

ANTIDIABETIC, ANTIHYPERLIPIDEMIC AND ANTIOXIDANT EFFECT OF ETHANOLIC EXTRACT OF *CURCUMA RAKTAKANTHA* J.K. MANGALY & M. SABU ON STREPTOZOTOZIN INDUCED DIABETIC RATS

RAJARATHINAM NATARAJAN INTHIRAKANTHI¹, NUNGAMPAKKAM SUBRAMANIAN MALATHY²,
NAGARAJAN ANUSUYA^{3*}

¹Department of Botany, Nirmala College for Women, Coimbatore- 641018, ²Department of Botany, P.S.G.R. Krishnammal College for Women, Coimbatore-641004, ³Department of Life Science, Manian Institute of Science and Technology (MISAT), Coimbatore - 641004, Tamil Nadu, India. Email: anusuya.nagarajan@gmail.com

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ABSTRACT

Objective: To evaluate the antidiabetic potential of *Curcuma raktakantha* (CR) rhizome using *in vitro* inhibitory assays and streptozotocin (STZ) induced diabetic animal model.

Methods: The α -amylase and α -glucosidase inhibitory activities of ethanolic extract of *Curcuma raktakantha* rhizome (ECR) were evaluated. The effect of ECR at doses of 150, 300 and 450 mg/kg b.w. on blood glucose, glycolytic and gluconeogenic enzymes, lipid profile, serum liver markers, antioxidants, were evaluated in STZ (50 mg/kg b.w.) induced diabetic rats and were compared with standard drug glibenclamide (600 μ g/kg b.w.).

Results: ECR proved to render an effective inhibition over α -amylase and α -glucosidase activities. The increase in levels of blood glucose, lipid profile, lipid peroxidation and activities of gluconeogenic enzymes along with a decrease in level of vitamin C, high density lipoprotein, activities of glycolytic enzymes and enzymatic antioxidants were observed in STZ induced diabetic rats. Oral administration of ECR for 30 days resulted in a dose dependent decrease in blood glucose. ECR also significantly restored the levels of all the above said biochemical parameters to near normalcy.

Conclusion: The results of the present study suggested that ECR possess potent antidiabetic and antihyperlipidemic effect by enhancing insulin release and thereby its activity.

Keywords: Streptozotocin; *Curcuma raktakantha*; α -amylase; α -glucosidase; Glycolytic enzymes; Gluconeogenic enzymes; Antioxidant; Antidiabetic.

INTRODUCTION

The chronic disease diabetic mellitus takes an ever-increasing proportion of national and international health care budgets as the number of people with diabetes is escalating worldwide. Inherited and/or acquired deficiency in the production of insulin by pancreas or the ineffectiveness of the insulin produced, causes diabetes mellitus. Such a deficiency results in increased concentration of glucose in the blood, which in turn damages many of the body's innate systems. Contributory factor in the pathogenesis of diabetes also comprises of oxidative stress [1]. Protein glycation and glucose autooxidation generates free radicals in diabetic patients, which in turn catalyses lipid peroxidation [2]. The antioxidant status of the diabetic is compromised and is unable to protect against oxyradical damage [3]. Sulphonylureas and biguanides, the two groups of oral hypoglycemic drugs, along with insulin are used for the treatment of diabetes. WHO has recommended the evaluation of traditional herbal treatments for diabetes, as the synthetic drugs presented undesirable side effects or contraindications. New alternatives, including plant extracts containing antioxidant substances, flavonoids and exogenous antioxidants are under clinical and experimental investigation in an effort to impede the progress of diabetic complications [4]. A number of plant species and plant products worldwide are known to have hypoglycemic [5], hypolipidemic [6] or both activities [7].

The genus *Curcuma* belonging to the Zingiberaceae family comprises 120 species of rhizomatous herbs which are used since ancient times as both spices and medicines in traditional Indian and Chinese medicines. The rhizomes of the *Curcuma* species are usually aromatic, carminative and are used to treat indigestion, hepatitis, jaundice, diabetes, atherosclerosis and bacterial infections [8]. They contain molecules credited with anti-inflammatory, antirheumatism, antimicrobial, antiviral, insect repellent, antidiabetic, hypocholestraemic, choleric, antifibrotic, antivenomous and antihepatotoxic properties as well as anticancerous properties [9]. *Curcuma raktakantha* Mangaly & Sabu is a small to large perennial plant with horizontal rhizomes largely distributed in Kerala, India.

The rhizomes are medium sized measuring 3 – 4 cm in length and 2.5 – 4 cm in width. They appear creamy to white colour internally (the younger are white) and whitish towards the periphery. The tubers are sessile and finger shaped possessing a pleasant aroma with traces of camphor and menthol. The chemical composition of the essential oil and the antioxidant and antibacterial activities of *C. raktakantha* oleoresins were reported by Dan et al [10] and Rajamma et al [11] respectively. Besides these, the other pharmacological properties of *C. raktakantha* have not yet been explored. In accordance with these data, the present study deals with the exploration of antidiabetic activity of ethanolic extract of *C. raktakantha* against streptozotocin induced Non-Insulin Dependent Diabetes mellitus.

MATERIALS AND METHODS

Chemicals

Streptozotocin (STZ) and glibenclamide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All the other chemicals used in this study were of analytical grade which were obtained from Himedia Laboratories Private Ltd, Merck and SD Fine Chemicals, Mumbai.

Plant material

The fresh rhizomes of *Curcuma raktakantha* were obtained from the National Bureau of Plant Genetic Resources Regional Station, Thrissur, Kerala (Accession No. IC088913). The rhizomes were washed thoroughly in tap water to remove adhering dust, shade dried at room temperature (25°C), powdered by milling and stored at 10°C for further studies.

Preparation of extract

The air dried, powdered rhizome was extracted with ethanol in a soxhlet apparatus. The extract was concentrated to dryness under reduced pressure, subsequently freeze dried and stored at -20°C until used for further experiments. The approximate extract yield was 18% w/w (dry weight basis).

In vitro antidiabetic activity

The *in vitro* antidiabetic activity of the ethanolic extract of *C. raktakantha* (ECR) was evaluated by performing α -amylase and α -glucosidase inhibition assay following the method of Miller [12].

In vitro inhibition of α -amylase

The enzyme α -amylase inhibitory activity was determined by premixing α -amylase (0.5 mg/ml) solution with various concentrations of ECR (50-250 μ g/ml) and 0.5% starch was added to start the reaction. The reaction was carried out at 37°C for 5 min and terminated by addition of 2 ml of DNS reagent. The reaction mixture was heated at 100°C for 15 min after which it was diluted with 10 ml of distilled water in an ice bath and its absorbance was measured at 540 nm. The IC₅₀ value was defined as the concentration of α -amylase inhibitor to inhibit 50% of its activity under the assay conditions.

In vitro inhibition of α -glucosidase

The enzyme α -glucosidase inhibitory activity was determined by premixing α -glucosidase (0.07 Units) with various concentrations of ECR (50-250 μ g/ml). Then 3 mM p-nitrophenyl glucopyranoside was added as a substrate. This reaction mixture was incubated at 37°C for 30 min and the reaction was terminated by addition of 2 ml of sodium carbonate. The α -glucosidase activity was determined by measuring the p-nitrophenyl release from p-nitrophenyl glucopyranoside at 400 nm. The IC₅₀ value was defined as the concentration of the sample extract to inhibit 50% of α -glucosidase activity under the assay condition.

Experimental animals

Male Wistar albino rats weighing 150 – 200 g were obtained from the small animals breeding station, Mannuthy, Kerala, India. All the animals were housed in clean polypropylene cages and maintained under standard environmental conditions (14 h dark/10 h light cycles; Temp 25 \pm 2°C; 35-60% humidity, air ventilation). The animals were fed with standard pellet diet (M/s. Hindustan Lever Ltd, Mumbai, India) and water *ad libitum*. The experimental protocol was carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and approved by the Institutional Ethical Committee (1454/po/c/11/CPCSEA).

Induction of Non - Insulin Dependent Diabetes Mellitus (NIDDM)

Non - Insulin Dependent Diabetes Mellitus was induced in overnight fasted rats by a single intraperitoneal injection of STZ (50 mg/kg b.w.) dissolved in citrate buffer (0.01 M, pH 4.5). The rats were given 5% (w/v) glucose solution orally on the first day to stave off hypoglycemic shock after STZ administration [13]. STZ injected animals exhibited massive glycosuria and hyperglycemia within 2 days. Three days after STZ induction, fasting blood glucose levels were determined using glucose oxidase - peroxidase reactive strips and a portable glucometer (Accu - sure, Roche Diagnostics, USA). Animals with blood glucose levels above 250 mg/dl were considered diabetic and selected for the study.

Experimental design

A total of 36 male Wistar rats (30 diabetic surviving rats and 6 normal rats) were used. The animals were divided into six groups containing six animals each as follows. Group I: Normoglycaemic control rats; Group II: STZ induced diabetic rats; Group III: Diabetic animals treated with standard drug glibenclamide (600 μ g/kg b.w.); Group IV: Diabetic animals treated with ECR (150 mg/kg b.w.); Group V: Diabetic animals treated with ECR (300 mg/kg b.w.); Group VI: Diabetic animals treated with ECR (450 mg/kg b.w.). The standard drug and ECR were administered orally to group III and group IV – VI animals respectively with oral gavage once in a day for 30 days. Third day after the induction of STZ was considered as the first day for extract administration in diabetic rats.

Soon after the last dosage, all the animals were fasted but had free access of water for 24 h. At the end of the experiment, the fasting blood glucose levels and body weight of the animals were measured before they were sacrificed. Later, the animals were euthanized and blood was collected via cardiac puncture. The blood samples were allowed to clot for 45 min and the serum was separated by centrifugation at 2500 rpm (4°C) for 10 min. Liver was excised, perfused with ice cold normal saline, dried with blotting paper, weighed, cut into pieces and 1 g of it was homogenized in 0.25 M Tris Hcl buffer of pH 7.5 to give a 10% homogenate. The homogenate was centrifuged at 10,000 rpm for 20 min at 4°C to obtain a clear supernatant which was used for various biochemical analyses.

Biochemical analysis

In the liver homogenate, the carbohydrate metabolizing enzymes namely glucose 6 phosphatase [14], hexokinase [15], phosphoglucosomerase [16] and fructose 1, 6 diphosphatase [17], antioxidants like superoxide dismutase (SOD) [18], catalase (CAT) [19], glutathione peroxidase (GPx) [20], glutathione - S - transferase (GST) [21], vitamin C [22] and lipid peroxidation (LPO) [23] were determined following the standard procedures. Serum total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL) and very low density lipoprotein (VLDL) were analyzed using commercially available diagnostic kits (Beacon diagnostics P. Ltd, India).

Statistical analysis

Data were analysed for statistical significance using one way analysis of variance (ANOVA) followed by Dunnet's multiple comparison test and results were expressed as mean \pm standard deviation (SD) using SPSS version 16.0.

RESULTS

In vitro antidiabetic activity

The ethanolic extract of ECR rendered an inhibitory effect on α -amylase and α -glucosidase with percentage inhibition ranging between 12 – 52% and 2 – 5% respectively. Their corresponding 50% inhibition concentrations were found to be 224.22 μ g/ml and 961.54 μ g/ml (Table 1).

Table 1: In vitro antidiabetic activity of ethanolic extract of *Curcuma raktakantha*

Sample Concentration (μ g)	α -amylase inhibition		α -glucosidase inhibition	
	% inhibition	IC ₅₀ (μ g/ml)	% inhibition	IC ₅₀ (μ g/ml)
50	12.71 \pm 5.90		2.38 \pm 0.80	
100	29.38 \pm 4.20	224.22	4.88 \pm 1.20	961.54
150	36.72 \pm 3.00		6.54 \pm 2.10	
200	43.50 \pm 4.20		10.31 \pm 3.40	
250	51.98 \pm 2.70		14.49 \pm 5.70	

Values are means of three independent analyses \pm standard deviation (n = 3).

In vivo antidiabetic activity

Effect of ECR on Body weight and blood glucose level

The body weight and fasting blood glucose levels measured on the final day of the experiment, for all the six groups of animals are given in the Figure 1 and Figure 2 respectively. A striking decrease

($P < 0.001$) in body weight was observed in the STZ induced rat when compared with the intact normal control rats. On oral administration of ECR at three different dose levels (150, 300 and 450 mg/Kg b.wt.) for 30 days, the restoration of the body weight was observed. Fasting blood glucose levels of untreated diabetic rats

were significantly higher ($P < 0.001$) than those in the normal rats. There was a significant decrease ($P < 0.001$) in the blood glucose levels in the diabetic groups treated with standard and ECR. Administration of higher concentrations of ECR effectively reduced the blood glucose levels.

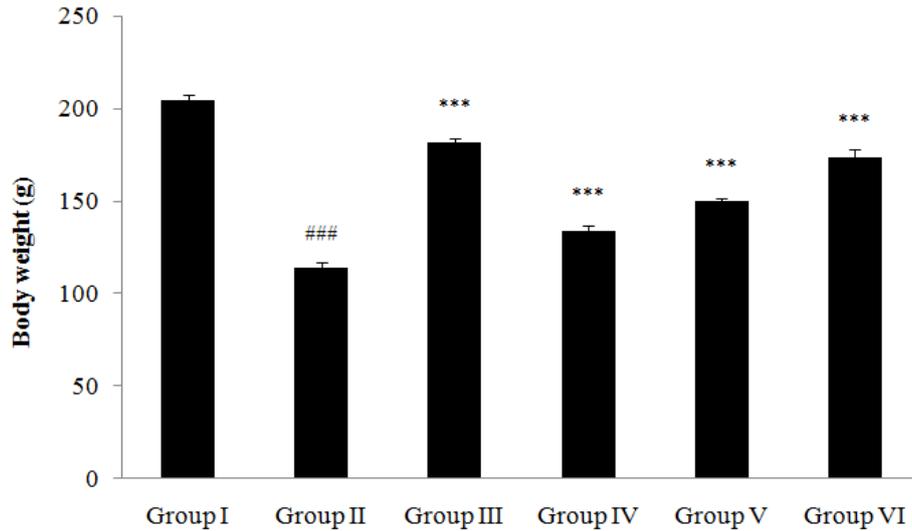


Fig. 1: Effect of ECR on body weight in experimental animals

Group I: Control rats; Group II: STZ induced rats; Group III: STZ + Glibenclamide (600 µg/kg); Group IV: STZ + ECR (150 mg/kg); Group V: STZ + ECR (300 mg/kg); Group VI: STZ + ECR (450 mg/kg).

#Decrease in body weight at $P < 0.05$ when group II compared to group I, ## $P < 0.01$, ### $P < 0.001$; *Increase in body weight at $P < 0.05$ when group III - VI compared to group II, ** $P < 0.01$, *** $P < 0.001$. Values are expressed as mean \pm SD (n=6).

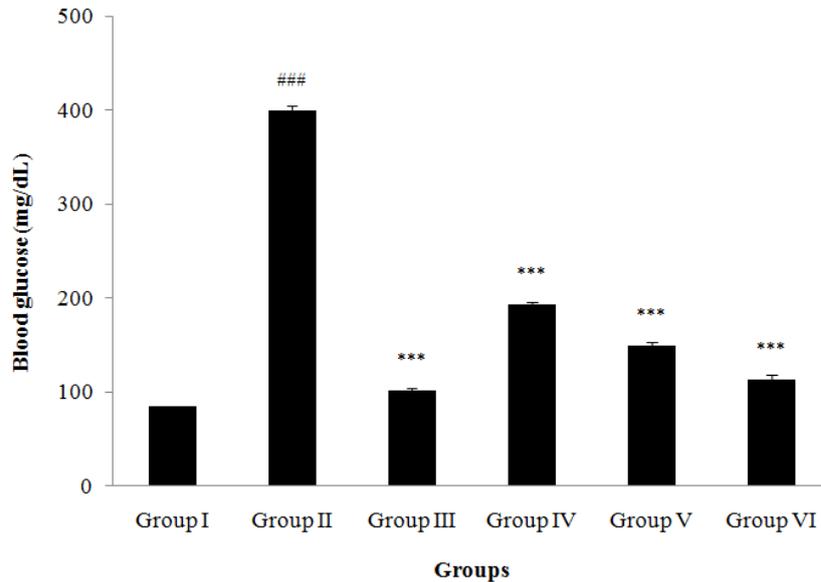


Fig. 2: Effect of ECR on blood glucose level in experimental animals

Group I: Control rats; Group II: STZ induced rats; Group III: STZ + Glibenclamide (600 µg/kg); Group IV: STZ + ECR (150 mg/kg); Group V: STZ + ECR (300 mg/kg); Group VI: STZ + ECR (450 mg/kg).

#Increase in blood glucose at $P < 0.05$ when group II compared to group I, ## $P < 0.01$, ### $P < 0.001$; *Decrease in blood glucose at $P < 0.05$ when group III - VI compared to group II, ** $P < 0.01$, *** $P < 0.001$. Values are expressed as mean \pm SD (n=6).

Effect of ECR on biochemical parameters

The STZ induced group II diabetic rats showed decreased activities of hexokinase and phosphoglucosomerase which significantly ($P < 0.001$) reversed to normal levels on treatment with higher doses of ECR for 30 days. The gluconeogenic enzymes (glucose 6 phosphatase, fructose 1, 6 diphosphatase) showed significant

($P < 0.001$) elevation in their activities in untreated diabetic rats when compared to that of normal rats. The administration of ECR altered these activities back to normalcy (Table 2). The effects of ECR on liver oxidative parameters in STZ induced diabetes are depicted in Table 3. Treatment over a period of 30 days exerted a protective effect over the significantly ($P < 0.001$) decreased levels of antioxidants, namely SOD, CAT, GPx and GST which was observed in

diabetes induced rats. The level of vitamin C was decreased in STZ induced diabetic group with a concomitant increase in lipid peroxide levels compared to that of control rats. Treatment with higher concentration of the ECR significantly ($P < 0.001$) increased the tissue vitamin C level and also significantly decreased the level of TBARS.

Serum TC, TG, LDL and VLDL were elevated with a decrease in HDL in STZ induced diabetic rats when compared to that of normal control rats. Glibenclamide and all the three concentrations of ECR significantly lowered the levels of TC, TG, LDL and VLDL with a simultaneous increase in HDL (Table 4).

Table 2: Effect of ECR on carbohydrate metabolizing enzymes of control and experimental rats

Groups	Glycolytic enzymes		Gluconeogenic enzymes	
	Hexokinase	Phosphoglucosomerase	Glucose 6 phosphatase	Fructose 1, 6 diphosphatase
Group I – Control rats	1.65 ± 0.18	13.61 ± 0.55	0.64 ± 0.05	0.50 ± 0.05
Group II – STZ induced rats	0.75 ± 0.11###	7.03 ± 0.53###	1.07 ± 0.07###	0.92 ± 0.04###
Group III – STZ + Glibenclamide (600 µg/kg)	1.34 ± 0.12***	11.45 ± 1.02***	0.76 ± 0.07***	0.54 ± 0.04***
Group IV – STZ + ECR (150 mg/kg)	0.87 ± 0.09	8.52 ± 0.22*	0.96 ± 0.07*	0.85 ± 0.04*
Group V – STZ + ECR (300 mg/kg)	0.99 ± 0.09*	9.25 ± 0.19**	0.79 ± 0.04***	0.66 ± 0.03***
Group VI – STZ + ECR (450 mg/kg)	1.30 ± 0.10***	12.37 ± 0.77***	0.73 ± 0.02***	0.58 ± 0.03***

Units: Hexokinase (µ moles of glucose 6 phosphate formed/min/mg protein); Phosphoglucosomerase (µ moles of glucose formed/min/mg protein); Glucose 6 phosphatase (µ moles of inorganic phosphorus released/min/mg protein); Fructose 1, 6 diphosphatase (µ moles of inorganic phosphorus released/min/mg protein).

#Change in activities at $P < 0.05$ when group II compared to group I, ## $P < 0.01$, ### $P < 0.001$; *Change in activities at $P < 0.05$ when group III - VI compared to group II, ** $P < 0.01$, *** $P < 0.001$. Values are expressed as mean ± SD (n=6).

Table 3: Effect of ECR on antioxidants and lipid peroxidation of control and experimental rats

Groups	SOD	CAT	GPx	GST	Vitamin C	LPO
Group I – Control rats	0.59 ± 0.04	7.75 ± 0.09	69.38 ± 0.88	6.26 ± 0.17	108.30 ± 4.05	0.32 ± 0.03
Group II – STZ induced rats	0.28 ± 0.03###	4.35 ± 0.13###	37.40 ± 0.65###	2.35 ± 0.14###	57.40 ± 1.71###	0.84 ± 0.04###
Group III – STZ + Glibenclamide (600 µg/kg)	0.54 ± 0.04***	6.71 ± 0.07***	61.67 ± 1.21***	5.56 ± 0.34***	91.42 ± 1.11***	0.36 ± 0.04***
Group IV – STZ + ECR (150 mg/kg)	0.33 ± 0.03*	5.34 ± 0.11***	48.45 ± 0.96***	3.56 ± 0.06***	61.55 ± 0.99*	0.60 ± 0.05***
Group V – STZ + ECR (300 mg/kg)	0.44 ± 0.04***	5.70 ± 0.05***	54.23 ± 1.09***	4.31 ± 0.10***	77.78 ± 0.58***	0.44 ± 0.04***
Group VI – STZ + ECR (450 mg/kg)	0.52 ± 0.02***	6.28 ± 0.08***	61.59 ± 0.98***	5.20 ± 0.10***	84.45 ± 0.76***	0.37 ± 0.02***

Units: SOD: Superoxide dismutase (Units/min/mg protein); CAT: Catalase (µ moles of H₂O₂ consumed/min/mg protein); GPx: Glutathione peroxidase (µ moles of GSH oxidized/min/mg protein); GST: Glutathione-S-transferase (µ moles of CDNB conjugation formed/min/mg protein); Vitamin C (µg/mg protein); LPO: Lipid peroxidation (µ moles of MDA formed/min/mg protein).

#Change in activities at $P < 0.05$ when group II compared to group I, ## $P < 0.01$, ### $P < 0.001$; *Change in activities at $P < 0.05$ when group III - VI compared to group II, ** $P < 0.01$, *** $P < 0.001$. Values are expressed as mean ± SD (n=6).

Table 4: Effect of ECR on lipid profile of control and experimental rats

Groups	TC	TG	HDL	LDL	VLDL
Group I – Control rats	64.44 ± 1.08	57.42 ± 1.09	15.16 ± 0.54	40.43 ± 0.92	11.48 ± 0.22
Group II – STZ induced rats	102.43 ± 1.17###	92.36 ± 1.01###	7.25 ± 0.21###	85.14 ± 0.95###	18.47 ± 0.20###
Group III – STZ + Glibenclamide (600 µg/kg)	66.95 ± 0.82***	61.21 ± 1.05***	10.81 ± 0.14***	47.25 ± 0.68***	12.24 ± 0.21***
Group IV – STZ + ECR (150 mg/kg)	85.61 ± 0.74***	78.34 ± 0.31***	8.20 ± 0.25**	71.54 ± 0.92***	15.67 ± 0.06***
Group V – STZ + ECR (300 mg/kg)	76.50 ± 1.02***	71.95 ± 0.50***	9.00 ± 0.06***	61.54 ± 0.87***	14.39 ± 0.10***
Group VI – STZ + ECR (450 mg/kg)	66.51 ± 1.05***	60.17 ± 0.75***	10.23 ± 0.20***	51.49 ± 0.47***	12.03 ± 0.15***

Units: TC: Total cholesterol (mg/dL); TG: Triglycerides (mg/dL); HDL: High density lipoprotein (mg/dL); LDL: Low density lipoprotein (mg/dL); VLDL: Very low density lipoprotein (mg/dL).

#Change in levels at $P < 0.05$ when group II compared to group I, ## $P < 0.01$, ### $P < 0.001$; *Change in levels at $P < 0.05$ when group III - VI compared to group II, ** $P < 0.01$, *** $P < 0.001$. Values are expressed as mean ± SD (n=6).

DISCUSSION

The ECR demonstrates potent *in vitro* antidiabetic activity by inhibiting α-amylase and α-glucosidase. This activity of ECR may be due to its action on carbohydrate binding regions of α-amylase and α-glucosidase that catalyse hydrolysis of internal α-1, 4 glucosidic linkages in starch, resulting in suppression of postprandial hyperglycemia. The presence of such inhibitors in food stuffs may be responsible for impaired starch digestion [24].

The antidiabetic and antihyperlipidemic effect of ECR is being reported for the very first time in STZ induced type II diabetic

model. Streptozotocin (STZ) induced diabetes model is the most frequently used one for the study of multiple aspects of the disease. STZ exert diabetogenic activity when they are administered intraperitoneally [25]. The toxic effect of STZ is not restricted to abolishment of pancreatic β-cells but also cause renal injury [26] and oxidative stress [27].

The STZ induced diabetic rats showed significant increase in fasting blood glucose and decrease in body weight. The weight loss is due to increased muscle wasting [28] and polyuria [29]. Treatment with standard glibenclamide and ECR restored the lost weight which was similar to earlier reports observed with administration of *C. longa*

extract [30]. ECR also significantly lowered the escalated blood glucose level which was observed in STZ induced diabetic rats.

The activities of glycolytic enzymes, hexokinase and phosphoglucosomerase were significantly decreased in diabetic rats. The first step in glycolysis is severely impaired in diabetic rats [31] due to decreased activity of hexokinase because of insulin deficiency. The decrease in phosphoglucosomerase activity may inhibit the proportion of glucose 6 phosphate getting metabolized via glycolysis [32]. Gluconeogenic enzymes namely glucose 6 phosphatase and fructose 1, 6 diphosphatase were found to show significantly elevated activities in liver of STZ induced diabetic rats. This increase in activity is due to the insulin insufficiency [33]. Treatment with ECR for 30 days improvised the impaired glycolytic enzymes activity and limited the activity of gluconeogenic enzymes.

STZ induction is associated with formation and accumulation of free radicals which leads to a number of deleterious effects [34]. Continuous exposure of the system to free radicals results in decreased activities of SOD, CAT, GPx and GST. Depression of these antioxidant marker enzymes under diabetic condition may be due to radical induced inactivation of glycosylation [35]. Decreased levels of vitamin C, a key non enzymatic antioxidant was observed in STZ induced diabetic rats which may be due to increased utilization in trapping the oxyradicals. Depletion in the antioxidant defense mechanism is also reflected by an increase in lipid peroxidation. However administration of ECR for 30days resulted in a significant restoration of the antioxidant system which is quite evident from the lowered levels TBARS, a lipid peroxidation marker.

STZ induction altered the lipid metabolism to a greater extent which was observed with an increase in TC, TG, LDL and VLDL with a decrease in HDL. Under normal circumstances insulin activates lipoprotein lipase and hydrolyses TG. Insulin deficiency results in failure to activate the enzyme thereby causing hypertriglyceridemia. The significant control of the levels of serum lipids in the ECR treated groups may be attributed to improvements in insulin levels [36]. These results manifests that the ECR possesses hypolipidemic activity which may be due to decreased fatty acid synthesis thereby preventing conditions like atherosclerosis and cardiovascular disorders.

C. raktakantha contains numerous polyphenols which have been shown to possess antioxidant activities. The pharmacological properties of *Curcuma* species is highly attributed to their main active component, curcuminoid [37]. The earlier reports on *C. raktakantha* also revealed the presence of ethyl p-methoxycinnamate, α - pinene, β - pinene, camphor, terpinyl acetate tumerone[10] and some oleoresins. The antidiabetic and antihyperlipidemic activity illustrated by ECR might be due to the presence of these beneficial components. The present study rationalize that the ECR has the potential to emerge as a novel remedy for the treatment of Non-Insulin Dependent Diabetes mellitus.

CONCLUSION

Both the *in vitro* and *in vivo* studies performed in the ethanolic extract of *Curcuma raktakantha*, reveals the effective protection against type II Diabetes mellitus. The current investigation advocates the usage of *Curcuma raktakantha* in the management of lipid and glucose levels. Further studies are required to understand the mechanism behind this action.

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