Evaluation of the Anti-Diabetic Activity of Ethanolic Extract of Carissa Streptozotocin Induced Diabetic Rats

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ABSTRACT :
The present investigation was undertaken to evaluate the phytochemical profile and antidiabetic potential of the stem of Carissa species. The study involved systematic phytochemical screening, physicochemical evaluation, acute toxicity assessment, and pharmacological analysis in streptozotocin (STZ)-induced diabetic rats. Physicochemical parameters including ash values, moisture content, and extractive values were determined, revealing higher alcohol-soluble extractive values compared to water-soluble ones. Soxhlet extraction using ethanol yielded a phytochemically rich extract containing phytosterols, flavonoids, phenols, proteins, amino acids, steroids, and terpenoids. Acute toxicity studies indicated that the extract was safe up to 2000 mg/kg, leading to the selection of 200 mg/kg and 400 mg/kg doses for further evaluation.
STZ administration produced hyperglycemia, oxidative stress, and altered antioxidant enzyme levels, as evidenced by elevated CK, LDH, TBARS, and reduced SOD and catalase activity. Treatment with the chloroform extract of Carissa significantly reduced fasting blood glucose levels in diabetic rats over 21 days, possibly through enhanced insulin secretion similar to Glibenclamide. The extract also restored antioxidant defence by increasing levels of glutathione, SOD, and CAT in pancreatic tissues. A marked reduction in glycosylated haemoglobin (HbA1C) and improvement in total haemoglobin levels were observed, indicating better glycemic control. Additionally, α-amyrin, a constituent of Carissa, reduced lipid peroxidation and improved antioxidant status by scavenging free radicals. The findings suggest that the ethanolic extract of Carissa stem possesses significant antidiabetic and antioxidant activity, supporting its traditional use and highlighting its potential for further development as a natural antidiabetic agent.
Keywords: alcohol-soluble,Carissa species,anti-diabetic, glycemic control.

I. INTRODUCTION

Diabetes is a significant clinical issue influencing a large number of individuals everywhere throughout the world. The human populace overall world seems to be amidst a contagion of diabetes. In the second century A.D. a medical specialist of Cappadocian, Aretaeuss, stated: "The epithet diabetes has been assigned to the disorder, being something like passing of water by a siphon". According to him, diabetes is just like a catastrophe of the flesh and limbs into urine. Diabetes the term means over-the-top urination in the sickness and mellitus implies "sweetened with honey", indicating the appearance of sugar in the urine of individuals affected by the disease.
Diabetes mellitus is known to be a metabolic disorder wherein an individual shows elevated blood glucose than a normal individual, either due to the body not secreting required insulin, or the body doesn't react to present insulin. The increased glucose shows the manifestation of polyuria (more than usual urination), polydipsia (increased thirstiness), and polyphagia (increased appetite). It is a group of composites, hormonal, and metabolic defects portrayed by high levels of sugar and glycosuria, with disruption of the metabolism of sugar, fat, and protein because of imperfections in insulin production, its function, or both.

In the world, with time day by day, there is an increase in the number of diabetic cases. In comparison to heart disease and malignancy it is the third epidemic and serious disease. Globally, it was predicted by the International Diabetes Federation (2011) that there were 239 million incidents of diabetes mellitus in the year 2010, and it is assumed that it will reach to 552 million in the year 2030.India leads the way to becoming the diabetic capital with the biggest number of diabetic patients. It observed that in 2010 there were around 50.8 million individuals living with diabetes in India, it will reach to 87.0 million by 2030[1].

Glucose is a simple carbohydrate found in food and is a fundamental supplement that acts as a prime source of fuel for the functioning of various cellular and biological activities in the body. Sugars are metabolized at the

small intestine absorbed by the intestinal cells into the circulation system and carried to all other parts of the body. As it can't reach in the cells alone; it required insulin for its transport. Absence of insulin, the cells cease for energy althoughthe being of sufficient glucose in the circulatory system. In particular sorts of diabetic condition, the cells are failed to use glucose causes to an unexpected circumstance of starvation amidst sufficient amount of unchanged glucose which is inefficiently discharged in the urine[1-3].

II. EXPERIMENTAL WORK

Materials

Plant material

Carissa plants were collected from our institution herbal garden and were identified by Dr. Madhav Chetty, Sri Venkateswara University, Tirupati (Andhra Pradesh). Carissa plants were collected during March 2024. The plants were washed thoroughly with distilled water to remove dust particles. Selected medicinal plant parts (crude drug) were cut into small pieces, cleaned, and shade dried at room temperature. After drying, the selected medicinal plant parts were subjected to size reduction to obtain a coarse powder, separately, using a mechanical grinder and then passed through sieve no. 40 to achieve the desired particle size. The powder was stored in well-closed glass jars. The uniform powder was subjected to standardization for different parameters.

Diabetes inducing chemical

In this study Streptozotocin was used to induce experimental diabetes. Streptozotocin was purchased from Sigma-Aldrich. It was used as freshly prepared aqueous solution for administration into the rats.

Experimental animals and their maintenance

The white laboratory rats (150-180 g) were used as the experimental animal for the hypoglycaemic activity study of the plants. The rats were used for experiment after an acclimatization period of 30 days to the laboratory environment. They were kept in plastic cages of 18 cm × 12 cm size under normal environmental condition of temperature and relative humidity. The cages were cleaned at alternative days. Animals were fed with normal commercial pellet diet and drinking water. Food and water were supplied regularly. Cleaned water bottle was provided to every plastic cage. The rats maintained in this experiment have showed satisfactory physical growth without any abnormality. Prior to each study, the animals were subjected to fasting for 12 hours but were allowed free access to water. The protocol for the study was approved by the Ethical Committee of Animal Research of Jeeva Life Sciences, Hyderabad.

One-touch commercial glucometer

The blood glucose levels of experimental rats were estimated by using a glucose test strip and a one-touch commercial glucometer. In this experiment, a GLUCOCARDTM 01 SENSOR blood glucose monitoring glucometer was used. The blood sample was dropped on the test strip of the glucometer. This test strip was inserted into the microprocessor of the electromagnetic digital blood glucometer and accordingly, the corresponding readings were recorded. The test strip of the glucometer contained Glucose Oxidase (GOD) and Hexaammineruthenium (III) chloride as the main reagent. The glucometer was imported and marketed in India by ARKRAY Healthcare Pvt. Ltd.

Experimental

Extraction

The Plant *Carissa* was washed thoroughly with water to remove the soil particles, shade dried, and powdered. 25gms of plant material was extracted by Soxhlet extraction apparatus using the ethanol solvents (1.5 L each time) with intermittent shaking. It was filtered using a Buchner funnel with Whatman No.1 filter paper. The filtrate was concentrated under reduced pressure at 40°C, and the extract was stored in a refrigerator at 4°C for use in subsequent experiments.

Experimental Animals[4]

Wistar Albino rats of either sex (150 to 200 g) were purchased from jeeva life sciences, Uppal. They were maintained under standard laboratory conditions at 25 ± 2°C, relative humidity (50 ± 15%) and normal photoperiod (12-hour light-dark cycle) were used for the experiment. Commercial pellet diet MFD, by Amrut trade corporation, Gwalior were given to the experimental animals throughout the study.

Acute toxicity test for LD_{50} determination

The acute toxicity study is aimed to establish the therapeutic index i.e., The ratio between the pharmacologically effective dose and the lethal dose, and also to perform the primary screening.

The ethanolic extract of *Carissa* was tested for its acute toxicity in rats. The study was designed on the basis of OECD (Organisation for Economic Co-operation and Development) guideline - 423. To determine acute toxicity of a single oral administration of the extract at four dose levels (5,50,300, 2000mg/kg body weight) were tried. The dose levels tested. 5,50, 300, 2000 mg/ml suspensions were prepared in 1% WA/ CMC (Carboxyl Methyl Cellulose) and given at the dose of 1 ml/kg body weight.

After administration of the extract, the animals were observed individually after dosing for 4 hours and there after 14 days. The parameters noted were grooming, hyperactivity, sedation, loss of righting reflex, respiratory rate and convulsion. All animals were observed twice daily for mortality during the period of study.

Selection of Dose[5](*OECD* guideline 423)

An acute oral toxicity test was carried out according to the OECD guideline No. 423. Wistar Albino Rats were kept for overnight fasting before drug administration. A total of three animals were used, which received a single oral dose of 2000 mg/kg, body weight of *Carissa Extract*. The animals were observed for a period of 24 hr for the changes in behaviour, hypersensitivity reactions etc. Mortality, if any, was determined over a period of 2 weeks. Hence in our studies, we selected 1/10 i.e. 200 mg/kg dose.

Preparation of Doses

Doses equivalent to 200 mg of the crude drug per kilogram body weight were calculated and suspended in 1% w/v Tween 80 solutions for the experiment.

Induction of experimental diabetes with Streptozotocin

It has been widely accepted that Streptozotocin selectively destroys the insulin-producing beta-cells in the pancreas; hence it is used to induce diabetes in laboratory animals. Streptozotocin, freshly dissolved in distilled water, was injected intraperitoneally at a dose of 55 mg/kg body weight (bwt) to rats. The induction of Streptozotocin diabetes was confirmed by measuring the blood glucose level. After 7 days (168 hrs), with diabetes mellitus, indicated by hyperglycemia, were used for the present study.

Experimental Design

In order to assess the anti-diabetic activity, the animals were divided in Four groups of six animals in each group.
Group 1: Normal control, 0.9% NaCl-treated animals
Group 2: Diabetic control, STZ -treated rats (40 mg/kg body weight)
Group 3: Treated with Ethanolic extract of *Carissa* (200 mg/kg body weight)
Group 4: Standard drug, Glibenclamide-treated rats (5 mg/kg body weight)

The test drug and reference drug was administered orally at two dose level for a period of 21 days from starting day of diabetes.

Blood collection and biochemical estimations in serum & Pancreas

On 22nd day, fasting blood samples were collected from the tail vein of all the groups of rats. Whole blood was collected for estimation of blood glucose by using the glucometer (Easy Gluco, More pen Laboratories Ltd.; New Delhi), glycosylated haemoglobin (HbA1C) (Murray et.al. 2003) and glutathione levels (Goldstein et al.1982). Serum was separated for estimation of specific serum marker enzymes, namely, lactate dehydrogenase (LDH)[117] (*karunanayke* et al.1990) and creatine kinase (CK)(Murray et al. 2000)..

Biochemical estimation in pancreatic tissue

After blood collection, all the animals were sacrificed and pancreas was dissected out. Tissue was washed with ice cold saline, weighed and minced; 10% homogenate was prepared in 0.15M ice-cold KCl for TBARS (thiobarbituric acid-reactive substances), a marker for lipid per oxidation[6] (Chen *et al.*2001) and protein estimation; in 0.02M EDTA for glutathione estimation[7](Goldstein *et. Al.*1982) and in phosphate buffer (pH 7.4) for superoxide dismutase (SOD)[8] (Koenig *et al.*1976) and catalase estimations[9] (Koenig *et al.*1976) using a Teflon tissue homogenizer. Decrease in levels of endogenous antioxidants with rise in TBARS levels was considered as oxidative stress.

Estimation of plasma glucose

Glucose content was estimated by the method of *Trinder*[10] (1969) using a diagnostic Kit (Sigma Diagnostics Pvt. Ltd., Baroda, India). 0.01mL of plasma, standard and distilled water (blank) were taken in three separate tubes, 1mL of the enzyme reagent was added to each tube, mixed and kept at 37°C for 15min. The colour developed was read at 510 nm against a reagent blank. Values are expressed in mg/dL plasma.

Estimation of glycosylated haemoglobin (HbA1c)[11]

Glycosylated haemoglobin in the blood was estimated by the method of Sudhakar Nayak and Pattabiraman (1981) 0.5 ml of saline washed erythrocytes were lysed with 5.5ml of water, mixed and incubated at 37°C for 15min. The contents were centrifuged and the supernatant was discarded, then 0.5ml of saline was added mixed and processed for estimation. To 0.2 ml of aliquot, 4mL of oxalate hydrochloric solution was added and mixed. The contents were heated at 100°C for 4h, cooled and precipitated with 2mL of 40% TCA. The mixture was centrifuged and to 0.5mL of supernatant, 0.05mL of 80% phenol and 3.0mL of concentrated sulphuric acid were added. The colours developed were read at 480 nm after 30 min. Values are expressed in mg/g of Hb.

Estimation of liver glycogen[12]

Liver glycogen was extracted and estimated by the method of Morales *et al.* (1975). 50 mg of fresh tissue was treated with 3 mL of 30% potassium hydroxide solution in boiling water bath for 15 min. The tubes were cooled and a drop of 1M ammonium acetate was added to precipitate glycogen and left it in a freezer overnight for complete precipitation. Glycogen was collected by centrifuging at 3000 rpm for 20 min. The precipitate was dissolved with the aid of heating and again the glycogen was precipitated with alcohol and 1 M ammonium acetate and centrifuged. The final precipitate was dissolved in saturated ammonium chloride solution by heating in a boiling water bath for 5 min. Aliquots of glycogen solution were taken up for suitable dilution and 4 mL of anthrone reagent was added by cooling the tubes in an ice bath.

The tubes were shaken well, covered with marble caps and heated in a boiling water bath for 20 min. After cooling, the absorbance was read at 640 nm against water as blank. Standard glucose solution was also treated similarly. The glycogen content was calculated from the amount of glucose present in the sample, by multiplying with the factor 0.91 and expressed as mg/100g of tissue.

Estimation of haemoglobin[13]

Haemoglobin content was measured by the method of Drabkin and Austin (1932). 0.0 2ml blood was mixed well with 5ml of Drabkin's reagent and allowed to stand for 10min. The colour developed was read in a spectrophotometer at 540 nm together with the standard solution of cyan methaemoglobin, against a reagent blank. Values are expressed in mg/dL blood.

Estimation of plasma thiobarbituric acid reactive substances (TBARS) [14]

Plasma TBARS was measured by the method of Yagi (1987). To 0.5 ml of plasma, 4mL of 0.83N H2SO4 and 0.5 ml of 10% phosphotungstic acid were added and mixed. After 5min, the mixture was centrifuged at 3000 rpm for 10min. The supernatant was discarded and the sediment was mixed with 2 ml of H2SO4 and 0.3 ml of 10% phosphotungstic acid. The mixture was shaken well and centrifuged at 3000 rpm for 10 min. The sediment was suspended in 4 ml of distilled water and 1mL of TBA reagent was added. The reaction mixture was heated to 95oC for an hour. After cooling, 5 ml of butanol was added; the mixture was shaken vigorously and centrifuged at 3000 rpm for 15min. The colour developed was extracted in the butanol layer and read in a spectrophotometer at 535 nm. Standard solutions in the concentration range of 1-5 nm and blank containing distilled water were processed along with test samples. Values are expressed as n moles/ml plasma.

Assay of superoxide dismutase (SOD)[15]

The activity of SOD was measured by the method of Kakkar*et al*. (1984). To 0.5 ml of tissue homogenate, 1mL of pre-chilled distilled water was added followed by the addition of 2.5 ml of pre-chilled ethanol and 1.5 ml of pre chilled chloroform. This mixture was shaken for 90sec at 4oC and then centrifuged at 160 rpm for 10min. The enzyme activity in the supernatant was determined as follows. The assay mixture contained 1.2 ml sodium pyrophosphate buffer, 0.1 ml PMS and 0.3 ml NBT and appropriately diluted enzyme preparation in a total volume of 3ml. The reaction was started by the addition of 0.2 ml NADH. After incubation at 30°C for 90sec, the reaction was stopped by the addition of 1mL glacial acetic acid. The reaction mixture was stirred vigorously and mixed with 4 ml of n-butanol. The contents were left aside for 10 min, centrifuged and the n-butanol layer was separated. The colour intensity of the chromogen in n-butanol layer was measured in a spectrophotometer at 560 nm. A system devoid of enzyme served as control. The enzyme concentration required to inhibit the chromogen produced by 50% in one minute under standard conditions was taken as one unit. Values are expressed in 50% NBT reduction/min/mg Hb or mg protein.

Estimation of glutathione peroxidase (GPx)[16]

The activity of GPx was measured by the method of Rotruck*et al*., (1973). 0.2 ml Tris buffer, 0.2mL EDTA, 0.1mL sodium azide and 0.5 ml erythrocyte lysate or tissue homogenate were mixed together. To this mixture, 0.2 ml GSH followed by 0.1 ml H2O2 were added. The contents were mixed well and incubated at 37oC for 10min along with a control containing all reagents except the homogenate or erythrocyte lysate. After 10min the reaction was arrested by the addition of 0.5 ml of 10% TCA. The contents were centrifuged at low speed and the supernatant was assayed for GSH by the method of Ellman. Values are expressed as µ moles of GSH utilized/min/mg Hb or mg protein.

Assay of HMG-CoA reductase[17]

The activity of HMG-CoA reductase was measured by the method of Rao and Ramakrishnan (1975). Equal volumes of fresh 10% tissue homogenate and dilute per chloric acid were mixed, kept for 5 min and centrifuged at 2000 rpm for 10min. To 1mL of filtrate, 0.5 ml of freshly prepared hydroxylamine reagent (alkaline hydroxylamine in the case of HMG-CoA) was added, mixed and after 5 min, 1.5 ml of ferric chloride was added and shaken well. Readings were taken after 10 min at 540 nm against a similarly treated saline-arsenate blank. The ratio of HMG-CoA to mevalonate was calculated. Lower ratio indicates higher enzyme activity and higher ratio indicates lower enzyme activity.

Histopathological study

On day 21 when the animals were sacrificed, the pancreas of one animal from each group was excised and stored in 10% formalin after washing with normal saline. Histopathological parameters were studied.

Statistical analysis

The values are expressed in mean ± SEM. The results were analyzed by using one-way analysis of variance (ANOVA) followed by Dunnet's "t" test to determine the statistical significance. p< 0.05 was chosen as the level of significance. Statistical analysis was performed using Graph Pad Prism Software 9.0 version.

III. RESULTS AND DISCUSSION

Streptozotocin induced antidiabetic activity

Effect on Blood glucose level

The induction of diabetes with streptozotocin significantly increased the blood glucose level (**p < 0.001**) in group II rats compared to normal rats. In the 21-day study, glibenclamide, the standard drug, restored the blood glucose level highly significantly by the 14th day (p < 0.001). The ethanolic extract (200 mg/kg) reduced the glucose level moderately and significantly on both the 14th and 21st days (p < 0.01).

Table 1: Effect of *Carissa Extract* on glucose level in streptozotocin induced diabetic rats

Group no.	Group	Blood sugar level				
		Before inducing diabetes	3	7	14	21
I	Normal control	80.3 ± 0.46	82.2 ± 0.17	81.4 ± 1.7	81.9 ± 0.57	80.11 ± 0.18
II	Diabetic control	82.4 ±0.81	241.7 ±1.89	274.8 1.43***	267.3 ±3.07 ***	290.1 0.24***
III	Ethanolic extract 200 mg/kg	79.4 ± 0.92	240.7± 1.69	225.3 ± 1.41	219.3±3.09**	217.1±0.34**
IV	Glibernclamide (5 mg/kg)	83.25±0.97	244.8± 2.54	199.4± 3.49**	169.3±2.77***	160.8±0.24***

Where- *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control vs treated groups

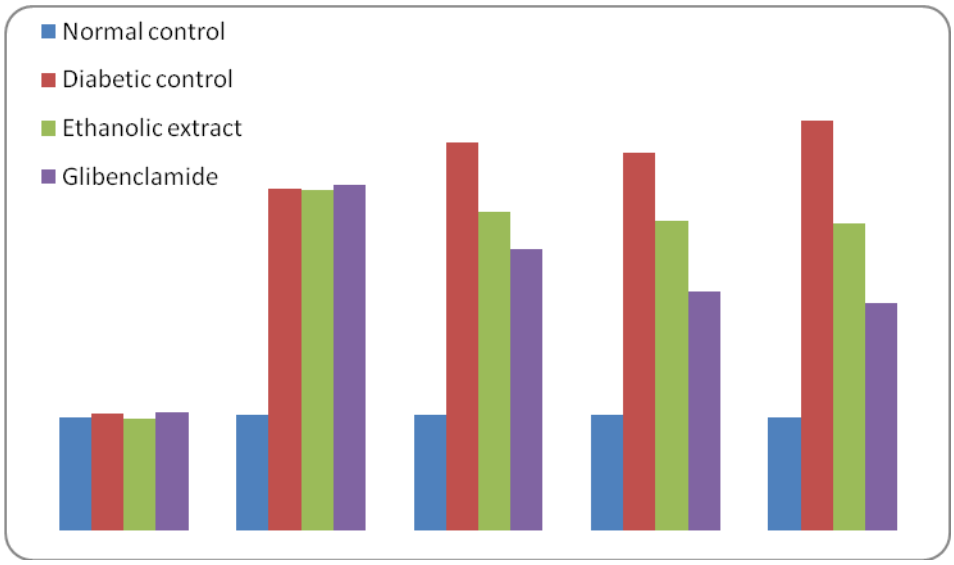


Figure: Comparative Effect of Ethanolic Extract and Glibenclamide on Blood Glucose Levels in Streptozotocin-Induced Diabetic Rats Over 21 Days

Figure 1 Effect of *Carissa Extract* on glucose level in streptozotocin induced diabetic rats

Effect on Different serum parameters

The levels of glycosylated haemoglobin and key enzymes (glutathione, CK, and LDH). The administration of streptozotocin significantly elevated the level of glycosylated haemoglobin in Group II (diabetic control rats).

Treatment with the **ethanolic extract of** *Carissa* significantly lowered the levels of glycosylated

haemoglobin after 21 days of administration.

The levels of blood **glutathione (GSH)** in diabetic rats (Group II) were significantly reduced **(p < 0.001**) when compared to those in normal control rats (Group I). Treatment with the **ethanolic extract (200 mg/kg)** for 21 days significantly restored GSH levels compared to the diabetic control group. **Glibenclamide** treatment also showed a highly significant (**p < 0.01**) increase in GSH levels when compared to Group II.

Furthermore, the levels of **creatine kinase (CK)**and**lactate dehydrogenase (LDH)** were significantly increased in Group II diabetic rats. However, treatment with the **ethanolic extract** for 21 days significantly reduced the CK levels (**p < 0.01**, at 200 mg/kg) compared to the diabetic group. The administration of the **ethanolic extract** also moderately significantly reduced (**p < 0.01**) serum LDH levels, with results comparable to glibenclamide treatment.

Table 2 Effect of *Carissa Extract* on glycosylated haemoglobin, blood glutathione, serum creatine kinase, serum lactate dehydrogenase

Group No	Group	Whole blood HbA1C (%)	Blood GSH (mg/dL)	Serum Creatinine Kinase (CK), (IU/L)	Serum LDH (IU/L)
I	Normal Control	5.12 ± 0.22	3.19 ± 0.15	69.14 ± 2.88	190.12± 5.40
II	Diabetic control	15.33±0.42***	1.10 ± 0.10*	153.32 ± 3.91**	311.44±8.11***
III	Ethanolic extract (200 mg/kg)	9.40± 0.11**	1.37 ± 0.25*	138.13 ± 0.69*	268.32±8.11**
IV	Glibenclamide (5 mg/kg)	6.17±0.43***	3.79± 0.22***	89.62 ± 2.67***	233.76±9.34***

Where- *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control vs treated groups

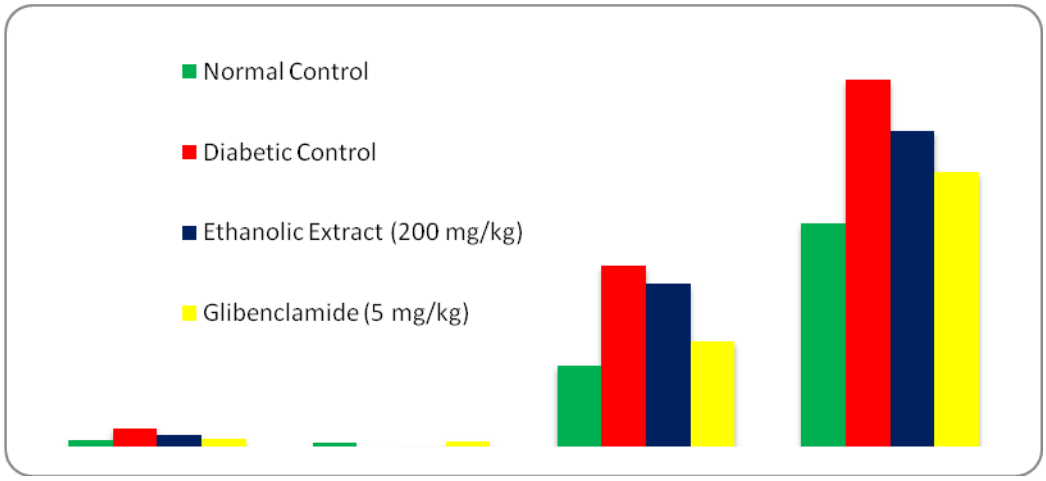


Figure 2 Effect on Different serum parameters

Effect of extracts on antioxidant enzymes

Effect of *Carissa* Ethanolic Extract on Lipid Peroxides, Catalase, Superoxide Dismutase, and Glutathione Levels

As the ethanolic extract showed good results in lowering blood glucose and improving blood biochemical parameters, it was selected for evaluating its effect on antioxidant enzymes. The extent of thiobarbituric acid reactive substances (TBARS) formed was significantly higher ($p < 0.001$) in the streptozotocin-treated diabetic group (Group II), indicating elevated lipid peroxidation. Treatment with ethanolic extract (200 mg/kg) significantly reduced the TBARS levels ($p < 0.001$), suggesting an antioxidant effect.

A significant reduction ($p < 0.001$) in the activity of superoxide dismutase (SOD) was observed in the pancreas of diabetic animals (Group II) compared to the normal control group (Group I). Diabetic rats treated with the ethanolic extract (200 mg/kg) showed a significant increase in SOD activity ($p < 0.01$), indicating restoration of the antioxidant defense system.

Total glutathione activity was also highly reduced in the pancreatic tissue of diabetic rats compared to

normal control animals. Treatment with the ethanolic extract (200 mg/kg and 400 mg/kg) significantly ($p < 0.01$) increased glutathione levels, indicating improvement in the antioxidant status of the tissue.

Table 3: Effect of *Carissa Extract* on lipid peroxides, catalase, superoxide dismutase and glutathione levels

Group No	Group	TBARS (Nmol MDA/mg protein)	CAT (nmol H2O2-consumed/min/mg protein)	SOD IU/mg protein	GSH level min/mg protein
I	Normal Control	0.489± 0.037	4.33± 0.089	3.59± 0.069	52.4±0.945
II	Diabetic control	4.921±0.562***	0.79± 0.020***	0.262±0.025***	11.58±1.034***
III	Ethanolic extract (200 mg/kg)	2.853±0.451*	2.10± 0.132**	3.44±0.272**	40.24±1.446***
IV	Glibenclamide (5 mg/kg)	1.223±0.041***	3.39± 0.156***	3.78±0.087***	44.65±1.656***

Where- *p<0.05, **p<0.01, ***p<0.001 compared with diabetic control vs treated groups

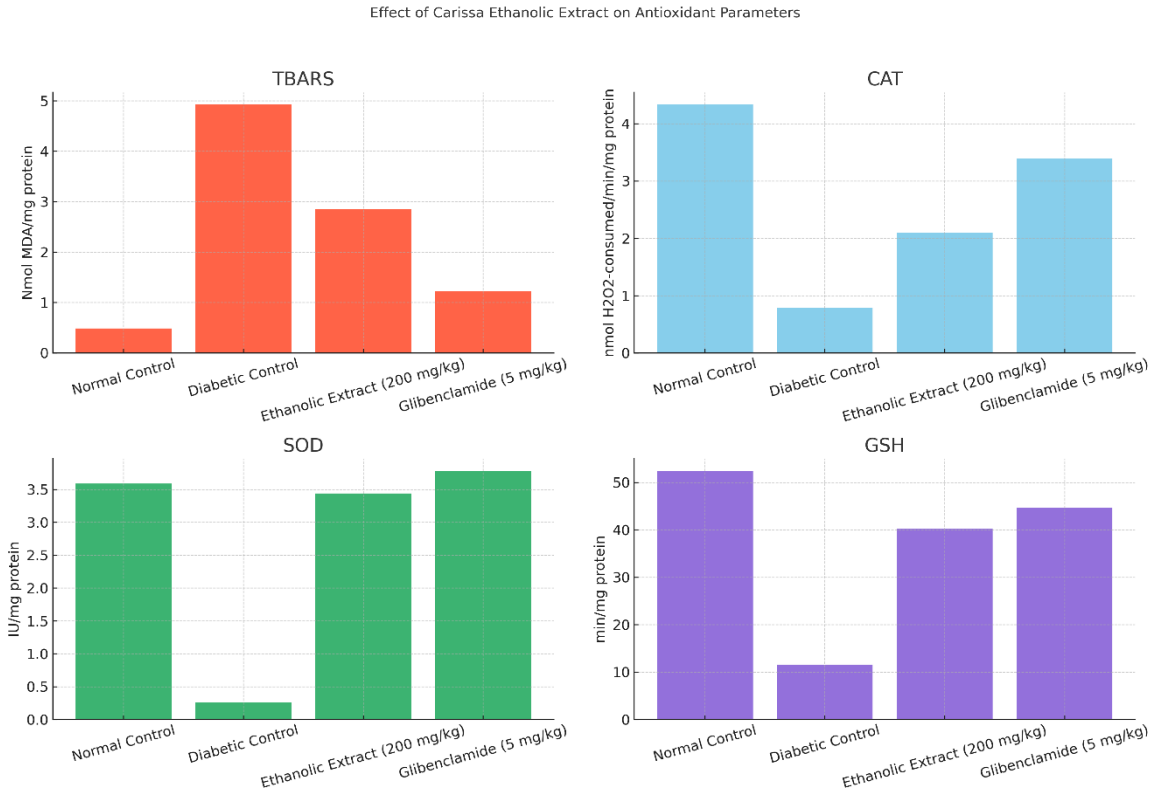
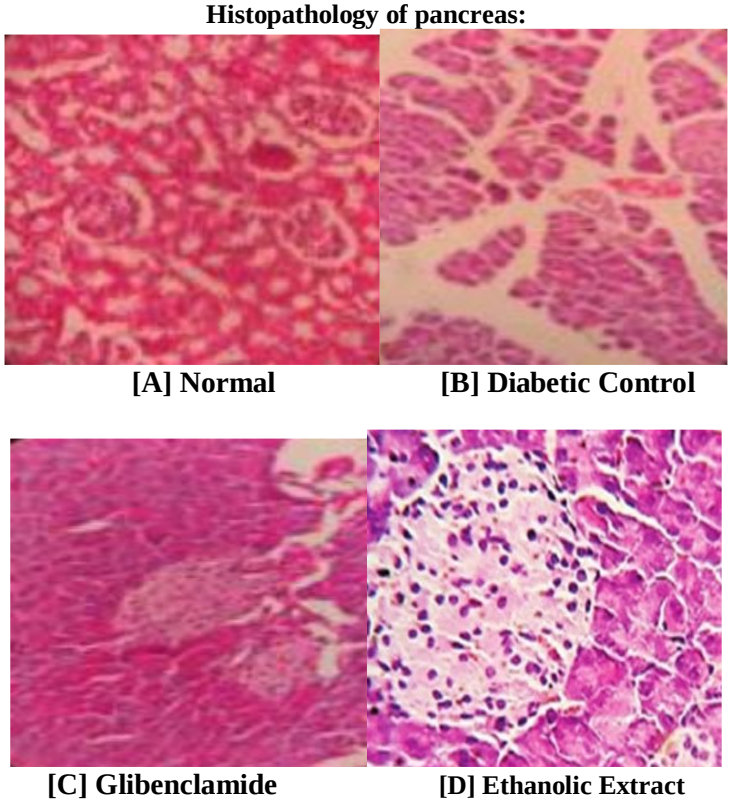


Figure 3: Effect of extracts on antioxidant enzymes

Histopathology of pancreas:



[A] Normal **[B] Diabetic Control**

[C] Glibenclamide **[D] Ethanolic Extract**

Figure 5.4: Histopathology of pancreas

[A] These figures were showed a normal architecture of pancreatic acini & normal islet of Langerhans in non-diabetic control.
[B] A reduction in β-cell number i.e., destruction of the islet of Langerhans
[C] With a slight haemorrhage in the pancreatic acini and a recovering from necrosis in the pancreatic acini in pancreas glibenclamide treated group
[D] With a slight haemorrhage in the islets of beta cells (red colour) in pancreas of Pet. ether extract treated group

Discussion

Non-insulin dependent diabetes mellitus (NIDDM) is a multifactorial disease, which is characterized by hyperglycemia and lipoprotein abnormalities. These traits are hypothesized to be responsible for damage to cell membranes through non-enzymatic glycosylation of proteins, auto-oxidation of glucose or increase metabolism of glucose by the sorbitol–polyol pathway. Cell damages will in turn, result in elevated production of reactive oxygen species or ROS. High levels of ROS have been found to play a role in the pathogenesis of NIDDM. Prolonged exposure to free radicals is a pivotal cause of tissue stress and injury. The free radical courses permanent damage to tissue structures results from an irreversible alteration in the molecular configuration of carbohydrates, lipids, proteins and even nucleic acid bases. In diabetes, the level of free radicals was reported to increase in streptozotocin and streptozotocin treated rats an elevated level of free radicals was detected in several tissues including the kidneys.

Conventionally, insulin dependent diabetes is treated with exogenous insulin and non-insulin dependent diabetes with synthetic oral hypoglycaemic agents like sulphonylureas and Biguanides. However, hormone fails as a curative agent for complications of diabetes and the major drawbacks of insulin therapy are the side effects like insulin allergy, lipodystrophy and lipoatrophy, insulin antibodies, altered metabolic control, autoimmunity and other late complications like morphological changes in kidneys and severe vascular complications.

Similarly, oral hypoglycaemic drugs have many side effects such as nausea, vomiting, cholestatic jaundice, aplastic and haemolytic anaemia, generalised hypersensitivity reactions, dermatological reaction etc.Traditionally, there are various herbs used for the treatment of diabetes mellitus, of which merely some have been evaluated as per the modern system of medicine.From these plants, only plant extracts have been prepared and evaluated for its anti-hyperglycaemic activity. Most of the reported plants seem to act directly on pancreas and stimulate insulin release in the blood. Some will favourably alter the activities of regulatory enzymes of glycolysis, gluconeogenesis and other pathways by acting directly on tissues like liver, muscle and fat (extra-pancreatic effect). Chemical constituents of these plants are known to possess wide range of medicinal properties.The

research was envisaged for antidiabetic activity of *Carissa Extract* procured by successive extraction methods and the preliminary phytochemical screening was done to know about the presence of secondary metabolites. The antidiabetic activity of all extracts has been evaluated by streptozotocin-induced diabetes.

Extractive values are chiefly used for the determination of exhausted or adulterated drug. The alcohol soluble extractives values were found to be higher than water soluble extractive value. Alcohol being a moderately non polar solvent, able to extract polar and non-polar components yields higher extractive value.

Literature review states that the presence of alkaloids, flavonoids, glycosides, terpenes, steroids, polysaccharides, phenols, coumarins and proteins in the plant extract contributes to pharmacological activities such as antidiabetic, hypoglycemic, antihyperlipidemic and antioxidant properties. Preliminary phytochemical evaluation report illustrates that petroleum ether extract of stem of *Carissa* showed the existence of triterpenoids, steroids and fatty acids, chloroform extract showed presence of saponins, phytosterols, flavonoids, phenols, steroids, terpenoids ethanolic extract showed the presence of alkaloids, flavonoids and glycosides, and aqueous extract showed the presence of carbohydrates, as phytoconstituents. Hence, keeping all this in view, research work was focused on the above-mentioned constituents in both extracts, for the evaluation of hypoglycaemic, antidiabetic and antioxidant potential. Toxicity study of a new compound must be done accurately for the selection of the dose, used for its pharmacological screening. This study is carried out on animals in the laboratory with a very sophisticated procedure. In this study, all the extracts at the dose of 2000mg/kg indexed neither visible signs of toxicity nor mortality and observations did not point out any proofs of substance related toxicity. The no-observed-adverse-effect level (NOAEL) was noticed at the dose of 2000mg/kg. The toxicity studies were determined by OECD guidelines 423.Based on the LD50 value, 1/10th (200 mg/kg) of its value was chosen for pharmacological studies.

Increased serum and tissue CK and LDH levels in diabetic rats indicate cardiac muscular damage[18]. Similar increase in the activity of these two enzymes in serum of the streptozotocin diabetic rats was observed in the present study. The quantity of enzyme released from the damaged tissue is a measure of the number of necrotic cells.

Further, streptozotocin -treatment in animals decreased the activity of marker enzymes in pancreatic tissue. SOD is an important defense enzyme which catalyzes the dismutation of superoxide radicals. Therefore, reduction in the activity of these enzymes (SOD) results in a number of deleterious effects due to the accumulation of superoxide anion radicals and hydrogen peroxides. Lipid peroxidation is one of the characteristic features of chronic diabetes. In the present study, a marked increase in the concentration of TBARS was observed in the pancreatic tissue of diabetic rats. Higher levels of lipid peroxides and low SOD activity indicate an oxidative stress condition. The antidiabetic effect *Carissa may* be due to increased release of insulin from the existing β-cells of pancreas similar to that observed after glibenclamide administration. Treatment of diabetic rats with the chloroform extract of *Carissa stem*significantly increased the levels of non-protein thiols in serum as well as in pancreatic tissues ofrats as compared to pathogenic diabetic rats. In present study, it was also observed that the levelof blood glutathione significantly ($p < .05$) increased, as well as pancreatic levels of glutathione in diabetic rats when treated with chloroform extract of *Carissa.* Further, the activities of SOD and CAT were also increased in the pancreatic tissues of test drug-treated diabetic animals. The antioxidant activity of the test drug might have been due to the inhibition of glycation of the antioxidant enzymes SOD and CAT. Glucose which forms Schiff's base with Proteins has been reported to have high affinity for proteins especially those containing transition metal ions. Increased Glycated Cu-Zn-SOD has been reported in diabetes.

A study on *Carissa* has shown that *Carissa* inhibits advanced glycation end products in streptozotocin diabetes in rats. In the present study too, decrease in the level of glycosylated hemoglobin in rats treated with the chloroform extract of *Carissa was* observed which is not reported earlier. Since the level of glycosylated hemoglobin has been shown to provide an index of blood glucose concentration during the previous 21 days period, it is being used increasingly in the clinical management of diabetes.

Furthermore, there was a significant attenuation of serum LDH and creatine kinase levels with the test drug treatment indicating the cardio protective effect of chloroform extract of *Carissa* . The treatment showed normal pancreatic β-cells. The protection might have been mediated through a *Carissa induced* increase in basal pancreatic SOD and catalase activities.

In our study, the diabetic rats had a decrease in the haemoglobin content with a proportionate increase in the glycosylated haemoglobin content. The increased glycosylated haemoglobin in the diabetic control rats indicates that erythrocytes are more prone to oxidative stress in diabetes. On the treatment of α-amyrin these changes were back near too normal. Therefore, at the end of the treatment the level of haemoglobin is improved and the glycosylated haemoglobin level is decreased. Glycogen is the primary intracellular storable form of

glucose and its level in various tissues especially in liver indicates direct reflection of insulin activity since it regulates glycogen storage by stimulating glycogen synthase and inhibiting glycogen phosphorylase.

Superoxide dismutase and catalase are the two major scavenging enzymes that remove radicals in vivo. Superoxide dismutase can catalyse dismutation of O2•− into H_2O_2, which is then deactivated to H_2O by catalase or glutathione peroxidase[21]. A decrease in the activity of these enzymatic antioxidants can lead to an excess availability of superoxide anion (O2•−) and hydrogen peroxide (H_2O_2), which, in turn, generate •OH, resulting in initiation and propagation of lipid peroxidation.

Glutathione peroxidase has a key role in enzymatic defense systems and reduces organic peroxides (H_2O_2, lipid or organic peroxides). The decrease may be due to the decreased availability of its substrate, GSH, which has been shown to be depleted during diabetes[19](Jain,*1995*). α-amyrin increased the activities of antioxidant enzymes in streptozotocin treated rats by inhibiting lipid peroxidation. The ability of α-amyrin to enhance the levels of antioxidants along with its anti-lipid–per oxidative activity suggests that this compound might be potentially useful in counteracting free radical mediated injuries involved in the development of tissue damage caused by streptozotocin -diabetic rats.

Glutathione is a major non-protein thiol in living organisms which plays a central role in co coordinating the antioxidant defense process in our body. It is involved in the maintenance of normal cell structure and function, probably through its redox and detoxification reaction[143]. Reduced glutathione functions as a free radical scavenger and in the repair of free radical caused biological damage[20]. Reduced glutathione is required for the recycling of vitamin C and acts as a substrate for glutathione peroxidase and glutathione reductase that are involved in preventing the deleterious effect of oxygen radicals. Diabetic rats exhibited a decreased level of reduced glutathione which might be due to increased utilization for scavenging free radicals and increased consumption by glutathione peroxidase and glutathione reductase. Treatment with α-amyrin significantly improved reduced glutathione level in the plasma and tissues of diabetic rats.

Diabetes is also known to be associated with an increase in the synthesis of cholesterol, which may be due to the increased activity of HMG-CoA reductase. HMG-CoA reductase catalyzes the rate limiting step in cholesterol biosynthesis, and its activity correlates closely with the rate of tissue cholesterol synthesis. Decreased HMG-CoA/mevalonate ratio indicates increased activity of the enzyme. Mounting evidence indicates that the activity of HMG-CoA reductase is increased in diabetic rats and deficiency of insulin is associated with increased HMG-CoA reductase activity.

Since, deficiency of insulin is associated with increase in HMG-CoA reductase activity and decrease in LPL activity in the diabetic state, increased insulin levels in the diabetic rats treated with α-amyrin might decrease the activity of HMG-CoA reductase and increase the activity of LPL.

Summary

The present investigation comprises of the phytochemical and pharmacological investigations of stem of *Carissa for* antidiabetic activity. The research work encompasses an in depth and systematic phytochemical and investigation of plant. The experimental work performed in this dissertation consists of three parts. First part consists of phytochemical screening and preliminary evaluation of anti-diabetic activity of plant stem in streptozotocin induced diabetes in rat.

The plant being authenticated and evaluated for different physicochemical properties which can be ash values, moisture content and extractive values etc. The acid insoluble ash value had been found to be less than the total ash value and water soluble ash value being found to be significantly less than total ash value within the proximate analysis. Alcohol soluble extractive value was significantly more than water soluble extractive value.

The Plant *Carissa* had been extracted successively by using soxhlet extraction method with ethanol and their color consistency and the percent yield had been determined. In preliminary phytochemical assessment, phytoconstituents like phytosterols, proteins, amino acids, flavonoids, phenols, steroids, terpenoids showed positive tests in the *Carissa Extract*.

In acute toxicity study, it was revealed that there were no signs of toxicity observed at a dose of 2000mg/kg body weight of *Carissa Extract*. So the 1/5th and 1/10th portion of 200 mg/kg were selected for further study.

When streptozotocin was given in rats it causes production of nitric oxide free radicals and alters the primary antioxidant defense mechanism mainly glutathione which counteract the oxidative stress. Induction of streptozotocin also causes increase in the level of serum CK and LDH. The level of lipid peroxide, TBARS was also increased whereas the level of SOD and catalase decreases indicates an oxidative stress condition. In present study, the chloroform extract of *Carissa* produced a marked decrease in blood glucose levels at 200 mg/kg and 400 mg/kg body weight in streptozotocin -diabetic rats after 21 days treatment. The antidiabetic effect *Carissa*

may be due to increased release of insulin from the existing β-cells of pancreas similar to that observed after glibenclamide administration.

There is a significant increase in levels of blood glutathione, as well as pancreatic levels of glutathione in diabetic rats when treated with chloroform extract of *Carissa*. Further, the activities of SOD and CAT were also increased in the pancreatic tissues of test drug-treated diabetic animals. The antioxidant activity of the test drug might have been due to the inhibition of glycation of the antioxidant enzymes SOD and CAT.

In the present study too, decrease in the level of glycosylated haemoglobin in rats treated with the chloroform extract of *Carissa* was observed which is not reported earlier. The diabetic rats showed significant decrease in the level of total haemoglobin and significant increase in the levels of HbA1C when compared with normal control rats. The levels of total haemoglobin and HbA1C were significantly reversed by the administration of α-amyrin in the diabetic rats.

Diabetic rats had elevated levels of thiobarbituric acid reactive substances (TBARS) and decreased activities of enzyme antioxidants (superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) in the plasma and tissues (liver and kidney).Oral administration of α-amyrin to diabetic rats resulted in decreased lipid peroxidation in plasma and tissues. The antioxidant status was also improved in diabetic rats treated with α-amyrin due to its antioxidant property effectively scavenged free radicals and decreased lipid peroxidation. A decrease in lipid peroxidation might be responsible for the increased activity of antioxidants and decreased oxidative tissue damage.

CONCLUSION

Our studies confirm that Ethanolic extract of *Carissa* shows anti-diabetic activity based on the results**.** Therefore, its traditional use, as mentioned above is justified and calls for further research, to optimize its anti-diabetic activity.

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