Evaluation of Antihyperlipidaemic Activity of Methanolic Extract of *Cuminum cyminum L.* Seeds in Diet Induced Hyperlipidaemic Rabbits

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Abstract

Objective: To evaluate antihyperlipidaemic activity of methanolic extract of seeds of C*uminum cyminum L.* in hyperlipidaemia in rabbits.
Methods: This study was conducted in Pharmacology Department of University of Karachi; the duration of study was 30 days. Healthy rabbits were taken and hyperlipidaemia was induced by giving high cholesterol diet for four weeks. All animals were divided into three groups, with each group having ten rabbits (n=10).The methanolic extract of seeds of *Cuminum cminum L.* was dissolved in an organic solvent DMSO (Dimethyl-sulphoxide). One group was control-receiving DMSO, while second group received methanolic extract of *Cuminum cyminum L.* (MECC) 500mg/kg and third group received Atorvastatin 2mg/kg.Blood samples were taken at 0, 15th and 30th day of dosing and sent for the tests to Punjwani Centre for Molecular medicine & Drug research (PCMD) Diagnostic Laboratory.
Results: The group that was given methanolic extract of *Cuminum Cyminum L*. showed decrease in High Density Lipoprotein (HDL) - cholesterol ratio (7.62 ± 4.01 to 4.45 ± 3.23) ($p \leq 0.05$), cholesterol (208.54 ± 5.71 to 130.26 ± 5.72) ($p \leq 0.01$), triglyceride (158.75 ± 5.23 to 136.14 ± 8.72) ($p \leq 0.001$) and Low Density Lipoprotein (LDL) (168.17 ± 5.43 to 135.42±11.39) ($p \leq 0.001$) and the group that was given Atorvastatin showed HDL-cholesterolratio(7.33 ± 3.27 to 3.12 ± 2.19) ($p \leq 0.01$), cholesterol (208.11 ± 6.72 to 98.75 ± 3.77) ($p \leq 0.001$), triglyceride (157.21 ± 6.72 to 141.25 ± 6.84) ($p \leq 0.01$) and Low Density Lipoprotein (LDL) (167.25 ± 4.32 to 127.67 ± 10.96) ($p \leq 0.001$) towards its normal levels from day 0 to 30th day of dosing on comparison to control, whereas triglycerides were significantly decreased by extract than atorvastatin.
Conclusion: The result of this study showed methanolic extract of *Cuminum cyminum L.* possesses antihyperlipidaemic activity.
Keywords: Hyperlipidaemia, Dimethyl Sulphoxide, *Cuminum cyminum*, LDL, HDL.
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Introduction

The incidence of coronary heart disease is increasing all over the world, due to lack of physical activities, change in life style and adulteration of food. Ischaemic heart disease is one of the causes of increased morbidity and mortality. Improved techniques are available for early diagnosis and therapy to control its complications and further are under study. Hyperlipidaemia is a major cause of cardiac ailments. Hence, by keeping the lipids at lower limits, the complications and progress of coronary heart disease can be controlled. The long-term treatment of anti-hyperlipidaemic drugs (Statin, Fibrates, Niacin etc.) leads to common side effects like myopathy, liver and kidney dysfunctions etc. Itneeds monitoring and regular adjustment of dosage therefore it leads to attraction towards alternative medicines, which have fewer side effects. It has been observed that cholesterol is the precursor for the development of atherosclerotic vascular lesions[1].

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Plants are a major source of biologically active chemicals[2]. The natural bioactive constituents of drugs are achieved from plants to prepare medicines whether to treat or to diagnose the diseases. While in alternative medicine whole plant or part of it is taken for treatment of various diseases. The plant phytochemicals flavonoids, polyphenols and sugar derivatives are responsible for the plants treating abilities. The quantities of these constituents make them specific in different diseases. It has been proven from number of plant studies that, it produces fewer side effects[3,4]. The usage of herbal medicine is increasing in old aged people and in chronic illnesses due to increased tolerability and affordability[5]. Culinary herbs are used for flavour, taste, aroma and colour of foods. Cumins (*Cuminum cyminum, Nigella sativa*) are the most popular spices in Indo-Pak foods.Bitter cumin (*Cuminum cyminum L*.) is widely used for seasoning in vegetarians and non-vegetarian food preparations. *Cuminum cyminum L*. is one of the constituents of Garam masala, belongs to Apiaceae family and is largely consumed by Asians. Cumin is used in Ayurvedic medicines commonly for gastric illnesses like diarrhoea, dyspepsia, and in carminative mixture and antispasmodic[6,7]. Dhandapani et al. has reported antidiabetic and antihyperlipidaemic activity in alloxan-induced diabetic rats[8]. The disease curing activity is due to its phytochemicals that are flavonoid, glycosides, apigenin, luteolin and essential oils including cuminaldehyde[9,10]. It has been observed from studies that phenolic contents of plants have antioxidant and disease curing activity[11,12]. There are many drugs available to control hyperlipidaemia, but long term usage safety and cost is the drawback to achieve compliance[13]. So, efforts are to search edible medicinal plants capable of ameliorating hyperlipidaemia. According to Ahmad et al it has been reported that hypoglycaemic activity of *Cuminum cyminum* occurs at the dose of 0.5mg to 1.5mg orally[14]. The approval was taken before starting the study by Board of Advanced Studies and Research (BASR), University of Karachi.

All around the world cardiac ailments are increasing day by day due to lack of physical exercise and adulteration of food. Hyperlipidaemia is one of the major causes of increasing cardiac illnesses. In this study, we are evaluating antihyperlipidaemic activity of methanolic extract of seeds of *Cuminum cyminum L*. in non-diabetic diet induced hyperlipidaemic rabbits.

Materials and Methods

Theseeds of *Cuminum cyminum L.* were collected from herbal market of Saddar, Karachi, Pakistan.The seeds were identified and authenticated by Pharmacy Department, Pharmacognosy, Hamdard University, Karachi, Pakistan. The sample was preserved in Herbal Museum of Pharmacognosy, University of Karachi (Sample # 00112) for future reference. The seeds of *Cuminum cyminum L.* were soaked in methanol for fifteen days. Extracts were obtained with the help of rotatory evaporator. Board of Advanced Studies and Research, University of Karachi approved the study, vide resolution # 10 (P) 11 dated: 21-02-2014 and 03-03-2014.

Rabbits, among other animals showed high tendency to develop hyperlipidaemia through exogenous high fatty diet and are used as hyperlipidaemic animal model to study changes due to hyperlipidaemia. It showed typical atherosclerotic changes as seen in humans[15]. Dietary high cholesterol intake of rabbits results in similar effects as in human due to increased level of cholesterol in blood[16]. Healthy rabbits irrespective of sex weighing from 1200-1500 gm were selected for the study. All rabbits were housed under standard conditions and kept for one week before starting the dosing to acclimatize with the surroundings. All animals were handled as per Helsinki's Resolution 1964. The high cholesterol diet was prepared by mixing cholesterol (USD grade, anhydrous, Sigma Chemical Co.) in locally available vegetable oil (Dalda Banaspati Ghee), the mixture was thoroughly coated on the pellets of rabbit chow. After one week of acclimatization, all animals were given high cholesterol diet and water ad libitum[15]. After four weeks, baseline lipid profile was done. All ani-

mals were divided into three groups; each group consisting of ten rabbits (n=10). The methanolic extract was insoluble in water so organic solvent dimethyl-sulphoxide (DMSO) was used. Solutions of methanolic extract of *Cuminum cyminum* (MECC) 500mg/ml and Atorvastatin 2mg/ml was prepared. The dosing given daily the control group was given 2 ml of DMSO, the MECC group received calculated amount of 500 mg/kg[11] and Atorvastatin group received calculated amount of Atorvastatin 2mg/kg[17] for 30 days.

The blood samples were collected from the middle ear vein of rabbits for lipid profile and immediately within an hour samples were sent to PCMD (Punjwani Centre for Molecular Medicine and Drug Research) diagnostic laboratory for determination of lipid profile. The tests were repeated at 0,15 and 30th day of dosing.

All the data obtained from the present study was analysed through SPSS version 20. All results were mentioned as mean ± SD (standard deviation). The one-way Analysis of variance (ANOVA) of variance were followed by post hocTukey's test and Least Significant Difference (LSD) for significance. An effect was defined as significant if $p<0.05$, very significant if $p<0.01$ and highly significant if $p<0.001$.

Results

Table 1 shows the values of components of lipid profile in (n=60) after 4 weeks of high cholesterol diet, which was continued till the end of the study (30 days).

Table 1. Induction of Hyperlipidaemia by high cholesterol diet in rabbits (n = 60).

Tests	Before Induction of Hyperlipidaemia	After Induction of Hyperlipidaemia
Cholesterol/HDL	4.14 ± 2.49	7.49 ± 4.51
Cholesterol	100.08 ± 4.29	208.54 ± 6.72
Triglyceride	100.25 ± 6.40	158.75 ± 6.42
HDL	17.53 ± 4.21	22.51 ± 3.71
LDL	44.52 ± 3.19	167.15 ± 5.53
VLDL	20.51 ± 4.22	26.95 ± 5.24

n=10, Values are mean ± SD (standard deviation)
HDL (high density lipoprotein)
LDL (low density lipoprotein)
VLDL (very low density lipoprotein)

Table 2 shows the effect of dosing in three groups from day 0 to 30th day of dosing. The significance is calculated by applying one-way ANOVA of variance followed by post hoc Tukey's test and LSD. In MECC group on comparison to day 0 regarding cholesterol/high density lipoprotein (HDL) ratio it showed significant ($p\leq0.05$) reduction at 15th and 30th day of dosing. Serum cholesterol level was decreased very significantly ($p\leq0.01$) at 30th day of dosing. The serum levels of triglyceride and low density lipoprotein (LDL) decreased highly significantly ($p\leq0.001$) at 30th day of dosing. While serum levels of HDL and very low density lipoprotein (VLDL) showed non-significant change till 30th day of dosing.

In the group, Atorvastatin comparison of readings from day 0 regarding cholesterol/HDL ratio it showed significant ($p\leq0.05$) and very significant ($p\leq0.01$) reduction at 15th and 30th day of dosing respectively. The serum level of cholesterol showed significant ($p\leq0.05$) and highly significant ($p\leq0.001$)reductionat day 15th and 30th of dosing respectively. Triglyceride levels decreased very significantly ($p\leq0.01$) at 30th day of dosing. The serum HDL level increased significantly ($p\leq0.05$) at 30th day of dosing. LDL level decreased very significantly ($p\leq0.01$) and highly significantly ($p\leq0.001$) at 15th and 30th day of dosing. The serum VLDL decreased significantly ($p\leq0.05$) at 30th day of dosing.

It is observed from results that the effect of methanolic extract of *Cuminum cyminum* (MECC) group and Atorvastatin group. The effect of Atorvastatin on serum cholesterol level is highly significant ($p\leq0.001$), while the effect of MECC on serum triglyceride ishighly significant ($p\leq0.001$) at 30th day of dosing.

Table 2. Effects of dosing on Lipid Profile (mg/dl) in the three groups

Lipid Profile	Drug	Day 0 (Mean±SD)	Day 15 (Mean±SD)	Day 30 (Mean±SD)
Cholesterol/HDL	Control	7.52 ± 3.65	7.14 ± 4.13	7.02 ± 3.41
	MECC	7.62 ± 4.01	4.72 ± 3.10*	4.45 ± 3.23*
	Atorvastatin	7.33 ± 3.27	4.92 ± 2.47*	3.12 ± 2.19**
Cholesterol	Control	208.11 ± 6.72	207.00 ± 4.31	207.00 ± 5.31
	MECC	208.54 ± 5.71	198.42 ± 4.72	130.26 ± 5.72**
	Atorvastatin	208.11 ± 6.72	167.25 ± 4.31*	98.75 ± 3.77***
Triglyceride	Control	158.08 ± 5.23	157.24 ± 9.45	157245 ± 9.45
	MECC	158.75 ± 5.23	152.33 ± 9.56	136.14 ± 8.72***
	Atorvastatin	157.21 ± 6.72	156.3 ± 10.35	141.25 ± 6.84**
HDL	Control	22.51 ± 3.41	22.21b ± 3.61	21.72 ± 4.03
	MECC	22.41 ± 3.51	20.23 ± 4.21	20.07 ± 2.74
	Atorvastatin	21.33 ± 3.46	23.22 ± 5.12	25.43 ± 3.43*
LDL	Control	167.47 ± 5.53	167.47 ± 11.23	167.83 ± 10.61
	MECC	168.17 ± 5.43	156.34 ± 9.28**	135.42 ± 11.39***
	Atorvastatin	167.25 ± 4.32	155.16 ± 10.05**	127.67 ± 10.96***
VLDL	Control	26.72 ± 4.53	26.22 ± 2.43	26.11 ± 2.44
	MECC	26.52 ± 5.23	25.22 ± 3.12	24.41 ± 4.26
	Atorvastatin	26.95 ± 3.34	23.76 ± 4.04	21.45 ± 2.16*

n=10, Values are mean ± SD (standard deviation), significance calculated by using one way
ANOVA followed by post hoc Tukey's test and LSD for significance
*p≤ 0.05, **p≤0.01, *** p 0.001are significant, very significant and highly significant respectively,as compared to day 0.
MECC (methanolic extract of Cuminum cyminum)
HDL(high density lipoprotein)
LDL (low density lipoprotein)
VLDL (very low density lipoprotein)

Discussion

Cuminum cyminum is a herbaceous plant widely distributed in South-East Asia and easily cultivated. Environment has marked effect on its production, which is decreased in environmental stress, especially deficiency of water or drought. Research analysis has proved that shortage of water causes significant (p≤0.05) reduction in yield of *Cuminum cyminum*[18,19]. So, the food products containing phenolic compounds have the potential to fight against different diseases[13,20,21].It has been reported through studies that the plants possess antihyperlipidaemic property by containing phenolic, beta sitosterol and flavonoid compounds in their phytochemicals. These phytochemicals that are present in *Cuminum cyminum L*., is responsible for its effectivity in different ailments[22]. Saravana kumar et al, has revealed that the bioactive principles in these plants cure the lipid dysfunctions and are helpful in maintaining their homeostasis[23]. Normal biochemical processes in humans produce free radicals and other reactive oxygen by-products. The increased or excess production of such free radicals leads to oxidative damage,which leads to disturbance in homeostasis of important biomolecules (e.g. lipids, proteins), eventually leading to many chronic diseases like hyperlipidaemia, atherosclerosis, cancer, diabetes and other degenerative dis-

eases in humans[24]. Similarly, like humans, in rabbits the protective activity of 7á-hydroxylase is inhibited by hypercholesterolaemia and leads to atherosclerotic changes[25]. Animal models like mice, rats, rabbits, pigeons, dogs are ideal to study antihyperlipidaemic drug activity, the mechanism of action and efficacy of these drugs. Rabbits are preferably used as animal model to study antihyperlipidaemic effect of drugs,because they have an increased capacity of storing cholesterol in tissues on being given high fat diet[26,27]. Our study is supported by Srivastava et al, who havealso reported that the methanolic seed extract of *Cuminum cyminum L*. has good antihyperglycaemic and antidyslipidaemic activity in diabetic and dyslipidaemic models[28]. In this study, we have observed the effect of *Cuminum cyminum L*. in hyperlipidaemia and compared it with Atorvastatin. It has been found to be effective in decreasing lipoproteins but its effect is more marked on triglycerides as compared to Atorvastatin. The antihyperlipidaemic activity on cholesterol is found to be highly significant in the case of Atorvastation, while *Cuminum cyminum* has a relatively decreased effect. The triglycerides, on the other hand, show highly significant reduction by *Cuminum cyminum* as compared to a less significant effect by Atorvastatin. Further studies are required to explore the mechanism of action of *Cuminum cyminum*. Our study also supports the other studies of antihyperlipidaemic activity of *Cuminum cyminum.*

Conclusion

The antihyperlipidaemic activity of methanolic extract of *Cuminum cyminum* showed that it is effective in hyperlipidaemia but more effective in lowering triglycerides serum level as compared to Atorvastatin.

Conflict of Interest

Authors have no conflict of interests and no grant or funding from any organization

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