



Original article

Combined efficacy of *Vigna radiata* (L.) R. Wilczek and *Amorphophallus paeoniifolius* (Dennst.) Nicolson on serum lipids in albino rats



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ABSTRACT

Coronary Artery Disease (CAD) is a major killer disease throughout the world. Dyslipidemia is a major contributor to the risk of CAD. Several dietary articles traditionally used in India and other South Asian countries reduced dyslipidemia. The present study was undertaken to evaluate the combined effect of Mung bean (*Vigna radiata*) and Elephant foot yam (*Amorphophallus paeoniifolius*) on serum lipids and atherogenic indices in albino rats and to compare it with a standard drug Cholestyramine. Thirty healthy albino rats of both sexes (150–200 g) were randomized to 5 groups of 6 animals each. The grouping were done based on the following criteria: Group I: Normal Control Group, Group II: (Standard Group): Cholestyramine resin 5 mg/kg bw, Group III: (Half Dose Group): Drug powder at 540 mg/kg bw, Group IV: (Effective Dose Group): Drug powder at 1080 mg/kg bw, and Group V: (Double Dose Group): Drug powder at 2160 mg/kg bw. Lipid profile was estimated at the beginning and after 30 days of treatment. The Effective and Double doses of the drug reduced Total cholesterol along with levels of Triglycerides, Low density lipoprotein and Very low density lipoprotein levels significantly ($p < 0.01$) along with a significant ($p < 0.01$) increase in high density lipoproteins (HDL) in rats. There was also significant ($p < 0.01$) improvement in atherogenic indices like Castelli Risk Index I, Non HDL C/HDL, Castelli risk Index II, TG/HDL, Atherogenic coefficient and Atherogenic Index of Plasma. The combination of powdered sprouted mung bean and yam powder have excellent lipid lowering potential.

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1. Introduction

The prevalence of CAD increased drastically in rural and urban India almost to the tune of two to four fold over the turn of the century. Though half of the Asian Indians are lifelong vegetarians, the CAD risk is similar when compared to non-vegetarians (Enas et al., 2007). In the South Asian populations, the escalated rate of disease burden due to CAD is primarily responsible to dyslipidemia (Enas et al., 1996). Dyslipidemia is referred to as derangements of one or more of the lipoproteins; elevated levels of total cholesterol,

low density lipoprotein (LDL) cholesterol and/or triglycerides, or low levels of high-density lipoproteins (HDL) cholesterol (Misra et al., 2004). Physical inactivity, body composition, genetic predisposition and diet are considered as the determinants of dyslipidemia in Asian Indians (Gaziano et al., 1997). Management of CAD involves the pharmacological management of risk factors like dyslipidemia, hypertension etc. along with the more powerful strategy of non-pharmacological management using diet modifications and nutrition management aimed at improving dyslipidemia along with improving the quality of life with proper care of physiological and nutritional health (Misra and Gulati, 2014). Dietary recommendations in dyslipidemia includes cereals, millets, pulses and legumes along with low glycemic index foods like flour, root vegetables such as Yam, Tapioca, and Colocasia. Traditional food items of Indian community had extensively utilized the wealth of natural nutritional sources. Traditional treatment systems like Ayurveda & Siddha incorporated and endorsed the use of these drugs as a dietary and a therapeutic agent in disorders originated from derangement of three humors (viz. vata, pitta and kapha).

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Among the commonly used articles of food items throughout the Indian subcontinent includes the green gram and the Elephant foot yam. Even the tribal populations scattered around India also included these items in their regular diet. The green gram (*Vigna radiata* (L.) R. Wilczek., Family Fabaceae, commonly called Mung-bean or *Mudga* contain balanced nutrients including protein, dietary fiber and bioactive phytochemicals (Venkateshwarlu et al., 2016). The phytosterols contained in the sprouted mung bean powder lowers blood fat by competitively bind to the enzymes responsible for esterification of cholesterol (Reeshma et al., 2011). The Elephant foot yam (*Amorphophallus paeoniifolius* (Dennst.) Nicolson, belonging to the family Araceae, commonly called surana or sweet yam is an underground tuber which is a staple vegetable of Indians. The corm of *Amorphophallus* is a rich source of phytosterols and has proved hepatoprotective activity comparable to that of Silymarin (Hurkadale et al., 2012) along with significant anti-oxidant property. However, these two plant sources have been utilized traditionally as a balanced diet in lowering lipid levels since antiquity. So, a combination of sprouted Mung bean powder and *Amorphophallus* corm provides a future perspective of non-pharmacological management of dyslipidemia. Considering the above facts, a study has been planned to evaluate the effect of the powder of sprouted mung bean and purified *Amorphophallus* corm on serum lipid levels in albino rats of Wistar strain receiving cholestyramine resin as standard drug.

2. Materials and methods

2.1. Drug

Seeds of Green gram were purchased from local market and were washed and cleaned thoroughly to remove any foreign matter and were soaked in twice the quantity of distilled water for 4 h. The seeds were again rinsed with distilled water and were kept in sterile petridishes in a single layer over sterile muslin cloth moistened with distilled water. The dishes were placed in a seed germinator (Single chamber) (Indosaw Germinator, Haryana, India) and incubated at 25 °C temperature and a relative humidity of 90% for a period of 48 h. After germination the seeds were dried in an oven maintained at 60 °C overnight and cooled to room temperature in desiccators. The seeds were later milled using a Mini Dal Mill Lab Model (Osaw Industrial Products, Haryana, India) at 20,000 rpm using size 30 mesh (Kumar and Singhal, 2009).

The consumption of raw Yam tubers produced several adverse reactions in the form of pruritus in oral cavity and throat. Keeping this in mind, the Yam was purified as per the accepted protocol of boiling in buttermilk as followed in the system of Ayurveda. Corms of yam were washed, peeled and sliced into thin uniform slices into a vessel containing distilled water. The pieces were boiled in an open vessel with butter milk till the pieces were properly cooked and soft. The excess butter milk was drained off and the pieces of yam were dried in an oven at 60 °C and were cooled in desiccators and were milled to a fine powder as in green gram.

The two powders were mixed in 1:1 proportion (w/w) and thoroughly mixed till the mixture was homogenized. The mixture was stored in airtight containers till the beginning of the study. The dose was fixed from a standard surface area conversion table (Paget, 1964). The corresponding human doses as per the references from *Sarngadhara Samhitha* (Murthy, 2005) of 12 g per day was converted to the corresponding animal dose of 0.108 g per 100 g body weight as the effective dose (ED). The doses 0.054 g and 0.216 g per 100 g body weight were considered as the Half dose (HD) and Double dose (DD) respectively. Drugs were administered after reconstituting in 10 ml of normal saline with the help of an intragastric tube.

2.2. Experimental design

The experiment was carried out after obtaining permission from the IAEC (No: IAEC/DRB18). A total of 30 albino rats of Wistar strain weighing between 150 and 200 g were used for the study. The animals were given a fortnight to acclimatize to the laboratory environment. The animals were housed in standard polythene cages with 12 h light and dark cycles and were fed on standard rat feed (Sai Durga Feeds, Bangalore, Karnataka). Water was given *ad libitum* throughout the experiment. All animals were taken care of as per the CPCSEA guidelines. All animals were weighed, randomized and properly marked for identification before the start of the study.

The experiment was completed in 30 days. Before the commencement of the study, the animals were divided into five groups of six animals each and they were fasted for 20 h by withdrawing food and not water. On the morning of the first day of experiment, blood samples were drawn from each animal under light ether anesthesia by retro-orbital puncture. Then, first dose of medicine was given to each animal depending on the group they belong to.

Group I: (Normal Control Group): Normal saline 10 ml/kg body weight/day.

Group II: (Standard Group): Cholestyramine resin 5 mg/kg body weight/day.

Group III: (Half Dose Group): Drug powder at a dose of 540 mg/kg body weight/day.

Group IV: (Effective Dose Group): Drug powder at 1080 mg/kg body weight/day. and

Group V: (Double Dose Group): Drug powder at 2160 mg/kg body weight/day.

The drugs were administered for a period of 30 days. On the last day of the study, the animals were fasted for 20 h by withdrawing food and not water. Blood was collected at the end of the study by retro-orbital puncture after light ether anesthesia.

2.3. Method of blood collection

Blood was collected from each animal at the beginning and end of the study. Blood was collected with the help of a capillary tube from the orbital sinus after engorging the retroorbital plexus by pressing the thumb behind the angle of the jaw. The blood was transferred to a tube and centrifuged for 10 min at 3000 rpm to collect the serum. After separating the serum, total Serum Cholesterol (TC), Triglycerides (TG), Low Density Lipoprotein Cholesterol (LDL), Very Low Density Lipoprotein Cholesterol (VLDL), High Density Lipoprotein Cholesterol (HDL), Castelli's Risk Index-I (CRI-I), Castelli's Risk Index-II(CRI-II), Non-HDL cholesterol/HDL ratio, Atherogenic Coefficient (AC) and Atherogenic Index of Plasma (AIP) were estimated (Kumar and Singhal, 2009). Serum LDL and VLDL were estimated using Friedwald's formula (Cordova et al., 2004). Atherogenic Index as well as percentage of protection was also calculated (Dhandapani, 2007).

2.4. Statistical analysis

Data obtained were subjected to One-way Analysis of Variance (ANOVA) followed by Dunnet's multiple comparison tests as post hoc test using SPSS Statistical package version 16.0. The chosen level of significance was 5% ($p < 0.05$).

3. Results

The results of all serum lipid estimation were reported as Mean \pm SEM (Standard error of mean) of 6 animals in each group.

The difference between the groups were analyzed using one-way ANOVA, followed by Dunnet's multiple comparison test as the post hoc test if the ANOVA showed significance at 5% level ($p < 0.05$). The different serum lipid parameters at the end of 30 days are as shown in Table 1. Table 2 shows the HDL Ratio, Atherogenic Indices and Percentage protection in each treated group.

Results reveal that there was a significant reduction in all the lipid parameters ($p < 0.05$) except HDL after 30 days of administration of the drug when compared to the normal animals. HDL showed significant elevation ($p < 0.05$) than the normal rats. The lipid lowering capability of the drug combination ($p < 0.05$) along with its potential to elevate HDL ($p < 0.05$) in rats when given at a dose of 1080 mg/kg body weight (Effective dose) and 2160 mg/kg body weight (Double dose) is proved. The Half dose of 540 mg/kg body weight showed insignificant results ($p > 0.05$) in lowering lipid levels as well as raising the HDL levels.

The Standard drug cholestyramine resin at a dose of 5 mg/kg body weight showed significant reduction ($p < 0.05$) in all serum lipid levels, but the drug was unable to elevate the HDL significantly ($p > 0.05$) when compared to the normal rates. The effective and double doses of the drug (1080 mg/kg and 2160 mg/kg body weight respectively) significantly reduced the Atherogenic Indices ($p < 0.01$) and significantly elevated the HDL Ratio ($P < 0.01$) and Percentage protection ($P < 0.05$) than the normal rats. The Hypolipidemic activity of the drugs were comparable to that of the Standard drug in all aspects and that it showed an enhanced capability in elevating the HDL levels than the standard drug. Figs. 1–3 represents the graphical representation of the effect of drug on lipid parameters, percentage protection, HDL Ratio and Atherogenic index in different groups respectively.

4. Discussion

Cholesterol build up in coronary arteries ends in fatal clinical events, which ranks higher than the mortalities from all types of cancers put together. The cholesterol homeostasis is maintained by the lipoproteins, which dismantles the excess intracellular cholesterol into packets of lipoproteins circulating in blood. This event is pivotal in the development of atherosclerotic plaques subsequently ending in coronary artery disease (Glomset, 1968). So, the cholesterol along with their lipoprotein levels helps in prediction and clinically gauging cardiovascular events. Among the lipoproteins, High density lipoproteins (HDL) promotes vascular health by extracting cholesterol from tissues and atherosclerotic plaques and delivers it back to the liver (Reverse cholesterol Transport) (Di Marco and Fernandez, 2015). The drug combination at a dose of 1080 mg/kg and 2160 mg/kg body weight significantly elevated the HDL levels in the experimental rats ($p < 0.05$). This result shows that the drug combination is a cardioprotective agent and accelerates the Reverse Cholesterol transport.

Low density lipoproteins (LDL) contributes to the formation of atherosclerotic plaques by binding to the connective tissue in the intimal layers of arteries (Tovar et al., 1998). Elevated levels of LDL cholesterol above 100 mg/dl is considered as the primary risk factor for CAD. The drug combination at both effective and double doses reduced the LDL levels significantly at $p < 0.01$ and $p < 0.05$ respectively. The result shows that the drug combination improves the atherosclerotic changes induced by the endothelium mediated vascular response in rats. Our study proves the significant result of the drug combination to reduce the LDL cholesterol significantly when compared to the normal rats.

Table 1
Effect of drug on serum lipid levels.

Group	Total cholesterol	Triglycerides	Hdl	Ldl	Vldl
Normal	141.0 ± 5.13	93.66 ± 2.71	30.83 ± 2.7	91.66 ± 5.7	18.5 ± 0.56
Standard	96.8 ± 3.6 ^a	80.5 ± 3.91 ^a	32.0 ± 3.57 ^d	48.83 ± 3.51 ^a	16.0 ± 0.68 ^b
Effective dose	103.0 ± 2.13 ^b	74.33 ± 4.68 ^a	49.3 ± 3.35 ^a	38.83 ± 3.5 ^b	14.83 ± 0.91 ^a
Half dose	115.83 ± 5.8 ^d	80.83 ± 5.12 ^d	38.3 ± 2.21 ^d	58.66 ± 7.3 ^d	16.2 ± 1.07 ^d
Double dose	98.50 ± 2.06 ^a	78.33 ± 6.06 ^a	39.0 ± 1.74 ^a	55.33 ± 9.5 ^a	15.66 ± 1.20 ^a
F Ratio	8.25 ^c	4.177 ^c	4.936 ^c	5.857 ^c	4.04 ^c
Df	4, 25	4, 25	4, 25	4, 25	4, 25

Values are expressed as Mean ± SEM, (n = 6), serum lipid levels are expressed in mg/dl. One-way ANOVA followed by Dunnet's Multiple comparison post hoc test have been performed with the normal group and Standard group.

- ^a $p < 0.05$.
^b $p < 0.01$.
^c $p < 0.001$.
^d $p > 0.05$.

Table 2
Atherogenic index and percentage protection in different groups.

	Cri i	Non-hdl c/hdl	Cri ii	Tg/hdl	Plasma ai	% protection
Normal	4.6 ± 0.15	3.6 ± 0.22	3.0 ± 0.37	3.0 ± 0.34	0.18 ± 0.04	0
Standard	3.0 ± 0.18 ^c	2.0 ± 0.18 ^c	1.5 ± 0.27 ^a	2.5 ± 0.1 ^d	0.24 ± 0.01 ^d	39.4
Effective dose	2.1 ± 0.07 ^c	1.1 ± 0.02 ^c	0.8 ± 0.13 ^c	1.5 ± 0.14 ^b	0.29 ± 0.01 ^a	51.2
Half dose	3.0 ± 0.19 ^c	2.0 ± 0.11 ^c	1.5 ± 0.09 ^b	2.1 ± 0.29 ^d	0.24 ± 0.02 ^d	45.8
Double dose	2.5 ± 0.08 ^c	1.5 ± 0.27 ^c	1.4 ± 0.12 ^b	2.0 ± 0.25 ^a	0.26 ± 0.01 ^d	33.7
F ratio	42.01	25.88	12.77	5.61	3.48	–
Df	4, 25	4, 25	4, 25	4, 25	4, 25	–

Values are expressed as Mean ± SEM, (n = 6). One-way ANOVA followed by Dunnet's Multiple comparison post hoc test have been performed with the normal group and Standard group.

- ^a $p < 0.05$.
^b $p < 0.01$.
^c $p < 0.001$.
^d $p > 0.05$.

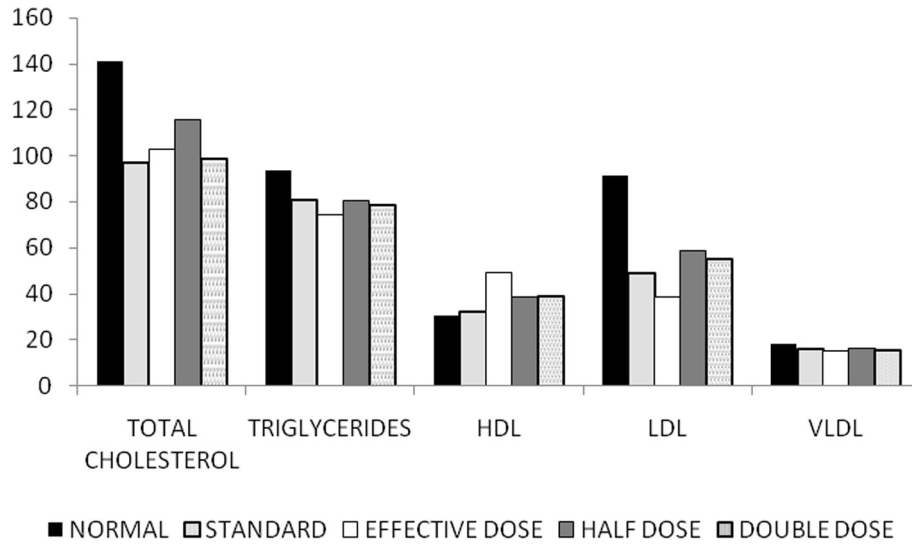


Fig. 1. Effect of the drug on serum lipid levels.

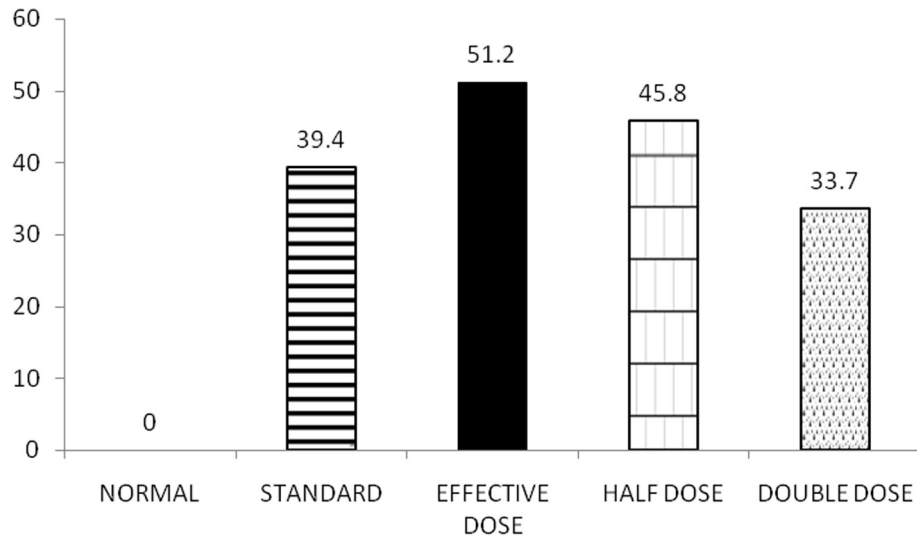


Fig. 2. Percentage protection of the drug in various groups.

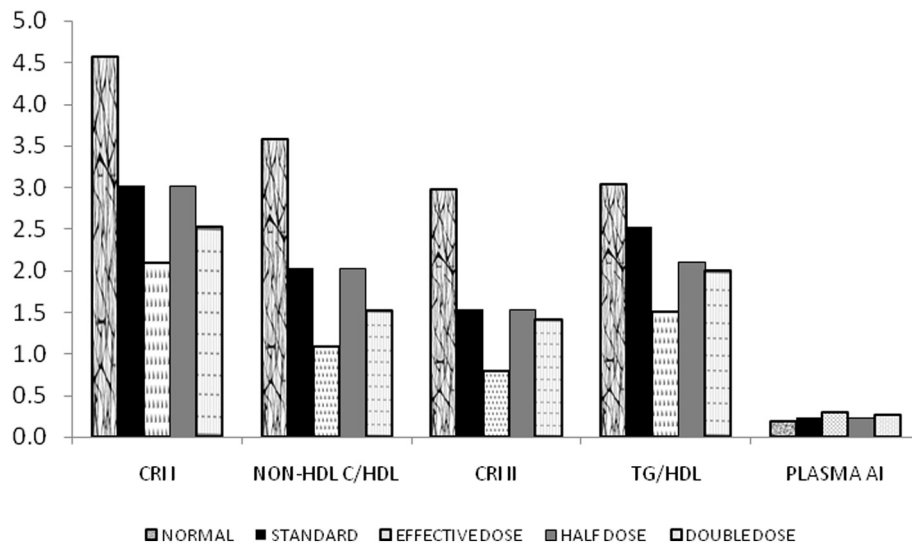


Fig. 3. Atherogenic indices in various groups after drug treatment.

Hypertriglyceridemia is considered as an independent risk factor for CVD. Fasting triglyceride measurement is important for evaluating the risk of heart disease especially in cases suffering from diabetes, glucose intolerance, insulin resistance syndrome and low HDL levels (Kanthé et al., 2012). Increased secretion of TG rich VLDL particles lead to increase in small dense LDL production associated with decrease in HDL with resultant atherosclerotic plaque development. The study drug combination at a dose of 1080 mg/kg and 2160 mg/kg body weight significantly reduced the serum triglyceride levels ($p < 0.05$). Our study proves the efficacy of the drug combination in reducing the independent risk factor for CVD, hypertriglyceridemia. The study also showed significant statistical reduction in VLDL levels ($p < 0.05$) in both effective and double doses.

Various indices and ratios of different lipoproteins also play an important role in the estimation of cardiovascular risks. These indices termed Atherogenic indices were calculated either by computing any of the following ratios; viz, Castelli Risk Index I (CRI-I), Non HDL C/HDL, Castelli risk Index II (CRI-II), TG/HDL, Atherogenic coefficient and Atherogenic Index of Plasma (AIP). A total cholesterol/HDL ratio (CRI-I) ≤ 3 is considered low risk, and a value above 4.5 is a moderate risk while a value >8 is considered high risk for developing coronary heart disease (Chia, 1991). The effective and double doses of the study drug had significantly reduced the CRI-I ratio ($p < 0.01$) when compared to normal and standard drugs. The Canadian Working group report considers CRI-I ratio as a secondary goal of therapy as it is more sensitive and specific index of cardiovascular risk than total cholesterol (Genest et al., 2003). The ratio of LDLc to HDLc is termed Castelli Risk Index-II (CRI-II), along with CRI-I, CRI-II are strong predictors of coronary heart disease (Castelli and Abbott, 1983). In this study both effective and double doses of the drug significantly reduced the CRI-II levels ($p < 0.01$). Non-HDL Cholesterol index is considered as a better predictor of cardiovascular disease risk than the Triglyceride levels. In our study, Non-HDL c/HDL ratio decreased significantly ($p < 0.01$) in effective dose as well as the double dose groups compared to the normal animals. The Atherogenic Index of Plasma (AIP) calculated as $(\log \text{ TG})/\text{HDLc}$ is considered a strong predictor for infarction (Gaziano et al., 2011). The study drug at effective dose reduced AIP significantly at $p < 0.05$. The double dose group, however, did not give a statistically significant result in AIP ratio. A Triglyceride based Atherogenic Index have more value in assessing cardiovascular risk than other indices (Kanthé et al., 2012). The drug combination significantly reduced the TG/HDL ratio by the effective and double doses at $p < 0.01$ and $p < 0.05$ levels respectively. The percentage of protection from atherogenesis provided by the formulation was computed using the concomitant increase in plasma HDL. A 1% decrease in HDL escalates the risk of heart disease by 3–4% (Dhandapani, 2007). Cholestyramine, the standard group offered 39.4% protection when compared to normal animals. The effective dose of the drug and the double dose offered 51.2% and 45.8% protection respectively.

Mung beans have proved efficacy in normalizing insulin sensitivity, plasma lipid profile and triglyceride levels by its protein isolates. It showed significant scavenging activity against reactive oxygen and nitrogen species and inhibited LDL oxidation. The multiple pharmacological activities like antioxidant, antimicrobial, anti-inflammatory, antitumor and regulation of lipid metabolism are attributed to the presence of high levels of phytoconstituents like proteins, amino acids, oligosaccharides and polyphenols (Randhir and Lin, 2004). Sprouted mung bean shows enhanced biological activities because of the activation of biosynthetic enzymes resulting in abundant availability of secondary metabolites after germination. Thus the nutritional and medicinal qualities of mung beans are improved by sprouting (El-Adawy et al., 2003). Though a

staple food across many countries, the consumption of mung bean is associated with flatulence in many due to the presence of Oligosaccharides, like raffinose, stachyose and verbascose present in raw seeds, which are removed to a greater extent by presoaking or germination as they are water soluble (Tang et al., 2014).

Amorphophallus is an edible corm used as a vegetable in tropical and sub-tropical countries with considerable medicinal properties. It has a wealth of phyto-constituents which includes sugars like glucose, galactose and rhamnose, glucomannan, flavonoids, phenols, coumarins, terpenoids, sterols and alkaloids. A diet rich in glucomannan reduced the levels of hormone, leptin, derived from adipocytes, which regulates body mass. The hypolipidemic effect of glucomannan derived from *Amorphophallus* was similar in action to statins; competitive inhibition of HMG-CoA reductase and reducing LDL synthesis. Thus the combination of Mung bean and Yam proves to act synergistically in reducing the lipid levels in dyslipidemia.

5. Conclusion

Considering the results obtained from this study, the combination of mung bean and yam significantly reduced the serum lipid levels as well as the atherogenic indices in albino rats in comparison to the bile acid sequestering agent, Cholestyramine as standard. Both mung bean and yam has been traditionally used as a food item throughout Asia. The combination provides a hope in reducing the high CAD risk among Asians by becoming a mainstay of non-pharmacological agents against dyslipidemia.

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