



ORIGINAL RESEARCH PAPER

Physiology

EFFECT OF TRITICUM AESTIVUM ON HEMATOLOGICAL PARAMETERS IN HIGH FAT DIET FED RABBITS

**KEY WORDS:** wheat grass, triticum aestivum, hemoglobin, neutrophils, oxidative stress.

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ABSTRACT

Wheatgrass has been used for therapeutic purposes since many decades and has shown encouraging results in the treatment of diseases including anemias and also as immunity booster. The present study was aimed to assess the effect of wheatgrass on hematological profile. Thirty rabbits were divided into three groups of ten rabbits each, group I receiving control diet, group II high fat diet and group III both high fat diet and wheat grass for a period of ten weeks. The fasting serum samples were analyzed for hemoglobin, packed cell volume (PCV), red blood cell count (RBC), total leucocyte count (TLC), differential leucocyte count(DLC) and blood indices were calculated and statistically analysed between the groups. There was significant improvement in hemoglobin concentration and RBC count in the group receiving wheatgrass and improvement in the inflammation induced by high fat diet as seen by total leucocyte count and neutrophil count reduction and increase in the lymphocytes in the group receiving wheatgrass.

**Introduction**

The history of herbs and plants used for medicinal purposes goes as far back as the history of mankind. Since ancient times, various herbs have been traditionally used for preventing and curing a wide array of diseases. In recent times also natives of villages and tribal areas rely mainly on these natural methods for maintaining healthy lives and dealing with ailments.<sup>1</sup> Wheatgrass known as *Triticum aestivum* Linn (Poaceae) or common wheat seed is used as a health improving adjuvant in several diseases as folk medicine.<sup>2</sup> It is early growth stage of wheat plant. During this stage this plant is much richer in vitamins, minerals & proteins as compared to the mature plant or seeds kernel.<sup>3</sup> According to ayurvedic texts, blood and wheatgrass juice are similar in the form of substance, quality and action.<sup>4</sup> Marwah et al have reported in their pilot study that thalassemia patients receiving wheat grass juice reported non-specific well being, improved appetite and reduced musculoskeletal aches and pain.<sup>5</sup> The present study was designed to study the effect of wheatgrass on hematological parameters of normal rabbits and those exposed to oxidative stress (high fat diet).

**MATERIAL AND METHODS**

**Animals**

Albino rabbits of either sex weighing 1.5-2.5 kg obtained from departmental animal house were used for this study. The rabbits were housed under controlled conditions of light (12 –h light-dark cycle) and temperature [(23 ± 2) °C] with free access to respective diets and water *ad libitum* for a period of 10 weeks. Commercially available dried wheat grass powder (URJA-777) was used in the study. Institutional Animal Ethical Committee (I.A.E.C.) approval (IAEC/PATHO/08/2352-58 dated 18.09.08) was obtained before the experiment and care was taken to handle the rabbits in humane manner.

**Experimental design:-**

The animals were divided into the following three groups, with 10 rabbits in each group:

- Group I: (Control): Standard chow diet
- Group II: High fat diet (HFD)
- Group III: High fat diet plus wheat grass powder (2gm/day)

The compositions of two diets were as follows<sup>6</sup>:

**Control Diet:-**

Wheat flour 22.5%, roasted Bengal gram powder 60%, skimmed milk powder 5%, casein 4%, refined oil 4%, salt mixture with starch 4% and vitamin and choline mixture 0.5%.

**High Fat diet:-**

Wheat flour 20.5%, roasted Bengal gram powder 52.6%,

skimmed milk powder 5%, casein 4%, refined oil 4%, coconut oil 9%, salt mixture with starch 4%, vitamin and choline mixture 0.5% and cholesterol 0.4%.

**Body weight** of animals of each group was recorded both before and after ten weeks period. After 10 weeks, animals were kept fasting overnight and blood samples were collected from marginal vein of pinna and subjected to following hematological tests using standard procedures and blood indices were calculated.<sup>7</sup>

**Hemoglobin (Hb) estimation** was done by Sahli s acid hematin method.

**Red blood cell (RBC) count & Total leucocyte count(TLC)** count was done using improved Neubauer's haemocytometer.

**Packed cell volume (PCV)** was done using wintrobe's tube.

**Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV) and Mean Corpuscular Hemoglobin (MCHC)** were calculated.

**Differential leucocyte count (DLC)** was done by preparing a blood smear and staining with Leishman's stain.

**Statistical analysis:** - Results were expressed as mean ± SD of 10 rabbits in each group. The data was analysed statistically using SPSS software version 14.0. All values were expressed as mean and standard deviation (SD) and p <0.05 was considered statistically significant.

**Observations and results:**

The rabbits were visibly healthy in all the groups. The average body weight increased in high fat diet fed rabbits compared with those in control group. Average body weight in group III rabbits was statistically significantly reduced as compared to group II.

**Table 1 Hb, RBC Count, PCV and blood indices**

Groups	Hb (gm%)	RBC count (cells/mm <sup>3</sup> )	PCV %	MCH (pg)	MCV(µm3)	MCHC %
Group I	10.45±1.8	4.16± .53	37.9± 4.6	25.13 ±.30	91.82± 1.1	27.59± .03
Group II	11.67 ±.72	4.59± .50	41.08± 2.3*	25.69 ± .32	90.66± 1.2	28.51± .28
GroupIII	13.43± .80*#	4.83± .26*	43.48± 1.4#	27.89 ± .24*	90.28± .62	30.92± .30*

\* p< 0.05 as compared to group I  
# p< 0.05 as compared to group II

**Table 2 Total Leucocyte Count (TLC) and Differential Leucocyte Count (DLC)**

Groups	TLC (cells/mm <sup>3</sup> )	Neutrophil %	Lymphocyte %	Monocyte %
Group I	4993± 899	46.2± 10.5	41.8 ±10.4	3.94± .8
Group II	5940 ±1058*	55.8± 8.8*	45.5± 9.5	3.72± .68
Group III	4325± 503#	49.1± 4.1#	63.31± 7.19*#	4.35± 1.1

\* p<0.05 as compared to group I

# p<0.05 as compared to group II

No change was observed in the percentage of monocytes in three groups.

### Discussion

Wheatgrass has been proven over many years to benefit people in numerous ways: cleansing the lymph system, building the blood, restoring balance in the body, removing toxic materials from the cells nourishing the liver and kidneys and restoring vitality.<sup>8</sup> In our study, in group III, supplementation with wheatgrass produced significant increase in Hb levels as compared to control and high fat diet fed groups. These results are in accordance with Shah et al also who reported significant increase in Hb levels when busulphan induced anemic rats were compared with the groups treated with fresh wheatgrass juice and methanol and acetone extracts of wheatgrass.<sup>9</sup> A study performed by Marwah et al concluded that the wheatgrass juice had the potential to lower the transfusion requirements in thalassemia patients.<sup>5</sup>

It has been postulated that chlorophyll content of wheatgrass juice is about more than 80%.<sup>10</sup> There is a striking similarity between the chemical structures of both the compounds except that the central atom in chlorophyll is magnesium while in hemoglobin it is iron which can account for increase in the levels of Hb in the wheatgrass fed animals.<sup>8</sup> Also wheatgrass extract induces the production of fetal hemoglobin (HbF) by 3-5 folds as measured by the cellular assay.<sup>11</sup> Our results were comparable with the results of a study by Bhikaji et al where there was significant increase in Hb levels in the group receiving fresh wheatgrass juice for 21 days.<sup>12</sup> Also in our study there was significant increase (p<0.05) in RBC count in wheatgrass fed group as compared to the control group. PCV was significantly increased in wheatgrass fed group as compared to the control group. Blood indices MCH and MCHC were significantly increased but there was no significant difference in the MCV in all the three groups. MCHC is the mean concentration of hemoglobin in a single RBC and considered to be more reliable indicator for increase in hemoglobin synthesis in RBC. It has been reported that the beneficial effects of wheat grass may be due to the action of natural antioxidants on red blood cell antioxidant function, and corresponding effects on cellular enzyme function and membrane integrity.<sup>13</sup> We observed significant increase in total leucocyte count in group II (high fat diet) while TLC was significantly reduced in the group III. There was a significant increase in percentage of neutrophils in the high fat diet fed group as compared to control; whereas in the group III, it was significantly reduced as compared to group II. Lymphocytosis was seen after supplementation of wheatgrass (Table 2). High fat diet induced oxidative stress probably caused state of low grade inflammation resulting in increased WBC count. Our results are consistent with Shabbir et al who have demonstrated obesity induced inflammation resulted in increased WBC counts and IL-6 levels.<sup>14</sup> There are other reports suggesting increase in IL-6 levels causing inflammation in obese and hyperlipidemic animals.<sup>15</sup> In our study predominant neutrophils in hyperlipidemic animals suggest onset of acute inflammation triggered by oxidative stress. It has been reported that inflammatory mediators such as GM-CSF play important role in differentiation and maturation of neutrophils during acute inflammation.<sup>16</sup> There are reports that oxidative stress leads to production of free radicals which can affect the content of circulating cells including WBCs in the blood.<sup>17</sup> Wheatgrass supplementation (group III) leading to significant reduction in the neutrophils indicate protective role of antioxidant defense mechanism against oxidative stress induced inflammation. Increased lymphocyte in the group receiving wheatgrass in our study reinforces the immunity boosting potential of wheatgrass as

observed in other studies (18). Wheatgrass contains many compounds such as bioflavonoids, Vit C and E, thiol containing amino acids, phenolic compounds, enzymes, P4D1 and Grass juice factor which may be responsible for these results.<sup>19,20</sup>

### Conclusion

Wheatgrass can be used as adjunct therapy in the treatment of anemias and to improve immunity however proper isolation of active principles is required.

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