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Research Article

EVALUATION OF ANTIDIABETIC ACTIVITY OF VARIOUS EXTRACTS OF *CASSIA AURICULATA* LINN. BARK ON STREPTOZOTOCIN- INDUCED DIABETIC WISTAR RATS

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ABSTRACT

The oral administration of various extracts (hexane, ethyl acetate, methanol and aqueous extracts) of *Cassia auriculata* bark were found to have potent antidiabetic activity that reduces blood sugar level in streptozotocin-(STZ) induced diabetic rats. In this study, the chronic (100 days) antihyperglycemic effect of the extracts at a dose of 250 mg/kg.b.wt of *C.auriculata* were investigated. Insulin was used as a reference drug at a dose of 3 I.U/kg.b.wt**.** Further, a significant elevation in the levels of fasting blood glucose, glycosylated heamoglobin (HbA1c), serum insulin, C-peptide and liver enzyme were observed. In the present investigation methanol extract was found to be more active, when compared with hexane, ethyl acetate and aqueous extracts. Histological observations made on the pancreatic tissue of control and experimental groups also revealed the beneficial effect of *C.auriculata* extracts.

Keywords: *Cassia Auriculata*, Streptozotocin

INTRODUCTION

Diabetes mellitus is a metabolic disease characterized by high blood glucose level resulting from defects in insulin secretion, insulin action or both[1]. It is a chronic disorder that affects the metabolism of carbohydrates, fats, proteins and electrolytes in the body, leading to severe complications which are classified into acute, sub-acute and chronic[2]. Acute complications include hypoglycemia, diabetic ketoacidosis, hyperosmolar and hyperglycaemic non-ketotic syndrome[3] while sub acute complications include thirst, polyuria, lack of energy, visual blurriness and weight loss[4]. Chronic hyperglycemia causes glycation of body proteins which in turn leads to complications that may affect the eyes, kidneys, nerves and arteries[5].The incidence of diabetes mellitus is on the rise to the world. Based on the World Health Organization (WHO) report, the number of diabetic patients is expected to increase from 171 million in year 2000 to 366 or more by the year 2030[6].

The management of diabetes involves both the non pharmacological and pharmacological approaches. The non pharmacological approach includes exercise, diet control and surgery, while the pharmacological approach includes the use of drugs such as insulin, and oral hypoglycemic agents. The present conventional drugs are not only costly but also associated with lots of adverse effects[7, 8]. Many herbal medicines have been recommended for the treatment of diabetes[9]. A variety of ingredients present in medicinal plants are thought to act on a variety of targets by various modes and mechanisms. They have the potential to impart therapeutic effect in complicated disorders like diabetes and its complications[10].

Though different types of oral hypoglycemic agents along with insulin are available for the treatment of diabetes mellitus, healers heavily relied upon medicinal plants and herbs to treat diabetes. Actually more than 12000 plants have been described to be experimentally or ethnopharmacologically used in the treatment of diabetes[11, 12]. Recently the medicinal values of various plants extracts have been studied by many scientists in the field of diabetic research[13, 14]. Various parts of herbs have been used for medicinal purpose including the treatment of diabetes mellitus. One such medicinal plant that is widely used to manage diabetes is *C.auriculata.*

C. auriculata is a shrub that is used as 'Avarai Panchaga Choornam' (mixture of five parts of the shrub i.e. roots, leaves, flowers, bark and unripe fruits) which establishes good control on sugar levels[15]. Pari and Latha 2002 showed the antihyperglycemic and hypolipidemic activity of aqueous extract of *C. auriculata* flowers in experimental diabetes using the rat model[16]. The antihyperglycemic effect of hydro-ethanolic and ethanolic extract of *C. auriculata* leaves were also reported in experimentally induced diabetic rats[17, 18]. In addition, *C. auriculata* has been shown to have antiviral, antispasmodic and antipyretic activity[19, 20].

However there is no previous report on the comparative study on different extracts of *C.auriculata* bark on Streptozotocin (STZ)-induced diabetic Wistar rats. Hence, the present study was aimed in assessing the anti-diabetic activity of various extracts of *C.auriculata* bark as a comparative biochemical and histological analysis on STZ-induced diabetic Wistar rats.

MATERIALS AND METHODS

Collection of plants and extraction

Bark of *C. auriculata* were obtained from local market and a voucher specimen of the plant has been deposited in the Department of Botany of our college for future reference. The bark was shade dried and powdered. The powder was defatted with petroleum ether (60-80°c) then extracted with hexane, ethyl acetate and methanol using soxhlet extractor. The extracts were dried under reduced pressure using a rotary vacuum evaporator. The percentage yield was 7% w/w and the extracts were kept in refrigerator for further use.

Experimental Animals

Male albino rats (Wistar strain, weighing 150–220 g) bred in the Animal Division of King's institute, Chennai were used in the present investigation.All the animals were kept and maintained under laboratory conditions of temperature (22+2 °C), humidity (45+5%) and 12 h day:12 h night cycle, and were allowed free access to food (standard pellet diet-(Sai Durga Feeds and Foods, Bangalore, India) and water *ad libitum*. The experimental protocol has been approved by the institutional animal ethics committee and by the regulatory body of the government (Reg. No 585/05/A/CPCSEA).

Induction of diabetes

Diabetes was induced by STZ (Sigma-Aldrich, St. Louis, USA). The animals were fasted overnight and diabetes was induced by a single intra peritoneal injection of a freshly prepared solution of STZ -60 mg/kg b.wt in 0.1 M citrate buffer (pH 4.5).Control rats were injected with citrate buffer alone[21]. On the third day of STZ-injection, the rats were fasted for 6 h and blood was taken by sinocular puncture. Rats with moderate diabetes having hyperglycemia (blood glucose of 250–400 mg/dl) were taken for the experiment. The blood glucose levels outside the specified range were excluded from the study. The rats were kept for 15 days to stabilize the diabetic condition[22].

In the experiment, a total of 42 rats (6 normal; 36 STZ-diabetic rats) were used. The rats were divided into seven groups comprising of six animals in each group as follows:

Group I: Normal control and were given only distilled water daily.

Group II: STZ- induced diabetic rats served as diabetic control and were given distilled water only.

Group III: STZ- induced diabetic rats treated orally with hexane extract of *C. auriculata* bark at the dose of 250 mg/kg.b.wt daily for 90 days, once a day.

Group IV: STZ- induced diabetic rats treated orally with ethyl acetate extract of *C. auriculata* bark at the dose of 250 mg/ kg b.wt daily for 90 days, once a day.

Group V: STZ- induced diabetic rats treated orally with methanol extract of *C. auriculata* bark at the dose of 250 mg/ kg b.wt daily for 90 days, once a day.

Group VI: STZ- induced diabetic rats treated orally with aqueous extract of *C. auriculata* bark at the dose of 250 mg/ kg b.wt daily for 90 days, once a day.

Group VII: STZ- induced diabetic rats given insulin at the dose of 3-IU / kg b.wt daily for 90 days, once a day.

After 90 days of extracts treatment, the animals were deprived of food overnight and sacrificed by decapitation. Blood was collected in two different tubes, i.e. one with anticoagulant, potassium oxalate and sodium fluoride for plasma, and another without anticoagulant for serum separation. The blood was then centrifuged at 3000 rpm for 20 min using refrigerated centrifuge at 4°C to separate the plasma and serum. Pancreas was immediately dissected, washed in ice cold saline, patted dry and weighed. The tissues were fixed in 10% formalin immediately after removal from the animal to avoid decomposition. Embedding in paraffin wax was carried out by removal of water using alcohol dehydration and infiltration of chloroform as a solvent for the wax.

Estimation of glucose and muscle and liver glycogen

Fasting plasma glucose was estimated using the method of Trinder[23]. Tissue glycogen was estimated in fed rats by the method of Plummer *et al*[24].

Estimation of glucose metabolizing enzymes

Hexokinase, glucose-6-phosphatase and fru.1, 6 bis phosphatase were assayed by standard protocols[25, 26, 27]. Glycogen Synthase and Glycogen Phosphorylase were estimated by the method of Leloir and Goldemberg; Cornblath *et al* respectively[28, 29].

Estimation of hemoglobin and glycosylated hemoglobin

Haemoglobin in the blood was estimated by the method of Drabkin and Austin[30]. Glycosylated hemoglobin was estimated using the diagnostic kit from Biosystems, Spain.

Determination of plasma insulin and C-peptide

Plasma insulin concentration was determined by radioimmunoassay kit (Diasorin, Saluggia, Italy). The kit included human insulin as standard and 125I-labelled human insulin antibody, which cross reacts similarly with rat insulin. C-peptide level was assayed by radioimmuno assay kit, Missouri, USA.

Histopathological studies

Light Microscopic studies (Paraffin Method- Humason[31])

The pancreas form the untreated and the experimental groups were blotted free of mucus, washed in physiological saline, cut into pieces of desired size and fixed in Bouin- Hollande fixative for 72 h. After fixation, the tissues were washed in 70% alcohol for 2 or 3 days to remove the excess picric acid and dehydrated in graded series of alcohol. The tissues were cleared using xylene. The cleared tissues were infiltrated with molten paraffin at 58 - 60°C through three changes (20 - 30 min) and finally embedded in paraffin. 3-5 µm thick sections of all the tissues were obtained using rotary microtome and stained in Ehrlich's hematoxylin with eosin as the counter stain. The slides were mounted using DPX mountant.

Statistical analysis

Statistical analysis was performed using SPSS software package Version 16.0. The values were analysed by one-way analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) [32]. All the results were expressed as mean ± S.D. for six rats in each group. $P < 0.05$ were considered significant.

RESULTS

Effect of *C. auriculata* crude extracts on plasma glucose level was measured in normal and experimental rats. STZ-treated diabetic rats showed significant increase in the levels of blood glucose when compared to normal rats. Oral administration of various extracts of *C.auriculata* 250 mg/kg b.w. showed gradual reduction on plasma glucose level and nearer to normal after 90 days treatment (table-1) in which methanol extract shows better reduction.

In the present study, a reduced level of body weight and elevated level of food and water intake were observed in STZ-induced diabetic rats. This condition was significantly reversed towards the normal after the oral administration of *C.auriculata* (bark) crude extracts for 90 days (tables- 2&3).

Table- 4 shows that the glycosylated hemoglobin level in diabetic control is 0.89± 0.07%, which is reduced to 0.45± 0.06% in diabetic *C.auriculata* methanol extract treated rats.

Table- 5 shows the levels of muscle glycogen and liver glycogen in normal and experimental animals. There was a decrease in the muscle glycogen and liver glycogen content of diabetic rats when compared to normal rats.

Table 1: Effect of oral administration of different extracts of *C. auriculata* on plasma glucose levels in normal and STZ-induced diabetic male albino Wistar rats for 90 days

Groups	Plasma glucose levels (mg/dl)				
	00th day	15th day	30th day	60thday	90thday
Normal	81.19 ± 6.6	83.69 ±6.7	87.07±6.2	86.35±6.6	85.81±6.6
Diabetic control	340.8 ± 25	381.68±20.47[a]	408.64±24[a]	421.84±21.3[a]	432.84±21.3[a]
Diabetic+hexane extract (250 mg/kg b.w.)	328.2 ±25	296.54±17.9[ab]	272.6±16.9[ab]	199.35±13.6[ab]	170.35±13.6[ab]
Diabetic+ethyl acetate extract (250mg/kg b.w.)	335.5±28.3	264.98±18.1[ab]	232.59±14.5[ab]	188.62 ±11.4[ab]	148.6±10.4[ab]
Diabetic+methanol extract (250 mg/kg b.w.)	342.63 ±28.3	248.24±18.1[ab]	198.64±10.5[ab]	182.9±10.4[ab]	106.9±9.4[b]
Diabetic+aqueous extract (250 mg/kg b.w.)	332.63 ±27.2	268.24±9.6[ab]	238.64±10.1[ab]	199.9 ±7.2[ab]	119.9±6.4 [b]
Diabetic+Insulin (3 IU/kg b.w.)	336.15 ±27.2	89.5±9.6 [b]	96 ±10.1[b]	93.65±7.2[b]	89.9±6.4[b]

Each value is mean± S.D. for six rats in each group, –: no significance.

a- $p < 0.05$ by comparison with normal rats. b -$p < 0.05$ by comparison with Streptozotocin diabetic rats

Table 2: Effect of oral administration of different extracts of *C. auriculata* on body weight in normal and STZ-induced diabetic male albino Wistar rats for 90 days

Groups	Before induction	After induction	After treatment
Normal	223.19±6.6	218.69 ±6.7	229.07±6.2
Diabetic control	220.8 ±6	171.68 ±10.47 [a]	148.64±10.1[a]
Diabetic+hexane extract (250 mg/kg b.w.)	224.2±7.3	156.54 ± 12.57[ab]	189.6±12.3[ab]
Diabetic+ethyl acetate extract (250mg/kg b.w.)	225.5±5	157.98±10.9[ab]	179.59±11.9[ab]
Diabetic+methanol extract (250 mg/kg b.w.)	212.63±8.3	169.24±12.1[ab]	178.64±14.5[ab]
Diabetic+aqueous extract (250 mg/kg b.w.)	232.63±8.3	158.24±11.1[ab]	181.64±10.5[ab]
Diabetic+Insulin (3 IU/kg b.w.)	230.15±10.2	141.5±12.6[ab]	228±12.1[ab]

Each value is mean± S.D. for six rats in each group, –: no significance.

a -$p < 0.05$ by comparison with normal rats. b -$p < 0.05$ by comparison with Streptozotocin diabetic rats

Table 3: Effect of oral administration of different extracts of *C. auriculata* on food and water intake in normal and STZ -induced diabetic male albino Wistar rats

Groups	Food intake (mg/day)	Water intake (ml/day)
Normal	52.19±4.6	238.69±6.7
Diabetic control	81.8±5[a]	331.68±10.4[a]
Diabetic+hexane extract (250 mg/kg b.w.)	70.2±4.3[ab]	276.54±12.57[ab]
Diabetic+ethyl acetate extract (250mg/kg b.w.)	76.5 ± 5[ab]	281.98±10.9[ab]
Diabetic+methanol extract (250 mg/kg b.w.)	52.63±3.3[b]	240.24±12.1[b]
Diabetic+aqueous extract (250 mg/kg b.w.)	67.63± 4.3[ab]	278.24±13.1[ab]
Diabetic+Insulin (3 IU/kg b.w.)	51.15±4.2[b]	231.5±12.6[b]

Each value is mean±S.D. for six rats in each group, –: no significance.

a -$p < 0.05$ by comparison with normal rats. b -$p < 0.05$ by comparison with Streptozotocin diabetic rats

Table 4: Effect of oral administration of different extracts of *C. auriculata* on haemoglobin and glycosylated hemoglobin in normal and STZ -induced diabetic male albino Wistar rats

Groups	Hemoglobin (mg/dl)	Glycosylated hemoglobin (% total Hb)
Normal	15.8±0.9	0.44±0.04
Diabetic control	6.9±0.5[a]	0.89±0.07[a]
Diabetic+hexane extract (250 mg/kg b.w.)	10.9±0.6[ab]	0.68±0.04[ab]
Diabetic+ethyl acetate extract (250mg/kg b.w.)	9.9± 0.9[ab]	0.63±0.04[ab]
Diabetic+methanol extract (250 mg/kg b.w.)	14.93±0.6 [b]	0.45± 0.06[b]
Diabetic+aqueous extract (250 mg/kg b.w.)	9.93 ±0.8 [ab]	0.74 ± 0.05 [ab]
Diabetic+Insulin (3 IU/kg b.w.)	15.75 ±0.7 [b]	0.43± 0.02 [b]

Each value is mean±S.D. for six rats in each group, –: no significance.

a -$p < 0.05$ by comparison with normal rats. b -$p < 0.05$ by comparison with Streptozotocin diabetic rats

Table 5: Effect of oral administration of *C. auriculata* extracts on muscle glycogen and liver glycogen, in normal and STZ-induced diabetic male albino Wistar rats for 90 days

Groups	Muscle glycogen (mg/g tissue)	Liver glycogen (mg/g tissue)
Normal	9.8±1.6	48.44±2.14
Diabetic control	3.9±1.1[a]	17.89±1.07 [a]
Diabetic+hexane extract (250 mg/kg b.w.)	6.9±1.3[ab]	29.58±2.13[ab]
Diabetic+ethyl acetate extract (250mg/kg b.w)	8.5±0.6[ab]	38.53±2.4[ab]
Diabetic+methanol extract (250 mg/kg b.w.)	9.63±0.89[b]	43.45±2.4 [b]
Diabetic+aqueous extract (250 mg/kg b.w.)	6.43±0.99[ab]	31.44±2.8[ab]
Diabetic+Insulin (3 IU/kg b.w.)	9.75±0.79 [b]	45.43±2.5[b]

Each value is mean±S.D. for six rats in each group, –: no significance.

a -$p < 0.05$ by comparison with normal rats. b -$p < 0.05$ by comparison with Streptozotocin diabetic rats

The activity of serum hexokinase was decreased while the activities of fru.1.6. bis phosphatase and glucose-6-phosphatase were increased in STZ-diabetic rats as compared to normal rats. Oral administration of *C.auriculata* (bark) crude extracts and Insulin for 90 days showed a significant effect ($p<0.05$) and improved all the activities of these enzymes to near normal in diabetic rats (table-6).

The activities of enzyme glycogen synthase was found to be decreased ($p<0.05$) whereas the enzyme glycogen phosphorylase activity was found to be significantly increased ($p<0.05$) in diabetic rats when compared to respective normal rats. Oral administration of *C.auriculata* (bark) crude extracts for 90 days to diabetic rats prevented the above changes in a significant manner when compared to diabetic rats (table-7).

The insulin and C-peptide levels of normal rats are 39.6± 2.8 µmol/ml and 263.07 ± 10.4 pM/ml, which is decreased to 8.2± 0.4µmol/ml and 45.8± 9.6 pM/ml (table-8). The insulin and C-peptide levels considerably increased after the treatment with the extracts of *C. auriculata* where methanol extract has given a better result (table-8).

Table 6: Effect of oral administration of *C. auriculata* extracts on glucose metabolizing enzymes, in normal and STZ-induced diabetic male albino Wistar rats for 90 days

Groups	Hexokinase (mg/dl)	Glu.6.Phosphatase (mg/dl)	Flu.1.6.bis Phosphatase
Normal	0.28±0.06	0.24±0.014	0.188±0.014
Diabetic control	0.09±0.001[a]	0.89±0.07[a]	0.69±0.07[a]
Diabetic+hexane extract (250 mg/kg b.w.)	0.19±0.03[ab]	0.18±0.014[ab]	0.12±0.009[ab]
Diabetic+ethyl acetate extract (250mg/kg b.w)	0.15±0.09[ab]	0.16±0.019[ab]	0.11±0.013[ab]
Diabetic+methanol extract (250 mg/kg b.w.)	0.23±0.09[b]	0.23±0.02[b]	0.18±0.011[b]
Diabetic+aqueous extract (250 mg/kg b.w.)	0.13±0.01[ab]	0.14±0.04[ab]	0.11±0.014[ab]
Diabetic+Insulin (3 IU/kg b.w.)	0.25±0.01[b]	0.23±0.09[b]	0.182±0.019[ab]

Each value is mean±S.D. for six rats in each group, –: no significance.

a -$p < 0.05$ by comparison with normal rats. b -$p < 0.05$ by comparison with Streptozotocin diabetic rats

Table 7: Effect of oral administration of *C. auriculata* extracts on glycogen metabolizing, in normal and STZ-induced diabetic male albino Wistar rats for 90 days

Groups	Glycogen synthase (µ mole of UDP released/ hr/mg protein)	Glycogen phophorylase (mg/g tissue)
Normal	849.8±76.6	648.44±52.14
Diabetic control	60 3.9±51.1[a]	817.89±71.07[a]
Diabetic+hexane extract (250 mg/kg b.w.)	716.9±61.3[ab]	529.58±42.13[ab]
Diabetic+ethyl acetate extract (250mg/kg b.w)	748.5±60.6[ab]	538.53±42.4[ab]
Diabetic+methanol extract (250 mg/kg b.w.)	829.63±70.89[b]	643.45±52.4[b]
Diabetic+aqueous extract (250 mg/kg b.w.)	776.43±60.99[ab]	531.44±42.8[ab]
Diabetic+Insulin (3 IU/kg b.w.)	839.75±60.79[b]	635.43±52.5[b]

Each value is mean±S.D. for six rats in each group, –: no significance.

a -$p < 0.05$ by comparison with normal rats. b -$p < 0.05$ by comparison with Streptozotocin diabetic rats

Table 8: Effect of oral administration of *C. auriculata* extracts on Plasma insulin, C-peptide in normal and STZ-induced diabetic male albino Wistar rats for 90 days

Groups	Plasma insulin (lmol/ml)	C-peptide (lmol/ml)
Normal	39.6 ± 2.8	263.07 ± 10.4
Diabetic control	8.2 ± 0.4[a]	45.8± 9.6[a]
Diabetic+hexane extract (250 mg/kg b.w.)	30.8± 2.04[ab]	246.8 ± 10.9[ab]
Diabetic+ethyl acetate extract (250mg/kg b.w)	31.2± 1.4[ab]	243.8± 9.6[ab]
Diabetic+methanol extract (250 mg/kg b.w.)	30.8± 2.04[b]	227.8± 10.9[b]
Diabetic+aqueous extract (250 mg/kg b.w.)	32.6±1.9[ab]	236.9± 7.4[ab]
Diabetic+Insulin(3 IU/kg b.w.)	9.1± 1.1[b]	48.08± 6.9[b]

Each value is mean±S.D. for six rats in each group, –: no significance.

a -$p < 0.05$ by comparison with normal rats. b -$p < 0.05$ by comparison with Streptozotocin diabetic rats

Hematoxylin and Eosin sections of Pancreas of untreated rats reveals that each of islets of langerhans is formed of numerous compactly arranged cells occurring as dense cords. Islets from the pancreas of the diabetic control rats possessed pyknotic nuclei, whereas some cells contain dark nuclei, and few cells at the periphery had round or ovoid nuclei. Hematoxylin and Eosin sections of Pancreas of the *C.auriculata* hexane, ethyl acetate and methanol treated diabetic rats show islet cells nearly similar to normal control. Insulin treated sections show islet cells similar to diabetic control.

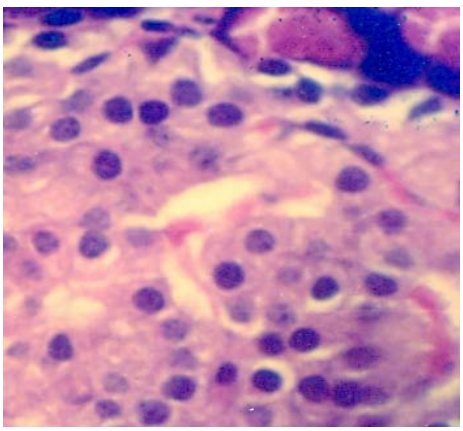

Fig. a) Hematoxylin and EosinSections of Untreated Rats

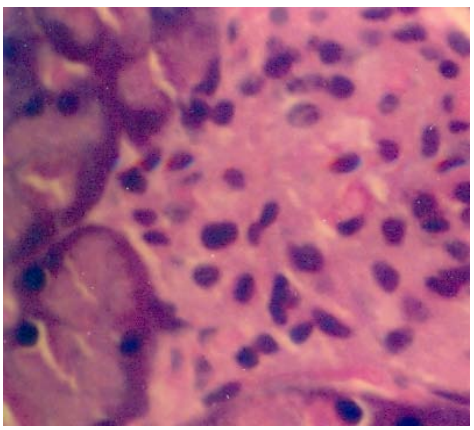

Fig. b) Hematoxylin and EosinSection of Diabetic Control Rats.

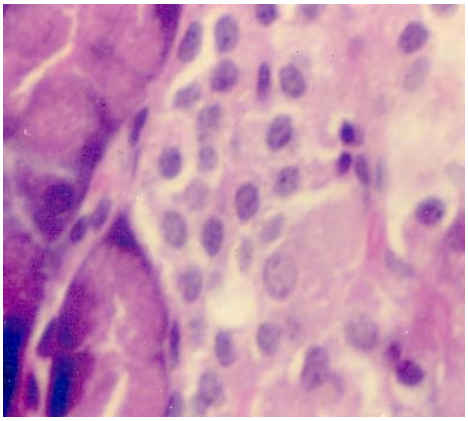

Fig. c) Hematoxylin and EosinSection of C. auriculata HexaneExtract Treated rats.

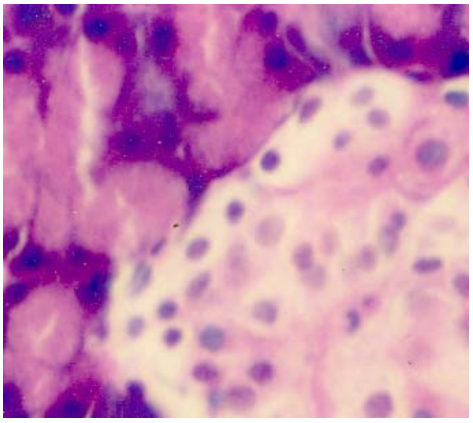

Fig. d) Hematoxylin and Eosin Section of C. auriculata Ethyl Acetate Treated Rats.

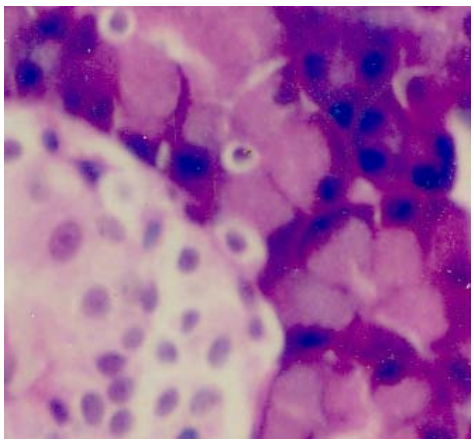

Fig. e) Hematoxylin and EosinSection of *C. auriculata* methanolExtract treated Rats.

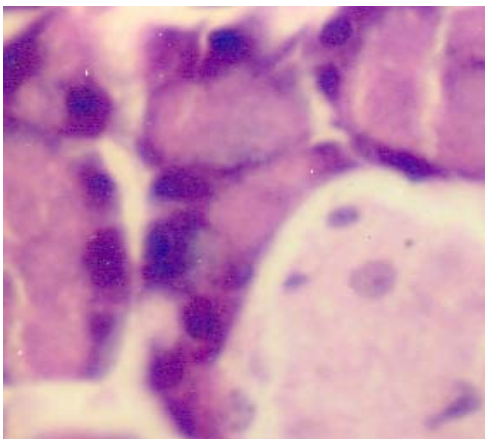

Fig. f) Hematoxylin and EosinSection of *C. auriculata* AqueousExtract Treated Rats.

DISCUSSION

STZ is a naturally occurring nitrosourea product of *Streptomyces achromogenes.* Usually, the intraperitonial injection of a single dose (60/mg/kg b. wt) of it exerts direct toxicity on β- cells resulting in necrosis within 48-72 h and causes a permanent hyperglycemia. STZ breaks nuclear DNA strand of the islet cells[33]. It interferes with cellular metabolic oxidative mechanisms[34].

In the present study STZ-induced diabetic rats was chosen as the animal model because it resembles many of the features of human diabetes mellitus[35]. The present study was conducted to assess the hypoglycemic effect of *C.auriculata* bark in STZ induced diabetic rats. In the present investigation, daily administration of hexane, ethyl acetate and methanol extracts of *C.auriculata* resulted in decrease in blood glucose levels in STZ-induced diabetic rats. Though all the three extracts proved to be effective, the methanol extract of *C.auriculata* had satisfactory capacity to restore back to near normal.

The decrease in body weight with diabetes mellitus has been attributed to the gluconeogenesis i.e., catabolism of proteins and fats, which is associated with the characteristic loss of body weight due to increased muscle wasting and loss of tissue proteins[36,37]. From the table-2 it is clear that diabetic rats treated with *C.auriculata* extracts showed an increase in body weight which proves that the *C.auriculata* extracts restored the muscle and tissue proteins.

Diabetes mellitus is known to impair the normal capacity of muscle and liver to synthesize glycogen[38]. The decrease of muscle and liver glycogen observed in this study may be due to lack of insulin in diabetic state or oxidative stress by diabetes may inactivate the glycogen synthetase. In the view of increased glycogen level in *C.auriculata* extracts treated animals, there may be three possible way of antidiabetogenic action, one possible way may be increased insulin level. Chakrabarti *et al.,* (2003) suggested that the restoration of the depleted glycogen in the liver and muscles might possibly be due to stimulation of insulin release from β–cells[39].

Liver is the site for glycolysis, a process where glucose is degraded and gluconeogenesis and these are the two important complementary events that balance the glucose load in our body[40]. Insulin increases hepatic glycolysis by increasing the activity and amount of several key enzymes including glucokinase. O'Doherty *et al*., (1999) said that glucokinase is an important regulator of glucose storage and disposal in liver[41]. It is the most sensitive indicator of the glycolytic pathway in the diabetic state[42].

In the present study, decreased activity of glucokinase was observed in STZ-induced diabetic rats. The enzymatic activity was increased with *C.auriculata* extracts treatment. These observations imply that entry of glucose into the cells is facilitated by *C.auriculata* extracts treatment, which in turn would stimulate the activity of this enzyme.

Fructose-1, 6-bisphosphatase and glucose-6-phosphatase are important regulatory enzymes in gluconeogenesis. Diabetic condition leads to an increase in the levels of these enzymes[43]. The increased activities of glucose 6-phosphatase and fructose-1, 6-bisphosphatase in liver of the STZ-induced diabetic rats may be due to insulin insufficiency. In the present study, a marked decrease in the activities of glucose-6-phosphatase and fructose-1, 6-bisphosphatase after the oral administration of *C.auriculata* extracts for 60 days indicates that gluconeogenesis is inhibited in extract-treated diabetic rats.

Leloir *et al.* (1959) suggested that glycogen synthetase is the key enzyme involved in the synthesis of glycogen from glucose in

skeletal muscle[44]. In resting muscle under aerobic conditions, glycogen phosphorylase is mainly responsible for glycogenolysis under most circumstances[45]. Reduction in glycogen synthase and a concomitant elevation in the glycogen phosphorylase activities during diabetic condition have been reported[46]. Our observations in the present study also go along with the above reports. Studies suggest that this fall in the enzyme level should be due to the low insulin in the diabetic state, which would result in the inactivation of glycogen synthetase system[47]. Lack of insulin may lead to glycogenolysis which results in the reduced liver glycogen content in diabetic rats. A significant increase in hepatic glycogen levels in STZ-diabetic rats after the treatment with *C.auriculata* extracts may possibly be due to the reactivation of glycogen synthase system as a result of increased insulin secretion from the regenerated β-cells.

The serum insulin decreases in diabetic animals[48, 49]. In the present study, serum insulin level of STZ-diabetic animals treated with extracts of *C.auriculata* extracts increased when compared to diabetic controls. In the present study, the drop in the circulating insulin and C-peptide levels in STZ-induced diabetic animals restored back to normal level after the treatment with *C.auriculata* extracts. Insulin is synthesized as a precursor molecule, proinsulin, in the β-cells of the pancreatic islets of Langerhans. Proinsulin is cleaved to give insulin, comprised of 2 polypeptide chains, A (21 amino acids) and B (30 amino acids), and C-peptide a single chain of 31 amino acids. When insulin is released in response to elevated glucose levels, equal amounts of C-peptide are also released. Since C-peptide level is increased in the reduced status in our present study it is very much clear that insulin secretion have also been occurred in the system. In this respect, we can come into a conclusion that the damaged β-cells are regenerated back by the treatment of *C.auriculata* extracts.

The increased level of insulin and C-peptide in *C.auriculata* extract treated diabetic rats may be due to the activation of remnant β-cells in the pancreas, which was in accordance with the observed histological observations(figure-1).It is interesting to note that the alterations in diabetic animals were reversed apparently near normal by *C.auriculata* extracts treatment. This trend in the pancreatic β-cells regeneration substantiates the increased in the insulin level of diabetic rats treated with *C.auriculata* extracts.

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