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Effect of Wheat Grass (Triticum aestivum) on Hemato-Biochemical Parameters and Body Weight in Rabbit

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Abstract:

This research work was conducted at animal laboratory in the Department of Physiology and Pharmacology, 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. Twenty rabbits age between 3-4 months 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 5 rabbits in each group. Group T_0 was kept for control group and 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 blood samples were collected and analyzed for hemoglobin, PCV, RBC, TLC, DLC, SGOT, SGPT and body weight from 0-45 days were calculated and statistically analysed. There was significant ($P>0.05$) increased in hemoglobin concentration, PCV, RBC, TLC, DLC count in the group receiving wheat grass compare to control group and highest value was found in group T_2 but statistically not significant ($P<0.05$). 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 ($P<0.05$). In this research wheat grass did not produce any significant ($P<0.05$) effect on body weight of rabbit . The results of this study reveals that 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, immunostimulatory effect and health improving adjuvant in liver disorders.

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1. Introduction

Herbs and plants have been used for medicinal purposes long before prehistoric period. 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. 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.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. In 2010 survey of 1000 plants was completed, wheat grass is one of them which contain the vital component chlorophyll (70%) that helps in building hemoglobin . Wheat grass is young grass, commonly known as *Triticum aestivum* Linn (Poaceae). 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). It is significant nutritious and medicinal value with rich source of chlorophyll. It is a natural source of iron. The 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 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 . 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). 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. 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). Examination of blood provides the opportunity to clinically investigate the physiological, nutritional and pathological status of a man and animal. For that research or study is on made evaluating or observing the following specific objectives:

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

2. Materials and Methods

2.1 Experimental site

This research work was conducted at animal Laboratory in the Department of Physiology and Pharmacology, Hajee Mohammad Danesh Science & Technology University, Dinajpur from 4th February to 21th March.

2.2 Experimental animal

Twenty New Zealand White rabbits age between 3- 4 months and weighting between 1200 to1500 gm were collected from local farm in Dinajpur.

2.3 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 tape. 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.

2.4 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.

2.5 Collection wheat grain and cultivation of wheat grass

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

2.6 Experimental animal grouping

Twenty rabbits were used to carry out this investigation. These rabbits were divided into 4 groups(T_0, T_1, T_2, T_3) containing 5 rabbits in each group. Rabbit of group T_0, were kept as control, fed only with commercial feed.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.

2.7 Collection, preparation and feeding of wheat grass

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.

2.8 Determination of body weight

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

2.9 Collection of blood

Blood samples were collected from ear vein of rabbit of both control and treated groups. For determining hematological and biochemical parameters 4 ml blood were collected from each rabbit after 15, 30, 45 days of experiment. Immediately after collection of 2 ml blood was transferred to sterile test tube containing anticoagulant at a ratio of 1:10 for hematological

examination. The rest (2 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 -20^0C in deep freeze until tested.

2.10 Hematological parameters

(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 (%)

2.10.1 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).

2.10.2 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).

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

2.10.3 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.

2.10.4 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).

2.10.5 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.

2.10.6 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.

2.11 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).

2.11.1 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.

2.11.2 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.

2.12 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.

3. Results and Discussion

All the control and treated rabbits were closely observed 45 days of treatment period and results were shown under following heading:

3.1 Effect of wheat grass on hematological parameters

3.1.1 Effect of wheat grass on hemoglobin

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.

3.1.2 Effect of wheat grass on Packed Cell Volume

Packed Cell Volume content was presented in (Table 2). 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.

3.1.3 Effect of wheat grass on Erythrocyte Sedimentation Rate

Erythrocyte sedimentation rate content was presented in (Table 3). 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.

3.1.4 Effect of wheat grass on Total Erythrocyte Count

Total Erythrocyte Count content was presented in (Table 4). In group T_1, T_2 and T_3 supplementation with wheat grass 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.

3.1.5 Effect of wheat grass on Total Leucocyte Count

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.

3.1.6 Effect of whea grass on neutrophil

Neutrophil content was presented in (Table 6). 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.

3.1.7 Effect of wheat grass on lymphocytes

Lymphocytes content was presented in (Table 7). 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.

3.1.8 Effect of wheat grass on Serum Glutamate Pyruvate Transaminase

SGPT content was presented in (Table 8). 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.

3.1.9 Effect of wheat grass on Serum Glutamate Oxaloacetate Transaminase (SGOT)

SGOT content was presented in (Table 9). 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.

3.1.10 Effect of wheat grass on body weight:

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.

Conclusion

The study was conducted to evaluate the effect of wheat grass (*Triticum aestivum)* on hemato-biochemical parameters and body weight in rabbit .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 stastically not significant. 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 hemoglobin and RBC related disorders. This results also suggest immunostimulant effects of wheat grass as it increase total and differential WBC counts. It can be concluded from the study that different dose (3gm, 5gm, 7gm/kg body weight) of wheat grass supplementation produce significant effect on hematological and biochemical parameters but 5gm/kg may be the best to obtain better result. This experiment was performed in small scale basis and modern equipment's were not so available. Before field application of wheat grass as antianemic agent further trial on a large scale basis is needed and also to make the findings more accurate and effective.

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Table 1: Effect of wheat grass on Hemoglobin

	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).

Table 2: Effect of wheat grass on Packed Cell Volume

	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).

Table 3: Effect of wheat grass on Erythrocytes Sedimentation Rate

	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).

Table 4: 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).

Table 5: 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).

Table 6: 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).

Table 7: 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).

Table 8: Effect of wheat grass on Serum Glutamate Pyruvate Transaminase

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).

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

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).

Table 10: 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).

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