Anti-hyperlipidemic effects of citrus fruit peel extracts against high fat diet-induced hyperlipidemia in rats


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INTRODUCTION

Hyperlipidemia leading to atherosclerosis, coronary heart diseases and cerebral vascular diseases has been ranked as one of the major risk factors for death worldwide (Nelson, 2013; Yu et al., 2000). The diets rich in calories, saturated fats and cholesterol, genetic status and lifestyle contribute to hyperlipidemia. It is characterized by the elevated levels of fatty substances in the blood, including very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL) and triglyceride (TG)-rich lipoprotein,
total cholesterol (TC) and decreased high-density lipoprotein (HDL) (Nelson, 2013; Shattat, 2014). Of note, the root cause for hyperlipidemia is the abnormal production of hepatic VLDL, leading to over-abundance and caloric surplus that leads to fat accumulation in the liver (Shattat, 2014).

Currently available lipid-lowering drugs lead to numerous adverse effects including hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function (Mahamuni et al., 2012; Kumar et al., 2013). Hence, the demand for the use of natural products as anti-hyperlipidemic agents is growing, due to their negligible side effects. Though, bioactive principles from citrus fruits are known to exhibit anti-hyperglycemic, anti-obesity, and hypercholesterolemic effects (Kurowska and Manthey, 2004; Ono et al., 2011), it is worth considering the unexploited and wasted citrus fruit peels to discover their health benefits. In this line, so far, citrus fruit peels have been shown to display significant antioxidant, anti-inflammatory, anti-proliferative, and cancer prevention activity (Fayek et al., 2017; Pallavi et al., 2017). However, there are no systematic and comparative studies on anti-hyperlipidemic effects of citrus fruit peels. Hence, the present study aims to evaluate the lipid-lowering potentials of fruit peel extracts of five different citrus species against high fat diet (HFD)-induced hyperlipidemia in rats.

MATERIALS AND METHODS

Collection and preparation of plant material

*Citrus reticulate* (C. reticulate; orange), *C. maxima* (pomello), *C. aurantifolia* (lime), *C. medica* (citron) and *C. aurantium* (sour orange) were obtained from a local market and authenticated by the Taxonomist, Department of Botany, Sahyadri Science College, Shimoga, Karnataka, India. The fruits were washed to remove the surface detritus, peels were collected, dried and subjected to extraction using 70% ethanol (Pallavi et al., 2017).

Animals

Male Wistar rats (100-150g) were housed under standard conditions, with 12 h light-dark cycle and had free access to either standard chow diet or HFD and required amount of water. HFD was prepared by mixing 2% cholesterol, 1% cholic acid and 1 ml of coconut oil with pulverized chow diet pellets (D’Souza et al., 2007). Animal care and handling were in accordance with the CPCSEA Ethical Guidelines, and the experiments were carried out according to the protocols approved by the Institutional Animal Ethical Committee (No. NCP/IAEC/CL/244/2013-14).

Induction of hyperlipidemia and treatment

Rats were randomly divided into 13 groups (n=6/group); including i) control rats untreated, ii) HFD-induced hyperlipidemic rats untreated, iii) hyperlipidemic rats treated with atorvastatin-5 mg kg⁻¹, iv) hyperlipidemic rats treated with lime-250 mg kg⁻¹, v) hyperlipidemic rats treated with orange-250 mg kg⁻¹, vi) hyperlipidemic rats treated with sour orange-250 mg kg⁻¹, vii) hyperlipidemic rats treated with pomello-250 mg kg⁻¹, viii) hyperlipidemic rats treated with citron-250 mg kg⁻¹, ix) hyperlipidemic rats treated with lime-500 mg kg⁻¹, x) hyperlipidemic rats treated with orange-500 mg kg⁻¹, xi) hyperlipidemic rats treated with sour orange-500 mg kg⁻¹, xii) hyperlipidemic rats treated with pomello-500 mg kg⁻¹, and xiii) hyperlipidemic rats treated with citron-500 mg kg⁻¹. Control group received regular chow diet, while all other groups received HFD. After 15 days, rats fed with HFD (group iv-xiii) received peel extracts via intraperitoneal injection (i.p). Rats receiving atorvastatin, the standard drug at a dose of 5 mg kg⁻¹ (i.p) served as reference drug control. On day 45, rats were euthanized by administering anesthesia followed by cervical dislocation. Blood was withdrawn by cardiac puncture, processed to collect serum and subjected to lipid profile analyses (TC, TG, LDL-C, VLDL-C, and HDL-C). Further, TC/HDL-C and LDL-C/HDL-C ratios, atherogenic index (AI) and percentage protection were calculated (D’Souza et al., 2007; Dhandapani, 2007). All the parameters were estimated using standard diagnostic kits. AI and percentage protection were calculated by the following formulae:

\[
\text{Atherogenic index (AI)} = \log(\frac{\text{TG}}{\text{HDL}})
\]

\[
\text{Protection} \% = \left(\frac{\text{AI of HFD group} - \text{AI of treatment group}}{\text{AI of HFD group}}\right) \times 100
\]

Histopathology

The liver samples were washed with saline and fixed in 10% buffered neutral formalin for 48h. Next, tissue samples were embedded in paraffin and 3-4μm thick sections were cut using a microtome, dehydrated in graded alcohol and stained in hematoxylin and eosin. The sections were examined microscopically for the evaluation of histopathological changes (Umbare et al., 2009).

Statistical analysis

The data are expressed as mean ± SEM of six animals in each group. The statistical analyses were performed using one-way ANOVA, followed by
Tukey’s pair-wise comparison test. The values of p<0.01 were considered as statistically significant.

RESULTS

The experimental rats fed with HFD demonstrated marked increases in serum levels of TC, TG, LDL-C and VLDL-C while decreased levels of HDL-C compared to untreated control rats. Among the extracts tested, lime, sour orange and pomello peel extracts (500 mg kg\(^{-1}\)) significantly decreased TC (p<0.01), whereas citron and orange moderately decreased TC. Besides, the lime peel extract showed significant decreases in serum levels of TG (p<0.01), while sour orange and pomello were effective merely at their 500 mg kg\(^{-1}\) dose (p<0.01). However, sour orange and pomello (250 mg kg\(^{-1}\)) and citron (500 mg kg\(^{-1}\)) moderately decreased TG levels.

Furthermore, lime and sour orange markedly decreased LDL-C and VLDL-C levels (p<0.01) at both the doses, while pomello and citron could only exhibit significant reductions at 500 mg kg\(^{-1}\) dose (p<0.05). However, orange peel extract was not significantly effective in reducing LDL-C and VLDL-C levels. Next, the serum HDL-C level was found to be decreased in HFD-fed rats when compared to controls. Conversely, lime, sour orange and pomello peel extracts (500 mg kg\(^{-1}\)) significantly augmented HDL-C levels (p<0.01), while orange and citron peel extracts moderately elevated HDL-C (p<0.05) (Figure 1).

The influence of citrus fruit peel extracts on TC/HDL-C and LDL-C/HDL-C ratios were analyzed using serum lipid profile data. Among the extracts, lime and sour orange at both the doses effectively decreased TC/HDL-C and LDL-C/HDL-C ratios (p<0.01), followed by pomello, citron and orange peel extracts (Figures 2 and 3). Furthermore, AI
a) Normal; b) HFD; c) atorvastatin-5 mg kg⁻¹; d) lime-250 mg kg⁻¹; e) lime-500 mg kg⁻¹; f) orange-250 mg kg⁻¹; g) orange-500 mg kg⁻¹; h) sour orange-250 mg kg⁻¹; i) sour orange-500 mg kg⁻¹; j) pomello-250 mg kg⁻¹; k) pomello-500 mg kg⁻¹; l) citron-250 mg kg⁻¹; m) citron-500 mg kg⁻¹.

**Figure 6: Effects of citrus fruits peel extracts on histopathology of liver from HFD-induced hyperlipidemic rats**
is a stand-alone index for cardiac risk assessment. Interestingly, lime and sour orange peel extracts markedly decreased AI at both 250 and 500 mg kg\(^{-1}\) doses. On the other hand, pomello (500 mg kg\(^{-1}\)) exhibited marked decrease in AI, while orange exhibited minimal effect (Figure 4). Correspondingly, the percentage of protection calculated based on AI indicated the maximum protection by lime peel extract followed by sour orange and pomello (Figure 5).

The analysis revealed that the accumulation of lipid droplets was drastically increased in HFD untreated rats with more mononuclear cellular and vascular congestion infiltration showing dispersed hepatocytes and sinusoids. On the other hand, the hepatic tissues from control and atorvastatin-treated rats showed normal central vein and radiating hepatocytes with normal nucleus with proper integrity and sinusoids. Of note, lime peel extract (500 mg kg\(^{-1}\)) improved hepatic architecture to near normal with less deposition of fat globules and fewer sinusoids. Besides, sour orange (500 mg kg\(^{-1}\)) also reduced the accumulation of lipid droplets and sinusoids (Figure 6). These histopathological observations substantiate the serum lipid profile data. Altogether, the lipid-lowering effect of lime and sour orange peel extracts seems to be effective when compared to the peel extracts of other citrus species.

DISCUSSION

Hyperlipidemia characterized by the decreased high-density lipoprotein levels, and elevated low density, very low density lipoproteins and serum total cholesterol, is one of the risk factor associated with the severity and frequency of the coronary heart diseases (Nelson, 2013; Shattat, 2014). Cholesterol is an important structural and regulatory element, and the variations in which increases LDL-C or reduces HDL-C, leading to cholesterol deposition in hepatic tissues, arteries and aorta (Nelson, 2013; Shattat, 2014). From the results, it is evident that HFD increases the serum total cholesterol, which is effectively reduced by the administration of citrus fruit peel extracts. Lime showed a significant decrease at 500 mg kg\(^{-1}\) followed by sour orange, pomello, whereas citron and orange recorded a moderate decrease at a similar concentration. Next, TG plays an essential role in the regulation of lipoprotein interaction. Notably, elevated serum TG is an important risk factor for atherosclerosis and cardiovascular diseases (Jones and Chambliss, 2000; Lorenzatti and Toth, 2020). From the results, it is apparent that HFD increases serum TG, while lime peel extract showed a significant decreases followed by sour orange and pomello at 500 mg kg\(^{-1}\). However, a significant decrease has been registered by sour orange and pomello at 250 mg kg\(^{-1}\) and by citron at 500 mg kg\(^{-1}\).

Moreover, the elevated HDL-C has been linked to the lower risk of coronary heart diseases due to its involvement in reverse cholesterol transport (Ali et al., 2012; Zakiev et al., 2017). In this study, it is observed that HFD decreased the serum HDL-C, which is markedly increased by the peel extracts of lime, sour orange and pomello at both 250 mg kg\(^{-1}\) and 500 mg kg\(^{-1}\). However, orange and citron peel extracts exhibited moderate effect on HDL-C levels. Importantly, LDL-C and VLDL-C are the accurate predictors of coronary heart disease, as they carry cholesterol towards tissues with atherogenic potential. Moreover, the elevated serum levels of LDL-C and VLDL-C along with TG are thought to be the best gauge of atherosclerosis (Lorenzatti and Toth, 2020; Bo et al., 2018). The peel extracts of lime, sour orange, pomello and citron at 500 mg kg\(^{-1}\) dose significantly decreased LDL-C and VLDL-C levels, while at 250 mg kg\(^{-1}\) dose, lime and sour orange could exhibit significant reductions in LDL-C and VLDL-C levels. However, orange peel extract significantly did not reduce the levels of LDL-C and VLDL-C.

Remarkably, TC/HDL-C and LDL-C/HDL-C ratios are regarded as major index of cardiovascular risk factors (Millán et al., 2009; Salam et al., 2013). Among the extracts, lime and sour orange peel extracts effectively decreased TC/HDL-C ratio at 500 mg kg\(^{-1}\) dose, while pomello exhibited moderate effect. However, orange and citron were found to be less effective in reducing the TC/HDL-C ratio. Similarly, lime and sour orange peels extract significantly decreased LDL-C/HDL-C ratio when compared to pomello and citron peel extracts. Furthermore, AI is a critical index that has recently been considered as a stand-alone index for cardiac risk assessment (Wu et al., 2018; Akici et al., 2020). Interestingly, lime peel extract markedly decreased atherogenic index value when compared to sour orange and pomello at both 250 and 500 mg kg\(^{-1}\) doses. On the other hand, citron manifested a moderate decrease, while orange exhibited minimal effect.

Correspondingly, the percentage of protection calculated based on AI indicated the maximum protection by lime peel extract followed by sour orange, pomello, citron and orange at 500 mg kg\(^{-1}\). Essentially, the increased accumulation of lipid droplets, vascular congestion and mononuclear cellular infiltration showing dispersed hepatocytes and sinusoids are salient features of HFD-induced hyperlipidemia (Hassan et al., 2018). Herein, lime peel
extract markedly improved hepatic architecture to near normal with less deposition of fat globules and fewer sinusoids. Besides, sour orange also reduced the accumulation of lipid droplets and sinusoids. However, pomello, citron and orange significantly did not improve the hepatic architecture. These histopathological observations substantiate the serum lipid profile data. Overall, the anti-hyperlipidemic effects of lime and sour orange peel extracts seem to be greater when compared to the peel extracts of other citrus species tested. Notably, though the anti-hyperlipidemic effects of lime and sour orange peel extracts are inferior to atorvastatin, the potential advantages of biocompatibility and safety warrant their therapeutic applications in hyperlipidemia.

Of note, synephrine, an alkaloid from citrus peels has been shown to reduce cholesterol synthesis in liver (Fayek et al., 2017). Further, the citrus fruit peel extracts contain flavonoids, polyphenols, alkaloids, glycosides, steroids, carotenoids, and terpenoids (Pallavi et al., 2017). Thus, the anti-hyperlipidemic effect of lime and sour orange peel extracts could be attributed to the presence of these bioactive principles and their synergistic action.

CONCLUSION

The results of this study scientifically validate the plethora of epidemiological surveys on anti-hyperlipidemic effects of citrus fruit peels. Moreover, the findings of present study inspire future attempts to develop new formulations and optimize composition of fruits in the diet, which could be cost-effective approach for the disease prevention and value-added approach in the management of hyperlipidemia and associated diseases.

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Conflicts of Interest

The authors declare that there are no conflicts of interest.

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