

Hematopoietic, Antioxidant, and Reproductive Modulatory Effects of *Triticum aestivum* Leaf Aqueous Extract in Female Albino Rats

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Abstract

Triticum aestivum (wheatgrass) is widely recognized for its rich phytochemical composition and medicinal properties, particularly in hematopoiesis, antioxidant defense, and reproductive health. This study aimed to evaluate the Hematopoietic, Antioxidant, and Reproductive Modulatory Effects of *Triticum aestivum* Leaf Aqueous Extract in Albino Rats. Twenty-four adult female albino rats weighing between 180g to 250g were used for this experiment. The experiment was divided into four groups with six albino rats each. Group 1 served as the control and was fed commercial feed only. Group 2, 3 and 4 were given the extract orally with a dose rate of 100mg/kg, 200mg/kg, and 300mg/kg body weight respectively daily for 30 days. The hematological, antioxidant, and hormonal biomarkers were assayed using the standard procedures. The administration of *T. aestivum* extract significantly ($P < 0.05$) influenced reproductive hormones, with the 300 mg/kg group showing an increase in progesterone, follicle-stimulating hormone and luteinizing hormone compared to control. Antioxidant enzyme activity also showed superoxide dismutase and glutathione peroxidase decreasing with an increase in dosage, with catalase showing increase again in the highest dose. Hematological analysis showed a significant ($P < 0.05$) increase in red blood cell count, packed cell volume and haemoglobin concentration while platelet count declined in a dose-dependent manner. These findings suggest that *T. aestivum* is recommendable in improving antioxidant defense, reproductive health and erythropoiesis. However, its dose-dependent effects on WBC and platelet levels necessitate further safety evaluation before therapeutic application to determine the most efficient dosage. A moderate dosage of the juice is recommended for consumption.

Keywords: *Triticum aestivum*, Wheatgrass, Hematopoietic, Antioxidants, Hormones, Fertility, Albino Rats.

Introduction

Plants are commonly commercialized as a food-based product but they also have other important roles making them an attractive thing to be explored by the researchers because of some medicinal properties (Sharifi-Rad *et al.*, 2018). These medicinal plants have been used for centuries as remedies and are the richest bio-resources of drugs of traditional

medicinal systems in pharmaceuticals, folk medicines, nutraceuticals and synthetic drugs (Furtado *et al.*, 2016). Previous research conducted showed that these medicinal plants contain bioactive constituents with certain phytochemicals that could be used in treatment of diseases and disorders like the tannins, flavonoids, terpenoids, alkaloids and phenolic compounds (Pieme *et al.*, 2006).

Pharmacologists, microbiologists, botanists, and natural products chemists are raking the earth for plants with phytochemicals and leads that could be developed for treatment of various diseases. Among the active compounds currently isolated from the higher plants and widely used in modern medicine, about 80 percent shows a positive correlation between their modern therapeutic use and the traditional use of the plants from which they are derived (Fabricant and Farnsworth. 2001).

Wheatgrass, the young grass or shoot of the common bread wheat plant (*Triticum aestivum*), a cereal grass of the Poaceae family, has been regarded as a power house of nutrients rich in chlorophyll, amino acids, minerals, vitamins, and enzymes (Choudhary *et al.*, 2021). The wheat plant is referred to as “alkama” in Hausa, alikama by the Yoruba and “oka wit” by the Igbos in Nigeria (Durosunmilola-Mohammed *et.al.*, 2019). The young shoots of the plant is mostly freshly juiced or dried into powder for animal and human consumption. Modern science has already accepted the potential of herbs as a source of new bioactive constituents and researchers have evaluated fertility effect of different herbs (Eissa *et al.*, 2020; Rana *et al.*, 2011).

In addition to supporting health and increasing body protection, wheatgrass may be potent to boost human fertility due to the presence of sufficient amounts of vitamin C, the presence of zinc, and quercetin according to the study by Fernandes *et al.*, (2011), who investigated the vitamin C effect on hyperglycemic rats and found that it declines the amount of abnormal sperm and raises testosterone in blood. Over the years, a large number of natural (plant) remedies have played different roles as hormonal boosters and aphrodisiacs in different cultures and civilizations to help enhance fertility (Enema *et al.*, 2018). Fertility as a concept is one of the most important aspects of human life and directly has a possible effect on the future of an organism and procreation. Infertility, which is described as the inability to achieve pregnancy after 12 months of unprotected intercourse, has also played a major role in the disintegration of many families worldwide (Mohan *et al.*, 2022). A study conducted by the World Health Organization in 2021 revealed that poor reproductive health is a significant cause of disability especially among women of 15 - 49 years in developing countries (World Health Organization, 2021). The normal physiological principle guiding female reproductive efficiency involves an interplay between the hypothalamus that secretes gonadotropin-releasing hormone (GnRH) in a pulsatile manner and the pituitary gland which is stimulated by the hypothalamus via the hypothalamo-pituitary-gonadal axis to regulate the secretion of luteinizing hormone (LH) and follicle stimulating hormone (FSH). These hormones play key roles in reproduction. In addition, the ovary that has both a methodical enzymatic system and steroidogenesis for

producing sex steroid hormones such as; estrogen and progesterone alongside a functional uterus that responds to these hormones is also key to reproduction (Nwogweze *et al.*, 2019). Female infertility is the major disorder which causes lack of conception and reproducibility. The cause of female infertility can be difficult to diagnose, but many treatments are available. Stressful life style, age, excess radiation, nutrition and genetical disorder have also given rise to female infertility (Ali and Hisham, 2016). Approximately one-third of the cases of infertility affecting couples are primarily attributable to the woman (female factors), one-third to the man (male factors), one third to an interaction between the two and 20% of those remaining unexplained (unknown factors) (Mohan, *et al.*, 2022).

Statement of the Research Problem

Infertility has remained a major health problem among couples and sexually active individuals who seek to procreate, therefore, the search for therapeutic solutions and remedies have remained endless. It is estimated that about 78 million people every year have reproductive issues relating to infertility, thereby making it a global health problem worldwide (WHO, 2021). In African countries where up to 90% of the population relies on the use of medicinal plants to help meet their primary health care needs especially in developing countries, the search for therapeutic plants is on the rise (Mohan *et al.*, 2022). Prevalence of infertility In Nigeria is about 10-30% and is becoming a burden and has been attached with stigma especially with the women (Durosinlorun-Mohammed *et al.*, 2019). Thus, production of fertility drugs by pharmaceutical industries has increased of recent due to increase in the rate of infertility caused by several factors including problems with egg sperm production, genetic factors, age or too much exposure to certain chemicals and toxins. The need to explore for additional sources of compounds harnessed into fertility drugs is therefore necessary. Some medicinal plants are known to possess anti-fertility effect through their action on the hypothalamo-pituitary-gonadal axis or direct hormonal effects on reproductive organs.

Aim and Objectives

Aim

This research aimed to determine the Hematopoietic, Antioxidant, and Reproductive Modulatory Effects of *Triticum aestivum* Leaf Aqueous Extract in Albino Rats.

Objectives

The specific objectives of the study include the determination of:

- i. the effect of *Triticum aestivum* leaf aqueous extract on the hematological parameters (RBC, WBC, HB, PLT, Differential count) of the albino rats.
- ii. the effect of *Triticum aestivum* leaf aqueous extract on the serum antioxidant enzymes, superoxide dismutase, catalase and glutathione peroxidase in the albino rats

- iii. the effect of *Triticum aestivum* leaf aqueous extract on some female reproductive parameters such as progesterone, follicle stimulating hormone and Luteinizing hormone of the rats

Literature Review

Wheatgrass: Nature's Remarkable Herb

Researchers have known for long that the cereal plant, wheat, (*Triticum aestivum*) of the family Poaceae, one of the world's largest cereal crop, at the young green stage, is many times richer in the levels of vitamins, minerals and proteins as compared to the seed kernel, or grain products of the mature plant (Meyerowitz, 1999). Wheatgrass (*Triticum aestivum*) is known as a "functional food" and the powerhouse of nutrients and vitamins serving both nutritional and therapeutic values, rich in vitamin K and Iron. The stems of wheatgrass are simple, hollow or pithy, glabrous, and the leaves are approximately 1.2 m tall, flat, narrow, 20-38 cm long and 1.3 cm broad. The spikes are long, slender, dorsally compressed and somewhat flattened. (Mohit *et al.*, 2014). Wheatgrass has been traditionally used, since ancient times, to treat various diseases and disorders. Presently, there are a number of wheatgrass suppliers in almost all cities of India that supply fresh wheatgrass, on daily basis for various ailments and as health tonic (Marwaha *et al.*, 2004). Wheatgrass can be taken in different forms like juice, powder and tablets, but the juice is found to be more potent. It is tropical plant known widely for its anti-anemic and hypoglycemic effect, and has been extensively used for diabetic studies in herbal medicine (Choudhry *et al.*, 2021).

Table 2.1: Taxonomical classification of *Triticum aestivum*

Taxonomical Rank	Taxon
Kingdom	<i>Plantae</i>
Division	<i>Magnoliophyta</i>
Class	<i>Liliopsida</i>
Order	<i>Cyperales</i>
Family	<i>Graminae/Poaceae</i>
Subfamily	<i>Pooideae</i>
Tribe	<i>Triticeae</i>
Genus	<i>Triticum</i>
Species	<i>Aestivum</i>
Common name	<i>Wheatgrass</i>

Source: Choudhary *et al.* (2021).

Fertility

Fertility is the natural capability to produce offspring. As a measure, fertility rate is the number of offspring born per mating pair, individual or population (Wall *et al.*, 2015). Human fertility depends on a number of factors including nutrition, sexual behaviour, consanguinity, culture, instinct, timing, and way of life, emotions and endocrinology. The

ability of a man or a woman to be fertile depends on a number of physiological factors (Nwogueze *et.al*, 2019). Both women and men have hormonal cycles which determines both when a woman can achieve pregnancy and when man is most virile. The female cycle is approximately twenty-eight days long, but the male cycle is variable. (Tuck *et al.*, 2019). Infertility primarily refers to the biological inability of a person to contribute to conception. Infertility in women may also refer to the state of a woman who is unable to carry a pregnancy to full term. There are many biological causes of infertility, including some that medical intervention can treat (Shahid *et al.*, 2022).

Fertility Hormones

Follicle stimulating hormone (FSH) a glycoprotein, is an important hormone for normal functioning of the reproductive system produced by the basophilic cells of the anterior pituitary gland. Gonadotropin-releasing hormone (GnRH), produced in the hypothalamus, controls the release of FSH from the anterior pituitary. FSH stimulates follicular development (Al-Fahham, 2016). It stimulates the growth of several ovarian follicles in the ovary before the release of an egg from one follicle at ovulation. It also stimulates the follicular cells in the ovary to secrete estrogen (Debnath, 2019). FSH helps control the menstrual cycle and production of eggs by the ovaries. It also increases oestradiol production (Al-Fahham, 2016).

Estrogen are primary female secondary sex hormone and play an important role in the normal sexual and reproductive development. They are made mostly in the ovaries. Estradiol, (E₂), is the most common type of estrogen (Delgado *et.al*, 2019). Estrogen stimulates proliferation of the endometrial lining and also the production of watery cervical mucus. Too high or too low estrogen can cause irregular periods, vaginal thinning and dryness. Estrogen also affects mood, thereby creating hormonal imbalance. In addition to regulating menstrual cycle, estrogens affect the reproductive tract, the urinary tract, the heart and blood vessels, bones, breasts, skin, hair, mucous membranes, pelvic muscle and the brain. (Obediah and Paago, 2018). Studies have also shown that too much estrogen may also negatively impact the genetic process that influences the implantation site, shortening the fertility window of opportunity. Estrogen dominance can cease ovulation a process known as anovulatory cycle common with women ages 30-50.

Luteinizing Hormone (LH), also known as lutropin or lutrophin, is another gonadotropin hormone. In women, LH plays an important role in puberty, menstruation and fertility and is also an important part of the menstrual cycle working in conjunction with follicle-stimulating hormone (Jiang *et al.*, 2014). The rise in estrogen tells the pituitary gland to stop producing FSH and to start making more LH. The shift to LH causes the egg to be released from the ovary, a process called ovulation. General, higher than normal levels of LH in a woman may mean the ovaries are absent or not functioning and in a young woman, high levels may mean that puberty is early and low levels of LH in the blood may indicate anorexia, an issue in the pituitary gland, stress, or damage to the hypothalamus in both men and women (Nam *et al.*, 2012). LH secretion surges due to high levels of estrogen and the

flow leads to the oocyte to complete meiosis and causes ovulation. LH transforms the follicle into the corpus luteum (Jiang *et al.*, 2014).

Progesterone belongs to a group of steroid hormones called the progestogens and is the major progestogen in the body and is secreted from the corpus luteum (Mohan *et al.*, 2022). It promotes the secretory phase of the uterine cycle. Without sufficient Progesterone, the endometrium cannot be prepared for implantation and the fertile ova cannot be converted into the corpus luteum (Mohan *et al.*, 2022). Low levels of progesterone can also cause irregular menstrual periods, spotting and headaches and could affect a person's ability to get pregnant. Progesterone also regulate secretions of gonadotropins and also stimulates the production of gamma-aminobutyric acid (GABA) (Cable *et al.*, 2022).

Antioxidants

The body has several mechanisms to counteract oxidative stress by producing antioxidants, either endogenous, or exogenous antioxidants whose role is to neutralize the excess of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention (Lien *et al.*, 2008). The actions of these antioxidant enzymes and non-enzymatic antioxidants correct the adverse effects of reactive oxygen species (ROS) (Pacher *et al.*, 2007). The major antioxidant enzymes directly involved are: superoxide dismutase (SOD), the first line of defense against free radicals, that catalyzes the dismutation of superoxide anion radical (O_2^-) into hydrogen peroxide (H_2O_2) by reduction; catalase (CAT) which transforms the H_2O_2 formed into water and oxygen (O_2); glutathione peroxidase (GPx) which also removes H_2O_2 by using it to oxidize reduced glutathione (GSH) into oxidized glutathione (GSSG) (Lien *et al.*, 2008). Glutathione reductase regenerates GSH from GSSG, with NADPH as a source of reducing power. Besides hydrogen peroxide, GPx also reduces lipid or nonlipid hydroperoxides while oxidizing glutathione (GSH).

Materials and Methods

Ethical Clearance

Ethical approval was obtained from the Ethical Committee, Nasarawa State University, Keffi.

Equipment

The equipment utilized in this research includes: Vortex meter, Spectrophotometer (UNICO 2150 series, USA), Compound Light Microscope (AmScope B120C-E1, China), centrifuge (NEWTRY, 800-1, China), plastic trays, scissors, mincer, syringes, blades, oral gavage. Microscope, Haemocytometer, RBC pipette, RBC diluting fluid (Sodium citrate).

Chemicals and Reagents

All reagents that were used are of analytical grade. The chemical reagents that were utilized in this study include Drabkin's reagent, Turk's solution, Hayem's solution, ammonium oxalate solution, DPPH (2,2-diphenyl-1-picrylhydrazyl), methanol, hydrogen peroxide,

phosphate buffer, sodium carbonate, EDTA (ethylenediaminetetraacetic acid), NBT (Nitroblue tetrazolium), DTNB (5,5'-dithiobis-(2-nitrobenzoic acid), also known as Ellman's reagent), trichloroacetic acid, thiobarbituric acid (TBA), acetic acid, sodium dodecyl sulfate (SDS), ethanol (70%, 90%, and 100%), xylene, paraffin wax, hematoxylin, eosin, DPX (Distrene Plasticiser Xylene), and 10% formalin.

Preparation of Plant Materials

Whole wheat seeds of about (800g), was gotten from Seed & Mech Hubs, Abuja, Nigeria, and was identified by an academic staff of the Department of Plant Science and Biotechnology, Faculty of Science of Nasarawa State University Keffi, Nigeria and the plant seedlings were prepared according to the method of (Wigmore, 1985).

Germination and cultivation of *Triticum aestivum* (Wheatgrass): Whole wheat seeds were washed properly with running tap water to remove dust and dead seeds and then soaked overnight for 12 hours to make seeds tender. Water was strained and the soaked seeds placed in wet cotton cloth and well covered for a period of 12 hours and water sprinkled over the cotton cloth during the germination period. After 12 hours of germination, the germinated wheat seeds were spread over soil in 12 plastic trays (20×40×10 cm) and further covered with soil and placed in a shady place (indirect sunlight) for growth of grass. Trays were planted at one-day interval to harvest a continuous supply of fresh grass of the same length.

Experimental Animal

Twenty-four adult female albino rats weighing between 180g to 250g were used for this experiment. The animals were gotten from the Zoology Department, University of Jos, Nigeria, and housed at the Animal House of Department of Biochemistry, Keffi in cages where the study took place. They were allowed to acclimatize for seven days before the start of the experiment and maintained at room temperature with a 12-hours light/dark cycle and allowed ad libitum to free food and water.

Sample Extraction

Young shoots were harvested right before splits and cut with scissors at 0.5-1.0 cm above the root. About 63g of fresh grass were weighed, cut into small pieces, washed, and placed in a strainer. The extract was then prepared by mincing the chopped grass in a mincer. The residue was then squeezed to get the aqueous extract, and fresh wheatgrass extracts were collected and stored at 4 °C within 24 hours to preserve. The subjects consumed the extract without any additives.

Experimental Design

The experiment was divided into four groups of six albino rats in each group. Group 1 served as the control and was fed with commercial feed only. Group 2, 3 and 4 were given *Triticum aestivum* leaf aqueous extract orally using an oral gavage with a dose rate of 100mg/kg,

200mg/kg, and 300mg/kg body weight, respectively daily for 30 days body weight of undiluted extract. Body weight, temperature and behavioural changes were observed throughout the experimental period.

Animal Grouping Extract Administration

The animals were grouped into four with six animals in each groups.

Group 1: Control

Group 2: Administered with 100mg/kg bw of the aqueous extract

Group 3: Administered with 200mg/kg bw of the aqueous extract

Group 4: Administered with 300mg/kg bw of the aqueous extract

Blood Sample Collection

At the expiration of administration period, after an overnight fast, blood samples were collected from the animals by cardiac puncture into plain bottles and EDTA bottles for hormonal assay and haematological parameters. Blood samples were left to clot at room temperature and then centrifuged at 3500 rpm for 5 minutes and serum, separated. Serum samples were kept in the deep freezer at -20°C till analysis

Haematological Assay

The haematological assay was determined by the method of Ochei and Kolhatkar (2000).

Determination of the Red blood Cell (RBC) count

Whole blood (20 μl) was added to 3.98 ml of 10 % sodium citrate and mixed well. After 5 minutes, the first few drops were discarded by holding the pipette vertically and the counting chamber was charged with the fluid. It was then allowed to settle for 3 minutes. By switching to low power (10X) objective, the centre large squares with 25 small squares were adjusted to light and then adjusted to high power (40X) objective. The red blood cells in the four corner squares and one central square were counted.

Determination of White Blood cells (WBC) count

Whole blood (20 μl) was added to 380 μl of diluting fluid (acetic acid, with gentian violet) and mixed. The counting chamber was charged with the well mixed diluted blood (after discarding the first five drops) with the aid of a pipette. Cells were allowed to settle in a moist chamber for 3 minutes. The four corners of the chamber were visualized under a low power (10X) objective microscope and the cells were counted in all the four marked corner squares.

Determination of the Packed Cell Volume (PCV)

Blood sample was taken with a heparinised capillary tube, cleaned and sealed with plasticine. The filled tubes were placed in the microhaematocrit centrifuge and spun at

10,000 rpm for 5 minutes. Spun tubes were placed into a specially designed scale and the PCV was read as a percentage.

Determination of the Haemoglobin (HB) Concentration

Whole blood (20 µl) was added to 4 ml of Drabkin's solution in a test tube in a 1:250 dilution. This was well mixed, allowed to stand for 10 minutes at room temperature and the absorbance was read with colorimetrically at 540 nm with Drabkin's solution as a blank.

Determination of Differential Count

The differential count was performed after the WBC blood count has been determined by the automated 3 part differential, and could serve to double check on the white blood cell count. The combination of polychrome methylene blue and eosin stains has selective staining properties. The differential staining allows one to identify the types of white blood cells on the smear.

Hormonal Assay

The serum collected from the animals were taken to a standard laboratory and used to analyze the levels of progesterone, follicle stimulating hormone and luteinizing hormone. The levels of hormones were evaluated by enzyme linked immunosorbent assay (ELISA) method with detection kit according to the manufacturer's instructions on using ELISA technique and as described by Amah-Tariah *et al.*, 2016.

Determination of Progesterone

The sample or standard solution was first added to the microplate and the diluted enzyme conjugate added next. The mixture was then shaken and incubated at room temperature for one hour. The plate is then washed removing all the unbound material. The bound enzyme conjugate is detected by the addition of substrate which generates an optimal colour after 30 minutes.

Results was then determined by measuring and comparing the absorbance reading of the wells of the samples against standards with a microplate reader at 650nm. The extent of colour development is inversely proportional to the amount of progesterone in the sample or standard.

Determination of Follicle Stimulating Hormone

All reagents were brought to room temperature before use. A volume of 50 µl standard and sample specimens was then dispensed into the appropriate wells. 100 µl of Enzyme Conjugate Reagent was also added into each well and mixed thoroughly for 30 seconds. The mixture was incubated at a room temperature of 18-25 °C for 45 minutes. Incubation mixture was then removed and the microtiter wells rinsed and flicked five times with distilled or deionized water.

100 µl of TMB reagent was then dispensed into each well and mixed gently for 10 seconds. Incubation was done at room temperature in the dark for 20 minutes. The reaction was

stopped by adding 100 µl of Stop Solution to each well and mixed gently for 30 minutes making sure that the blue colour changes to yellow colour completely.

The concentration of FSH is directly proportional to the colour intensity of the test sample. The optical density at 450 nm was read with a microtiter plate within 15 minutes.

Determination of Luteinizing Hormone

The desired number of coated wells was secured in the holder. 50 µl of standard and sample specimens were dispensed into appropriate wells. 100 µl of Enzyme Conjugate Reagent was dispensed into each well and mixed thoroughly for 30 seconds. The mixture was incubated at 37 °C for 2 hours. The incubation mixture was removed by dumping the plate contents into a waste container. Microtiter wells were rinsed and dumped five times with wash buffer.

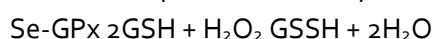
The wells were strike sharply onto the absorbent paper or paper towels to remove all residual water droplets. 100 µl of TMB reagent was dispensed into each well and mixed gently for 10 seconds. Incubation was done at room temperature in the dark for 20 minutes. The reaction was stopped by adding 50 µl of 2N HCL to each well. The mixture were mixed gently for 30 seconds, observing a colour change from blue to yellow. Optical density at 450 nm was read with a microtiter well reader.

Determination of Antioxidant Enzymes

Estimation of glutathione peroxidase was done following the modified method of Numan *et al.* (1990).

Principle

Glutathione peroxidase catalyses the following reaction:



Glutathione was measured by its reaction with DTNB to give a compound that absorbs at 412 nm.

Superoxide dismutase activity was determined by the method of (Kakkar *et al.*, 1984).

Principle

Superoxide dismutase uses the photochemical reduction of riboflavin as oxygen generating system and catalyses the inhibition of NBT reduction, the extent of which can be assayed spectrophotometrically.

Catalase activity was assayed spectrophotometrically following the method of Luck (1974) in the fresh leaves of the plant. A 20% homogenate of the leaves was prepared in phosphate buffer. The samples were read against a control without homogenate, but containing the H₂O₂-phosphate buffer. The time interval required for a decrease in absorbance by 0.05 units was recorded at 240nm.

Statistical analysis

All data were statistically analysed using the software program, SPSS version 25.0 following one-way analysis of variance (ANOVA) using Duncan multiple range Test. Values were considered statistically significance at $P < 0.05$ and presented as Mean $\pm$ SD (Standard deviation).

Results

Effect of *Triticum aestivum* Leaf Aqueous Extract on the Haematological Parameters

Table 1 below presents the effects of *T. aestivum* leaf aqueous extract on hematological parameters, including red blood cell count (RBC), white blood cell count (WBC), platelet count (PLT), packed cell volume (PCV), and hemoglobin (HB) levels. RBC count significantly increased ($P < 0.05$) in the 300 mg/kg group ($5.27 \pm 0.12 \times 10^{12}/L$) compared to the control ($4.53 \pm 0.08 \times 10^{12}/L$). However, no significant changes ($P > 0.05$) were observed in hemoglobin levels across the treatment groups except at 300 mg/kg (13.55 ± 0.05 g/dL) where it increased significantly ($P < 0.05$). The highest WBC count was observed in the control group ($9350.00 \pm 850.0 \times 10^6/L$), with significant decrease ($P < 0.05$) in the 100 mg/kg, 200 mg/kg, and 300 mg/kg groups. The lowest count was recorded at 300 mg/kg ($7000.00 \pm 200.00 \times 10^6/L$), indicating an immunomodulatory effect. Platelet count significantly decreased ($P < 0.05$) in a dose-dependent manner, with the lowest value observed in the 300 mg/kg group ($162.50 \pm 2.50 \times 10^9/L$). Conversely, PCV increased significantly ($P < 0.05$) in the treated groups compared to the control.

Table 1: Effect of *Triticum aestivum* Leaf Aqueous Extract on the Hematological Parameters

Groups	RBC($\times 10^{12}/L$)	WBC ($10^6/L$)	PLT ($10^9/L$)	PCV (%)	HB (g/dl)
Control	4.53 ± 0.08^a	9350.00 ± 850.0^c	179.00 ± 1.00^c	35.50 ± 0.50^a	11.95 ± 0.65^a
100mg <i>T. a</i>	4.73 ± 0.01^b	7300.00 ± 100.00^b	176.50 ± 1.50^c	41.00 ± 1.00^b	11.70 ± 0.50^a
200mg <i>T. a</i>	4.67 ± 0.01^b	8050.00 ± 550.00^b	169.00 ± 1.00^b	40.50 ± 0.50^b	11.80 ± 0.50^a
300mg <i>T. a</i>	5.27 ± 0.12^c	7000.00 ± 200.00^a	162.50 ± 2.50^a	41.50 ± 0.50^b	13.55 ± 0.05^b

Results are expressed as mean $\pm$ standard deviation (n=3). Mean values with different alphabets superscripts down the groups indicates significant differences at $P < 0.05$.

Table 2 below presents the effects of *T. aestivum* leaf aqueous extract on the differential counts of white blood cells (WBC), including neutrophils (N%), lymphocytes (L%), eosinophils (E%), monocytes (M%), and basophils (B%).

The control group had the highest neutrophil percentage ($61.50 \pm 1.50\%$), while the 200 mg/kg and 300 mg/kg groups showed significant ($P < 0.05$) reductions to ($53.50 \pm 1.50\%$) and ($53.00 \pm 1.00\%$) respectively. The lymphocyte percentage was significantly higher ($P < 0.05$) in the 100 mg/kg ($40.50 \pm 0.50\%$) and 200 mg/kg ($40.50 \pm 0.50\%$) groups compared to the

control ($39.50 \pm 0.50\%$). However, a significant reduction ($P < 0.05$) was observed at 300 mg/kg ($38.50 \pm 0.50\%$). The Eosinophil percentage significantly ($P < 0.05$) increased in the 100 mg/kg ($2.00 \pm 0.00\%$) and 300 mg/kg ($1.50 \pm 0.50\%$) groups compared to the control ($1.00 \pm 0.00\%$). The control group had the lowest monocyte percentage ($2.00 \pm 0.00\%$), while a significant increase ($P < 0.05$) was observed in the 100 mg/kg ($3.00 \pm 0.50\%$) and 200 mg/kg ($2.50 \pm 0.50\%$) groups. There were no significant differences in basophil count across all groups, with all values recorded at $0.00 \pm 0.00\%$.

Table 2: Effect of *Triticum aestivum* Leaf Aqueous Extract on the WBC Differential

Groups	N(%)	L(%)	E(%)	M(%)	B(%)
Control	61.50 ± 1.50^a	39.50 ± 0.50^b	1.00 ± 0.00^a	2.00 ± 0.00^a	0.00 ± 0.00^a
100mg <i>T. a</i>	62.50 ± 2.50^a	40.50 ± 0.50^c	2.00 ± 0.00^c	3.00 ± 0.50^b	0.00 ± 0.00^a
200mg <i>T. a</i>	53.50 ± 1.50^b	40.50 ± 0.50^c	1.00 ± 0.00^a	2.50 ± 0.50^b	0.00 ± 0.00^a
300mg <i>T. a</i>	53.00 ± 1.00^b	38.50 ± 0.50^a	1.50 ± 0.50^b	2.50 ± 0.50^b	0.00 ± 0.00^a

Results are expressed as mean $\pm$ standard deviation (n=3). Mean values with different alphabets superscripts down the groups indicates significant differences at $P < 0.05$. *T.a* = *Triticum aestivum*

Effect of *Triticum aestivum* Leaf Aqueous Extract on the Serum Antioxidant Enzymes

Table 3 below presents the effects of *Triticum aestivum* (wheatgrass) leaf aqueous extract on the levels of key antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) in serum. The findings indicate a dose-dependent reduction in antioxidant enzyme activities down the group, excluding catalase which seemed to increase non-significantly again at the highest dose.

The control group recorded an SOD level of 14.90 ± 0.17 U/mg. Treatment with 100 mg/kg and 200 mg/kg of *T. aestivum* extract resulted in slight but non-significant reductions ($P > 0.05$) to (13.10 ± 1.11 U/mg) and (13.11 ± 1.53 U/mg), respectively. However, at 300 mg/kg, a significant reduction ($P < 0.05$) to (10.05 ± 0.96 U/mg) was observed. For catalase, the control group had a level of 36.55 ± 1.97 U/mg. Treatment with 100 mg/kg and 200 mg/kg led to a significant reduction ($P < 0.05$) in CAT activity (32.47 ± 2.56 U/mg and 32.57 ± 1.78 U/mg, respectively). However, the 300 mg/kg group showed a non-significant increase in CAT activity to (33.99 ± 0.51 U/mg). The control group also recorded a GPx level of 22.96 ± 0.14 U/mg. No significant changes ($P > 0.05$) were observed at 100 mg/kg and 200 mg/kg (22.74 ± 1.03 U/mg and 22.34 ± 0.80 U/mg, respectively). However, at 300 mg/kg, a significant decrease ($P < 0.05$) to (20.94 ± 0.21 U/mg) was noted.

Table 3: Effect of *Triticum aestivum* Leaf Aqueous Extract on the Serum Antioxidant Enzymes

Groups	SOD(U/mg)	CAT(U/mg)	GPx(U/mg)
Control	14.90 ± 0.17^b	36.55 ± 1.97^b	22.96 ± 0.14^b
100mg <i>T. aestivum</i>	13.10 ± 1.11^b	32.47 ± 2.56^a	22.74 ± 1.03^b

200mg <i>T. aestivum</i>	13.11±1.53 ^b	32.57±1.78 ^a	22.34±0.80 ^b
300mg <i>T. aestivum</i>	10.05±0.96 ^a	33.99±0.51 ^b	20.94±0.21 ^a

Results are expressed as mean± standard deviation (n=3). Mean values with different alphabets superscripts down the groups indicates significant differences at P<0.05.

SOD= Superoxide Dismutase; CAT= Catalase; GPx= Glutathione peroxidase.

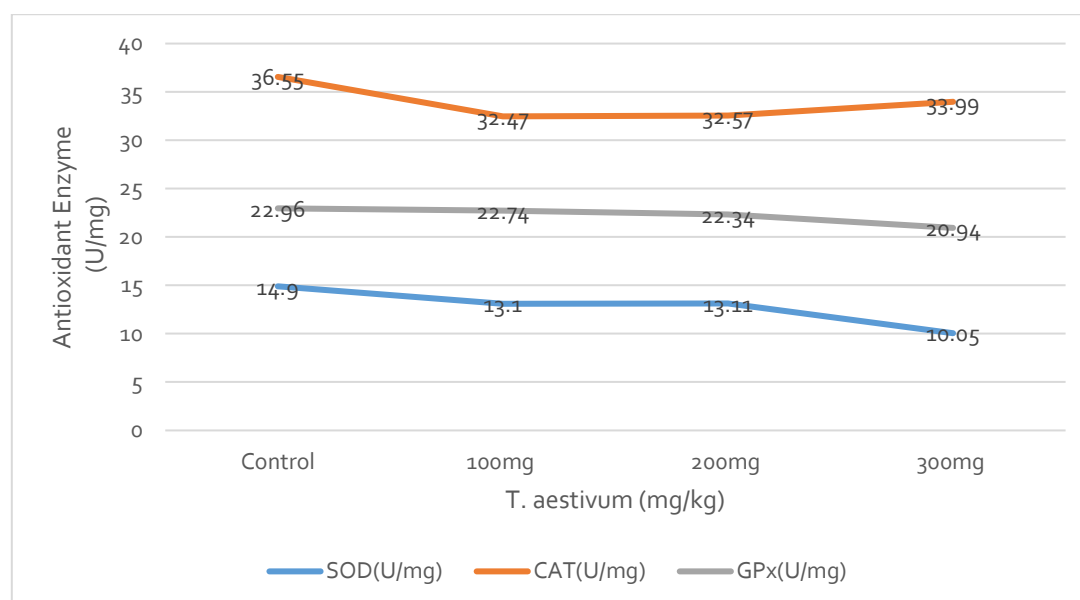


Figure 1: Effect of *Triticum aestivum* Leaf Aqueous Extract on the Serum Antioxidant Enzymes

Effect of *Triticum aestivum* Leaf Aqueous Extract Female Reproductive Hormones

Table 4 below presents the effects of *Triticum aestivum* leaf aqueous extract on female reproductive hormones, specifically follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone. The results indicate a dose-dependent increase in the levels of all three hormones, with significant differences observed across the treatment groups.

The control group recorded an FSH level of (2.54 ± 0.25 mg/ml). Administration of 100 mg/kg and 200 mg/kg of *T. aestivum* extract resulted in non-significant increases ($P>0.05$) to (2.95 ± 0.66 mg/ml) and (2.70 ± 0.39 mg/ml), respectively. However, at 300 mg/kg, FSH significantly increased ($P<0.05$) to (3.47 ± 0.07 mg/ml) suggesting a stimulatory effect at higher doses. The control group had an LH level of 1.44 ± 0.12 mg/ml. A non-significant increase ($P>0.05$) was observed in the 100 mg/kg group (1.47 ± 0.05 mg/ml), while significant increases ($P<0.05$) were noted at 200 mg/kg (1.82 ± 0.14 mg/ml, $P<0.05$) and 300 mg/kg (2.69 ± 0.32 mg/ml) indicating a dose-dependent rise in LH levels. The control group also had a progesterone level of (26.66 ± 0.91 mg/ml). The values progressively increased across the treatment groups, with a non-significant increase ($P>0.05$) at 100 mg/kg (27.48 ± 0.88 mg/ml) and significant increases ($P<0.05$) at 200 mg/kg (28.82 ± 0.52 mg/ml, $P<0.05$) and 300 mg/kg (30.48 ± 0.86 mg/ml).

Table 4: Effect of *Triticum aestivum* Leaf Aqueous Extract on Female Reproductive Hormones

Groups	FSH(mg/ml)	LH(mg/ml)	Progesterone(mg/ml)
Control	2.54±0.25 ^a	1.44±0.12 ^a	26.66±0.91 ^a
100mg <i>T. aestivum</i>	2.95±0.66 ^a	1.47±0.05 ^a	27.48±0.88 ^a
200mg <i>T. aestivum</i>	2.70±0.39 ^a	1.82±0.14 ^b	28.82±0.52 ^b
300mg <i>T. aestivum</i>	3.47±0.07 ^b	2.69±0.32 ^c	30.48±0.86 ^c

Results are expressed as mean± standard deviation (n=3). Mean values with different alphabets superscripts down the groups indicates significant differences at P<0.05.

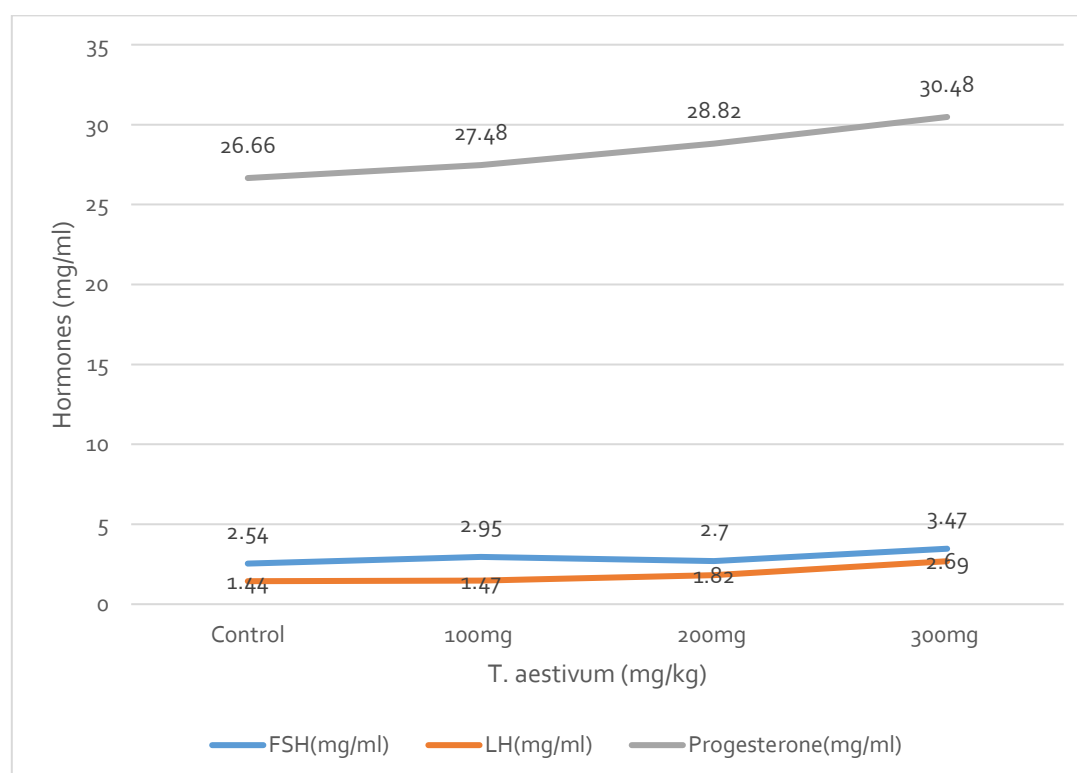


Figure 2: Effect of *Triticum aestivum* Leaf Aqueous Extract on Female Reproductive Hormones

Discussion

Haematological parameters are crucial indicators of physiological and pathological states, reflecting the impact of dietary and pharmacological interventions (Runjala and Murthy, 2020). The observed increase in RBC count and PCV in the treated groups suggests an erythropoietic effect of *T. aestivum*, possibly due to its rich content of iron, chlorophyll, and other hematopoietic bioactive compounds (Aldulaimi *et al.*, 2021). Similar findings were reported by Runjala and Murthy (2020), who observed increased RBC production following wheatgrass supplementation in anemic animal models. The decline in WBC count at higher doses may indicate an immunomodulatory effect of *T. aestivum*, which could be beneficial in reducing inflammatory responses. However, excessive reduction in WBCs may compromise immune function, necessitating further investigation into the safety profile of

wheatgrass at high doses. The significant reduction in platelet count aligns with previous findings suggesting that wheatgrass possesses mild anticoagulant properties, which may be beneficial in thrombotic disorders (Maruyama *et al.*, 2001; Khurana *et al.*, 2025). However, this effect should be carefully monitored in individuals with bleeding disorders. White blood cells (WBCs) are crucial components of the immune system, responsible for defense against infections, inflammation, and other pathological conditions. Neutrophils are the first line of defense in acute infections, while lymphocytes play vital roles in adaptive immunity, eosinophils mediate allergic responses, and monocytes contribute to phagocytosis and antigen presentation (Zendehbad *et al.*, 2014).

In this study, *T. aestivum* extract administration led to a dose-dependent decrease in neutrophil count, with the most significant reduction at 300 mg/kg. This finding suggests that wheatgrass may have anti-inflammatory properties, as neutrophils are commonly elevated during acute inflammatory responses. Conversely, lymphocyte count increased significantly at lower doses (100 mg/kg and 200 mg/kg), indicating potential immune-stimulatory effects of *T. aestivum* (Runjala and Murthy, 2020). However, the slight reduction in lymphocytes at 300 mg/kg suggests a possible immunosuppressive effect at higher doses. The significant increase in eosinophils at 100 mg/kg suggests that wheatgrass might influence allergic responses or parasitic defense mechanisms. This is consistent with previous studies demonstrating that wheatgrass contains bioactive compounds capable of modulating immune cell functions. The increase in monocyte count observed in the 100 mg/kg and 200 mg/kg groups suggests enhanced immune surveillance, which could be beneficial for host defence. However, excessive monocyte activation has been linked to chronic inflammation (Runjala and Murthy, 2020).

Antioxidant enzymes play a vital role in protecting cells from oxidative stress by neutralizing reactive oxygen species (ROS). Superoxide dismutase (SOD) catalyzes the conversion of superoxide radicals to hydrogen peroxide, which is then broken down by catalase (CAT) and glutathione peroxidase (GPx) (Yoon *et al.*, 2024). A decline in these antioxidant enzymes is often associated with increased oxidative stress and cellular damage (Kowalska *et al.*, 2014). The findings of this study demonstrate that *Triticum aestivum* leaf aqueous extract influences antioxidant enzyme activities in a dose-dependent manner. The significant reduction in SOD and GPx levels at higher doses (300 mg/kg) suggests a potential pro-oxidant effect of the extract at high concentrations, which may lead to oxidative stress rather than protection. Therefore a low dosage could be advised. Conversely, the observed decrease in CAT activity at lower doses (100 mg/kg and 200 mg/kg) followed by a slight increase at 300 mg/kg suggests a possible compensatory response to oxidative stress.

These results contrast with previous reports indicating that *T. aestivum* exhibits strong antioxidant properties due to its rich content of polyphenols, flavonoids, and chlorophyll (Yoon *et al.*, 2024). Studies by Kowalska *et al.* (2014), demonstrated that wheatgrass supplementation increased SOD and GPx activities in oxidative stress-induced animal models, suggesting an antioxidant protective role. However, our study suggests that at higher doses, *T. aestivum* may exert a paradoxical pro-oxidant effect, which has been

reported for some polyphenol-rich plant extracts when consumed in excess (Kulkarni *et al.*, 2006). The decline in SOD and GPx activities at higher doses of *T. aestivum* extract may be attributed to excessive accumulation of ROS, leading to enzyme inhibition. While *T. aestivum* contains potent antioxidants, an excessive intake may overwhelm cellular redox homeostasis, leading to oxidative stress instead of protection (Doan *et al.*, 2024). Additionally, variations in CAT activity suggest an adaptive response where increased ROS production at high doses may trigger compensatory upregulation of CAT (So *et al.*, 2024). Female reproductive hormones including follicle-stimulating hormone (FSH), luteinizing hormone (LH), and progesterone, play crucial roles in ovarian function, ovulation, and pregnancy maintenance. FSH regulates follicular development, LH triggers ovulation and corpus luteum formation, while progesterone is essential for the establishment and maintenance of pregnancy (Cristian-Alin *et al.*, 2025). The present study demonstrates that *Triticum aestivum* leaf aqueous extract significantly elevates FSH, LH, and progesterone levels in female albino rats in a dose-dependent manner. This suggests that *T. aestivum* may possess bioactive compounds that modulate hormonal secretion, potentially enhancing reproductive health. The observed increase in FSH and LH levels aligns with the findings of Eissa *et al.* (2020), who reported that wheatgrass (*Triticum aestivum*) extract enhances gonadotropin secretion in animal models. The phytochemical constituents of *T. aestivum*, including flavonoids, phenols, and alkaloids, may act as phytoestrogens, which have been shown to interact with estrogen receptors and modulate the hypothalamic-pituitary-gonadal axis (Ove *et al.*, 2024).

Similarly, the increase in progesterone levels is consistent with the findings of Cristian-Alin *et al.* (2025), who observed a positive effect of plant-derived bioactive compounds on progesterone secretion, promoting fertility and pregnancy maintenance. The significant increase in progesterone levels at higher doses suggests that *T. aestivum* may enhance luteal function, which is critical for reproductive success (Anna and Gladys, 2024). The stimulatory effect of *T. aestivum* on reproductive hormones could be attributed to its rich antioxidant profile. The presence of phenolic compounds and flavonoids (as shown in Table 4.2) may help protect ovarian follicles from oxidative stress, thereby improving hormonal regulation (Eissa *et al.*, 2020). Additionally, minerals such as zinc and selenium have been linked to improved ovarian function and hormone synthesis (Anna and Gladys, 2024). Some limitation to this study is lack of histological confirmation available as at the time of research.

Conclusion and Recommendations

Conclusion

The present study showed that *Triticum aestivum* leaf aqueous extract enhanced RBC production, significantly improved antioxidant enzyme activity, and showed modulation in female reproductive hormones assayed, indicating its potential as a natural supplement for anemia, oxidative stress, and reproductive disorders owing to the numerous nutritional values of *Triticum aestivum*. However, the dose-dependent reduction in WBC and platelet

counts suggests a possible immunosuppressive or thrombocytopenic effect at high doses. These findings align with previous reports on the hematological and therapeutic properties of *T. aestivum*, emphasizing its potential in complementary medicine. Nonetheless, further studies are necessary to determine its long-term safety and optimal therapeutic dosage for human applications.

Recommendations

Based on the findings from the present studies, the following recommendations were made:

1. Further studies should focus on identifying the optimal dose of *T. aestivum* to maximize its benefits while minimizing potential hematological risks.
2. More human studies should be conducted to validate its efficacy in treating anemia, oxidative stress, and reproductive health disorders.
3. Long-term toxicity evaluations should be performed to assess the safety profile of *T. aestivum*, particularly regarding its effects on WBC and platelet counts.

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