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**INVESTIGATION INTO THERAPEUTIC ROLE OF *TRITICUM AESTIVUM*  
(WHEAT) GRASS IN BUSULFAN INDUCE THROMBOCYTOPENIA**



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**ABSTRACT**

*Triticum aestivum*, wheatgrass is a cereal grass of Gramineae (Poaceae) family has been traditionally used, to treat various diseases like cancers, diabetes, gastritis, ulcers, pancreas and liver problems, anemia, skin problems and constipation etc. Wheatgrass is rich in chlorophyll, minerals like magnesium, selenium, zinc, chromium, antioxidants like beta-carotene (pro-vitamin A), vitamin E, vitamin C, antianemic factors like vitamin B12, iron, folic acid, pyridoxine and many other minerals, amino acids and enzymes, which have significant nutritious and medicinal value. Wheat grass is a rich source of phenolic and flavanoid content. Thrombocytopenia is a lower than normal number of platelets in the blood. Platelets play an important role in clotting and bleeding. The present study was planned to evaluate beneficial effects *T. aestivum* on busulfan induced thrombocytopenic animals. We have made the unexpected observation that fresh juice, methanol and acetone extracts of *T. aestivum* significantly increase Hb levels, RBC, total WBC and differential WBC counts in pancytopenic rats. Wheatgrass significantly increase platelet counts and reduce bleeding and clotting time in thrombocytopenic rats. In conclusion, our data suggest *Triticum aestivum* possess beneficial effect in thrombocytopenia and pancytopenia conditions by increase platelet counts. In addition it also produces immunostimulant effects.

**KEY WORDS**

*Triticum aestivum*, thrombocytopenia, pancytopenia

## **Introduction**

Thrombocytopenia is a lower than normal number of platelets in the blood. Platelets play an important role in clotting and bleeding. Platelets originate from megakaryocytes which are large cells found in the bone marrow. The number of platelets in the blood is referred to as the platelet count and is normally between 150,000 to 450,000 per micro liter (one millionth of a liter) of blood. Platelet counts less than 150,000 are termed thrombocytopenia. Platelet counts greater than 450,000 are called thrombocytosis. [1] al, 1993)

The function of platelets is very important in clotting system. They circulate in blood vessels and become activated if there is any bleeding or injury in body. Certain chemicals are released from injured blood vessels or other structures that signal platelets to become activated and join other components of system to stop bleeding. When activated, platelets become sticky and adhere to one another and to blood vessel wall at the site of injury to slow down and stop bleeding by plugging up the damaged blood vessel or tissue (hemostasis). [2]

Some of the most common and important causes of thrombocytopenias are outlined below. [3]

### ***1. Decreased platelet production***

Decreased platelet production is usually related to a bone marrow problem (agranulocytosis). These includes viral infections affecting bone marrow for example: parvovirus, rubella, mumps, varicella (chickenpox), hepatitis C, Epstein-Barr virus, and HIV; aplastic anemia, chemotherapy drugs and thiazide diuretics, cancers of bone marrow and blood (leukemia) or cancers of lymph nodes (lymphoma), long term alcohol, deficiency of vitamin B12 and folic acid can result in low platelet production by bone marrow.

### ***2. Increased platelet destruction or consumption***

Increased platelet destruction or consumption can be seen a number of medical conditions. They can be divided into immune related and non-immune related causes. Many medications can cause low platelet count by causing immunologic reaction against platelets, called drug-induced thrombocytopenia. Some examples may include: sulfonamide antibiotics, carbamazepine anti-seizure drug, digoxin, quinine,

quinidine, acetaminophen, and rifampin, heparin. Some rheumatologic condition, such as systemic lupus erythematosus, Transfusion of blood products, Thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) can cause platelet destruction.

**3. Splenic sequestration:** Common causes of thrombocytopenia due to splenic enlargement may include advanced liver disease (cirrhosis, for example, from chronic hepatitis B or C) and blood cancers (leukemias or lymphomas).

Modern science has already accepted the potential of herbs as a source of new bio-active constituents. There are numerous plants derived drugs of unknown chemical structure that have been found clinically useful in different alternative system of medicine including Ayurveda, Homeopathy and Unani system of medicine. The plants are rich reservoir of potential leads for drug discovery against various disorders. The recent development of the science of phyto-pharmaceuticals has generated new enthusiasm in herbal drug research to discover new medicines. <sup>[4]</sup>

Wheat, (*Triticum* species) a cereal grass of the Gramineae (Poaceae) family, is the world's largest edible grain cereal-grass crop. Wheat has been a food crop for mankind since the beginning of agriculture. The wheat plant is an annual grass. In early growth stages the wheat plant consists of a much-compressed stem or crown and numerous narrowly linear or linear-lanceolate leaves. For over fifty years, researchers have known that the cereal plant, at this young green stage, is many times richer in levels of vitamins, minerals and proteins as compared to seed kernel, or grain products of the mature cereal plant. <sup>[5]</sup>

The young germinated plant is a factory of enzyme and growth activity. In early stages of growth they store large amounts of vitamins and proteins in young blades. After jointing stage, nutritional level in leaves drops rapidly while fiber content increases rapidly. The jointing stage is that point at which the internodal tissue in grass leaf begins to elongate, forming a stem. This stage represents peak of cereal plant's vegetative development. <sup>[6]</sup>

Wheatgrass has been traditionally used, since ancient times, to treat various diseases and disorders. Presently, there are a number of wheatgrass suppliers, in almost all cities of India, supply fresh wheatgrass, on daily basis to their regular customers by

home-delivery system for various ailments and as health tonic. Dr. Ann Wigmore, U. S. A. founder director of the Hippocrates Health Institute, Boston, U.S.A. was one of proponents of 'Wheatgrass Therapy'. Dr. Wigmore reported that "wheatgrass" used in her program contain abscisic acid and laetrile, both of which may have anti-cancer activity. It was also reported that young grasses and other chlorophyll-rich plants are safe and effective treatment for ailments such as high blood pressure, some cancers, obesity, diabetes, gastritis, ulcers, pancreas and liver problems, fatigue, anemia, asthma, eczema, hemorrhoids, skin problems, halitosis, body odor and constipation. [7]

Scientific reports on nutritional analysis of wheatgrass have been published frequently in various journals. [6] [8] These reports and chemical analyses undertaken reveal that wheatgrass is rich in chlorophyll, minerals like magnesium, selenium, zinc, chromium, antioxidants like beta-carotene (pro-vitamin A), vitamin E, vitamin C, antianemic factors like vitamin B<sub>12</sub>, iron, folic acid, pyridoxine and many other minerals, amino acids and enzymes, which have significant nutritious and medicinal value. Clinically it was proved that different varieties of wheatgrass extracts are therapeutically used in treatment of anemia, thalassemia (major), cancer and bacterial diseases. [9]

There are a wide range of botanical sources and wide range of active constituents that might ultimately contribute to hemostatic action, including essential oils, flavonoids, saponins, and alkaloids. These effects should be expected to be observed within a few days of administering the herbs. In most of the Chinese medical reports, improvements in symptoms (such as spontaneous bleeding and petechia) were observed within about 10 days. Three groups of active constituents are known to have some hemostatic effects and may influence autoimmune processes:

- Anthraquinones, found in rubia and rumex and also an ingredient of rhubarb root (which has hemostatic effects, but is not included in the ITP formulas)
- Flavanoids, found in eclipta and agrimony, and also in scute (used to inhibit bleeding but rarely in the ITP formulas)
- Alkaloids, found in lotus (all plant parts), eclipta, and san-chi.

The role of essential oils (which usually dilate vessels; some might increase bleeding), triterpenes, and saponins found in several of the herbs remains unknown. One of the

most frequently-used herbs in the formulas, raw rehmannia, contains iridoid glycosides that have hemostatic effects. The same active constituents are found in gardenia, which is mentioned in a few of ITP treatments, as well as in scrophularia and cornus.

Compared to synthetic drugs, herbal preparations are frequently less toxic with fewer side effects. Therefore the search for more effective and safer treatment of thrombocytopenia has become an area of current research activity.

For thousands of years, mankind has known about the benefit of drugs from nature. Plant extracts, for the treatment of various ailments, were highly regarded by the ancient civilizations. Even today, plant materials remain an important resource for combating illnesses.

In the light of foregoing discussion the present study is planned for Investigation into therapeutic role of *Triticum aestivum* (Wheat) grass in busulfan induce thrombocytopenia.

### **Materials and methods**

Certified samples of species of wheat *Triticum aestivum*, was acquired from Wheat Research Center, Gujarat Krushi University, Junagadh, Gujarat, India. The authenticity of these certified samples was also confirmed by comparing their morphological characters with description mentioned in different standard texts and floras.<sup>[10]</sup> These wheat varieties were grown in plastic trays as per standard procedure described by Wigmore, 1985. Fresh juice of wheatgrass was prepared by standard procedure described by Wigmore, 1985. Dried powder (60 #) of wheatgrass was subjected for extraction with methanol and water using soxhlet apparatus. Solid powder obtained from successive extraction of methanol and acetone was subjected for investigation in therapeutic role of *Triticum aestivum* (Wheat) grass in busulfan induce thrombocytopenia model in rats.

All animals were housed at ambient temperature (22±10C), relative humidity (55±5%) and 12h/12h light dark cycle. Animals had free access to standard pellet diet and water given ad libitum. The protocol of the experiment was approved by the institutional animal ethical committee as per the guidance of the Committee for the

Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India (Protocol No. RKCP/COL/RP/09/01 dated 7th March, 2009).

### **Induction of thrombocytopenia**

Healthy wistar rats weighing 150-200 gm were used for study. Thrombocytopenia was induced in rats by busulfan according to methods of Evensen et al. 1968. <sup>[11]</sup> Busulfan at a final concentration of 10 mg/ml in polyethylene glycol was prepared and was infused doses of 25 mg busulfan/kg body weight each at 1, 5, 10 and 15 days of treatment period produce significantly reduction in platelet counts in rats, which shows severe bleeding conditions as found in thrombocytopenia. Busulfan is an alkylating agent with myeloablative properties and activity against non-dividing marrow cells and possibly, non-dividing malignant cells.

### **Treatment protocol**

The experimental animals were divided into five groups, n=6.

Group I : Normal healthy control

Group II : Diseases control, busulfan induced thrombocytopenic rats (25 mg busulfan/kg)

Group III: Busulfan induced thrombocytopenic rats treated with wheat grass fresh juice (5 ml/kg, p.o., day)

Group IV: Busulfan induced thrombocytopenic rats treated with methanol extract (50 mg/kg, p.o., day)

Group V : Busulfan induced thrombocytopenic rats treated with wheat grass acetone extracts (50 mg/kg, p.o., day)

All the studies were carried for a period of three weeks. After 21 days of treatment period blood samples were collected under fasting conditions and were subjected to estimations.

## Estimation of hematological parameters

Various hematological parameters like

- a. Hemoglobin content
- b. Total RBC
- c. Total WBC count
- d. Differential WBC count  
Neutrophil, Lymphocyte, Eosinophil, Basophil counts and Total lymphocyte count and
- e. Platelet count were estimated using fully automated hematology analyzer - Model XS-800i – Sysmex.

### **B. Determination of clotting time [Lee and white method] <sup>[12]</sup>**

During coagulation sol form of the blood is changed to gel form. The time elapsed between the moment of escape of blood outside the vessel and the observation of physical change is taken as clotting time.

*Procedure:* Blood was collected from animal by retro orbital plexus method under light anesthetic conditions. Immediately stop watch is started. Dip one end of capillary into blood drop gently without pressure. After every 30 seconds, using stopwatch, break a small piece of capillary. Repeat breaking at regular time intervals, till fibrin thread appears at broken end of capillary tube. Do not pull away the cut pieces lying apart and bristly. Record time interval between pricking finger and first appearance of fibrin thread at broken ends of capillary tube. That is clotting time of blood.

### **C. Determination of bleeding time**

The time elapsed between the moment blood escapes from vessel and the cessation of its flow is defined as the bleeding time. The usual bleeding time is 1-3 minutes and clotting time is 4-10 minutes. Bleeding time is prolonged during thrombocytopenia. The tip of finger is sterilized with spirit and bold prick is given so that blood flows freely. The stopwatch is immediately started. The blood is soaked on a filter paper (while soaking, the filter should not touch the skin of finger). This is repeated every

10 seconds till no blot appears on the papers. The time from the first appearance of the blood to the cessation of bleeding is the bleeding time.

## Result

### Hemoglobin level and RBC count

Disease control rats which received busulfan were found to be significant reduction in Hb and RBC count compare to normal healthy rat that indicates anemia in animals. Treatment with wheat grass fresh juice, methanol and acetone extracts produced significant increase in Hb level and RBC count in diseases rats. Decrease in blood Hb level and RBC count in rats was significantly prevented by treatment with fresh juice, methanol and acetone extracts of wheat grass. Thus wheat grass helps improve blood purification and to increase hemoglobin level and RBC count near to normal.(Table 1)

**Table 1: Beneficial effects of *Triticum aestivum* fresh juice, methanol and acetone extracts on Hb level and RBC count**

Blood parameters	Normal Control	Diseases Control	DWFJ	DWM	DWA
Hemoglobin (gm/dl)	10.9±0.98	8.1±0.75*	10.5±1.1 <sup>#</sup>	10.2±1.2 <sup>#</sup>	9.7±0.89
RBC (10 <sup>6</sup> /πl)	6.8±0.89	5.4±0.50*	6.7±0.59 <sup>#</sup>	6.1±0.47 <sup>#</sup>	6.3±0.7

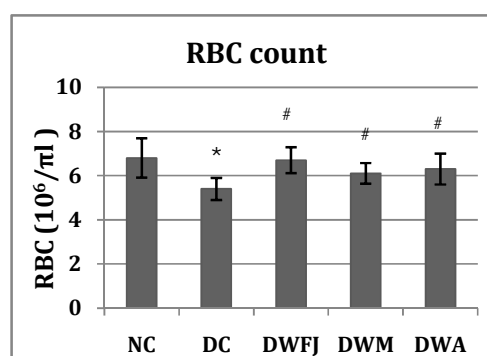
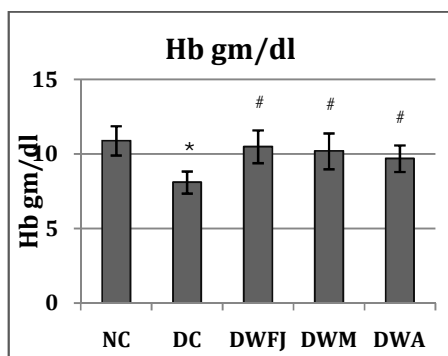


Fig 1: Beneficial effects of *Triticum aestivum* fresh juice, methanol and acetone extracts on Hb level and RBC count. Values are expressed as Mean ± S.E.M, \*- significantly different from normal control ( $p < 0.05$ ), # - significantly different from diseases control ( $p < 0.05$ ), DWFJ- Diseases rats treated with Wheatgrass Fresh Juice, DWM- Diseases rats treated with Wheatgrass Methanol extract, DWA- Diseases rats treated with Wheatgrass Acetone extract.



**Total WBC count**

Disease control rats which received busulfan were found to be significant reduction in total WBC and differential WBC counts like neutrophils, basophile, eosinophil, lymphocyte and monocyte counts compare to normal healthy rats. Treatment with wheat grass fresh juice, methanol extract produced significant increase in total WBC counts and differential WBC counts in busulfan induced pancytopenic rats. Treatment with acetone extract does not produce any significant increase in total WBC counts. Disease control rats shows pancytopenia (reduction in all blood cells count) compare to normal healthy control rats and treatment with wheat grass fresh juice and different extracts shows increase in WBC counts compare to disease control group. (Table 2)

**Table 2: Beneficial effects of *Triticum aestivum* fresh juice, methanol and acetone extracts on total and differential WBC counts.**

Blood parameters	Normal Control	Diseases Control	DWFJ	DWM	DWA
<b>WBC (<math>10^3/\pi l</math>)</b>	4.98 $\pm$ 0.49	3.26 $\pm$ 0.31*	4.78 $\pm$ 0.52 <sup>#</sup>	4.58 $\pm$ 0.43 <sup>#</sup>	3.89 $\pm$ 0.26
<b>Differential WBC</b>					
Neutrophil ( $10^3/\pi l$ )	2.63 $\pm$ 0.35	1.89 $\pm$ 0.28*	2.45 $\pm$ 0.09 <sup>#</sup>	2.01 $\pm$ 0.11 <sup>#</sup>	2.32 $\pm$ 0.12 <sup>#</sup>
Lymphocytes ( $10^3/\pi l$ )	1.52 $\pm$ 0.08	1.02 $\pm$ 0.07*	1.63 $\pm$ 0.08 <sup>#</sup>	1.26 $\pm$ 0.07 <sup>#</sup>	1.48 $\pm$ 0.09
Monocytes ( $10^3/\pi l$ )	0.42 $\pm$ 0.07	0.11 $\pm$ 0.08*	0.43 $\pm$ 0.06 <sup>#</sup>	0.28 $\pm$ 0.03 <sup>#</sup>	0.37 $\pm$ 0.03 <sup>#</sup>
Eosinophil ( $10^3/\pi l$ )	0.12 $\pm$ 0.01	0.02 $\pm$ 0.003*	0.09 $\pm$ 0.002 <sup>#</sup>	0.03 $\pm$ 0.0001 <sup>#</sup>	0.11 $\pm$ 0.01 <sup>#</sup>
Basophil ( $10^3/\pi l$ )	0.11 $\pm$ 0.002	0.04 $\pm$ 0.003*	0.12 $\pm$ 0.01 <sup>#</sup>	0.07 $\pm$ 0.004 <sup>#</sup>	0.09 $\pm$ 0.002 <sup>#</sup>

Values are expressed as Mean  $\pm$  S.E.M, \*- significantly different from normal control ( $p < 0.05$ ), # - significantly different from diseases control ( $p < 0.05$ ), DWFJ- Diseases rats treated with Wheatgrass Fresh Juice, DWM- Diseases rats treated with Wheatgrass Methanol extract, DWA- Diseases rats treated with Wheatgrass Acetone extract

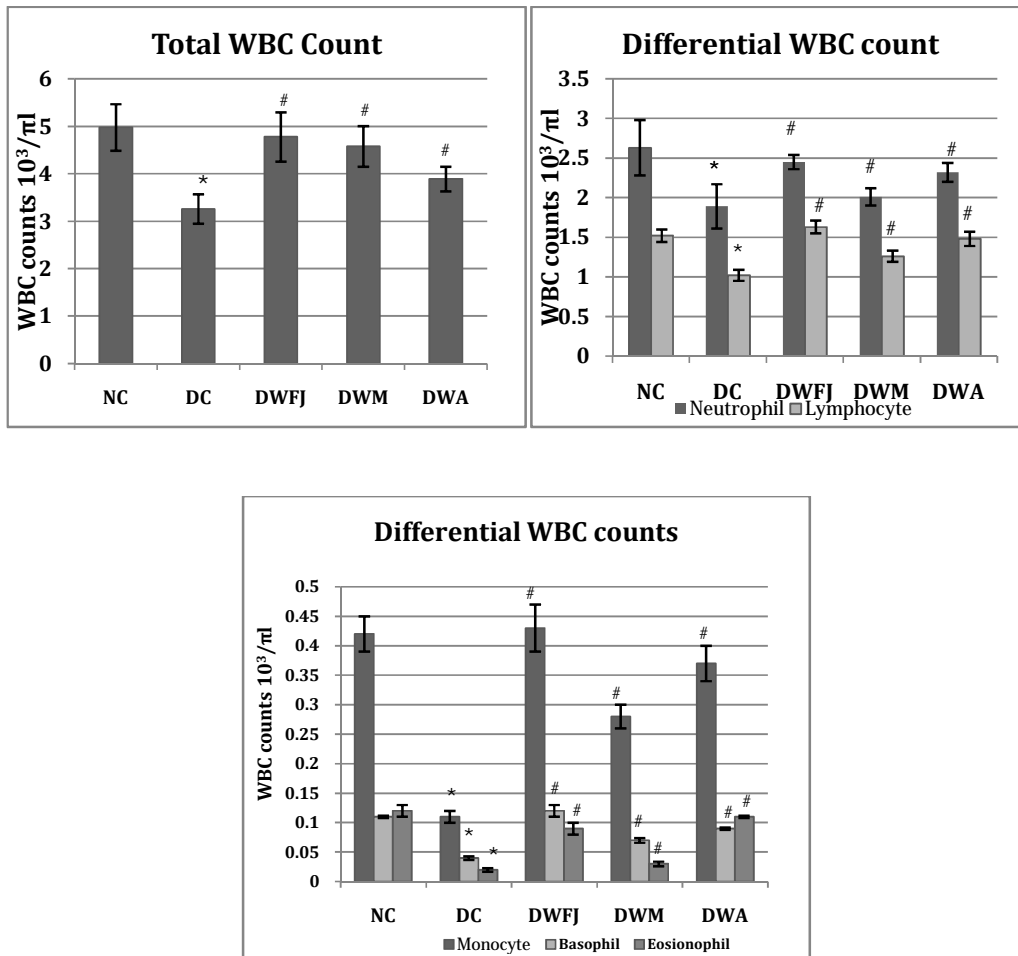


Fig: 2 Beneficial effects of *Triticum aestivum* fresh juice, methanol and acetone extract on Total and differential WBC counts.

### Platelet count

Disease control rats which received busulfan were found to be significant reduction in platelet count ( $523 \pm 46 \times 10^3/\pi l$ ) compare to normal healthy rat ( $905 \pm 82 \times 10^3/\pi l$ ) that indicates thrombocytopenia in animals. Treatment with wheat grass fresh juice, methanol and acetone extracts produced significant increase in platelet compare to diseases control rats. Decrease in blood platelet count in these rats was significantly prevented by treatment with fresh juice, methanol and acetone extract. (Table 3)

### Bleeding time and clotting time

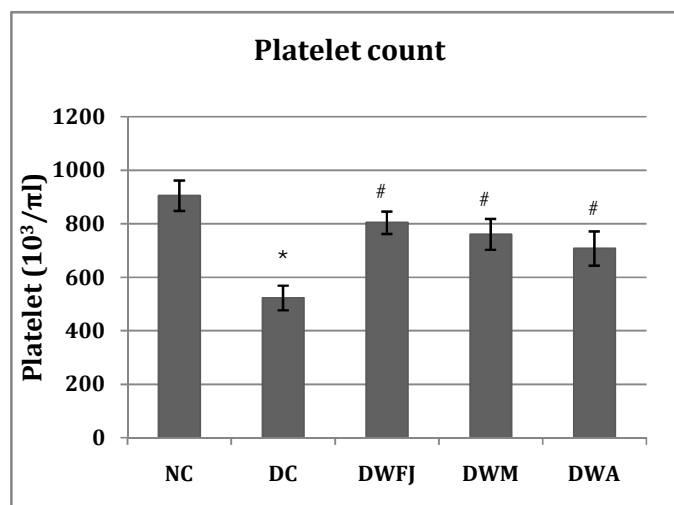
Disease control rats which received busulfan were found to be significant increase in bleeding and clotting time as a result of reduction in platelet counts compare to

normal healthy rats that indicates hemophilia and thrombocytopenia in animals. Treatment with wheat grass fresh juice, methanol and acetone extracts produced significant reduction in bleeding and clotting time in diseases rats. Increase in bleeding and clotting time in rats was significantly prevented by treatment with fresh juice, methanol and acetone extract of wheat grass. Thus wheat grass helps in reduction of bleeding and clotting time near to normal. (Table 3)

**Table 3: Effect of *Triticum aestivum* fresh juice, methanol and acetone extracts treatment on bleeding and clotting times on control and disease rats.**

Blood parameters	Normal Control	Diseases Control	DWFJ	DWM	DWA
Platelet ( $10^3/\pi l$ )	905 $\pm$ 102	523 $\pm$ 46*	804 $\pm$ 72 <sup>#</sup>	761 $\pm$ 58 <sup>#</sup>	708 $\pm$ 163 <sup>#</sup>
Bleeding time(sec)	80 $\pm$ 12	190 $\pm$ 18*	98 $\pm$ 13 <sup>#</sup>	106 $\pm$ 17 <sup>#</sup>	125 $\pm$ 15 <sup>#</sup>
Clotting Time (sec)	130 $\pm$ 22	390 $\pm$ 35*	150 $\pm$ 23 <sup>#</sup>	196 $\pm$ 24 <sup>#</sup>	214 $\pm$ 30 <sup>#</sup>

Values are expressed as Mean  $\pm$  S.E.M, \* - significantly different from normal control ( $p < 0.05$ ), # - significantly different from diseases control ( $p < 0.05$ ), DWFJ- Diseases rats treated with Wheatgrass Fresh Juice, DWM- Diseases rats treated with Wheatgrass Methanol extract, DWA- Diseases rats treated with Wheatgrass Acetone extract



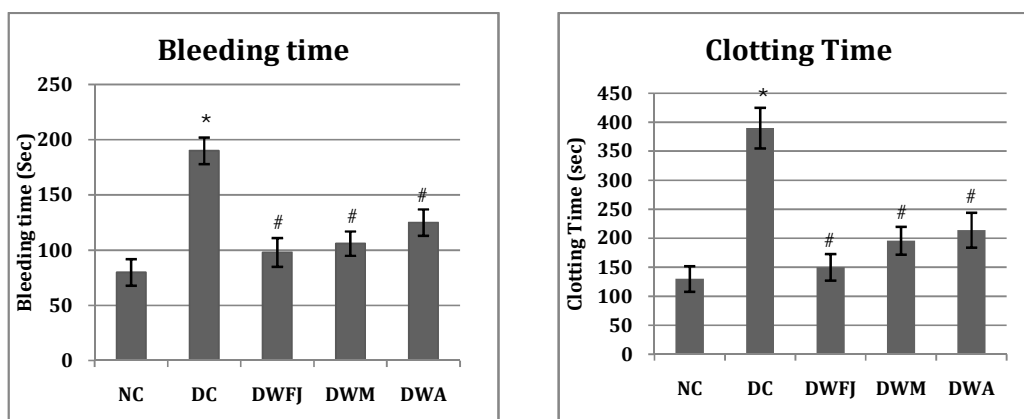


Figure 3: Effect of *Triticum aestivum* fresh juice, methanol and acetone extracts treatment on platelet count, bleeding and clotting times on control and disease rats.

### Discussion:

In our study, for evaluation of beneficial effects of wheatgrass in thrombocytopenia, busulfan was used to induce experimental thrombocytopenia. Busulfan is an alkylating agent with myeloablative properties and activity against non-dividing marrow cells and possibly, non-dividing malignant cells. Intraperitoneal injection of busulfan produced, significant reduction of platelet count in wistar rats, resulting in severe bleeding tendency as found in thrombocytopenia. A significant loss of blood cells was evident in diseases control rats over the period of three weeks. Three weeks treatment with fresh wheatgrass juice (5 ml/kg, p.o.), methanol and acetone extract (100 mg/kg/p.o./day each) produced a significant increase in all blood cell counts. Treatment with fresh juice, methanol and acetone extracts showed significant increase in hemoglobin, RBC, total and differential WBC and platelet counts in pancytopenia rats as compared to disease control group. Disease control rats showed significant increase in bleeding and clotting time indicating hemophilia and thrombocytopenia. Treatment with fresh juice, methanol and acetone extracts showed decrease in bleeding and clotting time period. Thus, data indicate beneficial effect of wheatgrass in thrombocytopenia and pancytopenia. Our results also suggest immunostimulant effects of wheatgrass as it increase total and differential WBC counts.

Chronic treatment with fresh juice, methanol and acetone extracts increased hemoglobin and RBC count indicating therapeutic usefulness of wheatgrass in anemia and other hemoglobin and RBC related disorders.

In conclusion our investigation reveals potential beneficial effect of wheatgrass in thrombocytopenia and pancytopenia. Wheatgrass is also possessing potent immunomodulatory effects.

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