

Study On Anti-Anaemic Activity Of *Xanthosoma Sagittifolium* (L.) Schott (Black Stem)

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Abstract

Background: Anaemia is a common health problem in developing countries such as India, especially among women and children, caused by iron deficiency. Side effects of traditional iron supplements have induced interest in the alternatives based on plants with improved safety profiles.

Aim: To assess the anti-anaemic activity of *Xanthosoma sagittifolium* (L.) Schott (Black Stem), an indigenous medicinal plant found to be very common in Manipur, employing an in vivo rat model.

Methods: Plant petiole methanolic extracts were processed and orally given to phenylhydrazine-induced anaemic albino rats in doses of 200 mg/kg and 400 mg/kg for 14 days. Hematological parameters like Hb, RBC, HCT, MCH, MCV, MCHC, and WBC count were assessed and statistically compared with control and standard drug (Vitafer forte) groups.

Results: The extract brought noteworthy, dose-dependent increases in hemoglobin, RBC count, and other red cell indices. The 400 mg/kg group had hematological recovery equivalent to the positive control group. Moreover, WBC count normalized, which might indicate reduction in inflammation or oxidative stress.

Conclusion: *X. sagittifolium* shows encouraging anti-anaemic and hematinic activity, possibly because of the high content of iron and phytochemicals. It has potential as a natural, inexpensive, and safer alternative to synthetic iron preparations, of particular value in resource-poor environments. Isolation of active constituents and clinical efficacy need to be confirmed through further studies.

Keywords: Anaemia, Hematinic activity, Iron supplementation, Medicinal plants. *Xanthosoma sagittifolium*.

1. Introduction

Iron is an important micronutrient required for many physiological functions, mainly used in the construction of hemoglobin—the oxygen transport pigment in red blood cells (RBCs) [1]. Hemoglobin enables the transport of oxygen from the lungs to body tissues and back carbon dioxide for expulsion. Iron is also needed for the construction of myoglobin, a pigment that temporarily stores oxygen in muscles for utilization upon exercise [2]. Iron deficiency, commonly a consequence of poor dietary intake or faulty absorption, results in compromised erythropoiesis and ultimately in anaemia syndrome characterized by decreased concentration of hemoglobin, decreased oxygen-carrying capacity, and accompanying systemic manifestations such as fatigue, shortness of breath, headache, and dizziness [3,4].

Anaemia is not an illness in its own right but a reflection of a multitude of underlying pathologies. It is most common in tropical and developing areas, posing major public health problems. Clinically, anaemia is characterized by a fall in hemoglobin concentration (e.g., <11 g/dL in lactating women), decreased RBC count, or abnormal RBC morphology [5]. The most common forms of anaemia are iron deficiency anaemia (IDA), haemolytic anaemia, vitamin B12 and folic acid deficiency anaemias, anaemias associated with inherited disorders such as sickle cell disease and thalassemia, and anaemia of chronic diseases [6]. Worldwide, anaemia due to iron deficiency is the most prevalent and avoidable nutritional disorder, with a majority in women of childbearing ages being affected by menstruation, pregnancy, delivery, and breastfeeding—factors that multiply iron requirements [7,8]. According to the World Health Organization (WHO), about 45% of pregnant women in developing nations suffer from anaemia. Anaemia in pregnancy is linked with increased risks of maternal and perinatal morbidity and mortality, such as premature birth and low birthweight babies. Postpartum haemodynamic alterations and blood loss also worsen the anaemic status [9-11].

At the cellular level, iron is crucial for hemoglobin formation via erythropoiesis. Inflammation may result in anaemia of inflammation (AI), a frequent type of iron-deficient erythropoiesis [12]. AI is controlled to a great extent by the hepatic hormone hepcidin, which is elevated during inflammation [13]. Hepcidin induces ferroportin (FPN), the major iron exporter in enterocytes, hepatocytes, and macrophages, to be internalized and degraded. This compromises iron release and absorption from stores, resulting in hypoferremia and functional iron deficiency even in the presence of sufficient body iron stores [14,15]. If not treated, anaemia can cause serious complications like cardiomegaly, as a result of decreased blood viscosity and the body's adaptive increase in cardiac output to compensate for tissue hypoxia. The management involves iron supplements, generally ferrous sulfate because of its high bioavailability [16]. Synthetic iron preparations, though, are usually linked with gastrointestinal side effects like nausea, constipation, abdominal cramps, and dark stools, which result in

non-compliance. In addition, iron supplement absorption is inhibited by the simultaneous use of antacids, calcium foods, and some dietary phytates [17]. These constraints have seen greater enthusiasm for natural and plant-based options with fewer side effects and greater nutritional value. Ethnobotanical practices justify the application of several medicinal plants with hematinic properties. Herbal treatments not only provide iron in bioavailable states but also contain other micronutrients and phytoconstituents that enhance hematopoiesis and health.

Iron from food sources comes in two forms: heme (mainly from animal sources) and non-heme (from plant sources) [18]. Heme iron is better absorbed and its absorption is not much affected by other food constituents. The absorption of non-heme iron, however, is affected by such absorption enhancers as vitamin C and such absorption inhibitors as tannins, phytates, calcium, and phosphates. Therefore, iron-containing medicinal plants, particularly when taken together with absorption enhancers, can be very effective natural drugs for the treatment of anaemia. Several plant extracts e.g., *Moringa oleifera*, *Carica papaya*, *Thymus vulgaris*, *Sauropus androgynus*, and *Psidium guajava* exhibited anti-anaemic and hemopoietic activities in preclinical research. For instance, chlorophyll from *Sauropus androgynus* increased hemoglobin and ferritin levels and lowered hemolysis markers. Likewise, *Carica papaya* leaf extract was found to have renoprotective and hemoprotective activity, indicating its potential application in inflammatory anaemias [19,20]. Research also points to the bioavailability of iron from natural sources such as blackstrap molasses and *Zanthoxylum usambarensis* bark extracts and *Erythrina abyssinica* bark extracts, showing their potential in controlling anaemia without the side effects that come with synthetic preparations.

Xanthosoma sagittifolium (L.) Schott, also referred to as black-stem taro, has attracted interest for its nutritional and medicinal value. The Araceae family member is historically utilized in a number of areas due to its suspected therapeutic benefits [21]. With a high content of micronutrients and phytochemicals such as iron and flavonoids, the leaves and stems of *Xanthosoma sagittifolium* can potentially provide hematinic advantage. Its anti-anaemic prospects, nonetheless, have yet to be comprehensively investigated using scientific research. The current study is concerned with assessing the anti-anaemic potential of the methanolic extract of *Xanthosoma sagittifolium* black stem using an in vivo model. Experimental subjects in this research are albino rats to find out if the extract has hematopoietic efficacy through the restoration of hemoglobin values and red cell indices. With this research, the viability of using *Xanthosoma sagittifolium* as a natural alternative to the use of traditional iron supplements in controlling anaemia can be critically examined and confirmed.

2. Materials and methods

2.1. Sample Collection and Authentication

Plant material for *X. Sagittifolium* was gathered from Thangmeiband in Imphal West, Manipur, in India. The plant was verified and the herbarium (1380/n-222) was given to the Institute of Bioresources & Sustainable Development (IBSD) in Takyel, Manipur. Petioles from *X. Sagittifolium* were cleaned with tap water at first, rinsed in double distilled water next and then dried in an oven at 600°C for one night. The petioles had to be dried, ground and put aside in airtight jars until needed. Tissue paper was used to clean the fresh petioles prior to measuring their moisture. For the free radical scavenging tests, the powdered sample was dissolved in pre-distilled chloroform and methanol one at a time.

2.2. Extraction and fractionation of the plant material

The stems were washed, then dried in the shade while shifting them often and ground to a rough powder using a motor. Afterwards, 2.0 kg of the coarse ground dried sample was treated with methanol for 72h to extract the ingredients. The helper group helped concentrate all the filtrate on a rotary evaporator set to 40°C so that the methanol extract could be recovered. Part of the concentrated methanolic extract was fractionated with chloroform, petroleum ether, methanol and water in order. After drying, the extracts were used as part of the experiment (Table 1).

Table 1. Fractionation of the extracts		
Plant part	Solvent	Weight of the extract
Petiole (stem) (2.0 kg)	Chloroform	18.8 g
	Methanol	21.1 g
	Water	9.0 g

2.3. In-vivo study

➤ Animal model

It was done on albino rats. Animals were kept on prescribed diet and water ad libitum. Animals were acclimatised for one week before research. All the experiments of animals were carried out based on Institutional Animal Ethical Committee (IAEC) protocols.

➤ Acute toxic studies

Acute toxicity tests (study of experiment with different doses as ranging from 1000 to 2000 mg per kg which didn't lead to animal death) were conducted. The animals are divided into groups of five, each containing eight animals. The four groups of animals (i.e. 32 animals) were induced by phenylhydrazine (PHZ). Blood was obtained in EDTA coated tube by telepuncture after administration of anaesthesia and estimation of various biochemical parameters such as Hb, RBC values etc. was done.

2.4. Induction of anaemia

Anaemia was induced into the Albino rats with phenyl hydrazine (PHZ) at a dose of 20 mg/ kg body wt through intraperitoneal injection. PHZ is a non-immunogenic drug with an effect of causing the red cell membrane changes, resulting in oxidative denaturation of haemoglobin. The denaturation effect is to shorten the life span of the erythrocytes. Damaged erythrocytes are cleared by the liver and spleen, and compensatory hemolytic anemia occurs. Anaemia induced by PHZ is a model, assisting in the research of hematinic effect. The PHZ-treated rats whose Hb level is below 9 g/L were taken as anaemic and included in the study.

The experimental animals were then divided into five groups of eight rats in each group. The plant extracts and reference drug – iron tonic in their respective dose level were administered to experimental animals daily for a period of 14 days.

- **Group 1 (Normal control):** received distilled water
- **Group 2 (Anaemic control):** received distilled water + PHZ (20 mg/kg)
- **Group 3:** received oral dose of plant extract (200mg/kg)+ PHZ (20 mg/kg)
- **Group 4:** received oral dose of plant extract (400 mg/kg)+PHZ (20 mg/kg)
- **Group 5: (Positive control):** received oral dose of reference drug 'Vitafer forte' syrup 5.0 mL (containing 200 mg ferric ammonium citrate)+PHZ (20 mg/ kg).

2.5. Blood Collection and Hematological Analysis

On Day-1, Day-7, Day-14, blood was drawn in EDTA coated tube by telepuncture after administration of anaesthesia. The samples were utilized for haematological parameters such as Hb, RBC, HCT, MCHC, MCV, MCH, WBC values using an automated blood cell counter. Another component of blood was drawn in plastic test tube and was kept at rest for 3 hours so that it became properly clotted. The blood clot samples were centrifuged for 10 minutes at 3000 rpm and clear serum samples aspirated and frozen -20°C stored to be used for biochemical parameter analysis.

2.6. Statistical Analysis

All the values were given as mean \pm standard deviation (SD). One-way ANOVA with post hoc Tukey's test was used to analyze differences between groups and the significance was taken to be <0.05 .

3. Results and discussion

3.1. Hematological parameters (Anti-anaemic activity) Studies

The result in Table 2 illustrates the impact of the methanolic extract of *Xanthosoma sagittifolium* on hemoglobin (Hb) concentration in test animals for 14 days. Group 1 (normal control) had hemoglobin levels that remained constant, reflecting normal physiological status. Group 2 (anaemic control) had low hemoglobin concentrations consistently, with only a slight increase on Day 14, reflecting sustained anemia without treatment. Conversely, Groups 3 and 4, that were given 200 mg/kg and 400 mg/kg of the plant extract respectively, had increasing hemoglobin levels, with Group 4 having a sharper increase from 8.93 g/dL on Day 1 to 12.33 g/dL on Day 14. This indicates a dose-related hematinic action of the extract. Group 5 (positive control) was also increased significantly, achieving levels close to normal on Day 14, attesting to the effectiveness of conventional treatment (figure 1). Other studies have also reported comparable hematinic activity with herbal extracts. For example, extract of *Xanthosoma sagittifolium* has provided hematological improvement in albino rats, such as elevated Hb and RBC values, attesting to its hematopoietic potential. Additionally, its antioxidant capacity and high iron content have been shown to counteract oxidative stress-induced anaemia. This is consistent with evidence showing that PHZ-induced hemolytic anaemia is caused by oxidative damage to erythrocyte membranes by ROS, which leads to RBC ageing and destruction, thereby lowering Hb levels [22].

Table 2. Effect of methanolic extract of *Xanthosoma sagittifolium* on hemoglobin level of experimental animals

Groups	Day -1	Day -7	Day-14
Group 1 (Normal control)	14.03 g/dL	13.86 g/dL	14.13 g/dL
Group 2 (Anaemic control)	8.43 g/dL	8.43 g/dL	8.56 g/dL
Group 3 (plant extract,200mg/kg)	8.83 g/dL	9.76 g/dL	10.26 g/dL
Group 4 (plant extract 400mg/kg)	8.93 g/dL	11.83 g/dL	12.33 g/dL
Group 5 (positive control)	9.33 g/dL	12.76 g/dL	13.13 g/dL

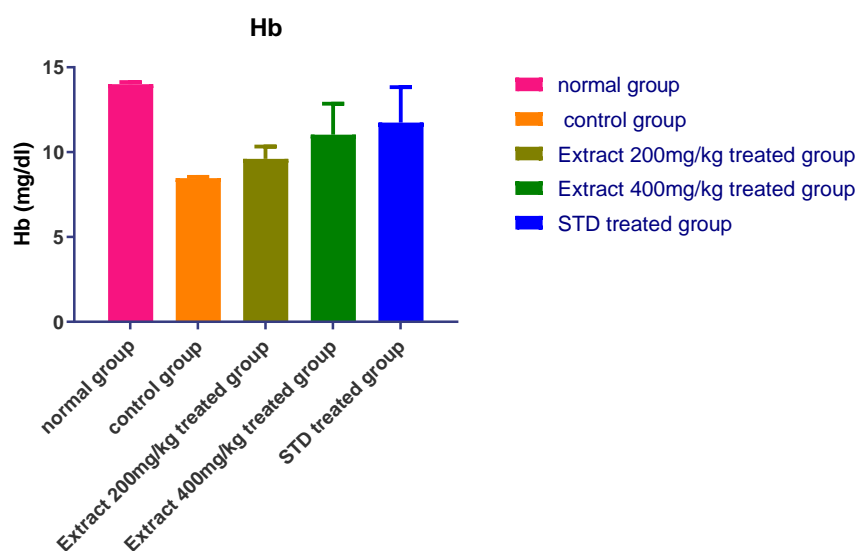


Figure 1. Graphical representation of Hb levels in all groups

3.2. Red Blood Cell (RBC) Count

Table 3 illustrates the impact of *X. sagittifolium* extract on RBC count over the course of 14 days. Group 1 had consistent RBC counts, Group 2 had a dramatic drop, reflecting deteriorating anemia. Groups 3 and 4 had dose-dependent increases in RBC counts, with Group 4 approaching values of Group 5 (positive control)(figure 2). These findings are consistent with earlier reports of plant interventions causing efficient erythropoiesis restoration in anaemic models. Similar increase in RBC was observed in rats treated with *Telfairia occidentalis* and *Moringa oleifera* extracts, reaffirming the importance of phytotherapeutics in anaemia control [22].

Table 3. Effect of methanolic extract of <i>Xanthosoma sagittifolium</i> on RBC level of experimental animals			
Groups	Day -1	Day -7	Day-14
Group 1 (Normal control)	7.53	7.53	7.56
Group 2 (Anaemic control)	6.93	5.13	4.86

Group 3 (plant extract,200mg/kg)	5.93	6.23	6.63
Group 4 (plant extract 400mg/kg)	5.63	6.53	6.73
Group 5 (positive control)	5.96	6.73	6.93

RBC normal range:

Men - 4.7 to 6.1 million cells per microlitre

Women - 4.2 to 5.4 million cells per microlitre.

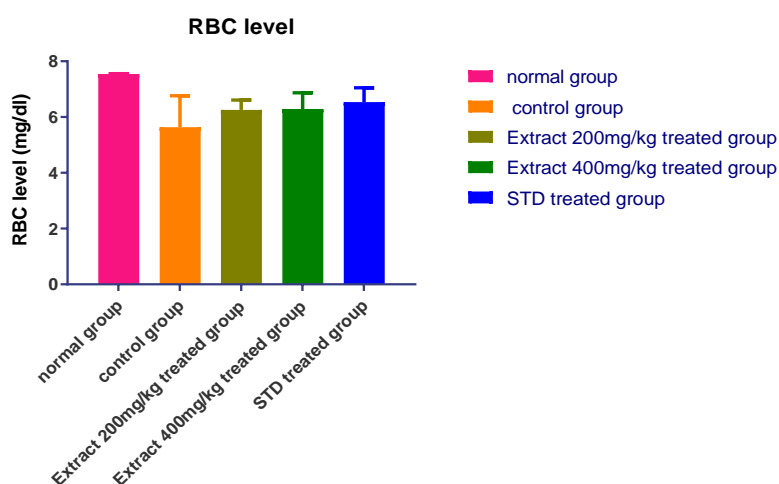


Figure 2. Graphical representation of RBC levels in all groups

3.3. Hematocrit (HCT)

In Table 4, HCT levels increased highly in treated groups, especially Group 4, which elevated from 36.43% to 42.46%. Such an improvement indicates increased RBC volume and validates the role of *X. sagittifolium* in enhancing oxygen-carrying capacity (figure 3). Comparable effects were reported with other medicinal crops, such as *Colocasia esculenta* and *Telfairia occidentalis*, that demonstrated hematocrit restoration in cases of anemia [23].

Table 4. Effect of methanolic extract of <i>Xanthosoma sagittifolium</i> on HCT level of experimental animals			
Groups	Day -1	Day -7	Day-14
Group 1 (Normal control)	44.86	45.33	45.16
Group 2 (Anaemic control)	31.06	30.86	30.43
Group 3 (plant extract,200mg/kg)	34.66	36.03	37.33
Group 4 (plant extract 400mg/kg)	36.43	38.83	42.46
Group 5	38.03	40.66	43.96

(positive control)			
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Hematocrit : proportion of the blood cell that consists of packed RBCs expressed as percentage by volume.

Range –Men – 42 to 54% in 100 mL of Blood

Women – 38 to 46% in 100 mL of Blood

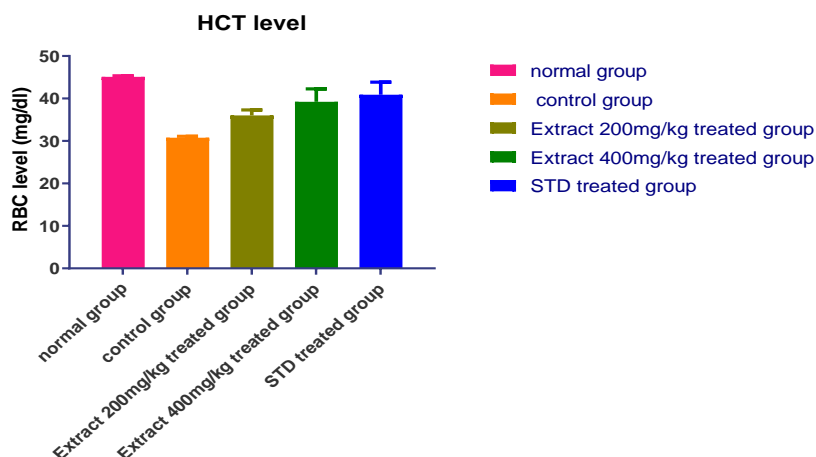


Figure 3. Graphical representation of HCT levels in all groups

3.4. Mean Corpuscular Hemoglobin Concentration (MCHC)

Table 5 findings show that *X. sagittifolium* extract enhanced MCHC in a dose-dependent pattern, indicating enhanced hemoglobin concentration per cell. The maximum rise was noted in Group 5 (positive control) but was followed closely by Group 4, indicating the extract's utility in correcting hypochromia (figure 4). These results are in line with previous studies that have shown the effectiveness of chlorophyll-containing plant extracts in increasing MCHC values [24].

Table 5. Effect of methanolic extract of *Xanthosoma sagittifolium* on MCHC level of experimental animals

Groups	MCHC(g/dL)		
	Day -1	Day -2	Day -3
Group 1 : (Normal control)	32.23	32.83	33.36
Group 2: (Anemic control)	22.26	23.76	25.06
Group 3: received oral dose of plant extract (200mg/kg)	23.83	24.96	25.63
Group 4: received oral dose of plant extract (400mg/kg)	24.26	25.13	26.16
Group 5: (Positive control)	25.33	26.83	28.56

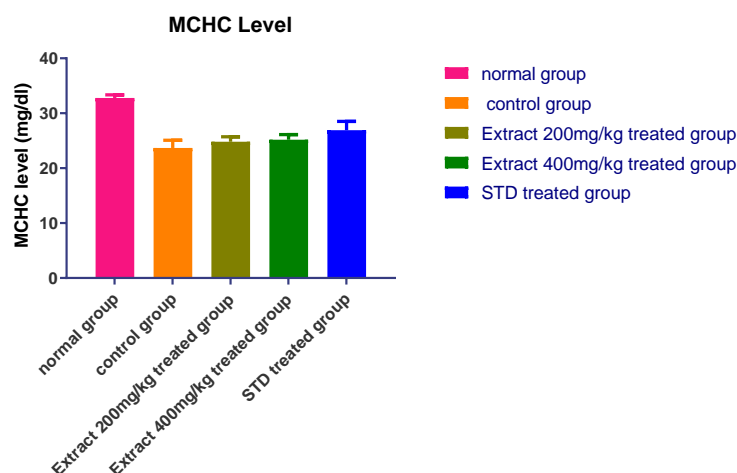


Figure 4. Graphical representation of MCHC levels in all groups

3.5. Mean Corpuscular Volume (MCV)

As evident from Table 6, MCV values significantly increased in Groups 3 and 4, showing a transition from microcytic to normocytic cell profiles. Group 4 had the highest recovery, from 63.63 fL to 69.36 fL (figure 5). This indicates that *X. sagittifolium* has an effect on red cell regeneration and normalization. Ubaluaet al., [25] have earlier described that the underground corm of *X. sagittifolium* has high iron, vitamins, and amino acids that enhance red cell maturation, supporting these observations.

Table 6. Effect of methanolic extract of *Xanthosoma sagittifolium* on MCV level of experimental animals

Groups	MCV fL (μm^3)		
	Day -1	Day -2	Day -3
Group 1 : (Normal control)	75.33	75.96	75.73
Group 2: (Anemic control)	52.93	47.66	45.76
Group 3: received oral dose of plant extract (200mg/kg)	56.76	58.93	62.83
Group 4: received oral dose of plant extract (400mg/kg)	63.63	65.96	69.36
Group 5: (Positive control)	65.93	68.76	72.83

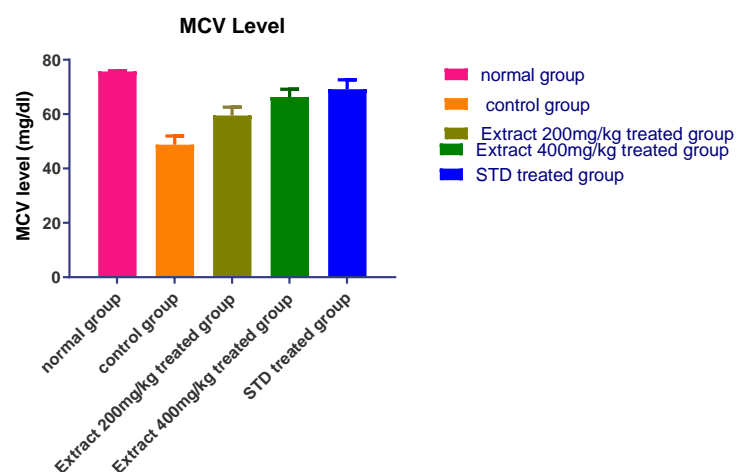


Figure 5. Graphical representation of MCV levels in all groups

3.6. Mean Corpuscular Hemoglobin (MCH)

In Table 7, MCH values improved in a dose-related manner. Group 2's high MCH could suggest macrocytosis but the correction in treated groups indicates augmented hemoglobin loading per RBC (figure 6). These findings replicate earlier observations in studies of herbal agents increasing MCH via erythropoietic stimulation [26,27].

Table 7. Effect of methanolic extract of *Xanthosoma sagittifolium* on MCH level of experimental animals

Groups	MCH (pg)		
	Day -1	Day -2	Day -3
Group 1 : (Normal control)	22.26	21.76	21.86
Group 2: (Anemic control)	29.13	29.43	30.83
Group 3: received oral dose of plant extract (200mg/kg)	25.43	26.43	28.26
Group 4: received oral dose of plant extract (400mg/kg)	23.06	23.86	25.53
Group 5: (Positive control)	24.13	23.53	23.43

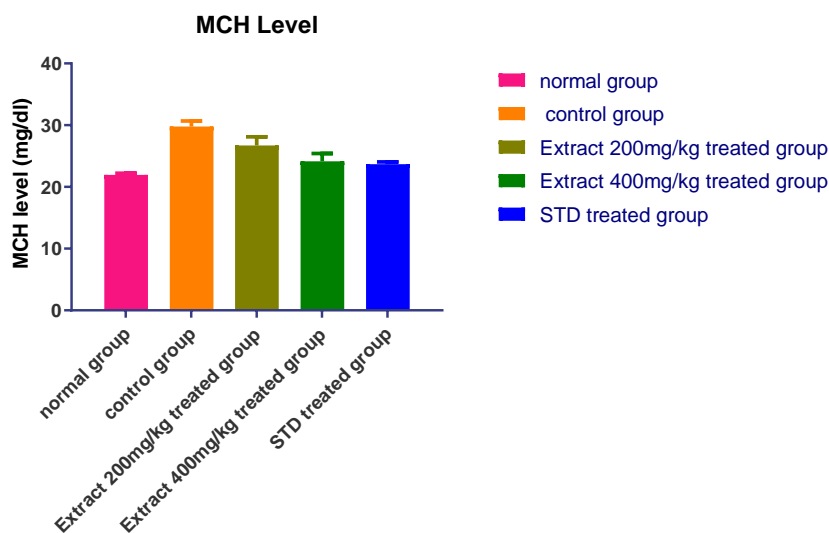


Figure 6. Graphical representation of MCH levels in all groups

3.7. White Blood Cell (WBC) Count

Table 8 presents the WBC profile, showing that Group 2 experienced elevated WBC counts due to PHZ-induced inflammation (figure 7). In contrast, Groups 3 and 4 exhibited reductions in WBC levels over time, indicating the immunomodulatory role of the plant extract. Gutiérrez et al. [22] noted that PHZ leads to inflammatory responses, while Fakunle et al. [26] showed that antioxidant-rich plant extracts reduce WBC counts by mitigating ROS-mediated stress.

Table 8. Effect of methanolic extract of *Xanthosoma sagittifolium* on WBC level of experimental animals

Groups	WBC ($10^3/\mu\text{L}$)		
	Day -1	Day -2	Day -3
Group 1 : (Normal control)	11.53	11.63	11.53
Group 2: (Anemic control)	16.66	17.13	18.46

Group 3: received oral dose of plant extract (200mg/kg)	14.53	15.66	16.93
Group 4: received oral dose of plant extract (400mg/kg)	12.56	13.66	14.46
Group 5: (Positive control)	12.36	12.93	13.66

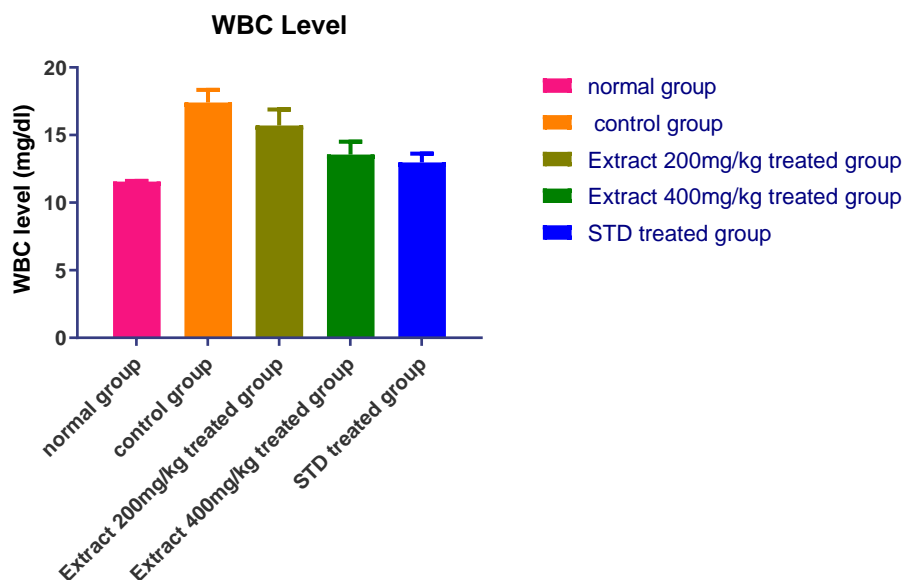


Figure 7. Graphical representation of WBC levels in all groups

Conclusion

The research emphasizes the potential anti-anaemic role of *Xanthosoma sagittifolium* (L.) Schott (Black Stem), a locally used and widely distributed plant in Manipur. The iron- and phytochemical-rich methanolic extract exhibited a notable hematinic activity in albino rats, pointing towards its capacity for hemoglobin level normalization and red cell indices improvement. This makes the plant a potential, nature-based solution to man-made iron supplements that tend to have unwanted side effects. The results support the inclusion of such ethnobotanically useful plants in controlling anemia, advocating for safer, nutritionally dense interventions most appropriate for populations in resource-poor or rural areas.

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