Dietary supplementation of bitter gourd reduces the risk of hypercholesterolemia in cholesterol fed sprague dawley rats

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Abstract: Functional and health endorsing benefits of various foods are often attributed to their phytochemistry. The bitter gourd holds potential in improving the health of the individuals owing to its incredible versatility in phytochemistry. However, the efficacy of different parts of bitter gourd needs attention of the researchers. In the current exploration, different parts of bitter gourd were evaluated for their cholesterol lowering potential in cholesterol fed Sprague dawley rats. For the purpose, four types of bitter gourd part *i.e.* whole fruit, seedless fruit, seeds, and seed extracts were used and compared with placebo in hypercholesterolemic rats. In placebo, momentous increase in serum cholesterol, triglycerides and LDL levels was observed. All parts attenuate the cholesterol 18.79 to 40.17% triglycerides 25.97 to 37.01% and LDL 14.49 to 26.09%. However, 1% extract powder was most effective in reducing the cholesterol and triglycerides. From the present study, it is deduced that bitter gourd extract can be supplemented in food products for the management of hypercholesterolemia. However, future studies in human subjects needs to be conducted for meticulousness of the present findings.

Keywords: Bitter gourd, hypercholesterolemia, cholesterol, triglycerides, antioxidants.

INTRODUCTION

In the last century, the science of pharmacology developed on scientific grounds and drugs discoveries & modern diagnostics tools enabled the medical specialists to identify the diseases and cure them. However, the safety and toxicological aspects of some drugs resulted in increased awareness in the consumers to focus on diet to prevent various ailments. The dietary regimens with phytochemical rich sources and their utilization in dietary staples are amongst the modern day popular trend in consumers. However, the biodiversity and array of phytochemicals with their distinct biological activities demands the attention of dietetics and pharmacists to research the health claims and their inclusion in daily diet with special focus on diet diversification (Tapsell *et al*., 2014).

Diet diversification focuses on dietary therapies to control various ailments and important to prevent various lifestyles related disorders e.g. obesity, diabetes and high cholesterol (Butt and Sultan, 2013). Amongst different lifestyle related disorders, cardiovascular disorders are of utmost importance and are responsible for mortality and morbidity. The increased levels of cholesterol and its bad fractions (low-density lipoproteins) are major causative agents for the onset of atherosclerosis and allied ailments. Hypercholesterolemia is generic term that is used to express the higher amounts of cholesterol present in the body. Although, family inheritance and lack of physical exercise are important for pathogenesis but poor dietary habits and reliance of population in respective dietary staples is playing major role nowadays. Moreover, recent evidence suggested that imbalance lipid profile also causes obesity, diabetes, inflammation, cancer and myocardial infraction (Masur *et al.*, 2008; Clifton *et al*., 2014; Moon *et al*., 2015).

There are several strategies that can used to address the menace of cardiovascular disorders but dietary regimens gained recognition in pharmacist and nutritionists to prevent the onset of such ailments. In this regard, several remedies can be used but plant based nutraceuticals and functional foods are of utmost importance. Bitter gourd is rich in phytochemicals and usually cooked in Asian cuisines owing to its specific aroma and flavor. It holds several health benefits that include antioxidants and antimicrobial potential that are effective to cure infections, diabetes mellitus, atherosclerosis, myocardial infraction, and obesity (Yibchok-Anun *et al.*, 2006). It also possesses antifungal, antioxidant, anticancer, hypotensive, antiobesity properties. Other reported medicinal benefits include alleviation of fever, HIV, skin diseases, detoxification of body, balancing of certain hormones, inflammation and exorcising worms from body (Chen and Li, 2005; Braca *et al*., 2008; Singh *et al.,* 2011; Santos *et al*., 2012; Zhang *et al*., 2015). Bitter gourd is one of these popular herbal remedies that is low in saturated fatty acid and is enriched with antidiabetic compounds (Chen and Li, 2005, Sathishsekar and Subramanian, 2005). Three groups of phytoconstituents are responsible for preventive actions. Mainly, charantin is one of those abundantly found in fruit and contribute to the hyperglycemic and hypercholesterolemic activities.

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Others are ρ-polypeptides known as plant insulin similar to insulin in composition and alkaloids (Harinantenaina *et al.,* 2006; Pitipanapong *et al.,* 2007; Cheng, 2008).

In this instant research treatise, authors attempted to utilize different parts of bitter gourd in cereal based cookies that include whole fruit, flesh, seeds and extracted fraction. These parts were used in the diets of Sprague dawley rats fed on high cholesterol diets and their impact was recorded against indices of lipid profile. The findings of the present research are useful for adding bitter gourd seed extract powder to balance the lipid profile and dyslipidemia.

MATERIALS AND METHODS

Bitter gourd was procured from Vegetables Research Institute, Ayub Agricultural Research Institute, Faisalabad. The raw material for cookies was purchased from local market. National Institute of Health (NIH), Islamabad provided infectious free Sprague dawley rats for this research intervention.

Preparation of bitter gourd and its extract

Fresh bitter gourds were washed and seeds were removed manually from samples. The whole fruits and seedless fruits were cut separately. Whole fruit, flesh and seeds of bitter gourd were dried in a dehydrator and then grinded to obtain fine powder. The extract was prepared by soaking pieces of fresh green whole fruits in water at a ratio of 10: 25 for one hour at room temperature. Then it was filtered and evaporated to dryness (Virdi *et al.,* 2003).

Housing of rats

The National Institute of Health (NIH) situated in Islamabad, Pakistan provided 25 Sprague Dawley infection-free rats that were further divided into five groups of five rats each. The ethical approval for conducting such studies was taken from Animal Care Committee (ACC), NIFSAT, University of Agriculture, Faisalabad. The standard conditions for the animal handling during the entire study durations were adjusted according to standard guidelines of Animal Institute of Nutrition (AIN), USA. These conditions include temperature (23.0±2.0ºC), relative humidity (55.0±5.0%), and 12-hr light-dark cycle.

Efficacy study

The rats were fed on their basal diet in the first few days to make them acclimatize to the new environment. High cholesterol diet containing 1.5% of cholesterol and 0.5% cholic acid was fed to all rats to raise their lipid profile. Later, rats received their respective experimental diets (for a period of 56 days). Cookies prepared from selected levels of control (D_1), 3% whole fruit (D_2), 3% flesh (D_3), 3% seeds (D_4) and 1% extract (D_5) were fed to the respective groups of rats for eight weeks. At the end of rodent modeling study, rats from each group were decapitated for blood collection through neck and cardiac puncture. Initially some rats were killed to calculate the base line values, while the rest were scarified at the end of study. The collected blood samples were analyzed for further assays and details are mentioned herein.

Feed & water intake and body weight

For measuring feed and water intake, special efforts were made to calculate the amount of feed given to the rats. The feed and water intake was measured daily (for statistical analysis, the one week data was pooled) by calculating the difference between feed/water fed to them and left over present in their respective bowls and bottles, respectively. However, the body weight was recorded on weekly basis.

Serum lipid profile

The serum lipid profile including cholesterol, high-density lipoproteins (HDL), low-density lipoproteins (LDL) and total triglycerides (TG) were measured. Serum cholesterol level was estimated following the procedures outlined by Stockbridge *et al.* (1989) that employ CHOD-PAP method. Likewise, HDL cholesterol contents were estimated using the protocols set by Assmann, (1979). TG levels were determined by following the method of Annoni *et al.* (1982) that employ liquid triglycerides (GPO-PAP) method. In the last, LDL cholesterol contents were determined using the standard formula outlined by McNamara *et al.* (1990).

STATISTICAL ANALYSIS

Statistical package i.e. Cohort V-6.1 (Co-Stat Statistical Software, 2003) was used for data analysis. The technique of analysis of variance (ANOVA) was applied to check the level of significance. Duncan's multiple range test (DMRt) further clarified the effects of diets (P value = 0.05) in a comprehensive manner (Steel *et al.,* 1997).

RESULTS

Globally, bitter gourd is common vegetable and is famous for its bitter taste and unique flavor. The results depicted that the feed and water intake is significantly affected by treatments and study intervals (table 1) in hypercholesterolemic rats. The graphical illustration in fig. 1 elucidated highest feed intake for D_1 (control) and feed intake was the lowest in seed part based cookies (D_3) among all diets. During study, D_1 group exhibited a progressive increase in feed intake, whereas trend varied in D_2, D_3, D_4 and D_5 groups. Generally, feed intake gradually increased up to 5^{th} week that decreased afterwards.

The maximum water intake ((fig. 1) was measured in D_3 (22.0mL/rat/day) followed by D_1 (21.7mL/rat/day) and D_4 (21.3mL/rat/day) While, the minimum intake was observed in D_2 (21.0mL/rat/day) and D_5 (21.0mL/rat/day). Whereas the trend observed at the termination of study

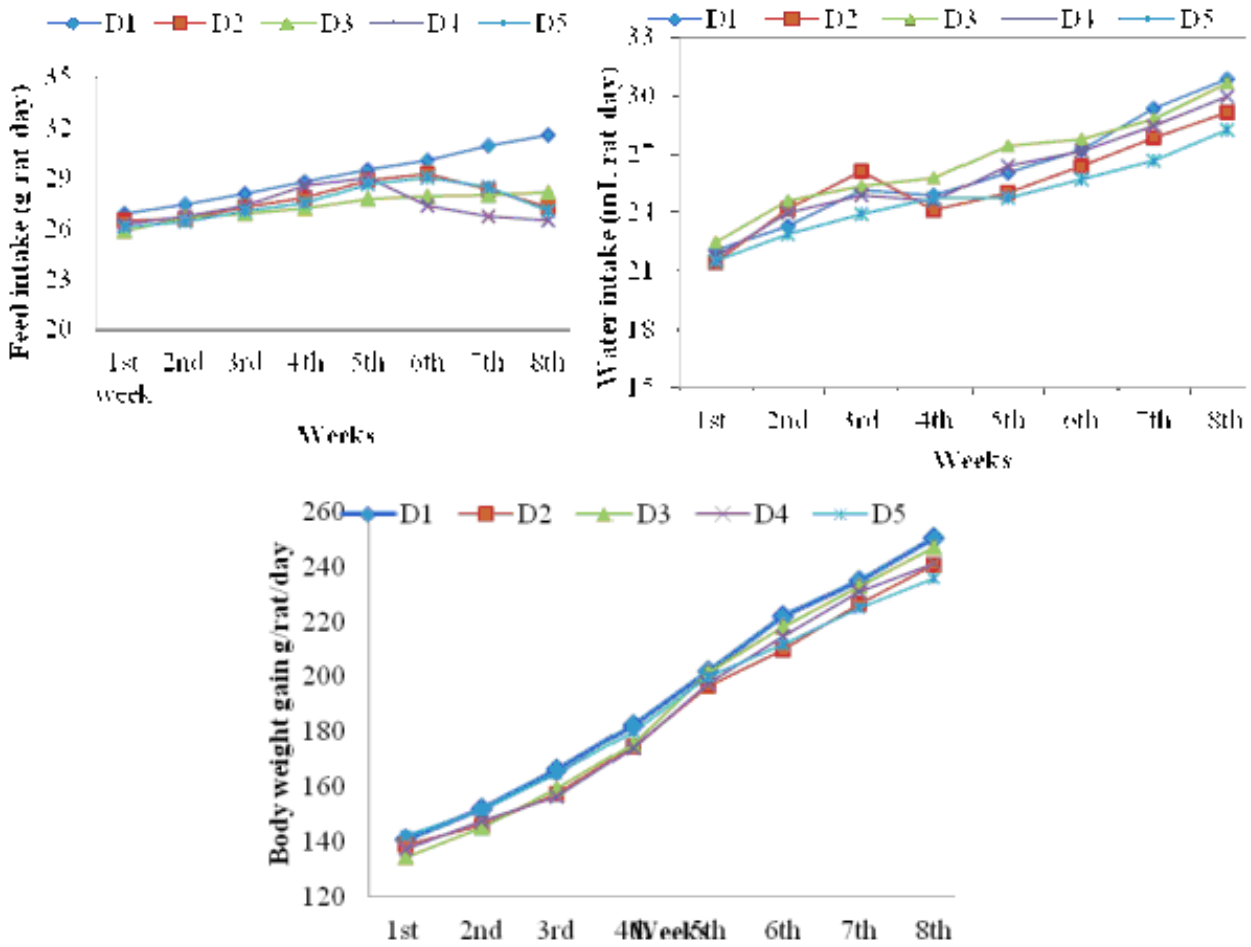

Fig. 1: Feed intake, water intake and body weight gain in placebo and experimental groups fed on different parts of bitter gourd

was slightly different i.e. D_1 (30.0mL/rat/day) and D_3 (30.0mL/rat/day) trailed by D_4 (29.4mL/rat/day), D_2 (28.6mL/rat/day) and D_5 (27.7mL/rat/day). The maximum gain in weight 253.01g was observed in group fed control diet. Groups of rats fed on diets containing whole fruit (D_2), flesh (D_3), seed (D_4) and extract (D_5) showed lower body weights *i.e.* 242.8, 249.7, 243.8 and 238.3g/rat, respectively (fig. 1). The results regarding feed intake and body weight showed that bitter gourd holds anorexic effects.

The statistical results (mean squares representing analysis of variance) and means regarding lipid profiles are shown in table 2 and 3, respectively. The statistical results for cholesterol (table 2) revealed that cholesterol varied significantly due to diets. In hypercholesterolemic rats the highest level of cholesterol (207.8mg/dL) was observed in D_1 (table 3). However, the lowest value for this trait was observed in D_5 (124.32mg/dL).

Means for serum triglycerides (table 3) revealed significant effect of diets and the maximum (197.68mg/dL) levels were recorded in rats fed on control cookies (D_1). Triglycerides decreased to 145.82mg/dL in flesh cookies group (D_3). While, the maximum reduction in this trait was observed in extract containing cookies (D_5) that was 124.09mg/dL. The statistical results pertaining to LDL (table 2) indicated that the diets supplemented with bitter gourd parts powder significantly affected low-density lipoprotein. Results (table 3) indicated a momentous decrease in LDL level; the maximum level was recorded for D_1 (68.88mg/dL) followed by D_2 (58.90mg/dL) and the minimum was recorded in rats fed on powdered extract i.e. D_5 (50.91mg/dL).

The results revealed that diets affected HDL level significantly in rats. HDL in hypercholesterolemic rats showed significantly the highest value for D_5 that was 54.25mg/dL (table 3). Those levels were significantly reduced in D_3 (50.11mg/dL) and D_2 (48.17mg/dL). However, the lowest level 45.59mg/dL for this trait was measured in D_1.

Overall, aqueous extract of *Momordica charantia* caused about 16% reduction in the body weight. The extract

powder fed @ 1% reduced cholesterol level by 40.17%, LDL by 26.09 and triglycerides 37.22%, whilst, improving the HDL contents improved 18.99% (fig. 2).

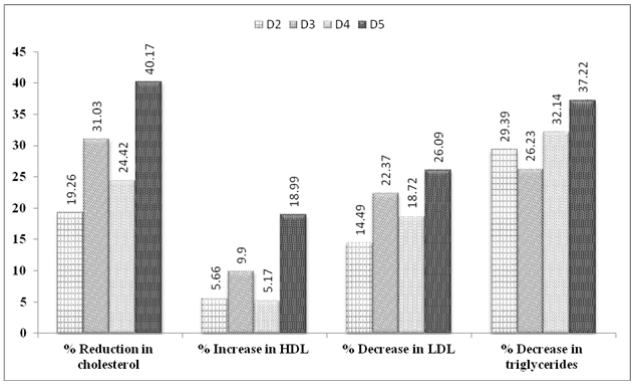

Fig. 2: % increase and decrease in serum lipid profile as a function of experimental diets with added bitter gourd fractions as compared to control

DISCUSSION

The question of linkage between diet and health has been addressed in thousands of research interventions. The recent developments in analytical tools and pharmacology have widened the scope for studying the biological activities of various foods. Amongst, plant based remedies are important and scientists over the globe made efforts to identify the phytochemicals rich sources that could be effective in curing some lifestyle related disorders. The communities living in developing societies are still dependent on traditional knowledge to prevent and cure various ailments using indigenous plants. Such dietary strategies in these communities are more effective in improving the lipid profile and decreasing the risk of lifestyle related disorders (Butt and Sultan, 2010). Bitter gourd is common vegetables used in Asian cuisines and famous for its bitter taste. There are several reports claiming the anti-diabetic and anti-obesity properties of their seeds (Chen *et al*., 2003; Chaturvedi *et al*., 2004) but limited research is available with reference to whole fruit, flesh, and extracted fractions.

In the present research, rats fed on experimental cookies containing different part of bitter gourd showed some favorable results. The experimental diets prepared with different part of bitter gourd improved the lipid profile. A gradual decrease in feed intake was observed in-group feeding on high fat bitter melon powder diet then high fat thiazolidinedione treated group (Huang *et al.,* 2008). The decreased feed intake gives rise to anorexic conditions thus resulting in reduced body weight that ultimately results in reduced deposition of fats. This whole phenomenon results in reduced risk of obesity and allied health disorders including diabetes mellitus and atherosclerosis (Story *et al.,* 2010). Previously, Chen *et al.* (2003) reported that supplementation of freeze-dried bitter melon juice (0.375, 0.75 and 1.5%) significantly reduced the weights of the insulinemic rats fed on high fat diet.

The reduction of total cholesterol and its bad fractions (low density lipoprotein) and improvement of good cholesterol (high density lipoprotein) might be attributed to variations in metabolism. One of the reasons could be linked with inverse association of vegetables consumption with that of body lipids (Bazzano *et al.,* 2002; Tapsell *et al*., 2014). Oral administration of aqueous extract of bitter gourd for eight weeks significantly decreased the serum cholesterol, triglyceride and LDL while increase the HDL level. The results are in accordance with Bano *et al.* (2011) and Temitope *et al.* (2013). According to these scientists, percent decrease in the cholesterol and triglycerides level was 21% & 20%, respectively (Matsui *et al.,* 2013).

Previously, Chaturvedi *et al.* (2004) also confirmed that its extracts reduce triglyceride and low-density lipid (LDL) levels and increases high-density lipid (HDL). Moreover, it was hypothesized that decline in cholesterol, LDL and triglycerides concentration is probably due to lowering effect of Apo-B secretion by the liver (Umesh *et al.,* 2005). Lipid lowering component of *Momordica charantia* decreased the liver secretion of Apolipoprotein B (Apo-B) and Apo-C III expression. These proteins are the lipoprotein of low-density "bad" cholesterol, which turns into LDL. At the same time, *M. charantia* increases the expression of Apo-A-1 that is the major protein component of high-density cholesterol (Bano *et al.,* 2011).

The feeding of bitter gourd extracts resulted in reduced feed intake that resulted in reduced body weight and this trend shows the anorexic properties of the bitter gourd. The anorexic effects of bitter gourd extract might be responsible for reducing total cholesterol and bad cholesterol (LDL). Moreover, bitter gourd seeds are rich source of dietary fiber and phytochemicals and thus can interfere in cholesterol metabolisms.

CONCLUSION

Present research investigation brightened the prospects that bitter gourd significantly attenuated hypercholesterolemia in terms of serum lipid parameters including triglycerides, total cholesterol and its bad fraction (LDL) in the experimental groups. In the nutshell, it can be concluded that bitter gourd especially its powdered extract and its flesh are effective in reducing the circulating total cholesterol and its bad fractions (LDL) significantly owing to interference in feed conversion and cholesterol metabolism. Further studies are still required to assess the phytochemistry of those functional ingredients responsible for health benefits.

Table 1: Effect of diets and study weeks on feed, water intake and body weight gain

SOV	df	Feed Intake	Water Intake	Weight gain
Diets	4	7.90**	0.56*	68.84*
Weeks	7	2.78*	30.67**	7644.51**
Error	28	1.04	0.065	9.53
Total	39			

Table 2: Mean squares for lipid profile showing the effects of placebo and experimental diets

SOV	Df	Cholesterol	HDL	LDL	Triglycerides
Diets	4	3239.46**	31.47*	145.03**	2480.04**
Error	10	77.88	7.32	10.12	68.22
Total	14				

The values presented in the table shows the mean squares (analysis of variance) and star are added to indicated the level of significance. *= Significant (P<0.05) **= Highly significant (P<0.01)

Table 3: Effect of different parts of bitter gourd on lipid profile (mg/dL)

Diets	Cholesterol	HDL	LDL	Triglycerides
D_1	207.80a	45.59d	68.88a	197.68a
D_2	167.78b	48.17c	58.90b	139.58c
D_3	143.33d	50.11b	53.47d	145.82b
D_4	157.06c	47.95c	55.99c	134.14d
D_5	124.32e	54.25a	50.91e	124.09e

Means carrying same letter in a column are non significant from each other D_1 =Control/placebo, D_2 =3% Whole fruit powder, D_3 = 3% Flesh powder, D_4 =3% Seed powder, D_5 = 1% Extract powder

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