Study of Antihyperglycemic, Antihyperlipidemic and Nephroprotective Activity of *Cassia Auriculata L.* Extract in Streptozotocin Induced Diabetic Rats.

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Abstract-

Present study was carried out to investigate antihyperglycemic, antihyperlipidemic and nephroprotective activity of hydroalcoholic extract of aerial parts of Cassia auriculata L. (HACA) in streptozotocin induced diabetes rats, to focus on its possible mode of action and identification of possible phytoconstituents responsible for the proposed activity.

Experimental diabetes was induced in wistar rats by single intraperitonial injection of streptozotocin (65 mg/kg). Animals were divided in six groups (n=6) and treated with variable doses of HACA for 4 weeks. At the end of 4 weeks, fasting blood glucose, oral glucose tolerance test (OGTT), blood urea nitrogen (BUN), serum creatinine, serum total proteins, serum albumin, lipid profile, glycosylated haemoglobin, was determined. Antioxidant enzymes of kidney were evaluated. Urine was analyzed for albumin, total proteins and creatinine clearance. Kidney of experimental animals was examined to determine structural changes. The results of our study demonstrate antihyperglycemic, antihyperlipidemic and nephroprotective potential of aerial parts of Cassia auriculata L. justifying its use in the indigenous system of medicine.

Keywords - *Streptozotocin, Cassia auriculata, Diabetes, Antihyperlipidemic, Antioxidant*

I. Introduction

Diabetes mellitus is a clinical syndrome characterized by hyperglycemia due to absolute or relative deficiency of insulin. Lack of insulin affects the metabolism of carbohydrate, protein and fat leading to significant disturbance of water and electrolyte homeostasis. [1,2]. Current research is focused on the development of newer drug leads from phytoconstituents of medicinal plants which have been used in traditional practices, so as to get more potential and effective agents with lesser side effects than existing hypoglycemic agents [3].

Cassia auriculata Linn. (Family:Caesalpiniaceae) is a tall, branched, bushy shrub growing wild throughout forest along roadside and in waterlands [4]. 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 and as astringent; leaves, flowers and fruits as anthelmintic; seeds for eye troubles and in diabetes [5]. Previous studies have proved that the chemical constituents such as flavonoids, bioflavonoids, alkaloids, tannins, saponins are promising agents for treatment of diabetes and its complications [6,7].

Different components of aerial parts of *Cassia auriculata L.* especially leaves [8], flowers [9] and stem [10] have been reported to possess antihyperglycemic activity which is attributed to presence of phytoconstituents such as alkaloids, anthraquinone glycosides, flavonoids, phenolic compounds, saponins, steroids and tannin [11]. However, till date no studies have so far been reported for diabetic complications. In the absence of any scientific evidence, we have attempted the present study for exploring nephroprotective potential of aerial parts of *Cassia auriculata L.* and to focus on its possible mode of action.

II. Materials and Methods

A. Preparation of hydroalcoholic extract

The aerial parts of *Cassia auriculata* Linn. were identified and authenticated by Botanical

Survey of India, Pune and a voucher specimen (V. No.CAAAAM 5) was deposited in the herbarium for future reference.Air dried aerial parts of *C. auriculata* were ground to coarse powder and 100 gm of powder was extracted in methanol: water (70:30) for 72 h at room temperature with intermittent shaking. The obtained hydroalcoholic extract was preserved in refrigerator till further use.

B. Experimental animals and chemicals

Wister rats of either sex (150-200 g) were procured from National Institute of Bioscience, Chaturshrungi, Pune. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Modern College of Pharmacy in accordance with the regulations of CPCSEA (884/ac/05/CPCSEA). Streptozotocin (STZ) was purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals used were of analytical grade.

C. Preliminary phytochemical study:

The HACA was screened for the presence of various phytoconstituents like alkaloids, glycosides, flavonoids, tannins, carbohydrates, amino acids and proteins [12].

D. Experimental Design

Experimental induction of diabetes

Rats were fasted overnight before being injected intraperitoneally with a single dose of freshly prepared solution of streptozotocin (STZ, 65 mg/kg) in ice cold citrophosphate buffer (pH 4.3). Rats showing fasting blood glucose more than 200 mg/dl were considered diabetic and used for the study [13].Treatment commenced on 7^{th} day of STZ administration [14] was considered as the first day of study.

Experimental groups

Study was carried for 28 days using following groups.

Group 1: Normal control rats administered cold citrophosphate buffer (pH 4.3).

Group 2: Diabetic control rats (STZ-(65 mg/kg i.p).

Group 3: Diabetic rats treated with 100 mg/kg of HACA

Group 4: Diabetic rats treated with 200 mg/kg of HACA

Group 5: Diabetic rats treated with 400 mg/kg of HACA

Group 6: Diabetic rats treated with 5 mg/kg of glibenclamide (Standard oral hypoglycemic drug)

E. Determination of Biochemical Parameters:

Fasting blood glucose and oral glucose tolerance was determined on 0, 7^{th}, 14^{th}, 21^{st} and 28^{th} day of study period using glucometer (Accu check, Germany) [15]. HbA1c % was determined in EDTA-blood samples obtained at the end of the 28^{th} day study using commercial assay kit (Crest biosystems, Goa, India).

Serum triglycerides (TG), total cholesterol (TC) and high density lipoprotein (HDL) levels were estimated using standard kits (Autozyme Diagnostics, India). Very low density lipoproteins (VLDL) and low density lipoproteins (LDL) levels were calculated using Friedewald formula [16].

VLDL= TG/5, LDL= TC – (HDL+ VLDL)

GSH activity was studied by method described by Kaur et al [17]. MDA activity was studied by method described by Kumar et al [18]. Catalase activity was studied by method described by Sahreen et al [19]. SOD activity was measured according to method of Marklund [20]. Serum total protein, urinary total protein, serum albumin and urinary albumin were estimated by using kit (Autozyme, India). Blood urea nitrogen, serum creatinine and urinary creatinine clearance was estimated using commercial kit (Crest biosystems, India).

Statistical analysis of data

All the data are presented as mean ± SEM of measurements made on six animals in each group. Statistical analysis was performed with one-way analysis of variance (ANOVA) followed by Dunnet's multiple test for comparison. A value of $p<0.05$ was considered to be statistically significant compared with the respective control.

III. Results

During preliminary phytochemical investigation HACA showed presence of flavonoids, phenolic compounds, tannins and alkaloids.

Treatment with HACA for 4 weeks exhibited a significant ($p<0.01$) decrease in fasting blood glucose in STZ diabetic rats as compared to diabetic control (Table 1). In diabetic rat's blood glucose level was reduced by 56.85.49%, 68.21% and 75.68% at 100, 200 and 400 mg/kg doses of the extract respectively. The standard oral hypoglycemic drug glibenclamide showed 69.41 % reduction in blood glucose level as compared to diabetic control group.

The protective effect of HACA on lipid profile has been shown in Table 2. There was a significant ($p<0.01$) decrease in T-CH, TG, LDL-CH, VLDL-

CH and significant ($p<0.01$) elevation in serum HDL-CH in diabetic rats when compared to normal rats. HACA (400 mg/kg) treated diabetic rats showed decreased levels of T-CH by 26.02 %, TG by 61.52

Table 1: Effect of oral administration of HACA on blood glucose level in STZ-diabetic rats

Experimental groups	Fasting blood glucose level (mg/dl)				
	0 day	**7thday**	**14th day**	**21thday**	**28th day**
Normal Control (NC)	87.5 ±3.86	86.83 ±4.33	78 ±2.2	81.33 ± 5.57	88.66 ±5.95
Diabetic Control (DC)	463.16 ±16$^{\#\#}$	549.5±18.12$^{\#\#}$	541.16 ±18.81$^{\#\#}$	503 ±16.94$^{\#\#}$	496.5 ±20.62$^{\#\#}$
DC+HACA (100 mg/kg)	556 ± 18.06	370 ±8.22	509.16 ±45.66**	268.83 ±5.89*	199.83±8.39**
DC+HACA (200 mg/kg)	331.83 ±51.94	495.66 ±15.35*	398 ±22.62**	301.76 ±35.74*	148 ±10.5**
DC+HACA (400 mg/kg)	433.66 ±18.44	319.5 ±17.97	283.16 ±26.59**	202 ±22.22**	114 ±5.73**
DC+GL (5 mg/kg)	585 ±8.4	484.83 ±14.15	392.5 ±18.3**	298.33 ±36.94**	141.66 ±40.62**
NC+ HACA (400 mg/kg)	98.66 ±5.46	101±5.71	102.5 ±5.75	97.16 ±4.28	95.66 **±7.12**

NC: Normal control; DC: Diabetic control; HACA: hydroalcoholic extract of *Cassia auriculata* aerial parts, GL: Glibenclamide; n=6, Values are mean ± S.E.M., $^{\#}p<0.05$, $^{\#\#}p<0.01$ as compared to normal control group; $^{*}p<0.05$, $^{**}p<0.01$ as compared to diabetic control group Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnet's multiple test for comparison.

Table 2: Effect of oral administration of HACA on lipid profile in STZ induced diabetic rats.

Experimental Groups	T-CH (mg/dl)	TG (mg/dl)	LDL (mg/dl)	VLDL (mg/dl)	HDL (mg/dl)
Normal Control (NC)	187.48± 11.8	65.44± 11.07	88.91± 4.50	13.89± 0.55	85.38±1.26
Diabetic Control (DC)	229.08± 22$^{\#\#}$	145.46±19.8$^{\#\#}$	179.5± 1.84$^{\#\#}$	29.09± 1.3$^{\#\#}$	2.47± 3.65$^{\#\#}$
DC+HACA (100 mg/kg)	169.47± 5.6**	84.25± 6.07*	116.72± 3.44*	16.84± 0.62**	34.22± .85**
DC+HACA (200 mg/kg)	151.99± 3 .8**	65.1± 4.09*	93.24 ± 3.5**	13.02± 2.2**	45.72± 1.56**
DC+HACA (400 mg/kg)	139.87± 3.2**	55.97± 7.85*	86.84± 5.30**	11.18±.70**	65.62± 2.6**
DC+GL (5 mg/kg)	145.68±1.3**	83.76±3.07*	76.58±1.47**	16.75±1.5**	52.3±0.70**

n=6, Values are mean ± S.E.M., $^{\#}p<0.05$, $^{\#\#}p<0.01$ as compared to normal control group; $^{*}p<0.05$, $^{**}p<0.01$ as compared to diabetic control group. HACA: Hydroalcoholic extract of aerial parts of Cassia auriculata; GL: Glibenclamide, T-CH: Total Cholesterol; TG: Triglycerides; HDL: High density lipoprotein; LDL: Low density lipoprotein; VLDL: Very low density lipoprotein. Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnet's multiple test for comparison.

%, LDL-CH by 90.61 % and VLDL-CH by 61.56 %. Whereas HACA (400 mg/kg) treated group showed a significant ($p<0.01$) increase in HDL-CH as compared to diabetic control group.

Nephroprotective activity:

Effect of HACA on nephroprotective parameters is shown in Table 3. Treatment with HACA for 28 days significantly ($p<0.01$) decreased glycosylated haemoglobin level in treatment group as compared to diabetic control group. Treatment with HACA significantly ($p<0.01$) increased serum total protein and albumin as compared to diabetic control group. On the other hand, total protein and albumin in urine was significantly ($p<0.01$) reduced by HACA treatment. Blood urea nitrogen and serum creatinine increased significantly ($p<0.01$) whereas creatinine clearance decreased steeply in diabetic control rats as compared to normal control group indicating a decreased glomerular filtration rate. Treatment with HACA (400 mg/kg) significantly ($p<0.01$) decreased the alteration in glomerular filtration

Table 2: Effect of HACA on nephroprotective parameters in STZ diabetic rats

Parameters	Normal control	Diabetic control	DC+ HACA (100 mg/kg)	DC + HACA (200 mg/kg)	DC+HACA (400 mg/kg)	DC+ GL (5 mg/kg)
HbA_{1c} (%)	5.41± 0.28	7.48± 0.04$^{\#\#}$	6.78± 0.09	5.48± 0.07**	6.46± 0.10*	5.2± 0.25**
Serum total protein (g/dl)	7.61± 0.49**	3.29± 0.617$^{\#\#}$	4.20± 0.75**	4.90± 0.25**	6.77± 0.47**	7.86± 0.45**
Serum albumin (g/dl)	8.56± 0.36**	3.02± 0.52$^{\#\#}$	4.22± 0.18	4.71± 0.21**	5.99± 0.21**	6.36± 0.12*
Serum creatinine (mg/dl)	6.31± 0.69**	15.07± 0.84$^{\#\#}$	11.62± 0.89**	10.42± 0.66**	8.31± 0.25**	8.91± 0.54**
BUN (mg/dl)	20.06± 0.98**	50.44± 3.32$^{\#\#}$	46.50± 0.70	43.50± 0.94*	39.96± 0.42**	29.85± 0.94**
Urinary total protein (g/dl)	8.14± 0.46**	12.87± 0.44$^{\#\#}$	9.95± 0.94**	9.35± 0.56**	7.94± 0.49**	7.86± 0.38**
Urinary albumin (g/L)	0.69± 0.03**	3.55± 0.11$^{\#\#}$	1.79± 0.05**	1.41± 0.07**	1.16 ± 0.12**	2.00± 0.09**
U.creatinine clearance (g/L)	0.96± 0.40**	4.67± 0.87$^{\#\#}$	2.73± 0.28**	3.27± 0.13**	4.31± 0.05**	2.51± 0.17**
Kidney SOD (U/mg protein)	5.671± 0.16**	3.35± 0.16$^{\#\#}$	4.186± 0.16**	5.043± 0.053**	6.151± 0.086**	6.11± 0.18**
Kidney Catalase (U/mg protein)	78.66± 1.68**	36.64± 1.54$^{\#\#}$	45.08± 1.16**	49.97± 0.60**	52.05± 0.42**	62.09± 1.20**
Kidney GSH (nmol/mg protein)	4.55± 1.42**	2.73± 0.06$^{\#\#}$	3.90± 0.06**	4.173± 0.05**	4.43± 0.06**	4.15± 0.05**
Kidney MDA (nmol/mg protein)	196.92± 2.509*	389.87± 6.58$^{\#\#}$	282.62± 3.71*	254.00± 3.39**	223.95± 3.011**	271.86± 9.51**

NC: Normal control, DC: Diabetic control , HACA: hydroalcoholic extract of *Cassia auriculata* aerial parts, GL: Glibenclamide; n=6, Values are mean ± S.E.M., $^{\#}p<0.05$, $^{\#\#}p<0.01$ as compared to NC; group $^{*}p<0.05$, $^{**}p<0.01$ as compared to DC: Data analysed by one way Analysis of Variance (ANOVA) followed by Dunnet's Multiple Test for comparison.

rate by decreasing serum creatinine and increasing creatinine clearance compared to diabetic control group. Diabetes resulted in significant decrease in antioxidant enzymes like GSH, catalase and SOD. Moreover the levels of MDA were significantly increased. HACA exhibited improvements in antioxidant enzymatic activity compared to diabetic control group and nearly normalized the levels of SOD, MDA, catalase and GSH.

IV. Discussion

In the present study, protective role of hydroalcoholic extract of aerial part of *Cassia auriculata L*. (HACA) in early diabetic nephropathy is evaluated by using STZ induced diabetic nephropathy in rats as the animal model. The present results suggest that HACA exhibit significant antihyperglycemic, hypolipidemic and nephroprotective effects in STZ induced diabetic rats. In the present study, induction of diabetic nephropathy by STZ was evidenced by elevated levels of urinary total protein, urinary albumin, serum creatinine, BUN and decreased creatinine clearance, which were taken as direct *in vivo* index for nephropathy in STZ diabetic rats [21].

In the present study, STZ induced diabetic rats exhibited significant increase in blood glucose level. Chronic treatment of diabetic rats with HACA reduced blood glucose level in duration dependent manner indicating its potent antihyperglycemic activity which contributes at least in part in delaying the progression of diabetic nephropathy. Hyperglycemia is also responsible for increased oxidative stress in the kidney which induces apoptosis that contribute to the development of diabetic nephropathy [22,23]. Hyperglycemia induced oxidative stress caused by free radical generation and decrease antioxidant defense system which, has been assessed to estimate the degree of oxidative stress [24]. Our study showed increased oxidative stress as demonstrated by increase in level of lipid peroxidation products such as MDA and decrease in SOD, GSH and catalase activity in kidneys of diabetic untreated group. HACA treatment restored the levels of MDA, SOD, GSH and catalase close to normal control values, which confirms that antioxidant potential of HACA is responsible for renal protective activity.

In the present study, urinary total protein and albumin, which are generally considered as markers of renal function [25], were increased and creatinine clearance was decreased in STZ diabetic rats. Decrease in urinary albumin, serum creatinine and BUN observed in HACA treated groups with improvement in urinary clearance of creatinine indicates that HACA ameliorated the loss of renal function and glomerular hyperfiltration in STZ diabetic rats. Magnitude of urinary protein level is further associated with a graded increase in the risk of progression to end stage renal disease and cardiovascular event [26]. Treatment with HACA in STZ diabetic rats showed significant reduction in urinary protein level which indicate that HACA may have ability to delay the end stage renal disease and associated cardiovascular complications.

HDL helps to scavenge cholesterol from extra hepatic tissues. Decreased HDL can contribute to the increased LDL cholesterol levels as there is a reciprocal relation between the concentration of LDL and HDL [46]. In our study, markedly increased levels of TG, T-CH, VLDL-CH, LDL-CH and decreased level of HDL-CH in STZ diabetic rats contributed to the pathogenesis of diabetic rats as reported in previous studies [27,28,29] This altered levels of TG, T-CH, VLDL-CH, LDL-CH and HDL-CH was reversed towards the normal control level by administration of HACA during treatment period.

In our study, increase in glycosylated haemoglobin was observed in STZ diabetic rats. It has been previously reported that the elevation of glycosylated hemoglobin beyond 7% generally leads to diabetic nephropathy. Treatment with HACA showed a marked improvement in the glycosylated haemoglobin levels which demonstrates its role in delaying the progression of diabetic nephropathy.

In conclusion, we demonstrated that the administration of HACA effectively ameliorated alterations in early diabetic nephropathy induced by STZ by virtue of its antihyperglycemic, antihyperlipidemic and antioxidant mechanism. The proposed mechanisms for renal protective activity of HACA are due to its major components viz. flavonoids and tannins. Further studies are required to isolate the major constituents, which will contribute in development of effective therapy for diabetic nephropathy.

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