3.1 Introduction

Diabetes is the chronic metabolic syndrome characterised by impaired insulin secretion or abnormal insulin utilization by peripheral tissues\(^1\). This may generate an array of disturbances in glucose and lipid homeostasis resulting in hyperglycemia or dyslipidemia; the key risk factors of diabetes\(^2\). Persistent hyperglycaemia (glucotoxicity) in diabetes causes increased production of oxygen free radicals from glucose autoxidation\(^3\) and protein glycosylation\(^4\) which leads to oxidative stress. This affects normal functioning of vital organs like pancreas, liver, kidney and eye leading to secondary complications like liver cirrhosis, retinopathy and microvascular complications\(^5\). So, there is increasing importance for therapeutics which protect these organs from deleterious effect of diabetes associated problems. In view of these, therapeutic agents that can bring about tight glycemic control as well as protection from oxidative stress are very much essential to lessen the diabetes complications\(^6,7\).

No detailed study on the effect of *Symplocos cochinchinensis* on hyperglycemia associated secondary complications like liver damage, retinopathy, glycation, microvascular complications, muscle damage etc. is available in the literature except the study of Sunil et al.\(^8,9\). Since diabetes is the multifactorial disease that affects the functioning of various vital organs leading to different dangerous complications mentioned above, it is essential to study the effect of test material on foresaid complications if we are aiming for the development of therapeutics in future. It is known that differential bioactivity of various compounds present in the plant extract may be helpful to protect the organs of different nature and function from the deleterious effects of hyperglycemia and oxidative stress\(^10\). Similarly bark of SC is known for its
composition of various bioactives\textsuperscript{11}. We had seen the effect of SC on various druggable targets like alpha glucosidase inhibition, glucose uptake, adipogenic potential, oxidative stress, pancreatic beta cell proliferation, inhibition of protein glycation, protein tyrosine phosphatase-1B (PTP-1B) and dipeptidyl peptidase-IV (DPP-IV). We found SC is effective in alpha glucosidase inhibition insulin dependent glucose uptake in L6 myotubes, pancreatic beta cell regeneration in RIN-m5F and reduced triglyceride accumulation in 3T3-L1 cells, protection from hyperglycemia induced generation of reactive oxygen species in HepG2 cells. So herein, studies have been conducted to check whether SC is effective against diabetes induced secondary complications. There is high demand for the medication options in the form of drugs/nutraceuticals/phytoceuticals for diabetic retinopathy, neuropathy, nephropathy etc. Hence, hydroethanol (70\% ethanol-water) extract of SC was evaluated against streptozotocin induced pathopysiological alterations of liver, kidney, pancreas, muscle and eye lens in Sprague Dawley rats with more emphasis on oxidative stress and glycation related parameters including histopathological changes in pancreas.

3. 2 Experimental details

3. 2. 1 Chemicals and reagents

All the chemicals and biochemicals were from Sigma (St. Louis, MO, USA).

3. 2. 2 Plant material

The plant material was processed as described in Chapter 2 section 2. 2. 2. SCE has been selected for \textit{in vivo} study due to its better activity with \textit{in vitro} assays, increased yield of more bioactive molecules and less toxicity of the solvent\textsuperscript{12}. Since its selective nature, 70\% ethanol is most suitable solvent for \textit{in vivo} pharmacological evaluation compared to other solvents; it will dissolve only the required bioactive constituents with minimum amount of the inert materials\textsuperscript{12}.

3. 2. 3 Induction of diabetes in animals and experimental design

Male albino rats of Sprague Dawley (SD) strain (160±20 g), bred at animal facility of CSIR-CDRI, Lucknow were selected for this study. The maintenance of animals is outlined in Chapter 2 section 2. 2. 15. Animals were made diabetic by single
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intraperitoneal injection of streptozotocin (60 mg/kg in 100 mM citrate buffer - pH 4.5) after overnight fasting. Animals showing fasting blood glucose level (BGL) >270 mg/dL after 72 h were selected, termed as diabetic. Normal animals received intraperitoneal injection of 100 mM citrate buffer (pH 4.5). Rats were randomly divided into 6 groups as given below:

Group 1: Normal control animals (NC) treated with vehicle (1.0% gum acacia) alone

Group 2: Normal animals treated with SCE 500 mg/kg body weight per day (mg/kg bwd) (N+SCE500 for toxicity evaluation of extract).

Group 3: Diabetic control animals (DC) treated with streptozotocin (60mg/kg).

Group 4: Diabetic animals treated with SCE 250 mg/kg bwd (D+SCE250).

Group 5: Diabetic animals treated with SCE 500 mg/kg bwd (D+SCE500).

Group 6: Diabetic animals treated with metformin 100 mg/kg bwd (D+Met100).

Animals of experimental groups were orally administered with SCEs (250 or 500 mg/kg bwd) or metformin (100 mg/kg bwd) dissolved in 1.0% gum acacia for 21 days. Metformin is the widely used antidiabetic to treat the cardinal symptoms of diabetes like polyphagia, polydipsia and polyuria due to its pleiotropic effect via various targets and it shows wide tolerance and less toxicity compared to other antidiabetics. Animals of control group were given an equal volume of 1.0% gum acacia. For all animal groups, food and water intake was determined daily; body weight was checked at every week. After the 21 days treatment, rats were sacrificed on day 22 by cervical dislocation under light ether anaesthesia. Schematic representation of experimental design is given as Fig. 3.1.

3.2.4 Oral glucose Tolerance Test (OGTT)

A glucose load (3 g/kg) was given to each animal orally 30 min after test sample or vehicle administration. BGL was determined at 30, 60, 90 and 120 min post-administration of glucose. OGTT was performed on day 7, 14 and 21.
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Figure 3.1. Schematic representation of experimental design, Normal and diabetic SD rats. STZ, Streptozotocin; FPG, Fasting plasma glucose, SCE, S. cochinchinensis (SC) ethanolic extract.

3. 2. 5 Homeostatic model assessment-insulin resistance (HOMA-IR)

HOMA-IR was calculated using the formula; HOMA-IR = (glucose × insulin)/405, where the concentration of glucose expressed in mg/dL and that of insulin in mU/L.

3. 2. 6 Biochemical parameters

Blood samples were collected from retro-orbital plexus of each animal in heparin coated
Figure 3. 2A-C. Effect of SCE on body weight, food and water intake. (A) Body weight, (B) food intake and (C) water intake. Data are expressed as the mean ± SD, n = 6. ‘a’ represents groups differ significantly from normal control group (p≤0.05). ‘b’ represents groups differ significantly from diabetic control group (p≤0.05). ‘ab’ represents groups differ significantly from both normal control and diabetic control group (p≤0.05). SCE, S. cochinchinensis (SC) ethanolic extract; NC, normal control group; N+SCE 500, normal control group treated with SCE 500 mg/kg bwd; DC, diabetic control group; D+SCE 250, diabetic control group treated with SCE 250 mg/kg bwd; D+SCE 500, diabetic control group treated with SCE 500 mg/kg bwd and D+Met 100, diabetic control group treated with metformin 100mg/kg bwd.

tubes for the separation of plasma and further analytical procedures on day 0 and 21, during the experiment. Plasma was separated by centrifugation at 3000xg for 20 min at 4°C, aliquots were made, one lot was used for the immediate analysis of triglyceride, total
cholesterol, HDL-C, LDL-C and the remaining portion was immediately frozen and stored at -80°C for further analysis. Plasma level of insulin, % HbA1c, aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, creatinine, albumin and total protein were measured. Insulin level was assayed using an ELISA kit from Mercodia (Uppsala, Sweden). All other plasma parameters were quantified using kits from Agappe diagnostics (Knonauerstrasse, Switzerland).

3.2.7 Evaluation of oxidative stress markers and glycogen content

Immediately after sacrifice of animal, liver, kidney and muscle tissues were excised, frozen in liquid nitrogen prior to storage at -80°C until further analysis. Hepatic and renal oxidative stress markers like reduced glutathione (GSH), total antioxidant activity, malondialdehyde (MDA), protein carbonyl content and antioxidant enzymes like superoxide dismutase (SOD, EC 1.15.1.1) and glutathione peroxidase (GPx, EC 1.11.1.9) were measured using kits from Cayman chemicals (Cayman, MI, USA). Liver and muscle glycogen content was measured using glycogen assay kit from Cayman chemicals.

3.2.8 Measurement of lens aldose reductase activity

Eye ball was removed to take out lens from animal. The lenses were enucleated by posterior approach. 10% lens homogenate (w/v) was prepared in 0.1 M phosphate buffer saline (pH 7.4). After centrifugation at 5000×g for 20 min at 4°C, the supernatant was collected and used for the determination of aldose reductase enzyme (AR, EC 1.1.1.21) activity according to the method described by Hayman and Kinoshita15. In brief, reaction mixture contains 0.7 mL of sodium phosphate buffer (67 mM, pH 6.2), 0.1 mL of NADP (25×10^{-5} M) and 0.1 mL of lens homogenate, in a final volume of 1 mL. The enzyme reaction was started by the addition of the 0.1 mL substrate (DL±Glyceraldehyde, 1 mM) and absorbance was recorded at 340 nm for 3 min at 30 sec time interval. Enzyme activity was expressed as change in OD/min/mg protein (ΔOD/min/mg protein).

3.2.9 Histopathology

Immediately after dissection, pancreas was taken out, cleaned and fixed in 10% neutral buffered formalin solution for the preparation of histopathological slides. After fixation, tissues were dehydrated in graded ethanol series, cleared in xylene and embedded in
paraffin wax. The solid sections were prepared at 5 mm thickness using a microtome, stained with haematoxylin-eosin (H&E). The sections were examined under light microscope and photomicrographs were taken.

3. 2. 10 Statistical analysis

Quantitative glucose tolerance of each group was calculated by the area under the curve (AUC) method using GraphPad Prism software version 3 (Graphpad Software Inc., La Jolla, CA, USA). All other results were analyzed using a statistical program SPSS/PC +, version 11.0 (SPSS Inc., Chicago, IL, USA). Data were presented as mean ± SD, n = 6. p≤0.05 was considered to be significant.

3. 3 Results

3. 3. 1 Fasting plasma glucose, body weight, food and water intake

More than 90% of STZ injected animals had developed symptoms of diabetes. There was no change in fasting plasma glucose of normal control animals (NC) during the experiment period but the diabetic control animals (DC) showed a progressive and significant increase in fasting plasma glucose (from 274 to 449 mg/dL) during 21 days period of the experiment. The summary of body weight, food and water intake are given in Fig. 3. 2.

3. 3. 2 Amelioration of glucose intolerance by SCE

The diabetic control animals showed severe glucose intolerance throughout the experiment period. Treatment with SCE 250 or 500 mg/kg bwd (SCE 250/500) and metformin 100 mg/kg bwd (Met 100) significantly (p≤0.05) reduced blood glucose profile on day 7, 14 and 21. Day 7 OGTT exhibited 24.64%, 30.23% and 42.96% reduction in glycemic response in SCE 250/500 and Met 100 treated groups respectively (Fig. 3. 3A and B). Day 14 OGTT showed 32.93%, 38.41% and 47.30% reduction in glycemic response in SCE 250/500 and Met 100 groups respectively (Fig. 3. 3C and D), while day 21 OGTT exhibited further improvement of 40.76%, 46.28% and 57.53% reduction in glycemic response in SCE 250/500 and Met 100 groups respectively (Fig. 3. 3E and F). Both extracts and metformin showed dose and duration dependent reduction in glycemic responses.
Figure 3. 3A-F. The improved glucose tolerance exhibited by streptozotocin-induced diabetic rats by SCE treatment on day 7, 14 and 21. (A) The glycemic response curve and (B) incremental AUC$_{0-120}$ min in normal rats, (C) The glycemic response curve and (D) incremental AUC$_{0-120}$ min in SLM model, (E) The glycemic response curve and (F) incremental AUC$_{0-120}$ min in STZ-S model. Data are expressed as the mean ± SD, n = 6. * represents groups differ significantly from control group (p≤0.05). SCE, S. cochinchinensis (SC) ethanolic extract; DC, diabetic control group; D+SCE 250, diabetic control group treated with SCE 250 mg/kg bwd; D+SCE 500, diabetic control group treated with SCE 500 mg/kg bwd and D+Met 100, diabetic control group treated with metformin 100 mg/kg bwd.

3.3 SCE improved plasma insulin level and insulin sensitivity

Plasma insulin level was depleted significantly (p≤0.05) in STZ rats, while SCE and metformin treatment improved the insulin levels (Fig. 3. 4A). Insulin sensitivity was
determined using HOMA-IR based on fasting plasma glucose and insulin levels. The insulin resistance of the DC animals was found to be increased 2 fold when compared to normal group on day 0 (Fig. 3. 4B). The diabetic control group showed around 3 fold increase in the insulin resistance on day 21 while the SCEs and metformin treatment in diabetic groups improved the insulin sensitivity comparable to that of normal control animals (Fig. 3. 4B).

Figure 3. 4A and B. SCE enhanced plasma insulin level and insulin sensitivity. (A) Plasma insulin level and (B) Homeostatic model assessment-insulin resistance (HOMA-IR). Data are expressed as the mean ± SD, n = 6. ‘a’ represents groups differ significantly from normal control group (p≤0.05). ‘b’ represents groups differ significantly from diabetic control group (p≤0.05). ‘ab’ represents groups differ significantly from both normal control and diabetic control group (p≤0.05). SCE, S. cochinchinensis (SC) ethanolic extract; NC, normal control group; N+SCE 500, normal control group treated with SCE 500 mg/kg bwd; DC, diabetic control group; D+SCE 250, diabetic control group treated with SCE 250 mg/kg bwd; D+SCE 500, diabetic control group treated with SCE 500 mg/kg bwd and D+Met 100, diabetic control group treated with metformin 100mg/kg bwd.
Figure 3. 5A and B. Effect of SCE in lens aldose reductase activity and % HbA1c level. (A) Aldose reductase activity in the eye lens and (B) % HbA1c level of various groups. Data are expressed as the mean ± SD, n = 6. ‘a’ represents groups differ significantly from normal control group (p≤0.05). ‘b’ represents groups differ significantly from diabetic control group (p≤0.05). ‘ab’ represents groups differ significantly from both normal control and diabetic control group (p≤0.05). SCE, S. cochinchinensis (SC) ethanolic extract; NC, normal control group; N+SCE 500, normal control group treated with SCE 500 mg/kg bwd; DC, diabetic control group; D+SCE 250, diabetic control group treated with SCE 250 mg/kg bwd; D+SCE 500, diabetic control group treated with SCE 500 mg/kg bwd and D+Met 100, diabetic control group treated with metformin 100mg/kg bwd.

3. 3. 4 Effect of SCE in lens aldose reductase activity and % HbA1c level

The aldose reductase activity was elevated significantly (p≤0.05) by 25.92% in the lens of diabetic animals after 21 days compared to the normal animals (Fig. 3. 5A). The lens aldose reductase activity was reduced by 14.66%, 15.99% and 17.32% respectively with SCE 250/500 and Met 100 for 21 days (Fig. 3. 5A). A significant elevation of % HbA1c was observed in diabetic control animals compared to the normal ones (p≤0.05). %
HbA1c level of diabetic control animals was found to be 8.95 and that of normal control rats was 4.60 on day 21 (Fig. 3.5B). SCE 250/500 or Met 100 treatment resulted in significant (p≤0.05) improvement in % HbA1c; 6.92, 6.61 and 6.32 respectively after 21 days (Fig. 3.5B).

3.3.5 SCE enhanced hepatic and renal function

ALT, the specific biomarker for hepatic damage was significantly (p≤0.05) enhanced (1.58 fold) in STZ diabetic rats compared to normal control (Fig. 3.6A). The second important indicator of hepatic injury, AST was also significantly (p≤0.05) elevated (1.59 fold) in diabetic rats compared to normal control (Fig. 3.6B). The decreased plasma albumin level of diabetic animals (0.44 fold) indicated the diminished liver and kidney function (Fig. 3.6C). The abnormal plasma concentration of total protein (0.40 fold decrease), urea (3.36 fold increase) and creatinine (2.46 fold increase) revealed the renal dysfunction in diabetic rats (Fig. 3.6D-F). The diabetic animals treated with SCE 250/500 exhibited significant (p≤0.05) improvement after 21 days in ALT, AST, albumin, urea, creatinine and total protein levels (Fig. 3.6A-F).

3.3.6 Beneficial effect of SCE in plasma lipid profile

DC animals showed significantly (p≤0.05) elevated plasma triglyceride (2.14 fold), total cholesterol (3.37 fold) and LDL-C (8.9 fold fold) concentration compared to normal control animals; 0.35 fold decrease in HDL-C level was also noticed in DC animals (Fig. 3.7A-D). Administration of SCE 250/500 for 21 days resulted in a significant (p≤0.05) decrease in the level of TG, TC and LDL (Fig. 3.7A-D). Effect of SCE 500 on lipid profile was comparable to that of metformin. Plasma HDL concentration was elevated significantly (p≤0.05) in both SCEs and metformin treated animals.

3.3.7 SCE showed protection against hepatic and renal oxidative stress

A marked increase in the carbonyl content (2.64 fold), MDA level (1.65 fold), and antioxidant enzyme activities; SOD (1.46 fold) and GPx (1.72 fold) were detected in hepatic tissue of diabetic animals compared to normal controls (Fig. 3.8C-F). Similarly, in renal tissue also there was a significant (p≤0.05) increase in the carbonyl content (1.57 fold), MDA level (2 fold), and antioxidant enzyme activities; SOD (1.37 fold) and GPx
Figure 3. 6A-F. SCE enhanced hepatic and kidney function. Plasma concentration of (A) alanine aminotransferase (ALT), (B) aspartate aminotransferase (AST), (C) albumin, (D) total protein, (E) urea and (F) creatinine in various groups. Data are expressed as the mean ± SD, n = 6. ‘a’ represents groups differ significantly from normal control group (p≤0.05). ‘b’ represents groups differ significantly from diabetic control group (p≤0.05). ‘ab’ represents groups differ significantly from both normal control and diabetic control group (p≤0.05). SCE, S. cochinchinensis (SC) ethanolic extract; NC, normal control group; N+SCE 500, normal control group treated with SCE 500 mg/kg bwd; DC, diabetic control group; D+SCE 250, diabetic control group treated with SCE 250 mg/kg bwd; D+SCE 500, diabetic control group treated with SCE 500 mg/kg bwd and D+Met 100, diabetic control group treated with metformin 100mg/kg bwd.
Figure 3. 7A-D. Beneficial effect of SCE in plasma lipid profile. Plasma concentration of (A) Triglyceride (TG), (B) Total Cholesterol (TC), (C) High density lipoprotein cholesterol (HDL-C), and (D) Low density lipoprotein cholesterol (LDL-C) in various groups. Data are expressed as the mean ± SD, n = 6. ‘a’ represents groups differ significantly from normal control group (p≤0.05). ‘b’ represents groups differ significantly from diabetic control group (p≤0.05). ‘ab’ represents groups differ significantly from both normal control and diabetic control group (p≤0.05). SCE, S. cochinchinensis (SC) ethanolic extract; NC, normal control group; N+SCE 500, normal control group treated with SCE 500 mg/kg bwd; DC, diabetic control group; D+SCE 250, diabetic control group treated with SCE 250 mg/kg bwd; D+SCE 500, diabetic control group treated with SCE 500 mg/kg bwd and D+Met 100, diabetic control group treated with metformin 100mg/kg bwd.
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Figure 3. 8A-F, SCE showed protection against hepatic oxidative stress. Hepatic (A) reduced glutathione (GSH) level, (B) total antioxidant activity, (C) super oxide dismutase (SOD) activity, (D) glutathione peroxidise (GPx) activity, (E) malondialdehyde (MDA) level and (F) protein carbonyl content. Data are expressed as the mean ± SD, n = 6. ‘a’ represents groups differ significantly from normal control group (p≤0.05). ‘b’ represents groups differ significantly from diabetic control group (p≤0.05). ‘ab’ represents groups differ significantly from both normal control and diabetic control group (p≤0.05). SCE, *S. cochinchnensis* (SC) ethanolic extract; NC, normal control group; N+SCE 500, normal control group treated with SCE 500 mg/kg bwd; DC, diabetic control group; D+SCE 250, diabetic control group treated with SCE 250 mg/kg bwd; D+SCE 500, diabetic control group treated with SCE 500 mg/kg bwd and D+Met 100, diabetic control group treated with metformin 100mg/kg bwd.
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(1.63 fold) (Fig. 3. 9C-F) and carbonyl content (1.58 fold) in muscle (Fig. 3. 10C) in diabetic animals compared to normal control animals. But, GSH and total antioxidant levels in hepatic and renal tissues were found to be decreased (Fig. 3. 8A-B and 3. 9A-B). Both the doses of SCE and metformin treatment reduced renal and hepatic carbonyl content, MDA level, SOD, GPx enzyme activities (Fig. 3. 8C-F and 3. 9C-F) and muscle carbonyl content (Fig. 3. 10C) which is comparable to normal range. SCE and metformin treatment also improved the antioxidant status of hepatic and renal tissues by protecting GSH and total antioxidant levels (Fig. 3. 8A-B and 3. 9A-B).

3. 3. 8 SCE promoted elevation in glycogen content

There was a significant depletion in glycogen content of liver (71%) and muscle (59%) of DC animals (Fig. 3. 10A and B) compared to normal control group (p≤0.05). Administration of SCE 250/500 or Met 100 for 21 days improved significantly the glycogen content in liver by 27.30, 36.58 and 43.83% respectively compared to diabetic animals (Fig. 3. 10A) and in muscle by 19.90%, 31.11% and 42.45% respectively (Fig. 3. 10B).

3. 3. 9 Protection of pancreatic islets integrity by SCE

Histological analysis of pancreas from normal control rat revealed the presence of well defined islets of Langerhans surrounded by acinar cells of exocrine pancreas (Fig. 3. 11a). The pancreas from normal control rat treated with SCE 500 exhibited more or less similar morphology to that of normal control animal, confirming the safety of SCE at higher dose (Fig. 3. 11b). But histopathology of pancreas of DC displayed pancreatic islets damage and presence of inflammatory and necrotic cells (Fig. 3. 11c). However, administration of SCE 250/500 for 21 days prevented the histopathological alterations in DC animals; endocrine cells were found to be in normal morphology (Fig. 3. 11e and f) and in metformin group the pancreas exhibited almost similar morphology to that of normal control without any change in endocrine cell population (Fig. 3. 11d).

3. 4 Discussion

Diabetes is a syndrome of metabolic derangement with diminished production of insulin (type 1) or impaired response to insulin and pancreatic beta-cell dysfunction (type 2) 1.
Both are characterised by hyperglycemia which leads to excessive urine production, compensatory thirst, increased fluid intake, unexplained weight loss, changes in energy metabolism, retinopathy, neuropathy, nephropathy and vascular complications\(^5\). The incidence of free radical-mediated toxicity is well documented in clinical diabetes\(^6\) and STZ-diabetic rats\(^7\). The elevated levels of toxic oxidants in diabetic animals are due to processes such as glucose autoxidation and lipid peroxidation\(^8\). In order to minimise the oxidative stress derived diabetic complications, a strict and careful glycemic control by the use of safe and efficacious therapeutic agents with antihyperglycemic and antioxidant potential is strongly recommended. Since hyperglycemia alter the functions of various vital organs via. depletion or imbalance of redox status, detailed study on various organs with respect to innate antioxidant and associated activity is very much essential for the evaluation of efficacy of test material.

From ancient times, diabetes has been treated with herbal medicines with the emphasis on maintaining normoglycemia and protection from damaging effects of this disease. In this regard, we studied the effect of SCE in the level of antioxidant enzymes (SOD and Gpx), oxidative stress markers like GSH, MDA, protein carbonyls etc. in the liver and kidney tissues of STZ diabetic rat model. Further we checked the effect of SCE in the serum % HbA1c level, lens aldose reductase activity and glycogen and protein carbonyl content in muscle. The present study also evaluated the extent of pancreatic islets damage and functional alteration and the protective property of SCE by histopathology and HOMA-IR analysis. This is the first detailed report on effect of SCE on hyperglycemia induced secondary complications of liver, kidney, eye and muscle. In this study, we selected STZ as the diabetogenic agent, as it specifically targets pancreatic beta cells by its alkylating property without direct damage to other tissues\(^9\). The selectivity for beta cells is associated with preferential accumulation of streptozotocin in beta cells after entry through the GLUT-2 glucose transporter receptor due to the chemical structural similarity with glucose\(^10\). Animals exhibited fasting hyperglycemia (>270 mg/dL) by a single intraperitoneal injection of streptozotocin after 72 h. Animals also displayed diabetes symptoms like increased food and water intake and loss of body weight within the first week itself. SCE administration for 21 days modulated the feeding and water intake pattern of diabetic animals in a similar way as noticed in metformin treated group (Fig. 3. 2B and C). The weight loss of diabetic animals was also limited to an extent by SCE and
Figure 3. 9A-F. SCE showed protection against renal oxidative stress. Renal (A) reduced glutathione (GSH) level, (B) total antioxidant activity, (C) super oxide dismutase (SOD) activity, (D) glutathione peroxidise (GPx) activity, (E) malondialdehyde (MDA) level and (F) protein carbonyl content. Data are expressed as the mean ± SD, n = 6. ’a’ represents groups differ significantly from normal control group (p≤0.05). ’b’ represents groups differ significantly from diabetic control group (p≤0.05). ’ab’ represents groups differ significantly from both normal control and diabetic control group (p≤0.05). SCE, S. cochinensis (SC) ethanolic extract; NC, normal control group; N+SCE 500, normal control group treated with SCE 500 mg/kg bwd; DC, diabetic control group; D+SCE 250, diabetic control group treated with SCE 250 mg/kg bwd; D+SCE 500, diabetic control group treated with SCE 500 mg/kg bwd and D+Met 100, diabetic control group treated with metformin 100mg/kg bwd.
Figure 3. 10A-C. SCE promoted improvement in glycogen content. (A) liver glycogen content, (B) muscle glycogen content (C) muscle protein carbonyl content. Data are expressed as the mean ± SD, n = 6. ‘a’ represents groups differ significantly from normal control group (p≤0.05). ‘b’ represents groups differ significantly from diabetic control group (p≤0.05). ‘ab’ represents groups differ significantly from both normal control and diabetic control group (p≤0.05). SCE, S. cochinchinensis (SC) ethanolic extract; NC, normal control group; N+SCE 500, normal control group treated with SCE 500 mg/kg bwd; DC, diabetic control group; D+SCE 250, diabetic control group treated with SCE 250 mg/kg bwd; D+SCE 500, diabetic control group treated with SCE 500 mg/kg bwd and D+Met 100, diabetic control group treated with metformin 100mg/kg bwd.
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metformin treatment (Fig. 3. 2A). From the OGTT results, it was evident that SCE treatment in these animals improved the glucose tolerance in a dose dependent manner from day 7 to day 21 (Fig. 3. 3A-F).

Insulin production becomes impaired in STZ-induced diabetic rats\(^{21}\). Our animals in the diabetic group showed impaired plasma insulin level (Fig. 3. 4A). But with SCE treatment plasma insulin level was elevated significantly. HOMA-IR analysis based on fasting measurements of glucose and insulin serves as reasonable and reliable method to measure the insulin sensitivity and beta cell function of rodent models as well as human subjects\(^{22}\). From the HOMA-IR results it is clear that the degree of insulin resistance is higher in diabetic group during the experiment period from day 0 to day 21. Both doses of SCE and metformin assisted in protecting the pancreas from its structural as well as functional alterations (Fig. 3. 4 and 3. 11a-f). This may be due to the insulinotropic effect of this extract. This improved pancreatic function in SCE treated STZ diabetic animals is in line with the beneficial effect of SCE in the pancreatic beta cell proliferation in cell line models that we have noticed in Chapter 2.

The excess prevalence of liver disease in diabetic population has stimulated interest in studying pathological changes in liver during hyperglycemia for control and management of diabetic complications\(^{23}\). AST and ALT levels were increased in diabetic control animals while treatment with SCE or metformin reduced plasma AST and ALT levels (Fig. 3. 6A and B). This indicates the hepatoprotective property of SCE in STZ diabetes. The enzymes activities of AST and ALT provide the link between carbohydrate and protein metabolism by the inter-conversion of the intermediates of metabolic pathways\(^{24}\). AST catalyzes the interconversion of aspartic and alpha-ketoglutaric acids to oxaloacetic and glutamic acids, while ALT catalyzes the interconversion of alanine and alpha-ketoglutaric acid to pyruvic and glutamic acids\(^{25}\). The ultimate result is the addition of keto acids into the Krebs cycle. The increased levels of AST and ALT activity can be regarded as a signal for enhanced gluconeogenesis\(^{26, 27}\). Because SCE decreases AST and ALT levels, it is acceptable that SCE reduces gluconeogenesis and can be further investigated for the treatment of diabetes mellitus. Negative nitrogen balance with elevated tissue proteolysis and declined protein synthesis can contribute to increased serum urea and creatinine levels, indicating impaired renal functions in diabetic animals\(^{28}\).
In the present study the diabetic control rats exhibited significantly higher plasma urea and creatinine levels compared to the normal control group. But SCE administration resulted in a lower plasma level of urea and creatinine in treated groups compared to the diabetic control group (Fig. 3. 6E and F). This shows protective property of SCE against renal damage.

In the pathogenesis of diabetic complications important risk factors include not only duration of hyperglycemia, but also dyslipidemia. Hyper-triglyceridemia and low level of HDL are the most common lipid abnormalities related to diabetes mellitus. Here also, the diabetic animals exhibited hyper-triglyceridemia, hypercholesterolemia, elevated LDL and low HDL level compared to the normal control group (Fig. 3. 7A-D). But in comparison with the diabetic control group, SCE treated groups showed a good TG-HDL ratio and lower TC and LDL levels indicating therapeutic potential of SC against lipid abnormalities associated with hyperglycemia.

The studies on oxidative stress and diabetic complications have shown that oxidative stress accelerates the development of pathological lesions linked to the state of hyperglycemia. GSH and antioxidant enzymes are the key agents which protect tissues from oxidative damage. GSH can participate in the elimination of reactive intermediates by reducing hydroperoxides in the presence of glutathione peroxidase. GSH is essential for the GSH redox cycle, which maintains adequate levels of reduced cellular GSH and a high GSH/GSSG ratio is essential for protection against oxidative stress. There was significant depletion in GSH and total antioxidant level in the hepatic and renal tissues of diabetic rats compared to the normal control group. The decrease in GSH level also represents its increased utilization due to oxidative stress. However, SCE treatment elevated the cellular antioxidant status as well as the GSH levels. ROS scavenging enzymes can respond to conditions of increased oxidative stress with a compensatory mechanism that increases the enzyme activity in diabetic rats. SOD, a prominent member of innate antioxidant system catalyses dismutation of superoxide (O$_2^-$) to oxygen and H$_2$O$_2$. Likewise glutathione peroxidase is involved in the reduction of deleterious lipid hydroperoxides to their corresponding alcohols and H$_2$O$_2$ then to water. Both SOD and GPx activities is found to be increased in diabetic conditions. In our study, also diabetic control showed elevated level of both hepatic and renal SOD and GPx activities.
Figure 3.11a-f. SCE showed protection of pancreas islets integrity. Representative microscopic scans of rat pancreas stained by Hematoxylin and Eosin (H and E 400x), (a) normal control group, (b) normal control group treated with SCE 500 mg/kg bwd, (c) diabetic control group, (d) diabetic control group treated with SCE 250 mg/kg bwd, (e) diabetic control group treated with SCE 500 mg/kg bwd and (f) diabetic control group treated with metformin 100mg/kg bwd. SCE, *S. cochinchinensis* (SC) ethanolic extract.

But administration of SCE was able to bring down these enzyme activities and was comparable with metformin in hepatic (Fig. 3. 8C and D) and renal tissues (Fig. 3. 9C and D). This result along with other parameters related to oxidative stress like % HbA1c, carbonyl content, pancreas damage etc. reveal the significant potential of SC to protect
the innate antioxidant system of body. Moreover, SCE exhibited significant antioxidant potential in in vitro cell free assays as well as it could protect cells from hyperglycemia induced oxidative stress in HepG2 high glucose model. Thus, the in vitro radical scavenging property of SCE was found to be effectively translated to these in vivo results also.

The protein carbonyls like advanced oxidation protein products, AGEs, % HbAlc etc act as "metabolic memory" markers resulting from the hyperglycemia and hyperlipidemia-induced oxidative stress. These increased chemical modification of proteins by carbohydrates and lipids in diabetes may bring about overload on metabolic pathways involved in detoxification of reactive carbonyl species. This increases the steady-state levels of reactive carbonyl compounds formed by both oxidative and non-oxidative reactions. The protein carbonyl content of diabetic hepatic (Fig. 3. 8F), renal (Fig. 3. 9F) and muscle tissue (Fig. 3. 10C) were found to be significantly higher compared to the normal animal. In addition an increase in lipid peroxide concentration in the tissues of diabetic animals has been observed. In line with this, the content of MDA in the diabetic liver (Fig. 3. 8E) and kidney (Fig. 3. 9E) was elevated than the normal control group in this study. Both protein carbonyl accumulation and lipid peroxidation in renal and hepatic tissues of diabetic rats was ameliorated by SCE treatment revealing potential capability of SCE against lipid peroxidation.

Diabetic subjects are more likely to be blind than non-diabetics of a similar age. Retinopathy is the commonest complication of diabetes and approximately 10% of diabetics at any one time will have sight-threatening retinopathy requiring specialist ophthalmological management. Although the complete etiology of diabetic retinopathy remains unknown, the polyol pathway has been associated with this. The organs like kidney, eye etc. that are susceptible to diabetic complications, exhibit insulin independent glucose uptake and possess the glucose metabolizing enzyme aldose reductase. Under hyperglycemia, increased glucose flux occurs in these tissues; through polyol pathway, aldose reductase catalyzes the conversion of glucose to sorbitol and that may cause accumulation of sorbitol. This may result in the development of hyperosmotic stress to the cells of retina which leads to the development of diabetic complications. The elevated % HbA1c level is considered as one of the important risk factor of proliferative
diabetic retinopathy. The increased level of advanced glycation end products (AGEs) and glycated hemoglobin are the dangerous consequences related to hyperglycemia. AGEs damage the cells in different ways, like by modifying intracellular proteins of endothelial cells or extracellular matrix proteins, or by spreading the precursors outside the cell and their complexes with serum albumins. SCE treatment reduced both lens aldose reductase activity (Fig. 3.5A) as well as elevated % HbA1c level (Fig. 3.5B) of diabetic animals revealing its beneficial effects against major secondary complications. In our in vitro cell free assays, SCE showed a moderate antiglycation and metal chelation properties which may contribute partly for reduction in % HbA1c level of SCE administered STZ animals.

The liver preserves normal blood glucose concentrations by storing glucose as glycogen and by generating glucose from glycogen breakdown or from gluconeogenic precursors. The liver equilibrates the uptake and storage of glucose via glycogenesis and regulates the release of glucose by activating glycogenolysis and gluconeogenesis. The major storage tissues such as liver, kidney, and skeletal muscle depend on insulin for glucose access. Glycogen deposition from glucose is altered in experimentally induced diabetic animals. Here also diabetic animals showed depletion in the amount of liver (Fig. 3.10A) and muscle (Fig. 3.10B) glycogen compared to the normal control. Considerable amount of glycogen was replenished in diabetic animals by SCE treatment.

Oxidative stress produced under diabetic conditions is likely involved in progression of pancreatic beta-cell dysfunction found in diabetes. Pancreatic beta-cells are vulnerable to damage induced by oxidative stress, possibly due to the low levels of antioxidant enzyme expressions. Integrity of pancreas is very much essential for the normal insulin secretion. These symptoms are clear in our study from the histopathology details of pancreas of DC (Fig. 3.11c). But, both the doses of SCE repaired most of the histopathological alterations in pancreas (Fig. 3.11e and f). The antioxidant potential of SCE may have protected the pancreatic beta-cells from the toxic effects of hyperglycemia in these cells. It is worth to mention that metformin, the positive control employed in this study protected all the vital organs from the deleterious effects of hyperglycemia in STZ diabetic rats.
3. 5 Conclusion

Overall results show that SCE can exert beneficial effects on liver, kidney, pancreas, eye lens and muscle against hyperglycemia induced secondary complications. Furthermore, SCE does not affect these cited parameters in normal control rats. These findings indicate that SCE only affects these markers in diseased conditions and suggests that this herb is safe for consumption by healthy subjects. On the basis of this, SC is recommended for further detailed research for the development of new chemical entities for the management of secondary complications of diabetes.
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