BRIEF COMMUNICATION

Bitter Melon (*Momordica Charantia*) Supplementation Has no Effect on Hypercholesterolemia and Atherosclerosis in Mice

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Abstract

Momordica Charantia, commonly known as bitter melon, has been reported to ameliorate diet-induced obesity and dyslipidemia. However, the effects of *M. Charantia* on atherosclerosis have not been determined. This study investigated the effects of *M. Charantia* on diet-induced atherosclerosis in LDL receptor deficient mice. Female mice (6-8 weeks old) were fed a saturated fat-enriched diet. In group 1, mice were fed this diet alone, while mice in groups 2 and 3 were fed the diet supplemented with *M. Charantia* either 0.1% or 1% by weight, for 12 weeks. No significant differences in body weights were observed among the 3 groups. There were also no significant differences in plasma cholesterol concentrations among the 3 groups. To determine the effects on atherosclerosis, lesion areas were measured on aortic intima by an *en face* technique. Neither dose of *M. Charantia* supplementation changed atherosclerosis development.

Key words: *Momordica Charantia*; atherosclerosis; hypercholesterolemia; Cholesterol

1. Introduction

Atherosclerosis is one of the major cardiovascular diseases with high morbidity and mortality rates in different populations with hypercholesterolemia and obesity as major risk factors.(1, 2) Lifestyle modification and healthy dietary habits reduce the risks of predisposition to atherosclerosis and overall cardiovascular health.(3) However, successful lifestyle changes are challenging. Complementary approaches and dietary adjuncts that identify potential therapeutic targets on hypercholesterolemic properties have substantial interests.

Momordica Charantia, colloquially known as bitter melon, is an herbal product that has been used traditionally for treatment of diabetes.(4) It has been reported in both human and animal studies that *M. Charantia* has beneficial effects on adiposity and lipid metabolism.(5-8) In one study, *M. Charantia* supplementation reduced plasma cholesterol and triglyceride concentrations in normolipidemic rats. Moreover, aqueous extracts of *M. Charantia* fruit improved lipid profile in normolipidemic diabetic rats. Although beneficial effects of *M. Charantia* on lipid metabolism in normolipidemic state have been demonstrated, there is a lack of information on anti-atherosclerosis effect in hypercholesterolemic animals.

Low-density lipoprotein receptor (*Ldlr*) deficient mice are a commonly used animal model to study atherosclerosis. (1) These mice have profoundly increased plasma cholesterol concentrations when fed a saturated fat-enriched diet. In a quest to determine the role of *M. Charantia* supplementation on atherogenesis, the present study

was performed to determine whether modest or substantial dietary supplementation of *M. Charantia* would attenuate atherosclerosis development in *Ldlr*$^{-/-}$ mice.

2. Materials and methods

2.1. Mice and diet

Female *Ldlr*$^{-/-}$ mice (B6.129S7-Ldlrt^{m1Her}/J; stock #002207) were purchased from The Jackson Laboratory (Bar Harbor, ME, USA). Mice were fed a saturated fat-diet (42% kcal/wt from milk fat diet no. TD.88137; Envigo, Madison, WI, USA) in group 1 for 12 weeks. The Western diet was also consumed by groups 2 and 3 with supplementation of a moderate (0.1% wt/wt; TD. 170736) or high (1% wt/wt; TD. 170735) amount of *M. Charantia* extract, respectively (n=10 per group). **Figure 1A** shows the study design. The mice were maintained in a light:dark cycle of 14:10 hours. Mice were 8 weeks old at the initiation of the study. All procedures were approved by the University of Kentucky Institutional Animal Care and Use Committee.

2.2. Measurement of plasma cholesterol concentrations

Plasma cholesterol concentrations were measured using an enzymatic kit (cat. no. 999-02601; Wako Chemicals USA, Richmond, VA, USA).

2.3. Quantification of atherosclerosis

Atherosclerosis was quantified on the intimal surface of the proximal aortic region (ascending aorta, arch, and from the aortic orifice of left subclavian artery to 3 mm below) or the entire aorta from the ascending to the iliac bifurcation by an *en face* method as described previously,(9) and in accord with the guidelines published by the American Heart Association. (1)

2.4. Statistical Analyses

SigmaPlot 13.0 (Systat Software Inc., San Jose, CA, USA) was used for all statistical analyses. To compare multiple groups that passed normality and equal variance tests, One Way ANOVA with Holm-Sidak post hoc test was used. Kruskal-Wallis One Way ANOVA on Ranks and Dunn's post hoc test was used for data that failed normality or equal variance test. $P<0.05$ was considered statistically significant.

3. Results

3.1. *M. Charantia* supplementation had no effect on body weights

M. Charantia has a bitter taste. Rodents are sensitive to inclusion of extra compounds to their diet, which can consequently affect food intake. To determine the impact on the inclusion of this supplement, mice were weighed weekly during the study period. All groups had steady weight gain that was not significantly different among the 3 groups (**Figure 1B**).

3.2. Plasma cholesterol concentrations were not affected by *M. Charantia*

Since hypercholesterolemia has a direct association with atherogenesis, plasma cholesterol concentrations were measured at the end of the study. All 3 groups had concentrations of approximately 2,500 mg/dl that were not significantly different among groups (**Figure 1C**).

3.3. *M. Charantia* had no effect on atherosclerosis

Atherosclerosis lesion areas were quantified on the intimal surface of the ascending aorta and arch region, or the entire aorta. All mice had atherosclerotic lesions but there was no significant difference among groups (**Figure 1D-E**)

4. Discussion

M. Charantia has been shown to promote beneficial effects on lipid metabolism. In this study, we investigated the role of *M. Charantia* supplementation on development of atherosclerosis in hypercholesterolemic mice. We hypothesized that supplementation of *M. Charantia* would have a dose-dependent anti-atherosclerotic effect in female *Ldlr*$^{-/-}$ mice. However, 12 weeks of *M. Charantia* supplementation did not have effect on plasma cholesterol concentrations or atherosclerosis development in this study.

M. Charantia extracts have lipid lowering effects on both diabetic and high fat diet-fed rats. (6, 8, 10-12) Aqueous extracts of *M. Charantia* not only improved glucose tolerance but also lowered plasma apolipoprotein B (apoB)-100 and apoB-48 concentrations in female C57BL/6 mice fed a high fat diet. (13) A 30-day administration of *M. Charantia* fruit extract also decreased triglyceride and LDL cholesterol concentrations in diabetic rats, (14) and *M. Charantia* (0.75% wt/wt) supplementation reduced plasma cholesterol concentrations in high fat diet fed rats. (8) With many chemicals contained in the extract, *M.Charantia's* active hypoglycemic and hypolipidemic compounds are unknown. The known isolated compounds such as charantin (a steroid glycoside) and a 166 residue insulin mimetic peptide have been reported to be potential compounds responsible for such beneficial effects. (15)

In agreement with the present study, there are inconclusive results in studies investigating the role of this traditional plant on dyslipidemia and hypercholesterolemia-induced atherosclerosis. Earlier studies reported no changes in either metabolic parameters or lipid metabolism after different doses of *M. Charantia* supplementation. (8, 10, 16) Rats fed diets for 14 days containing *M. Charantia* freeze-dried powder

supplemented with 1% wt/wt had no change in plasma cholesterol concentrations. (10) Also, a clinical study using encapsulated *M. Charantia* for a duration of 3 months did not report any significant difference in the lipid profile of patients. (17) Another randomized, double-blind, placebo-controlled trial also reported no significant effect on mean fasting blood sugar, total cholesterol, and weight after treatment with *M. Charantia* capsules.(18)

Although our own studies contrast some of the previously published results, these discrepancies may be attributed to different study designs used in combination with the different preparations, the range of doses, and administration modes of the *M. Charantia*. In addition, many recent reports indicate that treatment and outcomes of atherosclerosis exhibit sex differences in clinical studies and sex differences have been widely reported in mouse atherosclerotic studies. (19) Our studies used female mice which may be another compound factor to the divergent results. (19) Moreover, confirmation of extraction and purification of *M. Charantia's* active compounds and concentration measurement of such compounds in plasma would be helpful additions for future studies**.**

In summary, our study results indicate that supplementation of rodent diet with moderate (0.1% wt/wt) or high (1% wt/wt) doses of *M. Charantia* has no effects on hypercholesterolemia and atherosclerosis development. Further studies should investigate the effective dose of supplementation with measuring the bioactive compounds as a marker of consumption.

5. References

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6. Figure Legends

Figure 1: Female LDLR deficient mice were fed a high-fat diet supplemented with *M. Charnatia* for 12 weeks. (A) Body weights were measured weekly. (B) *M. Charantia* supplementation did not show any effect on total plasma cholesterol concentrations. (C) Atherosclerotic lesion development. (D-E) Percent of atherosclerotic lesion areas were measured on the intimal surface of ascending aorta and arch (D) and entire aorta. (E) Diamonds represent group means and error bars are SEMs. Circles represent individual mice. Ctrl; control, BM; Bitter melon. HFD; high fat diet.

7. Figures

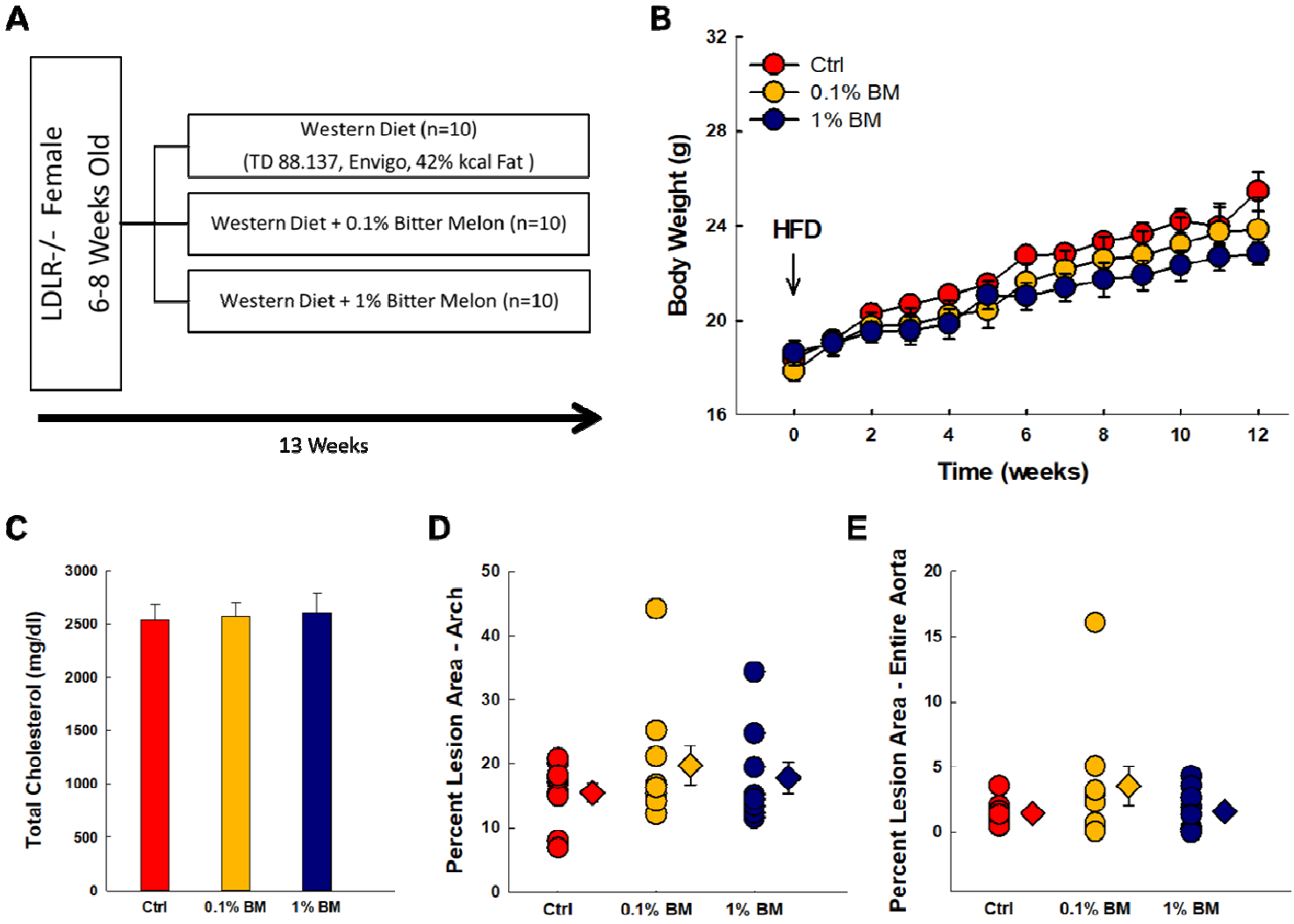

Figure 1