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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.

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 form *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 were 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 ± SEM (Standard error of mean) of 6 animals in each group.

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