Acta Poloniae Pharmaceutica – Drug Research, Vol. 68 No. 2 pp. 291–294, 2011 ISSN 0001-6837
Polish Pharmaceutical Society

HYPOLIPIDEMIC EFFECT OF FRESH *TRITICUM AESTIVUM* (Wheat) GRASS JUICE IN HYPERCHOLESTEROLEMIC RATS

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Abstract: Present study was aimed to elucidate hypolipidemic effect of fresh *Triticum aestivum* (common wheat) grass juice (GJ) in experimentally induced hypercholesterolemia in rats and to investigate its role in cholesterol excretion. Hypercholesterolemia was induced experimentally in rats by including 0.75 g% cholesterol and 1.5 g% bile salts in normal diet for 14 days. Hypercholesterolemic rats were administered fresh *Triticum aestivum* GJ at the dose of 5 mL/kg and 10 mL/kg and the standard drug atorvastatin 0.02% w/v in 2% gum acacia suspension at the dose of 1 mg/kg for 14 days by gavage. Blood samples were collected after 24 h of last administration and used for estimation of lipid profile. Fecal cholesterol levels were estimated using standard methods. Fresh GJ administration at 5 mL/kg and 10 mL/kg resulted in dose dependent significant decline in total cholesterol (TC), triglycerides (TG), low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) levels in hypercholesterolemic rats. Further, in comparison to atorvastatin, GJ administration at the dose of 10 mL/kg resulted in comparable decrease of TC, LDL-C, TG and VLDL-C levels ($p > 0.05$). Fecal cholesterol excretion was significantly ($p < 0.05$) enhanced by *Triticum aestivum* GJ administration. Phytochemical analysis revealed the presence of flavonoids, triterpenoids, anthraquinol, alkaloids, tannins, saponins and sterols in fresh wheat grass juice. The results of present study revealed hypolipidemic effect of *Triticum aestivum* GJ in hypercholesterolemic rats by increasing fecal cholesterol excretion. Fresh GJ could have potentially beneficial effect in atherosclerosis associated with hyperlipidemia.

Keywords: Triticum aestivum, grass, hypercholesterolemia, atorvastatin

Cardiovascular diseases are the most common cause of death worldwide. Abnormalities in plasma lipoprotein and derangement in lipid metabolism rank as the most firmly established and best understood risk factor for atherosclerosis and cardiovascular complications (1)**.** Approximately 10% of the global population is affected by dyslipidemia (2). Therapeutic approaches for prevention of atherosclerosis are largely based on the use of statins, which inhibit the rate limitng enzyme of cholesterol biosynthesis. Currently inhibition of intestinal cholesterol absorption by interfering with the sterol transporting system is reported as novel mechanism for lowering of serum cholesterol (3). This mechanism is complimentary to that of statins, which decrease the endogenous synthesis of cholestrerol. Statins are found less beneficial in persons where LDL receptors are low as in homozygous hypercholesterolemia (4). Myopathy is the most troublesome adverse effect of statins (5). Importance of natural products in modern medicine is increased recently. Natural products are important source of new drugs and lead compounds, suitable for further modification during drug development (6). *Triticum aestivum* L. (family: Poaceae) grass commonly known as wheat grass, is the freshly sprouted shoot of grain wheat that has been used as herbal medicine in present and past cultures and is highly valued for its therapeutic and nutritional properties but lacking scientific validation. Lipid lowering effect of fresh *Triticum aestivum* grass juice (GJ) in normal rats is reported (7). The present study is aimed to assess hypolipidemic potential of fresh *Triticum aestivum* grass juice in hypercholesterolemic rats. Effect of grass juice on fecal cholesterol excretion was evaluated to investigate mechanism of hypolipidemic activity.

MATERIALS AND METHODS

Growing of the grass

The grass of *Triticum aestivum* used in this study was grown indoors until required for experiments. Earthen pot was filled with 2.5 inches of

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growing medium composed of 3 parts of soil and one part of compost. Overnight soaked *Triticum aestivum* seeds were then evenly spread over it and further covered with 0.5 inch of soil. Small quantities of water were sprinkled evenly over soil and 3–4 h indirect sunlight was allowed daily for growth of grass. On the tenth day, when grass is about 6 inches tall, it is cut 0.5 inch above the surface of soil. To harvest continuous supply of fresh grass, pots were similarly planted at one-day interval (7).

Preparation of fresh grass juice

Twenty grams of above harvested fresh grass was grounded in a laboratory mortar and the juice was squeezed out through four layers of wet muslin cloth. The residue was twice resuspended in 3 mL of sterile water and similarly squeezed. The filtrate was made up to 20 mL (w/v) final volume with sterile water and administered as GJ. Each day the fresh juice was prepared prior to administration (7).

Animals

Randomly bred six to eight weeks old Wistar rats of both sexes, weighing 150–200 g, raised in the animal house of the Department of Pharmacology, Gajara Raja Medical College, Gwalior, (M.P.), India, were used for the study. These were maintained at 24 ± 2°C with 12 h light and dark cycle and kept on standard pellet diet (Pranav Agro Industries Delhi, India) and water *ad libitum*. The care and maintenance of animals was according to the approved guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPSCEA) in India. The Institutional Animal Ethics Committee approved the protocol.

Induction of hypercholesterolemia

Hypercholesterolemia was induced experimentally in rats by including 0.75 g% cholesterol and 1.5 g% bile acid in normal diet for 14 days (8).

Protocol for antihyperlipidemic activity

The experimental animals were divided into five groups of six animals in each group and received following treatments for 14 days by gavage.

The first group (NDB), served as baseline parameter and was treated with normal diet. The second group (HCG) served as hypercholesterolemic control and was administered with high cholesterol diet. The third, fourth and fifth group (HTA5, HTA10 and HSG) animals were given grass juice at the dose of 5 mL/kg, 10 mL/kg and standard hypolipidemic drug atorvastatin 0.02% w/v in 2% gum acacia suspension at a dose of 1 mg/kg, respectively, along with high cholesterol diet. All the above treatments were carried out each day in the morning under similar constant conditions, as far as possible.

Lipid profile measurements

After 24 h of the last dose administration the animals were anesthetized with diethyl ether and blood samples were collected by orbital puncture. These were allowed to clot and then were centrifuged at 3000 rpm/10 min and serum was used for estimation of total cholesterol (TG) by CHOD Pap, triglycerides (TG) by GPO Pap and high density lipoprotein-cholesterol (HDL-C) by direct method using commercial enzymatic kits (Randox, UK) and Photometer model BTR-830 (Biotech, Spain). Low density lipoprotein-cholesterol (LDL-C) and very low density lipoprotein-cholesterol (VLDL-C) were calculated using Friedewald's formula (9).

Fecal cholesterol excretion

Fecal matter was collected during last 3 days of the treatment period. The dried and powdered fecal matter was extracted with chloroform : methanol (2:1, v/v) mixture. The resultant extract was then analyzed for cholesterol content in a manner similar to that of serum. Cholesterol excreted in fecal matter (mg/g) was calculated (10).

Phytochemical screening

Fresh *Triticum aestivum* GJ was subjected to phytochemical tests for presence of bioactive compounds by standard methods as described by Harborne (11).

Statistical analysis

Statistical evaluation was done using one-way ANOVA followed by Student-Newman-Keul's multiple comparison tests. Differences with $p < 0.05$ were considered significant. Data are presented as the mean ± SD. All statistical analyses were performed by Sigma Stat software version 2.0, Jandel Scientific Inc. USA.

RESULTS

Supplementation of high cholesterol diet in rats (HSG) for 14 days resulted in significant ($p < 0.05$) increase in TC and LDL-C levels as compared to the rats treated with normal diet (NDC). TC and LDL-C levels were increased by 202% and 333%, respectively (Table 1). The levels of TC, TG, LDL-C and VLDL-C were decreased by 50, 22, 56 and 22 per-

Table 1. Effect of fresh grass juice of *Triticum aestivum* and atorvastatin on serum lipid profile of hypercholesterolemic rats.

Group	Total cholesterol	Triglycerides	High density lipoprotein-cholesterol	Low density lipoprotein-cholesterol	Very low density lipoprotein-cholesterol
NDB	69.25 ± 3.62	58.31 ± 4.80	14.36 ± 1.11	43.14 ± 3.63	11.66 ± 0.96
HCG	209.83 ± 14.66[a]	63.63 ± 11.93	10.21 ± 1.57	186.89 ± 17.56	12.72 ± 2.38
HTA5	104.21 ± 5.31[ab]	49.76 ± 4.89[a]	11.45 ± 1.04	82.81 ± 3.44[a]	9.95 ± 0.97[a]
HTA10	76.18 ± 4.87[ab]	39.50 ± 3.91[a]	11.75 ± 0.79	58.56 ± 4.10[ab]	7.89 ± 0.78[a]
HSG	72.26 ± 3.94[ab]	38.29 ± 7.86[a]	11.90 ± 0.85	52.74 ± 3.84[ab]	7.65 ±1.57[a]

Values are the mean ± SD, n = 6 in each group. The lipid profiles are given in mg/dL * $p < 0.05$ (Student-Newmen-Keul's multiple comparison test), [a] significant from control group (HCG), [b] significant from (HTA5) group; NDB = rats treated with normal diet, HSG = rats treated with high cholesterol diet. HTA5 = rats treated with high cholesterol diet and *Triticum aestivum* grass juice at the dose of 5 mL/kg. HTA10 = rats treated with high cholesterol diet and *Triticum aestivum* grass juice at the dose of 10 mL/kg. HSG = rats treated with high cholesterol diet and atorvastatin at the dose of 1 mg/kg

cent in rats treated with grass juice at the dose of 5 mL/kg (HTA5) and by 60, 38, 69 and 38 percent in rats treated with grass juice at the dose of 10 mL/kg (HTA10), respectively, in comparison with HCG . The decrease in TC, TG, LDL-C and VLDL-C levels were dose dependent and significant ($p < 0.05$). HDL-cholesterols shown an increase by 12 and 15 percent in HTA5 and HTA10, respectively, and were not significant ($p > 0.05$). Administration of standard drug, atorvastatin, resulted in a decrease of TC, TG, LDL-C, and VLDL-C by 66, 40, 72 and 40%, respectively, in HSG group and was significant ($p < 0.05$). HDL-C levels showed 6% non significant increase ($p > 0.05$).

Triticum aestivum grass juice induced 28 and 50% increase in cholesterol excretion at 5 mL/kg and 10 mL/kg doses, respectively, in fecal matter. The differences in cholesterol excretion were significant ($p < 0.05$) at the two test doses of GJ (Figure 1).

Phytochemical tests revealed the presence of flavonoids, triterpenoids, anthranol, alkaloids, tannins, saponins. and sterols in fresh grass juice.

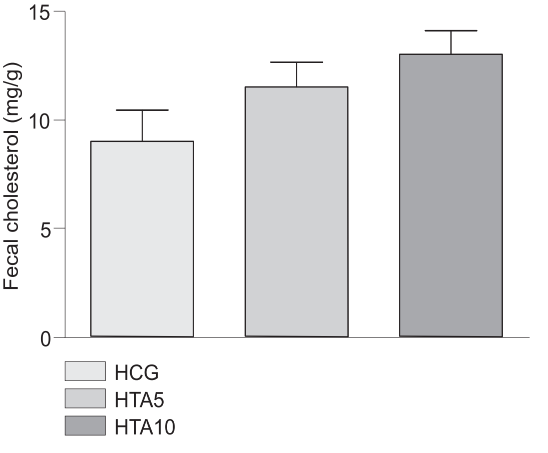

Figure 1. Effect of fresh *Triticum aestivum* grass juice on fecal cholesterol excretion in hypercholesterolemic rats. Each column represents the mean ± SD, n = 6. *$p < 0.05$ as compared with control group. HSG = rats treated with high cholesterol diet. HTA5 = rats treated with high cholesterol diet and *Triticum aestivum* grass juice at the dose of 5 mL/kg . HTA10 = rats treated with high cholesterol diet and *Triticum aestivum* grass juice at the dose of 10 mL/kg

DISCUSSION

High cholesterol diet-induced experimental hypercholesterolemic rat model has been used to study hypolipidemic effects of plant extract (8). The results of present study clearly show that oral fresh grass juice of *Triticum aestivum* has dose dependent significant hypolipidemic activity on diet-induced raised levels of TC, TG, LDL-C and VLDL-C as compared to control. Lipid levels at the dose of 10 mL /kg were comparable with that of standard drug – atorvastatin. These changes in lipid levels after grass juice treatment may be attributed to bioactive compounds that were demonstrated after phytochemical screening of fresh *Triticum aestivum* GJ. Bioactive plant compounds – flavonoids and triterpenoids, are reported to modulate lipid levels (12, 13). The presence of flavonoids and triterpenoids in *Triticum aestivum* grass juice might have contributed in lipid lowering effect of *Triticum aestivum* grass juice in similar manner. Tannins are reported to increase in activity of the endothelium bound lipoprotein lipase activity, which hydrolyzes triglycerides as reported by Tebib et al. (14). The presence of tannins in *Triticum aestivum* grass juice might be involved in triglyceride lowering activity but this need to be investigated by further studies. Cholesterol absorption inhibitors are novel class of drugs reducing cholesterol levels and ezetimibe is the first in this class (15). Cholesterol in the intestine can arise both from the diet and hepatic secretions. Further, inhibition of cholesterol absorption from intestine also decreases the delivery of cholesterol to the liver, thereby lowering serum as well as hepatic cholesterol. This, in turn, accelerates the uptake of LDL from plasma *via* LDL receptors and an increase in the clearance of plasma cholesterol (16). Plant sterols are also reported to decrease cholesterol absorption but through a different mechanism. Phytosterols compete with dietary and billiary cholesterol for incorporation into mixed miscelles in the intestinal lumen thus inhibiting their uptake (17). To study the effect of fresh *Triticum aestivum* GJ on intestinal absorption of cholesterol, fecal cholesterol levels were measured. A dose dependent significant increase in cholesterol content of fecal matter of hypercholesterolemic rats after *Triticum aestivum* GJ administration indicates interference in absorption of intestinal cholesterol. It is suggested that sterols found in grass juice might have decreased cholesterol absorption and increased cholesterol excretion thereby contributed in hypolipidemic activity of *Triticum aestivum*. Saponins are another highly active plant compounds reported to increase fecal cholesterol excretion (18). It is tempting to suggest that besides sterols, the presence of saponins in *Triticum aestivum* might have contributed in increasing fecal cholesterol excretion. The effect of fresh GJ on cholesterol synthesis is not investigated in the present study and the results found are encouraging to explore it in further studies.

Thus, it is concluded that fresh *Triticum aestivum* grass juice possess hypolipidemic activity in hypercholesterolemic rats and it increases cholesterol excretion. It might be useful with statins for the management of dyslipidemia. This is the first study which investigated the hypolipidemic activity of *Triticum aestivum* grass juice in hypercholesterolemic rats and its effect on cholesterol excretion. The results found are encouraging for further assessment to elucidate other mechanisms of hypolipidemic activity.

Acknowledgments

The authors wish to express their gratitude to Dr.R.Vijayraghvan Director and Mr. Dev Kumar technical officer Defence, Research and Development Establishment, Ministry of Defence, Government of India, Gwalior, for their unstinted help. We are also thankful to Dr. (Mrs.) Shaila Sapre, Dean, Gajara Raja Medical College and J.A. Group of Hospitals, Gwalior (M.P.), India for financial support.

REFERENCES

1. Singh R.B., Mengi S.A., Xu Y.J., Arneja A.S., Dhalla N.S.: Exp. Clin. Cardiol. 26, 1 (2002).
2. Shilpha S., Bairy K.: Indian J. Physiol. Pharmacol. 52, 319 (2008).
3. Davis H.R.: International Congress series 1262, 243 (2004).
4. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th edn., Hardman J.G., Limbird L.E. Eds., The McGraw Hill Co. Inc., New York 2001.
5. Chapman J.M., Carrie A.: Arterioscler. Thromb. Vasc. Biol. 25, 2441(2005).
6. Chin Y.W., Balunas M.J., Chai H.B., Kinghonn A.D.: JAAPA. 8, E239 (2006).
7. Kothari S., Jain A.K., Mehta S.C., Tonpay S.D.: Indian J. Pharmacol. 40, 235 (2008).
8. Visavadiya N.P., Narsinhacharya A.V.R.L.: Indian J. Pharmacol. 37, 376 (2005).
9. Friedewald W.T., Levy R.I., Fredrickson D.S.: Clin. Chem. 18, 499 (1972).
10. Souza T.D., Mengi S.A., Hassarajani S., Chattopadhyay S.: Indian J. Pharmacol. 39,196 (2007).
11. Harborne J.B.: Phytochemical methods: A guide to modern techniques of plant analysis. 2nd edn., Chapmann and Hall, London 1983.
12. Koshi A.S., Anita L., Vijayalakshmi N. R.: Food Chem. 72, 289 (2001).
13. Zhang Q., Chang Z., Yang J., Wang Q.: Phytother. Res. 22, 1040 (2008).
14. Tebib K., Besancon P., Rounanet J.M.: J. Nutr. 124, 2451 (1994).
15. Sudhop T., Lütiohann D., Kodal A., Igel M., Tribble D.L., Shah S., Perevoskaya I., von Bergmann K.: Circulation 106, 1943 (2002).
16. Patel S.B.: Curr. Cardiol. Rep. 6, 439 (2004).
17. Brufau G., Canela M.A., Refecas M.: Nutr. Res. 28, 217 (2008).
18. Matsui Y., Kumagai H., Masuda H.: Nihon Shokuhin Kagaku Kōgakkai (Food Sci. Technol. Res.) 12, 50 (2006).

Received: 23. 03. 2010