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EVALUATION OF ANTIDIABETIC EFFICACY OF *COCCINIA INDICA* IN RATS

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ABSTRACT

The present study was aimed to assess the hypoglycaemic, hypolipidemic and antioxidant activities of a common herb, *Coccinia indica*. The study was conducted in Sprague-Dawley strain of albino rats and the chemical, alloxan was used for the experimental induction of diabetes. Thirty two adult albino rats were divided into four groups of eight each. Group I served as normal control and group II was made as diabetic control. Group III and IV were made diabetic and administered orally with 200 mg/kg of ethanolic extract of C. *indica* leaves and 0.25 mg/kg of glibenclamide, respectively, for 45 days. There was a significant ($p<0.05$) reduction in blood glucose, serum cholesterol, triglyceride and lipid peroxides levels and elevation of reduced glutathione and liver glycogen in *C. indica* treated group when compared with the diabetic control. The results showed that the ethanolic extract of *C. indica* leaves possessed significant hypoglycaemic, hypolipidemic and antioxidant effects. The ethanolic extract of *C. indica* leaves was screened for the active ingredients and also for acute oral toxicity and sub acute toxicity. No toxic effects were revealed from the toxicity studies. The results indicate the safety and efficacy of *indica* in diabetes mellitus.

Key words : *Coccinia indica*, Alloxan, Antidiabetic, Antioxidant, Toxicity, Rats

INTRODUCTION

Many drugs are available to manage diabetes. In most instances these are expensive and may also have adverse effects like hypoglycemia and obesity. A number of plants have been used as a natural remedy for diabetes like *Tinospora cordifolia, Azadirachta indica, Trichosanthes dioica and Cocculus hirsutus.* Screening of herbs for hypoglycemic effect will be of great significance in this context. *Coccinia indica* (Bimba in Sanskrit) known as Ivy Gourd has a long history in ancient Indian medicinal system for its use in diabetes, bronchitis and skin diseases (Alagesaboopathi, 2009. Shaheen *et al.*, 2009). It is a climbing perennial herb, growing throughout India. Although studies on hypoglycemic effect of *Coccinia indica* have been reported earlier, the effect of *Coccinia* on oxidative stress in diabetes and also evaluation of its toxicity if any have not been done. Hence the present study was undertaken to identify the active principles and to evaluate the hypoglycemic, hypolipidemic, antioxidant and toxic effect of alcoholic extract of *C. indica* Wight and Arn. in rats.

MATERIAL AND METHODS

The ethanolic extract of *C. indica* leaves was tested for the presence of various active chemical constituents namely steroids, alkaloids, tannins, phenolic compounds, flavonoids, glycosides, diterpenes, triterpenes and saponins as per the procedure described by Harbone (1991). For

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evaluation of hypoglycemic, hypolipidemic and antioxidant effect thirty two adult Sprague-Dawley albino rats of both sexes (1:1), weighing 170-200 g, were selected for the study. The experiment was approved by the Institutional Animal Ethics Committee. The rats were procured from Small Animals Breeding Station, College of Veterinary and Animal Sciences, Mannuthy. The rats were divided into four groups of eight each.

Group 1 : Normal control, administered with 3% Tween 80 p.o @ 5 ml/kg b.wt. daily from 16th day to 60th day

Group 2 : Diabetic control, administered with single dose of 10% alloxan @ 130 mg/kg b.wt. subcutaneously on zero day. From 16th day to 60th day 3% Tween 80 was administered orally @ 5 ml/kg b.wt.

Group 3 : Diabetic rats were administered with ethanolic extract of leaf of *Coccinia indica* @ 200 mg/kg orally daily from 16th day to 60th day.

Group 4 : Diabetic rats were administered orally with glibenclamide @ 0.25 mg/kg, daily from 16th day to 60th day.

Rats of all groups except the normal control were made diabetic by subcutaneous injection of alloxan at the dose rate of 130 mg/kg body weight. On 16th day after induction of diabetes, those rats showing blood glucose level more than 200 mg/dl were selected for the study. The dose of *Coccinia indica* was selected bases on previous studies (Pari and Venkateswaran, 2003) and a reduced dose of glibenclamide (0.25 mg/kg) was selected to avoid the obesity forming tendency of glibenclamide (Ogbonnia *et al.* 2008). Blood was collected from retro-orbital plexus on 15th , 30th, 45th and 60th day and serum separated for the estimation of biochemical parameters like blood glucose, serum cholesterol and triglycerides for assessing hypoglycaemic and hypolipidemic effect. On 60th day all the animals were sacrificed and pancreas and liver collected immediately to estimate liver glycogen, reuduced glutathione and lipid peroxidation in pancreas and liver. The blood glucose level was estimated by O-toluidine method (Hyvarien and Nikila, 1962), serum cholesterol and triglyceride using biochemical kits (Ecoline kit from E.Merck India Limited). Liver glycogen, lipid peroxides and reduced glutathione were estimated using standard methods (Carroll *et al.* (1956), Ohkawa *et al.* (1979), Moron *et al.* (1979)).

The ethanolic extract of *C. indica* leaves was screened for acute oral toxicity (OECD Guidelines 423). Dose levels upto 2000 mg/kg body weight were administered orally and observed for 14 days. The sub acute toxicity was evaluated in five groups of adult wistar rats of six each. One group was kept as control and the remaining groups were administered with fine emulsified extract intraperitonealy at the dose rate of 50, 70, 90 and 100 mg/kg (Veerappan *et al.*, 2007, Turner, 1965). The biochemical parameters like blood glucose, total protein, ALT, AST, creatinine and hematological parameters like haemoglobin, packed cell volume, total erythrocyte and leucocyte count were evaluated and compared with the control. The sections of liver and kidney were also taken for preparation of histopathological slides and stained with Haematoxylin and Eosin.

Data obtained were analyzed and compared by analysis of variance (ANOVA) followed by Duncan's multiple range test (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

In the qualitative estimation of phytochemical constituents, presence of triterpenes and glycosides was detected. The *C. indica* treated group (Group III) showed a significant ($p<0.05$) decrease in blood glucose levels after 45 days of treatment when compared to diabetic control. The decrease in blood glucose level in *C. indica* (200 mg/kg) treated group was higher than that in glibenclamide (0.25 mg/kg) treated group (Table 1). The serum cholesterol and triglyceride values also showed a significant ($p<0.05$) decrease after treatment with *C. indica* which indicated its hypolipidemic effect (Table 1). There was a

Table 1 : Effect of ethanolic extract of *Coccinia indica* leaf on blood glucose (mg/dl), serum cholesterol (mg/dl) and serum triglyceride (mg/dl) in alloxan induced diabetic rats, mg/dl

Days	Parameter	Group I	Group II	Group III	Group IV
Day zero	Blood glucose	97.38±2.31	94.75±3.21	102.13±3.73	91.75±1.39
	Cholesterol	48.25±1.77	52.50±2.24	47.00±2.56	53.63±2.47
	Triglyceride	101.00±2.87	101.00±3.46	99.50±2.59	97.50±2.82
15th day	Blood glucose	93.38±2.88^{a}	296.25±14.18^{b}	293.13±10.62^{b}	289.75±11.10^{b}
	Cholesterol	49.12±1.72^{a}	88.25±2.80^{b}	*100.75±3.80^{c}	90.00±2.78^{bc}
	Triglyceride	100.00±3.40^{a}	162.25±7.69^{b}	154.63±2.59^{b}	157.75±5.71^{b}
30th day	Blood glucose	93.50±1.85^{a}	277.25±11.84^{d}	*197.63±6.62^{bc}	*195.38±3.95^{bc}
	Cholesterol	53.25±2.19^{a}	95.25±3.29^{c}	90.75±4.22^{bc}	88.13±2.47^{bc}
	Triglyceride	98.63±2.51^{a}	168.75±6.98^{c}	*152.13±4.97^{b}	*147.13±4.19^{b}
45th day	Blood glucose	100.87±2.87^{a}	249.00±9.67^{d}	*136.38±3.44^{b}	*169.75±2.91^{c}
	Cholesterol	53.38±1.53^{a}	87.88±1.96^{c}	*58.00±2.28^{ab}	*59.88±3.04^{ab}
	Triglyceride	96.50±3.13^{a}	163.00±6.69^{d}	*115.88±2.95^{b}	*128.50±2.85^{c}
60th day	Blood glucose	99.25±2.02^{a}	235.00±7.17^{d}	*116.00±2.65^{b}	*126.75±2.88^{c}
	Cholesterol	53.50±1.54^{a}	86.75±2.30^{c}	*50.25±1.35^{ab}	*50.88±1.04^{ab}
	Triglyceride	98.75±2.10^{a}	162.25±9.42^{b}	*105.88±2.82^{a}	*104.63±2.60^{a}

*P<0.05,significant at 5 per cent level. Compared with diabetic control.
For each parameter, means bearing the same superscript do not differ significantly

Table 2 : Effect of ethanolic extract of *Coccinia indica* leaf on liver glycogen(g%), reduced glutathione in pancreas and liver (μg / g) and lipid peroxides (nM / g) in pancreas and liver in alloxan induced diabetic rats on 60th day

Parameter	Group I	Group II	Group III	Group IV
Reduced glutathione (pancreas)	577.63±5.29^{d}	217.50±7.13^{a}	*463.13±17.37^{b}	*493.13±12.68^{c}
Reduced glutathione (liver)	492.50±7.01^{c}	66.25±7.72^{a}	*290.00±11.80^{b}	*313.75±20.71^{b}
Lipid peroxides (pancreas)	82.13±3.99^{a}	336.63±9.91^{d}	*166.50±9.71^{c}	*123.50±9.51^{b}
Lipid peroxides (liver)	140.88±3.49^{a}	392.75±12.31^{c}	*212.50±3.74^{b}	*197.38±7.99^{b}
Liver glycogen	4.71±0.29^{c}	2.40±2.40^{a}	*3.99±0.21^{b}	*3.78±0.16^{b}

*P<0.05,significant at 5 per cent level. Compared with diabetic control.
For each parameter, means bearing the same superscript do not differ significantly

significant ($p<0.05$) increase in reduced glutathione content and decrease in lipid peroxide levels in liver and pancreas of *C. indica* treated group which indicated the antioxidant property of the extract (Table 2). The liver glycogen level also showed a significant increase in *C. indica* treated group when compared to diabetic control. These results revealed the hypoglycaemic, hypolipidemic and antioxidant effect of ethanolic extract of *C.indica* leaves. It has been demonstrated that triterpenes have an insulin-like activity (Sakurai *et al.*, 2002).The hypoglycemic effect of *Coccinia indica* may be due to the insulin like activity of triterpenes present in it. The hypoglycaemic effect of glibenclamide can be explained by the action of sulphonyl ureas which cause stimulation of insulin secretion from pancreatic beta cells (Ravi *et al.*, 2004). An increase in liver glycogen content in *C. indica* treated group might have been due to the insulin like activity of *C. indica* which stimulate hepatic glucose uptake and also inhibit gluconeogenesis and glycogenolysis in liver. The hypolipidemic effect of *C. indica* may be due to the insulin-like activity of the active constituents like triterpenes which produced suppression of lipolysis and mobilization of free fatty acids from the fat deposits. This can be further supported by the findings of Lu *et al.* (2009) that the total triterpene acid fraction from *Folium eriobotryae* produced a good hypolipidemic profile. This reduced value of lipid peroxides obtained after treatment with *C. indica* and glibenclamide can be explained with the increase in values of reduced glutathione after the treatment. Reduced glutathione scavenges free radicals and renders protection against lipid peroxidation caused by free radicals (Chaturvedi and Segale, 2007). So the elevation in reduced

Table 3 : Effect of ethanolic extract of *C. indica* leaf extract on serum biochemical and hematological parameters

Parameter	Day	Group I	Group II	Group III	Group IV	Group V
Body weight (grams)	0	120.83±2.71	120.00±3.65	120.00±2.89	121.67±3.07	116.67±2.47
	14	124.17±2.71	120.83±2.71	124.17±2.01	125.83±2.01	123.33±2.11
Serum ALT level (U/L)	0	50.67±2.69	52.33±1.71	48.50±2.05	52.83±2.40	50.33±2.89
	14	49.33±2.39	52.33±2.09	53.83±1.51	49.00±3.34	49.50±2.16
Serum AST level (U/L)	0	157.83±9.12	157.33±6.46	159.83±5.71	158.67±7.89	159.33±6.17
	14	159.33±3.16	152.67±5.71	157.17±8.06	157.17±5.85	157.83±4.29
Serum Creatinine (mg/dl)	0	1.80±0.13	1.58±0.12	1.73±0.13	1.47±0.13	1.62±0.13
	14	1.57±0.12	1.67±0.12	1.62±0.14	1.64±0.15	1.70±0.13
Serum Glucose (mg/dl)	0	84.50±3.05	81.50±5.03	80.50±3.89	82.00±3.54	81.83±4.16
	14	84.17±3.79	78.83±3.1	83.67±2.69	84.33±4.26	81.5±4.14
Serum Total protein (g/dl)	0	4.33±0.21	4.17±0.17	4.00±0.00	4.33±0.21	4.17±0.17
	14	4.17±0.17	4.17±0.17	4.33±0.21	4.17±0.17	4.17±0.17
Haemoglobin (g/dl)	0	16.08±0.37	15.92±0.35	16.75±0.21	16.58±0.37	16.58±0.24
	14	16.67±0.25	16.58±0.35	16.83±0.31	16.25±0.38	16.42±0.35
Packed cell volume (mm)	0	48.83±0.95	50.67±1.02	47.67±0.88	50.00±1.44	49.33±0.87
	14	50.67±0.92	50.17±1.14	50.00±1.18	50.50±1.18	49.50±0.92
Total erythrocyte count ($10^6/\mu l$)	14	6.27±0.23	6.61±0.19	6.54±0.23	6.61±0.32	6.84±0.14
Total leucocyte count($10^3/\mu l$)	14	6.1±0.14	6.25±0.16	6.59±0.15	6.04±0.22	6.43±0.15

P>0.05, not significant at 5 per cent level. Compared with normal control.

glutathione by the treatment with *C. indica* and glibenclamide might have helped in the scavenging of free radicals produced by the oxidative damage caused by alloxan induced diabetes which caused cell membrane damage and death. This might have subsequently reduced the raised lipid peroxides by alloxan induced diabetes. The increase in reduced glutathione content by treatment with *C. indica* may be due to the antioxidant property of the triterpenes present in it. This can be supported by the findings of Geetha *et al*. (1998) that the triterpenes present in the stem bark of *Crataeva nurvala* increased the blood glutathione after the treatment.

Wed that the doses upto 2000 mg/kg were nonlethal and that all animals were alive, healthy and active during the observation period of 14 day post administration of highest dose of 2000 mg/kg body weight. In the sub acute toxicity study conducted, no abnormal behavior and change in biochemical and hematological parameters were observed after administration of extract for 14 days (Table 3). The sections were normal except for areas of sinusoidal congestion. The morphological structure of cells were normal in the sections as supported by the unchanged biochemical parameters. These toxicity studies indicated the safety of ethanolic extract of *C. indica* as a drug in the treatment of diabetes.

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