Asian journal of Phytomedicine
and
Clinical Research

Journal home page: www.ajpcrjournal.com

EVALUATION OF ANTI-OXIDANT AND ANTI-HYPERLIPIDEMIC ACTIVITY OF *CURCUMA AROMATICA IN* TRITON X-100 INDUCED HYPERLIPIDEMIA RAT MODEL

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ABSTRACT

Hyperlipidemia is the greatest risk factor of coronary heart disease. Currently available hypolipidemic drugs have been associated with number of side effects. Herbal treatment for hyperlipidemia has no side effects and is relatively cheap and locally available. Literature claims that *curcuma aromatica* is able to reduce hyperlipidemia. Based on this in herbal plant, *curcuma aromatica* was selected and the present study focus on the Anti-oxidant and Anti-Hyperlipidemic activity was investigated in Triton X-100 induced hyperlipidemic rat model. Twenty four rats were assigned randomly into four groups. The normal group was fed regular diet control group was treated with triton a single dose 100 mg/kg body weight i.p. Test group with triton and *curcuma aromatica*. Another group with triton treated with standard drug. The plasma levels of total cholesterol, triacylglycerol, LDL, VLDL, HDL levels and *in vitro* antioxidant parameter such as DPPH method-scavenging activity was examined. This may give results as inhibition of hyperlipidemic atherosclerosis by *curcuma aromatica* was associated with a decrease in plasma lipids and an increase in antioxidative abilities.

KEYWORDS

Anti-Hyperlipidemic, Anti-oxidant, Triton X-100 and Atorvastatin.

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INTRODUCTION

Hyperlipidemia it is a medical condition characterized by an elevation of any or all lipid profile and/or lipoproteins in the blood. This medical condition or problem divided into two subtypes which are: Primary hyperlipidemia, which is usually taken place as a result of genetic problem, i.e. mutation within receptor protein. Secondary hyperlipidemia will arises as a result of other underlining diseases like diabetes[1].

Alteration and/ or abnormality in the metabolism of lipid and lipoproteins is a very common condition that taken place within general population, Hyperlipidemia is the presence of high levels of cholesterol in the blood. It is not a disease but a metabolic derangement that can be secondary to many diseases and can contribute to many forms of disease, most notably cardiovascular disease[2]. There are several factors which play significant role in the incidence of this medical problem which are the following: family history, chronic diseases (diabetes mellitus, renal failure, nephritic syndrome and hypothyroidism), alcoholism and smoking, obesity and un healthy diets intake[3].

Hypercholesterolemia increases the levels of the lipid peroxidation product malondialdehyde in blood and the production of oxygen-free radicals (OFRs), which are induced by polymorphonuclear leukocytes. OFRs exert their cytotoxic effects by causing peroixdation of polyunsaturated fatty acids of membrane phospholipids, which can result in an elevation in membrane permeability and loss of cellular integrity. Furthermore, free radical-induced lipid peroxidation has been implicated in the pathogenesis of atherosclerosis and reactive oxygen species (ROS) are known to be the initiators of lipid peroxidation. Endothelial cells, smooth muscle cells, neutrophils, monocytes, and platelets may be the sources of ROS in atherosclerosis induced by hypercholesterolemia[4].

Cholesterol is the essential for human body but some types of cholesterol has very adverse effect on human body and leads to heart attack and death. The treatment of hyperlipidemia depends on the patient's cholesterol profile. Many antihyperlipidemic agents like statin, fibrates, niacin, bile acids sequestrates, ezitimibe reduce cholesterol level with different condition. Plants and many plant derived preparations have long been used as traditional remedies and in folklore medicine for the treatment of hyperlipidemias in many parts of the world. There are many plants and their products that have been reputedly and repeatedly used in Indian traditional system of medicine. We found that *curcuma aromatica* having high antioxidant and antihyperlipidemic property. Recently, the search for appropriate antihyperlipidemic agents have been again focused on plants because of less toxicity, easy availability and easy absorption in the body that may be better treatment than currently used drugs.

MATERIALS AND METHODS
Plant Material
Curcuma aromatica: Was obtained from local medicinal plants and herbs market[5].
Animals
Twenty four Wistar rats of 250 ± 5 g were obtained from National Institute of Nutrition Jamai Osmania, Hyderabad, Andhra Pradesh, India.
Chemicals
Triton purchase from otto chemicals Mumbai, total lipids (TL), triglycerides (TG), total cholesterol (TC), high density lipoprotein (HDL) kits were purchase from molychem Hyderabad.
Methods
Preparation of plant powder
Curcuma aromatica (rhizome) were collected and powdered.
Preparation of basal deit
Diet was formulated to meet recommended nutrients levels for rats. The normal group was fed regular diet and normal saline, control group was treated with triton a single dose 100 mg/kg body weight i.p. Test group with triton plus *Curcuma aromatica*. Another group with triton treated with standard drug.
Acute Oral Toxicity Studies
Curcuma aromatica the dose range of 100mg-2000mg/kg were administered orally to different group of rats comprised of ten rats in each group. Mortality was observed after 72 hours. Acute toxicity was determined according to the method of Litchfield and Wilcoxon. Extract was dissolved in 0.5% carboxymethyl cellulose and administered in a dose of 500mg/kg body weight[6].
Induction of hyperlipidemia
A single dose (100 mg/kg body weight i.p) of Triton X-100 dissolved in 0.15 N NaCl solution was used for induction of hyperlipidemia in the rats[7]. Hyperlipidemia was confirmed 72 hrs after triton injection by determining the blood cholesterol. The

quantities of individual drug (*curcuma aromatica*) to be administered were calculated and suspended in 0.5% CMC vehicle at a dose of 500 mg/kg b.w. The drug was administered continuously for 7 days orally using infant feeding tube. The results were compared with that of the standard drug Atorvastatin which was also given continuously for 7 days at a dose of 10 mg/kg b.w [8].

Experimental design

The animals were divided in to four groups consisting of six animals each. The animals were divided into following groups and receive respective treatments. Freshly prepared powders were administered daily for 4 weeks[9].

Group classification

Twenty Four healthy rats are divided into **four** groups

Group I: (n = 6) saline control

Group II: (n = 6) Disease control (triton induced)

Group III: (n = 6) Standard drug (Atorvastatin)

Group IV: (n = 6) treatment (triton + *Curcuma aromatica*)

[*]Dose Denominations will be fixed after Dose determination by Acute Oral Toxicity as per OECD Guideline 423.

Blood cholesterol and triglycerides profile were estimated before starting the treatment and end of the treatment period i.e.7 days.

ESTIMATION OF TOTAL BLOOD LIPIDS

Total Cholesterol estimation

Cholesterol Diagnostic kit.

Total Triglycerides estimation

Triglyceride Diagnostic kit.

Total HDL estimation

HDL Diagnostic kit.

Total VLDL estimation

VLDL Diagnostic kit.

Total LDL estimation

LDL Diagnostic kit.

Biochemical analysis

The serum was analyzed for total cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL), low density lipoprotein (LDL), very low density lipoprotein (VLDL) by using standard protocol methods. (Eon one Auto Analyzer) [10].

EVALUATION OF *IN VITRO* ANTIOXIDANT ACTIVITY

DPPH method

The free radical scavenging activity of methanolic extract of *Curcuma aromatica* was measured by the decrease in the absorbance of methanolic solution of DPPH and this activity was measured by spectrophotometric method[11]. 1mL of *Curcuma aromatica* at various concentrations (100, 200, 300, 400,500 and 600 µg/mL) were mixed with 1mL of methanolic DPPH (100µM). Similarly 1mL methanolic solutions of ascorbic acid (100 µg/mL) were mixed with 1mL of DPPH solution. A mixture of 1mL of methanol and 1mL of methanolic DPPH (100 µM) served as control. After mixing, all the solutions were incubated in dark for 35 minutes and absorbance was measured at 517 nm. The experiments were performed in triplicate and scavenging activity was calculated by using the following formula and expressed as percentage of inhibition[12].

Percentage of inhibition = Absorbance of control-(absorbance of test –absorbance blank)/Absorbance of control X 100.

Statistical analysis

All data are presented as mean ± SEM. Statistical analysis is done to investigate the relationship among the groups using one-way ANOVA. The P values ≤ 0.05 were considered as statistically significant.

RESULTS

DPPH free radical scavenging assay

DPPH Free radical scavenging activity, *Curcuma aromatica* were evaluated for their free radical scavenging activity with ascorbic acid as standard compound. The IC50 was calculated as well as ascorbic acid as standard and summarized in table and graphically represented (Table No.1 and 2, Figure No.1).

Histopathological studies

Liver, aortic strip of the scarified rats were taken and immersed in 10% formalin solution. The fixed specimens were then trimmed washed and

dehydrated in ascending grades of alcohol Paraffin, sectioned at 4-6 microns thickness and stained with Haemtoxylin and Eosin (Figure No.2).

DISCUSSION

The cardiovascular benefits of *Curcuma aromatica* use are described in ayurvedic books. Decreases in blood pressure and plasma lipid concentrations, especially triacylglycerols and low density lipoprotein-cholesterol have been demonstrated as a result of oral consumption of *Curcuma aromatica*. *Curcuma aromatica* has also been shown to indirectly modify the total cholesterol and high density lipoprotein cholesterol values. *Curcuma aromatica* was found to be non–toxic up to the dose of 5000 mg/kg and did not cause any death of the tested animals. Hyperlipidemia is associated with heart disease, which is the leading cause of death in the world.

The lowering of the levels of harmful lipids to satisfactory values has been confirmed by several experimental animal and interventional studies Specimens were then cleared in xylol, embedded in indicating lowered morbidity and mortality in coronary heart diseases[13]. The results are discussed under the lipid profile in serum. Lipid profile in serum indicates that increased phospholipids (PL), triglyceride (TG) and cholesterol levels were significantly reduced by treatment of 0.5g/day *Curcuma aromatica*. LDL and VLDL levels were significantly increased in triton injected animals to control rats. The results are shown in Tables. The *Curcuma aromatica* markedly lowers the levels of serum cholesterol and VLDL. The decrease in cholesterol may indicate increased oxidation of mobilized fatty acids of inhibition or lipolysis. The present investigation shows that all triton induced rats displayed hyperlipidemia as shown by their elevated levels of serum and liver cholesterol, triglyceride, PL, VLDL, LDL and the reduction in the HDL level. It can be concluded that 0.5g/day of *Curcuma aromatica* treatment was effective in reduction of cholesterol, PL, TG, VLDL, LDL in a dose dependant manner.

Table No.1: Effect of Anti-Hyperlipidemic activity of *Curcuma aromatica* in Triton X-100 induced hyperlipidemic rat model

S.No	Group No	Sample	T.C ± SD	DL ± SD	VLDL ± SD	HDL ± SD	TG ± SD
1	I	Normal control	62±0.60	62±0.60	62±0.60	62±0.60	62±0.60
2	II	Triton control	134±1.3***	134±1.3***	134±1.3***	134±1.3***	134±1.3***
3	III	Triton + Std	66±3.1	56±2.7***	56±2.7***	56±2.7***	56±2.7**
4	IV	Triton + Test	69±4.2***	24±5.3***	24±5.3***	25±5.3***	101±5.5***

TC – Total Cholesterol, TG – Triglycerides, LDL – Low density lipoprotein, HDL – High density lipoprotein, VLDL – Very low density lipoprotein. Values are expressed as Mean ± SEM of each group (n = 6). The results were analyzed by comparing with control group with one way ANOVA and statistically significant. ***= $p \leq 0.05$ and is significant.

Table No.2: DPPH free radical scavenging method

S.No	Concentration in µg/ml	Absorbance	% of inhibition	IC 50 VALUE µg/ml
1	100	0.058	95.7	-
2	200	0.055	95.9	-
3	300	0.053	96	50
4	400	0.042	96.9	-
5	500	0.040	97.8	-
6	600	0.023	98.3	-
7	Control	1.355	0.0	-
8	Standard	0.286	-	43.04

As shown in the table represent as mean ± standard deviation. IC50 values are significant when compared with standard.
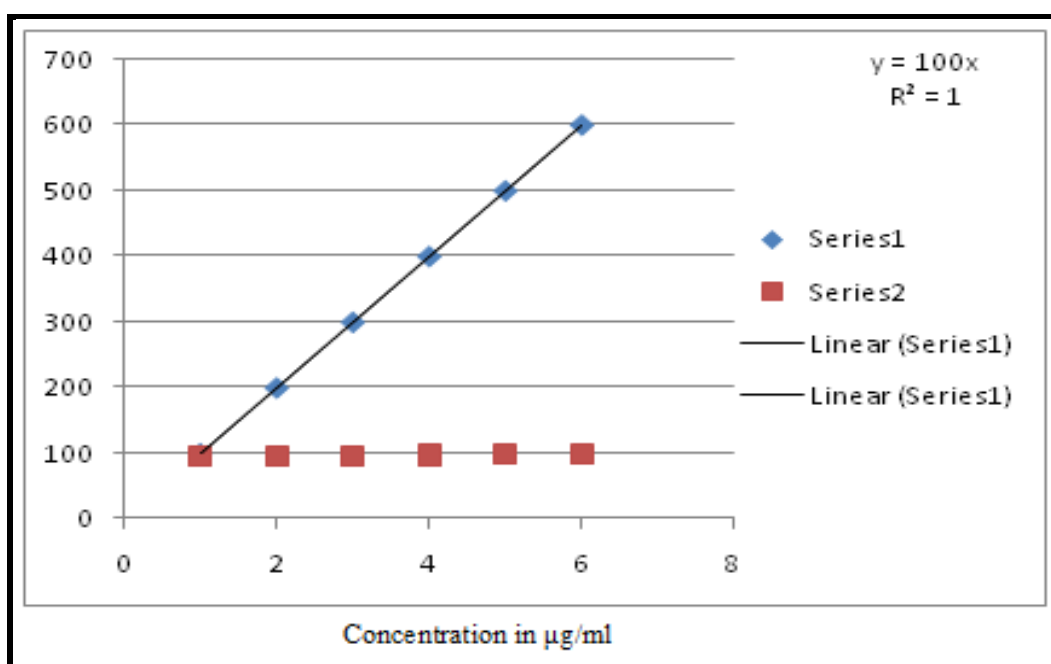
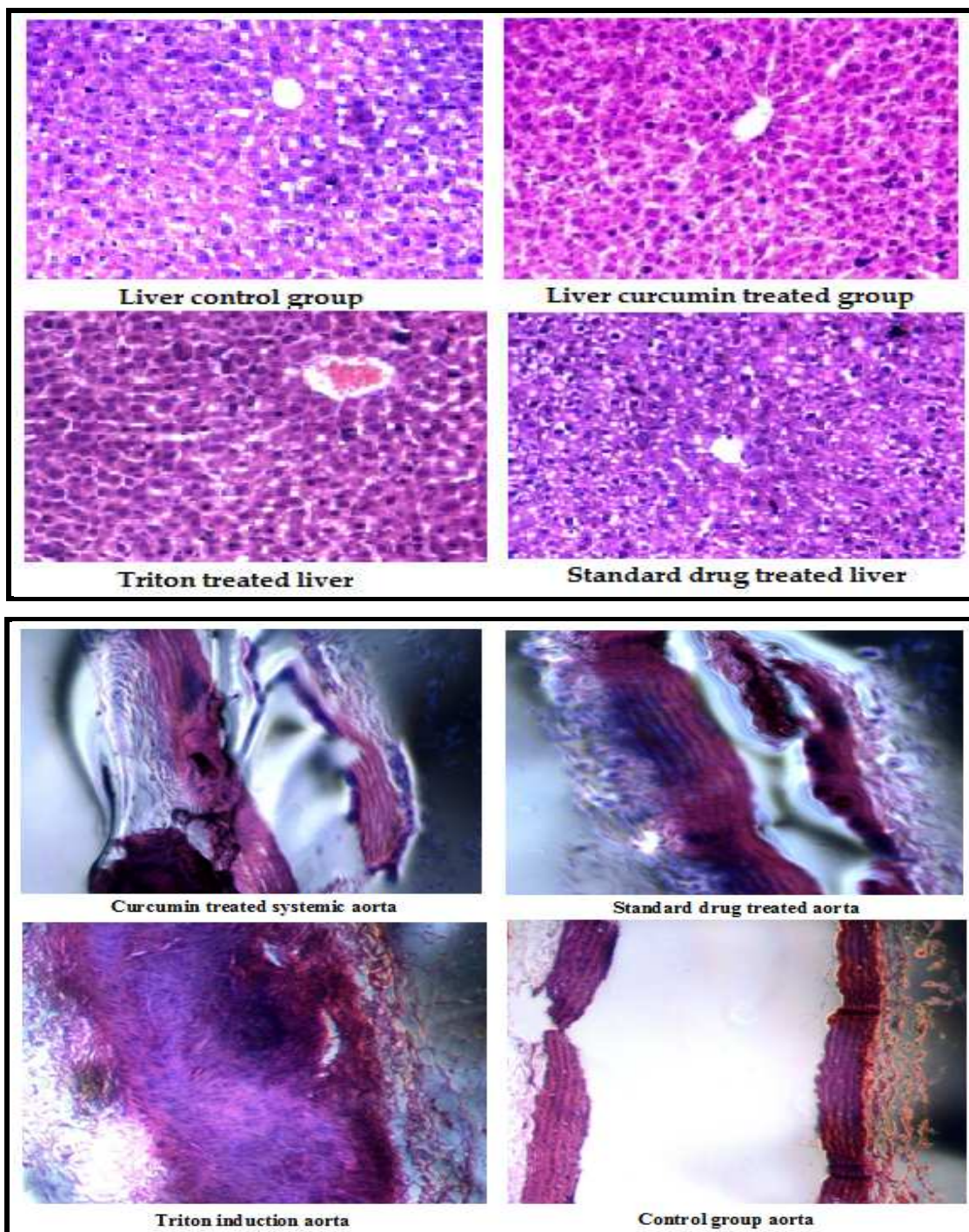**Figure No.1: DPPH free radical scavenging method**

Figure No.2: Histopathological studies

CONCLUSION

Curcuma aromatica exhibited significant anti hyperlipidemic activity in Triton X-100 induced hyperlipidemic rat model. It also showed significant improvements in lipid profile like Total cholesterol, Triglycerides, LDL, HDL and VLDL and also there was improvement in body weights which might be of great importance in the management of anti

hyperlipidemic activity. Though it is taken as dietary constituent in food which shows protective nature. It should be further tested against various animal models for the evaluation of anti hyperlipidemic activity. In order to elucidate the mechanism of action further active principles should be isolated and its nature should be studied. *Curcuma aromatica* also consists of various activities such as anti oxidant, anti cancer, and anti diabetes activities according to folklore books.

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