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EFFECT OF FEEDING *BRASSICA JUNCEA* SEEDS ON EXPERIMENTALLY INDUCED HYPERLIPIDEMIA

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Abstract

Cardiovascular Disease (CVD) is major cause of death worldwide. Atherosclerosis is principal underlying mechanism, stimulated by hyperlipidemia. Substantial evidences suggest that atherosclerosis can be prevented or at least retarded by controlling serum lipids, especially cholesterol, at initial stages. The study was aimed to investigate the antihyperlipidemic potential of *Brassica juncea* seeds in cholesterol-fed rabbits owing to expected lipid lowering effect of *B. juncea* seeds.

Age matched 18 rabbits were randomly divided into three equal groups control, hyperlipidemic and treated. Following one week acclimatization, control group kept on normal animal chow, 5% atherogenic diet was administered to hyperlipidemic group while treated were fed 10% dried *Brassica juncea* seeds along with atherogenic diet for eight weeks. Blood specimens were obtained and assayed for lipid profile (TC, HDL-C, LDL-C, TG, vLDL, TC/HDL-C). Data was analyzed using t-test at 0.05.

High cholesterol diet significantly increased plasma TC ($p<0.01$, 60.25%), LDL-C ($p<0.05$, 10.8%), TG ($p<0.05$, 19.9%) while rise in HDL-C (23.1%) and vLDL (18.4%) remained non-significant as compared with control. *Brassica juncea* seeds consumption showed significant lipid lowering activity TC ($p<0.05$, -24.7%), HDL-C ($p<0.05$, 36.7%), LDL-C ($p<0.05$, -34%), TG ($p<0.01$, -41%) and vLDL ($p<0.05$, -40.7%) in comparison to hyperlipidemic counterparts. A non-significant decrease in body weight was also observed (-11.5%).

The findings suggest that *B. juncea* seeds possess strong antihyperlipidemic effects and its dietary supplementation may be helpful as primary prevention against atherosclerosis and coronary heart disease.

Introduction

Coronary artery disease (CAD) is a major death-causing disease globally. Local prevalence of CAD is about 26.9%. One in 4 middle-aged adults in Pakistan has CAD (Jafar *et. al.*, 2005). Disease starts with a accumulation of fatty acid in the blood vessels walls that delivers blood to heart producing shortening of their diameter that can't keep up required blood supply to the heart because of process called atherosclerosis: the core contributor in such deaths (Galkina & Ley, 2009). According to World Health Organization, by A.D. 2020 non-communicable diseases would contribute for up to three quarters of deaths in developing countries and that CAD will lead the killers (WHO, 1997) and a report by Robert *et al*. (2005) showed that Asian Indian descent have markedly greater incidence of cardiovascular event as compared with rest of ethnic groups.

The well established risk factors for atherosclerosis are hypercholesterolemia, hypertension, diabetes, cigarette smoking, physical inactivity, obesity, male sex, age and family history (Jean-Charles *et al.*, 2004). Among these risk factors first five are potentially reversible, and there is evidence that their reversal reduces the complications of atherosclerosis.

Dyslipidemia is a cause of premature coronary artery disease, specifically, increased plasma cholesterol, higher low density lipoprotein (LDL) cholesterol, reduced high density lipoprotein (HDL) cholesterol and increase in triglyceride (TG) levels predispose to coronary disease. Atherosclerosis can be prevented and its genesis can be retarded as suggested by substantial evidence from basic and clinical research. To treat coronary artery disease, commonly two choices are available i.e. primary and secondary prevention trials. In both choices the main objective is to reduce serum levels of lipids, especially cholesterol. Thus the drugs that lower cholesterol concentrations decrease cardiovascular disease associated deaths are unambiguous. The use of 3-hydroxy-3-methylglutaryl coenzyme-A (HMG-CoA) reductase inhibitors (Statins) supports this conclusion in clinical trials over the last 11 years in different patient groups, including patients with or without clinical cardiovascular disease and patients with severe or only moderate hypercholesterolemia. However this pharmacological approach is still contentious in individuals without recognized CAD. And regrettably, sudden cardiac death at the time of first myocardial infarction is common, so it will never be an acceptable strategy to wait to treat until symptoms of CAD have developed as the first symptom of CAD is too often a sudden death. Exercise and dietary therapies are more important in non pharmacological approach. Diet plays a significant role in the primary and secondary prevention of CAD (Grover *et al.*, 2002).

In this context, with increasing concern to prevent atherosclerosis and/or decelerate atheroma formation, it becomes major goal to keep plasma cholesterol concentrations within normal range.

Brassica juncea commonly known as mustard belongs to the family Brassicaceae (Veliek *et al.*, 1995) has been recognized as a valuable medicinal plant and many researchers have reported therapeutic value of different parts of the plant (Khan *et al.*, 1997; Olivier *et al.*, 1999; Moghadasian *et al.*, 1999). It is reported as rubefacient, irritant; antimicrobial and antifungal (Dominiczak, 1998).

Musturd seeds (Urdu: *Rai*) are well known spice and added frequently in most of the dishes in the country. The main types include single or mixed white, black, or brown seeds. They have been used as culinary medicine since years but these effects are now being assessed on scientific basis. The objective of plan was to assess the outcome of *Brassica juncea* seeds feeding in cholesterol rich diet taking rabbits, in relation to its cholesterol reducing activity hence to identify its importance as a non-pharmacological mean for prevention of coronary artery disease in general.

Materials and Methods

Pure brown *Brassica juncea* seeds were bought from local market in Karachi and confirmed then from Department of Botany. They were used in grounded form in the study. Total 18 healthy New Zealand rabbits (*Oryctolagus cuniculus*) either sex were used in the study. The animals were two month old at the start of study with mean body weight of 1.75kg. The study was prospective analytical, done on experimental animals chosen randomly for different treatments. Initially all animals were acclimatized in a well ventilated room for one week provided normal rabbit chow and free access to water. During this period, the amount of chow consumed by a rabbit per day was measured carefully along with close monitoring of body weight and other physical parameters. This was practiced throughout the study period. After an over night fast, blood was drawn from the marginal ear vein and base line values for all parameters were measured along with body weights. Then experimental animals were randomly divided into three groups of equal size and designated as
Group I (n = 6): Control, Fed normal animal chow
Group II (n = 6): Fed hypercholesterolemic diet (HCD) i.e. 5% butter fat / 100g of daily diet (Moghadasian *et al*, 1999) & served as hyperlipidemic group.
Group III (n = 6): Fed hypercholesterolemic diet (HCD) i.e. 5% butter fat / 100g of daily diet plus 10% ground *Brassica juncea* seeds (Yadav *et al.*, 2004) continued for eight weeks.

Blood Collection: Blood samples were collected after eight weeks using sterile disposable syringe and needle. The blood was transferred into glass tubes and centrifuged at 3000 rpm. Plasma was collected in appendorfs and was kept in refrigerator until required for analysis. Simultaneously, body weights were also measured.

Biochemical Analysis: Plasma cholesterol and triglyceride levels were measured by the method of Trinder using enzymatic kit (Clonital Italy), HDL-C levels were measured by dextran sulphate Mg (II) method, using enzymatic kit (QCA, France). LDL-C concentration was determined by polyvinyl sulphate method using enzymatic kit (QCA, France).

Statistical Analysis: The data was analyzed through SPSS 17. A probability value $p<0.05$ at 95% confidence interval was selected level of statistical significance. While mean percent change (MPC) was calculated by dividing the difference of pre-post treatment with pre-treatment value and multiplying the result by 100.

Results and Discussion

Genesis of Hyperlipidemia in Cholesterol Fed Rabbits: The plasma total cholesterol, triglycerides, LDL-C and HDL-C levels in rabbits kept on normal diet alone (group I) remained stable and there was no significant variation in their values.

Conversely in the HCD treated animals (group II); a significant rise in TC ($p<0.01$, 60.25%), LDL-C ($p<0.01$, 23%), TG ($p<0.05$, 20%) was observed while increase in HDL-C and vLDL not touched level of significance though a mean percent increase of 23% and 18.4% respectively was recorded as compared with control group. There was also raised body weigh following HCD (18.8%) and TC/HDL-C ratio (29.9%), both statistically non significant. (Table 1 and Figure 1).

Effect of Brassica Juncea Seeds on Hyperlipidemic Rabbits: *Brassica Juncea* seeds showed significant antihyperlipidemic action. Its intake significantly reduced the levels of plasma cholesterol ($p<0.05$, -24.72%), triglycerides ($p < 0.01$, -41%), vLDL ($p<0.05$, -40.7%) and LDL – C ($p < 0.05$, -34%) as compare to hyperlipidemic rabbits. HDL-C raised ($p<0.05$, 37%). The reduction in total body weight of animals was non-significant (-11.5%) while TC/HDL-C ratio reduced significantly ($p<0.05$, -45%) in comparison with group II. (Table 1 and Figure 1).

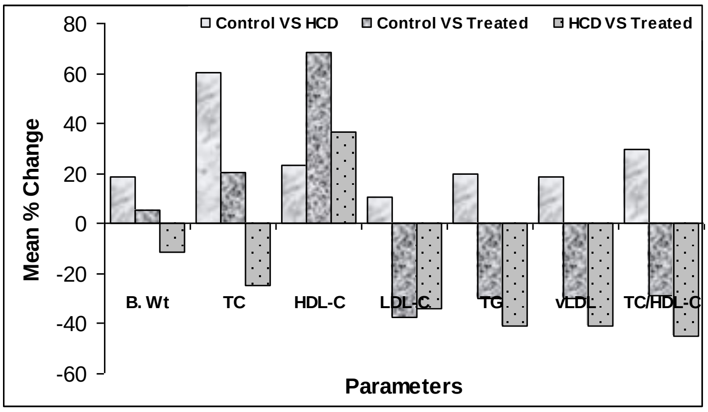

Fig. 1. Mean percent change from control and hyperlipidemic animals in body weight and lipid profile following oral administration of *Brassica juncea* seeds.

Table 1. Effects of *Brassica juncea* seeds oral administration on body weight and lipid profile in experimentally induced hyperlipidemia.

Parameters	**Control (n = 6)**	**HCD-FED[1] (n = 6)**	***B. Seed*-Treated[1, 2] (n = 6)**
Body Weight (Kg)	1.17 ± 0.08	1.39 ± 0.03^{NS}	1.23 ± 0.07$^{NS, NS}$
TC (mg/dl)	40 + 10.2	64.1 ± 7.0**	48.25 ± 6.5$^{NS, *}$
HDL-C (mg / dl)	19 ± 9.9	23.4 ± 5.6^{NS}	32.0 ± 4.5$^{**, *}$
LDL-C (mg / dl)	18 ± 4.5	37.5 ± 2.2**	24.75 ± 2.5$^{NS, *}$
TG (mg / dl)	133 ± 3.4	159.5 ± 3.5*	93.35 ± 2.7$^{*, **}$
vLDL (mg/dl)	26.6 ± 1.1	31.5 ± 0.8^{NS}	18.67 ± 0.7$^{NS, *}$
TC/HDL-C	2.105	2.735^{NS}	1.500$^{NS, *}$

Values shown as Mean ± S.D.
HCD: High Cholesterol Diet
1: As compared with control
2: As compared with HCD-Fed
NS: Statistically non-significant at chosen alpha $p>0.05$
*$p<0.05$ **$p<0.01$

Atherosclerosis has multi-factorial pathophysiology that could be stimulated by several etiological factors among which the most prevalent is hypercholesterolemia. It has been shown that a decrease in plasma cholesterol value following pharmacological drugs reduces cardiovascular risk rate (Gotto and Grundy, 1999), but as mentioned earlier usually it's too late to treat after the development of signs and symptoms. In addition to diet, medicinal plants application as one of non-pharmacologic approach in the prevention of lipid metabolism alteration has been extensively investigated world wide (Bhandari *et al.*, 1998).

Our results illustrated that raw Brassica seeds consumption by rabbits significantly lowered the risk of atherosclerosis by bringing fall in concentration of plasma total cholesterol, LDL cholesterol as well as an improvement in HDL-cholesterol levels that is in full agreement with previously described results by Khan *et al.* (1996a). This lipid lowering property of *B. juncea* may be due to its emulsification properties which were contained in its water soluble portion of proteins as reported by (Cui, 1997). Reduced plasma cholesterol concentration is also affected by improved function of LDL receptor, which accelerates LDL uptake from plasma (Ness *et al.*, 1996). These findings are in favour of former studies, showing that plant has cholesterol-reducing capacity (O'Brien and Reiser, 1979).

The same results described by Khan *et al.* (1996b) who observed reduction in cholesterol and phospholipids concentrations in experimentally induced colon carcinoma. In our study triglyceride levels were also significantly reduced. These lipid lowering properties of *Brassica juncea* help protect patient from myocardial infarction which is a manifestation of atherosclerosis. The evidence is supported by Indian Experiment of Infarct Survival Study about assessment of fish oil and mustard oil treatment effects, rich in n-3 fatty acids, in prospective patients of acute myocardial infarction. They reported a significant decrease in total heart abnormalities in patients consuming fish oil and mustard oil (de Lorgeril *et al.*, 1994).

The positive effect of *B. juncea* seeds on HDL-C also confirms its role in the protection against atherosclerosis. Substantial data demonstrated an inverse relation between HDL-C to coronary heart disease, the ultimate outcome of atherosclerosis (Castelli *et al.*, 1986). HDL mobilizes cholesterol from atheroma to be transported to the liver and be excreted in bile, thereby known as good cholesterol (Ridker *et al.*, 2001).

In our study we also observed the weight reducing ability of *Brassica Juncea* and it was revealed that although *Brassica juncea* lowered body weight but it was not statistically significant supported by previous studies (Khan *et al.*, 1996a).

Conclusion

The consumption of *Brassica* seeds may be potentially beneficial against atherogenesis hence protective against CAD as it possess quality of lowering the plasma cholesterol, triglycerides, LDL-C and improving HDL-C.

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