ANTI-DIABETIC EFFECTS OF TURMERIC IN ALLOXAN INDUCED DIABETIC RATS.
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ABSTRACT: OBJECTIVE AND BACKGROUND: Turmeric (Curcuma longa) is one of the common constituents of our daily food. The present study was undertaken to evaluate the anti-diabetic effects of ethanolic extract of Rhizomes of curcuma longa in alloxan induced diabetic rats and compared with Pioglitazone, which is the standard anti-diabetic agent. METHODS: Alloxan monohydrate is used to induce diabetes mellitus in albino rats in the dose of 120mg/kg i.p. and anti-diabetic activity of turmeric was studied by following study design. The rats are divided into 6 groups and each group contains 6 rats (N= 6) as follows.

Group I: Normal control
Given normal saline (10ml/kg/day).

Group II: Diabetic control
Given normal saline (10ml/kg/day).

Group III: Euglycemic rats
Given turmeric extract (300mg/kg/day).

Group IV: Diabetic rats
Given turmeric extract (300mg/kg/day).

Group V: Diabetic rats
Given turmeric extract (500mg/kg/day).

Group VI: Diabetic rats
Given Pioglitazone (6mg/kg/day)

Study was conducted for a period of 28 days and all the drugs were given orally once daily. Blood glucose levels were estimated at 1, 3, 5, 7 hrs. (acute study) and 7, 14, 21, 28 days (chronic study). The body weights of the rats in every groups recorded weekly and general behavior and health of the animal were monitored carefully. The data was analyzed statistically using student’s paired and unpaired t-test. RESULTS: Ethanolic extract of turmeric produced significant (p < 005) decreases in blood glucose levels on 7th, 14th, 21st and 28 days in diabetic
CONCLUSION: Present study revealed that curcuma longa possesses anti-diabetic activity can be used to treat diabetes patients.

KEY WORDS: Curcuma longa, Turmeric, Alloxan-induced rats, Pioglitazone, Diabetes mellitus.

INTRODUCTION: Diabetes mellitus is a chronic metabolic disorder characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both. It is associated with high morbidity and mortality from long-term micro vascular and macro vascular complication.

Diabetes mellitus is among the most common endocrine disorder in developed and developing countries and has become a major health problem in the modern world. India is the diabetic capital of the world. India leads the world with the largest number of diabetic patients i.e. 40.9 million in the year 2007 and is predicted to rise to 69.9 million by the year 2025. In the world, for every 10th second 2 persons will develop diabetes and 1 person dies from diabetes related causes. There is increased risk of atherogenic dyslipidemia and hypertension in diabetes; hence increases prevalence of coronary artery disease, heart failure, stroke in diabetic population.

Despite the availability of many medications to treat diabetes mellitus, still we are unable to check the growing incidence and prevalence of this modern pandemic. The currently available anti-diabetic drugs have number of adverse effects, so there is increased demand for herbal/natural products which are supposed to have no or less side effects. Also, following the recommendation of WHO expert committee on diabetes mellitus on beneficial uses of medicinal plants in the treatment of diabetes mellitus, research on medicinal plants has gained the momentum.

Indian traditional medicine is one of the richest medicinal systems among those available around the world. It is important to know that the oral hypoglycemic drug metformin was discovered from the traditional plant Galega ethlinasis or Galega officinalis. Earliest description of curative properties of medicinal plants found in Rig Veda (2500-1800 BC). Charaka Samhita and Sushruta Samhita give extensive description on various medicinal herbs. Turmeric is well known condiment in the world. It is a prime ingredient in curry powder and figures heavenly in Asian cuisines, aptly known as “Golden spice” of India. Turmeric plants were cultivated by Harappan civilization in 3000 BC. Turmeric is extensively used in Ayurveda, Unani and Siddha medicinal systems since from Vedic-ages and also as home remedy for various ailments. It is also used in Indian ritual and worship.

Turmeric is host of medical properties with its wide spectrum of actions such as Anti-inflammatory, anti-fungal, anti-mutagenic, anti-carcinogenic, anti-coagulant, anti-hepatotoxic, anti-fertility, anti-protozoa, anti-viral, anti-fibrotic, anti-venom, anti-ulcer, anti-hypertensive, anti-diabetic and hypo-cholesterolemic / hypo-lipidemic properties. Recently explored uses Alzheimer’s disease, Rheumatoid arthritis, Multiple sclerosis, Inflammatory bowel disease, Cataract and HIV.

The comparative study of anti-diabetic effects of turmeric extract with that of pioglitazone has not been documented. In the view of these reports, it seemed interesting to search for more efficient, safe and cost effective single anti-diabetic agent, which can also improve the lipid profile in diabetic patients. Hence the present is taken up to evaluate anti-diabetic activity of ethanolic extract of turmeric in alloxan induced diabetic rats and compared with the effects of pioglitazone, which is the standard anti-diabetic agent.
MATERIALS AND METHODS: STUDY CENTER: The present study was carried out in the Postgraduate Research Laboratory Department of Pharmacology, M R Medical College, Gulbarga after obtaining the permission from the Institutional Animal Ethics Committee (HKES/MRMCG/256/2008 Dated 03/11/2008) of M.R. Medical College, Gulbarga, Karnataka.

Materials
1. Adult albino rats weighing 180-200 grams of either sex.
2. Glucometer and Strips (Accu-chek Active Glucometer, Roche Diagnostics, Germany).
3. Needle, Syringes, Feeding tubes, Rat holder.
4. Soxhlet Apparatus for preparing the Ethanol extraction of Turmeric.
5. Drugs-
   a. Alloxan Monohydrate obtained from Otto Kemi Industry, Mumbai, India.
   b. Ethanol Extract of Rhizomes of Turmeric.
   c. Pioglitazone Hydrochloride (pure form) obtained from Bio-con Pharmaceuticals Bangalore.
   d. Ethanol (99.99%) of Changshu Yangyuan Chemicals, obtained from Venkatesh Chemicals, Gulbarga.
   e. Vehicle: Normal Saline (0.9%) and Tween80 (Hi Media Laboratories Pvt Ltd. Mumbai).
6. Proforma

Experimental Animals used in the study
- Study was carried out in healthy albino rats of Wister strain (Rattus norvegicus) of either sex.
- Body weight of all the animals was in the range of 180-200gms each.
- They were acclimatized to the laboratory conditions before carrying out experimental work in a well-ventilated animal house under natural photoperiod conditions for a period of 1 week.
- Total number of animals included in the study is 36, out of which diabetes was induced in 24 rats and 12 rats were euglycemic, housed separately in groups with distinct identity throughout the study in standard conditions of temperature and a 12 hours light-dark cycle.
- Sources: Animals were procured from Central Animal House, MR Medical College, Gulbarga, Karnataka.
- Diet: Animals were maintained on standard rat pellet diet. Water was given ad libitum during the entire period of the study.
- CPCSEA (Committee for the purpose of control and supervision of experiments on animals) Guidelines for laboratory animal facilities were strictly adhered.

INDUCTION OF DIABETES MELLITUS: A single dose (120mg/kg i.p) of freshly prepared solution of Alloxan Monohydrate (dissolved in Normal Saline, Citrate buffer, pH 4.5) was administered to overnight fasting rats for induction of type-2 diabetes mellitus in the rats. Control rats were similarly injected with normal saline. To prevent fatal hypoglycemia as a result of massive pancreatic insulin release, Alloxan administered rats were provided with 10% Glucose solution after 6 hours for next 24 hours. Fasting blood glucose level was checked after 48-72 hours when the animals became hyperglycemic reflected by glycosuria, hyperglycemia, polyphagia, polydipsia and progressive loss of body weight as compared with normal rats. Blood glucose levels between 200-350 mg/dl were selected as diabetic.
PREPARATION OF EXTRACT: Fine powder of dry rhizomes of Turmeric was purchased from local market and was packed into thimble of filter paper and put in Soxhlet extractor in 5 batches of 200gm each and subjected to continuous extraction with 99.99% ethanol for about 48 hours at 60°C till solvent in the siphon tube becomes colorless and it took around 8-10 cycles/200 gram powder. Small porcelain pieces were added to the flask to avoid bumping of solvent. The solvent so obtained was distilled off and was heated evaporated using water bath/magnetic heart stirrer to get concentrated thick extract which is later diluted in Tween80 and administered to the rat by oral route once daily.

EXPERIMENTAL DESIGN
The experiment was carried out for a period of 28 days. For this purpose, 36 healthy albino rats were selected and grouped as follows:

Group I: Normal control
   Given normal saline (10ml/kg/day).

Group II: Diabetic control
   Given normal saline (10ml/kg/day).

Group III: Euglycemic rats
   Given turmeric extract (300mg/kg/day).

Group IV: Diabetic rats
   Given turmeric extract (300mg/kg/day).

Group V: Diabetic control
   Given turmeric extract (500mg/kg/day).

Group VI: Diabetic rats
   Given Pioglitazone (6mg/kg/day)

Body weight of rats in every group was recorded weekly. Experimental rats were carefully monitored every day for general health and behavior.

Study was carried out in 2 phases to assess acute and chronic effect of Turmeric extract in euglycemic and hyperglycemic rats.

Acute study: After a single dose administration of Turmeric extract, blood samples were collected and estimated for blood glucose levels at the end of 0, 1, 3, 5 and 7 hours by using glucometer.

Chronic study: Treatment was continued for 28 days with once daily administration of Turmeric extract. Blood glucose levels were estimated at the end of 7, 14, 21, and 28 days.

ORAL ADMINISTRATION OF EXTRACT /DRUG: As ethanol extract of Turmeric and Pioglitazone were insoluble in water, they are suspended in 5% Tween80 (w/v), administered orally according to the dosage in the respective groups using an intra-gastric feeding tube.

Method of collection of blood samples: Tail cut method from tip of the rat tail.

Estimation of blood glucose
Blood glucose estimation was done by using Accu-check Active glucometer. It uses glucose oxidase specific strips and works on principle called as Reflectance Photometry. It is easy to use, quick to perform and reliable. There is a reasonable co-relation between laboratory results and those obtained with glucometer.
The test strip is inserted into the glucometer and the blood sample is directly placed on the strip. The result i.e., blood glucose level appeared on the screen within a few seconds in mg/dl.

STATISTICS: The results were analyzed by employing the Paired ‘t’ test and the Unpaired ‘t’ test.

RESULTS: The study was carried out to evaluate the effects of ethanol extract of Turmeric on blood glucose levels in Alloxan induced diabetic albino rats.

Table I Effect of ethanolic extract of Turmeric on blood glucose levels of euglycemic rats (Group III)

<table>
<thead>
<tr>
<th>Rat</th>
<th>0 hrs.</th>
<th>1hrs</th>
<th>3hrs</th>
<th>5hrs</th>
<th>7hrs</th>
<th>7days</th>
<th>14days</th>
<th>21days</th>
<th>28days</th>
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<tbody>
<tr>
<td>1</td>
<td>96</td>
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<td>2</td>
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<td>90</td>
<td>92</td>
<td>94</td>
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<td>3</td>
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<td>89</td>
<td>88</td>
<td>90</td>
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<td>90</td>
</tr>
<tr>
<td>Mean ±SD</td>
<td>92.5 ±4.9</td>
<td>90.8 ±1.9</td>
<td>91.66 ±2.68</td>
<td>89.2 ±4.6</td>
<td>89.6 ±2.9</td>
<td>90.5 ±2.92</td>
<td>91.3 ±1.49</td>
<td>90 ±2.4</td>
<td>88.58 ±3.29</td>
</tr>
<tr>
<td>‘t’ test value comparing with 0 hour</td>
<td>0.79</td>
<td>0.38</td>
<td>1.18</td>
<td>1.26</td>
<td>0.86</td>
<td>0.58</td>
<td>1.14</td>
<td>1.63</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

By the data analysis, it is very clear that Turmeric extract (300mg/kg) has no statistically significant effect on blood glucose levels of euglycemic rat.

By using paired ‘t’ test when we compared the initial values (at 0 hour) with 1 hour, 3 hour, 5 hour, 7 hour, 7 days, 14 days, 21 days and 28 days and p values at all the time intervals found to be > 0.05, i.e. statistically not significant.

Table –II Effect of ethanolic extract of Turmeric 300mg/kg on blood glucose levels of diabetic rats (Group IV)

<table>
<thead>
<tr>
<th>Rat</th>
<th>0 hrs.</th>
<th>1hrs</th>
<th>3hrs</th>
<th>5hrs</th>
<th>7hrs</th>
<th>7days</th>
<th>14days</th>
<th>21days</th>
<th>28days</th>
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<td>265</td>
<td>280</td>
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<td>2</td>
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<td>275</td>
<td>260</td>
<td>282</td>
<td>290</td>
<td>235</td>
<td>198</td>
<td>138</td>
</tr>
</tbody>
</table>
**Table III** Effect of ethanolic extract of Turmeric 500mg/kg on blood glucose levels of diabetic rats (Group V)

<table>
<thead>
<tr>
<th>Rat</th>
<th>0 hrs.</th>
<th>1hrs</th>
<th>3hrs</th>
<th>5hrs</th>
<th>7hrs</th>
<th>7days</th>
<th>14days</th>
<th>21days</th>
<th>28days</th>
</tr>
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<td>282</td>
<td>286</td>
<td>290</td>
<td>230</td>
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<td>125</td>
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<tr>
<td>2</td>
<td>302</td>
<td>286</td>
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<td>298</td>
<td>240</td>
<td>223</td>
<td>186</td>
<td>170</td>
<td>130</td>
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<td>3</td>
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<td>232</td>
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<td>269</td>
<td>292</td>
<td>294</td>
<td>258</td>
<td>196</td>
<td>175</td>
<td>152</td>
<td>98</td>
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<td>5</td>
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<td>284</td>
<td>208</td>
<td>220</td>
<td>200</td>
<td>180</td>
<td>145</td>
<td>105</td>
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<td>226</td>
<td>220</td>
<td>182</td>
<td>160</td>
<td>128</td>
<td>118</td>
</tr>
</tbody>
</table>

Turmeric extract (300mg/kg) has no significant effect on blood glucose levels of diabetic rats in acute study (i.e. 1hr, 3hr, 5hr, 7hr and 7 days). Turmeric controls the blood glucose only in the acute diabetic rats but not in the chronic diabetic rats in lower dose i.e.300mg/kg/day. By using paired 't' test, when we compared the initial values at 0 hour with 1 hr, 3 hr, 5hr, 7hr, 7 days and p value at all the time intervals found to be > 0.05, i.e. statistically not significant. In the chronic study, i.e. 14 days p < 0.01, 21 days and 28 days p < 0.001 i.e. very highly significant.
Turmeric extract (500mg/kg) has no significant effect on blood glucose levels of diabetic rats in acute study i.e. 1 hr., 3 hr., 5 hr., 7 hr. By using paired ‘t’ test when we compared initial values at 0 hr. with 1 hr., 3 hr., 5 hr., 7 hr. and p values at all the time intervals found to be > 0.05 i.e. statistically not significant. In chronic study i.e. 7 days to 28 days, p value < 0.001 i.e. very highly significant.

Table IV Effect of Pioglitazone 6mg/kg on blood glucose levels of diabetic rats (Group VI)

<table>
<thead>
<tr>
<th>Rat</th>
<th>0 hrs.</th>
<th>1hrs</th>
<th>3hrs</th>
<th>5hrs</th>
<th>7hrs</th>
<th>7days</th>
<th>14days</th>
<th>21days</th>
<th>28days</th>
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<td>300</td>
<td>294</td>
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<td>6</td>
<td>276</td>
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<td>206</td>
<td>202</td>
<td>112</td>
<td>128</td>
<td>113</td>
<td>108</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>282.03±19.13</td>
<td>271.66±18.16</td>
<td>254.86±26.12</td>
<td>206.6±9.6</td>
<td>202.28±13.22</td>
<td>120±7.6</td>
<td>119.5±4.89</td>
<td>110.16±7.44</td>
<td>101.5±7.8</td>
</tr>
<tr>
<td>‘t’ test value comparing with 0 hour</td>
<td>0.96</td>
<td>1.94</td>
<td>8.62</td>
<td>8.43</td>
<td>19.28</td>
<td>20.26</td>
<td>20.70</td>
<td>21.48</td>
<td></td>
</tr>
<tr>
<td>P value</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td>P &lt; 0.001</td>
<td></td>
</tr>
</tbody>
</table>

Pioglitazone has no significant effect on blood glucose levels of diabetic rats in acute study i.e. 1 hr. and 3 hr. By using paired ‘t’ test, when we compared the initial values at 0 hr. with 1 hr., 3 hr. p values found to be > 0.05 i.e. statistically not significant. But in chronic study after 21 days to 28 days pioglitazone has significant effect on blood glucose levels of diabetic rats in chronic study, p < 0.001, i.e. very highly significant.

Table V Recording of body weight changes in experimental rats at the end of every week

<table>
<thead>
<tr>
<th></th>
<th>0 days</th>
<th>7 days</th>
<th>14 days</th>
<th>21 days</th>
<th>28 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I</td>
<td>221±2.6</td>
<td>220±6.82</td>
<td>223±8.5</td>
<td>225±8.48</td>
<td>225±9.8</td>
</tr>
<tr>
<td>Group II</td>
<td>202±3.6</td>
<td>185±2.5</td>
<td>180±1.8</td>
<td>165±2.4</td>
<td>161±6.9</td>
</tr>
</tbody>
</table>
In comparison of body weight at 0 days to 28 days,
Group I, $t = 1.98, p > 0.05$, i.e. not significant
Group II, $t = 25.62, p < 0.001$, i.e. very highly significant
Group III, $t = 1.72, p > 0.05$, i.e. not significant
Group IV, $t = 3.98, p < 0.05$, i.e. significant
Group V, $t = 5.59, p < 0.01$, i.e. highly significant
Group VI, $t = 9.12, p < 0.001$, i.e. very highly significant

**DISCUSSION:** Diabetes Mellitus is the commonest endocrine disorder and is as old as mankind. Since Vedic period, many herbs have been in use for treating diabetes. The current arsenal of anti-diabetic drugs is prone to many grave side effects. Therefore a need to develop safer herbal preparations is felt. Turmeric is a well-known condiment, which is used in our daily diet and has many medicinal properties. The present study is taken up to evaluate the anti-diabetic effect of Turmeric which is rich in active ingredient Curcumin. From the earlier studies, it was found that Turmeric is known to possess anti-diabetic properties. Our standard anti diabetic drug, Pioglitazone, acts on PPAR-γ which is present primarily in adipose tissue and less in cardiac, skeletal, smooth muscle cells, islet β cells, macrophages and vascular endothelial cells. These are insulin sensitizers by nature and increase insulin mediated glucose uptake by 30-50% in patients with type 2 diabetes mellitus. Current use anti diabetic drugs like our standard drug Pioglitazone comes attached with various side effects including development of life threatening conditions like bladder cancer and cardiovascular abnormalities on long term administration. Main constituents of the extract viz Curcumin, Dimethoxy Curcumin, Bis-demethoxy Curcumin and Arturmerone have PPAR-γ ligand binding activity and thereby enhance the transcription of several insulin responsive genes and improve the insulin resistance in Type 2 Diabetes mellitus. From our study we also conclude that turmeric in itself has shown to possess a euglycemic property which is very useful and convenient for diabetic patients. Studies suggest that the anti-diabetic action of Turmeric needs some preparations of functional islet β cell mass. So it is very helpful in treating type 2 diabetes than type 1 diabetes mellitus.

After single dose administration, Turmeric extract reduced blood glucose levels in all groups but the reduction was not statistically significant, thus suggesting it requires a longer duration for the onset of action.

It is found that Turmeric extract at a dose of 300mg/kg, has not significantly reduced the blood glucose levels in euglycemic group (Group III), suggesting that it has no significant effect on blood glucose levels in healthy rats. Turmeric extract 300 mg/kg has significantly reduced the blood glucose levels in diabetic rats from 7th day onwards. But 500mg/kg has more efficient action, as reduction in blood glucose levels was statistically highly significant. Pioglitazone which is a standard anti diabetic drug, reduced blood glucose by a highly significant level in both acute and chronic study.

**CONCLUSION:** Results obtained from the present study proved that ethanolic extract of Turmeric has anti diabetic and euglycemic action, hence Turmeric is proved to be a promising
medicinal plant which can be used as an adjunct to drug and diet therapy for the management of diabetes mellitus.

REFERENCES:


