EFFECT OF WHEAT GRASS *(Triticum aestivum)* ON HEMATO-BIOCHEMICAL PARAMETERS AND BODY WEIGHT IN RABBIT

A THESIS

BY

MOST. TAHAMINA AKTAR
Registration No. 1605516
Session: 2016-2017
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MASTER OF SCIENCE (MS)
IN
PHARMACOLOGY

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Approved as to style and contents by

_____	_____
(Dr. Fahima Binthe Aziz)	**(Dr. Md. Mahmudul Hasan)**
Supervisor	**Co-Supervisor**

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DEPARTMENT OF PHYSIOLOGY AND PHARMACOLOGY
HAJEE MOHAMMAD DANESH SCIENCE AND TECHNOLOGY UNIVERSITY, DINAJPUR-5200, BANGLADESH

JUNE, 2018

DEDICATED
TO MY
BELOVED PARENTS

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ABSTRACT

This research work was conducted at animal research shed in the Department of Physiology and Pharmacology, at Hajee Mohammad Danesh Science & Technology University, Dinajpur for a period of 45 days. The experiment was undertaken to investigate the effect of wheat grass on hemato-biochemical parameters and body weight in rabbit. Wheat grass is rich source of minerals, vitamins, antioxidants, amino acids and many enzymes. The vital component of wheat grass is chlorophyll about 70% that helps in building hemoglobin in body. Twenty rabbits 4 months of age and weighing about 1200-1500 gm were acclimatized for 2 weeks and then randomly assigned into four groups (T_0, T_1, T_2 and T_3) and (n=5). Group T_0 was kept for control group and rabbit of T_1, T_2, T_3 group were kept as treated groups. Then chopped wheat grass supplementation was given to the group T_1, T_2 and T_3 at the dose rate of 3 gm, 5 gm, 7 gm/kg body weight respectively for 45 days. On day 15, 30, 45 the blood samples were collected and analyzed for hemoglobin, Packed Cell Volume (PCV), Red Blood Cell count (RBC), Total Leucocyte Count (TLC), Differential Leucocyte Count (DLC), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and body weight gain from 0-45 days were calculated and statistically analyzed. There was significant ($P>0.05$) improvement in hemoglobin concentration, PCV, RBC, TLC, DLC count and in the group receiving wheat grass compare to control group and highest value was found in group T_2 but statistically not significant ($P<0.05$). After treatment with chopped wheat grass there was significant ($P<0.05$) decrease in ESR, SGPT, SGOT level in all treated group compare to control and lowest value was found in group T_2 but statistically not significant ($P<0.05$). Wheat grass did not produce any significant ($P<0.05$) effect on body weight of rabbit. The results of this study reveals that wheat grass supplementation improved the hematological picture as well as liver function tests .This results of this study show the immunostimulant effects of wheat grass .Oral administration of wheat grass @ 5 gm/kg body weight (Group T_2) may be good for building blood, for adjuvant therapy in anemia treatment and health improving adjuvant in liver disorders.

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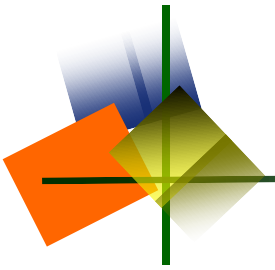
LIST OF ABBREVIATIONS

µl	:	Microliter
ALT	:	Aspartate Aminotransferase
AST	:	Alanine Aminotransferase
B. wt.	:	Body weight
ESR	:	Erythrocyte Sedimentation Rate
et al.	:	Associates
Fig.	:	Figure
gm	:	Gram
gm/dl	:	Gram per Desilitre
Hb	:	Hemoglobin
HbF	:	Fetal Hemoglobin
HCL	:	Hydrochloric acid
HDL	:	High Density Lipoprotein
ICDDRB	:	International Centre for Diarrhoeal Disease Research Bangladesh
J.	:	Journal
Kg	:	Kilogram
LDL	:	Low Density Lipoprotein
mg	:	Milligram
ml	:	Milliliter
mm	:	Milimeter
mm^3	:	Cubic Millimeter
No.	:	Number
NS	:	Non Significant
PBS	:	Phosphate Buffer Solution

PCV	:	Packed Cell Volume
RBC	:	Red Blood Cell
rpm	:	Revolutions per Minute
SE	:	Standard Error
SED	:	Standard Error Difference
SGOT	:	Serum Glutamate Oxaloacetate Transaminase
SGPT	:	Serum Glutamate Pyruvate Transaminase
SM	:	Sample Mean
SPSS	:	Statistical Package of Social Science
TC	:	Total Cholesterol
TEC	:	Total Erythrocyte Count
TLC	:	Total Leucocyte Count
U.S.	:	United States
U/L	:	Units Per Litre
Vol.	:	Volume
WBC	:	White Blood Cell
WHO	:	World Health Organization



Chapter 1

INTRODUCTION

CHAPTER 1

INTRODUCTION

Herbs and plants have been used for medicinal purposes long before prehistoric period. From ancient times, various herbs have been traditionally used for preventing and curing a wide array of diseases. In modern times also native villages and tribal areas rely mainly on these natural methods for maintaining healthy lives and dealing with ailments. They are a kind of alternative medicine that is inexpensive and has no side effects. For example: wheat grass, aloe vera, curcumin, alfalfa, garlic, ginger, German chamomile, grapefruit, green tea etc. Laboratory research on the health benefits of cereal grasses has increased over the past two decades in the United States and Japan (Bing, 1939). In 2002, the U.S. National Center for Complementary and Alternative Medicine of National Institutes of Health began funding clinical trials about the effectiveness of herbal medicines. In 2010 survey of 1000 plants was completed, out of which 356 had clinical trials published evaluating their pharmacological activities & therapeutic applications. One of these plants, wheat grass has been gaining much popularity and claim about its health benefits range from providing supplemental nutrition to detoxification of liver and blood. The consumption of wheat grass in the western world began in the 1930s as a result of experiments conducted by Charles F. Schnabel in his attempts to popularize the plant (Murphy, 2006) and began documenting a wide range of its health benefits, based on his observations in animals and humans. Wheat grass is young grass, commonly known as *Triticum aestivum* Linn (Poaceae). In the 1970s, Ann Wigmore renewed the popularity of wheat grass. Based on her personal health experience, Wigmore wrote books and lectured on the benefits of wheat grass, as part of a raw/living food diet. Wheat grass is used as a health improving adjuvant in several diseases as folk medicine

(Shemer , 2008). 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 (Schnabel, 1940). According to ayurvedic texts, blood and wheat grass juice are similar in the form of substance, quality and action (Shukla and Tripathi, 2005). Wheat grass juice 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 (Wigmore, 1985). Wheat grass juice has chlorophyll that neutralizes infections, heals wounds, overcomes inflammations and gets rid of parasitic infections the three most important effects of wheat grass on the human body are: blood purification, liver detoxification and colon cleansing (Miller, 1941, Kothari *et al.,* 2011). This is because wheat grass juice is the richest source of vitamins A, B, C, E and K, calcium, potassium, iron, magnesium, sodium, sulphur and 17 forms of amino acids (Kapil, 2012). Wheat grass juice contains all the nutrients the body requires and is considered a complete health tonic (Smith, 2000; Wigmore, 1986; Lam and Brush, 1950). Wheat grass juice has been shown to reduce blood transfusion requirements in patients with beta thalassemia (Marwaha *et al*., 2004). Wheat grass juice has been documented to be effective for an unrelated condition ulcerative colitis, in a randomied double blind placebo controlled trial from Israel (Ben-Arye *et al*., 2002). Wheat grass juice is great for blood disorders of all kinds (Ferruzzia and Blakesleeb, 2007).

WHO guideline denotes hemoglobin level in healthy male below 13 g/dl is abnormal and in female below 12g/dl is abnormal. This condition refers as anaemia. There are several types of anaemia. Nutritional or vitamin deficiency anaemia refers to a reduced red blood cell count due to poor diet habit, which is deficient in iron, proteins, vitamins like vitamin c, vitamin b12 along with folic acid (Sembulingam , 2005). Deficiency of above

nutrients can affect production and life expectancy of red blood cells. Anaemia is a wide spread public health problems associated with an increased risk of morbidity and mortality, especially in pregnant woman and children. India is among the countries with highest prevalence of iron deficiency anemia (IDA) in the world. Anaemia is one of the largest public health problems in Bangladesh. In 2011, the national prevalence of anaemia was 51 % in children under 5 years of age and 42% among non pregnant women. It develops mainly because of lack of proper food habits. Hemoglobin testing is the primary method of anaemia diagnosis (Kariyeva *et al.*, 2001). Wheat grass is rich source of minerals, vitamins, antioxidants, amino acids and many enzymes. It is significant nutritious and medicinal value with rich source of chlorophyll. It is a natural source of iron. P^H of blood and wheat grass juice are same. Chlorophyll and hemoglobin both are structurally very similar i.e. 7.4. That is the reason for quickly absorption of juice in blood as both are chromo protein. The vital component of wheat grass helps in building hemoglobin in our body. The presence of 70% chlorophyll, which is almost chemically identical to hemoglobin. The only difference is that the central element in chlorophyll is magnesium and in hemoglobin it is iron in hemoglobin (Wigmore, 1985). Chlorophyll in wheat grass is more useful in various clinical conditions involving hemoglobin deficiency and other chronic disorders ultimately considered as green blood.

Chlorophyll improves blood sugar problems (Smith, 2000). Blood is the major component of a body. The blood carries nutrient and materials to different parts of the body. The most abundant cells in vertebrate blood are red blood cells. These contain hemoglobin, an iron-containing protein, which facilitates oxygen transport by reversibly binding to this respiratory gas and greatly increasing its solubility in blood. Aro and Akinmoegun, 2012 and Aro *et al.,* (2013) reported that haematological parameters like haematocrit value, haemoglobin concentration, White Blood Cell count, Red Blood Cell

count among others are used in routine screening for the health and physiological status of livestock and even humans. Adejumo (2004) reported that haematological traits especially Packed Cell Volume (PCV) and Haemoglobin (Hb) were correlated with the nutritional status of the animal. Isaac *et al*., (2013) stated that PCV is involved in transport of oxygen and absorbed nutrient. Therefore, whatever affects the blood either drugs, pathogenic organism or nutrition will certainly affect the entire body adversely or moderately in terms of health, growth, maintenance and reproduction (Oke *et al*., 2007; Etim 2010). Haematological studies represent a useful process in the investigation of the extent of damage to the blood. Examination of blood provides the opportunity to clinically investigate the physiological, nutritional and pathological status of a man and animal. Evaluations of the haematological profile usually furnishes vital information on the body's response to injury of all forms, including toxic injury (Schalm *et al*., 1975; Coles, 1986 and Ihedioha *et al*., 2004).

Specific research objectives: For that research or study is made to observe the following specific objectives:

- To know the effect of wheat grass on live body weight in rabbit.
- To determine the effect of wheat grass on haemato-biochemical parameters in rabbit.

Chapter 2

REVIEW OF LITERATURES

CHAPTER 2

REVIEW OF LITERATURE

The purpose of this chapter is to provide a selective review of the research works accomplished in relation to the present study. Literatures effect of wheat grass rabbit model which is related to this study has been reviewed under the following heading.

2.1 Wheat grass

Wheat grass is Shoot of *Triticum aestivum* Linn. is called as a wheat grass, belonging to family: Gramineae. Triticum is a genus of annual and biennial grasses, yielding various types of wheat, native to south west Asia. Common or bread wheat, is widely cultivated almost all over the world. Nutritionally, wheat grass is a complete food that contains 98 of the 102 earth elements. One of the ingredients with major benefit in wheat grass is chlorophyll, which has the ability to draw toxins from the body like a magnet. Considered the "blood of plants", chlorophyll can soothe and heal tissues internally. Dr. Ann Wigmore, U.S.A. founder director of the Hippocrates Health Institute, Boston, U.S.A. was one of the proponents of 'Wheat grass Therapy'. Dr. Wigmore reported that "wheat grass" used in her program contain abscisic acid and laetrile, both of which may have anti-cancer activity. Wheat grass is a good source of mineral nutrients. It contains significant amount of iron, phosphorus, magnesium, manganese, copper & zinc. Wheat grass is a rich source of tocopherols with high vitamin E potency. Wheat grass stimulates metabolism, restores alkalinity to the blood, its abundance of alkaline minerals helps reduce over acidity in the blood. Wheat grass is also a detoxificant and helps restore healthy cells (Fahey *et al.,* 2005). Wheat grass, young grass of common wheat plant, is freshly juiced or dried into powder for animal and human consumption-both the forms provide chlorophyll, amino acid minerals, vitamins and enzymes (Walters ,1992).

2.2 Historological background

Wheat grass can be traced back in history over 5000 years, to ancient Egypt and perhaps even early Mesopotamian civilizations. It is purported that ancient Egyptians found sacred the young leafy blades of wheat and prized them for their positive effect on their health and vitality. The consumption of wheat grass in the Western world began in the 1930s as a result of experiments conducted by Charles Schnabel, food scientist who experimented with various mixtures of grain and feed and found that chickens fed on mixtures that contained a high proportion of wheat grass had grown better, were healthier. Further experimentation on other animals yielded the same results. Animals fed on wheat grass were undoubtedly healthier than those fed on other grains (Singh *et al.,* 2012). By 1940, cans of Schnabel's powdered grass were on sale in major drug stores throughout the United States and Canada. Ann Wigmore was also a strong advocate for the consumption of wheat grass as a part of a raw food diet. Wigmore, founder of the Hippocrates Health Institute, believed that wheat grass, as a part of a raw food diet, would cleanse the body of toxins while providing a proper balance of nutrients as a whole food. She also taught that wheat grass could be used to treat those with serious disease. Both of these claims are believed by many reputable health institutes to be entirely unfounded by facts, and possibly dangerous.

2.3 Phytochemistry

The name "green blood" of wheat grass is attributable to its high chlorophyll content which accounts for 70% of its total chemical constituents. Dr. Brusher calls it as Concentrated Solar Energy. Sachin *et al.,* (2013) reported that, wheat grass has been an integral part of Indian culture for thousands of years, and has been known to have outstanding healing properties. Some studies shows that wheat grass has chemical

constituents such as vitamins A, C, E and B complex. It contains a plethora of minerals (92) like calcium, phosphorus, magnesium, alkaline earth metals, potassium, zinc, boron, and molybdenum and iron. Iron is an essential element for life. Iron deficiency creates shortage of hemoglobin in blood. It is helpful in pregnancy, for excessive sweating, pale complexion, laziness and lethargy, and insomnia. Inorganic iron is often constipating, but the iron salts in wheat grass have no side effects. Wheat grass is also a source of vitamin B-17, also known as amygdaline, which some studies suggest can help ward off cancer. The various enzymes responsible for its pharmacological actions are protease, amylase, lipase, cytochrome oxidase, transhydrogenase, super oxide dismutase (SOD). The other notable feature of wheat grass is its high proportion of amino acids (17) such as aspartic acid, glutamic acid, arginine, ala-nine and serine. The major clinical utility of wheat grass juice is due to its antioxidant action which is derived from its high content of bioflavonoids like apigenin, quercitin and luteolin. Other compounds present, which make this grass therapeutically effective, is the indole compounds, apigenin and laetrile [Figure 1] and [Figure 2].

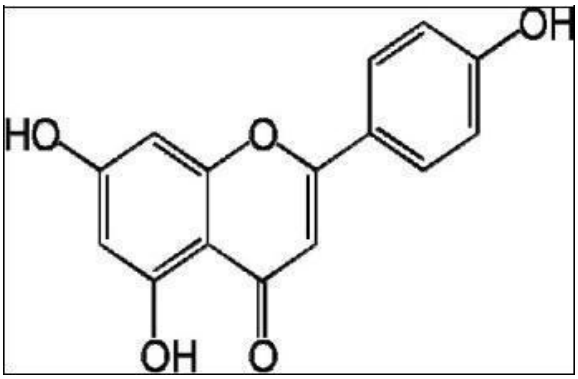


Fig 1: Structure of apigenin

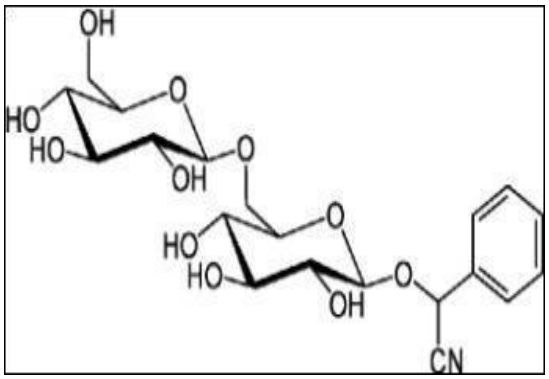


Fig 2: Structure of laetrile

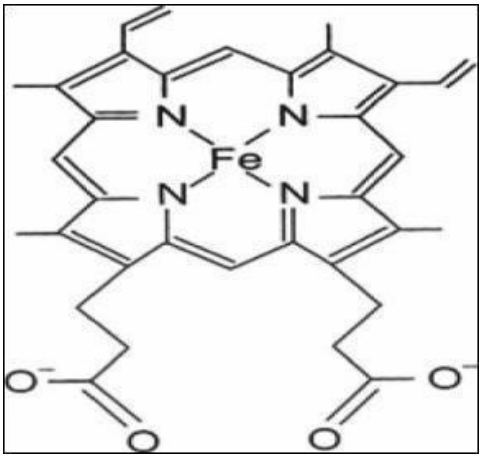


Fig 3: Structure of tetra pyrrole head of hemoglobin

Table 1: Nutritional value of Wheat Grass Juice per 100g (3.5oZ) (Roshan *et al.,* 2016)

Energy- 327 Kcal	Protein- 12.63g
Carbohydrates- 71.18g	
Sugar- 0.41g	Fat- 1.54g
Dietary fibre- 12.2g	
Iron- 126 mg	Calcium- 29mg
Magnesium- 3.985mg	Phosphorus- 288mg
Potassium- 363mg	Sodium- 2mg
Thiamine- 0.383mg	Riboflavin- 0.115mg
Niacin- 5.464 mg	Pantothenic acid- 0.954 mg
Pyridoxine- 0.3mg	Folate - 38µg
Vitamin E- 1.01mg	Choline - 3.12mg
Vitamin K- 1.9µg	Zinc (28%) 2.65 mg

2.4 Therapeutic potential

2.4.1 Chlorophyll as green blood

Wheat grass is rich in chlorophyll and enzymes. It contains more than 70% chlorophyll (which is an important dietary constituent). The analogy between chlorophyll and hemoglobin can be demonstrated with respect to the structure of their porphyrin heads. The structure of both the compounds depicts a striking similarity in having a tetra pyrrole ring structure, the only difference between the two being the nature of the central metal atom magnesium (Mg) in chlorophyll and iron (Fe) in hemoglobin. The apparent resemblance between the two is thus considered to be responsible for the therapeutic effects shown by chlorophyll in conditions involving deficiency of hemoglobin and because of this wheat grass is called 'Green Blood' (Padalia *et al.,* 2010). A 70-83% increase in red blood cells and hemoglobin concentratration was noted within 10-16 days of regular administration of chlorophyll derivatives (Kelentei *et al.,* 1958). It was reported that chlorophyll enhanced the formation of blood cells in anemic animals (Borisenko and Sofonova, 2001). Chlorophyll is soluble in fat particles, which are absorbed directly into blood via the lymphatic system. In other words, when the blood of plants is absorbed in humans it is transformed into human blood, which transports nutrients to every cell of the body. Chlorophyll present in wheat grass can protect from carcinogens; it strengthens the cells, detoxifies the liver and blood stream, and chemically neutralizes the polluting elements. Hemoglobin and its congeners are protein bodies which act as the oxygen carrier in higher animals by binding two electrons attached to the oxygen molecule, whereas chlorophyll is the active metabolic agent in plants which assimilates carbon from the carbon dioxide of the atmosphere by producing two electrons which are then transmitted through electron transport chain. The structural similarity between the two compounds is stipulated to be the reason behind the limited

use of chlorophyll as a blood substitute in conditions like chronic anemia, tissue hypoxia, thalassemia and other hemolytic disorders etc.

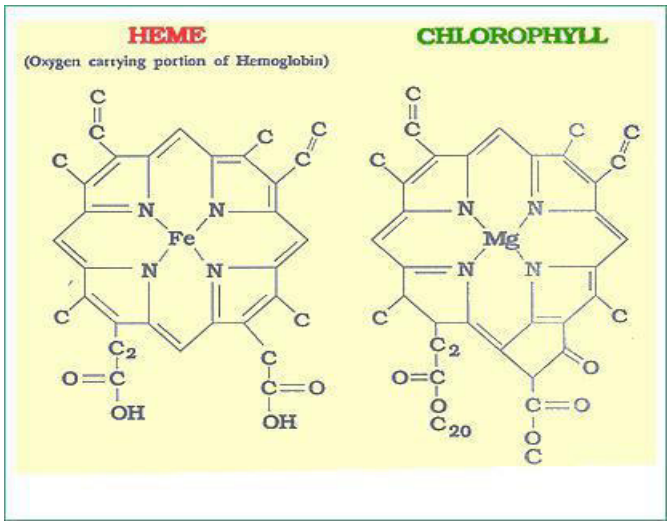


Fig. 4. Structure of hemoglobin and chlorophyll (Wigmore , 2011)

Bhikaji *et al.,* (2015) demonstrate the effect of wheat grass juice on hemoglobin level w.s.r. to Samanya Vishesha Siddhanta. For this study 30 subjects were selected in the study and divided into 3 groups on basis of their hemoglobin level. Group A having Hb%, 13gm% to 16gm%, group B having Hb% 10gm% to 13gm% and group C having Hb% below 10gm%. After 21 days the symptomatic improvement and increase in hemoglobin level in group C was noticed as compared to group A and group B. No significant therapy is there for chronic anaemias in modern science.. Result shows significant effect on the improvement of hemoglobin level in 21 days therapy

Yadav and Sethi (2017) demonstrate the effects of wheat grass on haematologital parameters in high fat diet rabbit. For this study 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 wheat grass 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 wheat grass.

Rana *et al*., (2011) reported that, wheat grass juice helps in building red blood cells and stimulates healthy tissue cell growth. 100 g of wheat grass powder is equal to 23 kg of fresh vegetables. Ideally, wheat grass should be taken about an hour prior to meal. This allows the body to fully metabolize it without competing with other foods, and it may also curb hunger. It is recommended that lot of water (at least a liter) should be consumed with the juice to reap its maximum nutritional benefits. Taking wheat grass as a supplement in the mid-morning or mid-afternoon is a great time for this "green" energy boost.

Hughes & Latner (1936) demonstrate that effects of pure chlorophyll, large doses of crude, unrefined chlorophyll and one with a magnesium free derivative, (which later came to commercial use as chlorophyllin.) Results showed that pure, refined chlorophyll in large doses has no effect on the speed of haemoglobin regeneration, very small doses markedly increases regeneration. Crude, unrefined chlorophyll is effective, even in large doses and magnesium free chlorophyll was shown to also aid regeneration when given in large doses. The haemoglobin levels in anaemic rabbits showed recovery after 12 days of a large dose of crude, unrefined chlorophyll.

Lalit *et al.,* (2014) investigate immunostimulatory effects of fresh etiolated wheat grass juice (FEGJ) in dexamethasone induced immunosuppressed albino rat through study of neutrophil adhesion percentage and phagocytic efficiency via carbon clearance test. For this study albino rats of both sexes were divided into four group (n=6). Group I (NC) was kept as normal control. Group II (ISC) animals were injected intra peritoneally with dexamethasone phosphate (5mg/kg) twice a day for 5 days and kept as immune suppressed control. Group III (FEGJ 20ml/kg) and Group IV (FEGJ 40 ml/kg) both were injected intraperitoneally with dexamethasone phosphate (5mg/kg) twice a day for 5 days. After this, from 5^{th} to 15^{th} day, animals were orally administrated with FEGJ at the dose of 20ml/kg and 40 ml/kg respectively. Results showed that In dexamethasone induced immune suppressed, FEGJ treated groups (FEGJ 20ml/kg and FEGJ 40ml/kg) significant increase in neutrophil adhesion percentage and significant decrease in mean carbon clearance time were observed.

Yadav *et al.,* (2013) showed the effect of *triticum aestivum* on physiological and biochemical parameters in high fat diet fed rabbit. For this study thirty rabbits were included that were divided into 3 group and ten of each. Group i received control diet, group ii high fat diet and group iii high fat diet along with wheat grass powder. The fasting blood sample analysis was done for various physiological and biochemical parameters. High fat diet induced derangement of liver functions (SGOT, SGPT, total protein and serum albumin) but the kidney functions remained unaffected. Result showed that wheat grass supplementation improved the hematological picture as well as liver function tests. Wheat grass can be used as health improving adjuvant in liver disorders.

2.4.2 Blood building activity in thalassemia major

Beta thalassemia is a genetically inherited disorder that arises due to abnormal beta globin chains which are required for the synthesis of adult hemoglobin (HbA). The characteristic deficiency of beta globin chains, seen in thalassemia results in the production of abnormal red blood cells (RBCs) having a preponderance of alpha globin chains.

This leads to destruction of such RBCs in the spleen and a decreased number of RBCs in the blood. Individuals with thalassemia may continue to produce gamma globin chains in an effort to increase the amount of fetal hemoglobin (HbF) and compensate for the deficiency of HbA (Susan *et al.,* 1993). Thus, induction of fetal hemoglobin in thalassemia can improve the patient's clinical condition. Drugs exhibiting this function like hydroxyurea are not used conventionally due to lack of specificity and greater degree of side effects (Fibach *et al.,* 1993). Wheat grass is an effective alternative to blood transfusion as the pH factor of human blood is 7.4 and the pH factor of wheat grass juice is also 7.4, which is why it is quickly absorbed into blood. Wheat grass has the potential to increase the hemoglobin (Hb) levels, increase the interval between blood transfusions, and decrease the amount of total blood transfused in thalassemia Major and intermediate Patients. Wheat grass sprout extract has been tested for its ability to induce fetal hemoglobin (HbF) production using advanced DNA technology. A rapid 3-5 fold increase in the production of HbF on consumption of wheat grass has been reported using a cellular assay. This has now been confirmed by the development of a specific assay method for HbF, which is based on detecting its production in human erythroleukemia cells using a fluorescent protein gene that replaces the genes for HbF.

The level and speed of induction of HbF by the wheat grass extract is significantly greater than any of the pharmaceutical inducers available. Chlorophyll extracted from the wheat grass plant or its synthetic derivative chlorophyllin has also been implicated in this clinical condition. The antioxidant mechanism of the various wheat grass constituents may be responsible for the beneficial effects. The enhanced anti-oxidative capacity of the RBCs may prolong the survival time of not only the newly formed cells, but also of the transfused RBCs (Radhey *et al.,* 2007).

Marwaha *et al.,* (2004) have reported in their pilot study that wheat grass juice reduces blood transfusion requirement in patients with thalassemia major. For this study thalasemic patients consumed about 100 ml of wheat grass juice daily. Each patient acted as his own control. Observations recorded during the period of intake of wheat grass juice were compared with one year period preceding it. Variables recorded were the interval between transfusions, pre transfusion hemoglobin, amount of blood transfused and the body weight. A beneficial effect of wheat grass juice was defined as decrease in the requirement of packed red cells (measured as gm/Kg body weight/year) by 25% or more. 16 cases were analyzed. Blood transfusion requirement fell by >25% in 8 (50%) patients with a decrease of >40% documented in 3 of these.

Shah *et al.,* (2011) investigate the therapeutic role of *triticum aestivum* (wheat grass) in busulfan induce thrombocytopenia .For this study 30 rats were divided into 5 groups and wheat grass juice given into busulfan induced thrombocytopenic rat. Result showed that fresh juice, methanol and acetone extracts of *T. aestivum* significantly increase Hb levels, RBC, total WBC and differential WBC counts in pancytopenic rats. Wheat grass significantly increase Fig.let counts and reduce bleeding and clotting time in thrombocytopenic rats.

Singh *et al.,* (2010) reported that in thalassemia major children taken wheat grass tablets 2-3,6,8 tablet per day in divided dose aged 1-3 years, 4-8 years and below 8 years respectively. Result shows that there is increase Hb level, increase interval between blood trasfussions, decreased amount of blood transfused.

2.4.3 Adjuvant therapy in anemia

Mathur *et al.,* (2017) use wheat grass juice for the treatment of anaemia in young women. Results showed that wheat grass juice has significant effect on blood haemoglobin level and helps to cure anemia. No side effect of juice was complained by any of the subjects. It was seen that wheat grass juice therapy decreased the total volume of blood transfused and increased the intervals between blood transfusions of the entire study cohort. These analyses suggested that not only is this therapy effective, but also that the benefit is related to the duration of the wheat grass juice therapy. The beneficial effects of this therapy have been attributed to its rich nutritional content that includes antioxidant vitamins (C & E) and bioflavonoids. The effects of the wheat grass juice therapy may be due to the action of natural antioxidants of Red Blood Cell (RBC) antioxidant function and corresponding effects on cellular enzyme function and membrane integrity. This thought is supported by studies that show decreased antioxidant capacities of RBCs of patients with hemolytic disorders as well as beneficial effects on RBC life span by supplementation of antioxidants in vivo longer lifespan. Aqueous extracts of wheat grass are good sources of antioxidants. Significant antioxidant activity was demonstrated by in vitro studies. The clinical studies conducted on human breast cancer have shown that chlorophyllin, a compound that is similar to chlorophyll produced synthetically, has capability to reduce the risk of breast cancer.

2.4.4 Hepatoprotective role of wheat grass

Triticum aestivum leaf extract affects liver enzyme activities as well as lipid peroxidation (Arya *et al.,* 2011. Jain *et al.,* 2007 reported the hepatoprotective role of fresh wheat grass juice has in CCl_4 treated rats. It showed a significant hepatoprotective effect with a dose of 100mg/kg/day in terms of SGOT, SGPT, ALP and Bilirubin in serum (Jain *et al*., 2007). Recently, the hepatoprotective effect of wheat grass tablets in CCl_4 treated rats has been investigated in a lab (unpublished data). Maximum hepatoprotection in this study has been observed with 80mg/kg /day dose of wheat grass tablets. This study indicated that wheat grass treatment prevented the increase in liver enzymes depending on the dose of wheat grass (Kamboj *et al.,* 2011). Decreased oxidative stress and increased antioxidant levels have also been observed with wheat grass treatment (Kamboj *et al.,* 2011). Three compounds (Choline, magnesium and Potassium), found abundantly in wheat grass, help the liver to stay vital and healthy. Choline works to prevent the deposition of fat. Magnesium helps to draw out excess fat in the same way. Magnesium sulfate (Epsom salts) draws pus from an infection, and potassium acts as an invigorator and stimulant (Wigmore 1985).

2.4.5 Detoxifying activity

The vitality of liver is of high concern for the overall well being of an individual as it is the major organ implicated in detoxification. In addition to the stimulating and regenerative properties of chlorophyll, other constituents of wheat grass juice like choline and its high mineral content are responsible for the therapeutic benefit. In a study conducted to observe the effect of choline on liver, it was seen that choline prevents the deposition of fats in the experimental animals liver when they were administered a diet rich in cholesterol (Best and Ridout, 1933). Choline promotes the removal of the esters

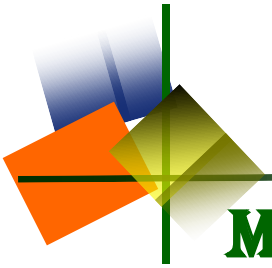
of both cholesterol and glycerol, with the effect on the glyceride fraction preceding that on the cholesterol esters. The lipotropic action of choline is attributed to its in vivo conversion to an active compound which is retained within the hepatic cells and enhances the oxidation of fatty acids and formation of tissue lecithins. The latter effect augments lipoprotein synthesis, which acts as a transport form of fatty acids in plasma and thus helps in removal of lipids from a fatty liver (Best *et al.,* 1931). It has been demonstrated experimentally that the dietary indoles like indole-3-carbinole and ascorbigen increase the activity of phase I and phase II xenobiotic metabolic enzymes in the liver and intestinal mucosa (Christine *et al.,* 2001). Thus the indole compounds of wheat grass may have a role in the deactivation of carcinogens.

An animal study by Kothari *et al.,* (2011) found that wheat grass reduced total cholesterol, LDL, bad cholesterol, and triglyceride levels in rats treated with wheat grass juice. Triglyceride levels fell by 38 percent in rats given the highest dose of the juice, 10 ml/kg consumed orally once daily for 21 days, which is equivalent to the results achieved by the common cholesterol lowering medication, atorvastatin (Locniskar , 1988).

A study by Sethi *et al.* (2010) found that supplementation with wheat grass in subjects consuming high fat diets resulted in the improvement of blood cholesterol levels. This study involved 30 animal subjects; authors had noted that the antioxidant effects of wheat grass appeared to be responsible for the decreasing of total blood cholesterol levels, and increasing good or HDL cholesterol, as well as the vitamin C blood levels. Therefore, the beneficial role of wheat grass in ameliorating hyperlipidemia and the associated oxidative stress has also been reported (Kothari *et al.,* 2008).

2.5 Wheat grass and general well being

Wheat grass loaded with vitamins A, C, and E acts as an anti oxidant and retards ageing of cells in the body that causes brain and heart problems. Components of wheat grass help in making menopause more manageable. Wheat grass is an effective tonic, beneficial for arthritis, skin allergies, graying or hair loss, weakness, kidney stones, weak eyesight, pyorrhea, or dental infections and fatigue. It is also super effective in serious cases of heart disease, acute stomach ache, infection of digestive system, gas, paralysis, asthma, constipation, diabetes, leucoderma, leukemia, and other cancers (Fahey *et al.,* 2005). It restores fertility and promotes youthfulness because the high magnesium content in chlorophyll builds enzymes that restore sex hormones. Wheat grass helps to detoxify the body by breaking impacted matter in the colon. Wheat grass juice is a fast and sure way to cleanse the body from environmental pollutants. Its high levels of enzymes and amino acids work like a natural cleanser to detoxify the liver, eliminate toxic heavy metals from the blood stream, rid the body of waste matter, and slow down the aging process (Wheat and Currie, 2008).



Chapter 3

MATERIALS AND METHODS

CHAPTER 3

MATERIALS AND METHODS

This research work was conducted at animal research shed in the Department of Physiology and Pharmacology, at Hajee Mohammad Danesh Science & Technology University, Dinajpur for a period of 45 days to evaluate the effect of wheat grass on hemato-biochemical parameters and body weight in rabbit.

3.1 Experimental site

The laboratory animal house at the Department of Physiology and Pharmacology was the Experimental Site.

3.2 Collection wheat grain and cultivation of wheat grass

Wheat grains were purchased from the local market in Dinajpur town at reasonable price.

Materials required

- Wheat seed
- Tray (2 x 3 feet)
- Soil
- Compost
- Paper towel
- Spray bottle

For cultivation of wheat grass three parts planting soil was mixed with one part of compost. Then it was place in 2 inch deep trays (2 x 3 feet). 2 cup of wheat seed was soaked for 24 hours then rinse. Then small amount of water was given to the soil mixture and the wheat seed was spread evenly over the moist soil. Wheat seed was covered with

a paper towel and the tray was place near a window to ensure proper ventilation for three days and it was keep away from direct sunlight. For the first three days, in the morning, water was given so that seeds are completely soaked in water. In the evening, lightly spray water with a spray bottle. On the fifth day, the young shoots was grow above 1 inch. Then water was given only once a day. Around 10-14 days the wheat grass was grown to 9-10 inch and it was ready for harvesting. At this stage, the wheat grass is at its nutritional peak. For obtaining fresh wheat grass same procedure was followed for other trays.

3.3 Experimental design

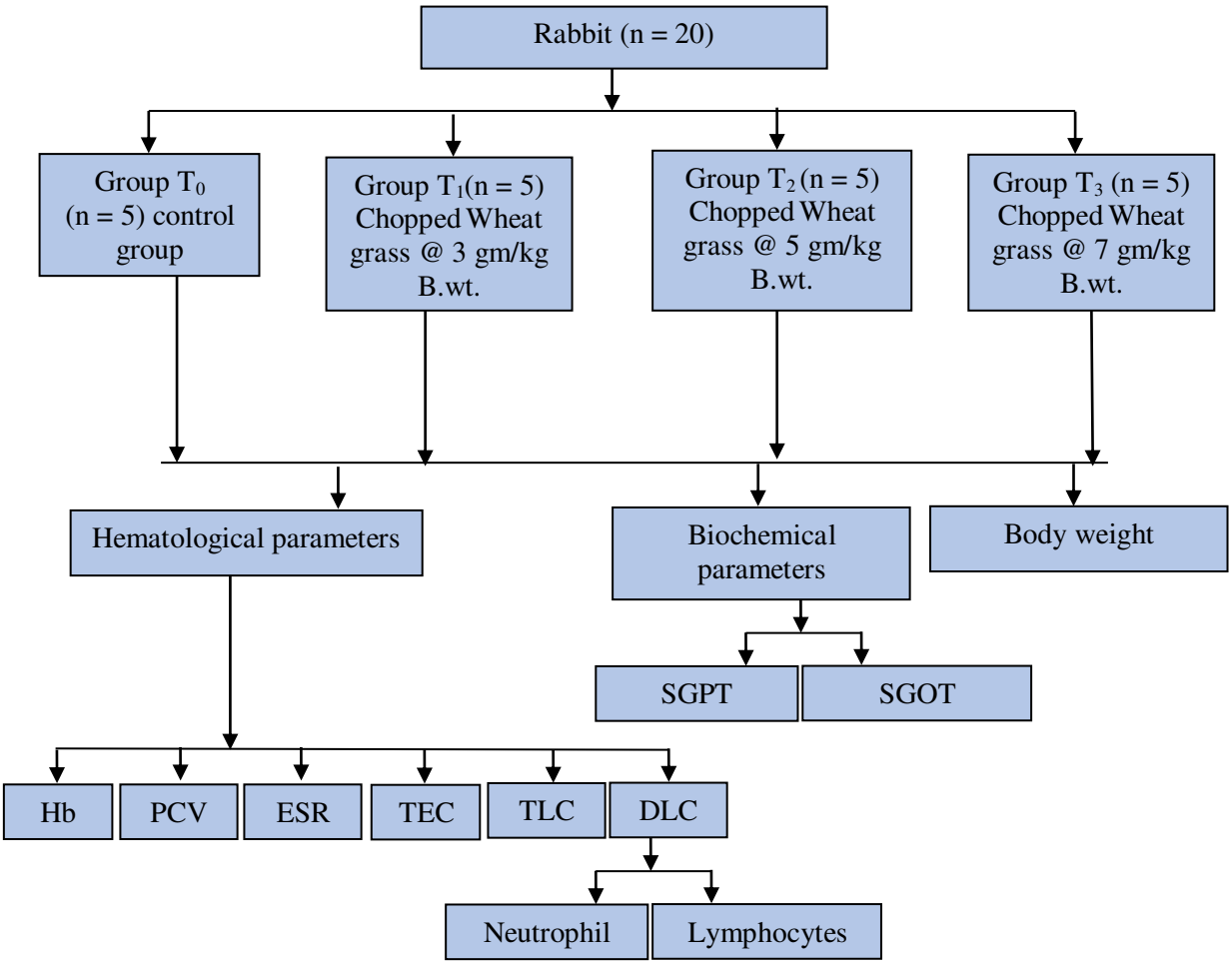


Fig. 5: Experimental layout

3.4 Experimental animal

Twenty New Zealand White rabbits aged about 4 months and weighting between 1200 to1500 gm were collected from local farm in Dinajpur.

3.5 Preparation of house

First the room as well as the wire cages were washed by sweeping and washing with tap water using hose pipe connected with the tap. The room was disinfected with a phenolic disinfectant (phenyl) and allowed to dry leaving the room unused with the electric fan and the bulb switched on. The room was properly ventilated and air conditioned. All the utensils required for the experiment such as feeder, water bottle, micro tube, syringe, needle etc. were collected and the laboratory was properly designed.

3.6 Acclimatization of rabbit

All the rabbit were housed at screen bottomed wire cages arranged in rows and kept in the departmental (Physiology and Pharmacology, HSTU) animal house. The animals were fed with pellet at a recommended dose of 150 g/kg as advised by ICDDRB. Drinking water was supplied adlibitum. The rabbits were maintained in this condition for a period of two weeks to acclimatize them prior to experimental uses.

3.7 Experimental animal grouping

Twenty rabbits were used to carry out this investigation. These rabbits were divided into 4 groups containing 5 rabbits in each group. The groups were designated and maintained as follows:

Group T_0: The rabbits were fed normal diet and given water adlibitum and then their body weight were recorded after acclimatization. Body weights, hematologial and

biochemical parameters were measured at the time when that of other groups was measured. This group was served as control group.

Group T_1: After acclimatization, body weights were measured .Then chopped wheat grass @ 3 gm/kg body weight was given to the rabbit up to 45 days. During that period on day 15, 30, 45 body weight, hematological and biochemical parameters were measured.

Group T_2: After acclimatization, body weights were measured. Then chopped wheat grass @ 5 gm/kg body weight (Yadav *et al*., 2017) given to the rabbit up to 45 days. During that period on day 15, 30, 45 body weight, hematological and biochemical parameters were measured.

Group T_3: After 2 weeks of acclimatization, body weights of rabbit were measured. Then chopped wheat grass @ 7 gm/kg body weight were given to the rabbit up to 45 days. During that period on day 15, 30, 45 body weight, hematological and biochemical parameters were measured.

3.8 Collection, preparation and feeding of wheat grass

Materials required

- Scissors
- Plastic tray for feeding of rabbit
- Electric balance

Wheat grass were collected from own cultivated trays. Wheat grass were cut 1 inch above the root with the help of scissors .Then wheat grass were measured separately by electronic balance and chopped with the help of scissors. Then chopped wheat grass was given to rabbit for feeding.

Fig. 6: Wheat grain

Fig. 7: Growing wheat grass

Fig. 8: Chopped wheat grass

Fig. 9: Feeding of wheat grass

3.9 Determination of body weight

3.9.1 Materials required

- Leather gloves
- Electric balance

Body weight was taken on day 0 (pretreatment), 15, 30, 45 (during treatment) with the help of electric balance.

Fig. 10: Recording of body weight

3.10 Hematological test

3.10.1 Collection of blood

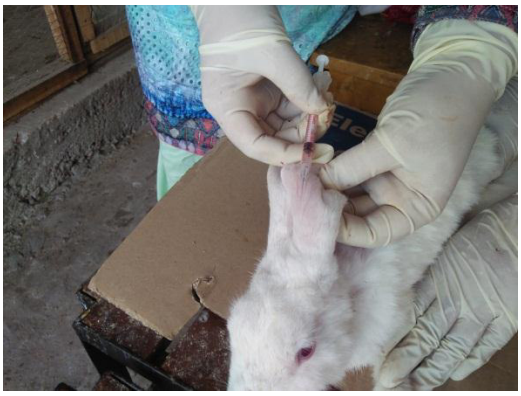


Fig. 11: Collection of blood

Fig. 12: Blood sample without anticoagulant

Fig. 13: Blood sample with anticoagulant

3.10.2 Materials required

- Leather gloves
- Syringe
- Ethanol
- Cotton
- Test tube with anticoagulant

Blood samples were collected from ear vein of rabbit of both control and treated groups. For determining hematological and biochemical parameters 3 ml blood were collected from each rabbit after 15, 30, 45 days of experiment. Immediately after collection of 1.5 ml blood was transferred to sterile test tube containing anticoagulant at a ratio of 1:10 for hematological examination. The rest (1.5 ml) blood of each rabbit was then transferred in separate sterile glass test tube. After clotting, the attachment of the clot to the wall of the test tube was detached by a long fine needle moving slowly in between the clot and test tube wall up to the bottom of the tube. Then the test tubes with clot were kept overnight at 4^0C in a refrigerator. In the next morning, the test tubes were centrifuged in a centrifuge machine (Hettich, Universal II, and Germany) at 1500 rotation per minute (rpm) for 15 minutes. The supernatant sera were collected by individual sterile Pasteur

pipette into corresponding marked sterile tubes and kept at -20^{0}C in deep freeze until tested.

(a) Hemoglobin Concentrations (Hb)

(b) Packed Cell Volume (PCV)

(c) Erythrocyte Sedimentation Rate (ESR)

(d) Total Erythrocyte Count (TEC)

(e) Total Leucocyte Count (TLC)

(f) Lymphocytes (%)

(g) Neutrophil (%)

3.10.3 Determination of hemoglobin concentrations

The N/10 hydrochloric acid was taken in a graduated tube up to 2 marks with the help of a dropper. Well-homogenized blood sample was then drawn into the Sahli pipette up to 20 cm. mark. The tip of the pipette was wiped with sterile cotton and the blood of the pipette was immediately transferred into the graduated tube containing hydrochloric acid. This blood and acid were thoroughly mixed by stirring with a glass stirrer. There was a formation of acid hematinic mixture in the tube by hemolysing red blood cells by the action of hydrochloric acid (HCL). The tube containing acid hematin mixture was kept standing in the comparator for 5 minutes. After that distilled water was added drop by drop. The solution was mixed well with a glass stirrer until the color of the mixture resembled to the standard color of the comparator. The result was read in daylight by observing the height of the liquid in the tube considering the lower meniscus of the liquid column. The result was then expressed in gm %. The above procedure was matched by the Helligehemo meter method as described by Lamberg and Rothstein (1977).

3.10.4 Determination of Packed Cell Volume (PCV)

The citrated well mixed blood sample was drawn into special loading pipette (Wintrobe pipette). The tip of the pipette was inserted up to the bottom of a clean, dry Wintrobe hematocrit tube. Then the Wintrobe tube was filled from the bottom by pressing the rubber bulb of the pipette. As blood came out, the pipette was slowly withdrawn but pressure was continued on the rubber bulb of the pipette so as to exclude air bubbles. The tip of the pipette was tried to keep under the rising column of blood to avoid foaming and the tube was filled exactly to the 10 cm mark. Then the Wintrobe hematocrit tube was placed in the centrifuge machine and was centrifuged for 30 minutes at 3000 rpm. Then, the hematocrit or PCV was recorded by reading the graduation mark; the percent volume occupied by the hematocrit was calculated by using the following formula as described by Lamberg and Rothstein (1977).

$$\text{PCV\%} = \frac{\text{Height of the red cell volume in cm}}{\text{Height of total blood in cm}} \text{ X } 100$$

3.10.5 Determination of Erythrocyte Sedimentation Rate (ESR)

The fresh anticoagulant blood was taken into the Wintrobe hematocrit tube by using special loading pipette exactly up to 0 marks. Excess blood above the mark was wiped away by sterile cotton. The filled tube was placed vertically undisturbed on the wooden rack for one hour. After one hour the ESR was recorded from the top of the pipette. The result was expressed in mm in 1st hour.

3.10.6 Determination of Total Erythrocyte Count (TEC)

For determination of TEC blood samples were drawn with red blood cell diluting pipette exactly up to 0.5 mark of the pipette. Outside of the tip of the pipette was wipped with

cotton. Then the tip of this pipette was immediately placed into the red cell diluting fluid (Hayem's solution) and the pipette was filled with the fluid up to 101 marks. The contents of the pipette were mixed thoroughly by shaking with 8-knot motion for 3-5 minutes. After discarding 2 or 3 drops of fluid from the pipette a small drop was placed to the edge of the cover glass on the counting chamber and the area under the cover glass was filled by the fluid introduced. One-minute time was spared to allow the cells to settle over the chamber uniformly and then the cells were counted from the recognized 80 small squares. After completion of counting total cells, the number of RBC recorded from the supplied samples were expressed as number of cells counted ×10, 000 and the result was expressed in million/cu. mm. of blood (Lamberg and Rothstein, 1977).

3.10.7 Determination of Total Leukocyte Count (TLC)

The principles involved in enumeration of Total Leukocyte Count were almost same to those of erythrocytes. Here the leukocyte diluting fluid was used N/10 HCl. Well mixed blood was drawn upto the 0.5 mark of while blood cell pipette. The diluting fluid was filled upto the 11 mark of the pipette and the contents were thoroughly mixed for 2 minutes. Two or three drops of contents were discarded and counting chamber was then filled in the same way as in the RBC count. The counting chamber was placed under the microscope and examined under low power objective (10 x). The leukocytes in the large squares (each 1 square mm) of the counting chamber were counted. The number of W. B. C. was calculated as follows: Number of WBC = No. of cell counted ×10 and the result is expressed in thousand per cu mm.

3.10.8 Determination of Differential Leucocyte Count (DLC)

For the counting of WBC Leishman's stained method was used .Making a blood smear then it was taken four clean and grease-free glass slides and select one as spreader. A

drop of oxalated blood on was place one end of a slide in the middle about 1cm from the end, by touching the slide to the top of the blood drop. With the help of a smooth clean edge of the spreader making an angle of 30^0-45^0 touch the blood drop, then the blood drop spreads across the length of the spreader, push the spreader to the other end of the slide with a smooth, quick, and controlled movement. A thin layered smear will be formed.2-3 smears of such were prepared. Blood smear was dry quickly by waving the film in the air. Staining procedure is Place the slides on the staining rack with the blood smear facing up. Pour 8-12 drops of Leishman's stain on the slide. The stain should just cover the smear, Leave it for 2 minutes. During this period, the alcohol in the stain fixes the cells (fixation time) after 2 minutes add double the amount of distilled water on the smear, with the help of a dropper without spilling until a greenish scum or metallic shiny layer is formed. Mix the stain and the water evenly by blowing gently or by shaking the slide Leave it for 8-10 minutes-(staining time). After 10 minutes overflow the stain with distilled water, and wash the slide gently and thoroughly under tap water. Shake off all water adhering to the slide and set the slide in an inclined position to dry. Focus under low power first and turn to oil immersion, count and identify the cells.

3.11 Biochemical parameters

The biochemical parameters were determined according to the method described by Deneke and Rittersdorf (1984) and Denecke *et al.* (1985) by using Reflotron® (Boehringer mannheim, Germany).

Following biochemical parameters were studied

(a) Serum Glutamate Oxaloacetate Transaminase (SGOT/AST).

(b) Serum Glutamate Pyruvate Transaminase (SGPT/ALT).

3.11.1 Determination of SGOT

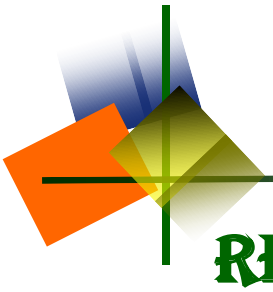
In brief, serum of the sample was 4-fold diluted in Phosphate Buffer Solution (PBS) with pH 7.4. Twenty five µl of diluted serum of blood was taken with the help of a capillary pipette avoiding any bubbles and was placed to the centre of the GOT test strip after removing the outer coverings of the strip. Care was taken in such a way that the tip of the pipette could not touch the application zone of the test strip. After opening the sliding cover of the machine, the test strip was placed on to the guide within 15 second and the test strip was forwarded until it locks into place. The sliding cover was closed properly. The GOT level of displayed on the monitor automatically after 1-2 minutes. The enzyme activity was expressed in U/L.

3.11.2 Determination of SGPT

SGPT is determined following the same procedure as done in case of SGOT. Serum sample was 4-fold diluted in Phosphate Buffer Solution (PBS) with pH 7.4. Twenty five µl of diluted serum of blood was taken with the help of a capillary pipette avoiding any bubbles and one drop was placed to the centre of the red application zone (xx) of the GPT test strip after opening the sliding cover of the strip. Within 15 second strip was placed on to the guide after opening the sliding cover of the machine and test strip was forwarded until it locks into place. Then the sliding cover was closed. The GPT level was displayed on the monitor within 2-3 minutes. The enzyme activity was expressed in U/L.

3.12 Data and statistical analysis

Collected data were analyzed using SPSS v.22 for Windows (SPSS Inc., Chicago, IL, USA). Statistically significant differences between group means were determined by analysis of variance (ANOVA). Mean values were considered significantly different at $P<0.05$. Data are expressed as mean ± SEM.



Chapter 4

RESULTS AND DISCUSSION

CHAPTER 4

RESULTS AND DISCUSSION

The experiment was conducted to study the effect of wheat grass on hematological, biochemical and body weight in rabbit. For this study 20 rabbits were randomly divided into four equal groups. Group T_0 rabbits were kept as control group. Next three groups (T_1, T_2 and T_3) were treated with (3 gm, 5 gm, 7 gm/kg body weight) of wheat grass for 45 days. All the control and treated rabbits were closely observed 45 days of treatment period and results were shown under different headings and subheadings.

4.1 Effect of wheat grass on hematological parameters

Table 2: Effect of wheat grass on hemoglobin

Day	Group				Level of Significance
	T_0 (Mean±SE) g/dl	T_1 (Mean±SE) g/dl	T_2 (Mean±SE) g/dl	T_3 (Mean±SE) g/dl	
Day 15	11.50±0.20	11.72±0.21	12.22±0.12	11.76±0.23	NS
Day 30	11.51^{a}± 0.22	12.46^{b}±0.19	13.34^{c}±0.24	12.60^{b}±0.19	*
Day 45	11.50^{a}±0.13	13.38^{b}±0.33	14.04^{bc}±0.04	13.20^{b}±0.26	*

Values with the different superscripts in the same row are statistically significant ($P<0.05$), NS = Non significant, * = Significant at 5% level of significance ($P<0.05$).

In this study, in group T_1, T_2 and T_3 supplementation with wheat grass (3 gm, 5 gm and 7 gm/kg B.wt.) do not produced any significant ($P<0.05$) effect in Hb levels as compared to control at day 15. At day 30 Hb (g/dl) was found significantly ($P<0.05$) highest in T2 (13.34 ± 0.24) which was followed by T3 (12.60 ± 0.19), T1 (12.46 ± 0.19) and T0 (11.51 ± 0.22) respectively and at day 45 Hb (g/dl) was found significantly ($P<0.05$)

highest in T2 (14.04 ± 0.04) which was followed by T3 (13.20 ± 0.26), T1 (13.38 ± 0.33) and T0 (11.50 ± 0.13) respectively. 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 wheat grass fed animals Wigmore, 1985. These results are in accordance with Bhikaji *et al*., (2015) where there was significant increase in Hb (8.46 + 1.18 to 11 + 1.46) level was found in group C receiving fresh wheat grass juice for 21 days.

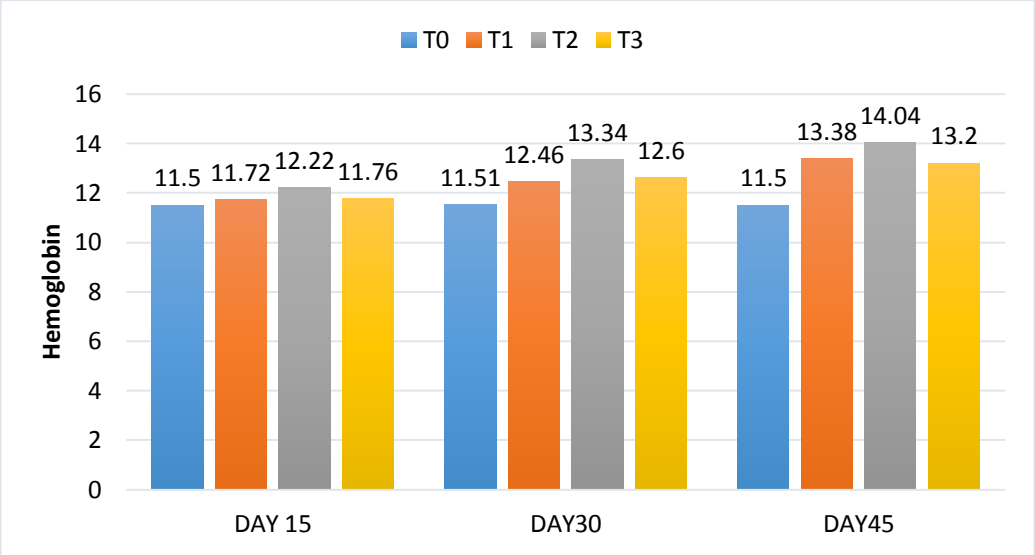


Fig. 14: Effect of wheat grass on hemoglobin

T_0 = control, T_1 = 3 gm/kg B.wt., T_2 = 5 gm/kg B.wt. and T_3 = 7 gm/kg B.wt.

Table 3: Effect of wheat grass on Packed Cell Volume

Day	Group				Level of Significance
	T_0 (Mean±SE) %	T_1 (Mean±SE) %	T_2 (Mean±SE) %	T_3 (Mean±SE) %	
Day 15	38.36 ±0.18	38.88 ±0.27	39.48 ±0.56	39.28±0.36	NS
Day 30	38.42^{a}±0.18	40.10^{b}±0.56	40.44^{b}±0.38	39.92^{ab}±0.56	*
Day 45	38.40^{a}±0.0.17	40.78^{b}±0.56	41.76^{b}±0.29	41.02^{b}±0.68	*

Values with the different superscripts in the same row are statistically significant (P<0.05), NS = Non significant, * = Significant at 5% level of significance (P<0.05).

Packed Cell Volume content was presented in (Table 3). In this study, in group T_1, T_2 and T_3 supplementation with wheat grass (3 gm, 5 gm, 7 gm/kg B.wt.) produced significant (P>0.05) increase in PCV levels as compared to control and highest level was found in group T_2 (41.76 ± 0.29) but statistically not significant. These results are in accordance with the results of a study by Yadav *et al.,* (2017) where there was significantly (P>0.05) increase in PCV level in the group receiving wheat grass.

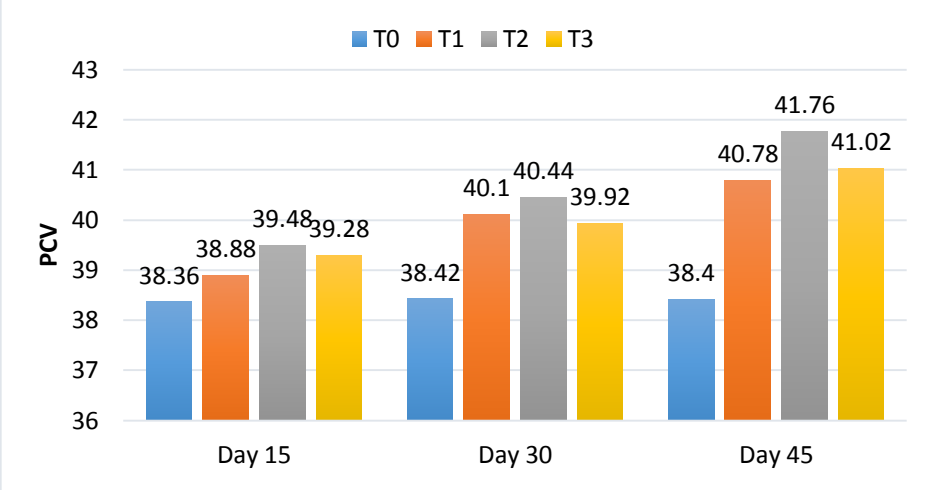


Fig. 15: Effect of wheat grass on Packed Cell Volume

T_0 = control, T_1 = 3 gm/kg B.wt., T_2 = 5 gm/kg B.wt. and T_3 = 7 gm/kg B.wt.

Table 4: Effect of wheat grass on Erythrocyte Sedimentation Rate

Day	Group				Level of Significance
	T_0 (Mean±SE) mm/1st hour	T_1 (Mean ±SE) mm/1st hour	T_2 (Mean ±SE) mm/1st hour	T_3 (Mean±SE) mm/1st hour	
Day 15	20.06±1.54	18.92±0.95	17.62±1.08	18.44±0.93	NS
Day 30	20.12±1.55	17.7±1.01	16.88±0.85	17.70±0.76	NS
Day 45	20.6^{b}±1.54	16.70^{a}±0.1	15.02^{a}±1.23	16.38^{a}±1.82	*

Values with the different superscripts in the same row are statistically significant (P<0.05), NS = Non significant, * = Significant at 5% level of significance (P<0.05).

Erythrocyte sedimentation rate content was presented in (Table 4). There is a relationship between PCV and ESR as PCV significantly (P<0.05) increase in T_1, T_2 and T_3 (Table 3) so ESR significantly (P<0.05) decrease in T_1, T_2 and T_3 group compare to normal and lowest level was found in group T_2 (15.02 ± 1.23) by wheat grass supplementation.

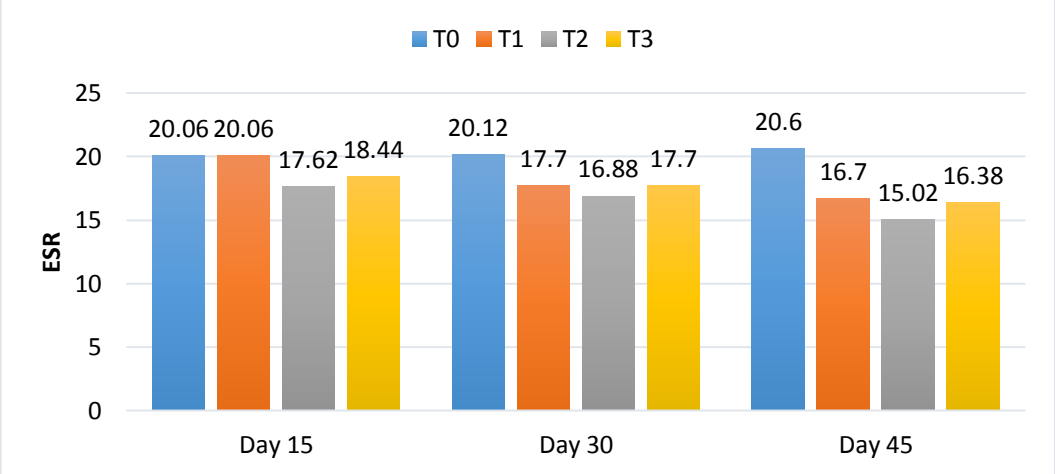


Fig. 16: Effect of wheat grass on Erythrocyte Sedimentation Rate

T_0 = control, T_1 = 3 gm/kg B.wt., T_2 = 5 gm/kg B.wt. and T_3 = 7 gm/kg B.wt.

Table 5: Effect of wheat grass on Total Erythrocyte Count

Day	Group				Level of Significance
	T_0 (Mean±SE) (cells10^3/µl	T_1 (Mean±SE) (cells10^3/µl))	T_2 (Mean±SE) (cells10^3/µl)	T_3 (Mean±SE) (cells10^3/µl)	
Day 15	4.1±0.08	4.25±0.09	4.32±0.97	4.27±0.94	NS
Day 30	4.09^a±0.07	4.46^b±0.05	4.58^b±0.12	4.38^b±0.09	*
Day 45	4.13^a±0.09	4.71^a±0.11	5.01^b±0.10	4.57^a±0.13	*

Values with the different superscripts in the same row are statistically significant (P<0.05), NS = Non significant, * = Significant at 5% level of significance (P<0.05).

Total Erythrocyte Count content was presented in (Table 5). In group T_1, T_2 and T_3 supplementation with wheat grass (3 gm, 5 gm, 7 gm/kg B.wt.) produced significant (P<0.05) increase in TEC as compared to control and highest level was found in group T_2 (at 30 day 4.58 ± 0.12 and 45 day 5.01 ± 0.10) than T_0, T_1 and T_3. These results are in agreement with the results of Yadav *et al.,* (2017); Shah *et al.,* (2011) where there was significantly (P<0.05) increase in TEC level in the group receiving wheat grass. Fernandes and Donovan have speculated that the effects of wheat grass juice therapy may be due to the action of natural antioxidants on red blood cell.

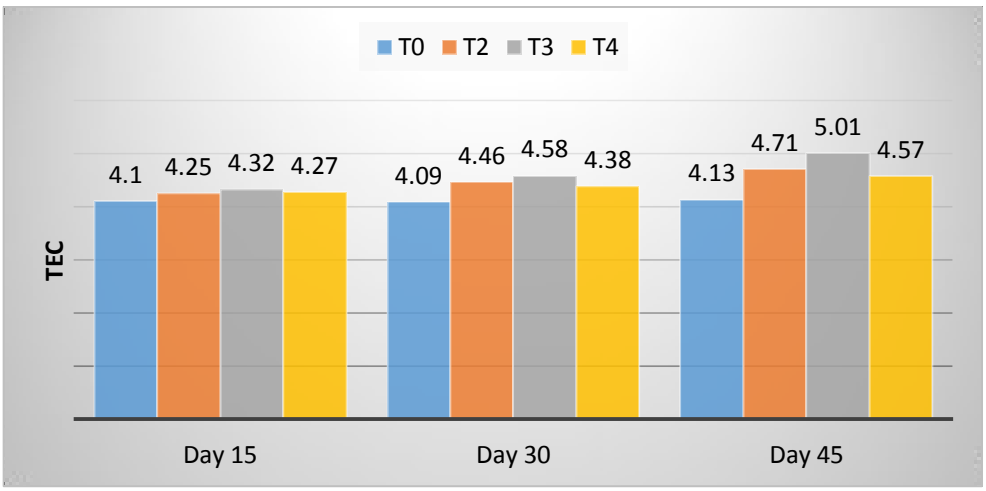


Fig. 17: Effect of wheat grass on Total Erythrocyte Count

T_0 = control, T_1 = 3 gm/kg B.wt., T_2 = 5 gm/kg B.wt. and T_3 = 7 gm/kg B.wt.

Table 6: Effect of wheat grass on Total Leucocyte Count

Day	Group				Level of Significance
	T_0 (Mean±SE) (cells10^3/µl	T_1 (Mean±SE) (cells10^3/µl))	T_2 (Mean±SE) (cells10^3/µl)	T_3 (Mean ±SE) (cells10^3/µl)	
Day 15	4.14±0.10	4.38±0.01	4.48±0.17	4.36±0.09	NS
Day 30	4.14^{a}±1.10	4.58^{b}±0.09	4.66^{b}±0.14	4.45^{ab}±0.08	*
Day 45	4.16^{a}±0.01	4.72^{b}±0.12	4.96^{b}±0.14	4.78^{b}±0.01	*

Values with the different superscripts in the same row are statistically significant (P<0.05), NS = Non significant, * = Significant at 5% level of significance (P<0.05).

In this research work there was significant increase TLC was found in group T_1, T_2, T_3 compared to normal group. These results are in accordance with Shah *et al*., (2011) also who reported treatment with wheat grass fresh juice, methanol extract produced significant increase in total WBC counts and differential WBC counts in busulfan induced pancytopenic rats.

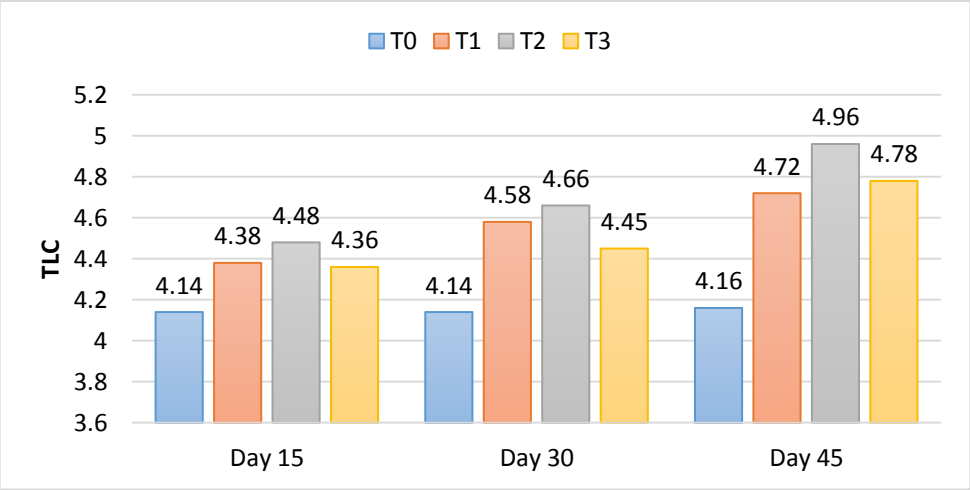


Fig. 18: Effect of wheat grass on Total Leucocyte Count

T_0 = control, T_1 = 3 gm/kg B.wt., T_2 = 5 gm/kg B.wt. and T_3 = 7 gm/kg B.wt.**Table 7:**

Table 7: Effect of wheat grass on neutrophil

Day	Group				Level of Significance
	T_0 (Mean±SE) %	T_1 (Mean±SE) %	T_2 (Mean±SE) %	T_3 (Mean±SE) %	
Day 15	31.90±0.917	32.64±0.81	33.22±0.70	32.56±1.34	NS
Day 30	31.94±0.91	33.46±0.86	35.02±0.61	33.58±0.75	NS
Day 45	31.90^{a}±0.92	34.46^{b}±0.52	35.70^{b}±0.58	34.88^{b}±0.52	*

Values with the different superscripts in the same row are statistically significant (P<0.05), NS = Non significant, * = Significant at 5% level of significance (P<0.05).

Neutrophil content was presented in (Table 7). The values of neutrophil were significantly increase in all treated groups (T_1, T_2 and T_3) compare to control group. These results are in accordance with Shah *et al.*, (2011) who reported treatment with wheat grass fresh juice, methanol extract produced significant increase in total WBC counts and differential WBC counts in busulfan induced pancytopenic rats.

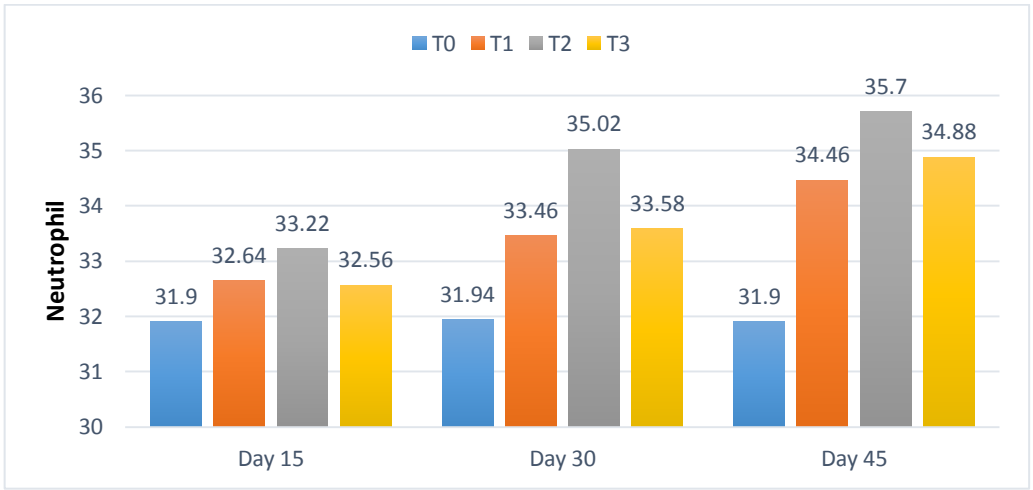


Fig. 19: Effect of wheat grass on neutrophil

T_0 = control, T_1 = 3 gm/kg B.wt., T_2 = 5 gm/kg B.wt. and T_3 = 7 gm/kg B.wt**Table 8:**

Table 8: Effect of wheat grass on lymphocytes

Day	Group				Level of Significance
	T_0 (Mean±SE) %	T_1 (Mean±SE) %	T_2 (Mean±SE) %	T_3 (Mean±SE) %	
Day 15	56.28±1.9	57.38±1.15	59.14±1.77	57.48±1.34	NS
Day 30	56.32±1.87	59.74±0.09	61.80±01.59	59.24±1.15	NS
Day 45	56.30^{a}±1.89	63.52^{b}±1.34	65.32^{b}±1.52	63.06^{b}±1.14	*

Values with the different superscripts in the same row are statistically significant (P<0.05), NS = Non significant, * = Significant at 5% level of significance (P<0.05).

Lymphocytes content was presented in (Table 8). The values of lymphocytes were significantly (P<0.05) increase in all treated groups (T_1,T_2 and T_3) compare to control group .Increased lymphocytes in wheat grass treated group in this study reinforces the immunity boosting potential of wheat grass which are accordance with Limbasiya *et al.,* (1988). These results are in agreement with the results of Yadav *et al.,* (2017); where there was significantly (P<0.05) increase lymphocyte level in the group receiving wheat grass.

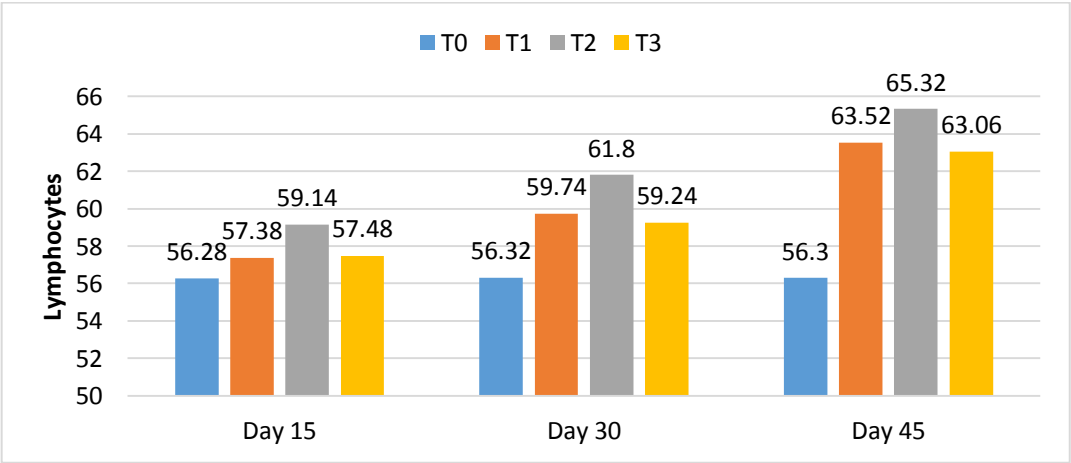


Fig. 20: Effect of wheat grass on lymphocytes

T_0 = control, T_1 = 3 gm/kg B.wt., T_2 = 5 gm/kg B.wt. and T_3 = 7 gm/kg B.wt.

4.2 Effect of wheat grass on biochemical parameters

Table 9: Effect of wheat grass on Serum Glutamate Pyruvate Transaminase (SGPT)

Day	Group				Level of Significance
	T_0 (Mean±SE) U/L	T_1 (Mean±SE) U/L	T_2 (Mean±SE) U/L	T_3 (Mean±SE) U/L	
Day 15	28.10±0.93	26.86±0.87	26.52±0.47	26.34±0.36	NS
Day 30	28.16^{b}±0.89	25.92^{ab}±0.54	25.08^{a}±0.37	26.0^{ab}±0.57	*
Day 45	28.16^{b}±0.89	24.58^{a}±0.47	23.86^{a}±0.12	24.34^{a}±0.39	*

Values with the different superscripts in the same row are statistically significant (P<0.05), NS = Non significant, * = Significant at 5% level of significance (P<0.05).

SGPT content was presented in (Table 9). In this study, in group T_1, T_2 and T_3 supplementation with wheat grass (3 gm, 5 gm, 7 gm/kg B.wt.) produced significant (P<0.05) decrease in SGPT levels as compared to control and lowest level was found in T_2 (23.86 ± 0.12) but statistically not significant (P<0.05). These results are in accordance with Jain *et al.*, (2007) who reported the hepatoprotective role of fresh wheat grass juice has in CCl_4 treated rats by decreasing liver enzymes in 21 days therapy.

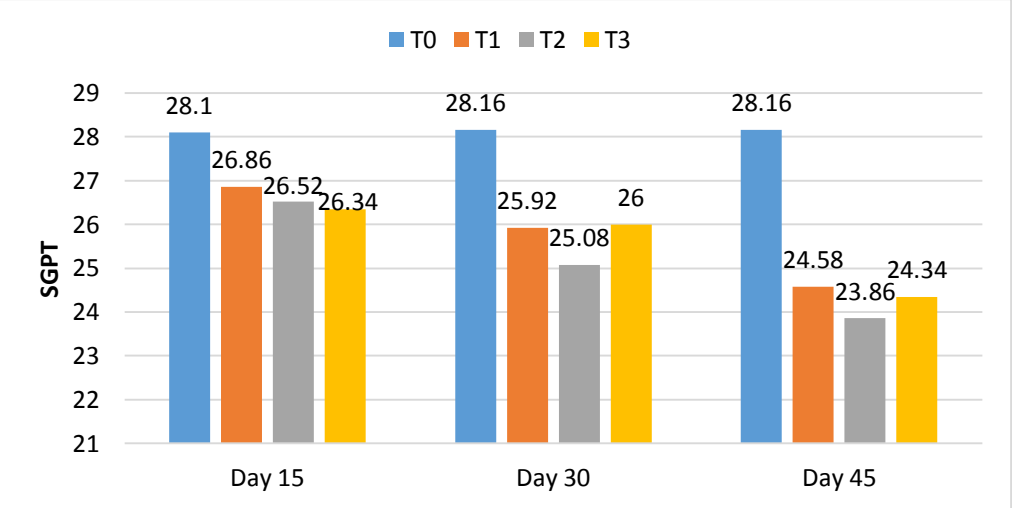


Fig. 21: Effect of wheat grass on Serum Glutamate Pyruvate Transaminase (SGPT)

T_0 = control, T_1 = 3 gm/kg B.wt., T_2 = 5 gm/kg B.wt. and T_3 = 7 gm/kg B.wt.

Table 10: Effect of wheat grass on Serum Glutamate Oxaloacetate Transaminase (SGOT)

Day	Group				Level of Significance
	T_0 (Mean±SE) U/L	T_1 (Mean±SE) U/L	T_2 (Mean±SE) U/L	T_3 (Mean±SE) U/L	
Day 45	66.74±1.44	65.68±1.16	65.08±0.94	65.78±1.15	NS
Day 30	66.68^{b}±1.46	63.58^{a}±0.46	63.28^{a}±0.30	63.94^{a}±0.57	*
Day 45	66.66^{b}±0.1.48	63.54^{a}±0.58	62.48^{a}±0.28	63.26^{a}±0.45	*

Values with the different superscripts in the same row are statistically significant (P<0.05), NS = Non significant, * = Significant at 5% level of significance (P<0.05).

SGOT content was presented in (Table 10). In group T_1, T_2 and T_3 supplementation with wheat grass (3 gm, 5 gm, 7 gm/kg B.wt.) produced significant (P<0.05) decrease in SGPT levels as compared to control and lowest level was found in group T_2 (62.48 ± 0.28) than T_1 and T_2. These results are in accordance with Jain *et al.*, (2007) who reported the hepatoprotective role of fresh wheat grass juice has in CCl_4 treated rats in 21 days therapy.

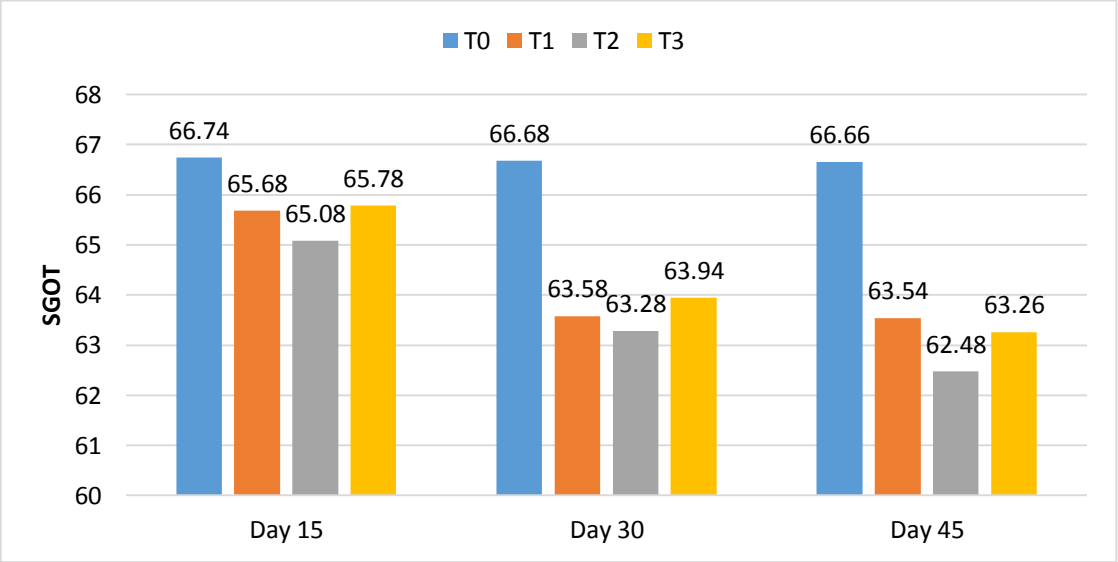


Fig. 22: Effect of wheat grass on Serum Glutamate Oxaloacetate Transaminase (SGOT)

T_0 = control, T_1 = 3 gm/kg B.wt., T_2 = 5 gm/kg B.wt. and T_3 = 7 gm/kg B.wt.

4.3 Effect of wheat grass on body weight

Table 11: Effect of wheat grass on body weight

Day	Group				Level of Significance
	T_0 (Mean± SE) gm	T_1 (Mean± SE) gm	T_2 (Mean± SE) gm	T_3 (Mean± SE) gm	
Day 0	1347±42.36	1335.6±43.34	1342.8±26.37	1348.2±34.3	NS
Day 15	1395±42.83	1386.4±46.32	1400.4±21.92	1395.6±33.2	NS
Day 30	1448.2±45.07	1428.40±42.80	1450.2±44.95	1442±28.31	NS
Day 45	1471±46.43	1480±21.90	1491±27.31	1495.6±25.47	NS

Values with the different superscripts in the same row are statistically significant (P<0.05), NS = Non significant, * = Significant at 5% level of significance (P<0.05).

In this research supplementation with wheat grass (3 gm, 5 gm and 7 gm/kg B.wt.) in group T_1, T_2 and T_3 do not produce any significant effect on body weight. Body weight of all treated group was increased day by day as like control group.

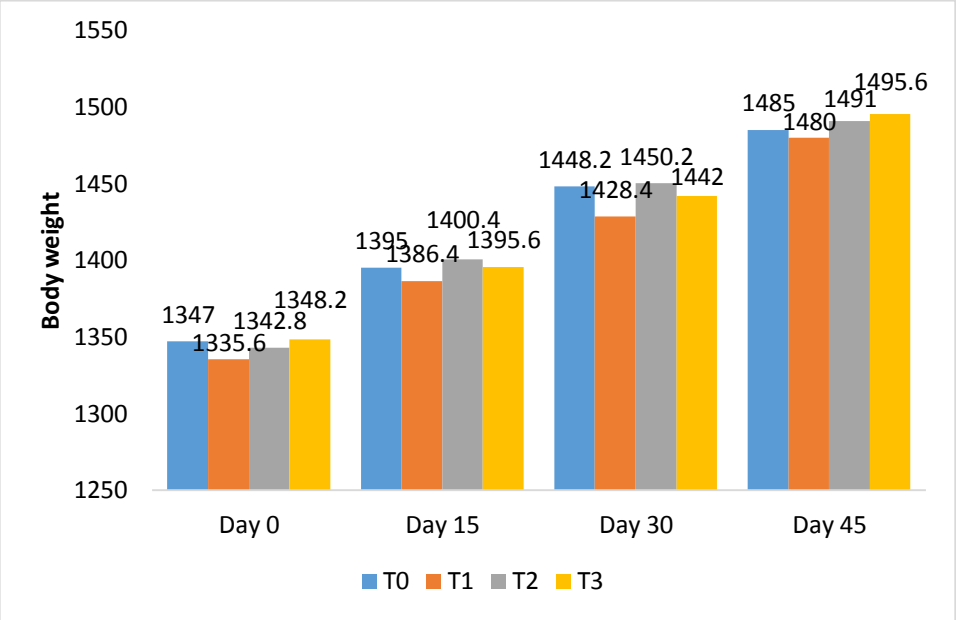
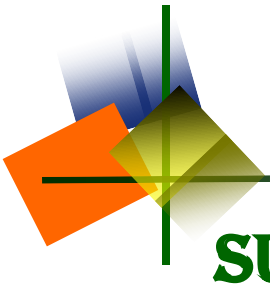


Fig. 23: Effect of wheat grass on body weight

T_0 = control, T_1 = 3 gm/kg B.wt., T_2 = 5 gm/kg B.wt. and T_3 = 7 gm/kg B.wt.



Chapter 5

SUMMARY AND CONCLUSION

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The study was conducted to evaluate the effect of wheat grass (*Triticum aestivum)* on hemato-biochemical parameters and body weight in rabbit. Twenty rabbits were randomly divided into 4 groups (n=5) to carry out this research work. One group (T_0) was kept for control and others three groups (T_1, T_2 and T_3) were considered as treated groups. Chopped wheat grass supplementation was given to the group T_1, T_2 and T_3 at the dose rate of 3 gm, 5 gm, 7 gm/kg body weight respectively for 45 days. During that period on day 15, 30, 45 under close observation the blood and serum samples were analyzed for hemoglobin, Packed Cell Volume (PCV), Red Blood Cell Count (RBC), Total Leucocyte Count (TLC), Differential Leucocyte Count (DLC), Serum Glutamate Oxaloacetate Transaminase (SGOT), Serum Glutamate Pyruvate Transaminase (SGPT) and body weight from 0-45 days . There was statistically significant ($P<0.05$) improvement in hemoglobin concentration, RBC, TLC, DLC count and in the group receiving wheat grass compare to control and highest level was found in group T_2 but statistically not significant ($P<0.05$) . There was significant ($P<0.05$) decrease in ESR, SGPT, SGOT level in all treated group compare to control and lowest value was found in group T_2 but statically not significant ($P<0.05$) . In this research work there is no any satisfactory result was found on the body weight of rabbit by feeding wheat grass. The results of this experiment supports the traditional usage of wheat grass for the treatment of anaemia and other blood related disorders. This results also suggest immunostimulant effects of wheat grass as it increase total and differential WBC counts. It can be concluded that wheat grass supplementation produce significant effect on hematological and biochemical parameters but 5gm/kg may be the best to obtain better result. Further study can be done to investigate the anthelmintic & antimicrobial effect of wheat grass.

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