Effect Of Celery (Apium Graveolens) Seeds Extract On Protease Inhibitor (Ritonavir) Induced Dyslipidemia

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Abstract: Objective: The present study was carried out to explore protective effects of ethanolic extract of Apium graveolens (celery seeds) on ritonavir, a protease inhibitor induced dyslipidemia. Materials & Methods: Thirty mice were divided into 5 groups. Group 1 mice served as healthy control. Group 2 mice were given drug ritonavir at doses of 33.33 mg/kg (200mg/day, human dose), group 3 received same dose of ritonavir and ethanolic extract of celery seeds(CSE) at the doses of 75mg/kg. Group 4 was treated with same dose of ritonavir and CSE at high doses i.e.150mg/kg. Group 5 mice were given ritonavir and hypolipidemic drug, fenofibrate. All groups of mice were given the drug and extract by oral gavage route for the period of 12 weeks. Blood lipid profile and liver lipids of all the groups were tested at the end of 12 weeks. Results: Blood lipid profile was found to be deranged in the group of mice treated with ritonavir. Concurrent treatment of ritonavir with low dose of CSE showed no significant improvement in blood lipid profile in group 3 mice but high dose CSE along with ritonavir with the same dose of ritonavir exhibited significant improvement ($p<0.05$) in group 4 mice. Effect of fenofibrate in group 5 was almost equally effective as that of high dose of CSE. There was a similar pattern of decrease in liver lipids in all the groups ($p<0.05$). Conclusion: Above results suggest that ethanolic extract of celery seeds possess potential for improving blood lipid profile & liver lipids deranged by ritonavir when given concurrently. Its efficacy approaches that of fenofibrate. Its intake along with ritonavir would be better in terms of cost and side effects as compared to fenofibrate. [Ahmed Q et al NJIRM 2012; 3(1) : 52-56]

Key Words: celery seed extract, protease inhibitor, dyslipidemia

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Introduction: Ritonavir, a protease inhibitor (PI), is a potent anti HIV drug and used in variety of treatment regimes. Addition of ritonavir to current therapy reduces HIV related mortality & disease progression by approximately 50% over a median of 6 months of follow-up[1]. It is used infrequently as a sole protease inhibitor in combination regimes because of side effects & potential for drug interactions. However, numerous clinical trials have shown benefit of ritonavir as a pharmacokinetic enhancer in various dual protease inhibitor combinations[2]. Apart from other reversible side effects, it causes dose dependent elevations in serum total cholesterol & triglyceride[3]. More than 50% of patients receiving combination regimes, develop dyslipidemia, a well known risk factor for serious cardiovascular complications including endothelial dysfunction & atherosclerosis[4].

Numerous studies both in animal models and humans have suggested that inflammation represents one of the major events in the pathophysiology of atherosclerosis[5].

Celery seeds (apium graveolens) extract used in ayurvedic medicine for centuries contain powerful healing factor, a compound known as 3n butylphthalideor (3nB). 3nB was discovered as the active component of celery in response to investigations by researchers seeking to explain some of the medicinal effects of celery including lowering of BP & cholesterol. It has been found to reduce formation of arterial plaques in experimental studies[6,7].

The isolated compounds from the seeds exhibited antioxidant & inhibitory effects of COX & topoisomerase enzyme[8]. The part of celery extract responsible for hypocholesterolemic action is the sugar or amino acid side chains, which mainly lowered the total cholesterol level by increasing bile acid excretion[9,10].

An increasing amount of attention has been paid to the use of complementary & alternative medicine as a part of treatment for HIV infection & the complications associated with HAART[11]. Keeping this in view, the present study has been designed to explore protective effects of celery seed extract on ritonavir induced dyslipidemia.

Material and Methods: Plant Material : Celery seeds were purchased from local market & confirmed by morphological identification. Biologically active extracts of seeds were produced by controlled ethanolic extraction, distillation , drying & further processed by supercritical fluid extraction by using soxhalet apparatus.

Drug : Ritonavir (Ritomune) tablets, 100mg, were arranged from pharmacy of this Institute by contact with Cipla company.

Animals : Thirty three mice weighing around 30-50 gm bred and maintained in our Institute's animal house, were used in the study. Study was conducted in the experimental animal laboratory of department of Pharmacology in this Institute. Animals were housed in polypropylene cages in room where temperature of 27°C ± 2°C &12 hrs light & dark cycles were maintained. Food (standard pellet diet) & water were provided ad libitum. The protocol was duly submitted and approved by Institutional Animal Ethical Committee.

Treatment : Toxicity testing: Before starting the study, one group of animals (n=3) was tested for CSE toxicity. They were given CSE at the doses of 300mg/kg/day for 15 days.No toxic effects were observed. The behavior of treated animals were found to be normal[12].

Male albino mice were divided into 5 groups. Each group comprised of 6 mice. The first group of mice considered as healthy control group and gavaged with 1ml of distilled water. Second group of mice (positive control) treated with ritonavir at doses of 33.33mg/kg that corresponds to human dose, 200mg/day. Third group of mice were given same dose of ritonavir supplemented with ethanolic extract of celery seed extract at doses of 75mg/kg. Fourth group received higher doses of extract i.e.150mg/kg with the drug and fifth group of mice were given fenofibrate, 33.33mg/kg (human dose, 200mg/day) along with ritonavir. Extract & drug was given by oral gavage tube daily for 12 weeks.
Collection of blood

Blood was collected by retro orbital sinus puncture at the end of 12 weeks, under mild ether anaesthesia. The collected samples were centrifuged for 10 minutes. Serum samples were collected & it is used for various biochemical experiments. Then animals were sacrificed and collected the liver.

Liver lipids: Total lipids were extracted from the liver according to Folch et al[13].

Biochemical Analysis : The serum were assayed for total cholesterol (TC), triglycerides(TG), HDL, LDL,VLDL. The serum cholesterol levels were determined by Zak's method. The TG, HDL, LDL & VLDL was calculated by using standard methods [14-17].

Statistical Analysis: Means and standard error of means (SEM) for each group were determined using graph pad prism software and compared for statistical significance using students't' test.

Result: The lipid profile in serum altered on administration of ritonavir for 12 weeks in group 2. The changes in lipid profile(all parameters) were significant ($p<0.05$) as compared to control mice, group 1(Table1). Group 3, who received ritonavir at same doses but supplemented with celery seed extract at low doses showed no significant changes with group 2 ($p>0.05$). Group 4, who were given ritonavir & high dose of extract, samples exhibited significant improvement ($p<0.05$) almost approaching the efficacy of hypolipidemic drug (Table 1). Group 5, received ritonavir and hypolipidemic drug, fenofibrate , results showed significant improvement in all the parameters ($p<0.05$) (Table 1).

Liver lipids also showed changes as was observed in serum. The liver lipids in control group (group 1) were slightly more elevated than that in serum and accordingly there were changes in all other groups (table 2).

Table 1 – Effect Of Ritonavir And Celery Seed Extract (CSE) On The Serum Lipid Profile Of Mice

Groups	Total Cholesterol(TC) (mg/dl)	Triglyceride(TG) (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL(mg/dl)
1	62.06 ± 1.46	73.69 ± 1.51	21.85± 1.16	23.64± 1.04	14.26 ± 0.68
2	85.67 ± 1.96*	89.96 ± 2.47*	17.84± 0.65*	37.61± 2.06*	30.34 ± 1.38*
3	78.50 ± 1.54	86.37 ± 1.92	18.34± 1.16	33.64± 1.38	29.06 ± 0.89
4	71.66 ± 0.63**	77.90 ± 0.92**	20.67± 1.04**	28.37± 1.13**	21.41 ± 0.92**
5	69.19 ± 3.32**	76.88 ± 3.23**	21.15± 1.20**	23.57± 1.60**	18.34 ± 1.30**

Table No. 2 – Effect Of Ritonavir And Celery Seed Extract (CSE) On The Liver Lipid Profile Of Mice

Groups	Total Cholesterol(TC) (mg/dl)	Triglyceride(TG) (mg/dl)	HDL (mg/dl)	LDL (mg/dl)	VLDL(mg/dl)
1	66.88 ± 3.43	62.00 ± 2.78	32.12± 2.03	25.81± 2.18	14.29 ± 1.17
2	105.43 ± 3.91*	86.78 ± 2.01*	31.59± 1.45	50.32± 3.27*	23.51 ± 2.06*
3	94.71 ± 4.16	85.15 ± 3.06	30.88± 1.34	44.51± 1.97	17.71 ± 1.95
4	68.10 ± 2.79**	67.48 ± 1.68**	37.08± 2.23	27.51± 1.57**	14.35 ± 0.79**
5	66.98 ± 1.92**	63.61 ± 1.72**	32.90± 1.92	27.52± 1.24**	13.87 ± 1.09**

Group 1 – Healthy mice, Group 2- Ritonavir treated mice, Group 3- Ritonavir treated mice + Celery seed extract (CSE) low dose, Group 4- Ritonavir treated mice + Celery seed extract (CSE) high dose, Group 5- Ritonavir, treated mice + Fenofibrate, Values are mean ± SEM , n=6,*p<0.05 Vs Group 1, **p<0.05 Vs Group 2

No significant change occurred in group 3 mice as compared to group 2(p>0.05). Group 4 exhibited significant changes (p<0.05) in all the parameters except HDL. Group 5 also showed significant changes in all the parameters except HDL(p<0.05)(table 2).

Discussion: Several studies have been carried out in past revealing that an increase in HDL cholesterol & decrease in TC, LDL,VLDL & TG is associated with a decrease in the risk of ischemic heart disease (IHD)[18]. Addition of hypolipidemic drugs along with ritonavir has resulted in drug interactions and side effects. So, development of other therapeutic interventions to combat HIV PI induced cardiovascular complications is urgently required. According to one study, daily administration of leptin significantly reversed the elevated plasma cholesterol level induced by ritonavir[19]. Another study stated that adiponectin (fat derived hormone) replacement therapy markedly ameliorates ritonavir induced elevations of triglyceride & free fatty acids but has little effect on hypercholesterolemia & hepatic cholesterol synthesis[20].

Herbal preparation has been used in many parts of the world since ancient times. In recent years, their popular alternative to modern medicine has increased considerably[21]. Weibin Zha et al.,[22] speculated potential protective effect of berberine , an isoquinoline alkaloid obtained from many herbs, on HIV PI induced inflammatory response in macrophages.

Extract of celery possesses a lot of medicinal properties. One of these is hypolipidemic activity. There are handful of experimental studies which have shown this effect. Significant lowering in serum TC, TG, LDL & VLDL & increase in HDL have been noticed in celery treated animals[9,10 &24] . Other researchers showed also that celery seed extract helped in the support of healthy BP & cholesterol levels because of its beneficial effect on prostaglandin levels. Le and Elliot[6] suggested that lipid lowering action of this natural product may be mediated through inhibition of hepatic cholesterol biosynthesis, increased faecal bile acid excretion & enhanced plasma lecithin: cholesterol acyltransferase activity and reduction of lipid absorption in the intestine.

In this study, lipid lowering action of ethanolic extract of celery seeds was exploited to reduce the elevated levels of TC, TG, LDL, VLDL caused by administration of doses that correspond to low dose of ritonavir i.e. 100mg twice a day. This dose is selected because ritonavir is very commonly used in boosted regime as a pharmacokinetic enhancer at low doses and it has been exhibited by a study that even low doses of ritonavir are sufficient to cause dyslipidemia[23]. Concurrent administration of ritonavir & high dose extract dose for 12 weeks resulted in near normal levels of lipids, however, there was no significant change in lipid profile when low dose of extract was used along with ritonavir
Addition of fenofibrate , exposes patients for more side effects and it is also a financial burden for the patient.

Conclusion: The present study concludes that celery seed extract has shown good antihyperlipidemic effect as also proved by Kamal M et al.,24 and could be of value in bringing the deranged parameters of serum lipid profile & liver lipids to baseline value in ritonavir treated patients.

Acknowledgement: We would like to thank Dr. Vinod Kumar, Dean of our Institution, for permitting us to publish this manuscript.

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