EVALUATION OF HYPOLIPIDEMIC EFFECT OF CITRUS LEMON

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ABSTRACT

Herbs have been used for several cardio vascular diseases, such as congestive heart failure, hypertension, angina pectoris, atherosclerosis, arrhythmia, cerebral and venous insufficiency. Atherosclerosis is the primary cause of heart disease and stroke. Hence blood lipid levels are probably the major determinant of the development of cardiovascular disease. Citrus fruit and juices have long been considered a valuable part of a healthy and nutritious diet. It is well established that some of the nutrients in citrus promote health and provide protection against chronic disease.
The present study has been specifically designed to investigate the hypolipidemic effects of citrus lemon juice in rabbits after high cholesterol diet for four weeks. The citrus lemon juice (1ml/kg/day) revealed a significant reduction in serum cholesterol, triglycerides; low density lipoprotein levels and resulted in an increase in high density lipoprotein. These results suggest that the hypocholesterolemic effects of citrus lemon juice may be due to its antioxidant effect.

Keywords: Citrus lemon, Hypolipidemic effect.

INTRODUCTION

Cholesterol is an amphipathic sterol, hydrophilic at the hydroxy end with a hydrophobic hydrocarbon side chain and thus can be incorporated into membrane bilayers. As well as being an essential structural component of cell membrane, cholesterol determines membrane lipid organization and structural integrity (Goldstein *et al*., 2006; Ikonen, 2006). Hypercholesterolemia is characterized by high levels of cholesterol in the blood .It is not a disease but metabolic derangement that can contribute to many disease, most notably cardiovascular disease. It is closely related to the terms hyperlipidemia and hyperlipoproteinemia .Elevated cholesterol in the blood is due to abnormalities in the levels of lipoproteins, the particles that carry cholesterol in the blood streams. This may be related to diet, genetic factors (such as LDL receptor mutations in familial hypercholesterolemia) and the presence of other diseases such as diabetes and an under active thyroid (Durrington etal, 2003).

Hypercholesterolemia is silent disorder that is usually diagnosed after a heart attack or stroke. Cardiovascular diseases have become a major clinical and public health problem in south Asian countries specifically India, Pakistan, Srilanka, Bangladesh and Nepal. This population carries increased risk even if they migrate to other countries (Iqbal *et al.*, 2005).The national cholesterol education program (NCEP) published a guideline in 1993 for screening and treating hyperlipidemia (Eric *et al.,* 2002). The NCEP program includes therapeutic life style changes such as reduced intakes of saturated fats to < 7% of total calories and < 200 mg/day cholesterol. 25% to 35% daily fat intake

provides low saturated and trans fats to body. Therapeutic options to reduce LDL also involve the use of plant steroids, 2gm/day and increased intake of viscous fiber (soluble fiber) about 10-25 gm/day. Similarly exercise and life style modification is also beneficial. However most of the allopathic drugs are toxic and generate free radicals in the body (Beldeu, 2006). Thus present study has been specifically designed to investigate the lipid lowering effect of citrus lemon with minimal side effects.

MATERIALS AND METHODS

Selection of Animals
The study was carried out on 18 healthy white rabbits of either sex weighing from 1000-1500 gm. Rabbits were selected as experimental animals since biochemical changes produced in rabbits are comparatively similar as observed in humans (Irena *et al*., 1979). Moreover sufficient amount of blood samples can easily be collected, easy to handle and economical.

Animal Groups
Animals were divided into two groups control and treated each containing 9 animals. Apparent health of these animals was monitored during the conditioning period under the laboratory environment for a week before administration of drug specifically noticing loss of hair, diarrhea, hematuria, skin ulceration and loss of activity. They were housed individually in cages, under controlled condition of temperature 23±2ºC. Diet and water was provided ad libitum.

Dosing
Animals of both groups were given high cholesterol diet (HCD) daily for a period of 30 days i.e. 0.125gm/kg cholesterol (Merck) in 0.5% corn oil purchased from local

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market. After 30 days animal of treated group were administered fresh citrus lemon juice orally for 30 days in the dose of 1ml/kg during the first phase of study, while animals of control group were administered water through same route equivalent to the volume of respective doses according to their body weight. During the second phase of study animals of treated group were further administered fresh citrus lemon juice up to 45 days and compared with control for the same period.

Sample Collection
Blood samples of 5 ml were collected thrice, first after 30 days of HCD then again after 30 and 45 days dosing of citrus lemon juice from ear vein of the animals in gel tube for evaluation of lipid profile.

Gross Toxicities
Overall gross toxicities such as skin ulceration, average weight variation, hematuria, alopecia, loss of activity, diarrhea, salivation, aggressive behavior and color of skin in different groups of animals was observed during the total period of experiment.

Animals of control group showed no gross toxicities, but there was an overall increase in average weight of control animals by 15 to 35 gm however animals at HCD comparatively showed more increase in average weight of the animals by 25 to 260 gm during the total period of experiment except in second week, where there was decrease in weight by 75 gm. Animals at HCD revealed slight gross toxicities, such as yellow skin, alopecia (especially at neck and foot), yellow urine with acidic pH and loss of activity that may be due to severe diarrhea.

Animals at citrus lemon (1ml/kg) after HCD did not reveal gross toxicities. However there was loss of weight of 30 to100 gm during the total period of study. However at the end of study there was 53 gm decrease in average weight.

MORTALITY RATE

Table 1 reveals mortality rate of animals kept on HCD for 30 days, treated with lemon and control animals for 45 days. Deaths were observed in animals at HCD during 2nd and 3rd weeks of dosing. However no death was observed in animals of control and treated groups.

Table 1. Comparison of Overall Mortality Rate (%)

Animals Groups	Mortality Rate
HCD	07/25(28.00)
Control	0/9(00.00)
Lemon	0/9(00.00)

No. of animals expired/total animals (percentage)

BIOCHEMICAL ANALYSIS

Serum samples collected at the end of dosing were utilized for assessment of lipid profile using standard reagent kits of Human Germany. All the values were expressed as the mean and standard error to the mean. Student test was performed and P values were observed. Values of $P<0.05$ were considered as significant and $P<0.005$ as highly significant. All statistical procedures were performed according to the method of Alcaraz and Jimenez (1989).

RESULTS

Table 2 reveals the comparative effects of citrus lemon on cholesterol, triglycerides, LDL and HDL at the dose of 1ml/kg citrus lemon for 30 and 45 days respectively in animals received high cholesterol diet for 30 days.

After 30 days animals showed highly significant decrease in cholesterol 150.8 ± 19.1mg/dl in comparison to control animals i.e. 345.3 ± 28 mg/dl. While after 45 days decrease in cholesterol continued and a highly significant decrease was observed i.e.16.9 ± 2.8 mg/dl in comparison to the value of control animals i.e. 232.6 ± 9.0 mg/dl. Similarly a highly significant decrease in LDL concentration was observed after 30 days i.e. 122.6 ± 13 mg/dl in comparison to control animals i.e. 273 ± 38 mg/dl. The decrease in the levels of cholesterol and LDL persisted even after 45 days. Where as HDL concentration was increased significantly after 30 days i.e. 7.27 ± 1.7 mg/dl than as compared to control i.e. 3.07 ± 0.26 mg/dl. However increase in HDL level became insignificant after 45 days i.e. 2.15 ± 0.31 mg/dl as compared to control i.e. 2.1 ± 0.32 mg/dl.

Decrease in triglycerides was insignificant after 30 days as compared to control animals. However a highly significant decrease was observed in triglyceride levels after 45 days i.e. 26.69 ± 1.4 mg/dl in comparison to control 40.3 ± 3.5 mg/dl.

Table 3 reveals the %age reduction of cholesterol, triglycerides, LDL and HDL at 1ml/kg dose of citrus lemon for 30 and 45 days respectively in animals received high cholesterol diet for 30 days.

DISCUSSION

Herbs have been used as medical treatments since the beginning of civilization and some derivatives (e g, aspirin, reserpine, and digitalis) have become mainstays of human pharmacotherapy. Herbs have been used for several cardiovascular diseases, such as congestive heart failure, hypertension, angina pectoris, atherosclerosis, arrhythmia and cerebral insufficiency. However, many herbal remedies currently in use have not undergone
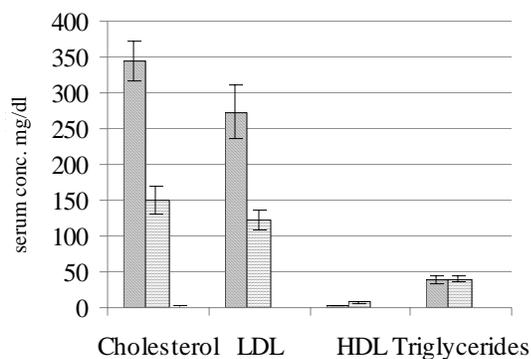
Fig. 1. Histogram showing comparative effects on lipid profile in HCD and citrus lemon animals after 30 days.
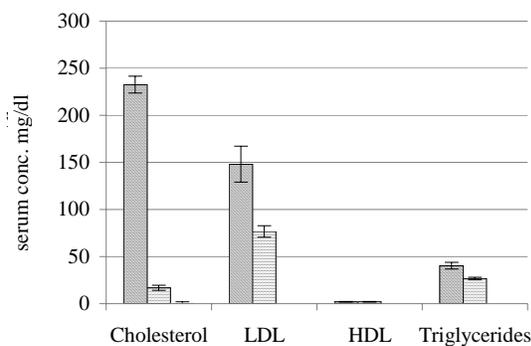

Fig. 2. Histogram showing comparative effects on lipid profile in HCD and citrus lemon animals after 45 days.

Table 2. Comparative effects of citrus lemon on lipid profile after high cholesterol diet for 30 and 45 days

No. of Days	Groups	Cholesterol Mg/dl	LDL-CHOL Mg/dl	HDL-CHOL Mg/dl	Triglycerides Mg/dl
30	Control	345.30±28.0	273.0±38.0	3.07±0.26	39.40±4.7
	Lemon	150.80±19.1**	122.6±13.0**	7.27±1.7*	39.40±4.2
45	Control	232.60±9.0	148.1±19.0	2.10±0.32	40.30±3.5
	Lemon	16.94±2.80**	76.8±6.10**	2.15±0.31	26.69±1.4**

n = 9, Average ± SEM, *p < 0.05 significant as compared to control, **p < 0.005 highly significant as compared to control

Table 3. Percentage reduction in lipid profile after 30 and 45 days administration of citrus lemon

No. of days	Groups	Cholesterol	LDL	HDL	Triglycerides
		%age			
30	Control	51	52.2	21.4	36.1
	Lemon	79	80.5	20.8	64
45	Control	68.5	73.8	40.4	41.2
	Lemon	97.7	87.8	65	67.1

careful pharmacological assessment, and some have the potential to cause serious toxic effects and major drug-to-drug interactions. The high prevalence of herbal use in current medical practice throughout the world has increased questions about the safety and efficacy of herbal drugs for cardiac disease and clinicians must be informed about the potential for benefit and harm. Thus continuous research is necessary to elucidate the pharmacological activities of the herbal remedies now being used to treat cardiovascular diseases (Nick *et al.*, 1998).

Vegetables, fruits and their constituents are potent effectors of biological system in humans (Lampe, 1990).The cholesterol-lowering effects of vegetables, fruits and their constituents have been examined in detail. Daily doses of these food showing cholesterol-lowering effects in human have been reported as follows; variety of vegetables (750g /day) and fresh apples (350-400g/day), guava fruit (0.5-1kg /day) , garlic (10-20g /day) and a low saturated fat diet combined with vegetables, protein (33g/day) and a high soluble fiber(18g/day) (Lampe,1990; Jenkins, 1999).

Atherosclerosis is the primary cause of heart disease and stroke. Hence, blood lipid content is probably the major determinant of the development of cardiovascular disease. Several authors have reported the hypolipidemic effects of Citrus juice (Monforte, 1995; Gorinstein, 2004) moreover, some studies suggest that a high dietary intake of orange or grape juice might reduce hypercholesterolemia, and this was postulated to be largely due to the principal Citrus flavanones, hesperidin from orange and naringenin from grapefruit, as their glycosides, hesperitin and naringin (Kurowska, 2000).

The present study has been specifically designed to investigate the hypolipidemic effects of Citrus lemon juice in rabbits after high cholesterol diet for 4 weeks. The Citrus lemon contains important natural components, including citric acid, ascorbic acid, minerals and flavonoids (Benavente *et al*, 1997) though health-related properties of citrus lemon have been associated with their content of vitamin C, but recently flavonoids have been also shown to play a role in this respect. Some researchers suggest that flavonoids have different biological functions, including antioxidative, anti-inflammatory, antiallergic, antiviral, antiproliferative, antimutagenic and anticarcinogenic activities (Middleton & Kandaswami, 1986; Godeberg, 1994, Meyer, 1994 and Rice, 1997).

The citrus lemon juice (1ml/kg/day) revealed a significant reduction in serum cholesterol, triglycerides, low-density lipoprotein levels and resulted in an increase in high-density lipoprotein(Fig 1 & 2). These results suggest that the hypocholesterolemic effect of citrus lemon juice may be due to its antioxidant effect since previous studies has suggested that antioxidant (vitamin C) administration in hypocholesterolemic rats improves endothelial function of coronary and peripheral vessels (Henry *et al.*, 1997). It is widely accepted that elevations in cholesterol and LDL plasma levels are major factors for coronary heart disease

There has been relationship between elevated LDL and atherosclerosis. Since LDL in the blood gets deposited in the blood vessel walls and becomes the major component of atherosclerotic plaque. Studies suggest that pathological process could be reversed by reducing the serum LDL level (Ross, 1993). Since citrus lemon juice has significantly reduced cholesterol and LDL levels therefore it may be concluded that citrus lemon might prevent atherosclerosis. Moreover there has been significant increase in serum HDL levels which is considered to be good cholesterol (Stein, 1999). The increase in HDL has shown to slow down the atherosclerosis process (Nofer, 2002). Therefore it can be safely concluded that citrus lemon juice could be considered as a valuable supplement to prevent coronary diseases.

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