ISSN: 0974-2115
www.jchps.com Journal of Chemical and Pharmaceutical Sciences

EVALUATION OF ANTI-DIABETIC AND NEPHRO PROTECTIVE ACTIVITY OF 95% ETHANOLIC EXTRACT OF CANTHIUM DICOCCUM WHOLE PLANT BY USING ALBINO RATS

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ABSTRACT

The anti-diabetic and nephroprotective activity of the Ethanolic extract of the whole plant of *Canthium dicoccum* (family: rubiaceae) was investigated on alloxan induced diabetic albino rats. A comparison was made between both plant extract and a known antidiabetic drug glibenclamide (5mg/kg^{-1}). The dried bark of *Canthium dicoccum* was subjected to extraction by continuous hot extraction method using ethanol as a solvent. Phytochemical estimation was done for the presence of phytoconstituents. Dose selection was made on the basis of acute oral toxicity study (200mg/kg^{-1}, 400mg/kg^{-1} bodyweight) as per OECD guidelines. Oral administration of extract of *Canthium dicoccum* for 21days resulted in significant reduction in blood glucose level. Alloxan induced diabetic rat model and oral glucose tolerance test (OGTT) model was used for evaluation of antidiabetic activity.The biochemical parameters were analysed.All rats in the diabetic groups had FBG levels well within the diabetic range (>150 mg dL^{-1}) at the initial stage of the experiment but after 21 days of treatment with extracts or glibenclamide the FBG significantly dropped in dose-dependent manner. The results suggest that the ethanol extracts of the*Canthium dicoccum* restored the metabolic changes in alloxan-induced diabetic rats**.**
Key Words: *Canthiumdicoccum,* glibenclamide, alloxan, anti-diabetic activity, nephro protective activity.

1. INTRODUCTION

Diabetes mellitus is a group of metabolic disorder characterized by hyperglycemia, glycosuria, hyperlipidemia, polyuria, polyphagia, polydipsia, negative nitrogen balance and sometime ketonemia, Hyperglycemia (high blood glucose level) that result from defects in insulin secretion, or defective response of insulin, or both. Diabetes is a chronic medical condition, meaning that although it can be controlled, it lasts a lifetime. The American Diabetes Association estimated that the US economy lost $ 58 billion, approximately half of the direct healthcare expenditure on diabetes in 2007, because of lost earnings due to lost work days, restricted activity days, lower productivity at work, mortality and permanent disability caused by diabetes. Diabetes induced by alloxan, it produce oxidative free radicals. This free radicals damage the β-Cells of pancreas. In present study *Canthium dicoccum*was selected for anti diabetic and nephro protective activity because it is having anti oxidant property. Due to this anti oxidant property the oxidative species were reduced so that β-Cells were recovered.

2. MATERIALS AND METHODS

Chemicals: Alloxan monohydrate, Glibenclamide, glucose, all other chemicals and reagents used were analytical grade.

Plant material: The plant of *Canthium dicoccum* plants were collected from the certified ayurvedic wholesaler The plant was identified and authenticated by Asst Prof. K. Dr. K. Madhava chatty, MSc, Med, Department of Botany, S.V. University, Tirupati.

Preparation of plant extraction: The collected bark was shade dried and powdered in mixer grinder to get coarse powder. The powdered plant material (500gms) was extracted with ethanol (95%v/v) by using soxhlet apparatus. The extract was air dried to evaporate solvent (Porohith, 2007).

Phytochemical screening: The preliminary phytochemical screening of methanolic extract of *Canthium dicoccum* was carried by using standard procedures. (The Ayurvedic Pharmacopoeia of India, 1996)

Acute Toxicity Study: toxicity studies were performed according to OECD guidelines.

Experimental model: Alloxan monohydrate was weighed individually for each animal according to their body weight and solubilised with saline just prior to injection. Diabetes was induced by injecting it at a dose of 150 mg/kg body weight intrapertonially. The animals were kept under observation and after 48 hrs blood glucose level was measured by One-touch glucometer. The diabetic rats (glucose level 200-300 mg/dl) were separated and divided into five different groups for experimental studies, with each group containing six animals. Present study has confirmed that the treatment of methanolic extract of *Canthium dicoccum* for a period of 21 days caused a significant decreased in BGL (Blood glucose level) of diabetic rats. 200&400 mg/kg of plant extract were screened for anti diabetic activity against alloxan induced

diabetic rats. It produced significant anti diabetic activity in a dose dependent manner. The animals treated with alloxan had high BGL. The anti diabetic activity exhibited by extract was compared with that of standard drug Glibenclamide. (Vogel HG, 1997; Syed M, 2003)

Experimental design: The rats were divided into five groups each consist of six rats. Significant hyperglycaemia was achieved within 48 hrs after alloxan (150 mg/kg body weight i.p) injection.
Group I- Served as normal control and did not receive any treatment
Group II- Served as diabetic control and received Inducer (alloxan-150mg/kg) and vehicle
Group III- Alloxan + Glibenclamide (5 mg/kg p.o.) and served as standard
Group IV- Alloxan + 95% ECD extract (200 mg/kg, p.o.)
Group V- Alloxan + 95% ECD extract (400 mg/kg, p.o.)
Statistical Analysis: The result of the study were subjected to one way analysis of variance (ANOVA) fallowed by Dunnet's test for multiple comparisions. Values with $p<0.05$ were consider significant.

3. RESULTS AND DISCUSSION

Phytochemical Screening: Phytochemical screening of methanolic extract of *Canthium dicoccum*showed the presence of various chemical constituents mainly alkaloids, amino acids, Proteins, glycosides, phytosterols and saponins. Extract may be responsible for antidiabetic and nephroprotective properties. The results obtained were comparable and satisfied the standard literature.

Acute oral toxicity studies: In the present study the ECD was subjected for toxicity studies. For the LD50 dose determination ECD was administered the dose level of 2000 mg/kg body weight and both doses did not produce any mortality.Hence one-fourth of the dose tested i.e. 200mg/kg and 400mg/kg body weight was selected for the study in order to ascertain a scientific base for the useful of this plant in the treatment of diabetes. It was decided to evaluate experimental design of antidiabetic activity by Alloxan induced model.

Anti-diabetic activity:

Alloxan induced diabetic model: The anti-diabetic effect of ECD in alloxan induced diabetic animals is presented. The results showed that after single dose treatment of the extract in individual group of alloxan induced diabetic rats, there was a significant reduction in serum glucose levels throughout the entire period of study (21 days) as compared to diabetic control group. *Canthium dicoccum* whole plant extract were screened for antidiabetic activity in rats Where Alloxan (150 mg/kg, i.p.) used as the diabetogenic agent.

In an Alloxan induced diabetic rats (Gr. II) significantly increased serum glucose level at '0'day ($P<0.001$), 1st day ($P<0.001$), 7th day ($P<0.001$), 14th day ($P<0.001$) and 21st day ($P<0.001$) were shown in the Table No.1.

Glibenclamide at an oral dose 5 mg/kg reduced serum glucose level at '0'day ($P<0.001$), 1st day ($P<0.001$), 7th day ($P<0.001$), 14th day ($P<0.001$) and 21st day ($P<0.001$) significantly when compared with control respectively.

Administration of ECD 200 and 400 mg/kg orally reduced significantly serum glucose level at '0'day ($P>0.001$), ($P>0.001$), at 1st day ($P<0.001$), ($P<0.001$), at 7th day ($P<0.001$), ($P<0.001$), 14th day ($P<0.001$), ($P<0.001$) and 21st day ($P<0.001$), ($P<0.001$) when compared to (Gr. II) control respectively.

Oral glucose tolerance test (OGTT) model: The anti-diabetic effect of MTT in glucose induced diabetic animals is presented. The results showed that after single dose treatment of the extract in individual group of glucose induced diabetic rats, there was a significant reduction in glucose levels throughout the entire period of study (1440 min) as compared to diabetic control group. In a glucose induced diabetic rats significantly increased serum glucose level at '0'hr ($P<0.001$), 1st hr ($P<0.001$), 2nd hr ($P<0.001$), 4th hr ($P<0.001$), 6th hr ($P<0.01$) and 24th hr (ns) were shown in the Table No.2.

Glibenclamide is administered as an oral dose 5 mg/kg reduced serum glucose level at '0'hr (ns), 1st hr ($P<0.001$), 2nd hr (ns), 4th hr ($P<0.001$), 6th hr ($P<0.001$) and 24th hr (ns) significantly when compared with control respectively. Administration of ECD 200 and 400 mg/kg orally reduced significantly serum glucose level at '0' hr ($P<0.001$) ($P<0.001$), 1st hr ($P<0.001$) ($P<0.001$), 2nd hr ($P<0.001$) ($P<0.001$), 4th hr ($P<0.001$) ($P<0.01$), 6th hr (ns) ($P<0.001$) and 24th hr (ns) (ns) when compared with control respectively.

The results obtained from the present investigation demonstrated that the plant extract of *Canthium dicoccum* constantly maintained significant reduction of the glucose level in alloxan and oral glucose mediated diabetic rats

throughout the experimental period suggesting the antidiabetic property of the title plant. Diabetes mellitus causes failure to use of glucose for energy that leads to increased utilization and decreased storage of protein responsible for reduction of body weight essentially by depletion of the body proteins. In the present study, it was observed that the extract reversed the weight loss of the diabetic rats. Alloxan has been shown to induce free radical production and cause tissue injury.The pancreas is especially susceptible to the action of Alloxan induced free radical damage. The dose of 150mg/kg of alloxan can induces an autoimmune process that results in the destruction of the β-cells of islets of Langerhans; it also results in the toxicity of beta cells with emergence of clinical diabetes within 2-4 days.

In this study, the plant extract, up to the highest dose (2000 mg/kg, p.o.) used in the acute toxicity test, did not cause any death to or acute toxicity symptoms in the albino rats. The LD50, therefore, may be greater than 4000 mg/kg (p.o.). This relatively high LD50 shows that the plant extract is non-toxic and/or safe in rats. Traditional medicine practitioners are known to use the plant for treatment in the form of infusion. However, this study did not ascertain the doses used by the practitioners for such treatments. The pharmacological screening results obtained in the present study.

Table.1. Effect of 95% ECD extract on fasting blood glucose level in Alloxan induced diabetic rats

	Normal	Control (Alloxan-150mg/kg)	Standard (Glibenclamide-5mg/kg)	ECD (200mg/kg)	ECD (400mg/kg)
0 Day	79.5±0.87	284.1±1.64 [a]	244.8±0.89***	286.3±0.69***	261±0.47***
1st Day	77.5±0.81	346±1.13 [a]	235.3±1.05***	230.6±0.45***	231.1±0.72***
7th Day	75±0.78	298±1.25 [a]	184.3±1.10***	178±0.53***	158.6±0.45***
14th Day	71.8±0.55	252.5±0.87 [a]	166.1±0.72***	162±0.78***	145.5±0.87***
21th Day	75.3±0.61	234.3±1.09 [a]	112.6±1.31***	126.1±3.77***	125.6±0.77***

All the values are mean± SEM, n=6, One way ANOVA followed by multiple compression Dennett's test ***P<0.001 as compare to control. [a]P<0.001 when compared with normal

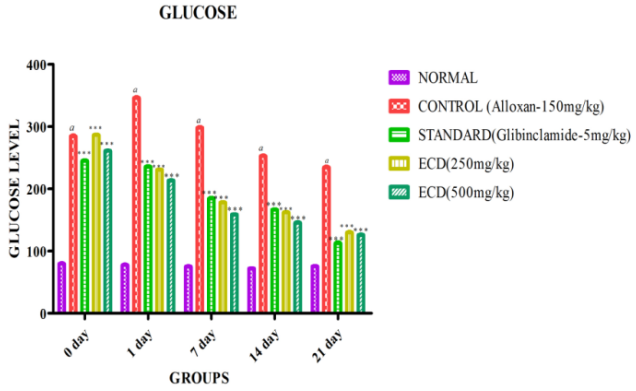

Figure.1.Effect of 95% ECD extract on fasting blood glucose level in Alloxan induced diabetic rats

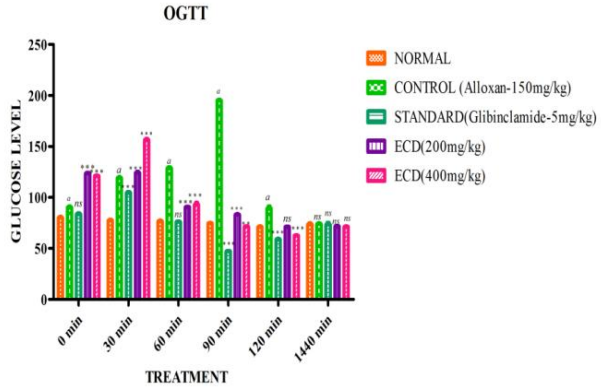

Figure.2. Effect of 95% MTT extract on OGTT in oral glucose induced diabetic rats

Table.2. Effect of 95% MTT extract on OGTT in oral glucose induced diabetic rats

	Normal	Control (Glucose,1gm/kg)	Standard (Glibenclamide,5mg/kg)	ECD (200mg/kg)	ECD (400mg/kg)
0hr	80.1±0.89	90±1.18 [a]	83.6±0.87[ns]	123.5±0.91***	121.1±0.44***
1hr	77.3±0.77	118.8±1.07 [a]	104.6±0.77***	124.1±0.93***	156.3±1.07***
2nd hr	76.5±0.81	128.6±1.01 [a]	76.17±0.44[ns]	90.6±0.30***	92.83±1.95***
4th hr	74.5±0.57	195±0.78 [a]	47.3±0.45***	83±0.62***	71.3±0.45**
6th hr	71.1±0.55	89±2.11 [a]	58.83±0.64***	71.6±0.43[ns]	62.5±0.57***
24th hr	73.6±1.02	74±0.67 [ns]	73.3±1.68 [ns]	71.7±0.30 [ns]	71.2±0.60[ns]

All the values are mean± SEM, n=6, ns= not significant One way ANOVA followed by multiple compression. Dennett's test ***P<0.001as compare to control. [a]P<0.001when compared with normal.

Table.3. Effect of 95% MTT extract on serum biochemical parameters in Alloxan induced diabetic rats

	CHOLESTROL (mg/dl)	TRIGLYCERIDE (mg/dl)	LDL (mg/dl)	HDL (mg/dl)	VLDL (mg/dl)	SGPT (U/L)	SGOT (U/L)	UREA (mg/dl)	URIC ACID (mg/dl)	CREATININE (mg/dl)	TOTAL PROTIEN (mg/dl)	ALBUMIN
NORMAL	145.30± 1.009	122.89± 0.79	55.69±0 .75	64.69± 0.83	24.58± 0.16	25.5± 0.33	26.03± 0.76	25±0.78	3.77± 0.07	0.8±0.0 1	7.4±0.11	4.9333± 0.060
CONTROL (Alloxan-150mg/kg)	299.76± 0.88^{a}	323.22± 0.86^{a}	31.79±1 .14^{a}	192.01 ±4.79^{a}	64.64± 0.17^{a}	51.25 ±0.73^{a}	56.35± 0.59^{a}	70± 0.67^{a}	10.82± 0.08^{a}	2.6±0.0 6^{a}	2.13±0.1 6^{a}	2.1666± 0.0871^{a}
STANDARD (Glibenclamide-5mg/kg)	155.73± 0.75***	141.38± 0.53***	63.198± 0.28***	68.28± 3.99^{ns}	28.27± 0.11*	12.57 ±0.69***	22.32± 0.38***	50± 0.67***	8.83± 0.09***	1.67±0. 01***	8.23±0.1 1***	5.2666± 0.0608**
ECD (200mg/kg)	178.36± 0.79***	195.78± 0.95***	63.19± 0.30***	76.01± 0.75^{ns}	39.17± 0.18***	22.43 ±1.85^{ns}	23.72± 0.54*	40.5± 0.46***	6.1± 0.08***	1.30±0. 01***	6.87±0.0 9*	3.3±0.04 08***
ECD (400mg/kg)	141.35± 0.80*	127.78± 0.73**	60.27± 0.52***	55.52± 0.99^{ns}	27.22± 1.58^{ns}	19.83 ±0.83**	19.85± 0.47***	33.67± 0.73***	4.37± 0.09***	0.93±0. 01*	7.77±0.1 1^{ns}	3.8333± 0.0561**

All the values are mean± SEM, n=6, ns=not significant one way ANOVA followed by multiple compression. Dennett's test ***P<0.001, **P<0.01, *P<0.05 as compared to control.^{a}P <0.001 when compared with normal.

Table.4.Effect of 95% ECD extract on urine biochemical parameters in Alloxan induced diabetic rats

	Urine Albumin	Urine total protien	Urine Creatinine
Normal	3.4333±0.0509	6.6333±0.0561	1.3666±0.1194
Control (Alloxan-150mg/kg)	6±0.0666^{a}	9.95±0.0935^{a}	0.6166±0.0760^{a}
Standard (Glibenclamide-5mg/kg)	5.4±0.0666***	9.1666±0.0408***	0.4666±0.03042^{ns}
ECD (200mg/kg)	4.9666±0.0732***	8.7±0.0608***	2.0833±0.0495***
ECD (400mg/kg)	3.8333±0.0871***	7.666±0.1194***	1.3666±0.0509***

All the values are mean± SEM, n=6, ns=not significant, one way ANOVA followed by multiple compression Dennett's test ***P<0.001 as compared to control. ^{a}P <0.001 when compared with normal.

Table.5.Effect of 95% ECD extract on body weight of Alloxan induced diabetic rats

	Normal	Control (Alloxan-150mg/kg)	Standard (Glibenclamide-5mg/kg)	ECD (200mg/kg)	ECD (400mg/kg)
0 Day	236.67± 8.05	238.33± 4.36^{a}	236.67±4.51**	238.33±4.36***	238.33±3.66***
1st Day	233.33± 6.94	241.67± 4.36^{c}	240±3.33***	236.67±3.85**	235±2.04**
7th Day	241.67± 6.42	225±3.91^{a}	230±3.33*	231.67±2.81***	228.33±2.81*
14th Day	243.33± 5.09	215±3.12^{b}	226.67±1.92**	226.67±1.92**	231.67±2.81**
21th Day	241.67± 6.42	206.67±3.85^{a}	231.67±2.81**	235±2.04***	240±3.33***

All the values are mean± SEM, n=6, one way ANOVA followed by multiple compression Dennett's test ***P<0.001,**P<0.01 as compared to control. ^{a}P <0.001 when compared with normal.

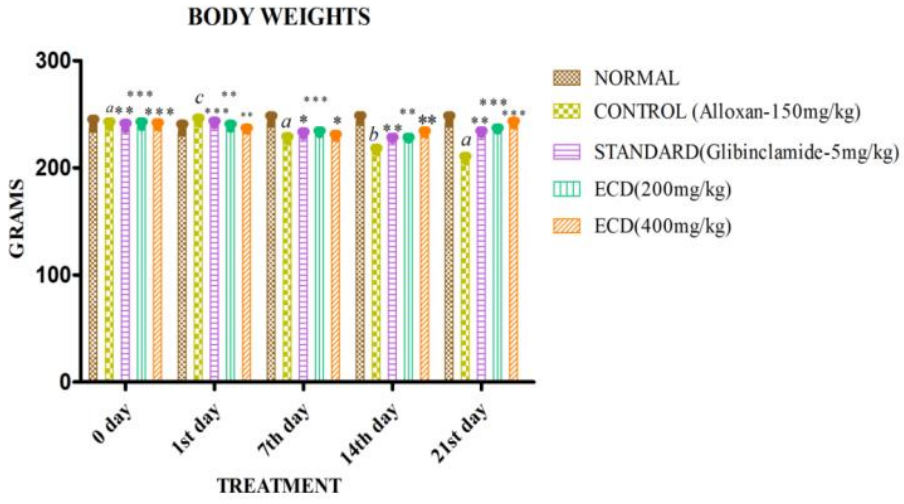

Figure.3. Effect of 95% ECD extract on body weight of Alloxan induced diabetic rats

4. CONCLUSION

The *canthium dicoccum* flower extract, showed a consistent effect on the Alloxan induced changes in the blood sugar level and the beta-cell population in the pancreas. From the above discussion it conclude that the *canthium dicoccum* extract at high doses (200, 400 mg/kg) exhibited significant anti-hyperglycaemic activity. So it can be used for the treatment of insulin dependent diabetes mellitus. The results of the present study indicated that *Canthium dicoccum* plant extract possesses significant antidiabetic activity and nephro protective activity against alloxan induced diabetic rats. Thus justifies the traditional use of this plant in the treatment of diabetes mellitus. Plant extract of the title plant possesses almost equipotent antidiabetic activity when compared with reference standard Glibenclamide.

REFERENCES

C.K.Kokate, A.P.Porohith and S.B. Gokhale (2007). Text Book of Pharmacognosy. 42 ndEdition, Pune : NiraliPrakashan, 2007, 108-109.

K.P. Sampath Kumar*, Debjit Bhowmik, Shweta Srivastava, Shravan Paswan, Amit sankar Dutta, Diabetes Epidemic in India: A Comprehensive Review of clinical Features, Management and Remedies, The Pharma Innovation, 1(2), 2012, 16-31.

Nathan D.M., Cleary P.A. and Backlund J.Y, Intensive diabetes treatment and cardiovascular disease in patients with Type 1 diabetes, The New England Journal of Medicine, 353 (25), 2005, 2643–2653.

Susanta Kumar Mondal, Goutam Chatraborty, Gupta M, Mazumder UK, In- vitro antioxidant activity of *Dispyrosmalabarica kostal* bark, Indian J Exp Biol, 44, 2004, 39-44.

The Ayurvedic Pharmacopoeia of India, Part I, Vol. IV, The Controller of Publications Civil lines, Delhi. 1996; 122-123.