Research Article
www.ijrap.net

ANTIOXIDANT AND ANTIDIABETIC ACTIVITY OF CURCUMA AROMATICA
Ammayappan Rajam Srividya*, Palanisamy Dhanabal, Parthkumar Bavadia, Vaithiyalingam Jagannathan Vishnuvarthan, Muthuredy Natarajan Sathish Kumar
JSS College of Pharmacy, Rockland’s, Ootacamund, Tamilnadu, India

Received on: 05/03/12 Revised on: 21/04/12 Accepted on: 02/05/12

*Corresponding author
A.R. Srividya, Assistant professor, Department of Pharmaceutical Biotechnology, JSS College of Pharmacy, Ootacamund TamilNadu, India.
E-mail: Pharmarsividya@yahoo.com

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 IC₅₀ value of 50.62±0.998 μg/ml, by lipid per oxidation method with the IC₅₀ Value of 75.0±0.87 μg/ml, hydrogen peroxide radical scavenging activity with the IC₅₀ Value 43.75±1.24 μg/ml, and ABTS radical scavenging method with the IC₅₀ 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 64.16 mg/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 complications1-3 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 complication3

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 water4

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 phytoconstituents5.

Quantitative phytochemical analysis
Phenol and flavonol are considered to be the most important phytoconstituents that are responsible for the pharmacological activities. Total phenol content and total flavonol content were estimated6.

In-vitro antioxidant evaluation
Antioxidant studies were performed by Diphenyl picryl hydrazyl radical scavenging method7, ABTS radical scavenging method7, Lipid per oxidation (LPO) assay8-10, and scavenging of hydrogen peroxide radical11,12.

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°C in an atmosphere of 100 % O₂ with shaking13,14.

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-Glucosidase 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 IC50 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°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 Streptozotocin19-21. 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 Glutathione20,22.

---

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

<table>
<thead>
<tr>
<th>Tests</th>
<th>Curcuma aromatica extracts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Petroleum ether</td>
</tr>
<tr>
<td>Alkaloids</td>
<td>-</td>
</tr>
<tr>
<td>Carbohydrates</td>
<td>-</td>
</tr>
<tr>
<td>Phytosterols</td>
<td>+</td>
</tr>
<tr>
<td>Fixed oil and fats</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>-</td>
</tr>
<tr>
<td>Protein and amino acids</td>
<td>-</td>
</tr>
<tr>
<td>Glycosides</td>
<td>-</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
</tr>
<tr>
<td>Volatile oils</td>
<td>+</td>
</tr>
<tr>
<td>Steroids</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>+</td>
</tr>
<tr>
<td>Total amount of phenol(%) mg/g of Ascorbic acid</td>
<td>90.8±1.76</td>
</tr>
<tr>
<td>Total amount of flavonol(%) mg/g of rutin</td>
<td>55±1.13</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>DPPH method</th>
<th>Lipid peroxide method</th>
<th>Hydrogen peroxide radical scavenging method</th>
<th>ABTS radical scavenging method</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC50 Values µg/ml</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Petroleum ether</td>
<td>229.5±1.12</td>
<td>247±1.67</td>
<td>137±1.78</td>
<td>8.067±1.12</td>
</tr>
<tr>
<td>Toluene</td>
<td>50.62±0.998</td>
<td>75±0.87</td>
<td>43.75±1.24</td>
<td>0.03±1.54</td>
</tr>
<tr>
<td>Chloroform</td>
<td>235.56±0.634</td>
<td>265±1.43</td>
<td>230±0.65</td>
<td>9.48±0.76</td>
</tr>
<tr>
<td>Ethyl acetate</td>
<td>118.75±0.667</td>
<td>136±1.09</td>
<td>69±1.08</td>
<td>0.13±0.87</td>
</tr>
<tr>
<td>Acetone</td>
<td>150.51±1.345</td>
<td>172±0.98</td>
<td>123.43±0.95</td>
<td>6.89±1.65</td>
</tr>
<tr>
<td>Ethanol</td>
<td>132.5±1.876</td>
<td>153±0.67</td>
<td>72.50±1.90</td>
<td>0.24±1.86</td>
</tr>
<tr>
<td>Water</td>
<td>427.75±1.436</td>
<td>447±1.16</td>
<td>270±0.01</td>
<td>11.67±1.98</td>
</tr>
<tr>
<td>Standard</td>
<td>2.75±0.097</td>
<td>-</td>
<td>36.16±0.90</td>
<td>11.25±1.43</td>
</tr>
</tbody>
</table>

*IC50* Values µg/ml

- Ascorbic acid
- rutin

---

**Ammayappan Rajam Srividya et al / LIRAP 3(3), May – Jun 2012**
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 0.383±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 uptake 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 uptake 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.

**Table 3: Glucose uptake by isolated rat hemi diaphragm**

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

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

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 Phytochemical 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 0.383±1.54 μg/mL. The results for the in vitro antioxidant activity were tabulated in the table 2.
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. 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 213±1.932 mg/dl when compared to the untreated control group (216.66±1.745) with the toluene extract of Curcuma aromatica was found to be effective in decreasing serum glucose level as 206.16±2.176 mg/dl when compared with the Glibenclamide (10mg/kg b.wt). 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 respectively. Catalase activity was found to be almost similar in the diabetic group treated with Glibenclamide (10 mg/kg b.wt) and toluene extract of Curcuma aromatica at the dose of 200 and 400 mg/kg b.wt.

CONCLUSION

Regulation of blood glucose level in diabetes can prevent the various complications associated with the disease. 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 homoeostatic 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 hemidiaphragm.

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 (H2O2) 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.

ACKNOWLEDGEMENT
The authors are thankful to J.S.S. Mahavidyapeeda, J.S.S. University, Dr. B. Suresh, Vice chancellor of J.S.S. University, Dr. K. Elango, Principal of JSS College of Pharmacy, Ooty for providing the facilities to carry out this research.

REFERENCES

Source of support: Nil, Conflict of interest: None Declared

Ammayappan Rajam Sridivya et al / IJRAP 3(3), May – Jun 2012