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ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF *CURCUMA AROMATICA*

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ABSTRACT
The objective of this paper is to find out the antidiabetic activity of *Curcuma aromatica*. In this research paper we dealt with antioxidant activities by DPPH method, ABTS method, Lipid peroxidation assay and scavenging ability of the extract for the hydrogen peroxide radical, Glucose uptake by rat hemi diaphragm method. Antidiabetic activity using healthy adult Wister rats were also carried out. Toluene extract of *Curcuma aromatica* showed the potent scavenging activity by DPPH method with the IC_{50} value of 50.62±0.998 µg/ml, by lipid per oxidation method with the IC_{50} value of 75±0.87 µg/ml, hydrogen peroxide radical scavenging activity with the IC_{50} value 43.75±1.24 µg/ml, and ABTS radical scavenging method with the IC_{50} value 0.038±1.54 µg/ml. After the treatment with the toluene extract of *Curcuma aromatica*, serum glucose level was found to be decreased from 278.53 to 116.5 mg/dl, total protein level increased from 3.09 to 5.78 mg/dl. There was a decrease in total cholesterol level from 292.33 to 134.50 mg/dl, decrease in serum triglyceride level from 85.66 to 64.16mg/dl when compared to diabetic control group. Toluene extract of *Curcuma aromatica* exhibited significant antioxidant and antidiabetic activities in both *in vitro* and *in vivo* models. So, it can be used as alternative herbal medicine in the treatment of diabetes and diabetic induced complication .
Key words: Antidiabetic, Antioxidant, *Curcuma aromatica*, α-Glycosidase activity

INTRODUCTION

As the number of people with diabetes multiplies worldwide, the disease takes an ever increasing proportion of national and international health care budget. It is projected to become one the world's main killers within the next 25 years. Regions with greatest potential are Asia and Africa, where DM rates could rise to two or three folds than the present rate. Apart from currently available therapeutic options, many herbal medicines have been recommended for the treatment of diabetes. Traditional plant medicine are used throughout the world for a range of diabetic complications[1]

Curcuma aromatica is a rhizome belonging to the family Zingiberaceae. This rhizome is used traditionally as tonic, carminative, externally in combination with astringent, bitters, and aromatics to bruises in sprains and in snake bite. They are also used for skin eruptions, infections and to improve the complexion. Present study was taken up to evaluate the antidiabetic activity of *Curcuma aromatica* and to establish its therapeutic potential in the treatment of diabetes and its complication[2]

MATERIALS AND METHODS

Collection and authentication

The dried rhizomes of *Curcuma aromatica* were purchased from Abram botanicals Tuticorin and the same was authenticated by Mr. P.S.S. Ramachandran, Abirami botanicals, Tuticorin. It was shade dried and coarsely powdered. A voucher Specimen was deposited in the Department of Pharmaceutical Biotechnology, JSS College of Pharmacy, and Ooty with the No – 0903.

Preparation of the plant extract

The coarsely powdered rhizome were subjected to successive Soxhlet extraction, using solvent petroleum ether, toluene, chloroform, ethyl acetate, acetone, ethanol and water[3]

Qualitative phytochemical screening

Different qualitative chemical tests were performed for establishing the profiles of the extracts for their nature of chemical composition and for identification of various phytoconsituents[4].

Quantitative phytochemical analysis

Phenol and flavonol are considered to be the most important phytoconsituents that are responsible for the pharmacological activities. Total phenol content and total flavonol content were estimated[5].

In- vitro antioxidant evaluation

Antioxidant studies were performed by Diphenyl picryl hydrazyl radical scavenging method[6], ABTS radical scavenging method[7], Lipid per oxidation (LPO) assay[8-10], and scavenging of hydrogen peroxide radical[11,12].

Glucose uptake by isolated rat hemi diaphragm

Wister albino rats of either sex weighed between 160-180 gm were selected. The animals were maintained on a standard pellet diet (water ad libitum) and fasted overnight. The animals were sacrificed by decapitation; diaphragm was dissected out quickly with minimal trauma and divided into two halves. The hemi diaphragm was rinsed in cold tyrode solution (without glucose) to remove any blood clot and placed in small culture tubes containing 2 ml of tyrode solution with 2 % glucose. This was immediately incubated for 30 minutes at 37^0 C in an atmosphere of 100 % O_2 with shaking[13-15].

Grouping of animals

Group I served as a control with the treatment of 2ml of tyrode solution with 2% glucose solution. Group II served as positive control treated with 2ml of tyrode solution with 2 % glucose and regular insulin (0.4units/ml). Group III, has been treated with 2 ml of Tyrode solution with 2 % glucose and toluene extract of *Curcuma aromatica* (2 mg/ml of Tyrode solution), Group IV has been treated with 2ml of Tyrode solution with 2 %

glucose with insulin and toluene extract of *Curcuma aromatica* extract.

α- Glycosidase inhibiting activity

α- Glucosidase inhibitors are among the available glucose lowering medications. This enzyme is located in the brush border of the small intestine and is required for the breakdown of carbohydrates to absorbable monosaccharide. The α- Glucosidase inhibitors delay but do not prevent the absorption of ingested carbohydrates but reduce the postprandial glucose and insulin peak. The α-glycosidase inhibitory activity was determined according to Matsui *et.al*. The assay media contained sodium phosphate buffer (0.1 M, ph 6.8), 4- nitro phenyl α-D glucopyranoside (4-NPGP), 0.1 U α – Glucosidase (from yeast) and plant extract or control drug in the range of 0.2 to 200 µg/ml of assay media, in the total volume of 1 ml. The assay was started by addition of 4- NPGP, the change in absorbance at 405nm was measured by spectrophotometer and IC_{50} values were calculated [16,17].

***In vivo* antidiabetic activity**

Animals

Healthy adult Wister rats of either gender was obtained from the central animal house from J.S.S college of Pharmacy, Ootacamund, Tamilnadu, India. The ethical committee clearance number given to this project was JSSCP/IACC/ M.pharm/pharm. biotech/ 09. The animals were kept in a well ventilated room and animals had exposed to 12 hrs day and night cycle with a temperature between 20 ± 5^0 C. The animals were housed in large spacious, hygienic polypropylene cages during the course of the experimental period. The animals were fed with water and rat feed ad libitum, supplied by this institution.

Induction of diabetes in animals

Non- insulin dependent diabetes mellitus was induced in overnight fasted rats by a single intraperitoneal injection of 50 mg/kg body weight of Streptozotocin[19-21]. Hyperglycemia was confirmed by elevated glucose level in plasma, determined at 72 hr. The rats with permanent NIDDM (250-350 mg/dl) were used for further studies.

Grouping of animals

Group 1 served as untreated group (maintained with ad libitum with water) and group II served as diabetic control (Streptozotocin treated), Group III served as diabetic group treated with Glibenclamide 10 mg/kg, Group IV served as diabetic group treated with toluene extract of *Curcuma aromatica* at the dose of 200 mg/kg b.wt) and Group V served as diabetic group treated with toluene extract of *Curcuma aromatica* at the dose of 400 mg/kg b.wt.

The extract was dissolved in Millipore water and administered for 21 days at a two different dose level i.e. 200 mg/kg and 400 mg/kg given orally. The blood was collected from tail vein under light Ketamine anesthesia and was centrifuged at 3000 rpm for 10 minutes. Serum glucose, serum triglycerides and serum total protein were analyzed. The parameters such as body weight and fluid intake were also taken into consideration for this study.

Estimation of in- *vivo* antioxidant enzymes levels

Tissue homogenization

Pancreas was excised by minimal trauma weighed accurately and was collected in ice- cold container containing 10 % potassium chloride solution in tissue homogenizer. Homogenate was taken for further in vivo study.

Estimation antioxidant enzyme level

Homogenate was used to estimate the important antioxidant enzymes such as Catalase, SOD, TBA-RS, and Glutathione[20, 22].

Table 1: Qualitative and quantitative phytochemical analysis of *Curcuma aromatica* extracts

Tests	*Curcuma aromatica* extracts						
	Petroleum ether	Toluene	chloroform	Ethyl acetate	Acetone	Ethanol	Water
Alkaloids	-	-	-	-	-	+	+
Carbohydrates	-	-	-	-	+	+	+
Phytosterols	+	+	+	-	-	-	-
Fixed oil and fats	+	+	-	+	+	-	-
Saponins	-	-	+	-	-	+	-
Tannins	-	-	-	-	-	+	+
Protein and amino acids	-	+	-	-	-	+	+
Glycosides	-	+	-	+	-	-	-
Flavonoids	+	+	+	+	+	+	+
Volatile oils	+	+	-	-	-	-	-
Steroids	-	+	-	-	-	-	-
Terpenoids	+	+	-	-	-	-	-
Total amount of phenols(%) mg/g of Ascorbic acid	90.8±1.76	265±1.08	98.9±1.78	231±1.46	188±0.98	83.5±0.13	77.3±1.69
Total amount of flavonol(%) mg/g) of rutin	55±1.13	175±1.56	45±0.98	132±0.67	110±1.09	103±1.05	36±1.45

+- presence - - absence

Table 2: Anti oxidant activity of *Curcuma aromatica* extracts

Plant extract	DPPH method	Lipid peroxide method	Hydrogen peroxide radical scavenging method	ABTS radical scavenging method
			IC_{50} Values µg/ml	
Petroleum ether	229.5±1.12	247±1.67	137±1.78	8.067±1.12
Toluene	50.62±0.998	75±0.87	43.75±1.24	0.038±1.54
Chloroform	235.56±0.634	265±1.43	250±0.65	9.485±0.76
Ethyl acetate	118.75±0.667	136±1.09	69±1.08	0.134±0.87
Acetone	150.55±1.345	172±0.98	123.43±0.95	6.896±1.65
Ethanol	132.5±1.876	153±0.67	72.50±1.90	0.244±1.86
Water	427.75±1.436	447±1.16	270±0.01	11.674±1.98
Standard	2.75 ± 0.09^a	-------	36.16 ± 0.90^b	11.25 ± 1.43^a
Standard				

a- Ascorbic acid b- rutin

Table 3: Glucose uptake by isolated rat hemi diaphragm

Group	Incubation medium	Glucose uptake (mg/g/30 minutes)
Group-1	Tyrode solution with 2 % glucose (control group)	30.75±0.21**
Group-2	Tyrode solution with 2 % glucose + Insulin (0.4 units/ml)	32.5±0.34**
Group-3	Tyrode solution with 2 % glucose+ Toluene extract of *Curcuma aromatica* (2mg/ml)	68.75±0.36**
Group-4	Tyrode solution with 2 % glucose+ Toluene extract of *Curcuma aromatica*(2mg/ml) + Insulin(0.4 units/ml)	87.±0.37**

Values are mean ± SEM, n=5,*p< 0.05, **p<0.01, ***p<0.001 as compared to control and standard.

Table 4: Effect of administration of toluene extract of *Curcuma aromatica* on body Weight and fluid intake

Group	Body weight (g)		Fluid intake (ml/day)
	Before treatment	After treatment	
Untreated control	194±1.88	220.5±1.839	22.047±0.247
Diabetic control	222.66±2.33	168.5±2.513	75.288±0.223
Diabetic + Glibenclamide (10 mg/kg)	216.66±1.745	236.33±1.96	53.610±0.375
Diabetic + Toluene extract of *Curcuma aromatica* 200 mg/kg	213±1.932	225.16±1.078	56.436±0.166
Diabetic+ Toluene extract of *Curcuma aromatica* 400 mg/kg	206.16±2.176	233.6±1.476	42.43±0.357

In normal control group, significant weight gain was observed in comparison to diabetic group. The extracts treated groups also shown significant weight gain as compared to diabetic groups

All the values are expressed as mean ±SEM (n=5) (P<0.01) significant as compared to standard (diabetic control)

Table 5: Effect of Toluene extract of *Curcuma aromatica* on α-Glycosidase activity

Name of the Extract	Glucose (mg/dl)
Toluene extract *of Curcuma aromatica*	41
Acarbose (Standard)	49

Table 6: *In- vivo* antidiabetic activity of Toluene extract of *Curcuma aromatica*

Group	Serum Glucose level (mg/dl)	Serum Triglycerides(mg/dl)	Total protein (mg/dl)	Total cholesterol (mg/dl)
Untreated control	88.83±1.014	54.83±1.138	6.254±0.28	120.66±1.29
Diabetic control	410±2.045$^{\#}$	85.66±0.88$^{\#}$	3.09±0.12$^{\#\#}$	292.33±1.64$^{\#\#}$
Diabetic + Glibenclamide (10mg/kg)	114.83±1.302**	74.83±1.35***	6.11±0.342***	118±1.09***
Diabetic + Toluene extract 200mg/kg b.wt	116.5±1.232**	64.16±1.53***	5.78±0.49***	134.50 ±1.62***
Diabetic + Toluene extract 400 mg/kg b.wt	113.85±0.986**	69.66±4.63***	5.12±0.24***	125.67±0.60***

All the values are expressed as mean ±SEM (n=5) *** P<0.001 as compared to diabetic control, ##P<0.01 as compared to untreated control.

Table 7: Effect of administration of Toluene extract of *Curcuma aromatica* on GSH, SOD, TBARS

Groups	GSH (µg/g) of protein	SOD (Unit/ min/ gm tissue)	CAT (µmol of H_2O_2/min/gm tissue)	TBARS nM MDA/mg of tissue
Untreated control	11.54±0.354	9.54±1.09	45.23±1.67	0.317±0.084
Diabetic control	2.17±0.22$^{\#\#}$	4.03±0.28#	28.65±1.98$^{\#}$	0.52±0.02$^{\#\#\#}$
Diabetic+ Glibenclamide (10 mg/kg)	11.01±0.77**	8.78±1.06**	42.62±0.97**	0.301±0.056***
Diabetic+ Toluene extract of *Curcuma aromatica* 200 mg/kg b.wt	7.31±0.109**	6.05±0.30**	33.43±3.25**	0.427±0.034**
Diabetic+ Toluene extract of *Curcuma aromatica* 400 mg/kg b.wt	10.36±0.099**	6.95±0.57**	39.34±1.85**	0.325±0.012***

All values are expressed as mean ± SEM (n=5), ***P<0.001, **P<0.01, *P<0.05, ### P<0.001, ##P< 0.01, #P<0.05 as compared to untreated control

RESULT AND DISCUSSION

Phytochemical analysis

Phytochemical analysis for the successive extraction of *Curcuma aromatica* with different solvents showed the presence of various constituents such as alkaloids, carbohydrates, phytosterols, fixed oil, fats, saponins, tannins, protein amino acids, glycosides, flavonoids, volatile oils, steroids and terpenoids. Total phenol (265±1.08 mg/g of Ascorbic acid) and flavonol content (175±1.56 mg/g of rutin) were present in toluene extract of *Curcuma aromatica* which was found to be maximum. The results for both qualitative and quantitative Phyto chemical analysis were tabulated in table 1.

In vitro antioxidant activity

Among all the extracts that were prepared by the successive extraction process from *Curcuma aromatica,* in vitro antioxidant studies revealed that Toluene extract of *Curcuma aromatica* showed potent scavenging activity by DPPH method with the IC_{50} value of 50.62±0.998 µg/ml, by lipid per oxidation method with the IC_{50} value of 75±0.87 µg/ml, hydrogen peroxide radical scavenging activity with the IC_{50} value 43.75±1.24 µg/ml, and ABTS radical scavenging method with the IC_{50} value 0.038±1.54 µg/ml. The results for the in vitro antioxidant activity were tabulated in the table 2.

Glucose uptake by isolated rat hemi diaphragm

In the presence of Insulin, isolated rat hemi diaphragm showed an increase in glucose up take from 30.75±0.21 to 32.5±0.34 mg/g/30 minutes. After the treatment with toluene extract of *Curcuma aromatica*, glucose uptake found to increase up to 68.75±0.36. In the presence of insulin and toluene extract, glucose up take was found to be maximum of 87±0.37 mg/g/30 minutes. The glucose uptake by rat hemi diaphragm was significantly more in all groups when compared to control. The combined effects of the extract and insulin was found to be P<0.01, which is significant. The results for the glucose uptake by the rat hemi diaphragm were tabulated in the table 3.

α-Glucosidase activity

Acarbose a carbohydrate inhibitor, when administered showed delayed in glucose absorption Acarbose, an α Glucosidase inhibitors, reduces intestinal absorption of carbohydrates and there by blunt the postprandial rise in plasma glucose in diabetic patients. However flatulence and abdominal bloating due to malabsorption limits its potential as favored medication[23]. The toluene extract of *Curcuma aromatica* was found to be effective in inhibiting the α-Glucosidase (59%) when compared to Acarbose (51%). The result are tabulated in table 5.

***In vivo* antidiabetic activity**

After the treatment with toluene extract of *Curcuma aromatica* 200 mg/kg b.wt and 400 mg/kg b.wt in diabetic induced rats, the body weight was found to be increased from 213±1.932 to 225.16±1.078 and from 206.16±2.176 to 233±1.476 respectively. Fluid intake was also found to increase up to 56 ml/day and 42 ml/day for the groups treated with the toluene extract at the dose of 200 mg/kg b.wt and 400 mg/kg b.wt respectively when compared to untreated group (22ml/day). Similar effect was observed in the diabetic group treated with Glibenclamide (10mg/kg b.wt). In this group the body weight was found to be increased from 216.66±1.745 to 236.33±1.96 and fluid intake was also found to be increased up to 53 ml/day when compared to the untreated control groups. The results were tabulated in the table 4.

Serum glucose level in the diabetic control group was found to be 410±2.045 mg/dl, where as the diabetic group treated with Glibenclamide (10mg/kg b.wt) and toluene extract of *Curcuma aromatica* showed the serum glucose level as 114.83±1.302, 116.5±1.232 and 113.85±0.986mg/dl respectively. In maintaining the serum blood glucose level, Glibenclamide and toluene extract of *Curcuma aromatica* (both 200 and 400 mg/kg b.wt) showed almost similar effect. The results produced by the toluene extract of *Curcuma aromatica* were found to be dose independent.

Serum triglycerides level were also found to be decreased from 85.66±0.88 mg/dl (diabetic control group) to 64.16±1.53, 69.66±4.63, in diabetic groups treated with toluene extract at the dose of 200 mg/kg b.wt and 400 mg/kg b.wt respectively. Toluene extract of *Curcuma aromatica* found to possess good activity in reducing the serum triglyceride level when compared to the diabetic group treated with Glibenclamide (74.83±1.35 mg/dl) at the dose of 10 mg/kg b.wt.

Total protein level was found to be increased from 3.09±0.12 mg/dl (diabetic control) to 5.78±0.49, 5.12±0.24 mg/dl in the groups treated with toluene extract of *Curcuma aromatica* at the dose to 200 and 400 mg/kg b.wt respectively. The diabetic group treated with Glibenclamide (10 mg/kg b.wt) almost restored the protein level 6.11±0.342 as that of untreated control groups (6.254±0.28). Toluene extract of *Curcuma aromatica* at both the doses (200 and 400 mg/kg b.wt) was found to effective in increasing the total protein level but when compared to Glibenclamide (10 mg/kg b.wt), it was found to be less effective in restoring the total protein level. The results were tabulated in the table 6.

Total cholesterol level was found to be decreased from 293.33±1.64 mg/dl (diabetic control) to 118±1.09 mg/dl (Glibenclamide treated groups), 134.50±1.62, and 125.67±0.60 in toluene extract of *Curcuma aromatica* at the dose of 200 and 400 mg/kg b.wt respectively. The extract was found to be more effective in reducing the total cholesterol at the dose of 400 mg/kg b.wt but less effective when compared to Glibenclamide at the dose of 10 mg/kg b.wt. All these results were tabulated in the table 6.

***In vivo* antioxidant enzyme level**

GSH enzyme level was found to be increased from 2.17±0.22 (diabetic control) to 7.31±0.109 10.36±0.099 µg/g of protein for the groups treated with toluene extract of *Curcuma aromatica* at the dose of 200 and 400 mg/kg b.wt respectively. Diabetic group treated with Glibenclamide (10mg/kg b.wt) was found to be effective and capable of restoring the GSH level similar to that of untreated control (11.54±0.354 µg/g of protein). The toluene extract at the dose of 400 mg/kg b.wt was found to be more effective in restoring GSH but less effective when compared to the Glibenclamide (10 mg/kg b.wt)

SOD activity was found to be increased from 4.08±0.28 (diabetic control) to 6.05±0.30, 6.95±0.57 in the groups treated with the toluene extract of *Curcuma aromatica* at the dose of 200 and 400 mg/kg b.wt respectively. Diabetic group treated with Glibenclamide (10 mg/kg b.wt) SOD activity was found to be doubled (8.78±1.06) when compared to the diabetic control. The extract at the dose of 400 mg/kg b.wt was found to be effective in increasing the SOD activity but it was found to be less effective when compared with the Glibenclamide. The results were expressed as unit/min/gm tissue.

Catalase activity was found to be increased from 28.65±1.98 (diabetic control) to 33.43±3.25, 39.34±1.85, 42.62±0.97 in the groups treated with toluene extract of *Curcuma aromatica* at the dose of 200 and 400 mg/kg b.wt, and Glibenclamide respectively. The extract at the dose of 400 mg/kg b.wt was found to be effective in increasing the CAT activity but less effective when compared to Glibenclamide, the results were expressed in µmol of H_2O_2/min/gm tissue

TBARS effect was decreased from 0.52±0.02 (diabetic control) to 0.427±0.034, 0.325±0.012 in the groups treated with toluene extract of *Curcuma aromatica* at the dose of 200 and 400 mg/kg b.wt. The extract at the dose of 400 mg/kg b.wt was found to be effective in decreasing the TBARS activity and the results were found to be almost similar in the diabetic group treated with Glibenclamide (10 mg/kg b.wt) 0.301±0.056 as well as in untreated control group (0.317±0.084). The results were expressed in MDA/mg of tissue. The results are tabulated in the table 7.

CONCLUSION

Regulation of blood glucose level in diabetes can prevent the various complications associated with the disease [24]. The long term maintenance of plasma glucose concentration under a variety of nutritional conditions, energetic demands is one of the most important and closely regulated processes in the mammalian species. Whole body homeostatic is the product of input from three primary tissue, the liver, skeletal muscle and β- cells of pancreas. The liver function as the primary source of

endogenous glucose production in the body under conditions of increased peripheral demand through the breakdown of glycogen store (Glycogenolysis) and synthesis of new glucose (Gluconeogenesis) from a variety of precursor molecule. The liver can also take up the glucose carbon as glycogen (Glycogenesis). One of the important sites of glucose uptake is isolated rat hemi diaphragm.

Oxidative stress, the imbalance between the cellular production of oxidants and antioxidant defense within the cells can play an important role in the multifactorial etiology of skeletal muscle, insulin resistance [24]. Plasma levels of hydrogen peroxide, one of the markers in oxidative stress, are higher in subject with type 2 diabetic compared to non- diabetic control. More definite evidence linking oxidative stress, insulin resistance comes from cell cultures and isolated muscle incubation studies. Prolong exposure to a low- grade oxidant stress (H_2O_2) markedly decrease insulin stimulated glucose metabolism. Medicinal plants are used in several countries to manage diabetes mellitus which are thought to be less toxic than allopathic hypoglycemic drugs, plant medicine are also easily available and affordable to many peoples [26]. Selection of scientific and systematic approach for the biological evaluation of plant products based on their use in the traditional system of medicine forms the basis for an ideal approach in the development of new drugs from the plant[17]. To obtain the maximum effect, therapy with the plant products should be continued for longer duration[26]. Two different doses (200 and 400 mg/kg b.wt) were selected, the studies were carried out for 20 days and the extract was administered as a single dose orally. Toluene extract of *Curcuma aromatica* exhibited significant antioxidant and antidiabetic activity in both in vitro and in vivo models. Therefore from the results it is significant that *Curcuma aromatica* can be used as alternative herbal medicine in the treatment of diabetes and diabetic complication.

Further studies with estimation of insulin, insulin receptors and higher in vivo models may find more insight into the mechanism of antidiabetic and antioxidant activity of Toluene extract of *Curcuma aromatica*.

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