STUDY OF THE ANTIHYPERLIPIDEMIC, ANTIOXIDATIVE AND ANTIATHEROGENIC ACTIVITY OF TRITICUM AESTIVUM LINN. IN RABBIT RECEIVING HIGH FAT DIET

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ABSTRACT
The aim was to study the antihyperlipidemic, antioxidative and antiatherogenic activity of Triticum aestivum Linn. in rabbit receiving high fat diet. Twenty rabbits of either sex were taken and divided into four groups five in each as - Normal Control- received normal diet, Hyperlipidemic control- received high fat diet, Test drug group - received high fat diet plus ethanolic extract of Triticum aestivum 500 mg/Kg /day orally and Standard Drug group- received high fat diet plus Atorvastatin 2.1 mg/Kg/day orally for 12 weeks and then animals were sacrificed. Blood sample were collected and lipid profile, catalase, superoxide dismutase and malondialdehyde levels were measured. The antiatherogenic effect was examined by histopathology of aorta. Data were statistically analysed by one way ANOVA followed by Dunnet’s multiple comparison test. Triticum aestivum significantly decreased serum lipids towards normal levels. It also increases catalase and superoxide dismutase activity and decreases malondialdehyde level and histopathological examination revealed prevention of atherosclerosis. Triticum aestivum has antiatherogenic, antioxidiant and anti atherogenic effects.

Key words: antihyperlipidemic, antioxidative, antiatherogenesis, Triticum aestivum, atorvastatin.

INTRODUCTION
Dys-lipidemia is a major contributor towards many chronic non-infectious diseases like atherosclerosis, diabetes, myocardial infarction, angina, stroke etc. Dys-lipidemia are mainly related to some genetic variations in lipid metabolism or dietary food habits or both which are highly prevalent in Indian sub-continent. Atherogenesis in human typically occurs over a period of many years, usually decades, the main cause being dyslipidemia. Generally after a prolonged “silent” period atherogenesis may become clinically significant. The fatty streak and thickening of intima in blood vessels represent the initial lesion of atherosclerosis. Triticum aestivum or common wheat is used as a health improving adjuvant in several diseases in India as folk medicine. Shoot of Triticum aestivum Linn. (Hindi Name-gehun, kanak, Sanskrit name- godhuma) is called as a wheat grass, belonging to family: Gramineae, which possess high chlorophyll content and essential vitamins(A, C, E, K), minerals (potassium, selenium, Iron, Zinc, boron and molybdenum), vital enzymes (Protease, amylase, lipase, cytochrome oxidase, transhydrogenase, superoxide dismutase), carotene, amino acids, dietary fibers. Phytochemical screening is also indicated the presence of alkaloids, tannins, saponins and sterols. Wheat grass has been shown to possess anti-cancer activity, anti-ulcer activity, antioxidiant activity, anti-arthritic activity, and blood building activity in Thalassemia Major. It has been argued that wheat grass helps blood flow, digestion and general detoxification of the body.

In modern practice, there are many drugs like statins and fibrates which are in use as hypolipidemic agent but the therapy is not cost-effective and as such these drugs do not fulfill the WHO guidelines of essential drugs. So, herbal drugs proved a boon here. As antihyperlipidemic activity of Triticum aestivum had not yet been elucidated in an exclusive hyperlipidemic model, the present study has been designed to evaluate lipid controlling, antiatherosclerotic and ant-oxidant activity of this plant.

MATERIALS AND METHODS

Plant
The leaves of Triticum aestivum were collected in the month of March from Dibrugarh and authenticated by Dr. M. Islam, Professor, Department of Life Science, Dibrugarh University. A voucher specimen (No. DU/LS/211) was deposited at Dibrugarh University.

Extract Preparation
The leaves of Triticum aestivum were air dried and powered. Material obtained from this was kept in percolator with ethanol for 48 Hours. The extract obtained from percolation was collected in a flask then evaporated by using controlled temperature until the solvent part was evaporated.

Drug and chemicals
Atorvastatin was obtained from Lupin LTD., Kartholi, Jammu. The kit for estimation of HDL-Cholesterol, Total Cholesterol and Triglyceride were obtained from CREST BIOSYSTEMS, Goa, India. Potassium Phosphate Buffer, Hydrogen Peroxide Solution and Tricarboxylic acid were obtained from Sigma Pvt. Limited, Bangalore, India. Thioarbituric acid was obtained from HiMedia Laboratories Pvt. Limited, Mumbai, India. Malondialdehyde bis was obtained from Merck Schuchardt OHG, Hohenbrunn, Germany.

High fat diet
This was prepared by mixing coconut oil and vanaspati ghee in a ratio of 2: 3 (v/v). It was given to the rabbits at a dose of 10 ml/Kg body weight per day mixed with food.

Animal
Healthy New Zealand white rabbit (Oryctolagus cuniculus) weighing from 1.5-2.5 kg of either sex were taken from Central Animal House, Assam Medical College (registration no. 634/02/a/CPCSEA dated 19/05/02). The animals were housed in standard cages and maintained under normal room temperature. The rabbits were fed with normal diet, high fat diet according to their group and water ad libitum. Before commencing the work permission from the Institutional Animal Ethical Committee was taken.

Acute oral toxicity studies
Acute oral toxicity test for the ethanolic extract of leaves of Triticum aestivum was carried out as per Organization for
Biochemical Estimations

Triglyceride

Lipid profile estimation

Test drug - received high fat diet and mixed food plus ethanolic extract of Triticum aestivum at a dose of 500 mg/Kg/ day per orally.

All the animals used for the experiment were kept under observation for daily food intake. The drugs were administered to the animals in the doses given above orally, once daily, for 12 weeks by means of intra-gastric feeding tube in the volume of 5ml/kg body weight.

At the end of the 12 weeks, all the animals were kept fasting for 18 hours. The rabbits were anaesthetized at the end of the experiment by Ether inhalation. The animals were dissected and blood was collected from inferior vena cava for biochemical examination. A 2 cm piece of abdominal aorta was then cut for making slide and histopathological examination to Pathology department. The sacrificed animals were sent in 10% formalin for making slides.

Experimental Design

Twenty number of rabbits of either sex were taken and divided into four groups, five rabbits (n=5) in each and treated as follows:

Normal Control - received normal diet.

Hyperlipidemic control - received high fat diet.

Test drug - received high fat diet mixed with food plus Atorvastatin at a dose of 2.1 mg/Kg/day p. o.

Standard Drug - received high fat diet plus mixed with food plus atorvastatin at a dose of 2.1 mg/Kg/day p. o. daily, for 18 hours.

At the end of the 2 weeks, all the animals were kept under observation for daily food intake. The drugs were administered to the animals in the doses given above orally, once daily, for 12 weeks by means of intra-gastric feeding tube in the volume of 5ml/kg body weight.

After collecting blood from inferior vena cava, blood was kept in different vials for biochemical analysis.

Lipid profile estimation

Total cholesterol was measured by CHOP/PAP method. Triglyceride was measured by GPO/PAP method. HDL-Cholesterol was measured by PEG precipitation method by using colorimetric method and LDL-Cholesterol was calculated by using Freidewald’s formula.

Catalase estimation

Catalase was measured in blood by Continuous Spectrophotometric Rate determination by Beers and Sizer method. Phosphate buffer (2.5 ml, pH 7.8) was added to the supernatant and incubated at 25°C for 30 minutes. After transferring into the cuvette, the absorbance was measured at 240 nm spectrophotometrically. Hydrogen peroxide (650 μl) was added and change in absorbance was measured for 3 minutes. Values were expressed as μmol/min/mg of proteins.

Superoxide dismutase estimation

SOD was assayed according to the method of Kakkar et al. The reaction was initiated by the addition of 0.2ml of NADH to serum and incubated at 30°C for 90 seconds and arrested by the addition of 1.0ml of glacial acetic acid. The reaction mixture was then shaken with 4.0ml of n-butanol, allowed to stand for 10 minutes and centrifuged. The intensity of the chromogen in the butanol layer was measured at 560nm in a spectrophotometer. One unit of enzyme activity is defined as the amount of enzyme that gave 50% inhibition of NBT reduction in one minute.

Malondialdehyde (MDA) estimation

MDA was estimated by Satoh K method. 75mg of Thiobarbituric acid (TBA) was dissolved in 15% TCA in 2.08ml of 0.2N HCL was added, the volume was made up to 100 ml using 15% TCA. 3.0 ml of this 2.08ml of 0.2N HCL was added, the volume was made up to 100 ml using 15% TCA. 3.0 ml of this reagent was added to 0.75 ml of serum of the rats. The test tubes were kept in a boiling water bath for 15 minutes. They were cooled and centrifuged for 10 minutes at 10000rpm. Absorbance of the supernatant was read against the blank at 535nm. The results were expressed in nmol/ml of serum.

Statistical analysis

The statistical significance between groups was analysed separately using One-way analysis of variance (ANOVA), followed by Dunnett’s multiple comparison test. The significance was expressed by ‘p’ values, as mentioned in the tables. ‘p’ values of <0.05 were considered as significant.

Table 1: Effects of Triticum aestivum on Lipid profile

<table>
<thead>
<tr>
<th>Groups</th>
<th>Total cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL (mg/dl)</th>
<th>LDL (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>38.74±3.428</td>
<td>76.02±5.702</td>
<td>11.23±1.108</td>
<td>10.52±1.602</td>
</tr>
<tr>
<td>Hyperlipidemic control</td>
<td>77.46±1.550</td>
<td>187.00±6.226</td>
<td>7.660±0.603</td>
<td>24.64±3.456</td>
</tr>
<tr>
<td>Test drug</td>
<td>48.82±3.536</td>
<td>112.8±5.434</td>
<td>16.08±0.329</td>
<td>10.04±0.7214</td>
</tr>
<tr>
<td>Standard Drug</td>
<td>60.89±2.160</td>
<td>118.4±5.483</td>
<td>18.20±0.860</td>
<td>16.22±0.7599</td>
</tr>
<tr>
<td>F</td>
<td>53.56</td>
<td>76</td>
<td>32.52</td>
<td>9.945</td>
</tr>
<tr>
<td>df</td>
<td>3.16</td>
<td>3.16</td>
<td>3.16</td>
<td>3.16</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are expressed as MEAN ± SEM (n=5).

One Way ANOVA followed by Dunnett’s Multiple Comparison test is done.

p <0.05, when compared to the Normal control Group.

p <0.05, when compared to the Hyperlipidemic control Group.

Table 2: Effects of Triticum aestivum on catalase, SOD and MDA

<table>
<thead>
<tr>
<th>Groups</th>
<th>Catalase (μmol/min/ml)</th>
<th>SOD (U/ml)</th>
<th>MDA (nmol/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Control</td>
<td>206.0±2.302</td>
<td>7.3±0.08</td>
<td>3.533±0.1291</td>
</tr>
<tr>
<td>Hyperlipidemic control</td>
<td>199.45±5.071</td>
<td>2.36±0.13</td>
<td>5.914±0.4934</td>
</tr>
<tr>
<td>Test drug</td>
<td>327.3±7.581</td>
<td>5.2±1.13</td>
<td>2.284±0.07709</td>
</tr>
<tr>
<td>Standard Drug</td>
<td>268.5±7.298</td>
<td>5.12±0.033</td>
<td>3.031±0.2013</td>
</tr>
<tr>
<td>F</td>
<td>448.7</td>
<td>448.7</td>
<td>151.6</td>
</tr>
<tr>
<td>df</td>
<td>3.16</td>
<td>3.16</td>
<td>3.16</td>
</tr>
<tr>
<td>P value</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Values are expressed as MEAN ± SEM (n=5).

One Way ANOVA followed by Dunnett’s Multiple Comparison test is done.

p <0.05, when compared to the Normal control Group.

p <0.05, when compared to the Hyperlipidemic control Group.
Table 3: Effects of *Triticum aestivum* on weight

<table>
<thead>
<tr>
<th>Group</th>
<th>Weight at the beginning of experiment</th>
<th>Weight at the end of experiment</th>
<th>Difference of weight (gm)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal control</td>
<td>1916.2</td>
<td>1927.4</td>
<td>11.2±1.182</td>
<td>Increased</td>
</tr>
<tr>
<td>Hyperlipidemic control</td>
<td>1939.6</td>
<td>2443.8</td>
<td>504.2±41.62*</td>
<td>Increased</td>
</tr>
<tr>
<td>Test drug</td>
<td>1930.8</td>
<td>1805.4</td>
<td>125.4±8.177*</td>
<td>Decreased</td>
</tr>
<tr>
<td>Standard</td>
<td>1889.0</td>
<td>1829.2</td>
<td>59.80±9.583*</td>
<td>Decreased</td>
</tr>
<tr>
<td>ANOVA</td>
<td>F</td>
<td>173.4</td>
<td>df</td>
<td>3, 16</td>
</tr>
<tr>
<td>p</td>
<td></td>
<td>&lt;0.0001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as MEAN ± SEM (n=5).

One Way ANOVA followed by Dunnett’s Multiple Comparison test is done for data of weight difference. *p <0.05, when compared to the Normal control Group. *p <0.05, when compared to the Hyperlipidemic control Group.

**Figure-1: Histopathology of aorta in low power view**

- a- NORMAL
- b- HYPERLIPIDEMIC CONTROL
- c- TEST DRUG
- d- STANDARD

A-tunica intima, B- tunica media, C- tunica adventitia

**Figure-2: Histopathology of aorta in high power view**

- a- NORMAL
- b- EXPERIMENTAL CONTROL
- c- TEST DRUG
- d- STANDARD

A-tunica intima, B- tunica media, C- tunica adventitia, D- foam cell
RESULTS

Acute toxicity test
There was no mortality among the animals. So the LD50 was calculated more than 2000 mg/kg body weight.

There was a significant (p < 0.05) decrease in serum cholesterol, triglyceride, low density lipoprotein (LDL) (table 1), malondialdehyde (MDA) (table 2) activity and weight (table 3) in test drug and standard group compared to Hyperlipidemic group which showed a significant (p < 0.05) increase as compared to normal control.

There was a significant (p < 0.05) increase in serum high density lipoprotein (HDL) cholesterol (table 1), catalase and SOD (table 2) activity in test drug and standard group compared to Hyperlipidemic group which showed a significant (p < 0.05) decrease as compared to normal control.

Histopathological examination of abdominal aorta shown that animals in Hyperlipidemic control group had intimal thickening, separation of tunica media from intima and lipid laden macrophages (foam cells) (figure 1b and 2b) while animals in test and standard group shows near normal histological architecture (figure 1c,d and 2c,d) compared to experimental group.

DISCUSSION

Antihyperlipidemic activity of this plant is mainly due to saponins and tannins. The mechanism of action of saponins and tannins is to inhibit lipid absorption and/or activation of fatty acid synthase, acetyl-CoA carboxylase and production of triglyceride precursors. Oxidants being derived from normal aerobic metabolism are also products of the inflammatory response. They are mostly of the nature of “Free radicals,” which are highly reactive molecules and can cause dyslipidaemia, coronary artery disease, atherosclerosis and so many other diseases. In human Catalase and superoxide dismutase are enzymes and act as antioxidants. This is due to its content - Vitamins C, E, carotene, selenium, bioflavonides etc. Thus antioxidant activity of this plant extract also prevent dyslipidaemia and atherogenesis.

MDA is considered as an important indicator of lipid peroxidation, which is found to be increased in rabbit received high fat diet. This might be due to lipid peroxidation. Rabbit treated with Triticum aestivum and atorvastatin showed protection against lipid peroxidation characterised by significant decrease in MDA level. Once produced, free radicals are removed by antioxidant defences including the enzymes catalase, glutathione peroxidase, and superoxide dismutase. Rabbits treated with Triticum aestivum and atorvastatin showed protection against lipid peroxidation characterised by significant increase in catalase and superoxide dismutase activity.

Histopathological examination also proved its anti-atherogenic effect. Thus extract of Triticum aestivum also prevents dyslipidaemia and atherogenesis.

Saponins are known to inhibit growth rate tannins were reported to be involved in growth regulations. The weight lowering potential of Triticum aestivum which could at least be partially attributed to the presence of saponins and tannins found in the plant. It can be concluded from the above study that ethanolic extract of Triticum aestivum has antihyperlipidemic, antioxidative and antiatherogenic activities.

REFERENCES