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RESEARCH ARTICLE

Assessment of Anti-oxidant and Anti-hyperglycemic Efficacy of the Seeds of *Cassia uniflora* Mill. by *In Vivo* Methods

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Abstract

Background: Diabetes is a chronic metabolic disorder which affects a large mass of the population today. Lifestyle changes have led to an increase in diabetic patients day by day. Metabolic disorders are a result of an excess of free radical species in the body. So the search for anti-diabetic or anti-hyperglycemic drugs with antioxidant activity is a requirement in the health sector. **Aim:** This research work aims to assess the anti-oxidant properties and anti-hyperglycemic efficacy of the seeds of *Cassia uniflora* Mill. **Methods:** The *in vivo* studies were performed using male Albino Wistar rats. The ethanol extract of the seeds of *C. uniflora* was extracted and subjected to determination of antioxidant activity by many *in vivo* methods that include reduced Glutathione (GSH), Glutathione Peroxide (GPX), Catalase (CAT), Superoxide Dismutase (SOD) and Lipid Peroxidation (LPO) enzyme-induced method. Similarly, the anti-hyperglycemic effect of the ethanol extracts was studied. Diabetes was induced in rats using streptozotocin and the animals were treated with ethanol extract of *C. uniflora* seeds. All the parameters, like the blood glucose level, hematological parameters, lipid profiling and liver function tests, were assessed to evaluate the anti-hyperglycemic activity. The histology of the pancreas of the animals was also studied. **Results:** The results of the antioxidant ability of the extract were related to the control drug, and the relation was found to be p< 0.05**. The hypoglycemic activity studies on the ethanol extract of the seed of *C. uniflora* Mill were matched with a group of the animals treated with the control drug, Glibenclamide. The blood glucose level was determined to be statistically **p<0.05**. The architecture of the pancreatic tissue revealed that the extract-treated animals showed improvement on the 28th day. **Conclusion:** The seeds of the plant *C. uniflora* possess anti-oxidant and anti-diabetic activity, which may be mediated through the phytochemical constituents of the plant.

Keywords: Anti-hyperglycemic, Antioxidant, Ethanol Extract, Glibenclamide, Histology, Hypoglycemic, Pancreas

1. Introduction

Cassia uniflora is a leguminous plant classified under the family *Fabaceae*; subfamily *Caesalpiniaceae*. It is commonly known as one leaf *Senna* which is a woody, annual, erect herb / under shrub, 15-20 cm in height with young branches. It is native to Mexico, Central America, South America and the West Indies. In India, it is found along the roadsides in Andhra, Telangana, Karnataka and Kerala. The synonym for *C. uniflora* is *Senna uniflora*. Medically the *C. uniflora* plant is reported to be used as a blood depurative[1]. The seeds have been reported to contain rich amounts of fibre, proteins and carbohydrates. It also contains several amino acids like lysine, glutamic acid, aspartic acid and arginine as reported by Geeta Ramachandra[2]. Abdul and Bakrudeen have reported the anti-inflammatory activity of *C. uniflora*[3]. The mucilage from *C. uniflora* is used as a natural gelling agent in the food and beverage, pharmaceutical and, cosmetic industries[4].

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C. uniflora was brought to India from South America and is currently spreading like a weed throughout the nation[5,6]. Analysis of the literature, both online and offline discloses that no other research has been undertaken in this plant. Hence this study attempts to assess whether *C. uniflora* has anti-diabetic and anti-oxidant activity.

Herbs have potentially active medicinal value. From the day man came into existence, diseases also began. Remedies for human ailments are present and distributed in nature all over the world and they are present mostly in plants. Plants possess high medicinal importance and need to be explored.

2. Materials and Methods

2.1 Materials

STZ, 3-(4, 5-Dimethylthiazol-2-yl), 2-diphenyl-1-picrylhydrazyl (DPPH), 2-fetal bovine serum, modified Eagle medium, 5-Diphenyltetrazolium Bromide (MTT) and Porcine Pancreatic α-Amylase (PPA) were obtained from Sigma-Aldrich. Himedia, Mumbai supplied

Folin-Ciocalteau reagent. Pellet food was obtained from Hindustan Lever, Mumbai.

2.1.1 Collection and Identification of Plant

Cassia uniflora seeds were gathered from Coimbatore district in Tamil Nadu in March. Dr. M.U. Sharif, botanist at the Southern Regional Center of the Botanical Survey of Coimbatore, Tamil Nadu, India, identified the seeds of *C. uniflora* and verified them.

2.1.2 Animals Used in the Experimental Research Studies[7,8]

Male albino rats were used in the *in vivo* studies. Well-fit rats weighing 150-200 g were chosen for the study. The animals were housed in cages maintained for 12 hours in the dark and the next 12 hours in light, alternating equally at a surrounding temperature of 25–30°C and 45–55% RH. The animals had access to an unending water supply and were fed with a regular pellet diet. The study was granted approval by the Department of Pharmacology, Institutional Animal Ethical Committee, KMCH College of Pharmacy in Coimbatore, Tamil Nadu and approved by the Committee for the Purpose of Control and Supervision on Experiments on Animals (CPCSEA), India. The Institutional Animal Ethics Committee (IAEC) approved the submitted experimental protocol (Proposal No. KMCRET/ReRc/Ph.D/69/2023).

2.2 Experimental Methodology

2.2.1 Extraction

Cassia uniflora seeds were cleansed with distilled water to remove dirt and debris. The moisture over the seeds was removed by a smooth water-absorbing cloth. The seeds were then shade-dried to get rid of extra moisture and also dried completely. The seeds were then pulverised in a blender to a coarse powder. About 50gm of the coarse powder was soaked in ethanol (EtOH) for 2 hours and then filled in a Soxhlet extractor and extracted for eight hours using ethanol solvent. The extract was evaporated in a rotary vacuum evaporator to concentrate to its original, maintaining a temperature of 40°C at very little pressure. The extract was dried to constant weight and stored in airtight containers.

2.2.2 Acute Toxicity Study

The Organisation for Economic Co-operation and Development's (OECD) guidelines were followed in conducting an acute oral toxicity study. The animals fasted for the whole night, and several groups of six rats each received EtOH extract of the seeds of *C. uniflora* extract at a dose starting from 100 mg/kg b.w (body weight) and reaching up to 2000 mg/kg b.w. Every 24 hours following the first hour, the animals were observed for indications of acute toxicity to check for any severe neurological and behavioural changes, such as writhing, convulsions, lethargy, restlessness or indicators of toxicity and death, if any.

2.2.3 Anti-diabetic Studies

The hypoglycemic impact of the extract on reducing the blood glucose was examined by testing the animals' tolerance to orally taken glucose after they had been fasting all night. Six animals were kept in one group, and likewise, five groups were formed using the selected animals. The rats were fasted all night and the rat tail vein's tip is used to collect blood to measure the initial fasting glucose of the animals. Diabetes was induced by injecting Streptozotocin (STZ) dissolved in a citric acid buffer to a pH of 4.5. Rats were allowed to fast overnight

and just 15 minutes before receiving intraperitoneal administration of 120 mg/kg b.w of nicotinamide (i.p.), a single intraperitoneal dose of 60 mg/kg streptozotocin was given. Type 2 diabetes (NIDDM) was developed knowingly in animals of the Group 2, 3, 4 v and Group 5. Rats which developed Diabetes Mellitus (DM) (indicated by increased levels of glucose in the blood of more than 250 mg/dl) after inducing Type 2 DM were used in the study. The description of groups is given in the next section.

Group 1: Control: Saline

Group 2: Diabetes control: STZ + Nicotinamide

Group 3: Drug Control: STZ + Nicotinamide + Glibenclamide

Group 4: STZ + Nicotinamide + 100mg EtOH Extract of seeds of *Cassia uniflora*

Group 5: STZ + Nicotinamide + 100mg EtOH Extract of seeds of *Cassia uniflora*

For 28 days the corresponding group of animals were injected with the vehicle (saline), STZ, Nicotinamide standard glibenclamide and EtOH extracts of *C. uniflora*. The levels of glucose or sugar and weight of the rats were monitored on days 0 hr, 72nd hr, 10th day, 15th day and on the 28th day. At the end of each experiment, rats were spared food overnight and then injected with anesthesia namely ketamine hydrochloride. The anesthetised rats were used for collecting blood from the retro-orbital sinus by using capillaries and led into an EDTA vacutainer for analysing blood parameters. The blood was also collected in plain vacutainers for analysing serum biochemical parameters. Blood samples for studying biochemical parameters were allowed to clot at room temperature when serum oozed out. The serum was separated by centrifuging at about 10,000 revolutions per minute for about 10 minutes. The used rats were then forgone. The pancreas and liver of the rats were removed washed with normal saline and stored for 12 hours for *in vivo* antioxidant ability analysis, carbohydrate metabolism, and liver profile studies. The isolated pancreas was used to study histopathological and immune histochemical studies. Different serum parameters, like protein, Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), serum Alkaline Phosphatase (ALP), Total Cholesterol (TC), High-Density Lipoprotein (HDL) and serum Triglycerides (TGL) were assessed as per the procedure given in the biochemical assay kits. The different haematological parameters studied were Red Blood Cell (RBC) count, White Blood Cell (WBC) count and Haemoglobin (Hb) level.

2.2.4 Antioxidant Studies

The antioxidant ability of the EtOH extract was studied by the evaluation of several anti-oxidative biomarker levels in the liver homogenate of the rats under study. At the end of each experimental day, rats were removed from the liver. The liver was then washed with normal saline solution and stored for 12 hours for further *in vivo* analysis, The SOD activity, CAT activity, glutathione peroxidase activity, reduced glutathione activity and lipid peroxidation efficacy were measured for the seed extract of *C. uniflora*. SOD activity was evaluated as per the protocol given by Kakkar *et al*[9]. The Sinha method was adopted for catalase activity analysis[10]. The glutathione peroxidase activity[11] was measured according to the process followed by Rotruck *et al*., Ellman's procedure was followed for Reduced Glutathione antioxidative study[12] and lipid peroxidation was estimated as described by Okhawa *et al*[13].

2.2.5 Histopathological Studies[14]

The pancreas of the treated and non-treated animals was studied to determine the activity of the extract to protect the pancreas from degeneration so that it can produce adequate amounts of insulin. The pancreas was instantly dissected out and washed with an ice-cold saline solution. A portion of the pancreas was fixed in a 10% neutral formalin fixative solution, dehydrated in alcohol and then embedded in paraffin. Sections of a 4-5 µm thickness of the pancreas were made by using a rotary microtome. The sections were stained with Hematoxylin-Eosin (H and E) dye to observe histopathological changes in the tissue of normal control, diabetic control and EtOH extracts of *C. uniflora*-treated groups of rats.

3. Results

3.1 Toxicity Studies

Acute oral toxicity studies involve oral administration of different doses (100–2000 mg/kg) of the extract. The acute toxicity trial study did not result in any harmful

effects on the behaviour, motor function, or general appearance of the animals. It demonstrated the extract's non-toxic qualities. In comparison to the groups treated with vehicles, all animals exhibited normal body weights and food consumption. The dosages for the subsequent studies were set at 100 and 200 mg/kg b.w.

3.2 Oral Glucose Tolerance

The impact of the seed extract on oral glucose tolerance is recorded in Table 1. After 30 minutes, blood glucose levels in the animals treated with extract 200 mg/kg body weight were considerably ($p < 0.001$) lower than at 100 mg/kg and stayed that way for nearly 180 minutes. Rats administered with the crude extract showed a notable decrease in blood glucose levels. The blood glucose levels significantly decreased starting on day 10th in extract-treated rats. The changes in the blood glucose levels of the treated and untreated animals are graphically shown in Figure 1. Similarly, the effect of the extract on the body weight of animals was observed regularly and recorded in Table 2. When compared to the control group, groups 4 and 5 treated with extract had a significant ($p < 0.001$) rise in their total body weight. Furthermore, the blood glucose level in the extract-treated animals demonstrates that the extract's efficacy persisted in the animal body until the cessation of therapy.

3.3 Hematological Parameters

The levels of the hematological parameters like RBC level, WBC level and Hb level were assessed in both treated and untreated control animals. Table 3. represents the hematological parameters in treated and untreated animals.

3.4. Serum Lipid Profile and Serum Liver Parameters

The Lipid profile in diabetes-induced rats showed a decrease in High-Density Lipoprotein Cholesterol (HDLC) and an increase in serum lipids such as TG and TC. The elevated levels of these lipids were reversed by oral administration of *C. uniflora* seed extract, with a dose-dependent rise in high-density cholesterol level that was significant ($p < 0.01$) as indicated in Figure 2. Similarly, important liver parameters like total protein and total bilirubin. ALP level, SGOT and SGPT levels to check the effect of the EtOH extract of *C. uniflora*

Table 1. Blood glucose levels of all the animals under study on 5 different days of the experiment

Group	Blood glucose level during the entire study				
	0 Hour	72 hour	10th day	15th day	28th day
Control	65.7±7.07	75.7±5.3	66.8±5.49	72±8.39	68.3±14.5
STZ and Nic	63.3±4.67	517±82	473±118	434±102	173±56.9
STZ and Nic + Std	68.7±9.23	465±43.2	453±56.8	181±62.9	98.7± 27.5
STZ and Nic + Ext 100mg	465±43.2	419±51.7	341±85.7	98.7±27.5	66.3±22.5
STZ and Nic + Ext 200mg	453±56.8	477±61.4	322±96.8	181±62.9	73.7±25.7

STZ- Streptozotocin; Nic- Nicotinamide; Std-Glibenclamide; Ext- EtOH extract *C. uniflora* seeds

Table 2. Changes in body weight of animals treated with EtOH extract of *C. uniflora* seeds

Group	Control	STZ+ Nic	STZ and Nic+Std	STZ+Nic+Ext100mg	STZ+Nic+ Ext200mg
Initial BW	148±2.83	166±3.46	150±4.21	136±5.95	143±4.61
7th day	159±3.59	149±4.94	141±3.27	137±3.64	143±2.21
14th day	173±5.96	117±25	149±30.9	133±29.3	166±14.3
28th day	149±30.2	102±35.9	142±28.6	123±42.7	108±34.7

STZ- Streptozotocin; Nic- Nicotinamide; Std-Glibenclamide; Ext- EtOH extract *C. uniflora* seeds
Values are given in the mean ± S.D. Statistical significance (p) was determined using a one-way ANOVA and Dunnett's test **P<0.05** was determined by comparing the treatment group to the control group.

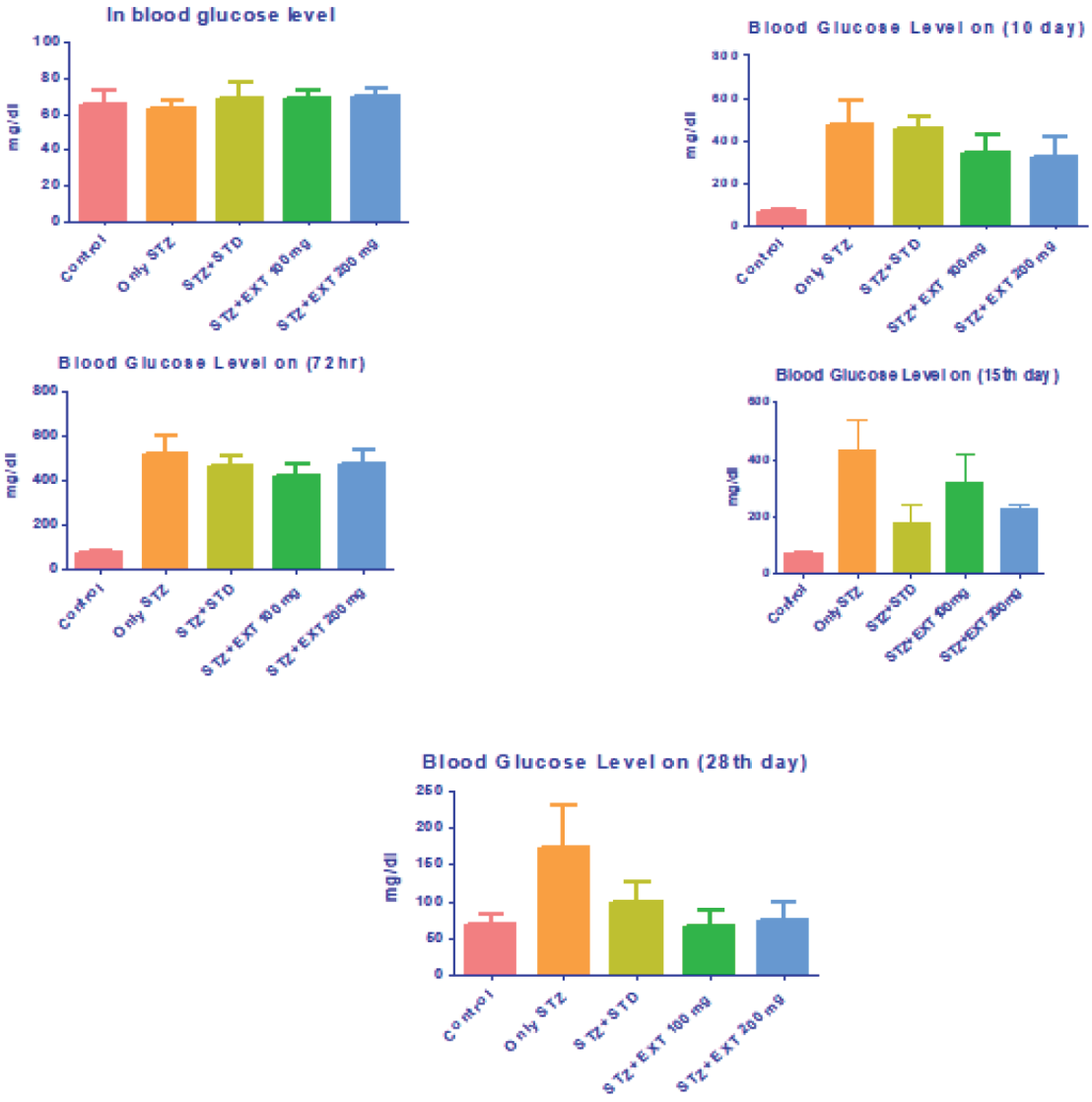


Figure 1. Effect of EtOH extract of *C.uniflora* seeds on blood glucose level of treated animals in comparison with normal, diabetic and standard control groups.

Table 3. Hematological parameters animals treated with EtOH extract of *C. uniflora* seeds

Group	RBC (X10^6/µL)	WBC (X10^3/µL)	Total Hemoglobin (g/dl)
Control	5.7±0.158	12.3±0.656	14.6±0.606
STZ and Nic	6±0.107ns	12.4±0.961	15±0.504
STZ and Nic+ Std	5.71±0.409	11.4±0.694	14±0.536
STZ and Nic + Ext 100mg	5.86±0.356	12±0.426	13.5±1.36
STZ and Nic + Ext200mg	5.73±0.319	9.42±1.54	13.7±0.924

seeds on the liver health of the animals under study. The results are shown in Figure 3.

3.5 Antioxidant Studies - Studies on Oxidative Stress Markers

For 30 days, the enzymatic antioxidant biomarkers levels of SOD, CAT, GPx, GSH and LOP were analysed and the results were tabulated in Table 4. A significant ($p < 0.05$) decline in the enzyme levels of STZ-induced diabetic rats on treatment with *C. uniflora* seed extract could be observed. Table 4 shows the modification in SOD, CAT, GPx, GSH, and LOP activity in both healthy rats and diabetes-induced rats after a 30-day treatment with

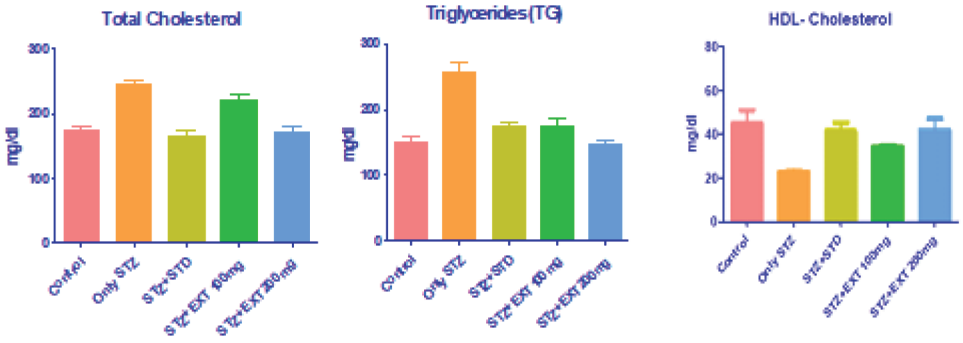


Figure 2. Serum lipid profile of animals treated with EtOH extract of *C. uniflora* seeds.

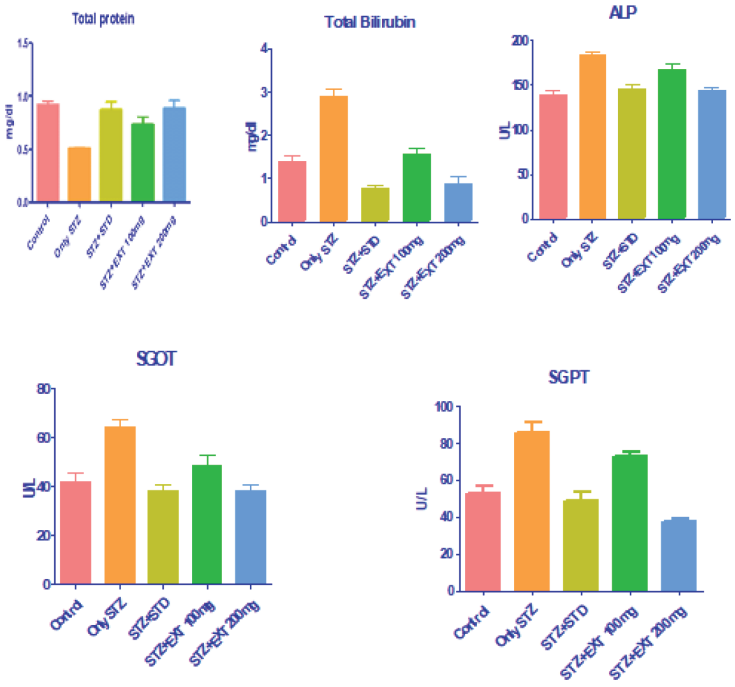


Figure 3. Effect of EtOH extract of *Cassia uniflora* seeds on serum liver parameter of diabetes-induced rats.

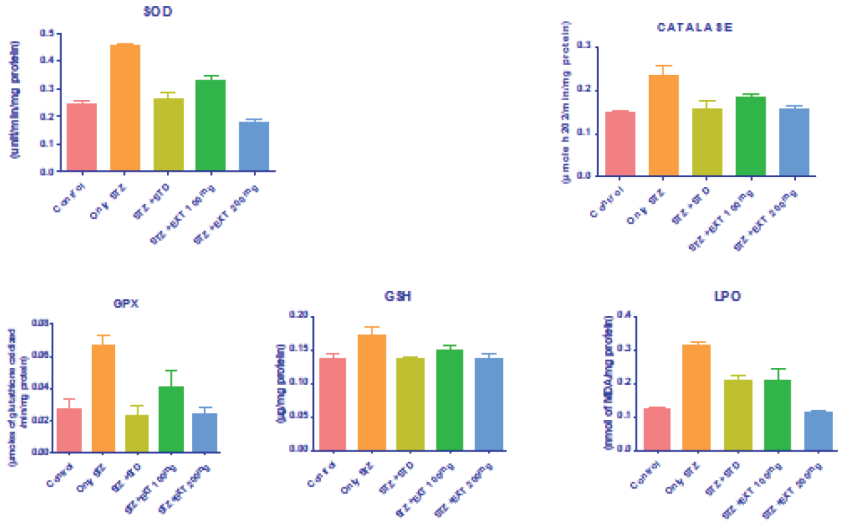


Figure 4. The antioxidant potency of EtOH of *C. uniflora* seeds on diabetic rats.

Table 4. Effect of EtOH extract of *C. uniflora* seeds on oxidative biomarker levels of diabetic rats

Group /Oxidative Biomarker	Control	STZ + Nic	STZ+ Nic +STD	STZ+ Nic +EXT 100mg	STZ+ Nic+ EXT 200mg
SOD (unit/min/mg protein)	0.245± 0.013	0.457±0.00635***	0.264 ± 0.0219ns	0.326 ±0.0222*	0.177 ± 0.0121
CAT (µ mole H202/min/mg protein**)**	0.149± 0.00549	0.233±0.0229**	0.158 ± 0.0179ns	0.185 ±0.00657ns	0.158 ± 0.00504nd
GPX (µmoles of glutathione oxidized/ min/mg protein)	0.0273± 0.0058	0.067±0.00643**	0.0233 ±0.00612ns	0.0413 ±0.0096ns	0.0243 ±0.00393nd
GSH (µg/mg protein	0.138± 0.00578	0.173±0.0128*	0.136± 0.00338ns	0.149 ±0.00751ns	0.138 ± 0.00639nd
LPO (n mol of MDA/mg protein)	0.125±0.00351	0.313±0.0115***	0.209 ±0.0153*	0.208 ± 0.0344*	0.114 ± 0.00722ns

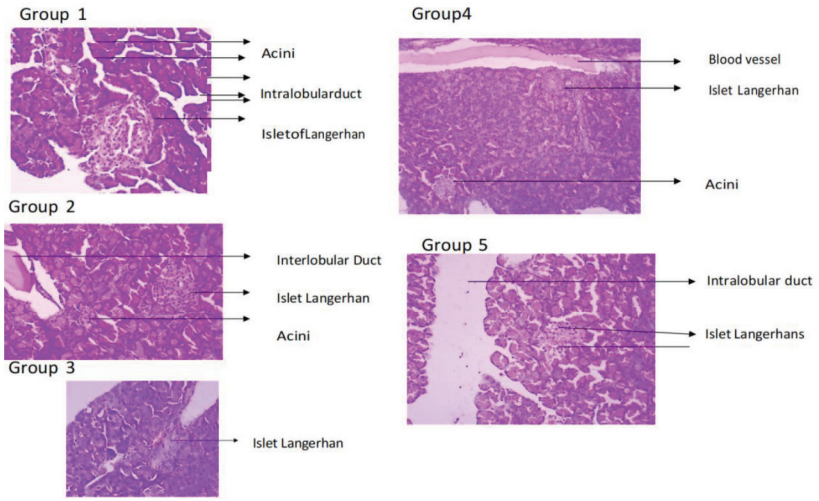


Figure 5. Histopathology of the pancreas of animals treated with *C.uniflora* seed extract compared with other groups of animals.

C. uniflora seed extract. A comparative observation is possible through a graphical representation in Figure 4.

3.6 Histopathological Examination-Pancreas

The five (1–5) groups' pancreas histological examinations are shown in the following figure 5. The exocrine components and the interlobular duct, which are encircled by supporting tissue, are well organised and have a normal shape, in contrast to the spherical Islets of Langerhans formations with a normal cell lining. The area of the mean islet in rats without diabetes was shown using image morphometry. However, abnormally formed acinar cells surrounding the islets were found in the diabetic rats' histological examination. The islets' cells were in a degenerative state, showing decreased islet cell size, decreased β-cell quantity, and asymmetrical vacuoles with intra-islet hemorrhage. The diabetic rats' mean islet area was lower than that of the control rats. The pancreatic sections of groups 4 and 5 underwent microscopical investigation that showed β-cell regeneration and size recovery, which indicated that the islets were protected. At larger doses, the regeneration of β-cells was more evident, whereas the mean islet areas varied among groups.

3.7 Statistical Observation

All the experiment results were converted to the mean of multiple readings in the experiments and given as mean ± Standard Error of Mean (SEM) for each value. The OGTT results were assessed using a two-way ANOVA and a Bonferroni post-test. Several study components were statistically analysed using a one-way analysis of variance (ANOVA) and Dunnett's multiple

comparison tests using the SOFTWARE Graph Pad Prism (version 5.5). All the outcomes were found to be statistically significant when the p-value was less than or equal to 0.05.

4. Discussion

A major fraction of people worldwide suffer from DM, a complicated metabolic condition characterised by hyperglycemia. Numerous biochemical and cellular metabolic processes are affected by the hyperglycemia linked to diabetes. Ultimately, an impact on the operation of numerous essential organs could be observed in a chronic condition. Reducing blood glucose levels over time will lower the chance of microvascular problems. So the goal of therapy for diabetic people should be broad such that the treatment and the drug regimen focuses not only on blood glucose level but also on protecting them from other complications like nephropathy, neuropathy and retinopathy. This current research work focuses on protection from inflammation of organs caused by free radicals and oxidative damage.

Because they come from nature, herbs and herbal products already contain antioxidants. When it comes to side effects, plant-based antioxidants are superior to synthetic antioxidants. To ascertain the safe dosage range and investigate the detrimental long-term consequences of the plant drugs, their extracts and constituents, toxicity evaluation was performed on test animals. In the acute toxicity testing, the extract was administered orally in a dose ranging between 100 and 2000 mg/kg producing no negative effects on the rats' overall appearance, behaviour or motor activity. Thus a minimum dose of 100 mg/kg to a highest dose of 2000 mg/kg body weight showed no toxicity thus proving that it is safe for consumption. Diabetes Type 2 was developed intentionally in the animals by injecting STZ after injecting nicotinamide[15]. In comparison to the groups treated with vehicles, all animals exhibited normal body weights and food consumption. The potential of the plant extract to produce toxicity in living systems can be evaluated using functional indices called hematological parameters[15]. They can also be used to illustrate how chemical compounds and plant extracts relate to blood. Such laboratory studies continue to be the cornerstone of moral and reasonable research, illness diagnosis, prevention and treatment, as they have shown to be extremely sensitive, accurate and dependable. In this investigation, the differential leukocyte count and WBC levels of the rats were considerably elevated by the different doses of ethanolic extract of the *C. uniflora* seeds. It is commonly known that WBCs protect the body from infections and tissue damage. Results suggest that the extract might contain substances which promote leucocyte formation and may strengthen the immune system. The maintenance of RBCs and Hb levels throughout the experiment suggests that the hemopoietic system of the rats benefited from the extract. Similar to the negative control, the different extract doses in the differential blood count experiment did not affect the counts of eosinophils, monocytes or basophils. All tested doses resulted in a considerable rise in neutrophil counts, although only 100, 200 mg/kg of the extract significantly raised the lymphocyte number. This suggested that lymphocytosis could be induced by the extract. In the analysis of lipid profile parameters, listed in Table 4 at all tested concentrations of the extract, the lipid parameter increased in a manner similar to the negative control ($P > 0.01$). This demonstrated that there was no discernible hypolipidemic impact from the extract[16].

The study's findings demonstrated that there were no apparent changes between the experimental group and the negative control found in any of the crucial biochemical components examined. None of the tested dosages of the extract had an impact on the levels of urea, creatinine, bilirubin, creatinine kinase, total protein, or ALP. This result showed that the extract was safe for the rats' kidneys, livers and hearts. STZ is commonly used to induce diabetes (Type I and Type II), which results in the formation of NO that selectively kills pancreatic β cells. This is dependent on the dosage administered to experimental animals. As a result, pancreatic cell concentration of pyridine nucleotides rapidly decreases, leading to β cell necrosis. Superoxide dismutase anions are produced by STZ via its impact on the mitochondria, which results in several diabetes complications. Consequently, the current investigation assessed the anti-oxidant, anti-diabetic, and qualities of the extract by using diabetic rats that were given STZ and Nicotinamide (NA) injections. The NA-STZ model is utilised here since NA partially protects beta

cells of the pancreas from the toxic effects of STZ, thus leading to partial destruction of the pancreas and saving the animal until the study is over. In diabetic rats created by NA-STZ, Glibenclamide is used as a reference drug to assess the efficacy of various novel anti-diabetic drugs. According to the results of an oral glucose tolerance assessment, the ethanolic extract exhibited dose-dependent hypoglycemic action; the highest hypoglycemic effect was observed at 200 mg/kg, which persisted for three hours. When compared to STZ-diabetic rats, the extract significantly lowered blood glucose levels in our investigation. This could be due to the phenolic compounds, flavonoids, and antioxidant chemicals in the extract, as well as the conventional medication Glibenclamide's activity of blocking ATP-sensitive K^+ channels to stimulate and cause the pancreatic β cells to renew or regenerate, to secrete insulin in the standard control rats. The phenolic chemicals in the extract may be what mimics insulin since earlier studies have demonstrated that they regulate hyperglycemia and operate on ATP-sensitive K+ channels[17].

Significantly losing weight is among the most notable features of diabetes brought on by STZ, mostly because of increased protein waste and poor glucose utilisation[18]. When the extract was given to diabetic rats, their body weight was lost. The improvement in glycemic management and subsequent increase in structural protein synthesis may be the cause of the weight gain. Even so, it is less than in normal control animals. Free radicals are hypothesised to attack the membrane using lipid peroxidation, ultimately resulting in membrane malfunction and damage, causing tissue damage in diabetes mellitus. One of the processes that may help prevent diabetes complications is a significant decline in lipid peroxidation levels combined with an increase in antioxidant ability. The plant's ethanol seed extract considerably reduced lipid peroxidation in our study. This may have been caused by the antioxidant properties of flavonoid and phenolic components, which are mostly present in the crude extract and were discovered through a first phytochemical examination.

The generation of Reactive Oxygen Species / Reactive Nitrogen Species (ROS/RNS) occurs due to either the non-enzymatic protein glycation, the resulting oxidative damage, or the glucose breakdown that results from the hyperglycemia linked to diabetes. According to some investigations, the glycation process produces oxygen-derived free radicals, which in turn damage cell membranes and induce lipid peroxidation in diabetics. Changes in lipid peroxidation linked to diabetic animals were found to be associated with a decline in the levels of several antioxidant enzymes, including glutathione peroxidase, catalases, and superoxide dismutase, which is essential in scavenging the hazardous intermediates that result from insufficient oxidation. Because these enzymes are not as active, biological systems have an excess of accessible hydrogen peroxide and superoxide anions (O^{2-}). This results in the production of hydroxyl radicals, which initiate and propagate lipid peroxidation[13]. The extract given to diabetic rats might have helped scavenge the free radicals that were produced by increasing the activity of these enzymes.

Increment in TC and TGL is linked to the development of microvascular and macrovascular problems, such as coronary artery disorders and atherosclerosis. The extract was given to diabetic rats for 30 days, during which time it significantly decreased serum triglycerides and total cholesterol and modulated irregularities in blood lipid levels.

5. Conclusions

The EtOH extract of *C. uniflora* seed improved blood glucose levels in the current research. It also corrected the increased cholesterol levels, triglycerides, and enzymes like ALP in the serum. Endogenous antioxidants like glutathione peroxidase, catalase, superoxide dismutase, and lipid peroxidase were all well-regulated and increased in level by it. The study's findings demonstrated that the antioxidant and antidiabetic properties of *C. uniflora* seed extract were good and significant. The research findings might be applied to the creation of novel anti-diabetic medications for the control and therapy of type II diabetes. To determine the molecular mechanism behind its anti-diabetic effect, more investigation is needed to discover the molecular targets and isolate bioactive phytoconstituents.

6. Author contribution

All the authors contributed to almost all the research related activities discussed in this paper. B. Vidhya

is the idea behind this plant, as this plant is widely growing in her native place. D. Kilimozhi designed the research work. B. Vidhya completed the experimental work from collection of plants, extraction and *invivo* studies. C. Rubina Reichal interpreted the results of the anti diabetic activity.

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