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Hypolipidemic Effect of *Triticum aestivum* in Albino Mice *Mus musculus* Fed with Fat Rich Diet

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Abstract: Wheatgrass refers to the young grass of the common wheat plant, *Triticum aestivum* Linn. (family Poaceae). Calorie-dense but high in nutrients, wheatgrass contains antioxidants such as glutathione, beta-carotene (pro-vitamin A), vitamin C, vitamin E, vitamin K, vitamin B, calcium, iron, magnesium, phytonutrients, and chlorophyll. Alanine, aspartic acid, glutamic acid, arginine, and serine are the key essential amino acids found in wheatgrass, which aids in supplying the body with an adequate quantity of protein. A Fat Rich diet (FRD) is a diet consisting of at least 35% of total calories consumed from fats, both unsaturated and saturated. Animal studies have explored the impact of Fat Rich diet in various aspects in Albino mice. Consuming large amounts of fat, particularly saturated and trans fats, can have detrimental health impacts. Coconut oil contain high amount of saturated fat. Vanaspati is a partially hydrogenated vegetable oil. It is clarified butter or ghee which contain trans fatty acid. In the present study FRD was made using a 2:3 ratio of edible coconut oil and vanaspati ghee mixture. In this study albino mice were divided into five groups (A-E; each group having 10 mice). Group A was employed as control. Groups B-E were given 10 ml of FRD/kg b wt/day for 30 days. Groups C, D and E were provided 10, 20 and 50 ml of wheatgrass juice/kg body wt/day, respectively for 30 days in addition to FRD. It was observed that Fat Rich Diet increases the serum cholesterol level. The hypolipidemic effect of *T. aestivum* at different doses were noticed in present study. Higher dose of *T. aestivum* has greater impact on serum cholesterol level. It has been seen that the serum cholesterol level of experimental group mice was lower than that of control group of mice when higher dose of *Triticum aestivum* were administered.

Keywords: Serum Cholesterol, Fat Rich Diet, Vanaspati ghee, Coconut oil, Wheatgrass, *Triticum aestivum*

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Introduction

Wheat, *Triticum aestivum* is one of the world's most consumed cereal grains in addition to its medicinal uses (Moshawih *et al.*, 2022).

Wheatgrass refers to the young grass of the common wheat plant, *Triticum aestivum* Linn., family Poaceae. It is also known as living food

(Rana *et al.*, 2011). It is mostly consumed as fresh juice or in powder form. Wheatgrass is powerhouse of nutrition and it is widely used as a food supplement. Wheatgrass has high proportion of amino acids such as aspartic acid, glutamic acid, arginine, alanine and serine and high content of bioflavonoids such as apigenin, quercetin and luteolin (Devi *et al.*, 2019). Wheatgrass is low in calories but rich in nutrients including antioxidants such as glutathione, vitamin C, vitamin E, vitamins K, vitamins B, calcium, iron, magnesium, phytonutrients and chlorophyll (Kapil, 2012). Wheatgrass has been reported to improve the blood parameters and vital organs after aluminium chloride toxicity (Adnan *et al.*, 2024). Wheatgrass juice improves blood markers, such as the content of blood albumin, globulin, total protein, calcium. It has superoxide scavenging and ferric reducing abilities. The presence of superoxide dismutase (SOD) helps in lowering the effect of radiations and thus digests the toxin (Cao *et al.*, 1996; Bar-Sela *et al.*, 2007). The pH of wheatgrass juice is similar to blood i.e., 7.4. Because of this wheatgrass juice gets quickly absorbed into the blood and helps in detoxification of the body, digestion, improves blood flow etc. (Virdi *et al.*, 2021).

A balanced diet must include fats however, consuming large amounts of fat, particularly saturated and trans fats, can have detrimental health impacts. An animal becomes obese when its energy intake exceeds its energy expenditure, which results in an increase in the energy storage found in body fat, especially in adipose tissues. Animal studies have explored the impact of Fat Rich diet in various aspects in Albino mice. The amount of fat, the composition of the FRD and the duration of the diet regime may cause different responses. Obesity is characterized by an increase in adipocyte size (hypertrophy) and/or number (hyperplasia) (Jéquier, 2002).

Prioritizing a balanced diet with an emphasis on unsaturated fats and understanding the difference between healthy and bad fats are crucial. Excessive consumption of saturated fatty

acids can raise LDL ("bad") cholesterol levels, increasing the risk of heart disease. Coconut oil contain high amount of saturated fat. Trans fats are a type of unsaturated fat. Trans fats are made when liquid oils are turned into solid fats. These are called partially-hydrogenated oils. It comes from either industrial or natural sources. Industrially produced trans fat can be found in Vanaspati ghee. It can increase LDL ("bad") cholesterol and lower HDL ("good") cholesterol levels. WHO's recommendation for adults is to limit consumption of trans fat to less than 1% of total energy intake, which is less than 2.2 g per day for a 2000-calorie diet. A Fat Rich diet (FRD) is a diet consisting of at least 35% of total calories consumed from fats, both unsaturated and saturated. Coconut oil contains maximum amount of saturated fatty acids (90.84%) so that it exhibits high resistance to oxidative reactions (Yousefi *et al.*, 2013). Coconut oil contains ~60% medium-chain fatty acids (carbon, C6:0–C12:0), a large proportion of which is lauric acid (C12:0) (Maki *et al.*, 2018). The portal vein absorbs MCFAs quickly, so that it may be more significant as an energy source through beta-oxidation than that of cholesterol synthesis (Swift *et al.*, 1990). Vanaspati is a partially hydrogenated vegetable oil. It is clarified butter or ghee. The process of hydrogenation involves adding hydrogen to fatty acid unsaturation sites at high pressure and temperature in presence of catalyst. Hydrogenation causes reduction in the unsaturation of oil and increases in the isomerization at double bond takes place. The trans isomers thus formed affect the physical and chemical properties of the final products as they have high melting point and greater stability (Lo *et al.*, 1983). In the present study, hypolipidemic effect of wheatgrass juice was tested on adult albino mice.

Materials and Methods

Collection of plant material and preparation of wheatgrass juice

Triticum aestivum was cultivated in the garden of University Department of Zoology for this experiment. When the grass reached a height of

around 6 inches, it was cut $\frac{1}{2}$ inch above the ground. 40 g of freshly picked grass were ground in a grinder with 20 ml of sterile water, and the juice was extracted using four layers of wet muslin cloth. The filtrate was made to 40 ml final volume with sterile water and administered as grass juice. Each day the fresh extract was prepared prior to administration (Rokhsana *et al.*, 2015).

Method of preparation of fat rich diet

Edible coconut oil and Vanaspati ghee were purchased from the market and mixed in a 2:3 v/v ratio (Shyamala *et al.*, 2003). It was administered at a dose of 10 ml/kg b wt with normal chow diet for 30 days.

Experimental design

This study used albino mice (*Mus musculus*), which were 40-50 days old and had an initial weight of 20-40 g. These animals were housed in polypropylene cages in the animal house of University Department of Zoology, Tilka Majhi Bhagalpur University, Bhagalpur, Bihar, India under controlled lighting and temperature conditions. In this study albino mice were divided into five groups (A-E; each group having 10 mice). Group A was employed as control. Groups B-E were given 10 ml of FRD/kg b wt/day for 30 days. Groups C, D and E were provided 10, 20 and 50 ml of wheatgrass juice/kg body wt/day, respectively for 30 days in addition to FRD. Mice were maintained in accordance with the experimental procedure and ethical considerations provided by University Department of Zoology, Tilka Majhi Bhagalpur University, Bhagalpur, Bihar.

Method of estimation of serum total cholesterol

After 30 days the albino mice from all groups were anesthetized and blood was collected into centrifuge tubes. The blood was centrifuged at 3000 rpm for 15 min and the supernatant (serum) was transferred to another test tube. Estimation of serum total cholesterol was done by Zak (1965) method. Three test tube were taken and marked as Test (T), Standard (S) and Blank (B). In each tube (test tube T, S and B) added 6 ml of glacial

acetic acid. In test tube T added 0.1 ml of serum and in test tube S added 0.1 ml of standard cholesterol. Now 4 ml of ferric chloride reagent (working solution) was added to each test tube i.e. T, S, and B. Then the Optical density was measured at 570 nm by using colorimeter.

Results and Discussion

Serum cholesterol levels in group B (10 ml FRD/kg b wt/day) mice increased significantly. (Table 1). The cholesterol levels were significantly lowered in mice (groups C, D and E) who were administered wheatgrass juice in combination with FRD (as compared to group B receiving only FRD) (Table 1). The presence of phytochemicals such as saponins, tannins, and sterols in wheatgrass juice seem to cause these changes. Such changes are already recorded for these compounds (Lee *et al.*, 2000). At higher dose of wheatgrass juice the effect was much more evident than that with lower dose. Kothari *et al.* (2008) also observed such changes in lipid parameters in dose related manner. According to (Durairaj *et al.*, 2014) the presence of phenolics in wheatgrass treatment may have contributed to the considerable reduction in lipid levels. This may have reduced the expression of important lipid-synthesis-related enzymes. The lipid-lowering properties of *Triticum aestivum* grass juice may have been facilitated by the presence of flavonoids and triterpenoids in a similar way. Tannins are reported to increase the endothelium bound lipoprotein lipase activity, which hydrolyzes triglycerides as reported by Tebib *et al.* (1994). 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. Plant sterols are also reported to decrease cholesterol absorption but through a different mechanism. Phytosterols compete with dietary and biliary cholesterol for incorporation into mixed micelles in the intestinal lumen thus inhibiting their uptake (Brufau *et al.*, 2008). It is suggested that sterols found in grass juice might

Table 1: Serum cholesterol levels in different groups of albino mice after 30 days of treatment

No. of Animals	Group	Dose	Total Cholesterol (mg/dl) Mean $\pm$ SE
10	A	Control	58.863 $\pm$ 3.09
10	B	FRD(10 ml/kg b wt/day)	140.495 $\pm$ 3.15*
10	C	FRD(10 ml/kg b wt/day) and <i>T. aestivum</i> ((10 ml/kg b wt/day).	112.647 $\pm$ 2.17*
10	D	FRD(10 ml/kg b wt/day) and <i>T. aestivum</i> (20 ml/kg b wt/day)	87.439 $\pm$ 1.77*
10	E	FRD(10ml/kg b wt/day) and <i>T. aestivum</i> (50 ml/kg b wt/day).	51.145 $\pm$ 2.70*

The f-ratio value is 382.99821; p-value is < 0.00001; *indicates significant value p <0.05 as compared to control

have decreased cholesterol absorption and increased cholesterol excretion there by contributed in hypolipidemic activity of *Triticum aestivum* (Kothari *et al.*, 2011). It has been found that saponins, another extremely potent plant ingredient, promote the excretion of cholesterol in the feces (Matsui *et al.*, 2006).

Tong *et al.* (2014) reported that wheat bran arabinoxylans reduced the total plasma- and LDL-cholesterol concentrations in Hypercholesterolemic hamsters fed with a high-cholesterol diet containing 0.5% arabinoxylans. By lowering the body's absorption of cholesterol, phytosterols lower cholesterol levels. Since cholesterol and plant sterols are both insoluble in water, they need to be emulsified with bile acids to create micelles that small intestine cells can absorb. Because phytosterols have a chemical structure similar to cholesterol, they compete with cholesterol for a space in micelles, limiting cholesterol absorption by small intestine cells (He *et al.*, 2018). In addition, the transporter protein (NPC1L1) plays an essential role in cholesterol absorption in the gut. NPC1L1 transports cholesterol molecules from the small intestinal mucosal surface to the cells (Borowicki *et al.*,

2010). According to Priyadarshani *et al.* (2013) mice that had been made hyperglycemic had elevated blood serum cholesterol levels. Leaf powder of *Moringa oleifera* was administrated to Diabetic mice. It significantly decreases the serum cholesterol level in Diabetic mice (Priyadarshani *et al.*, 2013).

Conclusion

In the present study, we observed that Fat Rich Diet (which was made up of coconut oil and vanaspati ghee) which contain high amount of saturated fat and trans fat, increase the serum cholesterol in albino mice. *Triticum aestivum* which is highly nutritious decreases the level of serum cholesterol in albino mice. Higher dose of *Triticum aestivum* is more effective as compared to lower dose of *Triticum aestivum*. Higher dose of *Triticum aestivum* has greater impact on serum cholesterol level and it decreases the serum cholesterol level efficiently. It has been seen that the serum cholesterol level of experimental group mice was lower than that of control group of mice when higher dose of *Triticum aestivum* were administrated. The presence of phytochemicals such as saponins, tannins, sterols etc. in wheat-grass juice seem to cause these changes.

Ethical Statement

Mice were maintained in accordance with the guidelines and ethical considerations provided by University Department of Zoology, Tilka Majhi Bhagalpur University, Bhagalpur, Bihar.

Author Contributions

Each author has contributed equally to the study's planning, analysis, writing, and editing. All authors have read and agreed to the published version of the manuscript.

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Conflict of Interest

The authors declare no conflicts of interest.

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