Academic Sciences Asian Journal of Pharmaceutical and Clinical Research

Vol 6, Issue 2, 2013 ISSN - 0974-2441

Research Article

HYPOLIPIDEMIC AND HEPATOPROTECTIVE EFFECTS OF *CASSIA AURICULATA* LINN BARK EXTRACTS ON STREPTOZOTOCIN INDUCED DIABETICS IN MALE WISTER ALBINO RATS

P. DAISY*AND FERIL G. JEEVA KANI

PG and Research Department of Biotechnology and Bioinformatics,Holy Cross College, Tiruchirappalli. 620002.
Email: daisylesslie@gmail.com

Received: 6 December 2012, Revised and Accepted: 13 January 2013

ABSTRACT

Diabetes mellitus (DM) is associated with change in lipid profile and impairment in the function of liver and kidney. Herbal remedies for DM always convalesce the above mentioned complications of DM. *Cassia auriculata*, is one such herbal plant which could recover DM.To assess hypolipidemic and hepatoprotective activity, Streptozotocin (STZ) induced-diabetic rats were treated with extracts of *Cassia auriculata* at the doses of 250 mg/kg, b.wt. and their influence on serum total cholesterol, triglycerides, HDL, LDL and VLDL, plasma enzymes (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and acid phosphatase) , total protein, urea, uric acid and creatinine were observed. Histological observation of both liver and kidney sections also studied. Oral administration of various extracts of *Cassia auriculata* showed significant elevation ($p<0.05$) in lipid profiles, plasma enzymes and kidney function test whereas, methanol treated was found to be more active when compared with hexane, ethyl acetate and aqueous extracts. Histological observations made on the liver and kidney tissue of control and experimental groups revealed that *Cassia auriculata* bark extracts has the effect of hypolipidemic, hepatoprotective and consequently may alleviate renal damage associated with STZ-induced diabetes in rats. It is evident from the study that extracts of *Cassia auriculata* improve liver and renal damages and bring about the hypolipidemic effect in Streptozotocin induced diabetic rats.

Keywords: Melatonin, Tulsi leaf extract, piroxicam, gastric ulceration, co-therapy.

INTRODUCTION

Diabetes mellitus is a disease in which homeostasis of carbohydrate, protein and lipid metabolism is improperly regulated by hormone insulin resulting in elevation of fasting and postprandial blood glucose levels[1]. The major chronic complications associated with diabetes include retinopathy, neuropathy, nephropathy, and atherosclerotic coronary artery disease and peripheral atherosclerotic vascular disease[2]. The number of people suffering from the disease worldwide is increasing at an alarming rate with a projected 366 million people likely to be diabetic by the year 2030 as against 191 million estimated in [3].

Although numerous oral hypoglycemic drugs exist alongside insulin, still there is no promising therapy to cure diabetes [4]. Over the last few decades the reputation of herbal remedies has increased globally due to its therapeutic efficacy and safety. Herbal drugs are widely prescribed today despite the fact that their biologically active compounds are unknown, due to its minimal adverse effects and low costs [5].Traditional herbal medicines have a long history of use and are generally considered to be safer than synthetic drugs. Traditional medicine inspired approaches remain important especially for the management of chronic diseases as well as to facilitate natural product drug discovery [6, 7].

The potential role of the medicinal plants as antidiabetic agents has been reviewed by several authors, supported by the ethno botanical surveys and traditional medicines of different cultures[8]. 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 *Cassia auriculata.*

Cassia auriculata (C.auriculata) Linn (Family: Caesalpiniaceae) commonly known as *Tanners Senna*, is distributed throughout hot deciduous forests of India and holds a very prestigious position in Ayurveda and Siddha systems of medicine. The plant is used in the traditional system of medicine for urinary disorders, female antifertility, leprosy, worm infestation, diarrhoea, disease of pittam; bark is used in skin conditions; bark as astringent; leaves, flowers and fruits as antihelmintic; seeds for eye troubles, diabetes[9,10,11].

We have already established the anti-diabetic activity of *C.aurriculata* extracts on STZ-induced diabetic male albino Wistar rats [12]. In the present study we aimed to investigate the effect of various extracts (Hexane, Ethyl acetate, Methanol and aqueous) of *C. auriculata bark* on STZ-induced diabetics in male albino Wistar rats.

MATERIALS AND METHODS

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 Streptozotocin (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 [13]. 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 [14].

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.

Determination of Kidney function test

Protein and urea were estimated by Lowry et al., [15] using bovine serum albumin as a standard and Fawcett and Scott[16] respectively. Uric acid in the serum was estimated using the diagnostic kit based on the enzymic method described by Caraway [17], and creatinine was estimated using a Diagnostic kit Dr. Reddys' Laboratories, Bachupally, Hyderabad, India.

Measurement of lipid profile

Serum total cholesterol, triglycerides serum HDL-cholesterol, and LDL-cholesterol were determined using Diagnostic kit, Beacon Diagnostics, Kbilpore, Navsari, India.

Determination of plasma enzymes

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities were assayed by the method of Reitman and Frankel [18]. Alkaline phosphatase (ALP) activity was measured at 405 nm by the formation of paranitrophenol from paranitrophenylphosphate as a substrate Kind and King [19]. Acid phosphatase (ACP) activity was measured using the method of Moss [20].

Histological studies

Light microscopic studies [Paraffin Method (Humason)] [21]

The organs from 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 58–60^0 C through three changes (20–30 min), and finally embedded in paraffin. 3–5-lm-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 17.0. The values were analyzed by oneway analysis of variance (ANOVA) followed by Duncan's multiple range test (DMRT) [22]. All the results were expressed as mean ± S.D. for six rats in each group. P Values\0.05 were considered significant.

RESULTS

Table 1 shows the effect of extracts of *C.auriculata* on the protein, urea, uric acid, and creatinine in normal and diabetic rats. In diabetic rats, there was a significant decrease in the protein level and an increase in urea, uric acid, and creatinine levels when compared with normal rats. When *C. auriculata* crude extracts was administered to diabetic rats for 90 days, the total protein content increased significantly and the urea, uric acid, and creatinine contents decreased respectively in which methanol extract shows better modification.

There was a significant decrease in the level of serum HDL-cholesterol and a significant increase in the levels of total cholesterol, triglycerides, and LDL-cholesterol in diabetic rats when compared with normal rats. Administration of extracts of *C.auriculata* for 90 days brought back the levels of serum lipids to near normal (Table 2).

The activities of plasma enzymes AST, ALT, ALP, and ACP significantly increased in diabetic rats when compared with normal controls. Oral administration of extracts of *C.auriculata* for 90 days significantly restored the enzyme levels to near normal (Table 3).

Table: 1. Effect of oral administration of different extracts of *Cassia auriculata* bark on serum urea, uric acid, creatinine and total protien in normal and Streptozotocin-induced diabetic male Albino Wistar rats for 90 days

Groups	Serum creatinine (mg/dl)	Serum Uric acid (mg/dl)	Serum urea (mg/dl)	Total protein (g/dl)
Normal	1.6 ± 0.09	0.99± 0.14	22.6 ± 2.1	8.99±0.6
Diabetic control	2.49± 0.09[a]	2.28± 0.14[a]	37.49± 2.2[a]	3.28±0.6[a]
Diabetic + hexane extract (250 mg/kg b.w.)	1.91±0.09[ab]	1.22± 0.14[ab]	28.71± 2.14[ab]	6.52±0.7[ab]
Diabetic+ethyl acetate extract(250mg/kg b.w)	1 .94±0.09[ab]	1.11±0.14[ab]	25.64±2.18[ab]	7.91 ± 0.71[ab]
Diabetic+methanol extract(250 mg/kg b.w.)	1 .91±0.09[ab]	1.18±0.14[ab]	28.61± 2.12[ab]	7.08±0.69[ab]
Diabetic+aqeous extract(250 mg/kg b.w.)	1 .91± 0.09[ab]	1.69±0.14[ab]	27.71± 2.19[ab]	6.99±0.64[ab]
Diabetic+Insulin(3 IU/kg b.w.)	1.91±0.09[ab]	1.64±0.14[ab]	26.61 ±2.17[ab]	7.04±0.69[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: 2. Effect of oral administration of different extracts of *Cassia auriculata* bark on Lipid profile in normal and Streptozotocin-induced diabetic male Albino Wistar rats for 90 days

Groups	Total cholesterol (mg/dl)	Triglycerides (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)
Normal	89.6 ±7.1	18.99±1.14	59.6±7.1	83.45±8.14	12.99±1.14
Diabetic control	271.49± 17.2[a]	53.58±4.16 [a]	21.49±2.2[a]	153.28±9.16[a]	53.28±4.16[a]
Diabetic+hexane extract(250 mg/kg b.w.)	178.71±12.14[ab]	43.52± 1.19[ab]	35.71±4.14[ab]	124.52±7.19[ab]	41.52±1.19[ab]
Diabetic+ethyl acetate extract(250mg/kg b.w)	155.64± 10.18[ab]	28.11± 1.16[ab]	38.64±5.18[ab]	118.11±7.16[ab]	30.11±1.16[ab]
Diabetic+methanol extract(250 mg/kg b.w.)	165.61±7.12[ab]	27.58±1.19[ab]	34.61±5.12[ab]	105.58±6.19[ab]	35.58±1.19[ab]
Diabetic+aqeous extract(250 mg/kg b.w.)	113.71±10.19[ab]	34.29±1.14[ab]	49.71± 5.19[ab]	111.29±7.14[ab]	27.29±1.14[ab]
Diabetic+Insulin (3 IU/kg b.w.)	95.61± 8.17 [ab]	32.64±1.12[ab]	55.61±6.17[b]	99.64±6.12[ab]	19.64±1.12[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 *Cassia auriculata* bark on AST, ALT,ALP and ACP in normal and Streptozotocin-induced diabetic male Albino Wistar rats for 90 days

Groups	AST (µg/dl)	ALT (µg/dl)	ALP (µg/dl)	ACP (µg/dl)
Normal	39.6 ±3.1	56.45±3.14	49.99±3.14	9.99±1.14
Diabetic control	72.49±3.2[a]	94.28±6.16[a]	88.28±6.16[a]	23.28±2.16[a]
Diabetic+hexane extract(250 mg/kg b.w.)	55.71 ± 3.14[ab]	72.52±3.9[ab]	65.52±4.19[ab]	18.52±1.19[ab]
Diabetic+ethyl acetate extract(250mg/kg b.w)	58.64 ± 3.18[ab]	61.11±5.16[ab]	54.11±4.16[ab]	14.11± 1.16[ab]
Diabetic+methanol extract(250 mg/kg b.w.)	52.61 ±2.12[ab]	68.58±4.19[ab]	55.58±4.19[ab]	15.58±1.19[ab]
Diabetic+aqeous extract(250 mg/kg b.w.)	46.71± 2.19[ab]	61.29 ± 4.14[ab]	63.29±4.14[ab]	14.29 ±1.14[ab]
Diabetic+Insulin(3 IU/kg b.w.)	47.61± 2.17[ab]	56.64±4.12[b]	50.64±3.12[b]	10.64±1.12[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 histological study performed on the kidneys of diabetic rats showed damage to the glomerulus, thickened basement membrane and edematous proximal convoluted tubule with increase in mucopolysaccharide deposits which were found to be absent in the diabetic kidneys treated with *C.auriculata* extracts. The treated diabetic rats however showed healing features, which resembled that of a normal kidney. (Figure.1)

Histopathologyical sections of liver of STZ-induced diabetic rats showed hepatocellular injury pronounced in loss of the normal architecture of the liver, cord-like arrangement of the normal hepatocytes were not well distinct, dilatation and inflammation in central and portal vein and also severe fibrosis and leucocytic infiltration around the portal veins. In contrast, liver section of rats treated with *C.auriculata* extracts showed improved hepatocellular architecture with signs of recovery, indicating the hepatoprotective effect. (Figure. 2)

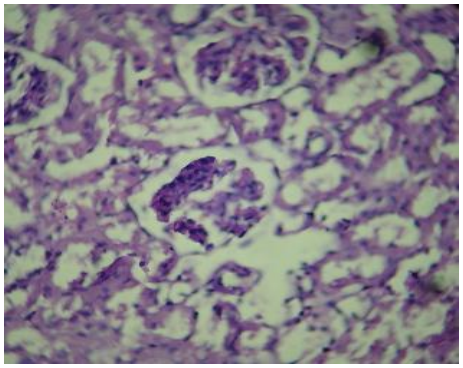

Fig. 1. a) Hematoxylin & EosinSections of Kidneys in Untreated Rats.

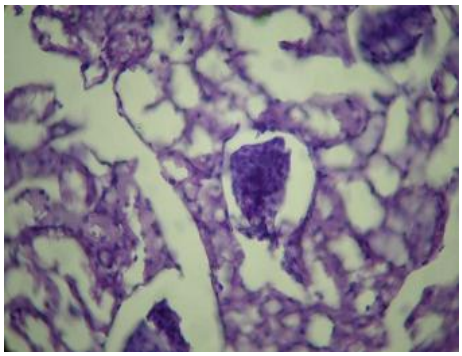

Fig. b) Hematoxylin & EosinSections of Kidney in Diabetic Controlled Rats

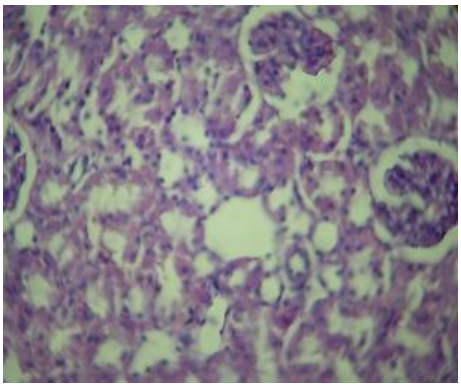

Fig. c. Hematoxylin & EosinSections of Kidney in c.auriculata methanol extract treated Rats.

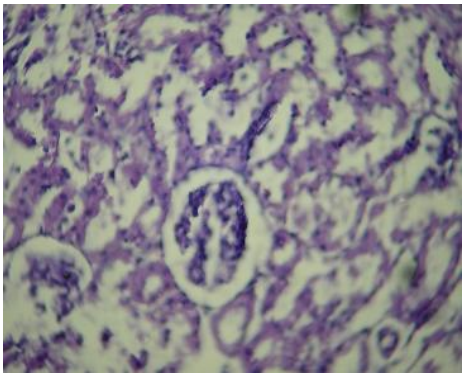

Fig.d) Hematoxylin & EosinSections of Kidney in Insulin Treated Rats.

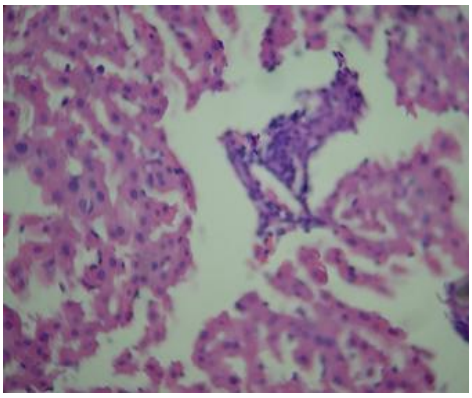

Fig. 2. a) Hematoxylin & EosinSections of Liver in Untreated Rats.

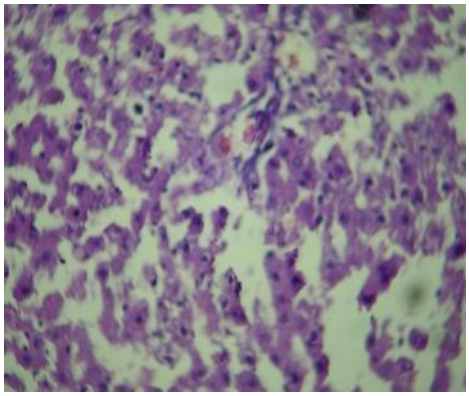

Fig.b) Hematoxylin & EosinSections of Liver in Diabetic Controlled Rats.

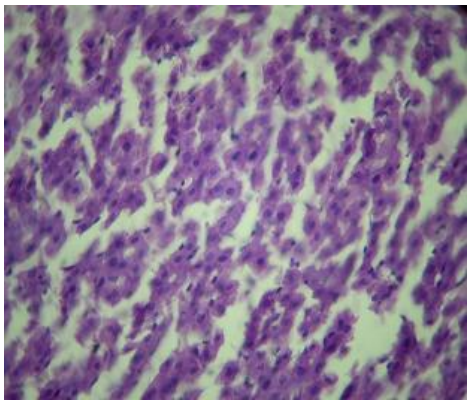

Fig.c) Hematoxylin & EosinSections of Liver in c. auriculata Methanol Extract Treated Rats

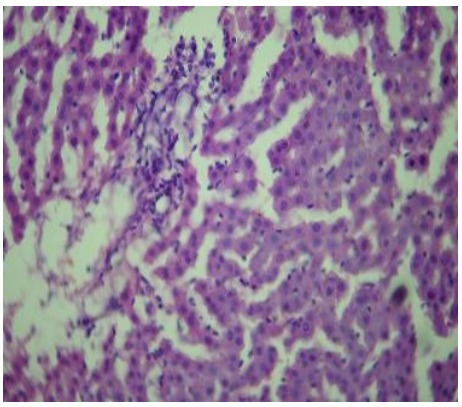

Fig.d) Hematoxylin & EosinSections of Liver in Insulin Treated Rats.

DISCUSSION

Diabetes mellitus is a group of metabolic disorders characterized by hyperglycemia and 1.3 % of the population suffers from this disease throughout the world [23]. These metabolic disorders include alteration in the carbohydrate, fat and protein metabolism associated with absolute or relative deficiencies in insulin secretions and/or insulin action. Hyperglycemia in the diabetes is associated with alteration of glucose and lipid metabolism and modification in liver enzyme level [24]. Streptozotocin-induced hyperglycemia has been described as a useful experimental model to study the activity of antidiabetic agents[25]. Streptozotocin selectively destroyed the pancreatic insulin secreting β cells, leaving less active cell resulting in a diabetic state [26].

The 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 [12]. Though all the three extracts proved to be effective, the methanol extract of *C.auriculata* had satisfactory capacity to restore back to near normal.

Lipids play an important role in the pathogenesis of diabetes mellitus. The level of serum lipids is usually raised in diabetes and such an elevation represents a risk factor for coronary heart disease [27]. Lowering of serum lipids levels through dietary or drugs therapy seems to be associated with a decrease in the risk of vascular disease [28]. The abnormal high concentration of serum lipids in diabetes is

mainly due to the increase in the mobilization of free fatty acids from the peripheral depots, since insulin inhibits the hormone sensitive lipase. In the present study, Streptozotocin induced diabetic rats had an elevation in the serum lipids. Oral administration of extracts of *C.auriculata* bark significantly decreased the serum cholesterol, triglycerides, LDL and VLDL and increased the HDL-cholesterol. The elevated hypertriglyceridemia was increased in the synthesis of triglyceride rich lipoprotein particles (very low density lipoprotein, VLDL) in liver diminished catabolism in diabetic rats [29]. Since insulin has a potent inhibitory effect on lipolysis in adipocytes, insulin deficiency is associated with excess lipolysis and increased influx of free fatty acids to the liver [30,31]. The increased levels of low-density lipoprotein (LDL) and very low density lipoprotein (VLDL) in the diabetic animals might be due to over production of LDL and VLDL by the liver due to the stimulation of hepatic triglyceride synthesis as a result of free fatty acid influx [30]. The high density lipoprotein (HDL) was significantly reduced in the diabetic rats which indicate a positive risk factor for atherosclerosis [32].

The diabetic hyperglycemia induces the elevation of plasma levels of urea, uric acid and creatinine, which are considered as the significant markers of renal dysfunction [33]. The results in Table 1 show significant ($p<0.05$) increase in the level of plasma urea, uric acid and creatinine in the diabetic rats when compared with respective control rats, while after the treatment of STZ diabetic rats with the bark extract of *Cassia auriculata* (250 mg/kg), the levels of urea, uric acid and creatinine were significantly ($p<0.05$) decreased. STZ-induced diabetes in rats had been shown to be associated with functional and/or morphological changes in the kidney[34]. All structural changes in kidneys resulting from STZ administration in rats can thus be attributed to altered metabolism in diabetes[35]. The improvement of renal morphology and function associated with STZ-induced diabetes and *C.auriculata* bark extract treatment in the present investigation could be attributed to its antidiabetic action resulting in alleviation of altered metabolic status in animals. However, the excellent recovery of renal function expected with treatment of *C.auriculata* bark extract can be explained by the regenerative capability of the renal tubules[36].

Liver enzymes such as AST, ALT, ACP, and ALP are marker enzymes for liver function and integrity[37]. These enzymes are usually elevated in acute hepatotoxicity or mild hepato-cellular injury, but tend to decrease with prolonged intoxication due to liver damage (Cornelius, 1979) 38. AST and ALT were used as markers to assess the extent of liver damage in Streptozotocin induced diabetic rats [39]. In this study, the administration of methanol extract to Streptozotocin induced diabetic rats reduces AST and ALT levels ($p<0.05$) efficiently than other extract treated rats. In addition to the assessment of AST and ALT levels during diabetes, the measurement of enzymatic activities of phosphatases such as acid phosphatase (ACP) and alkaline phosphatase (ALP) is of clinical and toxicological importance as changes in their activities are indicative of tissue damage by toxicants [40]. In the present study, serum ACP and ALP increased considerably in Streptozotocin- induced diabetic rats. Elevated level of these enzymes in diabetes may be due to extensive damage to liver in the experimental animals by streptozotocin. Administration of methanol extract of *C. auriculata* bark in STZ-induced diabetic rats produces a more significant ($p<0.05$) levels than the hexane, ethyl acetate and aqueous extract treated rats. Return of the above enzymes to normal serum values following *C.auriculata* extracts treatment may be due to prevention of intracellular enzyme leakage resulting from cell membrane stability or cellular regeneration [41]. In addition, it did not show any toxic effect and found to improve the liver function and thus the obtained results suggest that the extracts of *C.auriculata* possess hepatoprotective capacity.

CONCLUSION

From the above results it can be concluded that the extracts of *C.auriculata* improve the liver and renal damages and bring about the hypolipidemic effect in Streptozotocin induced diabetic rats. Further, isolation of compound and the molecular analysis about these effects are required to understand the mechanism.

REFERENCE

1. Tiwari AK, Rao JM. Diabetes mellitus and multiple therapeutic approaches of phytochemicals; Present status and future prospects. Curr Sci. 2002; 83: 30-8.
2. Kaczmar T. Herbal support for diabetes management. Clin Nutr Insights. 1998; 6: 1-4.
3. Wild S, Roglic A, Green, R, King H. Global prevalence of diabetes. Estimated for the year 2000 and projection for 2030. Diab. Care. 2004; 27: 1047–1054.
4. Sumana G, Suryawanshi SA. Effect of *Vinca rosea* extracts in treatment of alloxan diabetes in male albino rats. Ind J Exp Biol. 2001; 39: 748 – 758.
5. Valiathan MS. (1998). Healing plants. Curr Sci. 1998;75: 1122– 1126.
6. Patwardhan B, Mashelkar RA. Traditional medicine inspired approaches to drug discovery: can Ayurveda show the way forward? Drug Discov Today. 2009; 14: 804–11.
7. Patwardhan B, Vaidya ADB. Chorghade M. Ayurveda and natural products drug discovery. Curr Sci. 2004;86: 789–99.
8. Biesalski HK. Diabetes preventive components in the Mediterranean diet. European Journal of Nutrition, 2004; 43: 26-30.
9. Bates S H, Jones R B, Bailey C J. Brit J Pharm,2000; 130: 1944-1948.
10. Balasaraswati R, Sadasivam S, Ward M, Walker JM. An antiviral protein from Bougainvillea spectabilis roots; purification and characterisation. Phytochemistry, 1998; 47: (8); 1561-1565.
11. 11.Bolognesi A, Polito L, Olivieri F, Valbonesi P, Barbieri L, Battell M G, Carusi M V, Benvenuto E, Del Vecchio Blanc F, Di Maro A, Parente A, Di Loreto M, Stripe F. New ribosome-inactivating proteins with polynucleotide: adenosine glycosidase and antiviral activities from Basella rubra L. and bougainvillea spectabilis Willd. Planta, 1997; 203 (4), 422-429.
12. Daisy P, Feril G. Jeeva kani. Evaluation of antidiabetic activity of various extracts of Cassia auriculata Linn. Bark on Streptozotocin induced diabetic wistar rats. International Journal of Pharmacy and Pharmaceutical Sciences, 2012; 4: 312-318.
13. Akbarzadeh A, Norouzian D, Mehrabi MR, Jamshidi Sh, Farhangi A, Allah Verdi A, Mofidian SMA, Lame Rad B. Induction of diabetes by streptozotocin in rats. Ind J Clin Biochem, 2007; 22(2), 60–64.
14. Jyoti M, Vihas TV, Ravikumar A, Sarita G. Glucose lowering effect of aqueous extract of Enicostemma Littorale Blume in diabetes: a possible mechanism of action. J. Ethnopharmacol, 2002; 81: 317- 320.
15. Lowry OH, Rosebrough NJ Farr AL, Randall, R. Protein Measurement with the Folin Phenol reagement. J Biol Chem, 1951;193: 265–275.
16. Fawcett JK, Scott JE. A rapid and precise method for determination of urea. J Clin Pathol,1960; 13:156–159.
17. Caraway WT. Determination of uric acid in serum by carbonate method. Am J Clin Pathol,1955; 25:(7),840–845.
18. Reitman S Frankel SA. Colorimetric method for the determination of serum glutamate oxaloacetic and glutamate pyruvic transaminases. Am J Clin Pathol,1957;28:(1), 56–63.
19. Kind PRN, King EJ. Estimation of plasma phosphatases by determination of hydrolyzed phenol with amino-antipyrine. J Clin Pathol,1954; 7:322–326
20. Moss DW. (1984). Methods of enzymatic analysis. (3rd edn.). Verlag-chemic: Berlin, (pp 92-106). In Bergmeyer HU (ed) vol 4.
21. Humason, G.L. Animal tissue techniques. WH Freeman and Company, San Francisco.1979.
22. Duncan BD. Multiple range tests for correlated and heteroscedastic means. Biometrics, 1957; 13:359–364

23. Raghunathan M, Raghunathan N. Diabetes Mellitus and Vitamin D. Nutrition News, 1992;13: 4-6.
24. Jenson T, Stender, Deckert T. Abnormalities in plasma concentration of lipoprotein and fibrogen in type 1 (insulin dependent) diabetic patients with increased urinary albumin excretion. Diabetolgy,1998; 31:142- 146.
25. Szkudelski T. The mechanism of Alloxan and streptozotocin action in beta cells of the rats pancreas. Physiological Research, 2001; 50: 536-546.
26. Kamchouing P, Sokeng, DS, Moundipa FP, Watcho P, Jatsa BH, Lontsi D. Protective role of *Anacardium occidentale* extract against streptozocin- induced in rats. Journal of Ethnopharmacology, 1998;62: 55-99.
27. Al-Shamaony L, Al-khazrajoi SM, Twaij HAA. J Ethnopharmacol,1994; 43: 167–171.
28. Rhoads GG, Gulbranden, C.L., & Kagan, A. (1976). N Engl J Med, 294, 293–298.
29. Ginsberg HN. Lipoprotein physiology in nondiabetic and diabetic states.Relationship to atherogenesis. Diabetes care,1991; 14: 839- 855.
30. Coppack SW, Jenson MD, Miles JM. In vivo regulation of lipolysis in human. J. lipid Res, 1994;35:177-193.
31. . Ohno T, Horio F, Tanaka S, Terada M, Namikawa T, kitoh J .Fatty liver and hyperlipidemia in IDDM of Streptozotocin treated shrews. Life Sci, 2000; 66:125-131.
32. Bopanna KN, Kannan J, Sushma gangil, Blaraman R, Rathod SP. Antidiabetic and antihyperlipedemic effect of Neem, Lipedemic effect of Neem seed kernel powder on alloxon diabetic rabbits. Ind. J. Pharmacology,1997; 29: 162-167.
33. Almdal TP, Vilstrup H. Effect of streptozotocininduced diabetes and diet on nitrogen loss from organs and the capacity of urea synthesis in rats. Diabetologia,1987; 30: 952-956
34. Alderson NL, Chachich ME, Frizzell N, Canning P, Metz, TO, Januszewski AS. Effect of antioxidants and ACE inhibition on chemical modification of proteins and progression of nephropathy in streptozotocin diabetic rat. Diabetologia, 2004; 47: 1385-1395.
35. Rasch R. Prevention of diabetic glomerulopathy in streptozotocin diabetic rats by insulin treatment. Diabetologia, 1980; 16, 319-324.
36. Kissane JM. Anderson's pathology. 8th ed. (pp.754-9). Toronto: Washington University School of Medicine. 1985.
37. .Adaramoye OA, Osaimoje DO, Akinsanya AM, Nneji CM, Fafunso MA, Ademowo OG. Changes in antioxidant status and biochemical indices after acute administration of artemether, artemether–lumefan-trine and halofantrine in rats. J Compilation Basic Clin Pharmacol Toxicol,2008; 102: 412–8.
38. Cornelius CE. Biochemical evaluation of hepatic function in dogs. J Am Anim Hosp Assoc, 1979;15: 25–9.
39. Hye-Jin Hwang, Sang – Woo kim, Jong – Min lim, Ji - Hoon joo, Hyun – oh kim, Hyun – Mi kim, Jong – Wonyun. Hypoglycemic effect of crude epoxypolysaccharides produced by a medicinal mushroom *Phellinus baumii* in Streptozotocin induced diabetic rats. Life sci, 2005;76: 3069-3080.
40. Som Nath Singh, Praveen Vats, Shoba Suri, Radhey Shyam, Kumaria MML, Ranganathan, S, Sridharan K. Effect of an antidiabetic extract of *Catharanthus roseus* on enzymic activities in Streptozotocin induced diabetic rats. J Ethnopharmacol ,2001; 76: 269-277.
41. Thabrew M, Joice P.A comparative study of the efficacy of Pavetta indica and Osbeckia octandra in the treatment of liver dysfunction. Planta Medica, 1987; 53: (3),239–241.