CHAPTER IV

4. DEVELOPMENT OF BIOACTIVE MOLECULES LOADED CHITOSAN NANO PARTICLES: PREPARATION, CHARACTERIZATION AND ANTIDIABETIC STUDY IN RAT MODEL

4.1 Introduction

Bioactive molecules, endogenous peptides, protein and oligonucleotides are among the main drugs which attract much attention because of their great potentials in treating chronic diseases such as diabetes mellitus (Bowman and Leong, 2006). However, the extreme in vivo environment of human body has always limited the therapeutic applications of these substances (Devalapally et al., 2007). Nanoparticles composed of naturally occurring biodegradable polymers have emerged as potential carriers of various therapeutic agents for controlled drug delivery through the oral route. Polymeric nanoparticles have attracted much attention as delivery systems due to their ability in overcoming the physiological barriers and protecting and targeting the loaded substances to specific cells (Singh and Lillard, 2009).

Ethnopharmacological approach on the synthesis of nanoparticles is an exciting technology in creating symbiosis between nano and medical science. In this context, the functional chitosan encapsulated nanoparticles by incorporation of biomolecules and phytochemicals from medicinal plant extracts could be prepared as antidiabetic nanomaterials for its application in biomedicine and bionanotechnology. Experimental induction of diabetes mellitus in animal models is an essential step for understanding various aspects of hypoglycemia and ultimately finding new therapies and cure. An exciting result in diabetic treatments is to encapsulate the drug in a thermo-responsive biocompatible material that can be consumed orally with the intention of delivering drug to a pancreas in response to an external thermal activation source. Design and development of biodegradable controlled drug delivery of therapeutic entities with improved bioavailability is the main research aspect on which extensive work has been done in the recent past. One of the promising and exciting drug delivery systems was the above mentioned polymeric nanoparticles (Mulik et al., 2009). Chitosan, the abundant biodegradable
natural polymer with great potential for pharmaceutical applications due to its biocompatibility, high charge density, non-toxicity and mucoadhesion (Calero et al., 2010). It was reported that high molecular weight chitosan demonstrated the potential of reducing hyperglycemia and hypercholesterimia in streptozotocin induced diabetic rats (Yao et al., 2008). Chitosan-based nanoparticles are very important carriers for drug delivery and the supplement of antidiabetic drugs. Therefore it is feasible to develop chitosan nanoparticles encapsulated with bioactive molecules derived from two plant extracts for effective antidiabetic drug delivery.

In view of the above, the present research was mainly focused on fabrication and characterization of chitosan–TPP nanoparticles loaded with bioactive molecules of Gymnema sylvestre and Stevia rebaudiana plant extracts by ionic cross linking nanoprecipitation via dropping method. We also confirmed the successful encapsulation of bioactive molecules with chitosan by FT-IR spectroscopy and XRD. The shape, morphology and size of the synthesized chitosan nanoparticles were also determined by TEM and Zeta potential. In addition, the antidiabetic activity of the synthesized chitosan nanoparticles was also investigated using rat model system.

4.2 Materials and methods

4.2.1 Plant materials

Fresh leaves of Gymnema sylvestre were collected from ABS medicinal garden, Salem and Stevia rebaudiana leaves were collected from Horticulture Research Station, Tamil Nadu Agriculture Research Station, Yercaud, Salem, Tamil Nadu, India. The leaves were shade dried and ground into fine powder for further use.

4.2.2 Preparation of Gymnema sylvestre plant extracts

The coarse powder [100 g (w/v)] was extracted with 500 ml of hydro-methanol (20:80) using a soxhlet extractor for 12h at a temperature (64°C) not exceeding the boiling point of the solvents. The extract was filtrated using Whatman
filter paper (No. 1) and then concentrated at 40°C using a rotary evaporator. The residue was stored in freezer until further tests.

4.2.3 Extraction of sweet diterpene glycosides from S. rebaudiana

The coarse powder (100 g (w/v)) was extracted with 500 ml of hydro-methanol (30:70) using a soxhlet extractor for 12h at 64°C not exceeding the boiling point of the solvents. The extract was filtrated through Whatman filter paper (No. 1) and then concentrated at 40°C using a rotary evaporator. The residue was stored in the freezer until further tests.

4.2.4 Development of bioactive molecules loaded CNPs

Bioactive molecules loaded chitosan nanoparticles were prepared from nanoprecipitation method via dropping technique. A total volume of 150 ml chitosan (10 mg/ml in 1% acetone) was added drop wise to 50 ml aqueous Gymnema sylvestre leaf extracts (GSLE) /Stevia rebaudiana leaf extracts (SRLE) (10 mg/ml) having 0.5% (w/v) tripolyphosphate anions (TPP) with speed of one drop per second and constant stirring at 120 rpm and the nanoparticles suspension was allowed to stir for 30 min. Similarly, blank chitosan nanoparticles were prepared by repeating the above process. The nanoparticles were collected by centrifugation at 12,000 rpm for 15 min and washed thrice with distilled water. The washed nanoparticles were characterized using various methods. The final product was stored at 4°C until further use.

4.2.5 Characterization

4.2.5.1 Size measurements of nanoparticles

The particle size measurements for the determination of the average particle mean were performed on a Zetasizer (Nano ZS, Malvern) equipped with a 5 mW helium/neon laser to excite the samples. For the study, measurements of the nanoformulations were performed at 25°C in triplicate with 10-min runs. Scattered light was collected at an angle of 173° by a photon counting photomultiplier tube that was then directed towards a correlator to derive particle size from the correlator function. Results were expressed as the Z-average mean which was the harmonic intensity
averaged particle diameter. The results were presented in average mean diameters with an additional polydispersity index, which gave further information about the homogeneity of nanoparticles synthesis.

4.2.5.2 Fourier transform-infrared spectroscopy (FT-IR)

FT-IR analysis of pure plant extracts unloaded and loaded chitosan nanoparticles were recorded by a Perkin-Elmer FT-IR spectrometer, model Spectrum 1000. In order to collect the spectra, a small amount of each sample was mixed with KBr (1 wt% nanoparticles) and compressed to form tablets. The IR spectra of these tablets, in absorbance mode, were obtained in the spectral region of 400–4000 cm\(^{-1}\) using a resolution of 4 cm\(^{-1}\) and 64 co-added scans.

4.2.5.3 X-ray diffraction (XRD) pattern

X-ray powder diffraction patterns of chitosan nanoparticles and plant extracts loaded chitosan nanoparticle were obtained by a Phillips X-PERT PRO X-ray machine. The X-ray source was Cu K\(\alpha\) radiation (40 kV, 80 mA). Samples were scanned at a scanning rate of 4° min\(^{-1}\).

4.2.5.4 Morphological characterization of biomolecules loaded chitosan nanoparticles by TEM

The morphological characteristics of the nanoparticles were examined using a transmission electron microscope (TEM, Tecnai G20, FEI, Netherlands). A droplet of suspension was placed on a carbon film-covered copper grid (200 mesh) without being stained. Five minutes later, the excess liquid was removed by touching the edge of the copper grid with a piece of filter paper. The sample was then air-dried before observation by TEM.

4.2.6 In vivo study

4.2.6.1 Experimental animals

Healthy adult male Wistar rats (150–200g), in-house bred at the Animal House of Kovai Medical Centre Hospital College of Pharmacy, Coimbatore, Tamil Nadu, India were used for the study. Rats were housed in polypropylene cages lined
with husk in standard environmental conditions (temperature 25±2 °C, relative
humidity 55±10% and12:12 light: dark cycle). The rats were fed on a standard pellet
diet (Amrut rat and mice feed, Sangli, India) adlibitum and had free access to water.
The experimental protocol was subjected to the scrutiny of the Institutional Animal
Ethics Committee, Periyan University, Salem-636 011, Tamil Nadu, India and it was
cleared by same before beginning of the experiment (Approval No: 1085/AC/07/PU-
IAEC/2012/23) by prescribed guidelines of Committee for the Purpose of Control
and Supervision of Experiments on Animals (CPCSEA), Government of India.

4.2.6.2 Induction of experimental diabetes

The animals were fasted overnight and diabetes was induced by a single
intraperitoneal injection of a freshly prepared solution of streptozotocin (55 mg/kg
b.w.) in 0.1M citrate buffer (pH4.5) (Hemalatha et al., 2004). The animals were
allowed to drink 5% glucose solution to overcome the drug induced hypoglycemia.
On the third day of STZ-injection, the rats were fasted for 12 h and blood was
withdrawn by retro orbital puncture under light ether anesthesia. Rats with moderate
diabetes having hyperglycemia (that is, with blood glucose of 250–400 mg/dl) were
used for the experiment.

4.2.6.3 Acute toxicity and dose calculation

The acute oral toxicity of GSLE CNPs/SRLE CNPs in male rats was studied
as per OECD guideline 425 (Bala et al., 2010). Both GSLE CNPs and SRLE CNPs
were safe up to the dose of 1 g/kg b.w. p.o. for rats. Generally 1/5th to 1/10th of the
lethal dose was chosen for effective dose calculation. So, 100 mg/kg b.w. doses were
chosen in the study.

4.2.6.4 Animal grouping

In the experiment, a total of 48 rats (42 diabetic surviving rats and 6 normal
rats) were selected and used. The rats were divided into eight groups with six rats
each: group-I, normal control (untreated) rats; group-II, diabetic control rats; group-
III, diabetic rats given glibenclamide (10 mg/kg b.w.); group-IV, diabetic rats given
chitosan nanoparticles (100 mg/kg b.w.); group-V, diabetic rats given leaf extracts
of Gymnema sylvestre (100 mg/kg b.w.); group-VI, diabetic rats given chitosan based extracts of Gymnema sylvestre (100 mg/kg b.w.); group-VII, diabetic rats given leaf extracts of Stevia rebaudiana (100 mg/kg b.w.); group-VIII, diabetic rats given chitosan based extracts of Stevia rebaudiana (100 mg/kg b.w.). The experiment was carried out for 28 days, with oral administration of respective drugs.

4.2.6.5 Biochemical analysis

Blood glucose levels were measured on day 1, 7, 14, 21 and 28 of the study by glucose oxidase-peroxidase reactive strips and glucometer. The effects of the bioactive molecules loaded chitosan nanoparticles on diabetic rats were estimated on the 28th day after sacrificing the animals by decapitation. Blood was collected from the retro orbital puncture under light ether anesthesia for serum separation. Serum was separated by centrifugation 3500×g at 25°C for 10 min. Serum biochemical parameters i.e., glycosylated Hb, total protein (Lowry et al., 1951), SGOT, SGPT, alkaline phosphatase and changes in body weight were assessed in the diabetic animals treated with extracts and compared with diabetic control and normal animals.

4.2.6.6 Assay of antioxidants levels

The levels of lipid peroxidation (LPO) in tissues were estimated by the method of Okhawa et al. (1979). Reduced glutathione (GSH) was estimated by the method of Ellman (1959). Superoxide dismutase (SOD) was assayed by the method of Kakkar et al. (1984). The activity of catalase (CAT) was determined by the method of Sinha (1972).

4.2.6.7 Histological studies

At the end of the experiment, animals were sacrificed; the pancreatic and liver tissues from all groups of rats were subjected to histological studies. Pancreas was instantly dissected out, excised and rinsed in ice-cold saline solution and transferred to formaldehyde. About 5 μm thick sections were cut and stained with haematoxyline and eosin for histological examination (Singh and Kumar, 2011).
4.2.6.8 Statistical analysis

All the grouped data was statistically evaluated. Hypothesis testing method included one-way analysis of variance (ANOVA) followed by Duncan multiple range comparison tests. P-values of less than 0.05 were considered to indicate statistical significance. All the results were expressed as mean ± SEM for 6 animals in each group.

4.3 Results and discussion
4.3.1 Physiochemical properties of the fabricated nanoparticles

The chitosan nanoparticles were fabricated by ionic cross linking of positively charged chitosan with negatively charged TPP. The physiochemical properties of the CNPs, GSLE CNPs and SRLE CNPs such as particle size distribution, Zeta potential and poly dispersive index were analyzed by using dynamic light scattering technique. The average hydrodynamic diameter, size distribution and zeta potential were determined by particle size analyzer (Nano ZS, Malvern).

Table 21. Size and zeta potential value of chitosan and biomolecules loaded chitosan nanoparticles

<table>
<thead>
<tr>
<th>Sample</th>
<th>Size (nm) measured at pH 6.0 (Mean±SE)</th>
<th>Polydispersion index (Mean±SE)</th>
<th>Zeta potential (mV) at pH 6.0 (Mean±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitosan nanoparticles (CNPs)</td>
<td>280±2.5</td>
<td>0.474±0.35</td>
<td>23.39±0.8</td>
</tr>
<tr>
<td>Bioactive molecules loaded chitosan nanoparticles (GSLE CNPs)</td>
<td>374±3.8</td>
<td>0.680±1.05</td>
<td>28.20±1.3</td>
</tr>
<tr>
<td>Bioactive molecules loaded chitosan nanoparticles (SRLE CNPs)</td>
<td>327±4.8</td>
<td>0.509±0.09</td>
<td>12.40±1.5</td>
</tr>
</tbody>
</table>
Figure 21. Zeta potential graph of (a) Chitosan nanoparticles; (b) Gymnema sylvestre leaf extracts derived biomolecules loaded chitosan nanoparticles; (c) Stevia rebaudiana leaf extracts derived biomolecules loaded chitosan nanoparticles
Figure 22. Zeta particle size distribution graph of (a) Chitosan nanoparticles; (b) Gymnema sylvestre leaf extracts derived biomolecules loaded chitosan nanoparticles; (c) Stevia rebaudiana leaf extracts derived biomolecules loaded chitosan nanoparticles.
The drug loading, drug release and stability of the drug within the nanoparticles was influenced by particle size. Zetasizer characterization revealed that CNPs, GSLE CNPs and SRLE CNPs had an average size of 280 nm, 374 nm and 327 nm and overall positive charge and high encapsulation capacity (Fig. 22). This study clearly indicated that encapsulation of bioactive molecules into nanoparticles, allows the preservation of its biological activity leading to its prolonged action and oral adsorption in the diabetic rat model. Stability of CNPs and GSLE CNPs were studied by zeta potential measurements and the zeta potential value was found to be between -30 mV to +30 mV, indicating that the nanoparticles have good stability. In order to achieve highest antidiabetic activity, the polymeric nanoparticles must have more stable and it should not trigger any unwanted immune response. The zeta potential values (+23.39, +28.20 and +12.40) of the prepared nanoparticles (CNPs, GSLE CNPs and SRLE CNPs) was confirmed their stability (Fig. 21). The positive values obtained for zeta potential indicated that the nanoparticles surface was positively charged. This may be due to the availability of chitosan free NH$_3^+$ groups on the polymer surface (Rajan and Raj, 2012).

All prepared chitosan nanoparticles (CNPs, GSLE CNPs and SRLE CNPs) had polydispersity index observed below 0.5(PDI<0.5), where as SRLE CNPs has comparatively higher hydrodynamic diameter with higher PDI than the CNPs because of bioactive molecules enriched with chitosan nanoparticles. The polydispersity index below 0.5 showed high homogeneity of the nanoformulation SRLE CNPs. Similarly, chitosan nanoparticles had PDI below 0.5 produced high homogeneity was reported earlier (Wazed Ali et al., 2011; Fan et al., 2012). The positive zeta potential values of CNPs (2.39), GSLE CNPs (28.20) and SRLE CNPs (12.40) indicating the formation of bioactive molecules cross linked with chitosan nanoparticles and that too with good stability. Further, there also a chance of ionic band formation between negatively charged phosphate ions and positively charged bioactive molecules in the complexes.

4.3.2 FT-IR studies of polymeric systems and their nanoformulations

Fourier transform infrared spectroscopy (FT-IR) is a technique which is used to analyze the chemical composition of many organic chemicals, polymers, paints,
coatings, adhesives, lubricants, semiconductor materials, coolants, gases, biological samples, inorganics, and minerals. FT-IR can be used to analyze a wide range of materials in bulk or thin films, liquids, solids, pastes, powders, fibres, and other forms. FT-IR analysis can give not only qualitative (identification) analysis of materials, but, with relevant standards, can be used for quantitative (amount) analysis. FT-IR can be used to analyze samples up to ~11 millimetres in diameter and either measure in bulk or the top ~1 micrometer layer.

Chitosan nanoparticles were prepared by cross linking of chitosan and tripolyphosphate (TPP). FT-IR studies of CNPs, GSLE CNPs and SRLE CNPs were performed to characterize the chemical structure of nanoparticles.

![FT-IR spectra](image)

Figure 23. FT-IR spectra of (a) Chitosan powder; (b) Chitosan nanoparticles.

The FT-IR measurements were carried out to determine the biomolecules specifically bound on the chitosan nanoparticles that were involved in the stabilization. The present results showed sharp absorption peaks at about 3430.64, 2933.90, 2364.44, 2112.95, 1642.68, 1543.39, 1385.26, 1081.67, 891.81, 702.35, 645.84, 522.91 cm⁻¹ for CNPs and intense peaks were found at 3787.24, 3412.76,
2356.63, 1574.18, 1357.78, 1122.76, 831.35, 762.80, 656.60, 618.61 cm\(^{-1}\) for chitosan powder (Fig. 23).

For the Gymnema sylvestre leaf extracts prepared by soxhlet method sharp peaks observed were 3748.0, 3402.90, 2364.64, 2138.66, 1573.04, 1397.78, 1121.97, 1080.55, 924.34, 829.37, 760.61, 697.02, 656.56, 619.57 cm\(^{-1}\) and the characteristic peaks was noticed at 3404.24, 2927.23, 2364.64, 2140.78, 1736.09, 1631.04, 1539.11, 1450.22, 1380.18, 1306.62, 1155.31, 1075.19, 895.44, 712.44, 648.76, 527.08 cm\(^{-1}\) for GSLE CNPs. (Fig. 24). These results clearly showed that the biomolecules were loaded in the chitosan nanoparticles. FT-IR spectra of CNPs and GSLE CNPs are shown in Fig. 24 band at 3430.64 cm\(^{-1}\) indicates the combined peaks of the NH\(_2\) and OH group stretching vibration in chitosan.

![FT-IR spectra](image)

**Figure 24.** FT-IR spectra of a) Gymnema sylvestre leaf extracts; b) Gymnema sylvestre derived biomolecules loaded chitosan nanoparticles

In GSLE CNPs a shift from 3430 to 3404 cm\(^{-1}\) was shown and the peak of 3404 cm\(^{-1}\) becomes less wider, indicating reduced hydrogen bonding. The reduced amount of hydrogen bonding in the cross-linked nanoparticles complexes is due to
more open structure resulting from cross linking with biomolecules of Gymnema sylvestre as well as TPP. Division of combined peaks of amine and amide at 1631.04 and 1539.11 cm\(^{-1}\) also indicate the binding of proteins with O and N of those groups.

In the nanoformulations, characteristics peak shift was observed due to the potential interaction of protonated amine/amide groups and negatively charged TPP cross linking agent. The characteristic peaks representing to the spectrum of GSLE CNPs were appeared at 1736.09, 1631.04 and 1539.11 cm\(^{-1}\) ascribing to the aldehyde groups in which hydroxyl end groups of gymnemic acid was transformed after the oxidation reaction. The present results confirmed that bioactive molecules derived from aqueous leaf extracts of Gymnema sylvestre were loaded in the chitosan nanoparticles.

![Figure 25. FT-IR spectra of a) Stevia rebaudiana leaf extracts; b) Stevia rebaudiana derived biomolecules loaded chitosan nanoparticles.](image)

FT-IR analysis is an important tool for the identification of bioactive molecules derived from Stevia rebaudiana leaf extracts cross linked with chitosan nanoparticles. The present study confirms that bioactive molecules loaded in
chitosan nanoparticles (CNPs) due to the presence of characteristic peak in the SRLE CNPs and the SRLE while absent in the chitosan blank. The major characteristic peaks of the bioactive molecules of Stevia rebaudiana was 3396.2 (OH – stretching), 2942. 24 (Alkane CH stretching), 1737.42 (C=O stretch), 1638.87 (C=C stretch), 1079.07 (C-O-C stretch), 893 (C=C stretch) present in both SRLE and SRLE CNPs while this peaks are absent in CNPs (Fig. 25). Similar observation of characteristics peaks was notified recently in IR spectra of steviol glycosides isolated from the Stevia rebaudiana (Chaturvedula et al., 2012).

The presence of bioactive molecules (steviol glycosides derived from Stevia rebaudiana leaf extracts) characteristics peaks on the SRLE CNPs was direct confirmation of bioactive molecules cross linked with chitosan nanoparticles. Chitosan nanoparticles characteristic peaks of 3430.64 (-OH and –NH stretching), 1642.68 (amide I) and 528.68 (pyranoside ring stretching vibration) appeared in the CNPs, SRLE CNPs and chitosan where as absent in Stevia rebaudiana leaf extracts. Similarly, the characteristic peaks chitosan nanoparticles were reported recently (Hosseini et al., 2013). The present results strongly suggested that bioactive molecules loaded chitosan nanoparticles are synthesized successfully by ionic cross linking of nanoprecipitation method.

4.3.3 Crystallography assay

Crystallographic structure of CNPs, GSLE CNPs and SRLE CNPs were determined by XRD and were illustrated in Fig. 26. As compared with chitosan nanoparticles, in diffraction spectrum of GSLE CNPs, the characteristic peaks at 20 of 18° and 24° confirms the presence of bioactive molecules within chitosan nanoparticles (Fig. 26). It’s implied that the incorporation of bioactive molecules resulted in the change in the chitosan-TPP-GSLE packing structure. It’s well known that the width of X-ray diffraction peak was related to the size of the crystallite, the broadened peaks usually result from imperfect crystal (Jingou et al., 2011). Chitosan nanoparticles were comprised of a dense network structure interpenetrating polymer chains cross-linked to each other by bioactive molecules as well as TPP counter in ions. Similar observations were also reported by Wazed Ali et al. (2011).
Figure 26. XRD patterns of a) Chitosan nanoparticles; b) Gymnema sylvestre leaf extracts derived biomolecules loaded chitosan nanoparticles; c) Stevia rebaudiana leaf extracts derived biomolecules loaded chitosan nanoparticles

Crystallographic structure of CNPs and SRLE CNPs were determined by XRD and are presented in Fig. 26. XRD patterns of CNPs showed that was an amorphous to partially crystalline state. This observation was accordance with Papadimitriou et al. (2012) who reported a peak approximately 10° (20) corresponding to hydrated crystals and one at 27° (20) corresponding to anhydrous crystals. It is noteworthy to mention that the width of X-ray diffraction peak of chitosan nanoparticles may cause by the cross linking reaction between chitosan and TPP, which may destroy the crystalline structure of chitosan. As compared with chitosan nanoparticles, in diffraction spectrum of SRLE CNPs the characteristic peaks at 20 of 25° confirmed the presence of bioactive molecules within the chitosan nanoparticles. The present results confirmed that the incorporation of bioactive molecules resulted in a change in the chitosan nanoparticles packing structure. Similar observation of XRD patterns was noticed in oregano essential oil in chitosan nanoparticles (Hosseini et al., 2013).
4.3.4 Morphological characterization

The morphological characteristics of the CNPs, GSLE CNPs and SRLE CNPs were examined using the transmission electron microscope (TEM) technique.

Figure 27. TEM images of A) & B) Chitosan nanoparticles; C) & D) Gymnema sylvestre leaf extracts derived biomolecules loaded chitosan nanoparticles; E) & F) Stevia rebaudiana leaf extracts derived biomolecules loaded chitosan nanoparticles.
Morphological structures were depicted in Fig. 26A & B and it revealed that chitosan nanoparticles were spherical in shape with smooth surfaces. The chitosan nanoparticles (CNP) had spherical shape and size ranged between 50.24 to 119.81 nm (Fig. 27A & B). In GSLE CNPs size range was between of 91.71-160.49 nm (Fig. 27 C & D) whereas SRLE CNPs size ranges between 50.42 nm to 73.34 nm (Fig. 27 E & F). An aggregate of distinctive single particles with clear joining boundaries was formed and spherical along with the regular geometry of the proximate polyhedron (pentagon and hexagon) shaped particles. The increased size and multishape formation may be due to the entrapment of bioactive molecules on the chitosan nanoparticles to form a chelate like structure. The average size was smaller for prepared chitosan nanoparticles, when compared to that measured by DLS system as described. These differences may be due to the measurement conditions used in the both the techniques as well as the measuring technique but the principle was different in two cases. The inconsistency in the chitosan nanoparticles swell in aqueous media and DLS gave a hydrodynamic diameter of nanoparticles, while TEM showed an actual diameter of nanoparticles in dry state. Therefore, their structure was not in swelled stage resulting in lower size of the particles. Similar observation was also reported recently by Wazed Ali et al. (2011).

4.3.5 In vivo study

Evaluation of antidiabetic activity of biomolecules loaded chitosan nanoparticles was carried out in STZ induced diabetic rats.

4.3.5.1 Changes in body weight

Effect of GSLE CNPs on body weight

At the end of 28 days treatment, the body weight of diabetic control group decreased whereas treatment with bioactive molecules loaded chitosan nanoparticles (GSLE CNPs: 100 mg/kg b.w.) followed by GSLE (100 mg/kg) and glibenclamide (10 mg/kg) significantly increased the body weight to the normal level (Table 22).

Induction of diabetes by STZ leads to loss of bodyweight due to the increased muscle waste and loss of tissue proteins (Gupta et al., 2010). Decrease in
body weight due to derangement of metabolic pathways was a common feature in diabetes (Al-Shamaony et al., 1994). After 28 days of GSLE CNPs treatment, bodyweight gain was also observed in diabetic rats and the results were comparable with that of the standard drug glibenclamide. The reversal of weight loss in GSLE CNPs treated diabetic indicates that the restoration effect may be due to reversal of gluconeogenesis/glycogenolysis and increased synthesis of structural proteins.

Table 22. Effect of oral administrations of chitosan nanoparticles on body weight in normal and streptozotocin – induced diabetic male wistar rats for 28 days

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight in (g)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>bf induction (Mean±SE)</td>
</tr>
<tr>
<td>Group- I</td>
<td>161.0±6.1a</td>
</tr>
<tr>
<td>Group- II</td>
<td>173.7±4.3b</td>
</tr>
<tr>
<td>Group- III</td>
<td>175.5±5.7c</td>
</tr>
<tr>
<td>Group- IV</td>
<td>180.3±5.6d</td>
</tr>
<tr>
<td>Group- V</td>
<td>180.7±2.9e</td>
</tr>
<tr>
<td>Group- VI</td>
<td>178.7±1.3f</td>
</tr>
<tr>
<td>Group- VII</td>
<td>174.3±1.6g</td>
</tr>
<tr>
<td>Group- VIII</td>
<td>176.8±5.7h</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE for six rats in each group. One-way ANOVA repeated measures with Duncan’s multiple range tests was used to calculate statistical significance. *Mean values within the column followed by the same letter in superscript are not significantly different at P< 0.05 level.

Effect of SRLE CNPs on bodyweight

The body weight of the diabetic controls (group II) significantly decreased compared to the normal controls (group I). During the weekly of observation of the SRLE CNPs treated diabetic rats, there was a significant (P<0.05) weight gain on day 28, relative to day 0 as shown in Table 22. STZ – induced diabetes was characterized by a severe loss in body weight due to the overall muscle destruction (or) degradation of structural proteins (Salahuddin and Jalalpure, 2010). When diabetic rats were treated with SRLE CNPs, they showed improvement of body weight compared to the diabetic control and the results were comparable with that of the standard drug glibenclamide.
4.3.5.2 Effect of blood glucose

Induction of diabetes in the experimental rats was confirmed from the high fasting blood glucose level (>250 mg/dl). A significant (P < 0.05) increase in the blood glucose level (group II) was observed in diabetic rats when compared to controls (group I).

Table 23. Effect of oral administrations of chitosan nanoparticles on blood glucose levels in normal and streptozotocin – induced diabetic male wistar rats for 28 days

<table>
<thead>
<tr>
<th>Blood glucose levels (mg/dl)</th>
<th>Groups</th>
<th>0 day (Mean ±SE)</th>
<th>7th day (Mean ±SE)</th>
<th>14th day (Mean ±SE)</th>
<th>21st day (Mean ±SE)</th>
<th>28th day (Mean ±SE)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group - I</td>
<td>104.6±5.38&lt;sup&gt;a&lt;/sup&gt;</td>
<td>105.3±7.86&lt;sup&gt;a&lt;/sup&gt;</td>
<td>107.8±4.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>104.6±5.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>77.8±3.11&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Group- II</td>
<td>88.5±3.14&lt;sup&gt;b&lt;/sup&gt;</td>
<td>318.2±2.10&lt;sup&gt;b&lt;/sup&gt;</td>
<td>349.5±8.5&lt;sup&gt;b&lt;/sup&gt;</td>
<td>374.8±2.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>398.2±2.19&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Group- III</td>
<td>89.0±2.38&lt;sup&gt;b&lt;/sup&gt;</td>
<td>349.1±8.67&lt;sup&gt;b&lt;/sup&gt;</td>
<td>323.5±7.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>276.1±1.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>213.8±8.70&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Group- IV</td>
<td>94.0±4.12&lt;sup&gt;b&lt;/sup&gt;</td>
<td>355.2±11.7&lt;sup&gt;b&lt;/sup&gt;</td>
<td>395.5±7.24&lt;sup&gt;a&lt;/sup&gt;</td>
<td>331.2±7.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>297.0±1.9&lt;sup&gt;b&lt;/sup&gt;</td>
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<td></td>
<td>Group- V</td>
<td>104.3±3.41&lt;sup&gt;c&lt;/sup&gt;</td>
<td>368.1±7.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>329.8±9.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>285.8±2.9&lt;sup&gt;b&lt;/sup&gt;</td>
<td>195.6±3.9&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Group- VI</td>
<td>107.8±4.67&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>374.5±11.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>282.2±8.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>225.2±4.0&lt;sup&gt;c&lt;/sup&gt;</td>
<td>152.2±8.9&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Group- VII</td>
<td>111.7±1.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>362.8±5.37&lt;sup&gt;a&lt;/sup&gt;</td>
<td>316.6±1.0&lt;sup&gt;b&lt;/sup&gt;</td>
<td>246.2±8.5&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>202.6±9.4&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>Group- VIII</td>
<td>117.3±3.66&lt;sup&gt;c&lt;/sup&gt;</td>
<td>371.3±11.6&lt;sup&gt;a&lt;/sup&gt;</td>
<td>274.7±6.89&lt;sup&gt;a&lt;/sup&gt;</td>
<td>218.2±6.3&lt;sup&gt;c&lt;/sup&gt;</td>
<td>179.2±7.4&lt;sup&gt;cd&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE for six rats in each group. One-way ANOVA repeated measures with Duncan’s multiple range test was used to calculate statistical significance. *Mean values within the column followed by the same letter in superscript are not significantly different at P< 0.05 level.

Effect of GSLE CNPs on blood glucose level

Administration of aqueous extracts of GSLE CNPs at a dose of 100 mg/kg b.w. (group VI) to diabetic rats significantly (P < 0.05) decreased the blood glucose level again to near normal level (Table 23). Plant extracts have been shown to exert hypoglycemic activity through stimulation of insulin release like glibenclamide that was reported to enhance the activity of beta cells of pancreas resulting in increased secretion of large amount of insulin which in turn brings down blood glucose level (Nain et al., 2012). The present results suggested that the biomolecules loaded chitosan nanoparticles could be responsible for the enhanced stimulation of insulin and the observed restoration of metabolic activity.
Effect of SRLE CNPs on blood glucose level

Fasting blood glucose level and bodyweight was measured in normal and experimental rats on day 0, 7, 14, 21 and 28 of treatment. The antidiabetic effect of SRLE CNPs on the fasting blood glucose levels in diabetic rats are showed in Table 23. STZ – treated diabetic rats showed significant increase in the levels of fasting blood glucose levels when compared to the normal control rats (Group I). In the present study, daily administration of the SRLE CNPs (100 mg/kg b.w.) leads to the reduction of fasting blood glucose levels. At the end of the experiment (28th day) blood glucose level was 167.2±3.63 and 195.6±8.05 in SRLE CNPs and SRLE respectively. STZ has been extensively used to induce diabetes mellitus in experimental rat models. After administration of STZ damage the insulin secreting β-cells resulting in decreased endogenous insulin release that leads to the increased levels of glucose in blood. In the present study, after treatment of STZ – induced diabetic rats with SRLE CNPs (100 mg/kg), there was a significant reduction of fasting blood glucose levels was noticed.

4.3.5.3 Effect on biochemical parameters

The glycosylated haemoglobin (HbA1c) level of the diabetic control rats was significantly (P<0.05) increased compared to the normal control rats (Table 24).

Effect of GSLE CNPs on biochemical parameters

The level of HbA1c level was significantly (P<0.05) decreased in bioactive molecules loaded chitosan nanoparticles (GSLE CNPs) (100 mg/kg b.w.) treated groups of diabetic rats at the end of the study. It is noteworthy to mention that, the decreased level of total haemoglobin in the diabetic rats was mainly due to the increased accumulation of HbA1c level. During diabetes mellitus, the excess glucose present in the blood reacts with haemoglobin to form HbA1c. The increasing levels of HbA1c were directly proportional to the fasting blood glucose level. Treatment of GSLE CNPs to the diabetic rats had significantly reduced the glycosylation of haemoglobin which could be due to an improvement of insulin secretion (Jain et al., 2010).
Table 24. Effect of bioactive molecules loaded chitosan nanoparticles on biochemical parameter in streptozotocin induced diabetic rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>HbA1C (%)</th>
<th>SGOT(U/dl)</th>
<th>SGPT(U/dl)</th>
<th>ALP(U/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I</td>
<td>4.95±0.21a</td>
<td>19.50±1.31c</td>
<td>45.66±1.8b</td>
<td>119.66±3.1c</td>
</tr>
<tr>
<td>Group- II</td>
<td>8.75±0.64a</td>
<td>26.53±0.31a</td>
<td>63.66±1.7a</td>
<td>257.66±2.7a</td>
</tr>
<tr>
<td>Group- III</td>
<td>5.80±0.85a</td>
<td>23.93±1.04ab</td>
<td>47.66±3.1de</td>
<td>144.66±1.2bc</td>
</tr>
<tr>
<td>Group- IV</td>
<td>7.20±0.42b</td>
<td>25.83±2.30a</td>
<td>56.66±3.2b</td>
<td>205.00±2.5ab</td>
</tr>
<tr>
<td>Group- V</td>
<td>5.65±0.64d</td>
<td>23.57±0.96ab</td>
<td>48.33±2.1d</td>
<td>174.33±1.9bc</td>
</tr>
<tr>
<td>Group- VI</td>
<td>5.35±0.35d</td>
<td>19.60±1.25c</td>
<td>46.66±1.2c</td>
<td>144.66±2.8bc</td>
</tr>
<tr>
<td>Group- VII</td>
<td>6.40±0.28c</td>
<td>24.23±0.90ab</td>
<td>53.33±3.8c</td>
<td>187.00±1.2b</td>
</tr>
<tr>
<td>Group- VIII</td>
<td>6.20±0.42c</td>
<td>20.57±2.22bc</td>
<td>49.33±2.4d</td>
<td>149.00±1.2bc</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE for six rats in each group. One-way ANOVA repeated measures with Duncan’s multiple range tests was used to calculate statistical significance.

*Mean values within the column followed by the same letter in superscript are not significantly different at P< 0.05 level.

Biochemical parameters such as SGOT, SGPT, and ALP were significantly elevated in negative control group compared to the normal control group (Table 24). The elevation of biochemical marker enzymes such as SGOT, SGPT and ALP was observed in diabetic control rats and indicated the heptacellular damage (Jaeschkl et al., 2002). The present study also showed that the injection of STZ was induced hepatic damage that elevates intracellular enzymes, such as transaminases (SGOT and SGPT) and alkaline phosphatase (ALP). The diabetic complications such as increased gluconeogenesis and ketogenesis may be due to the elevated transaminase activity (Ghosh and Suryawanshi, 2001). After treatment with GSLE CNPs (100 mg/kg b.w. daily, 28 days) the hepatic damage was restored hepatocytes and the elevated transaminases were significantly reduced in diabetic rats. Total protein was found to be significantly decreased in STZ induced negative control group as compared to the normal control group. GSLE CNPs treated groups restored the protein content towards normal when compared to the control (Table 24). Total protein content was decreased in diabetic rats which may be due to the progressive protenuria (Latha and Daisy, 2011). GSLE CNPs treatment had normalized the total protein content level in diabetic rats suggested that curative role on kidney function.

The levels of SOD, CAT, GSH and TBARS activities in liver and kidney of experimental diabetic rats were showed in Table 25 & 26. There was a significant
decrease in the level of SOD, CAT and GSH activity in the STZ control group when compared with the control group.

Table 25. Effect of bioactive molecules loaded chitosan nanoparticles treatment for 28 days on superoxide dismutase, catalase, glutathione and lipid peroxidation in liver of control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (Units/mg protein)</th>
<th>CAT (μmol/min/mg protein)</th>
<th>GSH (μmol/min/mg protein)</th>
<th>TBARS (μmol/min/mg protein)</th>
<th>TOT PTN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group – I</td>
<td>10.15±0.43d</td>
<td>42.80±2.53e</td>
<td>43.40±2.94f</td>
<td>0.90±0.09g</td>
<td>6.84±0.21e</td>
</tr>
<tr>
<td>Group- II</td>
<td>4.62±0.39d</td>
<td>25.80±1.52e</td>
<td>11.83±3.36f</td>
<td>2.33±0.23a</td>
<td>3.90±0.14c</td>
</tr>
<tr>
<td>Group- III</td>
<td>9.19±0.57d</td>
<td>33.82±2.34bcd</td>
<td>29.30±2.96bcd</td>
<td>0.51±0.04d</td>
<td>6.37±0.18b</td>
</tr>
<tr>
<td>Group- IV</td>
<td>5.58±0.61d</td>
<td>31.64±2.24d</td>
<td>27.66±2.69ed</td>
<td>1.49±0.08b</td>
<td>4.57±0.30d</td>
</tr>
<tr>
<td>Group- V</td>
<td>7.74±0.80bc</td>
<td>38.37±1.95b</td>
<td>31.18±2.71bc</td>
<td>0.90±0.08c</td>
<td>5.87±0.21c</td>
</tr>
<tr>
<td>Group- VI</td>
<td>9.45±0.88a</td>
<td>40.19±0.58ab</td>
<td>34.57±1.53b</td>
<td>0.61±0.05d</td>
<td>6.56±0.10ab</td>
</tr>
<tr>
<td>Group- VII</td>
<td>6.75±0.51c</td>
<td>35.69±2.62bc</td>
<td>21.40±2.75e</td>
<td>1.06±0.17c</td>
<td>5.81±0.26c</td>
</tr>
<tr>
<td>Group- VIII</td>
<td>8.04±0.71b</td>
<td>37.14±1.24bc</td>
<td>24.42±3.08cd</td>
<td>0.92±0.13c</td>
<td>6.38±0.27b</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE for six rats in each group. One-way ANOVA repeated measures with Duncan’s multiple range tests was used to calculate statistical significance. *Mean values within the column followed by the same letter in superscript are not significantly different at P< 0.05 level.

Administration of GSLE CNPs had significantly increased the SOD, CAT and GSH level in liver and kidney of STZ-induced diabetic rats (Table 25, 26). Oxidative stress of diabetes is coupled to a decrease in the antioxidant status, which can increase the deleterious effects of free radicals. The SOD and CAT are the two major scavenging enzymes that remove free radicals. A decreased level of these antioxidant defense enzyme activities in liver and kidney tissues was observed in diabetic rats and this may cause a number of deleterious effects due to over accumulation of superoxide anion (O) and hydrogen peroxide (H₂O₂), which in turn generate hydroxyl radicals(OH), resulting in initiation and propagation of LPO. SOD protects from oxygen free radicals by catalyzing the removal of superoxide radical, which damage the membrane and biological structures. Catalase was proved to be responsible for the detoxification of H₂O₂, and protects the tissues from highly reactive hydroxyl radicals (Mahboob et al., 2005). This decreased CAT activity
could result from inactivation by glycation of enzyme (Yan and Harding, 1997). In the present study, GSLE CNPs treated groups showed a significant increase in the hepatic and renal SOD and CAT activities of the diabetic rats (Table 25 & 26). It indicated that active biomolecules derived from Gymnema sylvestre leaf extracts could reduce the potential glycation of enzymes or they may reduce reactive oxygen free radicals and enhance antioxidant enzyme activities. This result clearly showed that active biomolecules of Gymnema sylvestre loaded chitosan NPs (GSLE CNPs) contain a free radical scavenging activity, which could exert a beneficial action against pathological alteration of diabetes mellitus caused by the presence of superoxide radicals and hydrogen peroxide radical.

Glutathione is a tripeptide, intracellular antioxidant and protect the cellular system from adverse effects of lipid peroxidation. It is a direct scavenger of free radicals as well as a co-substrate for peroxide detoxification by glutathione peroxidases (Winterbourn, 1995). Increased oxidative stress, resulting from significant increase in aldehydic products of lipid peroxidation has probably decreased GSH content (Mohammed, 2008). Treatment with GSLE CNPs resulted in the elevation of the GSH levels, which protects the cell membrane against oxidative damage by regulating the redox status of protein in the membrane.

There was a significant elevation of TBARS in liver and kidney in STZ induced group when compared to the control group (Table 25, 26). It was found that administration of GSLE NPs significantly decreased TBARS levels in liver and kidney, which is an indication of the inhibition of oxidative damage of hepatic and renal tissues. Diabetes is strongly co-related with oxidative stress induction. Lipid peroxidation is one of the characteristic features of diabetes mellitus. Measurement of plasma thiobarbituric acid reactive substances (TBARS) was used as an index of lipid peroxidation and it helps to assess the extent of tissue damage. An increase level of TBARS and hydroperoxidases was reported in liver and kidney tissues of experimental diabetes mellitus (Ananthan et al., 2004; Nain et al., 2012). The result of the present study showed that GSLE CNPs significantly (P≥0.05) decreased the TBARS levels which ultimately reduce the risk of tissue damage in diabetic rats.
Effect of SRLE CNPs on biochemical parameters

The levels glyoxylated haemoglobin (HbA1C), Serum glutamic oxaloacetic transaminase (SGOT), pyruvic transaminase (SGPT) and Alkaline phosphatases (ALP) are shows in Table 24. The elevated levels of HbA1c observed in STZ-induced diabetic rats were significantly (P<0.001) reverted towards normal levels after the treatment of SRLE CNPs and glibencamide. In diabetic rats, increase in the levels of HbA1c was noticed due to the persistent increased level of glucose in bloods which produced in glycation of haemoglobin. In the present investigation, administration of SRLE CNPs significantly decreased the HbA1c levels in diabetic rats. The ability of SRLE CNPs to decrease HbA1c levels in diabetic rats showed its potentiality to prevent the diabetic associated complication.

Table 26. Effect of bioactive molecules loaded chitosan nanoparticles treatment for 28 day on superoxide dismutase, catalase, glutathione and lipid peroxidation in kidney of control and experimental groups of rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (Units/mg protein)</th>
<th>CAT (µmol/min/mg protein)</th>
<th>GSH (µmol/min/mg protein)</th>
<th>TBARS (µmol/min/mg protein)</th>
<th>TOT PTN (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group - I</td>
<td>11.04±0.53α</td>
<td>34.04±2.15α</td>
<td>41.40±3.87α</td>
<td>0.53±0.09α</td>
<td>5.46±0.20α</td>
</tr>
<tr>
<td>Group - II</td>
<td>4.63±0.69c</td>
<td>23.56±0.63c</td>
<td>19.04±2.07c</td>
<td>2.07±0.46c</td>
<td>3.64±0.16c</td>
</tr>
<tr>
<td>Group - III</td>
<td>8.83±0.70β</td>
<td>32.16±1.92bc</td>
<td>32.11±3.12bc</td>
<td>0.77±0.13bc</td>
<td>5.91±0.17bc</td>
</tr>
<tr>
<td>Group - IV</td>
<td>5.53±1.70bc</td>
<td>26.26±0.85d</td>
<td>23.34±2.17d</td>
<td>1.89±0.39d</td>
<td>4.08±0.16d</td>
</tr>
<tr>
<td>Group - V</td>
<td>7.82±1.28bc</td>
<td>31.48±1.17bc</td>
<td>31.98±3.32bc</td>
<td>1.01±0.19d</td>
<td>4.92±0.13b</td>
</tr>
<tr>
<td>Group - VI</td>
<td>9.65±1.40ab</td>
<td>33.40±0.41ab</td>
<td>35.04±0.76bc</td>
<td>0.98±0.06de</td>
<td>5.39±0.13ab</td>
</tr>
<tr>
<td>Group - VII</td>
<td>6.74±0.48cd</td>
<td>25.30±0.70de</td>
<td>27.43±3.27cd</td>
<td>1.49±0.25bc</td>
<td>4.42±0.46cd</td>
</tr>
<tr>
<td>Group - VIII</td>
<td>8.01±1.06bc</td>
<td>28.53±1.05c</td>
<td>33.97±0.80b</td>
<td>1.16±0.26cd</td>
<td>4.47±0.42c</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SE for six rats in each group. One-way ANOVA repeated measures with Duncan’s multiple range tests was used to calculate statistical significance. *Mean values within the column followed by the same letter in superscript are not significantly different at P< 0.05 level.

There was a significant increase in activities of SGOT, SGPT and ALP in diabetic rats. The liver was necrotized in STZ-induced diabetic rats. Therefore an increase in the activities of SGPT, SGOT and ALP in plasma might be mainly due to
the leakages of the enzymes from the cytosol into the blood stream which gives an indication the hepatotoxic effect of STZ (Kasetti et al., 2010). After treatment with SRLE CNPs, significantly reduced the levels of these enzymes (SGOT, SGPT and ALP) in plasma compared to diabetic control rats (Table 24).

Antioxidant profiles (SOD, CAT, GSH and LPO) of liver and kidney tissues of normal and diabetic rats are showed in Table 25 & 26. There was a significant reduction in activities of SOD, CAT and GSH in diabetic rats. Antioxidants play a vital role in preventing cells from being exposed to oxidative damage. In diabetes mellitus, high glucose can inactivate antioxidant enzymes SOD, CAT and GSH by glycating these proteins thus producing induced oxidative stress, which in turn, causes lipid peroxidation (Kennedy and Lyons, 1997). A marked increase of LPO in STZ treated rats leading to tissue injury and failure of the endogenous antioxidant defense mechanisms to prevent over production of free radicals. Endogenous enzymatic antioxidant (CAT, SOD) and nonenzymatic antioxidant (GSH) act as reducing agents and detoxified highly reactive oxygen and nitrogen species. After administration with SRLE CNPs (100 mg/kg b.w.) the enzyme activities levels were near to normal indicating the efficacy of SRLE CNPs in attenuating the oxidative stress in diabetic liver and kidney.

4.3.5.4 Histology of pancreas and liver

Both liver and pancreas tissues were used for histological study and it was found that GSLE CNPs was non-toxic and alleviated from the toxic effects of STZ treatment in diabetic rats. The STZ injection caused diabetes mellitus, which may had resulted from the destruction of beta cells of the islets of langerhans of the pancreas followed by insulin deficiency and may have led to a variety of derangements in metabolic and regulatory processes.

Microscopically examined pancreas section of the control group showed normal pancreatic acini, ducts and islets. There is no evidence of inflammation, destruction of islets (Fig. 28A). However, in the diabetic control group II, few of the islet cells showed destruction and apoptosis associated with inflammation and nuclear debris (Fig. 28B).
Figure 28. Histology of pancreas in experimental rats after 28 days of treatment; (A) Normal control; (B) Diabetic control; (C) Diabetic + Glibenclamide (10 mg/kg); (D) Diabetic + Chitosan NPs (100 mg/kg); (E) Diabetic + GSLE (100 mg/kg); (F) Diabetic + GSLE CNPs (100 mg/kg); (G) Diabetic + SRLE (100 mg/kg); (H) Diabetic + SRLE CNPs (100 mg/kg).
Figure 29. Histology of liver in experimental rats after 28 days of treatment; (A) Normal control; (B) Diabetic control; (C) Diabetic + Glibenclamide (10 mg/kg); (D) Diabetic + Chitosan NPs (100 mg/kg); (E) Diabetic + GSLE (100 mg/kg); (F) Diabetic + GSLE CNPs (100 mg/kg); (G) Diabetic + SRLE (100 mg/kg); (H) Diabetic + SRLE CNPs (100 mg/kg).
In the standard-treated group III, there was a mild infiltrate of lymphocytes at some foci and the acini were lined by round to oval shape cells with moderate cytoplasm and small round to oval shape nuclei (Fig. 28C). In the CNPs (100 mg/kg b.w.) treated group IV, the pancreas appeared normal pancreatic acini and also few islets showed mild inflammation. Most of the islets were unremarkable (Fig. 28D). In the GSLE (100 mg/kg b.w.)-treated group V, mixed inflammation in the islets of langerhans of lymphocytes, neutrophils and plasma cells were noticed (Fig. 28E);

However, in GSLE CNPs (100 mg/kg b.w.) treated group VI, the pancreas with normal hyperplasia of Islet was recovered, mild congestion of pancreatic parenchyma and number of islet were found to be increased. The islets were normal in size and shape. There was no evidence of inflammation or destruction of Islets (Fig. 28F). Results showed that the SRLE CNPs treated groups enhanced the regeneration of islets of langerhans in the pancreas and restoration of normal cellular size of the islet with hyperplasia (Fig. 28H). In the present study, the damage of pancreas in streptozotocin treated diabetic control rats and regeneration of islets of langerhans by glibenclamide was observed (Fig. 29C). The comparable regeneration and restoration of normal cellular size of the islet with hyperplasia was also observed SRLE CNPs treated groups (Fig. 29H). The hypoglycemic effect may be attributed to the regeneration of islet of langerhans which may confirm the efficiency of the given SRLE CNPs in the management of diabetes.

Histology of liver in experimental rats was determined after 28 days of treatment. Normal control group of rat’s liver showed normal hepatic cells and architecture. There was no necrosis or fibrosis (Fig. 29A). Diabetic control-clear hepatocyte cellular necrosis and extensive vacuolization with vanishing of nuclei and disordered liver structure were noticed in STZ induced rat. The portal tract showed mild to moderate inflammation composed of lymphocytes and plasma cells. Diabetic significantly attenuated STZ-induced hepatocyte cellular necrosis and fibrotic changes were observed in rat liver (Fig. 29B). Diabetic glibenclamide (10 mg/kg) section of liver showed moderate inflammation of few portal tracts composed of lymphocytes and neutrophils with focal ductulitis (Fig. 29C). Diabetic CNPs (100 mg/kg) section of liver indicated the minimal portal tract inflammation and lobular inflammation of
hepatocytes (Fig. 29D). Diabetic GSLE and GSLE CNPs (100 mg/Kg) liver revealed normal portal tracts and lobular architecture were maintained (Fig. 29E and 29F). There was no evidence of inflammation or necrosis. Histological studies of liver confirmed that GSLE CNPs significantly enhanced the lobular architecture and central vein in STZ induced diabetic rat model system. After administration of SRLE CNPs reversed these changes to near normal (Fig. 29H).

Histopathological study of liver showed that both SRLE CNPs and glibenclamide reduces hypertrophy of hepatocytes and lymphocyte infiltration in liver (Fig. 29C). The effects of streptozotocin on glucose homeostasis reflect the toxin-induced abnormalities in β-cell function. The therapeutic advantage of SRLE CNPs was also reflected in the pancreatic histology of rats supplemented with SREL CNPs where the damage induced on the Islet and β-cells was minimal and preserved the Islet architecture of the pancreas.

4.4 Summary

The bioactive molecules derived from GSLE loaded chitosan nanoparticles (GSLE CNPs) were successfully fabricated by nanoprecipitation method via dropping technique and its enhanced antidiabetic activity was studied by using STZ induced diabetic rats. GSLE CNPs had significantly reduced the blood glucose content and showed enhanced levels of antidiabetic activity. The level of serum enzymes (SGOT, SGPT, ALP), antioxidant enzymes (CAT, GSH, SOD) and lipid peroxidation treated groups were restored towards normal level as compared to the diabetic control groups. Results strongly confirmed that GSLE CNPs treatment had significant antidiabetic activity that standard drug (glibenclamide) in STZ induced diabetic rats. An enhanced level of antioxidant as well as serum enzymes activities and reduced lipid peroxidation in diabetic rats strongly suggested the potential antidiabetic activity of GSLE CNPs which trigger multiple defense mechanisms in a coordinated manner to alleviation STZ induced antioxidative stress in rat model.

SRLE CNPs had significantly reduced the blood glucose content and showed enhanced levels of antidiabetic activity. The results strongly confirmed that SRLE CNPs treatment had significant antidiabetic activity than standard drug
(glibenclamide) in STZ induced diabetic rats. Therefore, normal blood glucose level, the restoration of various biomarker as well as antioxidative defense enzymes indicated that SRLE CNPs had higher antidiabetic activity which provides a scientific rationale to consider as potential antidiabetic agent. This novel SRLE CNPs could be used as safe and effective antidiabetic nanomedicine for diabetes mellitus.

Therefore, normal blood glucose level and the restoration of various biomarker as well as enzymes indicates that GSLE CNPs and SRLE CNPs had very good antidiabetic activity provides a scientific rationale for the use as an antidiabetic agent. This newly formulated GSLE CNPs and SRLE CNPs showed very high potential to be used as antidiabetic nanomedicine for safe and effective use in in vivo.