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Evaluation of Anti-hyperlipidemic Potential of Combinatorial Extract of Curcumin, Piperine and Quercetin in Triton-induced Hyperlipidemia in Rats

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ABSTRACT

The present study was undertaken to assess the hypolipidemic, hypocholesterolemic and hypotriglyceridemic potential of the combinatorial extract consisting of Curcumin with Piperine and Quercetin (CPQ extract) using Triton induced hyperlipidemia in rats. CPQ extract was subjected to preliminary qualitative phytochemical investigation by using standard procedures. Acute oral toxicity study for the CPQ extract was carried out using OECD guidelines 423.Comparative single- dose oral bioavailability studies of 100 mg kg^{-1} of curcumin and combination consisting of Curcumin with Piperine and Quercetin (CPQ) were carried out in rats under fed conditions. Hyperlipidemia was induced by single intraperitoneal injection of Triton X-100 (100 mg kg^{-1}). CPQ extract was orally administered using three different doses (50,100 and 200 mg kg^{-1} orally), immediately as well as 24 h after triton injection. Blood was collected at 24 and 44 h by retro-orbital sinus puncture method in EDTA coated vials and biochemical estimation was studied using enzymatic assay kit. Bioavailability studies show significant increase in serum curcumin concentration on the inclusion of piperine and quercetin in the CPQ. Triton administration increased level of plasma cholesterol (PTC), Triglycerides (PTG), LDL (199.5±10.2, 301.83±7.78 mg dL^{-1}, 163.36±4.79) respectively as compared with normal control group. However oral administration of combinatorial extract "Curcumin with piperine and quercetin" (100 mg kg^{-1}) significantly ($p<0.05$) reduced PTG (61.26%), LDL (66.69%) and PTC (48.26%) and increased the plasma HDL level by 24.45% as compared to triton control group. Treatment with Combinatorial extract of curcumin exhibited quite competitive potential when compared with the reference drug Lovastatin which indicates that CPQ extract could be explored as an alternative therapeutic agent in the treatment of hyperlipidemia.

Key words: Hyperlipidemia, bioavailability, herbs, triton, triglycerides

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INTRODUCTION

Hyperlipidemia has been ranked as one of the greatest risk factors contributing to the prevalence and severity of coronary heart diseases[1]. Coronary heart disease, stroke, atherosclerosis and hyperlipidemia are the primary cause of death[2].

Hyperlipidemia is characterized by elevated serum total cholesterol, low density, and very low-density lipoprotein and decreased high-density lipoprotein levels. Among these hypercholesterolemia and hypertriglyceridemia are closely related to ischemic heart disease[3]. The main aim of treatment in patients with hyperlipidemia is to reduce the risk of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease[4]. Currently available hypolipidemic drugs have been associated with a number of side effects. Herbal treatments for hyperlipidemia have no side effects and is relatively cheap and locally available.

World ethnobotanical information reported that a number of herbal medicines from plants and vegetables are used for controlling hyperlipidemia and related complications in patients[5]. Curcumin is one such ayurvedic remedy that has been mentioned in many Indian medical literatures for many pharmacologic activities including anti-inflammatory properties, powerful antioxidant activity, and cancer-preventive properties[6,7]. In the traditional Indian system of medicine it has been used in several ways namely (i) as an ingredient in the preparation of medicinal oils, ointment and poultice, (ii) in diabetes and leprosy, (iii) for stomachache, carminative, tonic, laxative, antirheomatic, blood purifier, vermicide, antiseptic and cure for liver

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ailments. The raw juice is used to tear in gallstones, gall bladder complaints and dental-troubles and for sore throat and common cold parasitic skin diseases and pile cure. A recent study showed that curcumin lowered blood glucose and glycated hemoglobin levels by lowering oxidative stress in diabetic rats[8,9]. Furthermore, several studies suggested that curcumin has hypocholesterolemic properties[10,11]. Arafa[10] reported that the hypocholesterolemic effect of dietary curcumin (0.5%, w/w) in high-cholesterol-fed rats could possibly be medicated through a local effect on cholesterol absorption or excretion through the bile, but not due to its antioxidant effect. Meanwhile, Srinivasan and Manjunatha[12,13] showed the hypolipidemic and antioxidant effects of curcumin (0.2%, w/w) in high-fat (30%)-fed rats.

Despite having wide spectrum of pharmacological actions, the medicinal properties of curcumin cannot be utilized due to its low bioavailability[14]. Therefore in view of the foregoing, there is an extensive need for such combinatorial extracts of herbs containing curcumin, piperine and quercetin, as Piperine is an alkaloid found naturally in plants as *Piper nigrum* L., commonly known as black pepper is established as a bioavailability enhancer of drugs. It is an inhibitor of glucuronidation metabolism hence increases bioavailability (serum concentration) of oral curcumin by 154% by in rats and 2000% in humans[15] and if a second bioavailability enhancer such as quercetin is added, an increase in absorption is possible to a greater extent as it aid in the inhibition of metabolic conversion of curcumin by inhibition of sulfotransferase enzymes. Thus the present study was undertaken for the first time to investigate the hypolipidemic, hypocholesterolemic and hypotriglyceridemic potential of the combitorial extracts consisting of curcumin with quercetin and piperine using triton induced hyperlipidemia in rats.

MATERIALS AND METHODS

Experimental animal: Swiss albino mice and Wistar rats of either sex were used for the experiment. Animals were maintained in the Animal House of School of Pharmacy and Technology, NMIMS (temperature 25±2°C; relative humidity 75%±5%). During the experiments animals were provided with standard rodent pellet diet (Amrut feed) water *ad libitum*. All studies were conducted after obtaining prior approval from the institutional animal ethical committee in accordance (IAEC) (Approval no: CPCSEA/SPTM/P-57/2009).

Drugs and chemicals used: The different reagents used for the estimation of plasma cholesterol, triglyceride, LDL and HDL were procured from Transasia-Biomedicals Limited, Mumbai. Quercetin dehydrate and Triton was procured from Sigma Aldrich, Bangalore. All other chemicals were of analytical grade.

Collection and authentication of plants: The rhizomes of *Curcuma longa* Linn, fruits of *Piper nigrum* and fresh leaves of *Allium cepa* were collected in the months of March-June from the local market of Mumbai, Maharashtra state, India, and was authenticated by Department of Raw and Crude drug material, National Institute of Science Communication and Information Resource (NISCAIR), New Delhi.

Preparation of combinatorial extract of curcumin: Extraction and isolation of curcuminoid, piperine and quercetin from *Curcuma longa* Linn, *Piper nigrum* and *Allium cepa* was done using petroleum ether, chloroform and ethanol as a solvent. The % yield obtained was found to be about 6.18, 9 and 0.1% (w/w) curcumin, piperine and quercetin respectively. The Combinatorial extract was prepared by suspended curcumin: piperine: quercetin in a ratio (94:1:5) in 5% Gum Acacia and 0.5% tween 80. Required quantity of gum acacia was weighed and taken in a mortar. It was triturated with very little quantity of tween 80 to form slurry. Accurately weighed quantity of curcumin, piperine and quercetin was added to the slurry and trituration continued. The slurry was transferred to a measuring cylinder and the volume was made with water. The suspension was transferred to a vial and shaken thoroughly.

Phytochemical screening: CPQ extract was screened for the presence or absence of secondary metabolites such as presence of flavonoids, carbohydrate, alkaloids, steroids, glycosides, saponins, phenolic etc. by using standard procedures[16,17].

Acute toxicity study: Swiss albino mice of both sexes were randomly divided into three groups, each containing six animals. The combinatorial extractwas administered orally at doses of 5, 50, 300 and 2000 mg kg^{-1} body weight (OECD, 423). Distilled water was administered to control group. The general behavior of the mice was continuously monitored for 1 h after dosing, periodically during the first 24 h with special attention given during the first 4 h and daily thereafter, for a total of 14 days. Changes in the normal activity of mice and their body weights were monitored and the time at which signs of toxicity or death appeared recorded.

Comparison of the Pharmacokinetic profile of the Curcumin and CPQ: Twelve rats were divided into 2 groups consisting of 6 rats each. Each of them was given a single dose of "curcuminoid alone" and "CPQ "orally. The dose was kept constant for all the extracts as 100 mg kg^{-1} from the literature and preliminary studies done. The extracts were prepare just before administration and were administered within 1 h of

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preparation. The blood samples were collected at Pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, 8 and 10 h. Blood samples were collected from the retro orbital plexus at the said intervals. Blood was allowed to clot at room temperature for about 1 h, then centrifuged at 3000 rpm for 15 min and serum was separated. A simple analytical method was developed for determining concentrations of total curcuminoids extract as curcumin in blood serum. A standard curve for curcumin was prepared in serum at excitation and emission wavelengths of 232 and 614 nm, using a spectrofluorimeter.

Lipid lowering activity in triton induced hyperlipidemia[18,19]: Hyperlipidemia was induced in Wistar albino rats by single intraperitoneal injection of freshly prepared solution of Triton-X-100 (100 mg kg^{-1}) in physiological saline solution after overnight fasting for 18 h. The rats were divided into eight groups, each group consisting of six animals. The experimental design and schedule of treatment was followed as:

Group I: Triton-X-100 (100 mg kg^{-1}, b.wt.) induced hyperlipidemic rats fed with 5% gum acacia and 0.5% tween 80

Group II: Triton-X-100 induced hyperlipidemic rats treated with standard lovastatin (10 mg kg^{-1} orally) simultaneously with triton injection and 24 h later

Group III: Triton-X-100 induced hyperlipidemic rats treated with curcumin (50 mg kg^{-1} orally) simultaneously with triton injection and 24 h later

Group IV: Triton-X-100 induced hyperlipidemic rats treated with curcumin (100 mg kg^{-1} orally) simultaneously with triton injection and 24 h later

Group V: Triton-X-100 induced hyperlipidemic rats treated with curcumin (200 mg kg^{-1} orally) simultaneously with triton injection and 24 h later

Group VI: Triton-X-100 induced hyperlipidemic rats treated with combinatorial extract of curcumin (50 mg kg^{-1} orally) simultaneously with Triton injection and 24 h later

Group VII: Triton-X-100 induced hyperlipidemic rats treated with combinatorial extract of curcumin (100 mg kg^{-1} orally) simultaneously with Triton injection and 24 h later

Group VIII: Triton-X-100 induced hyperlipidemic rats treated with combinatorial extract of curcumin (200 mg kg^{-1} orally) simultaneously with Triton injection and 24 h later

Biochemical analysis in plasma: At the end of experiment, rats were kept overnight fasting. They were anaesthetized under light ether and blood samples were collected from retro orbital plexus using glass capillary in EDTA coated tubes after 18 and 44 h of triton injection using micro centrifuge tubes. Plasma was separated by centrifugation at 2000 rpm for 5-10 min. The plasma was then analyzed for total Cholesterol, Triglycerides, LDL and HDL using semi-auto analyser.

Statistical analysis: One way analysis of variance (ANOVA followed by Bonferroni's test) were performed by comparison of values for triton treated group with control, triton and extract treated groups with triton. All the values are expressed in terms of Mean±SD of (n = 6). P values <0.05 were considered as statistically significant. The statistical analysis was carried out by the Graph Pad INSTAT 3.0 software.

RESULTS

Preliminary phytochemical screening: The result indicated the presence of flavonoids, carbohydrate, alkaloids, steroids, glycosides and phenolic compound in CPQ extract.

Acute toxicity study: The combinatorial extract of curcumin was found to be non toxic up to the dose of 2 g kg^{-1} and did not cause any mortality or symptoms of toxicity. According to Organization for Economic Cooperation and Development (OECD, 423) guidelines for acute oral toxicity, An LD50 dose of 2000 mg kg^{-1} and above is categorized as "unclassified" and hence drug is found to be safe. So further dosing to find out LD50 of combinatorial extract of curcumin was not performed.

Comparison of the pharmacokinetic profile of the curcumin and CPQ: The mean plasma concentration-time profile of curcumin following single oral administration is shown in Fig. 1. After the administration of 100 mg kg^{-1} of curcumin and CPQ,

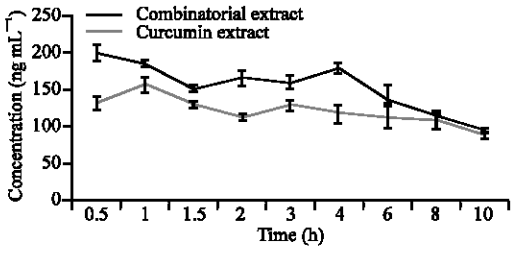

Fig. 1: The mean plasma concentration-time profile of curcumin following single oral administration of curcumin and CPQ. The values are expressed as Mean±SEM (n=6)

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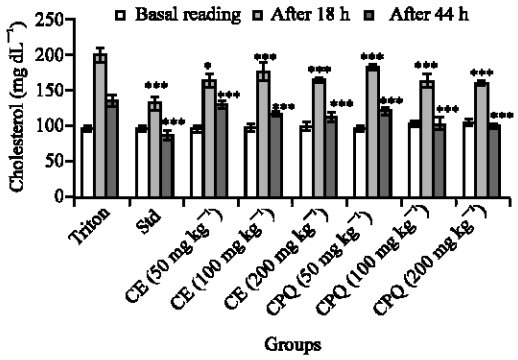

Fig. 2: Effect of CPQ on plasma cholesterol in triton induced Hyperlipidemia. Values are Mean±SD of six rats ***p<0.001, **p<0.01 and *p<0.05 versus control

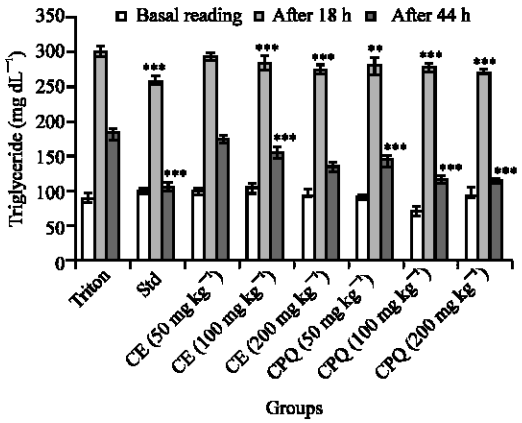

Fig. 3: Effect of CPQ on plasma triglyceride in triton induced Hyperlipidemia. Values are Mean±SD of six rats ***p<0.001, **p<0.01 and *p<0.05 versus control

plasma level of curcumin reached the Cmax of 157.34±10.60 ng mL^{-1} at the Tmax of 1.0 h while with that of CPQ the maximum curcumin concentration reached is 199.50±11.24 at 30 min. The inclusion of piperine and quercetin in the extract showed a rapid absorption of curcumin immediately after oral ingestion. There was a significant increase in the peak serum concentration at 0.5 h with CPQ as compared to curcumin alone. A sluggish second phase peak was also appeared at 6 h time point. This plasma pharmacokinetic profile suggests that to some extent quick curcumin absorption taken place from stomach followed by second more prominent absorption at the intestine. The observed second peak can be explained by the slow enterohepatic circulation and intestinal reabsorption. While comparing the overall profile by considering serum concentration between the groups, piperine and quercetin addition in combination with curcumin shows significant increase ($p<0.01$) in serum curcumin concentration in the concentration versus intensity profile.

Lipid lowering activity of combination consisting of curcumin with piperine and quercetin (CPQ) in triton induced hyperlipidemia: The acute administration of triton X-100 caused marked increase ($p<0.001$) in the plasma levels of Cholesterol, Triglycerides (TG), Low density Lipoprotein (LDL)-199.5±10.2; 301.83±7.78 mg dL^{-1}; 163.36±4.79, respectively and reduction in HDL level as compared with normal control group (Fig. 2-5).

Effect of CPQ on plasma lipid profile in rats: Treatment with Combination consisting of curcumin with piperine and quercetin (100 mg kg^{-1}) caused significant reversal ($p<0.001$) in the levels of triglyceride

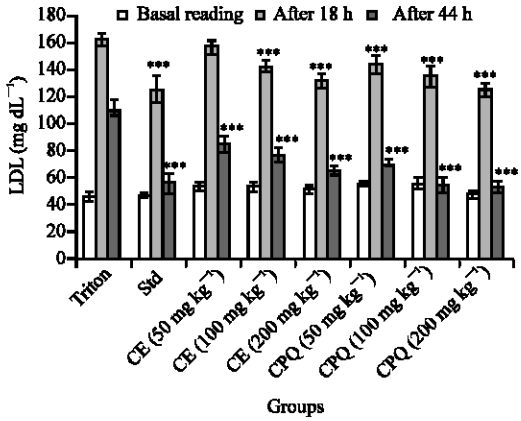

Fig. 4: Effect of CPQ on plasma LDL in triton induced Hyperlipidemia. Values are Mean±SD of six rats ***p<0.001, **p<0.01 and *p<0.05 versus control

(TG), Low density lipoproteins (LDL), cholesterol and significant increase ($p<0.01$) in HDL level (Fig. 2-5) as compared to triton-injected animals. The lipid lowering activity of combinatorial extract (curcumin with piperine and quercetin) was comparatively more than that of curcumin extract alone as it results in significant decrease in plasma levels of triglyceride and cholesterol ($p<0.05$).

DISCUSSION

Hyperlipidemia is associated with heart diseases, 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 animals and interventional studies indicating lowered

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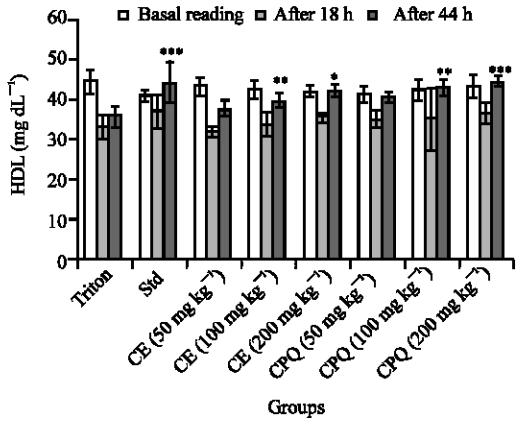

Fig. 5: Effect of CPQ on plasma HDL in triton induced Hyperlipidemia. Values are Mean±SD of six rats ***p<0.001, **p<0.01 and *p<0.05 versus control

morbidity and mortality in coronary heart diseases. The present study was planned, as there have been reports that currently available hypolipidemic drugs have been associated with a number of side effects. The consumption of synthetic drugs leads to hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function[20]. Therefore, to overcome these adverse effects there is an urgent need for the development of new hypolipidemic drugs from natural resources. In recent year many natural products have been screened for lipid lowering activity[21]. An indigenous approach for hypercholesterolemia having no side effects and being relatively cheap, locally available would be a choice for people in developing countries. Curcumin obtained from the root of the plant *Curcuma longa* Linn has enormous potential for a variety of diseases. Bioavailability of curcumin is a major concern which limits its therapeutic utility. Various studies have reported that curcumin undergoes extensive reduction, most likely through alcohol dehydrogenase, followed by conjugations like sulfation and glucuronidation at various tissue sites mainly in liver and intestine[22-24]. Although, advanced drug delivery systems (nanoparticles, liposomes, micelles and phospholipid complexes) may increase the bioavailability, but the simultaneous administrations of adjuvants, which can block metabolic pathways of curcumin, are still the major means to improve bioavailability of curcumin. Piperine, a known inhibitor of hepatic and intestinal glucuronidation, increases the bioavailability of many drugs including curcumin[15,25]. Quercetin (extracted from red onions) inhibits sulfotransferase enzymes. Therefore in view of the foregoing, there is an extensive need for combinatorial extract "Curcumin with piperine and quercetin" which may enhance bioavailability of oral curcumin by inhibiting the enzymes responsible for the metabolism of curcumin. Interestingly, the results of bioavailability studies shed new light on the inclusion of piperine and quercetin in the combination consisting of CPQ because they showed rapid absorption of curcumin immediately after oral ingestion and shows significant increase in serum curcumin concentration in the concentration versus intensity profile.

The present investigation revealed that all triton induced rats displayed hyperlipidemia as shown by their elevated levels of plasma cholesterol, triglyceride, LDL and the reduction in the HDL level. The large increase in plasma cholesterol and triglycerides due to Triton X-100 injection results mostly from an increase of VLDL secretion by the liver accompanied by a strong reduction of VLDL and LDL catabolism. Triton X-100 acts as a surfactant and has been widely used to block clearance of triglyceride-rich lipoproteins to induce acute hyperlipidemia in several animals[26]. This model is widely used for a number of different aims[27] particularly, in rats and has been used for screening natural or chemical hypolipidemic drugs[28].

Our present study clearly shows that combination consisting of curcumin with piperine and quercetin at a dose of 100 mg kg^{-1} significantly lowered levels of triglyceride (TG), Low density lipoproteins (LDL), cholesterol and significant increase ($p<0.01$) in HDL level (Fig. 2-5) as compared to triton-injected animals. CPQ extract at a dose of 50 mg kg^{-1} did not exhibits lipid lowering effect whereas a combination consisting of curcumin with piperine and quercetin (CPQ) at a dose of 100 mg kg^{-1} gave results comparative to 200 mg kg^{-1} dose of the CPQ extract in triton induced hyperlipidemic rats..This data suggests that cholesterol-lowering activity of the CPQ can be result from the rapid catabolism of LDL cholesterol through its hepatic receptors for final elimination in the form of bile acids[29]. It is well known that HDL-Cholesterol levels have a protective role in Coronary artery disease[30]. Similarly increased level of LDL-cholesterol results in increased risk for the development of atherosclerosis[31]. However, this hypothesis needs to be validating by an experimental study. The study exhibited that elevated blood cholesterol, triglycerides, LDL and decreased HDL which occur in hyperlipidemia, was significantly reduced by the administration of combination consisting of curcumin with piperine and quercetin (CPQ). Hence the hypolipidemic activity of this of CPQ could be attributed to the presence of the piperine and quercetin in the extract which act as a valuable source of polyphenolic compounds. This finding is in agreement with previous reports showing that flavonoids and anthocyanins a heterogeneous group of ubiquitous plant

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polyphenols, have exhibited a variety of pharmacological activities, including the anti-atherogenesis effect[32]. Our findings which indicates the treatment with CPQ exhibited hypolipidemic activity and also paves way towards unravelling its mechanism of action in view of their lipid profiles analogous to standard reference drugs.

CONCLUSION

It can be concluded that combinatorial extract of curcumin 100 mg kg^{-1} treatment was effective in lowering cholesterol, TG, LDL levels and the results were compared to the group of animals treated with lovastatin. Treatment with Combinatorial extract of curcumin exhibited quite competitive potential when compared with the reference drug Lovastatin which indicates that CPQ extract could be explored as an alternative therapeutic agent in the treatment of hyperlipidemia. The results are encouraging enough for further studies aimed at understanding the mechanism of action and to establish the efficacy of the combinatorial extract of curcumin as a hypolipidemic drug.

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