracts exhibited a significant fall in elevated blood glucose levels compared with their respective control groups. Induction of necrosis to the pancreatic beta cells is due to the cytotoxic action of alloxan and this pathway is mediated by reactive oxygen species (ROS) generated in the body and responsible for oxidative stress, with a tremendous increase in cytosolic calcium concentration [17]. The traditional knowledge obtained through herbal medicines employed in therapeutic strategies to cure different illnesses is known as herbal medicine, or phytotherapy including the facets of herbal medicine[18]. Experimental studies reported in some literature reveals that the petroleum ether extracts from *Gliricidia sepium* (250, 333and 500 mg/kg) exhibited a remarkable reduction in the blood glucose level in alloxan-induced diabetes in rats. As per the spectroscopic determination of total phenolic, flavonoid, and tannin contents in the plant, it was found the 70% EEGSL possesses 3.682 mg/G, 14.51 mg/G, and 186 mg/G of the respective constituents from 70% EEGSL. The changes in body weight of the different groups of animals during the period of study are given in Table no.01 and represented in the graph in Fig No.01 which shows and indicates an increase in the mean body weight (± SEM) of normal rats. This shows that the group of normal rats obtained body weight during the treatment period of 15 days. A marked increase in fasting blood glucose level was observed in diabetic control compare to normal control rats. Hydro-alcoholic extract of Gliricidia sepium leaves shows a major anti-hyperglycemic activity on 5th, 10th, and 15th days post-treatment in alloxan-induced diabetic rats. The result is shown in Table No.02, and the graph in Fig No. 02. The mean glucose levels (± SEM) in the diabetic control group of rats was found to be 252.8±24.17, 384.1±9.555, 313.1±17.99, and 175.1±6.349 mg/dl on 0, 5th, 10th, and 15th day respectively, which was found to be significantly ($p \leq 0.05$) higher when compared with the normal rats. The biochemical parameters like serum urea, serum creatinine, serum protein, serum triglycerides, HDL, LDL, VLDL, serum cholesterol, and pancreas weight are normal in the negative control group during the treatment period. The same parameters are significantly increased in the positive control group ($p\leq 0.05$) compared to the negative control group. These parameters in other groups (Standard, lower dose, medium dose, and high dose) show significant control in comparison to a positive control group with the mean (± SEM) is shown in Table No 03. This 70% EEGSL contains a higher concentration of total polyphenolic, flavonoids, tannins and

hence this is selected for further studies like antioxidant and antidiabetic activity. In the present study, it has been observed that the plant possesses tannins and flavonoids and because of the presence of these constituents, it was reported to have anti-diabetic properties. Hence the anti-diabetic properties may be assigned to these constituents that are present in 70% EEGSL. In our study, the major finding observed with 70% EEGSL treated diabetic rats indicates the predominant exocrine pancreatic tissue comprises of acini with draining ductules. Within the substance of the exocrine pancreas, the endocrine component was found as scattered nodules. Compared to diabetic controlled rats no insulitis was observed.

CONCLUSION:

The present data generated in this work states that it may be concluded that, the plant possesses an appropriate quantity of the tannin content and reducing sugars with very good hypoglycemic activity. The 70% EEGSL successfully shown Antidiabetic activity, we credited its Tannin and Reducing sugars that are present in the study plant.

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