

Effects of *Citrus aurantifolia* peel essential oil on serum cholesterol levels in Wistar rats

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ABSTRACT

The present study seeks to evaluate the effects of *Citrus aurantifolia* peel essential oil on serum triglyceride and cholesterol levels in *Wistar* rats. Thirty *Wistar* rats were divided into 5 groups: control, sham, and 3 experimental groups. The animals were treated in 2 phases: first, except for control group, which received normal saline, the rest of the groups were fed with a high cholesterol regimen to induce hyperlipidemia; then, the 3 experimental groups were treated with *Citrus aurantifolia* peel essential oil in 3 different doses: 25, 50, and 100 μ lit/kg. Results: The sham group demonstrated a significant rise in mean serum triglyceride, cholesterol, and LDL level in comparison with the control group ($p < 0.05$) proving the effectiveness of hypercholesterolemia induction. The results of experimental groups treated with peel essential oil in 50 and 100 μ lit/kg doses demonstrated a significant reduction in triglyceride, cholesterol, and LDL ($p < 0.01$). Application of the peel essential oil of *Citrus aurantifolia* showed significant decrease of cholesterol in rats in doses 50 and 100 μ lit/kg. It is suggested that for cardiovascular risk reduction, the essential oil may be investigated for the same effects in human beings.

Keywords: Hypercholesterolemia; Citrus Peel Extract; Lipid; Wistar Rat.

INTRODUCTION

Current predictions estimate that by the year 2020 cardiovascular diseases (CVD), particularly atherosclerosis, will become the leading global cause of total disease burden [1]. Recently, mortality caused by ischemic heart disease, comprise 7,063,000 deaths annually (12.6% of all causes of death), ranks first among the 10 leading causes of death world wide [2]. Ischemic heart disease itself, is caused by high serum cholesterol level. Hyperlipidemia represents a determinant for development of atherosclerosis and an important risk factor for cardiovascular disease [3] which is also the cause of 3,880,000 deaths annually throughout the world [2].

Considering the low rate of successful treatments, their high prices, and concerns on their availability, it has been suggested that the wise approach toward CVD is primary prevention and early treatment of hyperlipidemia [4]. This strategy seems to have been proven to be useful as the rate of CVD has increased in recent decades [4, 5].

High serum triglyceride, a high level of LDL, and low serum HDL has proven to be associated with coronary artery disease [5]. Dyslipidemia, either primary (hereditary) or

secondary (due to life style), is a modifiable risk factor [5 & 6] whose resolution reduces the total CVD risk score [7].

There is an undeniable tendency in the general population for herbal medicine and it is wise to direct this tendency toward major health problems if they prove to be effective. Citrus fruit peels have long been a source of herbal products especially in Chinese medicine [8]. In vitro studies indicate that the flavonoid component of citrus essential oil has cholesterol-lowering effects in cultured liver cells [9] and recent studies favor their effects in dyslipidemia both in animal studies and human clinical trials [10-14].

In this study we treated previously induced dyslipidemia in rats with evaluating how the lipid profile might respond to this herbal essence. The ingredients of *Citrus aurantifolia* peel essential oil have been investigated to be limonene, alpha-terpineol, gamma-terpinene, terpinolene, linalool and cineole [8].

MATERIALS AND METHOD

Thirty male *Wistar* rats were purchased from Pasteur Institute of Iran (Tehran, Iran) at 6 weeks age. They were kept in animal room at 22-25° C temperature and were fed with

commercial pellets until weighing 250-260g. They were randomly divided afterwards in to 5 groups: experimental-1 (E-1), experimental-2 (E-2), experimental-3 (E-3), sham and control group (n=6). The first 4 groups (E-1, E-2, E-3, and sham) received cholesterol (Merck Labs) for 4 weeks in two simultaneous ways: mixed with their daily pellet (2g cholesterol dissolved in 6 ml Oleic acid/100g pellet) and a separate diet of twice weekly (0.4 g dissolved in 1.2 ml Oleic acid) drip-fed. The Oleic acid was purchased from Merck Labs. The control group received a cholesterol-free diet and was force-fed with normal saline. After 4 weeks of cholesterol treatment, cholesterol was withdrawn from the diets. The E-1, E-2 and E-3 experimental groups started to be force-fed with the *Citrus aurantifolia* of *Rutaceae* family peel essential oils (Barij Essence pharmaceutical Co., Kashan, Iran) for 6 weeks, once daily, dissolved in distilled water, 25 μ lit/kg, 50 μ lit/kg, and 100 μ lit/kg respectively (according to the information provided by provider). The sham group received normal saline once daily with the same method of experimental groups while the control group did not receive any treatment.

After the 70th day, the food was withheld for 12 h and the rats underwent general anesthesia by having them breathe evaporated diethyl ether in a closed chamber for 1 to 3 minutes and blood samples were collected from the inferior vena cava with a 5 ml syringe carrying a 24 F needle. The rats were treated in this study according to The International Guiding Principles for Biomedical Research (1985). The serum was extracted from the centrifuged blood samples at 3000 rpm for 15 minutes and the sera were kept in -4 °C until analyzed.

Cholesterol (Chol), High Density Lipoprotein (HDL), and Low Density Lipoprotein (LDL), as well as TG (CPO-PAP), were all analyzed enzymatically (CHOD-PAP) using kits purchased from Pars Azmun Co (Tehran, Iran).

The results were analyzed using SPSS 16.0 (SPSS Inc; Chicago, Illinois, USA). The descriptive results were reported as mean and standard deviation. In order to interpret the results differences between those in experimental groups and in sham and control groups, Kruskal Wallis test was used. Mann-Whitney U test was applied to compare each experimental group with sham. The sham group and the control group were also

compared using Mann-Whitney U test. P value was considered significant at < 0.05 level.

RESULTS

The level of cholesterol (Chol), triglyceride (TG), high density lipoprotein (HDL), and low density lipoprotein (LDL) for each group is described in Table-1. Comparing the sham and the control groups, serum level of Cholesterol, TG, and LDL were markedly higher in the sham group ($p < 0.05$), but the lower level of HDL in the sham group was not statistically significant.

Cholesterol and LDL levels were significantly lower in all experimental groups in comparison with the sham group. Triglyceride levels in all experimental groups were lower than that in the sham group; however, this difference was statistically significant only in E-2 and E-3 ($p < 0.01$). Although the HDL levels were higher in all experimental groups both versus the shams and the controls, the result of Kruskal-Wallis test of heterogeneity in all 5 groups opposed this inference ($p = 0.110$).

The difference supposed to be made by the cholesterol regimen was first inspected in the results of the sham group whose variables were compared with those of the controls. This indicated that the cholesterol regimen had caused a significant rise in serum cholesterol, triglyceride and LDL, but this comparison did not show marked decrease in HDL mean value of the sham group. Then, results of all experimental groups (E-1, E-2, and E-3) were compared corresponding with variables in the sham group.

TG did not reduce significantly in the E-1 group which was treated with 25 μ lit/kg of essential oils but the significant decrease was observed in TG, Cholesterol and LDL in the other two experimental groups which may reduce cardiovascular risk [7, 15].

CONCLUSION

The National Cholesterol Education Program (NCEP) in 2001 presented a guideline that a fall in HDL cholesterol less than 40 mg/dl HDL is a risk factor, but HDL cholesterol ≥ 60 mg/dl denotes a negative risk factor as it removes one risk factor for CVD from total scores [7]. In Kurowska's study the serum HDL decreased significantly in rabbits which had been given orange juice, but the

decreased HDL level in rabbits given grapefruit juice was not significant [12]. Kurowska (2000) showed that human participants who took 750 ml/day orange juice showed significant increase in serum HDL cholesterol as much as 20%. Not only the HDL level in subjects of our study did not decrease, but showed a mild increase in experimental groups, and none of them was statistically significant.

Kurowska et al (2000) concluded that 4 weeks treatment of mild-moderate hypercholesterolemic patients with 750 ml/d of orange juice raises the HDL level and hence decreases LDL/HDL ratio [10]. Unlike the human study, orange juice decreases the LDL level in rabbits.

Kurowska et al (2000) postulated that incompatibility of the results in human and animal studies using the same substance with different results might be due to the low amount of the juice or its components consumed by the participants [10]. This rationale could be correct since the concentration of active components matters and reaching to effective dose of component by taking juices solely requires more than normal servings of orange juice, which could cause non-compliance. This picture is not encountered in treatment with essential oils, as they are rich in active components and need not to be administered in large quantities to be effective.

Cesar et al (2010), showed that consuming orange juice as a daily juice for 60 days (750 ml/d) could help hypercholesterolemic patients to decrease their risk factors of cardiovascular by reduction of LDL and cholesterol, but HDL and triglyceride remained unchanged, although free-cholesterol transfer to HDL increased [16].

In conclusion, the effect of *Citrus aurantifolia* peel essential oil on the gross indicators of dyslipidemia seems promising in suggested doses of 50 μ lit/kg and 100 μ lit/kg, but further studies are required to evaluate the usefulness of this herbal essential oil in normal human and dyslipidemic patients regarding clinical framework.

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