

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

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Abstract:

Context: An experimental study was designed to observe the effect of wheatgrass (*Triticum aestivum*) juice on serum triglyceride on experimentally induced hypercholesterolaemic rats.

Materials & Methods: The experiment 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 90-120 days, and weighing from 140-200 grams were used for the 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 triglyceride levels were measured. Rest of the rats were sacrificed at 57th day and their serum triglyceride levels were measured.

Results: The mean serum concentration of total cholesterol of 10ml/kg grass juice fed normal rats was decreased by 26.79% than that of the laboratory diet fed control group. The result was statistically significant ($P<0.001$). The mean concentration of serum triglyceride of the group of rats treated with 10ml/kg grass juice and 1% cholesterol diet were decreased by 38.04% than that of the hypercholesterolaemic control group, which was also statistically significant ($P<0.001$). The mean serum concentration of triglyceride of the hypercholesterolaemic rats treated with 10ml/kg grass juice for 28 days were decreased by 25.73%, and in hypercholesterolaemic rats treated with 20ml/kg grass juice for 28 days were decreased by 34.81%, both of which were statistically highly significant ($P<0.001$). The mean serum concentration of triglyceride of the hypercholesterolaemic rats treated with 0.14mg/kg Atorvastatin for 28 days were found to be decreased by 32.90% than that of hypercholesterolemic control group, which was statistically highly significant ($P<0.001$).

Conclusion: The present study provides the initial step for demonstrating the lipid lowering effects of wheatgrass (*Triticum aestivum*) juice at hyperlipidaemic state. Further studies with high technical back-up are recommended.

Key words: Wheatgrass Juice, Hyperlipidaemia, Serum triglyceride, Long Evans Rat.

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Introduction:

Excess in serum triglyceride acts as a risk factor of atherosclerosis like cholesterol¹. Fresh wheatgrass (*Triticum aestivum* Linn.) juice showed potential lipid lowering effect on normal rats in different experimental settings²⁻⁴. It is used as a health improving adjuvant in several

diseases including coronary artery disease in India as folk medicine². In the Western world, it is used as merely nutritional supplement to curative agent for last 50 years³. In the molecular level, chlorophyll is very similar to haemoglobin. This allows wheatgrass to quickly move into the blood system through the liver to

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distribute its positive effects⁵. However, to the best of our knowledge, 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 lowering effect on excess serum triglyceride by wheatgrass (*T. aestivum*) juice on experimentally induced hypercholesterolaemic rats.

Materials:

1. Animals: The experiments was carried out upon Long Evans Rats (*Rattus rattus*), which were obtained from Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka. A total number of 48 adult male rats, aging 2-3 months and weighing about 140-200 gm. 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 each.

2. Drugs and chemicals:

- a) Fresh common wheat (*Triticum aestivum*) grass juice: The grass of *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. 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 Jesmin (2006)⁶.

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).

Methods:

The experiment 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 90-120 days, and weighing from 140-180 grams, 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 Amin (2009)⁷.

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 Jesmin (2006)⁶.

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 grass juice on serum cholesterol of normal rat.

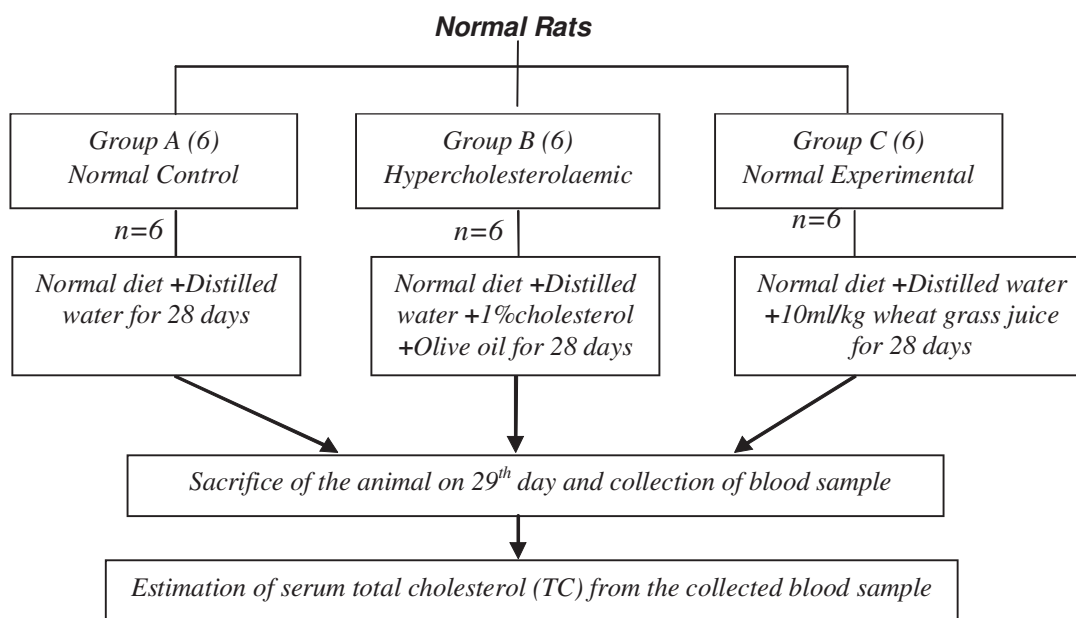


Fig.-1: Flow Chart of Experiment-I

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 Amin (2009)⁷.

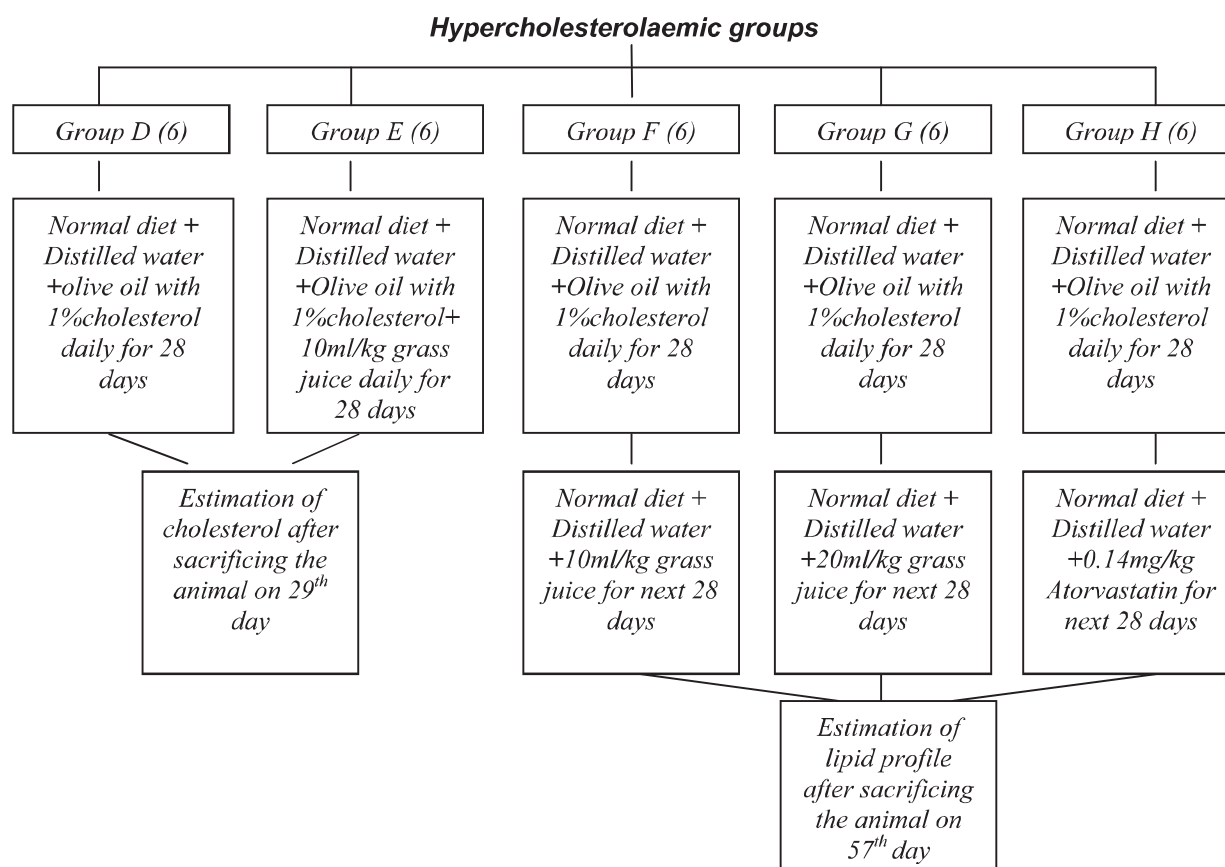


Fig. 2: Flow Chart of Experiment-II

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 ketamin 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:

Effect of 1% cholesterol diet on serum triglyceride of rats: The mean concentration of serum total cholesterol was found to be increased by 49.85% 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 serum triglyceride of normal rats: The mean serum concentration of total cholesterol of 10ml/kg grass juice fed normal rats was decreased by 26.79% than that of the laboratory diet fed control group. The result was statistically significant ($P < 0.001$) (Table-I, Fig. 3).

Experiment-II:

Effect of grass juice (10ml/kg) when taken along with 1% cholesterol diet: The mean concentration of serum triglyceride of the group of rats treated with 10ml/kg grass juice and 1% cholesterol diet were decreased by 38.04% 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 triglyceride of the hypercholesterolaemic rats treated with 10ml/kg grass juice for 28 days were decreased by 25.73%, 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 triglyceride of the hypercholesterolaemic rats treated with 20ml/kg grass juice for 28 days were decreased by 34.81%, 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 triglyceride of the hypercholesterolaemic rats treated with 0.14mg/kg Atorvastatin for 28 days were found to be decreased by 32.90% than that of hypercholesterolemic control group, which was statistically highly significant ($P < 0.001$) (Table-II, Fig. 4).

Table-I
Serum triglyceride levels in different group of rats in Experiment I

Group	n	TG (mg/dl) (Mean±SD)	P value	Percent change (Mean)
A	6	70.65±3.05		
B	6	105.62±3.81	B vs A 0.0001 ^{***}	+49.85
C	6	51.62±2.26	C vs A 0.0001 ^{***}	26.79

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

Group A : Control

Group B : Hypercholesterolaemic

Group C : Laboratory diet plus grass juice

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

The serum triglyceride of the hypercholesterolaemic rat group treated with 20ml/kg grass juice for 28 days was found to decrease more by 11.88% 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 triglyceride with the dose level of 20ml/kg of grass juice (34.81%) was found almost similar to that of Atorvastatin (32.90%). The result statistically was not significant (Table-IV, Fig. 4).

Table-II

Serum triglyceride levels in different group of rats in Experiment II

Group	n	TG (mg/dl) (Mean±SD)	P value	Percent change (Mean)
D	6	105.62±3.81		
E	6	65.37±2.91	E vs D 0.0001***	38.04
F	6	78.33±3.99	F vs D 0.0001***	25.73
G	6	68.82±2.23	G vs D 0.0001***	34.81
H	6	70.72±4.06	H vs D 0.0001***	32.90

Comparison of serum triglyceride 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

Serum triglyceride 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	TG (mg/dl) (Mean±SD)	P value	Percent change (Mean)
F	6	78.33±3.99		
G	6	68.82±2.23	0.0001***	11.88

Comparison of serum triglyceride 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

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

Group	n	TG (mg/dl) (Mean±SD)	P value	Percent change (Mean)
G	6	68.82±2.23		
H	6	70.72±4.06	0.339 ^{ns}	+2.93

Comparison of serum triglyceride 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

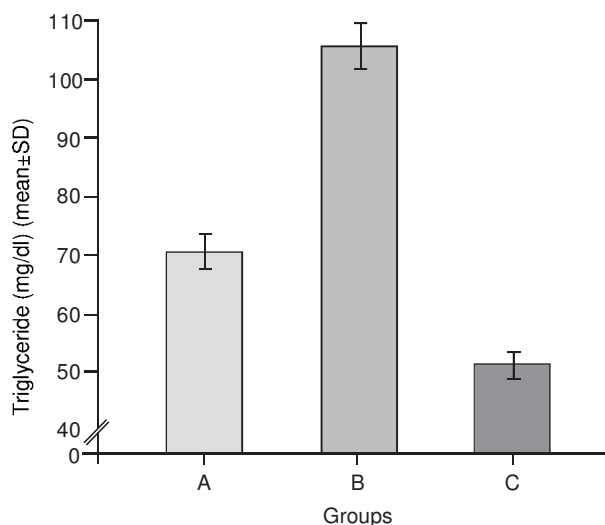


Fig. 3: Serum triglyceride levels in different group of rats in Experiment-I

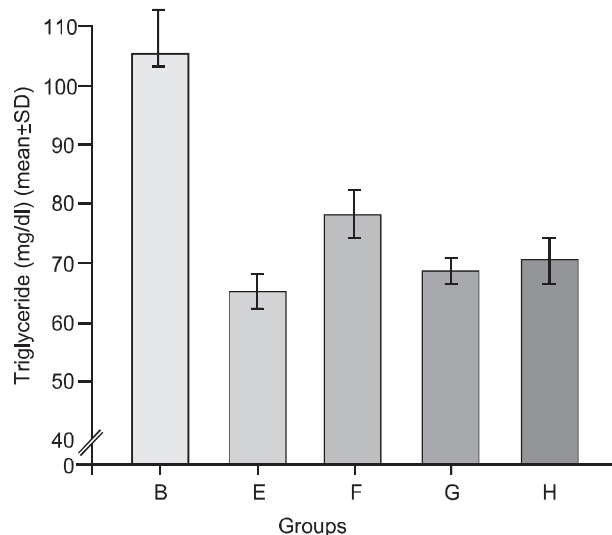


Fig. 4. Serum triglyceride levels in different group of rats in Experiment II.

Discussion:

The present study was carried out to evaluate the effect of fresh juice of *Triticum aestivum* grass on serum triglyceride levels. 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 ($P<0.001$) increase in serum TCL, LDL and TG levels. Besides, serum HDL level significantly ($P<0.01$) decreased. Akter (1991)⁸, Lata et al. (1991)⁹, Anita & Okokon (2005)¹⁰, Jesmin (2006)⁶ and Amin (2009)⁷ 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 10 ml/kg grass juice had shown significant reduction in serum triglyceride. The result was in full agreement with that of Kothari et al. (2008)². Administration of grass juice along with cholesterol diet also showed significant reduction of serum triglyceride ($P<0.001$) than the hypercholesterolaemic group. It indicates preventive role of grass juice against increased triglyceride in blood. Moreover, the lipid lowering effect of grass juice in the present study was found to be dose dependant. 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. (2008)² also observed such changes in lipid parameters in dose related manner. In the present study, the reduction of serum triglyceride with the dose level of 20ml/kg of grass juice (34.81%) was found almost similar to that of Atorvastatin (32.90%). 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 juice².

Conclusion:

The present study provides the initial step for demonstrating the lipid lowering effect of fresh grass juice of *Triticum aestivum* at hyperlipidaemic state. To the best of our knowledge, any study on the lipid lowering effect of wheat grass juice in hyperlipidaemic condition has not been done in our country or in abroad 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 their mechanism of action. Toxicological studies should also be undertaken before any clinical use.

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