

# Comparative Study of Hypolipidemic effects of Ethanolic extract of Rhizomes of *Curcuma longa* (turmeric) Versus Pioglitazone in Alloxan induced Diabetic Rats

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## ABSTRACT

**Background:** Rhizomes of *Curcuma Longa* belonging to the genus, *Curcuma* is widely used for medicinal purpose. Its ethanolic extract has been used traditionally as a hypolipidemic. **Objectives:** The present study was undertaken to evaluate the hypolipidemic effects of ethanolic extract of Rhizomes of *Curcuma longa* in Alloxan induced diabetic rats with high fat diet and compared with of Pioglitazone, which has anti-diabetic and action. **Materials and method:** Alloxan monohydrate was used to induce diabetes mellitus in albino rats in the dose of 120 mg/kg Intraperitoneally and hyperlipidemia was induced by feeding animals with high fat diet orally. The body weights of the rats in every group was recorded weekly. Six groups of 6 animals in each received normal saline for normal control, normal saline for diabetic control, turmeric extract (TE) 300 mg/kg/day for euglycemic rat, Diabetic rat with high fat diet 10 mg/kg/day (turmeric extract 300 mg/kg/day), diabetic rat with high fat diet 10 ml/kg/day (turmeric extract 500 mg/kg/day), Diabetic rats with High fat diet 10 ml/kg/day (pioglitazone respectively for 4 weeks). After overnight fasting, 2 ml of blood was collected in from orbital sinuses of all animals. Various biochemical parameters were estimated like blood sugar and lipid profile. The data was analyzed statistically using student's paired and unpaired t-test. **Results:** Turmeric extract significantly raised HDL levels both in healthy and diabetic rats. TE (500 mg/kg) is more efficient as compared to 300 mg/kg dose, as TE (500 mg/kg) has also reduced VLDL, LDL levels in Group V which was statistically highly significant ( $p < 0.001$ ). **Conclusion:** Present study revealed that the turmeric has hypolipidemic action and can be safely used in the treatment of mild to moderate cases of hyperlipidemia.

**Key words:** *Curcuma Longa*, Turmeric, Alloxan, Diabetic Rats, Hypolipidemic.

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## INTRODUCTION

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 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.<sup>[1]</sup>

The most frequent serum lipid abnormality in Type 2 diabetes mellitus (DM) is an elevation of serum triglycerides to 1.5-3.0 times as compared to sex, age, and body weight matched non-diabetic controls. More than half of all patients

with type 2 DM have established Coronary artery disease (CAD) and once atherosclerotic disease is established, diabetes worsens the prognosis. In comparison with patients

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who do not have diabetes, patients with type 2 diabetes mellitus have a twofold to fourfold increased risk of CAD. Because of the additive cardiovascular risk of hyperglycemia and hyperlipidemia, lipid abnormalities should be assessed aggressively and treated as part of comprehensive diabetes care.<sup>[2]</sup>

The most common pattern of dyslipidemia is hypertriglyceridemia and reduced HDL cholesterol levels. DM itself does not increase levels of LDL, but the small dense LDL particles found in type 2 DM are more atherogenic because they are more easily glycosylated and susceptible to oxidation. There is increased risk of atherogenic dyslipidemia and hypertension in diabetes, hence increased prevalence of coronary artery diseases, heart failure, and stroke in diabetic population.<sup>[3]</sup> Atherosclerosis is the major cause of premature death in the diabetic patients, whether it is Type 1 or Type 2 diabetes.

Patients with type I diabetes mellitus generally do not have hyperlipidemia if they remain under good glycemic control. Diabetic ketoacidosis is frequently accompanied by hypertriglyceridemia due to an increased hepatic influx of free fatty acids from adipose tissue. Patients with type II diabetes mellitus are usually dyslipidemic, even when under relatively good glycemic control. Triad of lipid abnormalities, namely increased triglycerides, LDL and decreased HDL has been termed “Diabetic Dyslipidemia”. Over the last few years the changes in the lifestyle, particularly the westernization of the diet and a relatively sedentary lifestyle have led to an increased frequency of lifestyle related disorders such as hyperlipidemia, diabetes mellitus, and atherosclerosis.<sup>[4]</sup>

The importance of traditional systems of medicine and of certain traditional medical practices has now been recognized all over the world. Today, it is required to have an intelligent and pragmatic approach to evaluate selective drugs of herbal origin. Therefore, it should really be a matter for Pharmacologists to obtain information from traditional healers, about their remedies and to extract the active principles for development into drugs.<sup>[5]</sup>

Turmeric is well known condiment in the world. It is a prime ingredient in curry powder and figures heavily in Asian cuisines, known as “Golden spice” of India. Turmeric plants were cultivated by Harappan Civilization in 3000 BC. *Curcuma longa* is an herbaceous perennial plant, belonging to the Zingiberaceae family. Turmeric is extensively used in Ayurveda, Unani and Siddha medicinal systems since Vedic-ages and also as home remedy for various ailments. It is also used in Indian

rituals and worship. Turmeric has many medicinal properties and having wide spectrum of actions.<sup>[6]</sup> Such as: anti-inflammatory, Anti-fungal, Anti-mutagenic, Anti-carcinogenic, Anti-coagulant, Anti-hepatotoxic, Anti-fertility, Anti-Protozoal, Anti-viral, Anti-fibrotic, Anti-venom, Anti-ulcer, Hypotensive, Anti-diabetic,<sup>[7,8]</sup> Hypo-cholesterolemic<sup>[9]</sup> and Hypo-lipidemic properties.<sup>[10]</sup>

The comparative analysis of hypo-lipidemic effects of turmeric extract with that of pioglitazone has not been documented/reported. Keeping in view of the above ideas, the present study has been undertaken to evaluate the effect of Ethanolic extract of Rhizomes of *Curcuma Longa* (Turmeric) on the serum lipids level in the Alloxan induced diabetic rats fed with high fat diet and compare with the effects of pioglitazone, which is antidiabetic agent with hypo-lipidemic action.

## MATERIALS AND METHODS

The present study was carried out in the postgraduate research laboratory, department of Pharmacology, Mahadevappa Rampure Medical College, Gulbarga after obtaining the permission from the Institutional Animal Ethic Committee. (IAEC: HKES/MRMCG/256/08 dated 3/11/2008).

### Materials

- Experimental animals used in the study:

Study was carried out in healthy albino rats of Wistar strain (*Rattus norvegicus*) of either sex weighing 180-200 g 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 one week. Total number of animals included in the study was thirty six, out of which diabetes was induced in 24 rats and 12 rats were Euglycemic. Animals were procured from Central Animal House, M.R. Medical College, Gulbarga and Karnataka. Animals were maintained on standard rat pellet diet. Water was given *ad libitum* during the entire period of the experiment. Guidelines of the Committee for the purpose of control and supervision of experiments on animals (CPCSEA) for laboratory animal facilities are strictly followed.<sup>[11-14]</sup> (CPCSEA NO: 142/99 DATED 11-07-1999).

**Measurement of blood sugar:** Glucometer and strips (“Accu-chek Active” Glucometer, Roche Diagnostics, Germany) were used to assess blood sugar levels.

## Drugs/Chemicals

- Alloxan Monohydrate obtained from Otto-kemi Industry, Mumbai, India.
- Ethanolic Extract of Rhizomes of Turmeric.<sup>[11,12]</sup>
- Pioglitazone HCL (Pure form) obtained from BIOCON Pharmaceuticals, Bangalore.
- Ethanol (99.9%) of Changshu Yangyuan Chemicals, obtained from Venkatesh Chemicals, Gulbarga.
- Vehicle: Normal saline (0.9%) and Tween 80 (Hi Media Laboratories Pvt Ltd, Mumbai).
- High Fat Diet: Mixture of Coconut Oil (from Marico Industries Ltd., Mumbai) and Vanaspati Ghee procured from Ruchi Industries, Mumbai.

## Induction of Diabetes Mellitus

A single dose (120 mg/kg, Intra-peritoneal route) of freshly prepared solution of Alloxan Monohydrate (dissolved in Normal Saline, citrate buffer, pH 3) was administered to overnight fasted rats for induction of type 2 Diabetes Mellitus. Alloxan is a uric acid derivative and is highly unstable in water at neutral pH, but reasonably stable at pH 3. Alloxan acts by selectively destroying the pancreatic beta islets leading to insulin deficiency, hyperglycemia and ketosis.<sup>[15]</sup> 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 h for the next 24 h. Fasting blood glucose level was checked after 48-72 h when the animals become hyperglycemic reflected by glycosuria, hyperglycemia, polyphagia, polydipsia and progressive loss of body weight as compared with normal rats. Rats with blood glucose levels between 200-350 mg/dl were selected as diabetic.<sup>[16]</sup>

## Preparation of High Fat Diet for inducing Hyperlipidemia

Edible Coconut oil and Vanaspati ghee mixed together in the ratio of 2:3 v/v as per the method of Shymala MP *et al*,<sup>[17]</sup> at a dose of 10 ml/kg body weight, was fed to the animals per oral daily in addition to a normal diet for 8 weeks.

## 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 200 gm each and subjected to continuous extraction with 95% ethanol for about 48 h at 60°C till solvent in the siphon tube becomes colorless and it took around 8-10 cycles/200 g powder. Small porcelain pieces were added to the flask to avoid bumping of solvent. The solvent so obtained was distilled off and was heat evaporated using water bath/magnetic heart stirrer to get concentrated thick extract which is later diluted in tween 80 and administered to the rats by oral route once daily.<sup>[18]</sup>

**Experimental Design:** For the study the animals were weighed, numbered and randomly divided into 6 groups (n=6). Group I: Normal control-given normal saline (10 ml/kg/day), Group II: Diabetic control-given normal saline (10 ml/kg/day), Group III: Euglycemic rats-Turmeric extract (300 mg /kg/day), Group IV: Diabetic rats with High fat diet (10 ml/kg/day) Turmeric extract (300 mg /kg/day), Group V: Diabetic rats with High fat diet (10 ml/kg/day)-Turmeric extract (500 mg /kg/day), Group VI: Diabetic rats with High fat diet (10 ml/kg/day)-Pioglitazone (6 mg/kg/day).

Body weights of rats in every group were recorded weekly. The experimental rats were carefully monitored every day for general health and behavior. The study was carried out in two phases to assess acute and chronic effects of turmeric extract in hyperlipidemic rats.

**Acute study:** After the single dose administration of turmeric extract, blood samples were collected and estimated for blood glucose and lipid level at the end of 0, 1, 3, 5, and 7 days.

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

**Oral administration of extract/Drug:** As ethanolic extract of Turmeric and pioglitazone are insoluble in water, they were suspended in 5% Tween 80(w/v) administered per orally according to the dosage in respective groups using an intra-gastric feeding tube.

## Method of Collection of Blood

1) For Blood glucose-Tail cut method from the tip of rat tail-1 drop of blood.<sup>[19]</sup>

2) For Lipid profile-2 ml of blood was collected from the orbital sinus with the help of a capillary tube by pressing

the thumb behind the angle of the jaw resulting in the engorgement of retro-orbital plexus.<sup>[20]</sup>

Estimation of blood glucose: Blood glucose estimation is done by using Accucheck 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 correlation between laboratory results and those obtained with glucometers.<sup>[21,22]</sup> 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 will appear on the screen within few seconds in mg/dl.

Estimation of lipid parameters: 2 ml of blood was collected in plain bulbs without anticoagulant, from orbital sinuses<sup>[23]</sup> of all animals. Plain bulbs containing blood are kept at room temperature for 30-45 min for serum separation. After separation of serum from blood, the various biochemical parameters were estimated in the Bio-chemistry Laboratory of Basaveshwar Teaching and General Hospital, attached to M.R. Medical College, Gulbarga, and Karnataka.

1. Total Serum Cholesterol-It was estimated by using Erba Kit manufactured by Transasia Bio-Medicals Ltd.<sup>[24]</sup>

2. Serum Triglyceride-It was estimated by using a kit manufactured by AGAPPE Diagnostics.<sup>[25]</sup>

3. High Density Lipoprotein Cholesterol (HDL)-It was estimated by using Erba Kit Manufactured by Transasia Bio-Medicals Ltd.<sup>[24]</sup>

4. Low Density Lipoprotein Cholesterol (LDL). It was estimated by using Erba Kit manufactured by Transasia Bio-Medicals Ltd.<sup>[24]</sup>

**Statistical analysis:** The results are expressed as mean ± SEM. Data were subjected to one-way analysis of variance (ANOVA) followed by Dunnett’s test by keeping the significance level as p<0.05.

## RESULTS AND 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. It is found that diabetes mellitus is invariably associated with dyslipidemia. So it becomes mandatory to take care of dyslipidemia in diabetic patients along with blood sugar levels as it is known to increase the morbidity and mortality if neglected. Therefore there is need for drug with both antidiabetic and hypo-lipidemic activity. In this regard, turmeric plays crucial role.

**Table 1: Effect on Serum lipid level at the end of 4 weeks**

Groups	Serum TC (mg/dL)	TG (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	VLDL (mg/dL)
Group I	79.6 ± 2.86	78.13 ± 4.5	29.5 ± 1.71	44.93 ± 4.04	15.9 ± 1.36
Group II	144.8 ± 8.12	136.33 ± 3.32	19.5 ± 1.71	82.12 ± 3.86	20.98 ± 1.26
Group III	79.6 ± 2.86	75.86 ± 3.45	34.66 ± 1.98	42.15 ± 1.49	15.75 ± 1.49
Group IV	103.66 ± 4.24	109.0 ± 7.98	22.66 ± 1.69	67.36 ± 3.74	19.64 ± 2.21
Group V	83.5 ± 4.32	94.33 ± 5.15	35.0 ± 3.16	59.78 ± 3.25	18.02 ± 1.62
Group VI	119.16 ± 6.12	107.0 ± 6.3	32.5 ± 2.5	78.88 ± 4.78	19.68 ± 1.45

Results expressed as mean ± SD (n=6).

**Table 2: Effect on body weight at the end of every week**

Groups	0 days	7 days	14 days	21 days	28 days
Group I	221 ± 2.6	220 ± 6.82	223 ± 8.5	225 ± 8.48	225 ± 9.8
Group II	202 ± 3.6	185 ± 2.5	180 ± 1.8	165 ± 2.4	161 ± 6.9
Group III	210 ± 5.84	211 ± 3.8	213 ± 4.3	213.3 ± 5.2	213.4 ± 6.96
Group IV	211 ± 5.32	210 ± 3.86	209 ± 2.86	206 ± 4.36	206 ± 3.2
Group V	210 ± 3.6	208 ± 3.6	213 ± 2.78	214.8 ± 3.6	215.2 ± 2.96
Group VI	208 ± 3.2	190 ± 3.2	212 ± 2.3	218 ± 3.9	221.2 ± 6.52

Results expressed as mean ± SD (n=6).

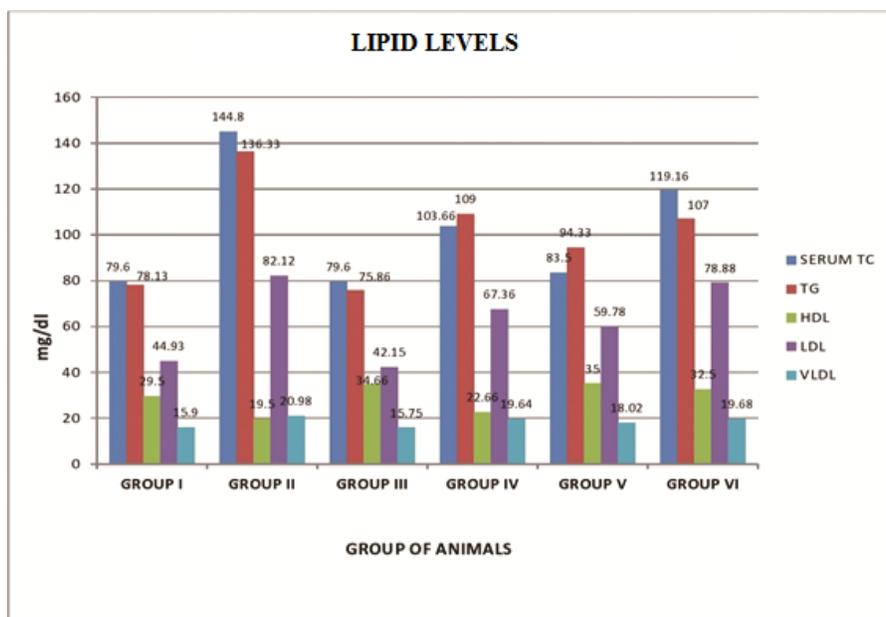


Figure 1: Effect on mean serum lipid parameters the end of 4 weeks.

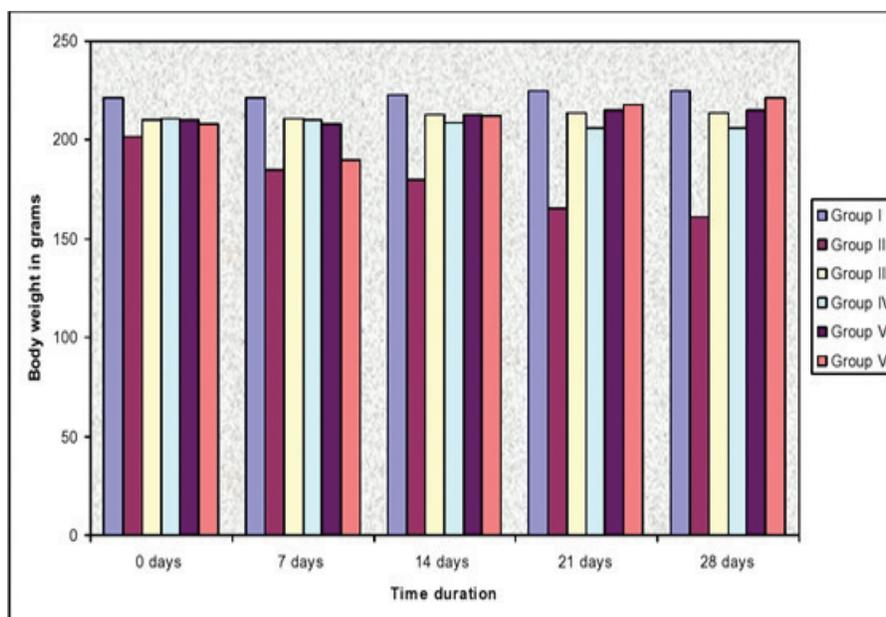


Figure 2: Effect on body weight changes at every week.

Turmeric is well known condiment, which is used in our daily diet and has many medicinal properties.

Earlier studies have found that, turmeric is known to possess hypolipidemic activity,<sup>[26-28]</sup> Hypolipidemic effect probably results from increased elimination of cholesterol in the form of bile acids, as turmeric increases production and secretion of bile acids.<sup>[29]</sup> And also increased hepatic cholesterol 7 $\alpha$  hydroxylase activity suggest higher rate of cholesterol catabolism.<sup>[30]</sup>

In the present study, turmeric extract has significantly raised HDL levels both in healthy and diabetic rats and significantly reduced serum TC, TG, LDL levels in diabetic rats as shown in Table 1 and Figure 1.

Pioglitazone, though being standard antidiabetic drug, has hypo-lipidemic action. It is clearly evident from results that, it has raised HDL and reduced TC, TG levels, but found to have no effect on LDL and VLDL levels It acts by increasing expression of ABCA1, which transport extra

hepatic cholesterol into HDL and also like partial agonist of PPAR  $\alpha$ , thereby exhibiting 'fibrate like effect'. Looking at body weight record chart of experimental rats, it is clear that total body weight of diabetic rats is significantly reduced at the end of 28 days. Standard antidiabetic drug pioglitazone has also significantly improve the body weight of diabetic rats at the end of 28 days as shown in Table 2 and Figure 2.

## CONCLUSION

Antidiabetic efficacy of pioglitazone is more effective than turmeric extract, but the hypolipidemic efficacy of turmeric extract was more than that of pioglitazone. Hence ethanolic extract of rhizomes of turmeric (*C. longa*) is proved to be the promising medicinal plant (rhizome) which can be used as an adjunct to drug and diet therapy for the management of diabetes mellitus and dyslipidemia.

## Limitations of the study

This study did not evaluate the molecular and biochemical basis of hypolipidemic action of turmeric and further studies are required to find out the molecular mechanism.

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