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Short Communication

Antioxidant effects of *Citrus aurantifolia (Christm)* juice and peel extract on LDL oxidation

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Abstract

BACKGROUND: We studied the antioxidant effects of fresh juice and peel extract of *Citrus aurantifolia (Christm)*.

METHODS: Low density lipoprotein (LDL) was separated from one hypercholesterolemic human serum by modified Bronzert and Brewer procedure. Oxidation of LDL was measured at 234 nm against 0, 5, 10, 20, 25, 30 and 40 µl of fresh lime juice and 0, 5, 10, 15 and 20 µl of peel polyphenolic extract solution in DMSO.

RESULTS: 5 µl of lime juice didn't change LDL oxidation. 10 µl of juice inhibited LDL oxidation, and with increasing the juice concentration, LDL was oxidized faster. The higher concentrations of peel extract prevented LDL oxidation better than the lower ones.

CONCLUSIONS: Both juice and peel demonstrated antioxidant properties, but the excessive consumption of lime juice seems not to be beneficial. Regarding the intensity and type of flavonoids, lime juice and peel may show different effects.

KEYWORDS: Antioxidant, *Citrus Aurantifolia (Christm)*, Juice, LDL Oxidation, Peel.

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There are strong evidence that oxidative modification of low density lipoprotein (LDL) plays an important role in initiating vascular inflammation and atherosclerosis lesion formation.[1-3]

The results of some researches suggest that a diet rich in vegetables and fruits may alter the atherogenicity of LDL particle [4] and protect its oxidation.[5] It has been found that this effect of fruits is attributable to antioxidants like vitamins and phenolic phytochemicals.[6] Citrus family is a large group of fruits which contains various bio functional nutrients as flavonoids, carotenoids and ascorbic acid.[7] One of the members of this group is lime fruit that has various species which is found in some countries like India, China and etc. *Citrus aurantifolia (Christm)* is the most widespread significant cropped and consumed lime species in Iran. This fruit which is found as a healthy fruit for a long time has antioxidative activity.[8]

Although beneficial effects of the existing flavonoids in some types of fruits have been studied till now, the effects of whole fresh juice and also peel extract of Iranian species have not been examined on oxidation of LDL in vitro. Therefore, we decided to study antioxidant effects of fresh juice and peel extract of this type of lime fruit.

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Methods

Fruits of *Citrus aurantifolia (Christm)* were collected from gardens near the Shiraz city, Fars province, Iran in summer of 2008, and identified by a botanist at the Biology Department, Science Faculty of Isfahan University, Isfahan, Iran. A voucher specimen (5527) was deposited at the Herbarium of the Isfahan University. The whole peal of fruits was dried at room, pulverized and polyphenolic extract was prepared. LDL was separated from one human serum sample by a double step density gradient ultracentrifugation by modificated Bronzert and Brewer method.[9] At first step (VLDL separation) a discontinued gradient was formed from bottom to top with 6 ml of serum and 3 ml of 1.006 g/cm3 solution A. The centrifugation was carried out in a Beckman Coulter 90Ti rotor in a Beckman Optimal X-100 ultracentrifuge at 60000 rpm×10h at 16°C. Immediately after centrifugation, the LDL fraction was collected, and dialyzed for 24 h at 4°C in dark place against 0.01 mol/L phosphate buffered saline (PBS) buffer. The buffer was changed three times during the dialysis period and purified LDL was stored at -80°C.

Protein concentration of the LDL sample was determined by Lowry method.[10] The separated LDL sample was used for studying antioxidant effects of both juice and peel extract, and oxidation process was done in completely similar status. 10 µl LDL (protein concentration of 150 µg/ml) was diluted in 827 µl PBS buffer. Then, after adding 250 µl CuSO4 solution (5 µM), oxidation of LDL was started. Oxidation was followed by measuring the absorption of conjugated dienes at 234 nm at 10-min intervals for nearly 500 minutes at 37°C in a Shimadzu UV/VIS spectrophotometer. LDL oxidation was measured against 0, 5, 10, 20, 25, 30 and 40 µl of fresh lime juice and 0, 5, 10, 15 and 20 µl of peel poly phenolic extract in DMSO solution (Stock solution : 3.4 mg/ml). The juice and extract used were filtered with 0.45 µm syringe filter (Orange Scientific No. 1520014).

Results

The study results are presented in Figures 1 and 2 (oxidation curves). The LDL oxidation didn't change using 5 µl fresh lime juice (Figure 1). But, 10 µl of juice inhibited LDL oxidation, and after increasing lime juice concentration, LDL was oxidized faster and lag time of oxidation curve was shorter. According to Figure 2, peel extract inhibited LDL oxidation with increasing the concentration. The higher concentration of peel extract prevented LDL oxidation better than lower ones and lag time of oxidation curve became higher.

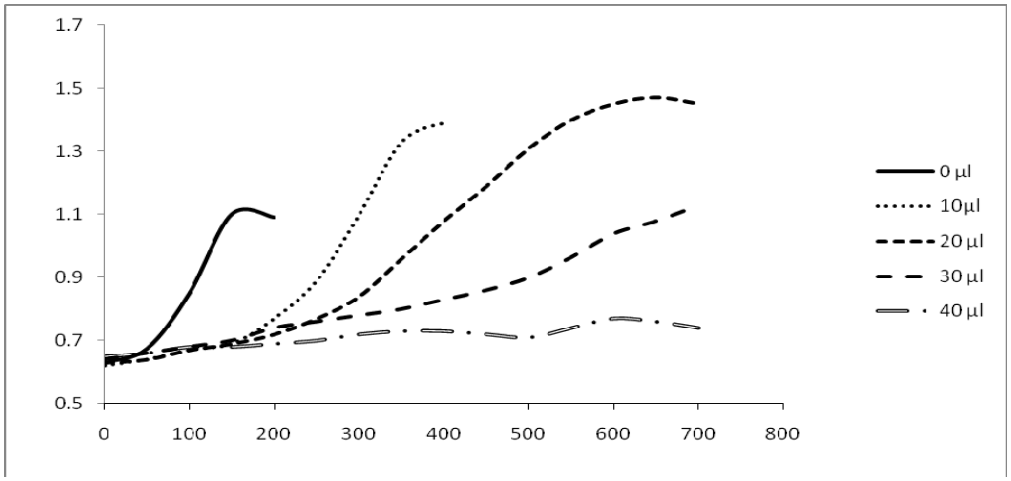

Figure 1. Effect of different concentrations of fresh lime juice on LDL oxidation

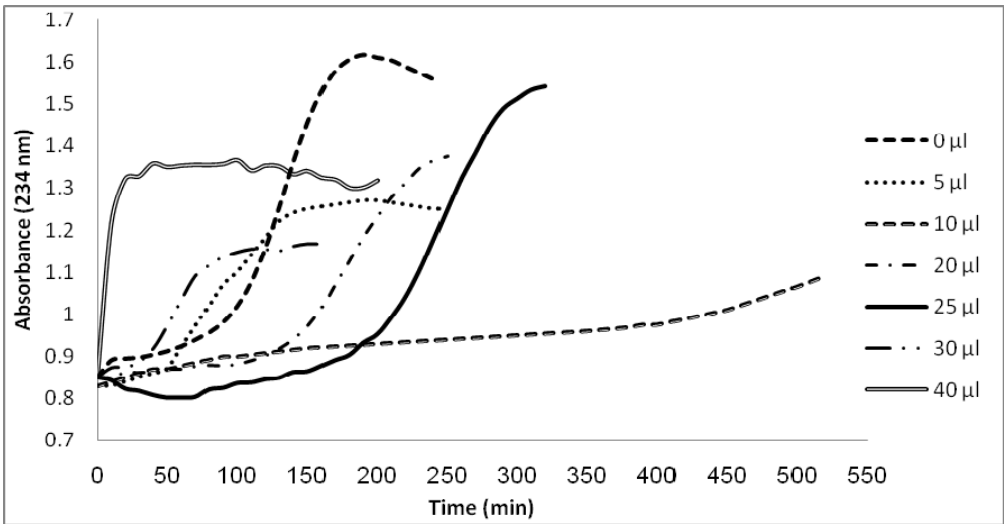

Figure 2. Effect of different concentrations of lime peel extract on LDL oxidation

Discussion

In this study it is found that the peel and fresh juice of lime have different effects on LDL oxidation. The low concentration of lime juice (5 µl) did not show considerable effect on LDL oxidation, *in vitro*. But, antioxidant activity of lime juice was observed only after applying increased concentration of 10 µl with LDL oxidative inhibition. Beyond this concentration, LDL oxidation was decreased. Therefore, it was demonstrated that peak stimulation for lime juice is 10 µl for 150 µg/ml LDL. Additionally, oxidation curve is a proof for a dose-independent response of antioxidant effect found for the lime juice. It reflects that flavonoids composition and other component of lime juice do not show similar effects.

Regarding the extract, the peak stimulation was 20 µl (158 mg/ml of stock extract) with antioxidant activity to inhibit LDL oxidation. Comparing the two figures, it seems that flavonoids contents of the extract show their expected and natural effects but some or all flavonoids in lime juice reveal inverted-U response. Regarding the intensity and type, lime juice and peel appear to include flavonoids with different effects in this study. Results of several studies on the antioxidant effects of flavonoids have controversies [11]. For instance, naringin, one of the flavonoids in lime juice, showed the chemo-preventive effect in an experiment and has proved mild antioxidative effects on lipid peroxidation in another study.[2] On the other hand, although in some reports, an antioxidant effect has been demonstrated for this flavonoid, flavonoids containing phenol B rings, such as naringin, hesperidin, and apigenin have been implicated in the initiation of atherosclerosis and carcinogenesis.[12]

Moreover, these flavonoids act as independent pro-oxidants in autoxidation reactions catalyzed by transition metal.[2] Also, it should be noted that aglycones of some flavonoids like naringin and hesperidin have less antiperoxidative potential compared to their corresponding aglycones.[13] In addition, a study has reported a weak antiperoxidative capacity for naringin when no decrease was observed in plasma and hepatic TBARS levels after its supplementation with a high-cholesterol diet.[2]

As mentioned before, flavonoids showed different antioxidant effects. For example, a research in Japan showed that tea flavonoids reduced oxidizability of LDL *in vitro* and *in vivo*.[14] Vitamin C is another effective component in lime juice.[15] It is a predominant reducing agent known to act as an antioxidant *in vitro* and *in vivo*.[16] Vitamin C protects human plasma lipids and LDL against peroxidative damage induced by various types of oxi-

dants.[3] Compared to other endogenous antioxidants in plasma like vitamin E which is soluble in human lipids, this vitamin inhibits lipid peroxidation.[17-20] So, the inverted-U dose response for lime juice maybe related to the simultaneous effect of vitamin C together with some or all flavonoids in lime juice or inversely, they may dilute an intensive inverted-U dose response in one of its components. Perhaps, this response is attributable to some unknown effective components in limes cultivated in Iran, different from ones cited so far, or this property might belong to 5000 trace plant flavonoids have not been determined till now. The found effect was obtained with fresh hand-squeezed lime juice applied immediately. This is aligned with the 50 percent-decreased effects for the industrially processed juices compared to the hand-squeezed ones other studies reported.[15]

The results obtained from the extracts are logic. It seems that all flavonoids in the extract have antioxidant role and increasing their concentrations causes antioxidant role to be elevated.

Conclusion

We concluded that in contrast to lime peel, the excessive consumption of lime juice is not only beneficial but also harmful due to an inverted-U dose response and only intermediate effective concentration showed antioxidant effect. Therefore, the recommendation for consumption of this fruit especially its peel seems useful for all people but *in vivo* human studies are needed for any dietary recommendation.

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Conflict of Interests

Authors have no conflict of interests.

Authors' Contributions

MB was the coordinator of the study, analyzed the data and prepared the manuscript. JM and GhN have assisted in designing the study, coordinated all the experiments and participated in preparing the manuscript. SA and HN provided assistance in the study design and participated in preparing the manuscript.

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