LIPID LOWERING AND ANTIOXIDANT EFFECT OF WHEAT WHOLEGRAIN EXTRACT IN HYPERCHOLESTEROLEMIC RABBITS

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ABSTRACT
Objective: The present study was designed to examine the hypocholesterolemic and antioxidant activity of Triticum aestivum (Wheat) whole grain extract in cholesterol fed rabbits. Methods: Ethanolic extract of wheat whole grain was administered to induced hypercholesterolemic rabbits at a dose of 500 mg/kg.b.wt/day. The animals were divided into control, hypercholesterolemic, wheat whole grain extract- regular and parallel treatment groups for 60 days experimentation. The serum lipid profile, Lipid peroxidation (LPO), antioxidant parameters like Catalase (CAT) and Superoxide dismutase (SOD) were estimated and histopathological study of thoracic aorta was carried out. Results: Cholesterol feeding caused a significant increase (P ≤ 0.001) in serum total cholesterol, low density lipoprotein-cholesterol and triglycerides, when compared with the control group. The changes in the antioxidant parameters were observed as an increase in serum LPO and reduction in CAT and SOD activity in the cholesterol fed rabbits. The serum lipid profile parameters and level of lipid peroxidation was reduced significantly whereas CAT and SOD activity were elevated after the treatment with 70% ethanolic extract of wheat whole grain. Histopathology of aorta exhibited significant increase in lumen size by reduced cholesterol deposition and regressed atherosclerotic lesions as compared to the hypercholesterolemic group. Conclusion: These outcomes recommend that wheat whole grain extract reduces elevated cholesterol levels thereby controlling atherosclerosis. It has a potent antioxidant activity which may be responsible for its hypolipidemic effect.

KEYWORDS: Wheat whole grain, atherosclerosis, antioxidant, hypercholesterolemia.

INTRODUCTION
Alterations in the concentration of major lipids like serum total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C) and triglycerides (TG) give useful information on the lipid metabolism and predisposition of the heart to atherosclerosis and its associated coronary heart diseases. Atherosclerosis is recognized as a multisystemic, diffuse, and chronic inflammatory disorder which involves the vascular, metabolic and immune systems of the body, with various local and systemic manifestations. Lipoprotein oxidation appears to contribute significantly to the pathogenesis of atherosclerotic cardiovascular disease.

Wheat (Triticum aestivum), is the world’s most consumed cereal grain. Whole wheat grain consists of the pericarp, the seed coat, the endosperm and germ or embryo. Wheat and wheat products contribute substantially towards proteins, vitamins and minerals when consumed as a major component of diet. Phytochemicals are compounds found in plants that have a beneficial effect on health and play active role in the amelioration of disease. Whole wheat provides a rich source of bioactive phytochemicals namely phenolic acids, carotenes, tocopherols, alkylresorcinols, benzoazinoids, phytosterols, and lignans. Epidemiological evidences indicate that whole-grain consumption substantially lowers the risk of chronic diseases such as cardiovascular diseases, diabetes and cancer, and plays a role in body weight management and digestive health. It is recommended by various dietary guidelines worldwide to increase whole grain consumption by replacing refined grains. High fat diet brings about enormous modifications in the antioxidant defence mechanism against the process of lipid peroxidation.

It is well documented that flavonoids and polyphenols are plant derived antioxidants which are known to significantly increase Superoxide dismutase and Catalase activities. The total antioxidant activity of wheat grain extracts and their phenolic acid contents is highly correlated. Owing to the side effects of synthetic medications to treat hyperlipidemia, patients are increasingly using herbal alternatives for preventive and therapeutic purposes. The present investigation was carried out to evaluate the hypocholesterolemic,
MATERIALS AND METHODS

Extraction of Plant Material
Unpolished wheat wholegrain was procured from local market, Jodhpur, Rajasthan state, India and extracted with 70% ethanol for 24 to 36 hours by soxhlet extraction method. Then ethanol was separated under reduced pressure to obtain a brownish crude residue which was dissolved in distilled water and orally administered to the animals.

Model Animals
New Zealand albino male rabbits were used as atherogenic animal models. Weight and age of animals were 1.25-1.50 Kg. and 10-12 months, respectively. The temperature was maintained at 24 ± 1°C; humidity at 40-60% and the daily light cycle was 12 h. The rabbits were kept in in metallic wire gauges with ample space. The regular diet food was supplemented with green leafy seasonal vegetables and water ad libitum. The Institutional Animal Ethical Committee approved the protocol. (IAEC, Reg. No. 1646/G0/ERE/S/12/PCSEA).

EXPERIMENATION

Induction of hypercholesterolemia: Rabbits were rendered hypercholesterolemic by oral gavage of cholesterol powder at the dose of 500 mg/kg body weight/day dissolved in 5 ml of coconut oil for 15 days.[10]

Experimental Design
The experimental period comprised of 60 days and the rabbits were divided into the following groups (n=5)

- Group I: Control- Placebo treated for 60 days.
- Group II: Hypercholesterolemic control- Cholesterol feeding (atherogenic diet + 500 mg cholesterol powder/kg.b.wt./rabbit/day in 5ml coconut oil) for 60 days.
- Group III: Wheat wholegrain extract regular treatment group- Cholesterol feeding for 15 days followed by wheat wholegrain extract treatment (500 mg/ kg.b.wt./rabbit/day) for 45 days.
- Group IV: Wheat wholegrain extract parallel treatment group- Cholesterol feeding for 15 days followed by concomitant treatment of cholesterol and wheat wholegrain extract (500 mg/kg.b.wt./rabbit/day) for 45 days.

Assessment of serum lipid profile
At the end of the experiment, all the rabbits were sacrificed under prolonged anaesthesia and blood was collected through direct cardiac puncture. Serum was separated by centrifugation and stored at -20°C until analysed. Serum total cholesterol, TG and HDL-C were analysed using Biochem Analyser RX-50 and commercial diagnostic kits (Siemens Healthcare Dignostics, USA) and LDL-C, VLDL-C were calculated using Friedewald’s formula.[11]

Assessment of antioxidant parameters
Serum lipid peroxidation was estimated by measuring the thiobarbituric acid reactive substances (TBARS) and was expressed in terms of malondialdehyde (MDA) content, according to the method of Okhawa.[12]

Assays for measuring serum Catalase and Superoxide dismutase activity were carried out by Aebi’s and Marklund’s method respectively.[13,14]

Histopathology of aorta
The ascending aorta (2-3 cm length) was cut at the origin and excised from the heart each animal and kept in 10% formalin fixative. The processed aorta samples were ultra-sectioned (5-6 μm thickness), stained with haematoxylin and eosin (HE) and examined under a light microscope for histopathological study.

Statistical analysis
All biochemical estimations were expressed in terms of Mean ± SEM. Statistical differences between the means of the various groups were evaluated using one-way analysis of variance (ANOVA) followed by Tukey’s test using GraphPad Prism 7.0 software. The graphical representations were made using MS Excel 2013.

RESULTS

Serum lipid profile
Chronic feeding of cholesterol in rabbits caused significant elevations (P≤ 0.001) in the total serum cholesterol, TG, LDL-C, VLDL-C and HDL-C when compared to control group. Significant reductions (P≤ 0.001) were observed in regular wheat wholegrain extract treated rabbits while slightly significant reductions in serum lipid profile parameters were noted (P ≤ 0.05) in the animals given parallel wheat wholegrain extract treatment, when compared to the hypercholesterolemic group (Table 1).
Table 1: Serum lipid profile of cholesterol fed rabbits treated with wheat wholegrain extract (Mean ± SEM).

<table>
<thead>
<tr>
<th>Treatment Groups</th>
<th>Total Cholesterol (mg/dl)</th>
<th>HDL Cholesterol (mg/dl)</th>
<th>LDL Cholesterol (mg/dl)</th>
<th>VLDL Cholesterol (mg/dl)</th>
<th>Triglycerides (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A (Intact Control)</td>
<td>53.52 ± 2.04</td>
<td>22.03 ± 3.68</td>
<td>19.92 ± 1.47</td>
<td>12.01 ± 1.74</td>
<td>60.03 ± 3.71</td>
</tr>
<tr>
<td>Group B (Hypercholesterolemic)</td>
<td>1664.85 ± 82.39 **</td>
<td>175.05 ± 13.28 **</td>
<td>1369.11 ± 72.51 **</td>
<td>53.19 ± 3.14 **</td>
<td>265.97 ± 15.69 **</td>
</tr>
<tr>
<td>Group C (Wheat wholegrain extract - Regular)</td>
<td>118.6 ± 8.90 *###</td>
<td>37.49 ± 3.50 *###</td>
<td>49.06 ± 4.01 *###</td>
<td>27.05 ± 2.49 **</td>
<td>135.25 ± 11.49 **</td>
</tr>
<tr>
<td>Group D (Wheat wholegrain extract - Parallel)</td>
<td>1222.71 ± 58.16 ***###</td>
<td>145.28 ± 13.48 ***###</td>
<td>1028.44 ± 47.66 ***###</td>
<td>48.99 ± 3.85 ***ns</td>
<td>232.84 ± 19.22 ***###</td>
</tr>
</tbody>
</table>

Gr. B to D compared with Gr. A
P ≤ 0.05 = * P ≤ 0.01 = **
P ≤ 0.001 = *** Non-significant = ns

Gr. C and D compared with Gr. B
P ≤ 0.05 = # P ≤ 0.01 = ##
P ≤ 0.001 = ### Non- significant = ns

Effect on antioxidant parameters
High level of MDA formation (P ≤ 0.001), which is the indicator of lipid peroxidation, was seen in the hypercholesterolemic group when compared with the control group. Besides significant reduction in serum MDA levels, significant increase in serum SOD and CAT activities were seen (P ≤ 0.001) in the regular wheat wholegrain extract treated rabbits when compared to the hypercholesterolemic group (Figure 1).

**Figure 1:** Effect of *Triticum aestivum* (wheat) wholegrain extract on (i) Lipid peroxidation in nano moles MDA/mg protein in serum and (ii) Superoxide dismutase and Catalase activity of various treatment groups in U/mg protein (Mean of 5 values ± SEM), LPO: lipid peroxidation, WWE-R: wheat wholegrain extract- regular, WWE-P: wheat wholegrain extract- parallel.

Effect on aortic plaque formation
The aorta of hypercholesterolemic rabbits showed fibro-fatty plaque with foam cells and spaces within the intima, tunica and media. Administration of wheat wholegrain extract in regular treatment showed almost normal histo-architecture and significant regression in aortic lesions. The rabbits given parallel treatment of cholesterol and wheat wholegrain extract showed presence of atheroma but in much reduced state than that of the hypercholesterolemic group (Figure 2).
(i) Intact Control. (ii) Hypercholesterolemic control.


Figure 2 (i-iv): Photomicrographs (100x HE) of thoracic aorta of treated rabbits. (i) Intact control- Normal aortic wall consisting of tunica adventitia, tunica media and tunica intima. (ii) Hypercholesterolemic control- Intima shows marked increase in thickness with large number of foam cells, focal hyalinization in media and formation of atherosclerotic plaque (iii) Wheat wholegrain extract regular treatment- intact endothelium with restored histo-architecture (iv) Wheat wholegrain extract parallel treatment- thickened intima contained foam cells and cholesterol deposition showing some degree of atherosclerotic lesions.

DISCUSSION

The formation and distribution of atheroma in the rabbit model resembles atherosclerosis in humans to a great extent, so New Zealand white male rabbits were used for the present investigation. Hypercholesterolemia caused atheromatous changes in the aorta. The lipids deposited in the atherosclerotic lesions are mostly derived from plasma LDL, which is modified by oxidative processes, resulting in an enhanced uptake by the scavenger receptor of macrophages leading to foam cell formation. Wheat wholegrain extract treated rabbits showed significant regression in the plaque which was similar to that of the observations made by Purohit and co-workers who showed antiatherosclerotic activity of bees wax treatment in albino rabbits. LDL-C carries cholesterol from the liver to the blood stream. Therefore, when LDL-C is reduced, the amount of cholesterol being carried will also reduce because the uptake of LDL from plasma via LDL receptors increases thereby increasing the clearance of plasma cholesterol. High level of HDL-C protects endothelial cells from the cytotoxic effects of oxidized LDL. The concentration of HDL was found to increase by the wheat wholegrain extract treatment. The reduction in serum lipid profile was comparable to the reduction observed by treatment of wheat germ oil in cholesterol fed rabbits. Trowell gave the high-fibre food hypothesis and summarised that a diet rich in whole grains protected against coronary heart disease. Absorption of dietary cholesterol from the intestine is an important part of cholesterol homeostasis and represents the first step that allows dietary cholesterol to exert its metabolic effects. Inhibition of cholesterol absorption from intestine also decreases the delivery of cholesterol to the liver, thereby lowering serum as well as hepatic cholesterol. The higher the concentration of water-soluble dietary fibre ingested, the better its efficacy in reducing blood cholesterol. Triglycerides are the main storage form of fatty acids. The decrease in serum TG by the ethanolic extract of wheat wholegrain may be due to reduced lipolysis. This
may deplete the store of fatty acids. Also, the reduced serum TG level in the treated animals could be co-related to elevated lipoprotein lipase activity which is in agreement with previous studies.\textsuperscript{[23]}

In the present study we found significant increase in MDA (malondialdehyde) formation in the serum of cholesterol fed rabbits. It is the indicator of oxidative stress in hypercholesterolemic state which implicates in development and progression of atherosclerosis.\textsuperscript{[26]} Hypercholesterolemia also leads to significant decrease in the levels of antioxidant enzymes (SOD and CAT) which in turn generates hydroxyl and peroxyl radicals resulting in the beginning and proliferation of lipid peroxidation.\textsuperscript{[27]} The wheat wholegrain extract supplementation exhibited significant decrease in the levels of LPO indicating a probable role in scavenging reactive oxygen species (ROS) generated due to cholesterol overfeeding. A significant increase was seen in the activity of SOD and CAT in the serum of the wheat wholegrain treated rabbits. It can be attributed to the influence of flavonoids and polyphenols indicating the free radical scavenging activity of the herbal extract.\textsuperscript{[28]}

CONCLUSION

The present study concludes that Triticum aestivum (wheat) wholegrain extract administration to hypercholesterolemic animals reduced serum cholesterol, regressed the atheromatous plaques in the aorta and also improved antioxidant enzyme activities by virtue of its phytochemical constituents. Therefore, it can be used as an effective herbal therapeutic agent to treat cardiovascular problems.

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