

EFFECTS OF WHEATGRASS (*TRITICUM AESTIVUM*) JUICE ON SERUM CHOLESTEROL OF EXPERIMENTALLY INDUCED HYPERCHOLESTEROLAEMIC MALE LONG EVANS RAT

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ABSTRACT

An experimental study was designed to observe the hypocholesterolaemic effect of wheatgrass (*Triticum aestivum*) juice on experimentally induced hypercholesterolaemic rats, which was carried out in the Department of Pharmacology & Therapeutics, Dhaka Medical College, Dhaka, from July, 2009 to June, 2010. A total number of 48 healthy adult male Long Evans rats (Norwegian strain), age ranging from 2-3 months, and weighing from 140-200 grams were used for the present study. 6 rats were taken in each group of total 8 groups treated differently with 1 control group. Rats of the first 5 groups were sacrificed at 29th day and their serum total cholesterol levels were measured. Rest of the rats were sacrificed at 57th day and their serum total cholesterol levels were measured. The mean serum concentration of total cholesterol of 10ml/kg grass juice fed normal rats was decreased by 11.64% than that of the laboratory diet fed control group ($P < 0.01$). The total cholesterol of the group of rats treated with 10ml/kg grass juice and 1% cholesterol diet were decreased by 36.59% than that of the hypercholesterolaemic control group ($P < 0.001$). The mean total cholesterol of the hypercholesterolaemic rats treated with 10ml/kg grass juice for 28 days were decreased by 29.10%, and in hypercholesterolaemic rats treated with 20ml/kg grass juice for 28 days were decreased by 39.46% ($P < 0.001$). The mean total cholesterol of the hypercholesterolaemic rats treated with 0.14mg/kg atorvastatin for 28 days were found to be decreased by 38.86% than that of hypercholesterolemic control group, which was statistically significant ($P < 0.001$).

Keywords: Wheatgrass juice, Hypercholesterolaemia, Serum cholesterol, Long Evans rat.

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INTRODUCTION

The importance of hypercholesterolaemia as a risk factor of coronary heart disease had been recognized during the late 1950s.¹ The aim of treating hyperlipidaemia is to prevent or reduce the risk and complication of cardiovascular disease.^{2,3} Lipid lowering therapy reduces the risk of coronary heart disease (CHD) in high risk individual.³

The use of herbal supplements has increased dramatically over the past 30 years.⁴ Herbalism has a long tradition of use outside of conventional medicine. People all over the world used hundreds to thousands of indigenous plants for treatment of ailments since prehistoric times.⁴ Herbal medicine -- also called botanical medicine or phytomedicine -- refers to using a plant's seeds, berries, roots, leaves, bark, or flowers for medicinal purposes. Recently, the World Health Organization estimated that 80% of people worldwide rely on herbal medicines for some part of their primary health care.⁴

Fresh wheatgrass (*Triticum aestivum* Linn.) juice had shown lipid lowering effect on normal rats.⁵⁻⁷ It is used as a health improving adjuvant in several diseases including coronary artery disease in India as folk medicine.⁵ In the molecular level, chlorophyll is very similar to haemoglobin, which allows wheat grass to move quickly into the blood system through the liver to distribute its positive effects.⁸ However, to the best of our knowledge, experimental study on the lipid lowering effect of wheatgrass juice in hyperlipidaemic condition has not been done in our country previously. Hence, the current study was designed to observe the hypolipidaemic effect of wheatgrass (*T. aestivum*) juice on experimentally induced hypercholesterolaemic rats as a continuation of search for newer, cheaper and less toxic natural herb for the treatment of hyperlipidaemia.

MATERIALS AND METHODS

1. Animals: The experiments was carried out upon Long Evans Rats (*Rattus rattus*) (Norwegian strain), which were obtained from Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. A total number of 48 adult male rats, aged 2-3 months and weighing

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about 140-200 grams were included in this study. They were kept in the animal house of the Department of Pharmacology & Therapeutics, Dhaka Medical College, Dhaka. The rats were kept in cages, acclimatized at 26-29°C and humidity, with a 12 hour light and dark cycle. They were allowed free access to standard laboratory diet and water *ad libitum*. Animals were randomly distributed into eight (8) groups having six (6) rats in each group.

2. Drugs and chemicals:

- a) Fresh common wheat grass (*Triticum aestivum*) juice: The wheatgrass (*T. aestivum*) used in this study was grown in the roof top garden. When grass was about 6 inches tall, it was cut ½ inch above the surface of soil. Twenty grams of harvested fresh grass was grounded by grinder with 10ml of sterile water and the juice was squeezed out through four layers of wet muslin cloth. The filtrate was made to 20 ml final volume with sterile water and administered as grass juice^{9,10}. Each day the fresh juice was prepared prior to administration.
- b) Distilled water;
- c) Standard laboratory diet;
- d) Fatty mixture: 1% cholesterol diet was prepared by dissolving 1gm of cholesterol in 100ml of olive oil, according to Jesmin⁹.

3. Kits for the estimation of serum cholesterol: Serum cholesterol was estimated by cholesterol liquicolor CHOD-PAP-Method (enzymatic colorimetric test with lipid clearing factor).

The experiment was carried out in the Department of Pharmacology & Therapeutics, Dhaka Medical

College, Dhaka, from July, 2009 to June, 2010. As described earlier, a total number of 48 healthy adult male Long Evans rats were used for the present study.

Experiment design:

Group A: This control group consists of six rats received standard laboratory diet and distilled water for 28 days.

Group B: This group of rats was fed standard laboratory diet, distilled water and 10ml/kg 1% cholesterol diet through feeding tube for 28 days, according to Amin¹⁰.

Group C: This group was fed standard laboratory diet and 10ml/kg grass juice.

Group D: This hypercholesterolaemic control group received standard laboratory diet and 10ml/kg 1% cholesterol diet for 28 days.

Group E: This group had received 1% Cholesterol diet along with 10ml/kg fresh wheat grass juice to observe the preventive effect of grass juice.

Group F: This group received 10ml/kg 1% cholesterol diet for 28 days followed by 10ml/kg fresh grass juice for next 28 days.

Group G: This group was fed 10ml/kg 1% cholesterol diet for 28 days followed by 20ml/kg fresh grass juice for next 28 days.

Group H: This group received 10ml/kg 1% cholesterol diet for 28 days followed by 0.14 mg/kg atorvastatin (a lipid lowering agent) for next 28 days, according to Jesmin⁹.

Experiment I

This part of the experiment was carried out on first three groups of rats to observe the effect of 1% cholesterol diet and wheatgrass juice on serum cholesterol of normal rat.

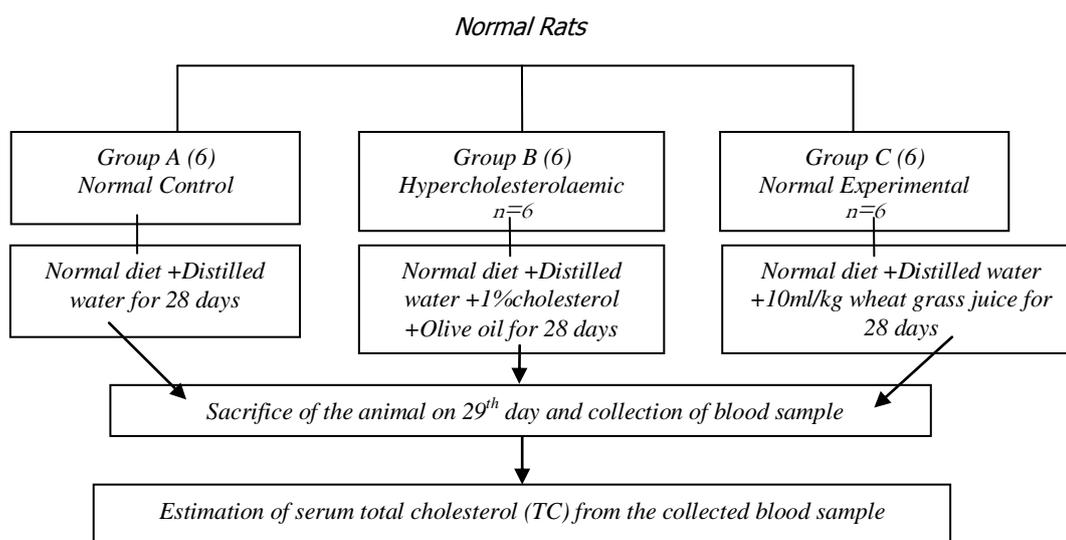


Fig. 1: Flow Chart of Experiment-I

Experiment II

The rats of the experiment-II were fed 1% cholesterol diet 10ml/kg or 1ml/100gm of body weight for 28 days in order to elevate plasma cholesterol level, according to Amin¹⁰.

Hypercholesterolaemic groups

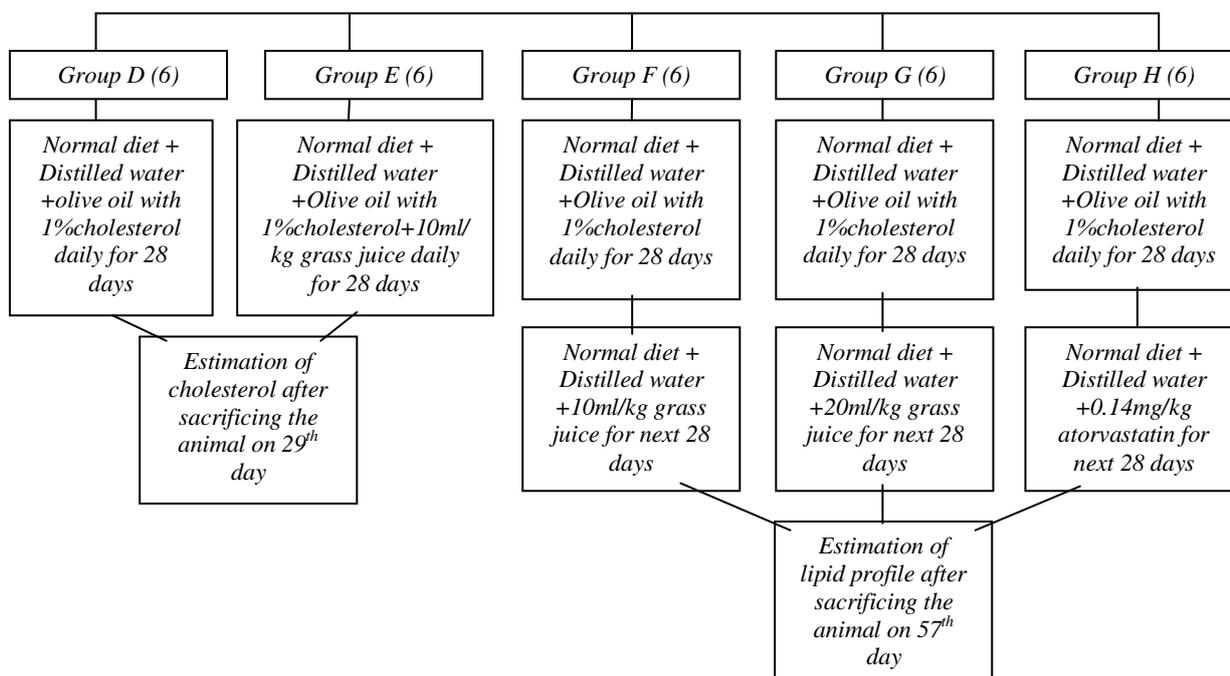


Fig. 2: Flow Chart of Experiment-II

Each rat was fasted for 18 hours before sacrifice and collecting blood sample. They were given only water *ad libitum* during the fasting period. All animals were sacrificed under ketamine anaesthesia and blood samples were taken directly from the heart. Samples were collected in the test tubes. These were kept slanting position till blood had clotted. Serum was separated from the clot after centrifugation in the centrifuge machine. Serum was collected in small test tubes and kept at 0°C. Then the serum was analyzed by the lipid profile kit.

Ethical Clearance: The present study was approved by the Ethical Review Committee of Dhaka Medical College, Dhaka.

RESULTS

Experiment-I:

Hyperlipidaemia was induced in rats by administration of 10ml/kg body weight olive oil with 1% cholesterol through feeding tube for 28 days. Hyperlipidaemia was evident with a significant increase in serum TC, LDL and TG levels ($P<0.001$) and decreased serum HDL level ($P<0.01$).

Effect of 1% cholesterol diet on lipid profile of rats: The mean concentration of serum total cholesterol was found to be increased by 58.27% in rats treated with 1% cholesterol diet compared to the rats fed normal diet, which was statistically highly significant ($P<0.001$) (Table-I, Fig. 3).

Effect of wheat grass juice on the lipid profile of normal rats: The mean serum concentration of total cholesterol of 10ml/kg grass juice fed normal rats was decreased

by 11.64% than that of the laboratory diet fed control group. The result was statistically significant ($P<0.01$) (Table-I, Fig. 3).

Experiment-II:

Effect of grass juice (10ml/kg) when taken along with 1% cholesterol diet: The mean concentration of serum total cholesterol, LDL-C and triglyceride of the group of rats treated with 10ml/kg grass juice and 1% cholesterol diet were decreased by 36.59% than that of the hypercholesterolaemic control group, which was statistically significant ($P<0.001$) (Table-II, Fig. 4).

Effect of grass juice (10ml/kg) on hypercholesterolaemic rats: The mean serum concentration of total cholesterol of the hypercholesterolaemic rats treated with 10ml/kg grass juice for 28 days were decreased by 29.10%, which was statistically highly significant ($P<0.001$) (Table-II, Fig. 4).

Effect of grass juice (20ml/kg) on hypercholesterolaemic rats: The mean serum concentration of total cholesterol of the hypercholesterolaemic rats treated with 20ml/kg grass juice for 28 days were decreased by 39.46%, which was statistically highly significant ($P < 0.001$) (Table-II, Fig. 4).

Effect of atorvastatin (0.14mg/kg) on hypercholesterolaemic rats: The mean serum concentration of total cholesterol of the hypercholesterolaemic rats treated with 0.14mg/kg Atorvastatin for 28 days were found to be decreased by 38.86% than that of hypercholesterolemic control group, which was statistically highly significant ($P < 0.001$) (Table-II, Fig. 4).

Comparison of the effects of two different doses (10ml/kg and 20ml/kg) of grass juice: The serum total

cholesterol of the hypercholesterolaemic rat group treated with 20ml/kg grass juice for 28 days was found to decrease more by 14.54% than that of the hypercholesterolemic rat group treated with 10ml/kg grass juice for the same period, which was statistically highly significant ($P < 0.001$) (Table-III, Fig. 4).

Comparison of the effects of the wheat grass juice (20ml/kg) to that of with atorvastatin (0.14mg/kg): In the present study, the reduction of serum total cholesterol with the dose level of 20ml/kg of grass juice (39.46%) was found almost similar to that of atorvastatin (38.86%). The result was not statistically significant (Table-IV, Fig. 4).

Table-I
Total cholesterol levels in different group of rats in Experiment-I

Group	N = 6	TC (mg/dl) (Mean±SD)	P value (Mean)	Percent change
A		61.80±3.45		
B		97.48±2.97	B vs A 0.0001***	+58.27
C		54.47±1.58	C vs A 0.001**	-11.64

Comparison of total cholesterol levels between groups were done by unpaired Student's 't' test. ** = Significant at $P < 0.01$, *** = Significant at $P < 0.001$.

- Group A : Control
- Group B : Hypercholesterolaemic
- Group C : Laboratory diet plus grass juice

Table-II
Total cholesterol levels in different group of rats in Experiment-II

Group	N = 6	Total Cholesterol (mg/dl) (Mean±SD)	P value (Mean)	Percent change
D		97.48±2.97		
E		61.78±2.83	E vs D 0.0001***	-36.59
F		69.06±2.03	F vs D 0.0001***	-29.10
G		58.93±3.39	G vs D 0.0001***	-39.46
H		59.52±2.29	H vs D 0.0001***	-38.86

Comparison of total cholesterol levels between groups were done by unpaired Student's 't' test. *** = Significant at $P < 0.001$.

- Group D : Hypercholesterolaemic
- Group E : 1% cholesterol diet plus grass juice
- Group F : 1%Cholesterol followed by 10 ml/kg grass juice
- Group G : 1%Cholesterol followed by 20 ml/kg grass juice
- Group H : 1%Cholesterol followed by atorvastatin

Table-III

Total cholesterol levels in the groups of rats taken two different doses (10 ml/kg and 20 ml/kg) of grass juice in Experiment-II

Group	n	TC (mg/dl) (Mean±SD)	P value	Percent change (Mean)
F	6	69.06±2.03		
G	6	58.93±3.39	0.0001***	-14.54

Comparison of total cholesterol levels between group G and group F was done by unpaired Student's 't' test. *** = Significant at P<0.001

Group F : 1%Cholesterol followed by 10 ml/kg grass juice

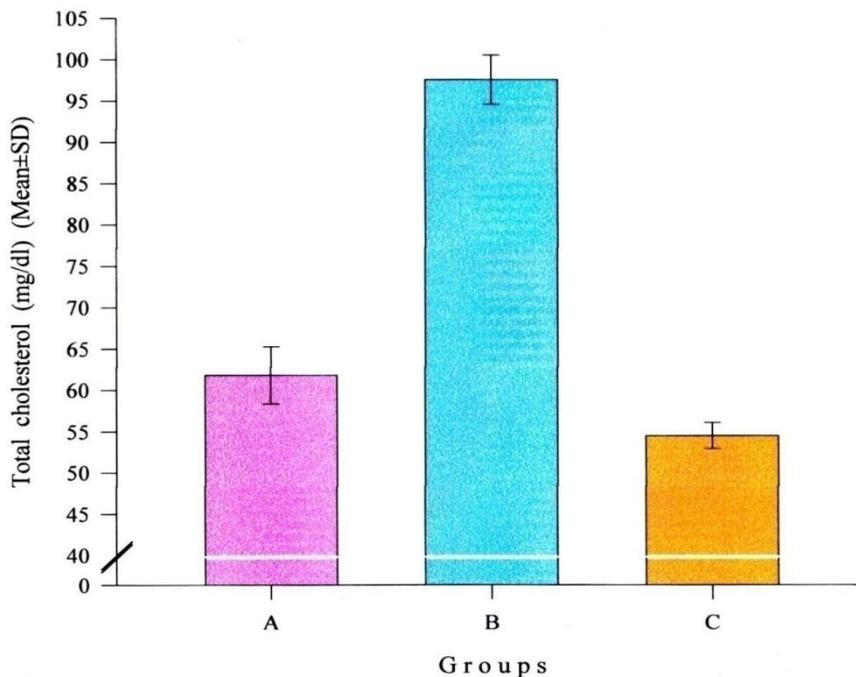
Group G : 1%Cholesterol followed by 20 ml/kg grass juice

Table-IV

Total cholesterol levels in the groups of rats taken higher dose (20 ml/kg) of grass juice to that of Atorvastatin in Experiment-II

Group	n	TC (mg/dl) (Mean±SD)	P value	Percent change (Mean)
G	6	58.93±3.39		
H	6	59.52±2.23	0.742 ^{ns}	+1.25

Comparison of total cholesterol levels between group H and group G was done by unpaired Student's 't' test. ns = Not significant.



Group G : 1%Cholesterol followed by 20 ml/kg grass juice

Group H : 1%Cholesterol followed by atorvastatin

Fig. 3. Serum total cholesterol levels in different group of rats in Experiment-I.

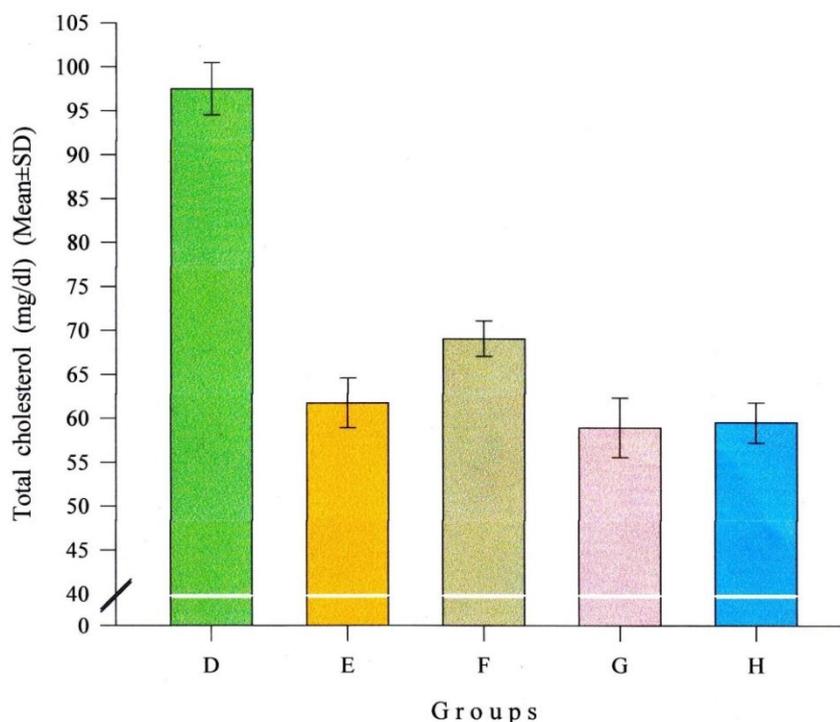


Fig. 4. Serum total cholesterol levels in different group of rats in Experiment-II.

DISCUSSION

In the present study, hypolipidaemic effect of wheat grass juice was tested on adult Long Evans rats. Hyperlipidaemia was induced in rats by administration of 10ml/kg body weight olive oil with 1% cholesterol through feeding tube for 28 days. Hyperlipidaemia was evident with a significant increase ($P < 0.001$) in serum TCL, LDL and TG levels. Besides, serum HDL level significantly decreased ($P < 0.01$). Jesmin⁹, Amin¹⁰, Akter¹¹, Lata et al.¹² and Anita & Okokon¹³ achieved similar effect on lipid levels by administering similar hyperlipidaemic diet. The results of their studies are more or less in agreement with that of the present study. The group of normal rats that took 10ml/kg grass juice had shown significant reduction in serum total cholesterol. The result was in full agreement with that of Kothari et al.⁵. Administration of grass juice along with cholesterol diet also showed significant reduction of serum total cholesterol ($P < 0.001$) than the hypercholesterolaemic group. It indicates preventive role of grass juice against hypercholesterolaemia. Moreover, the lipid lowering effect of grass juice in the present study was found to be dose dependent. In higher dose level (20ml/kg) of grass juice the lipid lowering effect was much more evident than that with lower dose (10ml/kg). Kothari et al.⁵ also observed such changes in lipid parameters in dose related manner. In the present study, the reduction of serum total cholesterol with the dose level of 20ml/kg of grass juice (39.46%) was found almost similar to that of atorvastatin (38.86%). However, the present study did

not address the mechanisms by which grass juice produce lipid lowering effect. In fact, it is yet to be defined. However, several speculations have been made. The presence of alkaloids, tannins, saponins and sterols, as had been revealed by the preliminary phytochemical analysis may responsible for the lipid lowering effect of the wheat grass juice⁵. It was already recorded that, saponins have lipid lowering effects¹⁴ and tannins have been shown to affect lipid profile favourably through their effects on the lipoproteins, lipoprotein lipases and tissue lipids¹⁵. In addition to this, antioxidant property might also be beneficial in the prevention of atherosclerosis and its sequelae as Kulkarni et al.¹⁶ found that the phenolic and flavonoid contents of wheatgrass inhibit significantly ascorbate- Fe^{++} induced lipid peroxidation in rat liver mitochondria.

CONCLUSION

The present study provides the initial step for demonstrating the lipid lowering effect of fresh wheatgrass (*T. aestivum*) juice in hyperlipidaemic rat. To the best of our knowledge, no study was done on the lipid lowering effect of wheatgrass juice in hyperlipidaemic condition in our country previously. Therefore, the result could not be correlated further with those of others. Further investigations are recommended to reconfirm and identify the hypolipidaemic active principles and elucidate its mechanism of action.

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