

WHEAT BRAN EXTRACT MIMICS STATIN LIKE ACTION IN HYPERLIPIDEMIC RABBITS: AN EXPERIMENTAL STUDY

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ABSTRACT

Objective: The study was aimed to evaluate the hypolipidemic and antioxidant effect of *Triticum aestivum* (wheat) bran extraction cholesterol-fed rabbits.

Methods: The induced hyperlipidemic rabbits were treated with 70% ethanolic extract of wheat bran at a dose of 500 mg/kg body weight/day. The experimental protocol was designed for control, hyperlipidemic, wheat bran extract, and statin-treated groups for 60 days. The estimations for serum lipid profile, lipid peroxidation (LPO), and ferric reducing ability of plasma (FRAP) were done, and histopathological study of thoracic aorta was carried out.

Results: Oral administration of wheat bran extract caused significant reductions in the serum lipid profile by 94.30% (total cholesterol), 96.73% (low-density lipoprotein cholesterol), and 58.28% (triglycerides). It significantly decreased the LPO and enhanced the antioxidant capacity (FRAP) activity as compared to the hyperlipidemic rabbits. Histopathology revealed that wheat bran extract treatment prevented the accumulation of cholesterol and regressed atheromatous plaques significantly in the aorta as compared to the hyperlipidemic group, and the results were comparable to that of the standard drug statin.

Conclusion: The study exhibited that wheat bran extract controls atherosclerosis just like statin and can be used as a regular dietary supplement to manage cholesterol levels and prevent cardiovascular problems.

Keywords: Wheat bran, Atherosclerosis, Antioxidant, Hyperlipidemia.

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INTRODUCTION

Cardiovascular diseases (CVDs) are one of the leading causes of mortality in both the developed and developing countries. Elevated serum cholesterol level along with generation of reactive oxygen species (ROS) plays a key role in the development of CVD. Hyperlipidemia is a metabolic disorder and its major consequence is atherosclerosis which can be described as a chronic process involving the interplay of inflammation and oxidative stress. Alterations in the serum lipid profile due to raised concentration of total cholesterol (TC), low-density lipoprotein (LDL), very LDL (VLDL), and triglycerides (TGs) with a concomitant lower high-density lipoprotein (HDL) in blood circulation are the hallmark for atherosclerosis [1]. Medicinal plants since time immemorial have been in use for the treatment of various diseases worldwide. Herbs and phytochemicals play a major role in the discovery of new therapeutic agents and have received considerable attention toward the utilization of bio-friendly traditional medicine to meet healthcare needs [2]. *Triticum aestivum*, commonly called as Wheat, belongs to family *Poaceae* and is the most widely consumed grain in the world. By weight, the wheat kernel is composed of an outer bran (14–16% of the grain), the germ or embryo (2–3%), and the central endosperm (mainly starch: 81–84%) [3]. Botanically, the term “wheat bran” refers to the outer layers of the wheat grain comprising the aleurone, pericarp, and seed coat. The bran fractions produced as a by-product of wheat milling are rich in fiber, minerals, Vitamin B6, thiamine, folate, Vitamin E and are a good source of secondary metabolites such as phenolic acids, flavonoids, lignans, and phytosterols [4]. Several studies have shown that high-fat diet increases oxidative stress in plasma and tissues and act as a significant risk factor of CVDs [5]. Oxidation of LDL, recruitment of macrophages, and subsequent uptake of LDL-derived cholesterol by them are the major cellular events contributing to the development of atherosclerotic plaques [6]. A number of medicinal plants have shown

their beneficial effect on the CVD by virtue of their lipid-lowering, antianginal, antioxidant, and cardioprotective effects [7,8]. Wheat and wheat products contribute significantly toward proteins, vitamins, and minerals when consumed as a major component of diet [9]. Wheat bran is used as a source of dietary fiber and helps in the prevention of colorectal cancer, hemorrhoids, gallbladder disease, hiatal hernia, CVD, obesity, and some gastrointestinal diseases [10]. It is also used for treating constipation, irritable bowel syndrome [11], Type 2 diabetes [12], cholesterol, and high blood pressure [13]. Synthetic medications like statins are used extensively to treat hyperlipidemia but cause several side effects. Hence, the patients are increasingly switching over to herbal products for preventive and therapeutic purposes. The present investigation was carried out with the aim of extrapolating the antiatherosclerotic effect of wheat bran extract in induced hyperlipidemic rabbits.

METHODS

Extraction of plant material

Wheat bran was acquired from Morarka organic foods Ltd., Jaipur, and extracted with 70% ethanol for 36 h by Soxhlet extraction method. Then, ethanol was separated under reduced pressure to obtain a brownish residue which was resuspended in distilled water and administered orally 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 $24 \pm 1^\circ\text{C}$, humidity at 40–60%, and the daily light cycle was 12h. The rabbits were kept in in metallic wire cages 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 (Reg. No. 1646/GO/ERe/S/12/CPCSEA).

EXPERIMENTATION

Induction of hyperlipidemia

Rabbits were rendered hyperlipidemic 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 [14].

Experimental design

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

1. Group A: Control - placebo treated for 60 days.
2. Group B: Hyperlipidemic control - cholesterol feeding (atherogenic diet + 500 mg cholesterol powder/kg.b.wt./rabbit/day in 5 ml coconut oil) for 60 days.
3. Group C: Wheat bran extract treatment group - cholesterol feeding for 15 days followed by wheat bran extract treatment (500 mg/kg.b.wt./rabbit/day) for 45 days.
4. Group D: Statin treatment group - cholesterol feeding for 15 days followed by statin treatment (atorvastatin - 0.25 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 anesthesia and blood was collected through direct cardiac puncture. Serum was separated by centrifugation and stored at -20°C until analyzed. Serum TC, TG, and HDL-cholesterol were analyzed using Biochemistry Analyzer RX-50, and commercial diagnostic kits (Siemens Healthcare Diagnostics, USA) and LDL-cholesterol, VLDL-C were calculated using Friedewald's formula [15].

Assessment of antioxidant parameters

Serum lipid peroxidation (LPO) was estimated by measuring the thiobarbituric acid reactive substances and was expressed in terms of malondialdehyde (MDA) content, according to the method of Ohkawa *et al.* [16].

Serum total antioxidant capacity was measured by the ferric reducing ability of plasma (FRAP) (ferric reducing antioxidant power) assay carried out by according to the modified method of Benzie and Strain [17].

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 ultrasectioned (5–6 μm thickness), stained with hematoxylin and eosin (HE), and examined under a light microscope for histopathological observations.

Statistical analysis

All biochemical estimations were expressed in terms of mean \pm standard error of the mean (SEM). Statistical analysis was carried out by one-way ANOVA followed by Tukey's multiple comparison tests. Data were analyzed using GraphPad Prism-7 software and graphical representations were made using MS Excel 2013.

RESULTS

Effect on serum lipid profile

Chronic feeding of cholesterol in rabbits caused significant increase ($p \leq 0.001$) in the serum lipid profile parameters when compared to the control group. The administration of wheat bran extract (500 mg/kg.b.wt.) caused significant reduction by 94.30% (TC), 84.07% (HDL-C), 96.73% (LDL-C), and 58.28% (TG) while the standard drug (atorvastatin) treatment group showed reductions by 90.71%, 76.66%, 94.03%, and 39.52% in TC, HDL-C, LDL-C, and TG, respectively, as compared to the hyperlipidemic rabbits (Table 1).

Effect on LPO

Changes in the lipid peroxides in serum were studied for the effect of wheat bran extract treatment in hyperlipidemic rabbits. High level of MDA formation ($p \leq 0.001$) which is the indicator of LPO was seen in the hyperlipidemic group when compared with the control group. The inhibition of LPO was significant ($p \leq 0.01$) in both the treated groups, wheat bran and standard drug statin, when compared with the hyperlipidemic group (Fig. 1).

FRAP

To measure the total antioxidant capacity, FRAP value was calculated in terms of $\mu\text{M/L}$ ascorbic acid equivalents. Induction of hyperlipidemia in rabbits caused highly significant decrease ($P \leq 0.001$) in the serum FRAP values. These were restored to near normal values by the treatment of wheat bran extract and statin indicating a positive influence of wheat bran extract on the enzymatic antioxidant defense in hyperlipidemic rabbits (Fig. 2).

Effect on aortic plaque formation – Fig. 3 (i-iv)

The aorta of animals given *T. aestivum* bran extract showed almost normal histoarchitecture. However, the aorta of hyperlipidemic rabbits showed fibrofatty plaque with foam cells and spaces within the intima, tunica, and media. Administration of wheat bran extract showed significant protection from aortic lesions. Aortic plaques were regressed significantly and lumen size was also restored near to normal. This effect was comparable to that of the standard drug statin.

DISCUSSION

New Zealand white male rabbits fed with cholesterol were used for the present study. The formation and distribution of atherosclerotic lesions in the rabbit model resembles atherosclerosis in humans to a great extent [18]. In the present study, administration of cholesterol increased the levels of serum TC, HDL-C, LDL-C, TG, and VLDL-C in rabbits, in accordance with what has been reported previously [19]. The oxidative modification of LDL is predominantly responsible for the initiation and progression of atherogenic changes in the aorta [20]. Atorvastatin has been known to exercise its lipid-lowering effect by competitive inhibition of HMG-CoA reductase, which is the key enzyme for cholesterol biosynthesis. Studies have revealed that terpenoids, steroids, flavonoids, and phenolic compounds are responsible to prevent binding of HMG-CoA to HMG-CoA reductase, either by working individually or synergistically within the plant extract [21]. Our results of wheat bran extract showing similar effect as that of statin in hyperlipidemic rabbits are in agreement with the study by Reddy

Table 1: Serum lipid profile of cholesterol-fed rabbits treated with wheat bran extract

Treatment groups	TC (mg/dl)	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)	TG (mg/dl)
Group A (intact control)	53.52 \pm 2.04	22.03 \pm 3.68	19.92 \pm 1.47	12.01 \pm 1.74	60.03 \pm 3.71
Group B (hyperlipidemic)	1664.85 \pm 82.39c	175.05 \pm 13.28c	1369.11 \pm 72.51 c	53.19 \pm 3.14c	265.97 \pm 15.69 c
Group C (wheat bran extract)	94.79 \pm 7.16 a, g	27.87 \pm 2.43 d, g	44.73 \pm 2.97a, g	22.19 \pm 1.78 a, f	111.17 \pm 9.01a, g
Group D (statin)	154.65 \pm 15.37b, g	40.85 \pm 2.23 a, g	81.62 \pm 7.47 b, g	32.17 \pm 1.68 b, f	160.85 \pm 13.36 b, f

Values expressed as mean \pm SEM (n=5). Group B to D compared with Group A, where $p \leq 0.05$ =a, $p \leq 0.01$ =b, $p \leq 0.001$ =c, and non-significant=d. Groups C and D compared with Group B, where $P \leq 0.05$ =e, $P \leq 0.01$ =f, $P \leq 0.001$ =g, and non-significant=h. SEM: Standard error of the mean, TC: Total cholesterol, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, VLDL: Very low-density lipoprotein, TGs: Triglycerides

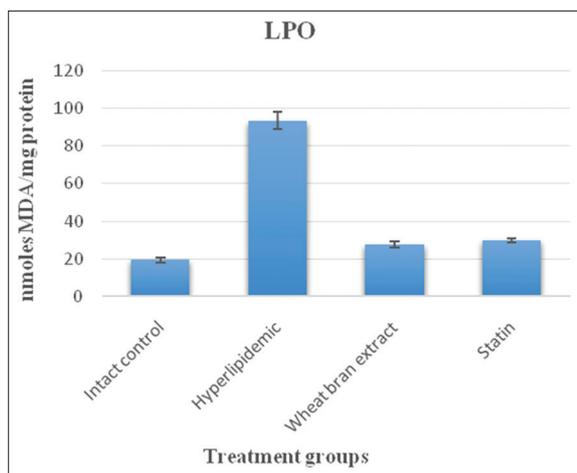


Fig. 1: Effect of *Triticum aestivum* (wheat) bran extract on lipid peroxidation in nanomoles malondialdehyde/mg protein of various treatment groups (mean of 5 values \pm SEM)

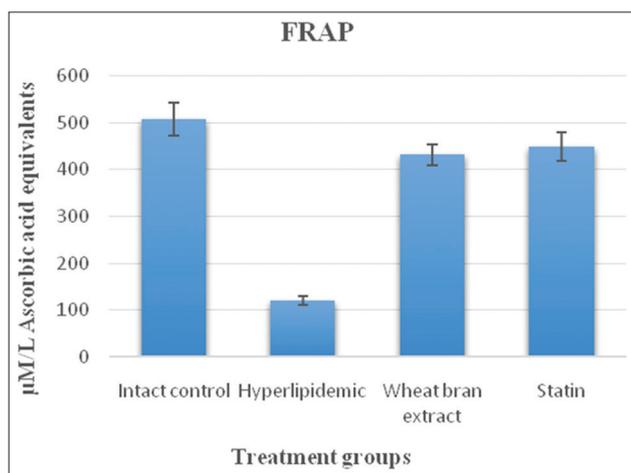


Figure 2: Effect of *Triticum aestivum* (wheat) bran extract on FRAP values in $\mu\text{M/L}$ ascorbic acid equivalents of various treatment groups (Mean of 5 values \pm SEM)

et al. who showed *in vitro* and *in vivo* HMG-CoA reductase inhibition by *Cassia fistula* methanolic and ethyl acetate extracts on high-fat-induced hypercholesterolemic rats [22]. Importantly, it was demonstrated that the *T. aestivum* bran extract reduced plaque formation, cholesterol, and triglyceride levels in rabbits at degrees comparable to that of atorvastatin. The atheromatous changes observed in the aorta of hyperlipidemic and wheat bran-treated rabbits were similar to that of the observations made by Purohit and Ram who noticed regression in atheroma by *Prosopis cineraria* bark extract treatment in albino rabbits [23]. The inhibition of atherosclerotic plaque formation might be mediated by the improvement of antioxidant status, lipid metabolism, and anti-inflammation response [24].

Carlson *et al.* suggested that increasing dietary fiber in the diet was associated with a lesser risk of metabolic syndrome [25]. Wheat bran is a by-product of flour milling and is composed of the pericarp and the outermost tissues of the seed which include the aleurone layer. It has an effect on fecal bulking, delays gastric emptying, and accelerates small bowel transit [26,27]. The physiological effects of wheat bran can be split into nutritional effects due to the nutrients present in it, mechanical effects mainly on the gastrointestinal tract due to the fiber content and antioxidant effects resulting from the phytonutrients such as phenolic acid and alkylresorcinols present in the bran [28]. The phenolic antioxidants present in wheat bran have been shown to inhibit

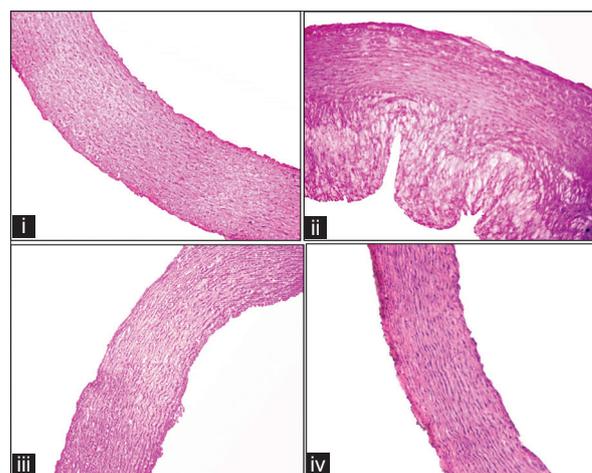


Figure 3: Photomicrographs (100 \times H and E) of thoracic aorta of treated rabbits - (i) Intact control: Thoracic aorta exhibiting normal histology with aortic wall consisting of tunica adventitia, tunica media, and tunica intima. (ii) Hyperlipidemic: Aortic endothelium with the formation of atherosclerotic plaque which comprises a large number of foam cells and luminal adhesion of mononuclear cells. Intima shows marked increase in thickness with focal hyalinization in media. (iii) Wheat bran extract: Intact endothelium with high degree of restoration and mild proliferation of smooth muscles in tunica media. (iv) Statin: Thoracic aorta with restored histoarchitecture of the aortic wall

LDL oxidation, possibly by binding with apolipoprotein B [29]. Phytic acid, lignans, and phenolic acids like ferulic acid have been frequently reported as the phytochemicals in wheat bran responsible for health benefits. Consumption of wheat bran fiber in diet is also reported to cause a decrease in plasma cholesterol and TG [30]. In the current investigation, chronic cholesterol feeding in rabbits caused a significant increase in LPO as compared to the control group. LPO refers to the oxidative deterioration of polyunsaturated lipids and MDA is the most abundant product of LPO which indicates tissue damage [30]. Our findings are in agreement with the study by Shalan *et al.* [31], which demonstrated that wheat bran extract causes significant lipid-lowering and antioxidative activities in hypercholesterolemic rats.

Phytochemicals present in the herbal extracts are known to possess strong free radical and ROS scavenging activity which protects LDL from oxidation [32]. Higher values of FRAP signify higher antioxidant power because the FRAP value is based on reducing ferric ion, where antioxidants act as reducing agent. Significant improvement was observed in the antioxidant status in terms of FRAP value of the serum of rabbits treated with *T. aestivum* bran extract similar to the observations by Raneva and Shimasaki [33], who studied effect of green tea catechins on atherogenic diet fed mice. Phytosterols are naturally occurring plant sterols that structurally resemble cholesterol. They possess hypolipidemic effect by reducing intestinal cholesterol absorption, thereby enhancing fecal cholesterol excretion and reducing serum LDL cholesterol concentrations [34]. Investigations have shown that intake of fiber also reduces the subsequent ingestion of saturated fat [35]. Wholegrain and bran intakes were associated with reduced CVD-specific mortality in women with diabetes mellitus, suggesting a potential benefit of whole grain intake in reducing mortality and cardiovascular risk in diabetic patients [36]. The antioxidant action of ethanolic extract of wheat bran may be accredited to the presence of bioactive phytochemicals which provide maximum conjugation with reactive free radical species, thus preventing oxidative stress-related ailments like atherosclerosis.

CONCLUSION

The results of the present study conclude that *T. aestivum* (wheat) bran extract mimics statin like action in hyperlipidemic rabbits and its oral

administration could significantly manage serum lipid profile and can be used as a safe and effective hypocholesterolemic and antioxidative agent.

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AUTHORS CONTRIBUTION

The complete research work was suggested and designed by Ashok Purohit. All experimentations were carried out by Rashi Chadha. Authors drafted and approved the final manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interests.

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