4. RESULTS

All over the world plant based products are popular for treating several ailments for centuries. The herbal plants are proven to provide symptomatic relief and also prevent the secondary complications in Diabetes. Some plants are proven to help in regeneration of β- cells and in overcoming insulin resistance. Some herbs maintain normal blood glucose level along with its antioxidant activity and cholesterol lowering action. Hence, the present study was carried out to analyse the potential of crude and SNP extracts of *C. anisata* for its antioxidant, antibacterial and hypoglycemic activity under *in vitro* and *in vivo* conditions.

4.1. Preliminary phytochemical screening of *C. anisata*

The leaf and roots extracts of crude powder of *C. anisata* obtained from solvents of varying polarity such as hexane, EC, acetone, ethanol and aqueous were employed to preliminary phytochemical screening. The detailed analyses of phytochemicals in above solvents are shown in Plate – 6a, Table 3, Plate - 6b, and Table 4.

The screening revealed that the leaf extracts of *C. anisata* showed presence of alkaloids in all the five extracts, followed by carbohydrates which was found to be absent in hexane extract alone. Coumarins, saponins, gums and mucilages were found to be present only in aqueous and ethanolic extracts. Proteins and aminoacids showed the maximum presence in acetone, combined ethylacetate/chloroform and hexane extracts. Aqueous, ethanolic and hexane extracts showed the presence of flavonoids. Tannins, anthocyanin and leucoanthocyanin were completely absent in all the extracts.
The root extracts of *C. anisata* showed the maximum presence of alkaloids and carbohydrates in all the extracts. Except in acetone extract, flavonoids and coumarins were found to be present in all the other extracts. Proteins and aminoacids were found to be present only in acetone extract. Ethanolic extract alone showed the presence of gums and mucilages. Saponins, tannins, anthocyanin and leucoanthocyanin was found to be absent in all the root extracts.

4.2. DPPH – free radical scavenging assay

A simple reliable DPPH method was carried out to evaluate the antioxidant activity of different leaf, root, SNP leaf and SNP root extracts of *C. anisata*. The scavenging activity of the plant extracts through the annihilation of the DPPH radicals was tabulated. The percent inhibition of DPPH radical scavenging activity was shown in the Table 5a, 5b and 5c. From the table it was observed that, minimum DPPH scavenging activity for *C. anisata* leaf extract was found in hexane extract (56.35%) and maximum activity was found in ethanolic extract (70.2%), where standard showed 75.6% at 500µg/ml (Table 5a) (Fig. 3a). The *C. anisata* root extracts showed maximum scavenging activity in ethanolic extract (71.25%) and minimum activity was observed in aqueous extract (65%), where standard showed (75.75%) at 500µg/ml (Table 5b) (Fig. 3b).

The SNP leaf extract showed 71.60% inhibition scavenging activity when compared with standard that showed 77.38% inhibition. The SNP root extract showed maximum activity of 74.07% and standard showed 75% inhibition of scavenging activity (Table 5c) (Fig. 3c). The significant inhibition was found to be at 100 µg/ml (P<0.0001) for ethanolic root extract,
followed by ethanolic leaf extract at 400 µg/ml (P<0.001) and SNP root extract at 300 µg/ml (P<0.0001). All other extracts showed DPPH scavenging activity but it was low compared with standard.

4.3. Determination of antibacterial activity

An antibacterial activity for the different extracts of *C. anisata* were determined using agar well diffusion method and disc diffusion method. The data pertaining to the antibacterial potential of the extracts were presented in Table 6a, 6b, 6c and 6d. The leaf and root extracts of *C. anisata* showed antibacterial activity for atleast two of the microorganisms tested. The SNP leaf and root extract showed antibacterial activity against all the organisms tested in this study.

The ethanolic leaf extract showed antibacterial activity against all the organisms tested except *K. pneumonia*, where standard showed highest antibacterial activity of 22±1.15 mm against *S. epidermis*. The highest zone of inhibition was found to be for *P. vulgaris* in ethanolic extract with 21±0.57 mm (P<0.0001). The least activity was found to be against *P. vulgaris* in acetone extract with 4±1.2 mm (P<0.01) zone of inhibition (Plate – 7) (Table – 6a). The acetone extract showed significant activity against *C. perfringens* with 9.166±0.72 (P<0.0001), followed by EC extract against *P. vulgaris* with 5.333±0.33 mm (P<0.01).

The ethanolic root extracts of *C. anisata* showed antibacterial activity against all the organisms tested. The significant activity was found to against *S. epidermis* (P<0.0001), *S. aurues* (P<0.0001) and *P. aeruginosa* (P<0.0001). Followed by the acetone root extracts that showed inhibition against, *P. aeruginosa* (P<0.001) and *K. pneumonia* (P<0.01). The combined ethylacetate/chloroform extract showed significant activity against *P. aeruginosa* (P<0.0001) (Plate – 8) (Table – 6b).
The SNP leaf and root extracts showed antibacterial activity in the concentration ranging from 50 µg – 150 µg/ml. All the extracts showed antibacterial activity against all the tested microorganisms. In which, the SNP leaf extract showed maximum zone of inhibition against *P. aeruginosa* (12±0.5) (P<0.0001), followed by *Bacillus subtilis* (P<0.0001), *Staphylococcus aureus* (P<0.0001), *Proteus vulgaris* (P<0.0001) and *P. rettgeri* (P<0.001) with each 10 mm in diameter and *E. coli* with 8±0.6 mm in diameter, where standard showed 15±0.57 mm in diameter against *E.coli* (Plate – 9) (Table – 6c).

The maximum zone of inhibition for SNP root extract was observed against *E. coli* (P<0.01) and *P. rettgeri* (P<0.01) with 15 mm in diameter each, followed by *P. aeruginosa* (P<0.0001), *B. subtilis* (P<0.0001), *S. aureus* (P<0.0001) and *P. vulgaris* (P<0.0001) with 14±2.5 mm, 12±1.52, 12±2.1 and 9±0.57 mm zone of inhibition respectively and standard showed 20±1.52 mm zone of inhibition against *P. rettgeri* (Plate - 10) (Table – 6d).

4.4. *In vitro* hypoglycemic activity of *C. anisata*

4.4.1. Alpha amylase inhibition assay

The *in vitro* alpha amylase inhibition of various extracts of *C. anisata* was determined and the results were tabulated (Table 7a, 7b and 7c). The figure showed the percent inhibition of alpha amylase of leaf and root extracts of *C. anisata*. The screening of various extracts showed that the ethanolic leaf extract has significantly higher percentage of alpha amylase inhibition of 80.65% (P<0.01) and least inhibition was found to be for EC extract with 63.2%, whereas the standard showed 83.64% (Table – 7a) (Fig. 4), at 500 µg/ml. The ethanolic leaf extract was found to be highly significant at 100 µg/ml (P<0.001) and at 200 µg/ml (P<0.001).
The ethanolic root extracts of *C. anisata* showed highest percent inhibition of 78.97% and least inhibition was observed with 63.34% for hexane extract, whereas the standard showed 85.89% at 500 µg/ml (Table – 7b) (Fig. 5). The SNP leaf and root extract exhibited 80.32% (P<0.01) and 83.60% (P<0.001) inhibition respectively at 500 µg/ml, in which the standard percent inhibition was found to be 85.24% (Table – 7c) (Fig. 6). All the extracts showed concentration dependent increase in α-amylase inhibition activity.

4.4.2. Glucose uptake by Yeast cells

Glucose transport across the yeast cell membrane was studied in an *in vitro* system comprising of yeast cells along with varying concentrations of glucose solution in the presence of the plant extracts. The indicator of the glucose uptake by yeast cells was the amount of glucose remained in the medium after a specific time. The percentage increase in the glucose uptake was calculated for leaf extract and it was found to range between 20% to 85% in 5 mM of glucose (Table 8a), 30% to 80% in 10 mM of glucose (Table 8b) and 25% to 89% in 25 mM of glucose with extract concentration ranging from 100 – 2000 µg/ml (Table 8c).

The percent increase in glucose uptake for root extract was found to be between 40% to 83% in 5 mM of glucose (Table 9a), 44% to 89% in 10mM of glucose (Table 9b) and 48% to 89% in 25 mM of glucose concentration (Table 9c). In 5mM of glucose concentration SNP leaf showed glucose uptake between 30% to 50%, 40% to 69% in 10mM of glucose and 42% to 62% in 25 mM of glucose. The glucose uptake for SNP root was found to be between 35% to 61% in 5 mM of glucose, 42% to 70% in 10 mM of glucose and 44% to 64% in 25 mM of glucose concentration (Table 10a, 10b and 10c). The
results indicated that *C. anisata* had greater efficiency in increasing the glucose uptake by yeast cells as compared to standard drug metformin. The glucose uptake was found to increase in a dose dependent manner in all extracts.

The leaf extracts (combined ethylacetate/chloroform and hexane) of *C. anisata* showed linear increase of glucose uptake from 5 mM to 25 mM glucose concentration. Comparatively, the aqueous, acetone and ethanolic leaf extracts showed non-linear increase in glucose uptake from 5 mM to 25 mM (Fig. 7), where the combined ethylacetate/chloroform and hexane extracts showed linear increase in glucose uptake. Individually, the highest uptake was found to be at 25 mM glucose concentration for ethanolic leaf extract with 88.37% (P<0.01) and it was highly significant at 100 µg/ml (P<0.0001). The root extracts (ethanol, hexane and EC) of *C. anisata* showed linear increase in glucose uptake by yeast cells from 5 mM to 25 mM. The aqueous and acetone extracts showed maximum uptake at 10 mM glucose concentration (81.03% and 77.58%) respectively (Fig. 8). The ethanolic root extract showed maximum uptake at 25 mM (P<0.01) concentration at 2000 µg/ml and at 500 µg/ml it was found to be highly significant (P<0.001).

The SNP extracts of leaf and root showed maximum uptake of glucose by yeast cells at 10 mM glucose concentration with 68.28% & 69.51% (P<0.001) (Fig. 9). These extracts also showed non-linear increase in glucose uptake. Further studies are needed to validate these variations in glucose uptake by yeast cells. The significant uptake for SNP root was at 1000 µg/ml (P<0.0001) in 10 mM glucose concentration.

From the above studies, the ethanolic leaf and root extracts of *C. anisata* was found to be active compared to all other extracts and hence that extracts alone selected for further studies.
4.4.3. *Glucose diffusion inhibitory assay*

The ability of the *C. anisata* extracts to additionally retard the diffusion and movement of glucose in the intestinal tract can be evaluated by glucose diffusion inhibitory assay. The effect of ethanolic leaf, root, SNP leaf and SNP root extracts of *C. anisata* on retarding glucose diffusion across the dialysis membrane was shown in **Plate - 11**. GDRI, an *in vitro* index was used to predict the effect of fibres present in the extract, which is responsible to delay the glucose absorption in gastro intestinal tract, was calculated and the results obtained were shown (**Table – 11a to 14b**). The glucose movement was monitored from 30 mins to 180 mins. The rate of glucose in the dialysate was measured using standard calibration curve for known concentration of glucose (**Fig. 10a**).

In all the four extract the GDRI (Glucose Dialysis Retardation Index), was found to decrease with increase in time. Higher GDRI index indicates the higher retardation index of glucose by the sample. The higher GDRI index was found in the ethanolic extracts of SNP root (78.33%) (P<0.001), SNP leaf (75.47%) (P<0.001), ethanolic root (75%) (P<0.001) and ethanolic leaf (63.01%) (P<0.001) respectively at 30 mins (**Fig. 10b**). 1ml of sample from dialysate was collected in intervals and glucose estimation was done by DNS method (**Table 11a, 11b, 12a, 12b, 13a, 13b, 14a and 14b**). The concentration of glucose ranges from 100 µg to 600 µg/ml in all extracts (**Fig. 10c**). This results show that the concentration of glucose inside the dialysis membrane gradually decreased due to the adsorption ability of plant extracts.
4.4.4. *In vitro glucose adsorption assay*

The glucose adsorption capacity of ethanolic extracts of *C. anisata* at different glucose concentration (5 mM, 10 mM & 25 mM) was investigated in this study and the results were presented in the Table 15a, 15b, 15c and 15d.

The adsorption capacity of the extracts was found to be directly proportional to the glucose molar concentration and the glucose binding capacity was found to increase with increased molar concentration of glucose (5 mM, 10 mM and 25 mM). The rate of glucose adsorbed was calculated using standard calibration curve for known concentration of glucose (Fig. 11a). Among the extracts assessed, high amount of glucose was bound in 25 mM of glucose concentration for ethanolic root extract with 45 mMol/L at 25 mM (P<0.0001), followed by ethanolic leaf extract with 43.75 mMol/L (P<0.0001) and SNP root extract with 41.5 mMol/L (P<0.0001) at 1000 µg/ml. From this result it is clear that as the concentration of extract increases, the glucose adsorption capacity also increases. The ethanolic root extract (P<0.0001) showed the highest adsorption rate followed by leaf extract (P<0.0001) (Fig. 11b), SNP root (P<0.0001) and SNP leaf extract respectively (Fig. 11c).

The ethanolic leaf and root extracts of *C. anisata* was further used for the synthesis of SNPs and the effect of SNPs on antioxidant, antibacterial and hypoglycemic activity under *in vitro* and *in vivo* conditions was also studied. In this view a detailed investigation was carried out to check its potential for use in the synthesis of SNPs, along with characterization and its hypoglycemic effect.
4.5. Optimization and synthesis of SNPs

Among various concentration and methods used, room temperature (10-15 mins) and sunlight irradiation (10 mins) was found to be effective for ethanolic leaf (Plate - 12) and root extracts (Plate - 13) respectively. 1 ml of the homogenized extracts was shown maximum synthesis of SNPs.

4.5.1. Visual observation

A change in color from green to reddish brown for leaf (Plate – 12f) and from beige to brown for root (Plate – 13e) was observed in the solution after respective irradiation.

4.6. Characterization of SNPs

The synthesized SNPs from ethanolic leaf and root extracts were characterized to confirm its synthesis. The different characterization methods were as follows:

4.6.1. UV spectrophotometric analysis

The color change of leaf and root ethanolic extracts of C. anisata arised due to the excitation of SPR with the SNPs. The UV-Vis profile for crude ethanolic leaf and root extracts showed peaks at 270.04 nm, 536.76 nm, 612.13 nm, 665.45 nm with the absorption 2.1041, 0.29930, 0.32196 and 0.45481 (Fig. 12a); 317.06 nm, 350.61 nm with the absorption 3.6322 and 3.5583 (Fig. 12b) respectively. The SPR of SNPs produced a peak centred near to 488.99 nm (Fig. 12c) and 432.97 nm (Fig. 12d) for leaf and root, with absorption 0.86693 and 1.5557 respectively. These results indicated the successful conversion of silver nitrate (Ag+) to Silver (Ag0).

4.6.2. FTIR analysis

FTIR analysis was used to identify the functional groups of the active components in the extracts that are responsible for reducing and capping the
synthesized SNPs. Eight IR bands were identified for leaf, root and SNP extracts which was tabulated. The absorption peak of 1631.77 cm\(^{-1}\) and 1630.84 cm\(^{-1}\) for SNP leaf and SNP root in the infrared region of the electromagnetic spectrum exhibited the binding of amide linkage with SNPs that may be assigned to the carbonyl stretch in proteins and clearly indicated the presence of protein as capping agent for SNPs. This absorption peak was found to be absent in crude ethanolic leaf and root extracts of *C. anisata*, which strongly indicates that protein acted as a capping agent for SNPs.

The crude ethanolic leaf extract showed FTIR peak values 651.59 cm\(^{-1}\), 762.94 cm\(^{-1}\), 830.05 cm\(^{-1}\), 1079.70 cm\(^{-1}\), 1402.65 cm\(^{-1}\), 1588.02 cm\(^{-1}\), 2926.82 cm\(^{-1}\) and 3375.51 cm\(^{-1}\) which are attributed to the stretching vibrations of C-Br, C-Cl, CH out of plane, C-N, C-O, NH\(_2\) in plane bend, C-H and dimer O-H respectively (Fig. 13a) (Table 16a). The crude ethanolic root extract showed absorption peaks centered around 1121.53 cm\(^{-1}\) and 1400.13 cm\(^{-1}\), which are attributable to the stretching vibrations of C-O group and 697.05 cm\(^{-1}\), 830.90 cm\(^{-1}\) for CH out of plane. Other characteristic stretching modes are found at 652.53 cm\(^{-1}\) for C-Br stretch, 762.94 cm\(^{-1}\) for C-Cl stretch, 1036.09 cm\(^{-1}\) for C-N stretch, 1574.21 cm\(^{-1}\) for C=C stretch and 3389.71 cm\(^{-1}\) for N-H stretch (Fig. 13b) (Table 16b).

FTIR spectral data for the biosynthesized SNP leaf extract are attributable to -C C-H, C-O, N-O symmetric stretch, N-H bend, -C C-, RCO\(_2\)H and O-H / H-bonded stretch exists in the region of 667.22 cm\(^{-1}\), 1015.41 cm\(^{-1}\), 1360.46 cm\(^{-1}\), 1631.77 cm\(^{-1}\), 2083.51 cm\(^{-1}\), 2833.19 cm\(^{-1}\), 3432.71 cm\(^{-1}\) and 3464.31 cm\(^{-1}\) respectively (Fig. 13c) (Table 17a).

FTIR spectral data for the biosynthesized SNP root extract were attributed to the N-H stretch at 3432.97 cm\(^{-1}\), H-C=O stretch exists in the
region of 2832.62 cm\(^{-1}\), peaks at 663.20 cm\(^{-1}\), 774.15 cm\(^{-1}\), 1015.96 cm\(^{-1}\), 1361.75 cm\(^{-1}\) and 1630.84 cm\(^{-1}\) can be assigned to C-H bend, C-Cl stretch, C-O stretch, N-O symmetric stretch and RNH\(_2\) stretch (Fig. 13d) (Table 17b).

4.6.3. **FESEM analysis**

This analysis was carried out to measure the size and shape of the SNPs. The synthesized SNPs showed spherical form and with a size distribution ranging from 50 nm to 66 nm, with an average size 60.67 nm for SNP leaf (Plate - 14). The size distribution ranging from 23 nm to 44 nm, with average size 32.75 nm was found to be for SNP root.

4.6.4. **EDS analysis**

The EDS analysis was performed to know the percentage of silver in the sample at 3KeV. The EDS spectra showed the different types of elements with their weight percentage such as silver (55.24%), carbon (18.97%), chlorine (13.43%), oxygen (9.61%) and copper (2.76%) in SNP leaf (Fig. 14a) and in SNP root it was found to be silver (24.72%), carbon (38.78%), oxygen (28.25%), chlorine (6.37%) and calcium (1.88%) respectively (Fig. 14b).

4.6.5. **XRD analysis**

XRD analysis was used to confirm the crystalline nature of the SNPs. Different diffraction intensities were recorded from 20° to 80° in 2θ angles. This was compared with the standard ICDD files. The biosynthesized SNPs by using extracts of *C. anisata* was further confirmed by the characteristic peaks observed in the XRD image at 2θ angles with minimum and maximum range at 27.48°-76.44° for SNP leaf (Fig. 15a) and 27.64° - 76.53° for SNP root (Fig. 15b).
4.7. GC-MS analysis

Preliminary GC-MS analysis was carried out for crude ethanolic leaf (Fig. 16a) and root extract (Fig. 16b) of *C. anisata* to determine the phytoconstituents responsible for the *in vitro* and *in vivo* activities that was carried out. The numbers of compounds obtained were compared with NIST library that contains more than 62,000 known compounds based on retention time and molecular mass.

The active principle with their retention time, molecular formula, molecular weight and concentration (%) for leaf and root were presented in Table 18 and 19. The GC-MS analysis of ethanolic leaf and root extract of *C. anisata* was shown in Fig. 17 – Fig. 30.

Amongst, the ethanolic leaf extract analysis showed the presence of 18, 19-seco-15a-yohimban-19 oic acid, 20, 21-didehydro-16a-(hydroxymethyl)-, methyl ester with peak area 7.754% and retention time 21.75. The crude ethanolic root extract analysis showed the presence of corynan-17ol, 18, 19-didehydro-10-methoxy- with retention time 22.67 and peak area 80.387%, in which both the compound was found to be indole alkaloid and more or less similar in structure with molecular formula $C_{21}H_{24}N_2O_3$ and $C_{22}H_{28}N_2O_3$.

Several other compounds in leaf and root extracts are reported to possess antimicrobial, antitussive activity, in the treatment of asthma, hepatitis, liver diseases, coronary heart diseases, allergy, rheumatoid arthritis, gout, antioxidant, antacids, and anti-inflammatory activities.

4.8. Column chromatography

From the GC-MS analysis, it was found that indole alkaloid was present in higher concentration in ethanolic leaf and root extracts. Hence an
attempt was made to prepare crude alkaloid fractions of leaf and root, which was subjected to column chromatography separately (Plate - 15). Several fractions were obtained, combined together and made it as single fraction for both leaf and root. The fractions were labeled as LF1 - LF20, RF1 - RF20 for leaf and root respectively. All this fractions were tested for the presence of alkaloid by carrying out Wagners, Dragendorffs and Mayers test. The fractions that showed positive for alkaloid were further used to investigate the hypoglycemic activity under in vivo.

4.9. Hypoglycemic study of c. anisata extracts under in vivo

The hypoglycemic study on different extracts of C. anisata was studied using alloxan induced diabetic rats weighing 150-200 gm. Different parameters such as body weight, liver weight, pancreas weight, estimation of glucose, protein, tissue cholesterol, triacylglycerol, biochemical parameters in serum and liver were studied. The histopathological studies were carried out for pancreatic tissues from all groups.

4.9.1. Effects of different extracts of C. anisata on body weight

Body weight was recorded every 3\textsuperscript{rd}, 7\textsuperscript{th}, 15\textsuperscript{th} and 30\textsuperscript{th} day. The data are shown in Table 20. There was significant gradual decrease in the body weight of diabetic rats (Group II) (119.83±0.31 g to 103.33±0.71 g), when compared with normal rat (125.33±0.33 g to 139.33±0.33 g). The normal rats treated with leaf (200 mg/kg bw) (Group – IV), root (200 mg/kg bw) (Group – VI), SNP leaf (10 mg/kg bw) (Group – VIII) and SNP root (10 mg/kg bw) (Group – X) did not showed any major variations in body weight when compared with normal control (Group – I). The diabetic rats treated with SNP root (10 mg/kg bw) (P<0.0001), SNP leaf (10 mg/kg bw) (P<0.001), root (200 mg/kg bw) (P<0.01), SNP root (5 mg/kg bw) (P<0.01) and SNP leaf (5 mg/kg bw)
(P<0.01) showed gradual increase in the body weight and was found to be 138±0.26 g, 137.25±0.26 g, 136.83±0.17 g, 136.50±0.26 g and 136.81±0.31 g respectively on 30th day. The diabetic rats treated with alkaloid fraction of leaf/root (Group – XI) (1 mg/kg bw) also showed gradual increase in body weight (136.25±0.26) when compared with normal rats (139.33±0.33). The diabetic rat treated with standard glibenclamide (1 mg/kg bw) (139.15±0.26) (Group – XII), did not show any variations in body weight when compared with normal control (Fig. 31).

4.9.2. Effects of different extracts of C. anisata on liver and pancreas weight

Liver and pancreas weight was recorded at 30th day and the results were tabulated (Table 21). There was a significant decrease in the liver and pancreas weight of diabetic rats (Group – II), when compared to normal control rats (Group – I). The liver and pancreas weight was found to be 4.60±0.08 g and 1.54±0.00 g in normal control, in diabetic rats it was 2.17±0.03 g and 0.63±0.02 g respectively. The diabetic rats treated with SNP root (10 mg/kg bw) (P<0.0001), SNP root (5 mg/kg bw) (P<0.001), followed by SNP leaf (10 mg/kg bw) (P<0.01) showed gradual increase in the liver and pancreas weight, when compared with normal control. The maximum increase in liver and pancreas weight was found to be for SNP root extract (10 mg/kg bw) with 4.20±0.02 g (P<0.0001) and 1.48±0.02 g respectively. The diabetic rats treated with alkaloid leaf/root fraction (1 mg/kg bw) (Group – XI), and Glibenclamide (1 mg/kg bw) (Group – XII) slightly increased in the liver and pancreas weight with 3.50±0.02 g, 1.22±0.02 g and 3.62±0.01 g and 1.23±0.01 g respectively when compared with normal rats (Fig. 32).
4.9.3. Effects of different extracts of *C. anisata* on blood glucose level

The serum blood glucose level was monitored every 3rd, 7th, 15th and 30th day (Table 22). The alloxan induced diabetic rats (Group – II) resulted in a significant increase in serum blood glucose level (219.17±0.60 to 228.17±0.60 mg/dl) in comparison with the normal control group (102.50±0.62 to 103.77±0.60 mg/dl). The serum glucose level was gradually increased from day 3 to day 30. After the administration of different extracts of *C. anisata* to diabetic rats during 30 days a significant decrease in the blood glucose level was observed. The maximum decrease was found to be in SNP root (10 mg/kg bw) (P<0.0001) extract (133.75±0.37 mg/dl), SNP leaf (10 mg/kgbw) extract (160.50±0.37 mg/dl) (P<0.001) followed by SNP root extract (5 mg/kg bw) (P<0.01) respectively. The diabetic rats treated with alkaloid leaf/root fraction (1 mg/kg bw) and glibenclamide (1 mg/kg bw) showed blood glucose level of 189.75±0.37 mg/dl and 174.25±0.37 mg/dl respectively on 30th day (Fig. 33).

4.9.4. Effects of different extracts of *C. anisata* on serum cholesterol and triglycerides

The serum cholesterol levels in diabetic rats showed a significant rise in comparison to the values in control rats (245.17±1.35 vs 122.00±0.68 mg/dl) (Group I). Serum triglycerol levels were also elevated in diabetic rats compared to control rats (182.17±0.40 and 91.67±0.21 mg/dl respectively) (Table 23). On the other hand, the diabetic rats treated with SNP root extract (10 mg/kg bw) (P<0.0001), SNP root extract (5 mg/kg bw) (P<0.0001), SNP leaf extract (10 mg/kg bw) (P<0.001), SNP leaf extract (5 mg/kg bw) (P<0.001) and root extract (200 mg/kg bw) (P<0.01) showed gradual decrease in serum cholesterol level with 123.50±0.43, 124.33±0.42, 126.67±0.21,
127.17±0.17 and 128.33±0.21 mg/dl respectively. The diabetic rats treated with alkaloid leaf/root fraction (1 mg/kg bw) and glibenclamide (1 mg/kg bw) showed decrease in the cholesterol level but when compared with normal control rats it was less potent (128.83±0.40 and 125.17±0.31 mg/dl respectively).

The diabetic rats treated with SNP root extract (10 mg/kg bw) (P<0.0001), SNP root extract (5 mg/kg bw) (P<0.0001), SNP leaf extract (10 mg/kg bw) (P<0.001), SNP leaf extract (5 mg/kg bw) (P<0.001) and root extract (200 mg/kg bw) (P<0.01) showed gradual decrease in serum triglycerol level with 93.67±0.33, 94.83±0.40, 95.50±0.34, 96.17±0.31 and 98.83±0.54 mg/dl respectively. The diabetic rats treated with alkaloid leaf/root fraction (1 mg/kg bw) and glibenclamide (1 mg/kg bw) showed decreased in the triglycerol level when compared with normal control rats (102.50±0.22 and 95.17±0.31 mg/dl respectively) (Fig. 34).

4.9.5. Effects of different extracts of C. anisata on protein

Significant decrease in the protein level was observed in diabetic rats (3.16±0.04 mg/dl) (Group II) when compared with control rats (6.20±0.03 mg/dl) (Table 23). Different extracts administered in diabetic rats showed gradual increase in protein levels. The maximum increase in protein level was found to be for SNP root extract (10 mg/kg bw) (P<0.0001), SNP root (5 mg/kg bw) (P<0.0001) and SNP leaf extract (10 mg/kg bw) (P<0.0001) with 6.36±0.02, 5.96±0.01 and 5.37±0.03 mg/dl respectively. The diabetic rats treated with alkaloid leaf/root fraction (1 mg/kg bw) did not show any increase in the protein level (3.84±0.01 mg/dl). The standard drug glibenclamide (1 mg/kg bw) showed 4.46±0.02 mg/dl of protein, which was found to be less when compared with normal control (Fig. 35).
4.9.6. Effects of different extracts of C. anisata on serum AST, ALT and ALP

Serum activities of AST, ALT and ALP act as a biomarker of liver toxicity. In this study the level was elevated in alloxan induced diabetic rats (Group II) (76.33±0.67, 65.00±0.37 and 66.67±0.49 U/L) respectively. when compared with control rats (Group I) (31.50±0.43, 18.50±0.22 and 30.17±0.31 U/L) respectively (Table 24).

Treatment of diabetic rats with SNP root (10mg/kgbw) (P<0.0001), SNP root (5 mg/kg bw) (P<0.0001) and SNP leaf (10 mg/kg bw) (P<0.001) significantly reduced the activity of these biomarkers with respect to control rats. The reduced AST level was found to be 32.17±0.31 (P<0.0001), 35.00±0.37 (P<0.001) and 35.33±0.33 U/L (P<0.001) respectively for SNP root (10 mg/kg bw); ALT level was 16.17±0.31 (P<0.0001), 16.50±0.22 (P<0.0001) and 17.17±0.17 U/L (P<0.001) for SNP root (5 mg/kg bw) and ALP level was 32.00±0.26 (P<0.0001), 32.68±0.16 (P<0.0001) and 33.35±0.17 U/L (P<0.001) for SNP leaf (10 mg/kg bw) respectively. The glibenclamide (1mg.kgbw) treated diabetic rats decrease the level of ALT biomarker (18.17±0.17 U/L) when compared with normal control, the AST and ALP biomarker was found to be decreased (38.00±0.26 and 34.50±0.34 U/L respectively) when compared with normal rats. The alkaloid leaf/root fraction (1 mg/kg bw) also showed decrease in the level of biomarkers when compared with diabetic rats (Fig. 36), in which the level of ALP was significantly decreased (P<0.0001).
4.9.7. Effects of different extracts of *C. anisata* on gluconeogenic enzymes

The activity of gluconeogenic enzymes (Fructose-1, 6-bisphosphatase and Glucose-6-phosphatase) were found significantly elevated in diabetic rats (Group II) (148.61±1.39 and 78.47±1.16 respectively) when compared with normal control rats (Group I) (48.61±1.39 and 27.08±0.76 respectively) (Table 25). Diabetic rats treated with different extracts showed gradual decrease in this level, but the extracts especially SNP root (10 mg/kg bw) (P<0.0001) and SNP root (5 mg/kg bw) (P<0.0001) brought back the activity of Glucose-6-phosphatase to near normal level (28.82±0.35 and 29.97±0.17 respectively). The SNP root (10 mg/kg bw) (P<0.0001) brought back the activity of fructose-1, 6-bisphosphatase to 52.08±1.42, when compared with normal control. The diabetic rats treated with glibenclamide (1 mg/kg bw) have decrease the level of gluconeogenic enzymes to 65.14±0.62 when compared with normal control (Fig. 37). The combined alkaloid leaf/root fractions reduced the levels of fructose-1, 6-bisphatase and Glucose – 6 – phosphatase to 73.61±0.50 and 34.03±0.16 respectively.

4.9.8. Effects of different extracts of *C. anisata* on glucokinase

The activity of glucokinase in liver and pancreas of control and diabetic animals are shown in Table 26. The activity of this enzyme in liver and pancreas was found to be significantly decreased (34.65±1.41 and 24.75±1.41 respectively) in diabetic rats when compared with normal control (127.60±1.10 and 101.20±1.10 respectively). But the different extracts treated in diabetic animal for 30 days showed increase in this enzyme level. The maximum rise was found to be in SNP root (10 mg/kg bw) (P<0.0001), SNP root (5 mg/kg bw) (P<0.0001) and SNP leaf (10 mg/kg bw) (P<0.001) in liver and pancreas, when compared normal control. 119.35±1.57, 118.80±0.85 and
117.70±0.70 in liver (P<0.01); 106.15±1.57, 105.60±0.85 and 104.50±0.70 in pancreas respectively. All other extracts showed gradual increase in the enzyme level but was not active when compared with normal control. The standard glibenclamide treated rats showed the enzyme activity of 119.90±1.10 and 106.70±1.10 respectively in liver and pancreas, when compared with normal control (Fig. 38). The alkaloid leaf/root fraction significantly increase the pancreatic glucokinase activity to 98.45±1.01 (P<0.01).

4.9.9. Effects of different extracts of C. anisata on LPO

The activity of LPO was found to be increased in diabetic rats 60.7±0.4 (Group II), when compared with normal control 16.2±0.5. The different extracts of C. anisata treated in diabetic rats showed gradual decrease in this level, in which SNP root (10 mg/kg bw) (P<0.0001) and SNP leaf (10 mg/kg bw) (P<0.001) showed significant increase with 16.5±0.1 and 17.4±0.1 respectively (Fig. 39). The standard treated diabetic rat showed LPO (17.4±0.1), when compared with normal control (Table 27). The alkaloid leaf/root fraction also reduced the LPO to 18.3±0.1 (P<0.001).

4.9.10. Effects of different extracts of C. anisata on GSH

The activity of GSH was significantly decreased in diabetic control rats (3.3±0.1) when compared with normal control (13.3±0.3). The treatment of diabetic rats with different extracts showed gradual increase in this level by 30th day. The SNP root (10 mg/kg bw) (P<0.0001) and SNP root (5 mg/kg bw) (P<0.001), showed maximum increase with 13.1±0.2 and 12.7±0.3 respectively as of normal (Table 27). The standard treated rats increased the GSH level to (12.0±0.1), when compared with normal control (Fig. 39). The alkaloid leaf/root fraction showed 10.8±0.2.
4.9.11. Effects of different extracts of C. anisata on SOD

The activity of SOD was significantly increased in diabetic control (20.8±0.4 U/mg protein) when compared to normal control (9.1±0.1 U/mg). The SNP root (10 mg/kg bw) (P<0.0001) and combined alkaloid fractions of leaf and root (1 mg/kg bw) (P<0.0001) treated diabetic rats significantly decreased the SOD level to 9.5±0.1 U/mg from 20.8±0.4 U/mg followed by SNP root (5 mg/kg bw) (P<0.001) and crude leaf extract (100 mg/kg bw) (P<0.01) respectively (Table 27). The diabetic rat treated with glibenclamide (1 mg/kg bw) showed 10.8±0.1 U/mg of SOD activity (Fig. 39).

4.9.12. Effects of different extracts of C. anisata on insulin

The concentration of insulin was significantly decreased in the alloxan induced diabetic rats (Group II) (3.43 ± 0.1527 µU/ml) when compared with control (Group I) (7.2 ± 0.2645 µU/ml) (Table 28). Alloxan causes destruction on beta cells of islets of langerhans that leads to reduction in insulin release causing hyperglycemia, hyper cholestero lemia, high levels of alkaline phosphate and transaminase. The results of the study showed the ability of C.anisata extracts in elevating plasma insulin level in a dose dependent manner but it was low when compared with normal and standard glibenclamide treated animals showed (6.9 ± 0.20 µU/ml). The SNP root extract at a concentration of 10 mg/kg bw showed maximum increase 6.36 ± 0.2516 µU/ml (P<0.0001), followed by crude root extract (200 mg/kg bw) 6.26 ± 0.3214 µU/ml (P<0.0001) and combination of alkaloidal leaf and root extract (1 mg/kg bw) 6.16 ± 0.1527 µU/ml (P<0.0001) respectively. The other extracts also increased the insulin level when compared with diabetic control but was found to be less potent (Fig. 40).
4.10. Histopathological study

The staining of H & E of pancreatic sections showed changes of pancreatic islets in alloxan induced diabetic rats (Plate - 16).

In normal group, the islets of langerhans were unevenly scattered in the pancreatic tissue with varying size. The acinar cells were stained strongly and arranged in lobules. The islet cells were embedded within the acinar cells in normal control (Plate – 16a). In diabetic control the number of islets and β cells was found to be decreased and the acinar cells was found to be abnormal (Plate – 16b), when compared with normal control. The diabetic animals treated with extracts (Plate – 16c to 16i) and glibenclamide (Plate – 16l) showed increase in the number of islets by restoring normal cells as compared with that of control animals. Of which crude root extract (200 mg/kg bw) (Plate – 16f) and SNP root extract (10 mg/kg bw) (Plate – 16j) showed the better restoration and maximum rise in β cells followed by combined alkaloid fractions of leaf/root extract (1mg/kg bw) (Plate – 16k) when compared with the diabetic control.