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Research Article

In vivo Analysis of *Clausena anisata* (Willd.) Hook. f. ex Benth Crude and SNP Extracts Against Antioxidant and Hypoglycemic Activity

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ABSTRACT

Type II diabetes mellitus which is a chronic metabolic disorder is characterized by insulin resistance. Due to the serious side effects of synthetic antidiabetic drugs, the search for safer and more efficacious hypoglycemic agents is still being continued. The study was carried out to evaluate the antioxidant and hypoglycemic effects of *C. anisata* (*Rutaceae*) leaf and root crude extracts and extracts mediated synthesized Silver Nanoparticles (SNPs) under *in vivo* conditions. The synthesis of SNPs from ethanolic leaf and root extract of *C. anisata* was characterized by UV-Vis, FTIR, FESEM, XRD and EDS. The average size was found to be 60.67 nm for SNP leaf and 32.75 nm for SNP root. Two different doses 100 mg/kg bw, 200 mg/kg bw of each crude leaf and root extracts and 5 mg/kg bw and 10 mg/kg bw of SNP leaf and root extracts were used to treat alloxan-induced diabetic rats for 30 days. On administration of different extracts of *C. anisata* the activities such as serum glucose, triglyceride, cholesterol, liver biomarkers, gluconeogenic enzymes, antioxidants were significantly reduced and the activity of glucokinase, protein, serum insulin level, body weight, liver and pancreas weight was significantly increased in alloxan-induced diabetic rats on the 30th day. Glibenclamide (1mg/kg bw) was used as a standard positive control. The extracts had a beneficial effect on regeneration of β-cells of the pancreas in alloxan-induced diabetic rats. Among the extracts, SNP root extract (10 mg/kg bw) was potent for *in vivo* hypoglycemic activity. This possible effect is due to natural bioactive compounds present in *C. anisata* extracts that acted synergistically or independently in enhancing the antioxidant and hypoglycemic activities.

Keywords: Silver Nanoparticle, *C. anisata*, Diabetes, hypoglycemia, glucose, insulin, triglyceride, alanine transaminase and aspartate transaminase.

INTRODUCTION

Medicinal plants with active constituents such as alkaloids, steroids, carbohydrates, flavonoids, glycosides, terpenoids, polysaccharides, peptidoglycans and coumarins are known to cure several ailments such as to strengthen immune system, inflammation, kidney and urinary tract ailments, cancer, dengue, malaria, diabetes[1], scurvy, arthritis, liver problems, cardiac diseases, laxative, asthma, obesity etc. The prescribed drugs (25%) that are currently marketed are isolated from plants or semi synthetic analogues of phytochemicals. Several thousands of different chemical constituents are present in crude plant extracts that are known to interact in complex ways and produce physiological actions in body[2]. Diabetes a multifactorial metabolic disease with hyperglycemia, including abnormal carbohydrate, protein and fat metabolism leading to several complications and hence needs a combined therapeutic approach[3,4]. Insulin injections are given in case of total lack of insulin; the post prandial hyperglycemia is managed at digestive level by using acarbose, miglitol and voglibose drugs[5]. Out of the two major types of diabetes, diabetes (type II) is more prevalent due to the loss of β-cell function that results in insulin resistance. The regeneration or stabilization of β-cell must occur, in order to prevent the loss of β-cells[6]. The postprandial hyperglycemia will cause non-enzymatic glycosylation of proteins that leads to micro and macro vascular complications. It is essential to control the postprandial plasma glucose level in the early treatment of diabetes and also to reduce micro and macro vascular complications[7]. Eventhough there are several drugs to tightly regulate blood glucose, to reduce microvascular and macrovascular complications, the main undesirable effects of this anti-diabetic drug that are currently available are brain damage, swelling, erythema, abdominal pain, weight gain, metallic taste, vitamin B12 deficiency, heart failure and gastrointestinal disturbances[3]. Due to these side effects of oral hypoglycemic agents and oxidative stress in complicating diabetes[8] it become necessary to search herbal remedy for the treatment of type II diabetes mellitus and oxidative stress[5,9]. N-D-glucose serves as a transport and short term storage form of energy. The glucose gets oxidized and provide energy to sustain life. The aldehyde group in glucose is found to be more reactive and many

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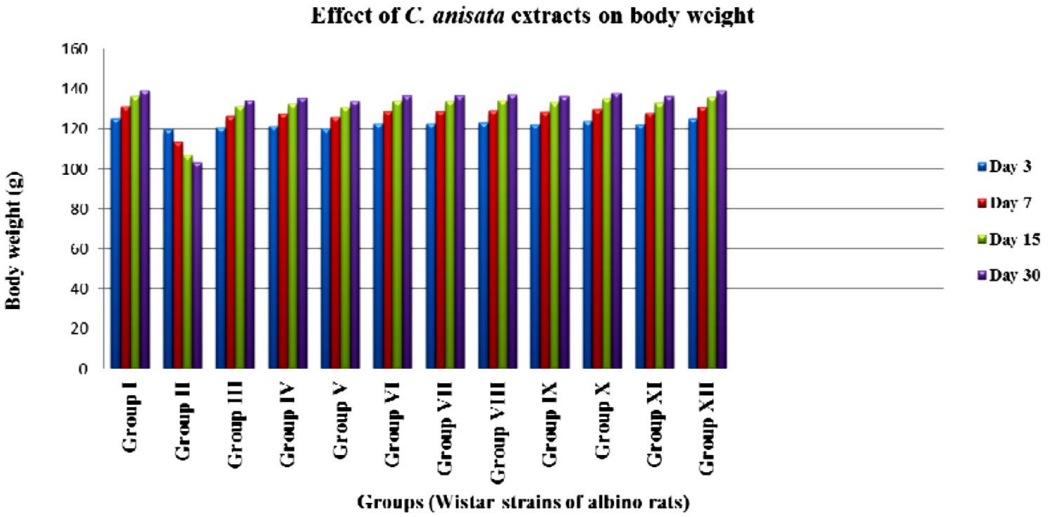

Figure 1: Effects of *C. anisata* extracts on body weight.

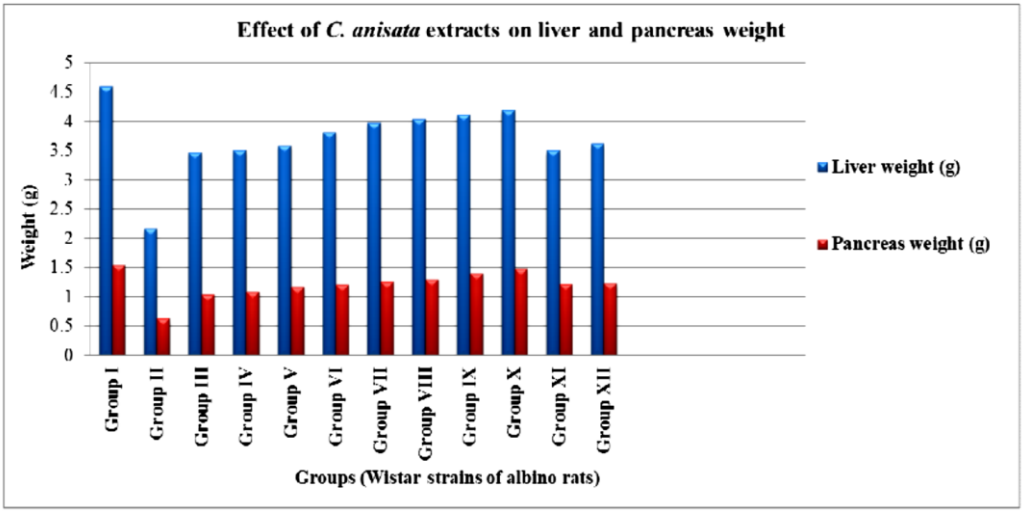

Figure 2: Effects of *C. anisata* extracts on liver and pancreas weight.

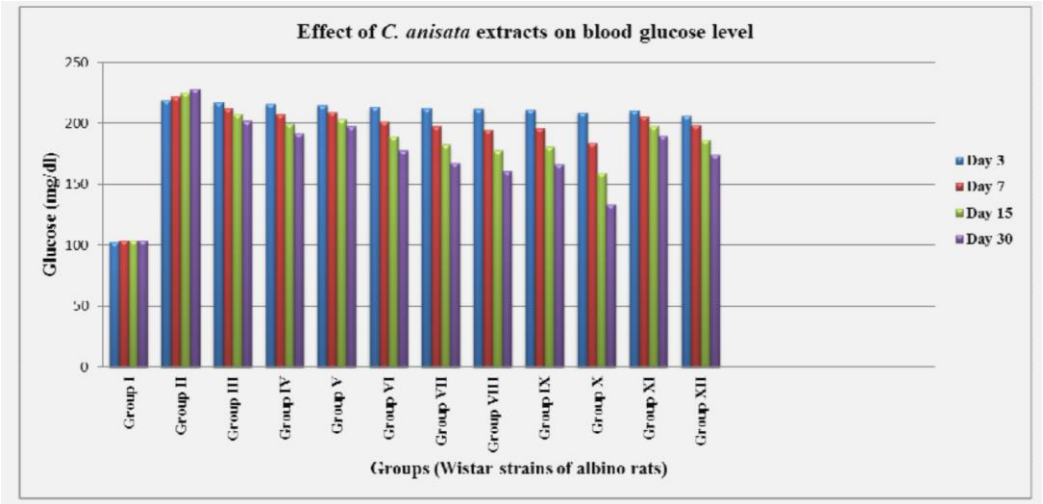

Figure 3: Effects of *C. anisata* extracts on blood glucose level.

nucleophilic agents like alcohol, amine and carbanions are prone to attack the aldehyde group[10].

When there is an elevation in glucose control (fasting blood sugar in diabetes – 126mg/dl and above; random blood sugar in diabetes – 200 mg/dl and above) it leads to chronic hyperglycemia[11]. Due to defects in insulin secretion, insulin action or both, it results in chronic hyperglycemia, that culminate to a metabolic disorder

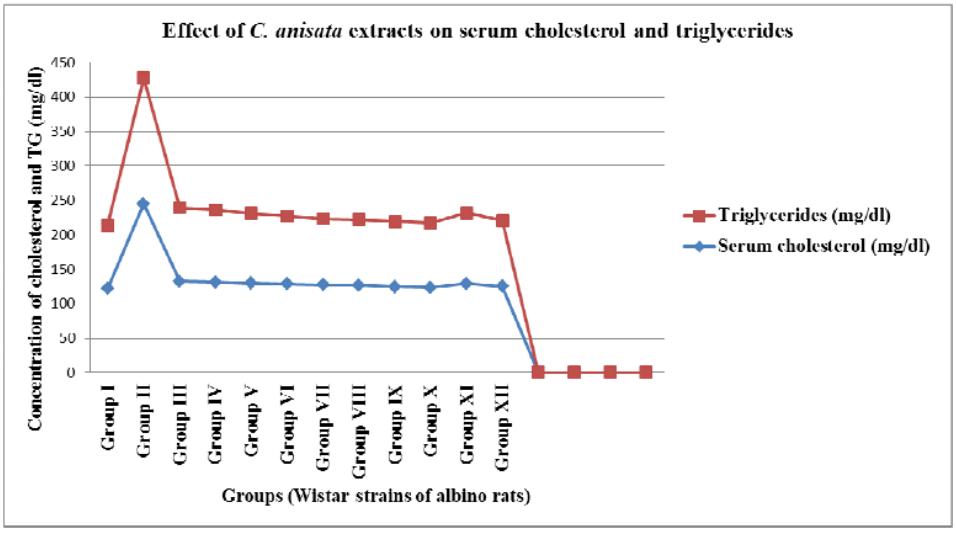

Figure 4: Effects of *C. anisata* extracts on triglyceridesvand serum cholesterol.

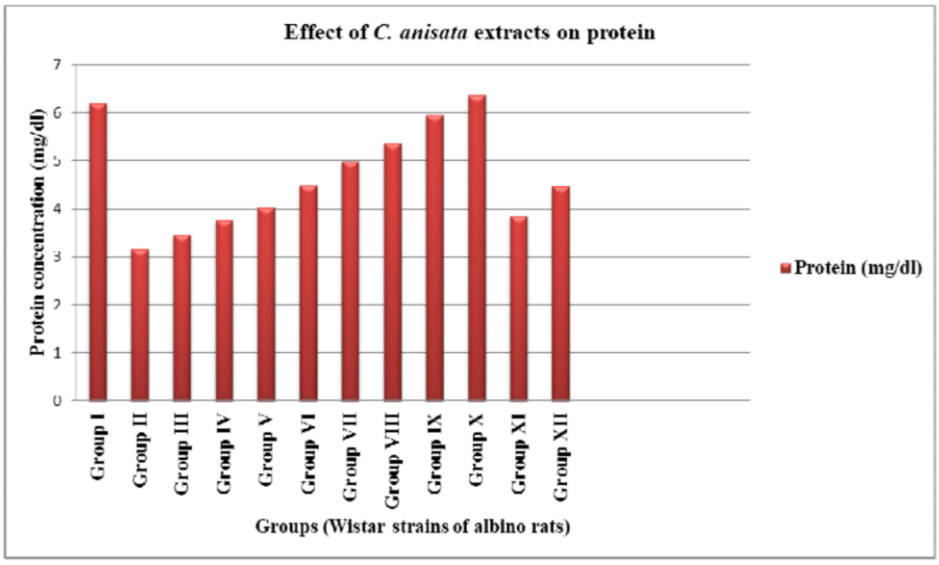

Figure 5: Effects of *C. anisata* extracts on protein.

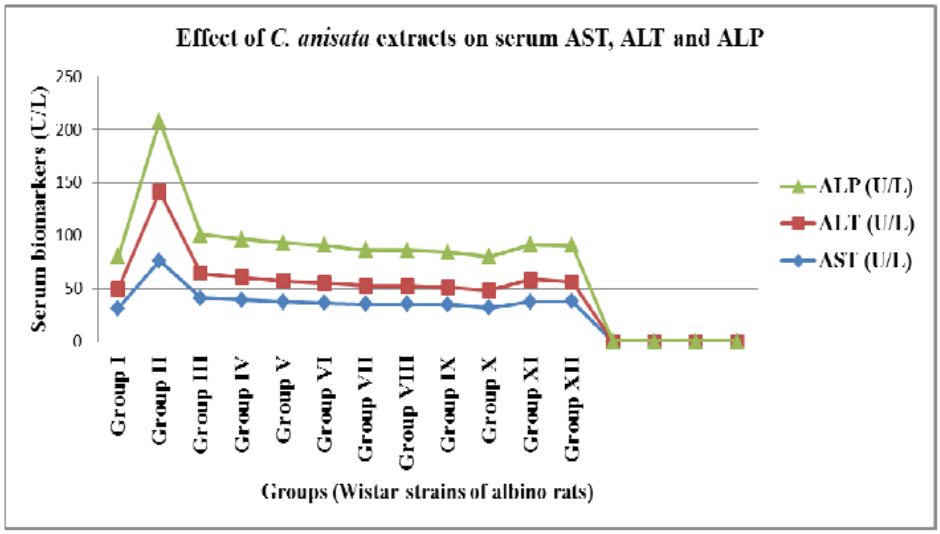

Figure 6: Effects of *C. anisata* extracts on serum AST, ALT and ALP.

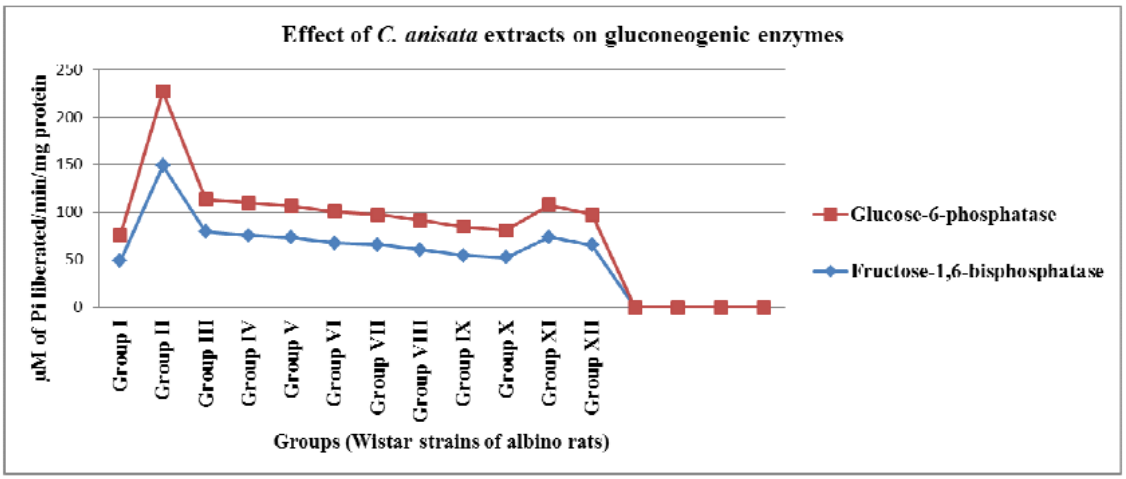

Figure 7: Effects of *C. anisata* extracts on gluconeogenic enzymes.

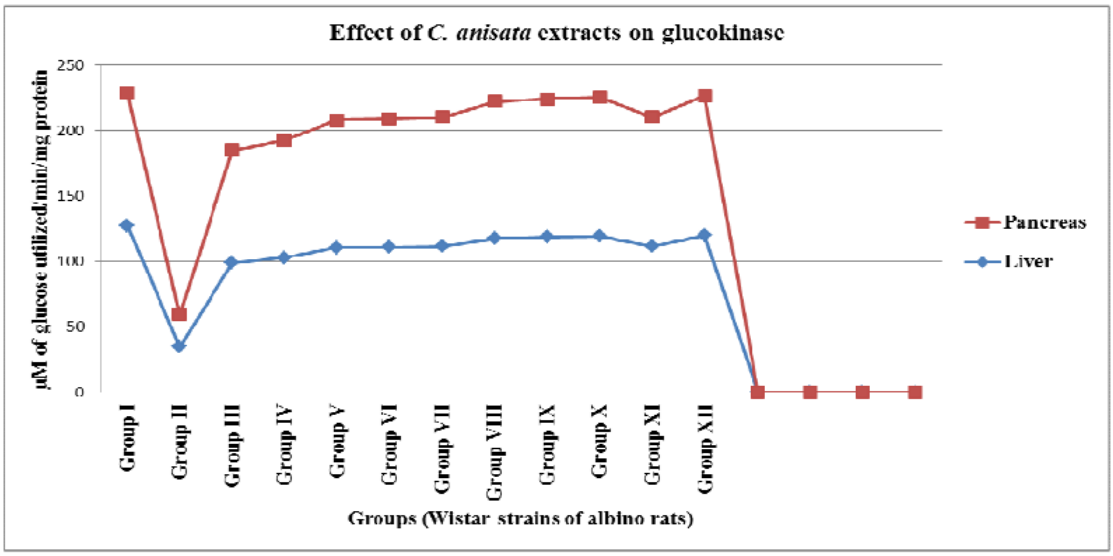

Figure 8: Effects of *C. anisata* extracts on glucokinase.

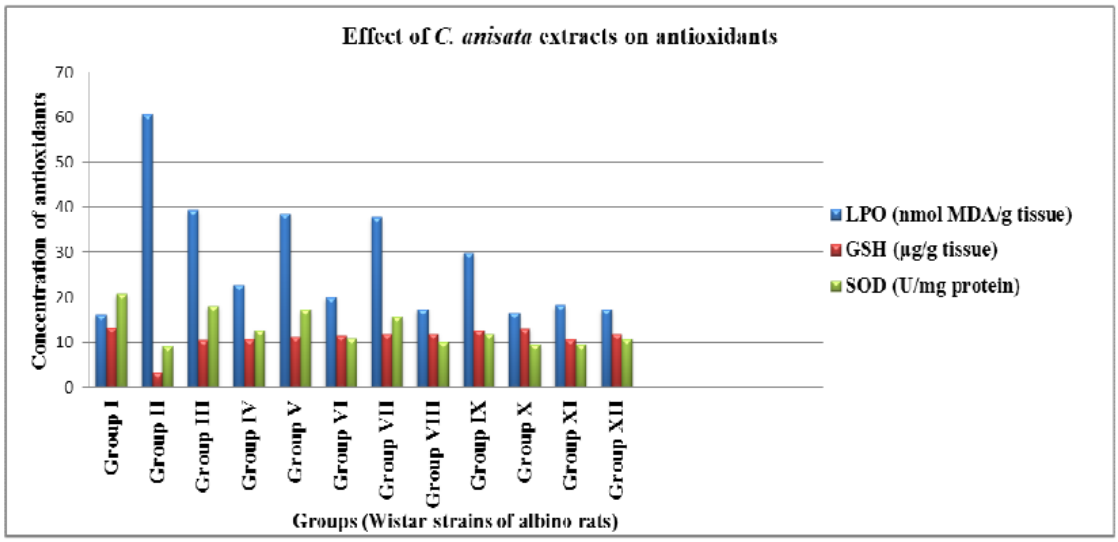

Figure 9: Effects of *C. anisata* extracts on LPO.

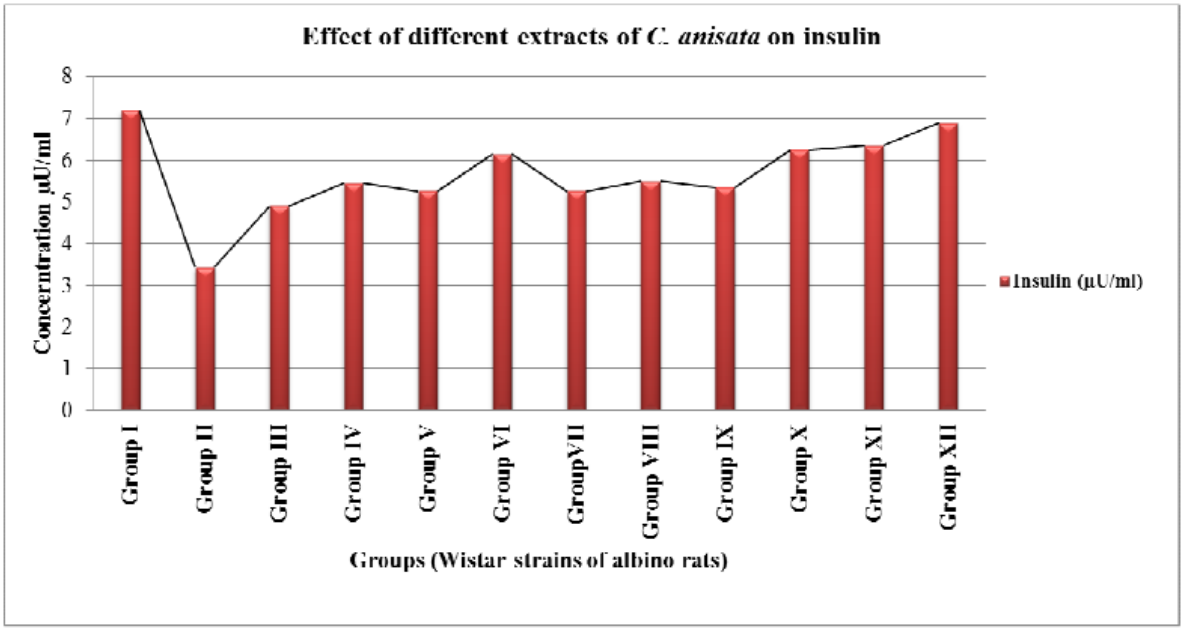

Figure 10: Effects of *C. anisata* extracts on insulin.

called Diabetes Mellitus. There are several synthetic drugs like biguanides, sulfonylurea, insulin, insulin analogues, meglitinides, thiaazolidinediones, DPP-4 inhibitors, pramlintide and GLP-1[12] inhibitors are available but due to its side effects and cost, traditional treatment with plants having medicinal potential becomes an alternative option for health conscious and for financially deprived populations. Through chemical, physical and biological methods nanoparticles can be produced. However, the ecofriendly method for synthesizing nanoparticles is biological method (plant extracts)[13]. Particle with a size less than 100nm are called nanoparticles. Silver which is harder than gold, act as antimicrobial agent and silver colloids are used to treat various diseases in medicine[14,15]. Nanoparticles (Nps) are also used in detergents, shampoos, soaps, toothpastes, cosmetics and pharmaceutical products and hence it can be come into contact with human system[15] and has good conductivity, chemical stability, catalytic activity, anti-inflammatory, antifungal and antiviral activities[16]. Plant mediated synthesized NPs are biodegradable, non-toxic and biocompatible that show quick action by entering into cell membrane and act as an alternative system of herbal medicine to treat diabetes[17].

MATERIAL AND METHODS

Plant Collection and Preparation of extract

Plant parts (leaf and root) were procured in the months of October to November (2013) from Manamettupatti, Viralimalai Taluk (Pudukottai District, Tamilnadu). Dr. S. John Britto, Director, The Rapinat Herbarium and Centre for Molecular Systematics, St. Joseph's College (Campus) Tiruchirappalli - 620002 authenticated the plant as *Clausena anisata* (Willd.) Hook f. ex. Benth.

The powedered leaf and root was sequentially extracted using ethanol in the ratio of 1:10 by maceration method. The combined filtrate obtained from the maceration was then concentrated to dryness under controlled temperature 40 - 50°C using Rota evaporator (Yamato, Japan (Model-RE801)).

Animals

Healthy Wistar strains of albino rats weighing 150 – 200 gm were procured from Biogen, Bangalore, India. The rats were maintained under temperature 25±2°C and dark/light cycle 14/10 h, and were allowed free access to standard dry pellet diet and water *ad libitum*. The rats were accustomed to laboratory conditions for 7 days and all procedures described were approved by the University Animal Ethical Committee.

Biosynthesis and characterisation of Silver Nanoparticles

The biosynthesis and characterisation of silver nanoparticles from *C. anisata* leaf and root ethanolic extract was reported in previous paper [18] [19]. Based on GC-MS analysis an attempt was made to extract crude alkaloid from leaf and root extracts of *C. anisata* using column chromatography followed by acid-base titration method and the positive crude alkaloid fraction of leaf and root alone was used in animal studies.

Animal studies

Induction of Diabetes in rats

Diabetes in rats was induced by single intraperitoneal injection of alloxan monohydrate (120 mg/kgbw). The persistent hyperglycemia after 3 days was determined in urine sugar levels by BQR method. The plasma glucose level of >140 mg/dl were included in the study. Treatment with plant extracts was started after 48 hours of alloxan injection.

Experimental design

Wistar strains of albino rats weighing 150-200 gm were obtained from Biogen, Bangalore and randomly divided into twelve groups (Group I – XII). The animals were placed in well ventilated cages and fed with commercial pelleted rat chew and water ad libitum. The rats in various groups were treated as follows:

Group I Normal control: Animals were given 0.9% saline through oral from day 1 to day 30.
Group II Diabetic control: Animals were treated with alloxan in normal saline at a dosage of 120 mg/kg bw through ip
Group III Group II treated with *C. anisata* ethanolic leaf extract 100 mg/kg bw ip for 30 days
Group IV Group II treated with *C. anisata* ethanolic leaf extract 200 mg/kg bw ip for 30 days
Group V Group II treated with *C. anisata* ethanolic root extract 100 mg/kg bw ip for 30 days
Group VI Group II treated with *C. anisata* ethanolic root extract 200 mg/kg bw ip for 30 days
Group VII Group II treated with *C. anisata* ethanolic SNP leaf extract 5mg/kg bw ip for 30 days
Group VIII Group II treated with *C. anisata* ethanolic SNP leaf extract 10mg/kg bw ip for 30 days
Group IX Group II treated with *C. anisata* ethanolic SNP root extract 5mg/kg bw ip for 30 days
Group X Group II treated with *C. anisata* ethanolic SNP root extract 10 mg/kg bw ip for 30 days
Group XI Group II treated with *C. anisata* combined alkaloid leaf / root fraction 1 mg/kg bw ip for 30 days
Group XII Group II treated with standard drug Glibenclamide 1 mg/kg bw ip for 30 days.
The animals were sacrificed after the experimental period by cardiac puncture under mild chloroform anesthesia. From the collected blood, serum was separated by centrifugation for 20 mins at 2000 rpm; liver and pancreas were dissected out and washed in ice-cold saline. They were homogenized in 0.1 M phosphate buffer, pH 7.4 and used for various biochemical experiments as described below.

Evaluation of body weight, liver weight and pancreas weight

The animals were observed for gain or loss of weight at 3rd, 7th, 15th and 30th day. The liver and pancreas weight were recorded on 30th day. The changes in the weight were expressed in grams.

Estimation of glucose

The blood glucose content was estimated by [20] at a period of 3rd, 7th, 15th and 30th day.

Extraction of lipids

The lipid was extracted from the homogenate by following method[21].

Estimation of tissue cholesterol

The tissue cholesterol was estimated as described by[22]. The colour developed was read at 560 nm.

Estimation of triacylglycerol

Triglycerol content was estimated by following method[23].

Biochemical parameters in serum

Estimation of alanine transaminase, aspartate transaminase and alkaline phosphatase

The alanine transaminase, aspartate transaminse and alkaline phosphatase in the sample were estimated by following method[24].

Biochemical parameters in liver

Determination of lipid peroxidation activity (LPO)

Lipid peroxidation in liver was determined colorimetrically by Thiobarbituric acid reactive substance (TBARS) by the method of[25].

Determination of superoxide dismutase activity (SOD)

Superoxide dismutase activity was determined by following method[26]. The enzyme level was expressed as U/mg of protein.

Determination of reduced glutathione activity (GSH)

The GSH activity in the sample was determined by following method[27].

Assay of glucokinase

The glucokinase activity in the sample was determined by following[28] and expressed as μM of glucose utilized/min/mg of protein.

Assay of glucose-6-phosphatase

The glucose-6-phosphatase activity in the sample was determined by following[24].

Assay of fructose-1, 6-bisphosphatase

The fructose-1, 6-bisphosphatase in the sample was estimated by following[29]. The suspension was centrifuged at 3000 rpm for 10 mins. The phosphorus content in the supernatant was determined according to the method described by[30].

Estimation of protein

The protein in the sample was determined by following method[31].

Estimation of insulin

The blood that was collected was centrifuged at 3000 g for 10 minutes. Plasma insulin level was assayed by insulin ELISA kit[32]. The concentration of the insulin was expressed as μU/ml.

Histopathological study on pancreas

The histopathological studies on pancreas were studied by following method[33]. From all groups the pancreatic tissues were subjected to histopathological studies. The whole pancreas was collected in 10% formalin solution and immediately processed by paraffin technique. 5μm thickness section was cut and stained by hematoxyllin and eosin (H & E) stain. After staining, the sections were observed under light microscope. The number of islets of langerhans was measured by comparing with control.

Statistical analysis

All experiments were carried out in triplicates. Results were expressed as mean ± standard deviation. The significant differences between mean values were determined by one-way analysis of variance (ANOVA) followed by Tukey's post hoc analysis.

RESULTS

Different parameters such as body weight, liver weight, pancreas weight, estimation of glucose, protein, tissue cholesterol, triacylglycerol, biochemical parameters in liver and serum were studied. The histopathological studies were carried out for pancreatic tissues from all groups.

Effects of C. anisata extracts on body weight

On 3rd, 7th, 15th and 30th day the body weight was recorded. The gradual decrease in the body weight was observed in 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

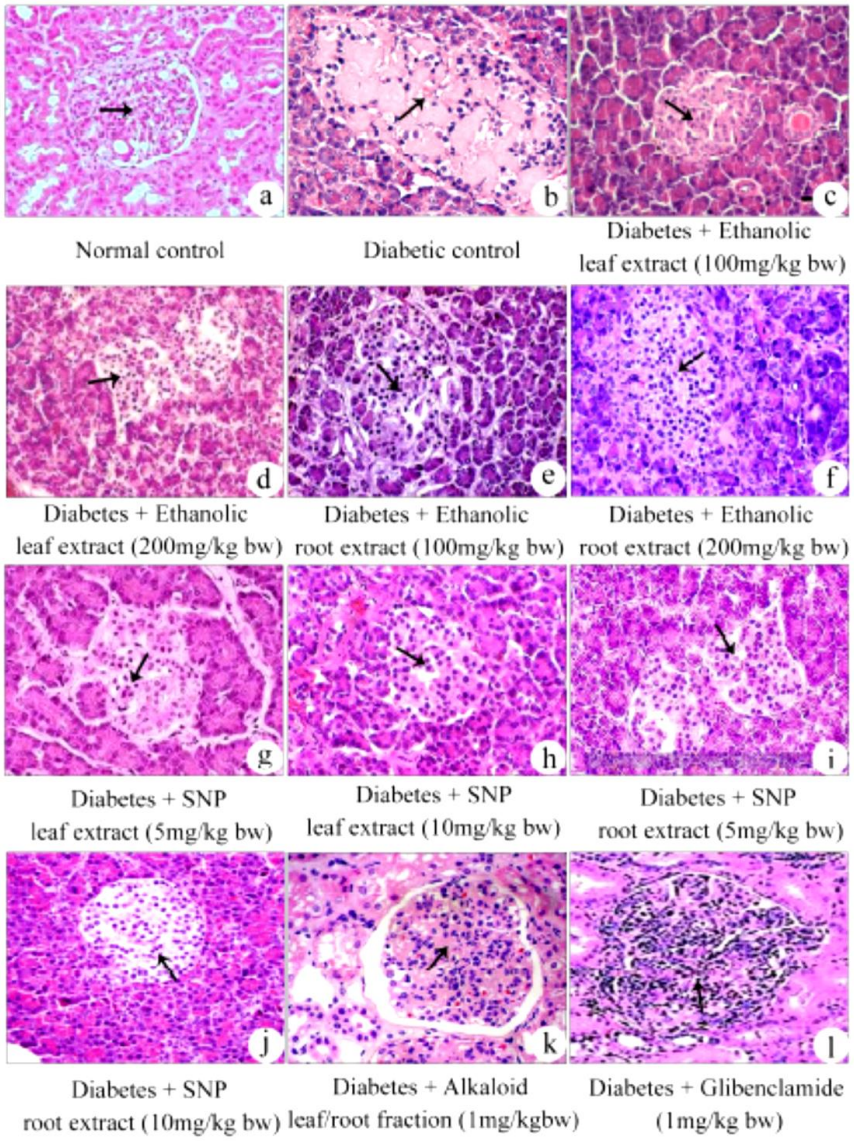

Figure 11: H & E staining of pancreatic sections.

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 significant 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 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. 1).

Effects of C. anisata extracts on liver and pancreas weight

Liver and pancreas weight was recorded at 30th day. There was a gradual 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 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) ($P<0.01$) treated diabetic rats 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 Glibenclamide (1 mg/kg bw) (Group – XII) showed moderate increase in the liver and pancreas weight with 1.22±0.02 g and 1.23±0.01 g respectively when compared with normal rats (Fig. 2).

Effects of C. anisata extracts on blood glucose level

The blood glucose level in serum was monitored every 3rd, 7th, 15th and 30th day. The alloxan induced diabetic rats

(Group – II) showed 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. The administration of different extracts of *C. anisata* to diabetic rats during 30 days showed decrease in the blood glucose level was observed. The maximum decrease was 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). The diabetic rats treated with glibenclamide (1 mg/kg bw) showed blood glucose level of 174.25±0.37 mg/dl respectively on 30th day (Fig. 3).

Effects of C. anisata extracts on triglycerides and serum cholesterol

The diabetic rats showed a significant rise in serum cholesterol level in comparison to control rats (245.17±1.35 vs 122.00±0.68 mg/dl) (Group I). Serum triglycerol levels were also increased in diabetic rats compared to control rats (182.17±0.40 and 91.67±0.21 mg/dl respectively). The diabetic rats treated with SNP root extract (10 mg/kg bw), SNP root extract (5 mg/kg bw), SNP leaf extract (10 mg/kg bw, SNP leaf extract (5 mg/kg bw) 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), SNP leaf extract (10 mg/kg bw), SNP leaf extract (5 mg/kg bw) 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 glibenclamide (1 mg/kg bw) treated rats and normal rat showed 102.50±0.22 and 95.17±0.31 mg/dl respectively (Fig. 4).

Effects of C. anisata extracts on protein

There was significant decrease in the protein level in diabetic rats (3.16±0.04 mg/dl) (Group II) when compared with control rats (6.20±0.03 mg/dl). 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), SNP root (5 mg/kg bw) and SNP leaf extract (10 mg/kg bw) with 6.36±0.02, 5.96±0.01 and 5.37±0.03 mg/dl respectively. The standard drug glibenclamide (1 mg/kg bw) showed 4.46±0.02 mg/dl of protein (Fig. 5).

Effects of C. anisata extracts 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. Treatment of diabetic rats with SNP root (10mg/kgbw), SNP root (5 mg/kg bw) and SNP leaf (10 mg/kg bw) reduced the activity of these biomarkers when compared with 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), 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 (Fig. 6).

Effects of C. anisata extracts on gluconeogenic enzymes

The gluconeogenic enzymes such as fructose-1, 6-bisphosphatase and glucose-6-phosphatase were found significantly increased in diabetic rats (Group II) (148.61±1.39 and 78.47±1.16 respectively) when compared with control rats (Group I) (48.61±1.39 and 27.08±0.76 respectively). Diabetic rats treated with different extracts showed gradual decrease in this level, but the extracts especially SNP root (10 mg/kg bw) and SNP root (5 mg/kg bw) 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) brought back the activity of fructose-1, 6-bisphosphatase to 52.08±1.42, when compared with control. The glibenclamide (1 mg/kg bw) treated rats have decrease the level of gluconeogenic enzymes to 65.14±0.62 when compared with control (Fig. 7).

Effects of C. anisata extracts on glucokinase

The glucokinase activity 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 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), SNP root (5 mg/kg bw) and SNP leaf (10 mg/kg bw) in liver and pancreas, when compared with control rats. The activity was found to be 119.35±1.57, 118.80±0.85 and 117.70±0.70 in liver; 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. 8).

Effects of C. anisata extracts on LPO

The level of LPO was found to be elevated 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. The standard treated

diabetic rat showed LPO (17.4±0.1), when compared with normal control (Fig. 9).

Effects of C. anisata extracts on GSH

The GSH activity 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) and SNP root (5 mg/kg bw), showed maximum increase with 13.1±0.2 and 12.7±0.3 respectively as of normal. The standard treated rats increased the GSH level to (12.0±0.1) (Fig. 9).

Effects of C. anisata extracts on SOD

The SOD activity 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) 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) and crude leaf extract (100 mg/kg bw) respectively. The diabetic rat treated with glibenclamide (1 mg/kg bw) showed 10.8±0.1 U/mg of SOD activity (Fig. 9).

Effects of C. anisata extracts on insulin

The insulin concentration was decreased in the alloxan induced diabetic rats (Group II) (3.43 ± 0.1527 μU/ml) when compared with (Group I) (7.2 ± 0.2645 μU/ml). Alloxan causes destruction on pancreatic beta cells that leads to reduction in insulin release causing hyperglycemia, hyper cholesterolemia, high levels of alkaline phosphate and transaminase. The results showed the ability of *C.anisata* extracts in increasing plasma insulin level but it was low when compared with control and glibenclamide treated animals that showed (6.9 ± 0.20 μU/ml). The SNP root extract 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 ($P<0.0001$) respectively (Fig. 10).

Histopathological study

The staining of H & E of pancreatic sections showed changes in pancreatic islets in alloxan induced diabetic rats (Fig- 11). In normal group, the islets of langerhans were scattered in the pancreatic tissue with varying size. The acinar cells were arranged in lobules. Within the acinar cells, the islet cells were found to be embedded in normal control (Fig- 11a). In diabetic control the number of β cells was found to be decreased and the acinar cells was found to be abnormal (Fig- 11b), when compared with control. The diabetic animals treated with extracts (Fig- 11c to 11i) and glibenclamide (Fig- 11l) showed increase in the islets number by restoring normal cells as compared with that of control rats. Of which crude root extract (200 mg/kg bw) ((Fig. 11f) and SNP root extract (10 mg/kg bw) (Fig- 11j) showed the better restoration and maximum rise in β cells when compared with the allaoxan induced rats.

DISCUSSION

The body weight was found to be increased in animals treated with SNP extracts when compared with those treated with the crude extracts. The results also corroborated with [9] that showed the treatment with *D. indica* methanolic leaf extract at a dose of 250 mg/kg bw and 500 mg/kg bw in alloxan induced diabetic rats significantly increased the body weight of animals. This may be due to the reversal antagonism of SNP extracts in controlling muscle wasting and preventing protein loss. The weight loss may be due to loss of tissue proteins that acts as an important sign in diabetes[34].

This result agrees with previous results of[34] that have employed different plant extract and also reported loss of body weight. The decrease in blood glucose level may be due to insulin secretion by pancreatic beta cells and increase in the blood glucose transport to peripheral tissues. Thus the results corroborated with[13], the insulin secretion had decreased the blood glucose in alloxan induced diabetes rats treated with gold nanoparticles synthesized from aqueous extracts of *Cassia fistula*. Another study stated that *W. fructicosa* crude extract treatment in alloxan induced diabetic rats had decreased blood glucose level on 7th, 14th and 21st day of[35] and administration of curcumin for 2 months decreased the blood glucose level to 110.46±5.589 mg/dl and post prandial glucose level to 160.982±6.784 mg/dl as compared with diabetic group[36].

From the results obtained, SNP extracts was effective after glibenclamide in decreasing the blood glucose. At the end of the experimental period, the values did not reach the normal value but prolong intake of extracts may bring down the values to normal[37] reported the similar lipid lowering effects of *Costus pictus* extracts to be more effective against oxidative stress, blood glucose and serum markers.

Triacylglycerol and cholesterol are the principal lipids carried by lipoproteins. Decreased level of triacylglycerol and cholesterol in liver will leads to decreased synthesis of lipoproteins[38]. Hypercholesteremia and hypertriglycerimedia are occured in diabetic rats. The elevated level of cholesterol will develop atherosclerosis[39]. The blood glucose reduction in alloxan induced diabetic rats was similar to the study carried out by[40] in which the *Phyllanthus niruri* methanolic extract of aerial parts decreased the blood glucose level in alloxan induced diabetic rats

The results of this study was corroborated with another study where *D. indica* methanolic extract increased protein level in diabetic rats gradually[9]. The balance between protein levels in tissue depend on synthesis and catabolism from the body. The estimation of protein acts as a clinical marker in diabetic nephropathy. The decrease in protein synthesis in all the tissue is due to deficiency of insulin in alloxan induced diabetic rats[41].

Hence, the administration of *C. anisata* extracts may enhance the protein synthesis by stabilizing the endoplasmic reticulum. Increase in insulin mediated amino acids uptake, enhancement of protein synthesis or inhibition of protein degradation results in increase in protein level[42].

The decrease in liver enzyme was noticed after the administration of different extracts of *C. anisata* as compared to diabetic rats. It implies the normal functioning and protective effect of *C. anisata* liver and

supports hepato protective nature of *C. anisata*. The liver enzyme activities were related to the diabetic complications such as retinopathy and neuropathy[43]. The rise in serum AST and ALP is due to the liver damage.

The results obtained are in line with those of[37] showed an inhibitory effect by different plant extracts on transaminase, when treated with *D. indica* methanolic extract and showed decrease in increased level of liver enzymes in a dose dependent manner.

The final step in gluconeogenesis and glycogenolysis was catalysed by Glucose-6-phosphatase, that hydrolyse glucose-6-phosphate and maintains the blood glucose homeostasis. The fructose-1, 6- bisphosphatase converts fructose-1, 6-bisphosphate to fructose-6-phosphate in Calvin and gluconeogenesis cycle.

The activity of glucose-6-phosphatase, fructose-1, 6-diphosphatase, pyruvate carboxylase and phosphoenol pyruvate carboxy kinase[44]. Increase in the above enzymes is due to increased synthesis that leads to increased glucose production in diabetes. The plant extracts treated animals regulate the cAMP or inhibiting gluconeogenesis by modulating and regulating the activities of these two enzymes[41].

The activity of glucose-6-phosphatase and fructose-1, 6-bisphosphatase in gluconeogenesis process was decreased by insulin. *C. anisata* extracts reduces this two enzymes that may be due to increased insulin secretion which is responsible for the repression of the gluconeogenic enzymes which is already reported in[44] who evaluated the antidiabetic activity in bark extract of *T. arjuna* using alloxan induced diabetic rats.

It was observed that the *C. anisata* SNP root extract could increase the GSH and LPO activity in the liver tissue of diabetic rats. The *C. anisata* extracts will reduce the damage caused in pancreas and stimulate the secretion of insulin by beta cells[45]. LPO affects the cellular integrity, in which antioxidant could retain the detoxification machinery[46], Oxidative stress (LPO) occurs in cells and tissues at low level. The generation of free radicals will act on lipids causing lipid peroxidation. Enzymatic and non-enzymatic antioxidants will reduce the free radical concentration in cells and prevent excessive oxidative stress[47].

The free fatty acids are catabolize into acetyl coA in liver and the excess acetyl coA is converted to cholesterol, triglycerides and ketone bodies resulting in ketosis. The abnormal concentration of serum lipoprotein in the diabetic control rats is due to the increase mobilization of free fatty acids from peripheral fat depots by glucagons. The alloxan-induced diabetes increases fatty acids synthesis in plasma that promote the liver to convert some fatty acids into triglycerol, phospholipids and cholesterol which may be discharged into the blood as lipoproteins[38].

On 30 days administration of *C. anisata* extracts to diabetic induced rats significantly reversed these values to near normal in all the parameters studied. That shows the ability *C. anisata* in insulin secretion which decreases the total cholesterol, total triglycerides and increase in HDL level. The result was in line with[40] in which the *Phyllanthus niruri* methanolic extract in normal and alloxan induced diabetic rats reduced the total cholesterol and triglyceride level in dose dependent manner. The dietary or drug therapy reduction of total cholesterol and triglyceride level seems to be beneficial in preventing diabetic complications and also it improve lipid metabolism.

The result obtained in insulin assay, was in accordance with[48] where the ethanolic bark extract of *F.* showed increase in blood glucose level and significant decrease in plasma insulin level in diabetic animals as compared to control. After treatment with extract the blood glucose and plasma insulin level was found to be normal in diabetic rats.

The possible mechanism of *C. anisata* for its hypoglycemic action is due to insulin secretion from pancreatic beta cells and increased transport of glucose to peripheral tissue. The methanolic extract of *Vinca rosea* (500 mg/kg bw) was effective in

regeneration of beta cell after 14 days of treatment[49]. This revealed the restoration of size of islet along with beta cell repair by *Vinca rosea* extract.

The *C. anisata* SNP root extracts reduced the serum triglycerides, which was high in alloxan diabetic control. The extract also found to have beneficial effect on glucokinase activity and plasma insulin. Based on the overwhelming evidences, it was clear that hyperglycemia is coupled with hyperlipidemia which leads to cardiovascular diseases. These findings strongly suggests that phytoconstituent of plant origin have antidiabetic effects.

CONCLUSION

Based on the above results, among all extracts employed the SNP synthesized using the root extract of *C. anisata* showed maximum potent of antioxidant and hypoglycemic activities under *in vivo* conditions that may be due to the active phytoconstituents which was responsilble for the activities.

The SNP root extract, has effective hypoglycemic activity in alloxan induced diabetic rats, by lowering blood glucose level, triglycerides, cholesterol, AST, ALT, ALP, fructose-1, 6-bisphosphatase, glucose-6-phosphatase, LPO and SOD. And increase in body weight, reduced glutathione, the activity of glucokinase in liver and pancreas, protein level, liver and pancreas weight was also found. The histopathological sections of pancreas showed a reduction in number of islets in diabetic rats when compared to normal group. The crude root extract and SNP root extract has restored the size of islets along with beta cell repair. The recovery of beta cell was found to be dose dependent. The phytoconstituents of *C. anisata* extracts may be acting synergistically with antioxidant properties along with hypoglycemic effects in exerting an overall antidiabetic action in this study, and that should be chemically analyzed and their chemical structure should be understood in order to develop an effective diabetic therapeutic agent in future.

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