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Research Article

Combating Iron Deficiency Anaemia: Insights From Solvent-Extracted Fractions of *Theobroma cacao*

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ABSTRACT

Herbs have been used from time immemorial to treat and manage different diseases, including nutrient-related disorders. This study seeks to compare the anti-anaemic effects of different solvent fractions of the stem bark of *Theobroma cacao* in treating Iron-deficiency anaemia. The effect of administration of ethanol, ethyl acetate and N-hexane extract fractions of *Theobroma cacao* stem bark on some haematological parameters, serum ferritin of iron-deficient rats was investigated. Forty (40) albino rats with an average weight of 60.0 g were used. Ten (10) of the rats were placed on iron-sufficient feed, twenty-five (25) were fed iron iron-deficient diet, while the remaining five (5) were given commercial (standard) feed. The body Weight, packed cell volume (PCV), and Red Blood Cell Count (RBC) of the iron-deficient rats reduced significantly after eight (8) weeks of feeding, which confirms the presence of iron-deficient anaemia. The iron-deficient groups showed increased hematologic parameters when administered with ethanol, ethyl acetate, and hexane fractions of *Theobroma cacao* extracts, respectively, for two (2) weeks. Their hematologic parameters also compared closely with those Iron Sufficient and Commercial Feed Groups. Phytochemical screening of the extract revealed Saponins and Glycosides across board, ethanolic and ethyl acetate fractions contain tannins and phlobatannins, while ethanol and hexane fractions exclusively contain Terpenoids and Flavonoids, respectively. The results revealed that the administration of ethanol, ethyl acetate and n-hexane extracts of *Theobroma cacao* reversed iron-deficiency anaemia and improved the weight of the animals.

Keywords: Anaemia; Hematology; *Theobroma cacao;* Ferritin; Medicinal Plants; Packed Cell Volume

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INTRODUCTION

Iron deficiency anaemia remains the most widespread nutritional disorder worldwide affecting women of reproductive age and children under five, being mostly vulnerable (Safiri *et al.*, 2021). Among children, the condition is defined by haemoglobin levels below 105 g/L for those aged 0–23 months, and below 110 g/L for those aged 24–59 months (WHO, 2011; WHO, 2024). Presently, it is estimated that about 40% of children between 6 and 59 months suffer from anaemia worldwide (WHO, 2023). In Africa, the situation is alarming as more than 60% of children are anaemic, and over 40% of these are classified as severe anaemia (WHO, 2024). In Sub-Saharan Africa, the prevalence of anaemia among children under 5 varies from 42% in Eswatini to 91% in Burkina Faso (Soares and Clements, 2011).

The consequences of anaemia in children is far-reaching with the subject experiencing stunted growth, impaired motor and cognitive development, increased vulnerability to infections, and increased morbidity and mortality (WHO, 2023). Early acquired mental deficits are believed to be irreversible, and the consequences can persist even after treatment, emphasizing the importance of early detection and prevention (Brunt *et al.*, 2012).

Despite significant efforts by African governments and various stakeholders to combat anaemia, this condition still represents a critical public health challenge among children under five years old and pregnant women (Seifu and Tesema, 2022). Conventional strategies such as iron supplementation that have been previously used yielded limited success, especially in regions of Africa where affordability and accessibility are major barrier as many in this region struggle to afford basic meals, let alone micronutrient supplementation.

This has led to an increased reliance on local herbs including *Sorghum bicolo*r, *Mangifera indica* and *Theobroma cacao* that have been verified to be potent in treating iron deficiency anaemia and the most effective dose (25 mg) of the latter in aqueous extract has been verified (Modupe *et al.*, 2018; Oladiji *et al.* 2007). However, the effectiveness of herbs often depend on the method of extraction and particularly the solvent (s) used in extracting their bioactive compounds. Despite the establishment of the potency, safety and effective dose of *Theobroma cacao* in managing iron deficiency anaemia, there is paucity of information on the most effective solvent for extracting their bioactive compounds and their effects on weight and haematological indexes of diet- induced iron deficiency anaemia, hence the study was conducted.

MATERIALS AND METHODS

Plant Materials and Authentication

Fresh stem barks and leaves of *Theobroma cacao* were obtained from a farm in Ile-0luji town, Ondo State, Nigeria. Authentication of the plant was done in the Department of Plant Biology, University of Ilorin, Ilorin, Nigeria were voucher specimen was deposited in the herbarium. The voucher number given was UIH0785.

Experimental Animals

Forty (40) albino rats (*Rattus norvegicus*) of both sexes with an average weight of 50.0 g±10.0 g were used for this study. The animals were obtained from the animal house in the department of Biochemistry, University of Ilorin. The rats were housed in cages under standard conditions (25-29˚C, 12 hour light and 12 hour darkness cycles) and fed rat chow. They were also given water *ad libitum.* This study was conducted in accordance with the ethics of animal experimentation of the Department of Biochemistry, University of Ilorin.

Feed Materials/Components

Yellow maize (*Zea may*) and locust bean (*Parkia biglobosa*) seeds were bought from local sellers at Owode market, Offa, Nigeria; Soybean Oil and Vitamin Mix were products of Grand Cereals and Oil Mills Limited, Bukuru, Jos, Nigeria. The Mineral Mix components used were products of Sigma Chemical Company Limited, London.

Preparation of Extract

The method described by Yakubu *et al* (2005) was used in the preparation of plant extract with slight modification. Briefly, the stem bark of the plant was air dried to a constant weight then pulverized into powder. A 200g of the pulverized sample was extracted in 1 ml of ethanol for 72 hours after which it was filtered using filter paper (Whatman No. 1). The filtrate was concentrated using Rotary Evaporator (Brand, company and serial No) at 40^0C and further exposed to air to fully dry. The residue was soaked in n-hexane and ethyl acetate respectively and the procedure for ethanol extraction was repeated in each case. An aliquot of the three fractions were stored in Eppendorf bottles and later used for phytochemical screening.

Feed Formulation

The method adopted for the formulation of iron sufficient and iron deficient feed was that of Folayan (1997) as modified by Oladiji *et al.*, (2007). In this method, a locust bean seed (prepared in a certain characteristic manner-figure 1) was used as the source of protein, among other things. The components of the diets were thoroughly mixed and made into pellets to ensure good handling by the animals. It was produced weekly and packed into air-tight polythene bags to prevent rancidity, auto-oxidation of the oil and microbial contamination.

Qualitative Phytochemical Screening

N-hexane, ethyl acetate and ethanol fractions of the extract were screened for phytochemicals using well documented procedures: the presence of alkaloids and phlobatannins were determined according to the method described by Harborne (1973) while the method described by Odebiyi and Sofowora (1978) was used for flavonoids and tannins. Steroids and phenolics were qualitatively determined by the method of Trease and Evans (1989) while glycosides and saponins were determined by the methods of Sofowora (1993) and Wall *et al.* (1954) respectively.

Table 1: Feed Components of Iron Sufficient and Iron Deficient Diets

Feed components	Iron sufficient (g/kg)	Iron deficient (g/kg)
Locust bean seed	750	750
Maize flour	40	40
Corn husk	20	20
Soybean oil	40	40
Sucrose	100	100
Methionine	5	5
Lysine	5	5
Mineral mix	30	30
Vitamin mix	10	10
$FeSO4{\cdot}7H_2O$	35.06mg/kg	8.50 mg/kg

Experimental Design and Anti-Anaemic Study

Forty (40) animals were housed in metabolic cages of dimensions 33cm × 20.5cm × 19cm under standard conditions (12-hr light:12-hr dark cycle; 28°C±3°C and 40–55% humidity). They were also given water ad libitum. This study was conducted in accordance with the ethics of animal experimentation of the Department of Biochemistry, Faculty of Life Sciences, University of Ilorin.

The method described by Oladiji *et al.*, (2007) was adapted for the anti-anaemic study.

The animal grouping consisted of an initial three (3) groups:

A – 10 Rats maintained on iron sufficient diet designated as **IS**.

B – 25 Rats maintained on iron deficient diet designated as **ID**.

C – 5 Rats maintained on commercial feed and designated **CF**.

Animals in groups **A** and **B** were maintained on their respective diets for **eight (8) weeks.** This was to induce iron deficiency anaemia in the animals in group B, and verify with the animals in group A which had sufficient iron in their diet. At the end of the eight (8) weeks feeding period, five (5) rats each from **IS** and **ID** groups were sacrificed and their haematological indices were determined. Upon the confirmation of the anaemic state of the **ID** animals, the remaining rats in group B were further grouped into **four (4)**, with **five (5) rats** in each group. One of these groups was maintained on the iron deficient feed for two weeks without treatment. The remaining three (3) groups were orally treated with ethyl acetate **(EA)**, n-hexane **(NH)** and ethanol **(ET)** fractions of the *Theobroma cacao* stem bark extract daily, for two (2) weeks. The grouping was as follows:

B1- Iron deficient rats fed with iron deficient feed for two weeks (iron deficient feed all through) designated **IDF**

B2- Iron-deficient rats orally treated with ethyl acetate fraction of the extract for two weeks designated as **EA**

B3- Iron deficient rats orally administered on a daily basis for two weeks with n-hexane fraction of the extract designated **NH**

B4- Iron deficient rats orally served ethanol fraction daily for two weeks designated **ET**.

The rest of the animals in group A were still fed with iron sufficient feed for two weeks (iron sufficient all through) designated as **ISF,** while those in group C were placed on commercial feed and still retained their designation as **CF**.

The extracts were administered to the animals in the various groups orally. They were sacrificed after the second week of extract administration.

Blood Collection, Preparation and Haematological Study

The blood samples were collected following the procedure described by Oladiji *et al.,* 2007 and was prepared and analyzed using the method described by Lewis *et al.* (2006) and Dacie and Lewis (1991) for haematological parameters such as packed cell volume, red blood cells count, white blood cell count and platelet using automated haematological analyzer (BioBase BK-3200). Serum ferritin was assayed using the method described in the commercial kit procured for the purpose.

Data Analysis

Results were expressed as the mean ±SEM of five determinations. The data were analyzed using Duncan Multiple Range Test and complemented with Student's t-test. The differences were considered statistically significant at $p < 0.05$. All the analyses were done using SPSS version 16.0 software (SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Identification of important bioactive compounds from medicinal plants through phytochemical screening provides a valuable link in validating the therapeutic potential of medicinal plants against disease management (Parihar, 2024; EL-saadony *et*

al., 2025 & Chintada and Golla, 2025). In this study, the qualitative phytochemical screening of solvent-extracted fractions of *Theobroma cacao* (SEFTC) extracts revealed notable variation in the distribution of bioactive constituents across the three solvent fractions (Table 2). Among the phytochemicals screened for, saponins and glycosides were found in all three solvent fractions, underlining their universal presence in the plant matrix. The ethanol and ethyl acetate fractions reveal the presence of tannins, and phlobatannins in addition to saponin and glycoside that cut across all the fractions while terpenoids and flavonoids were exclusively present in ethanol and hexane fractions respectively. This result positioned ethanol as most effective solvent for extracting both polar and non-polar phytochemicals from *T. cacao.* Some of these phytochemicals have earlier been indicted to play a role in curbing anaemia. For instance, saponins are known to enhance iron bioavailability by facilitating iron transport across the intestinal membrane while the role of flavonoids in counteracting oxidative stress emanating from a surge in erythropoiesis and iron turnover in IDA complications is also well documented (Cotoraci *et al., 2021; Ma et al.,* 2025). Tannins and phlobatannins have been indicted in hematinic effect in anaemic condition (Fagbohoun *et al.*, 2022; Kolawole *et al.,* 2021). In general, the presence of these important phytochemicals in various fractions of the extract aligns with previous reports that established the association of the compounds with hematinic and iron absorption-enhancing properties, positioning them as potential contributors to the anti-anaemic effects of *T. cacao* (Nyirenda, *et al.*, 2021; Yembeau *et al.*, 2022).

Table 2: Qualitative Phytochemical Composition of Solvent-Extracted Fractions of *T. cacao* Stem Bark

Test/sample	Hexane	Ethanol	Ethyl acetate
Saponins	+	+	+
Tannins	-	+	+
Flavonoids	+	-	-
Glycosides	+	+	+
Steroids	-	-	-
Phlobatannins	-	+	+
Terpenoids	-	+	-
Alkaloids	-	-	-
Anthraquinones	-	-	-

Key: + = present, − = absent

The weight progression of experimental rats fed with formulated and commercial feed for 8 weeks are shown in Figure 1. At the beginning of the experiment (week 0), the rats had comparable body weights (~60 g) across all groups, ensuring uniformity prior to dietary induction of experimental anaemia. Compared with other groups, the rats fed with the control diet showed the most robust growth, with a steady increase in body weight reaching almost 130 g by week 8 that the iron deficiency induction lasted. The group fed with IDF displayed noticeable stunted growth, with body weights peak of about 70 g while intermediate growth trajectory was observed in ISF group with body weights increasing progressively to just over 110 g by week 8.

As against ISF, the observed growth impediment in the IDF group alongside the recorded decrease in PCV, RBC with corresponding increase in WBC (Figure 2) are characteristics of iron deficiency anaemia that might lead to diminished erythropoietic activity and reduced oxygen-carrying capacity as well as suppression of red cell production due to iron deficiency. This underscores the systemic impact of iron deficiency anaemia, which extends beyond haematological disturbances to significantly hindrance in somatic development. However, the remarkable increase in WBC count in the IDF group relative to the ISF group might be due to an inflammatory or immune response commonly associated with iron-deficient states and such increase in WBC levels of iron deficient subjects have been linked to systemic stress and heightened vulnerability to infection due to compromised immune function. This agrees with the findings of Oladiji *et al.* (2007) and Olusegun *et al.* (2018) who established iron deficiency anaemia in rats using growth and haematological indexes following induction with IDF.

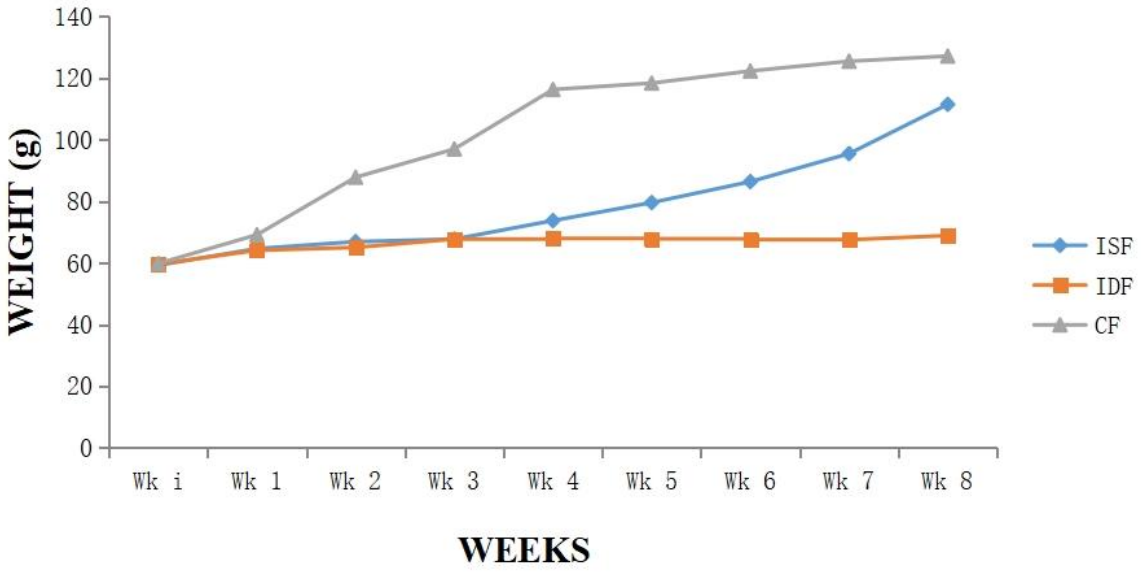

Figure 1: **Weight Progress in Iron Sufficient and Iron-Deficient Feed Fed Rats for 8 weeks**
Values are expressed as Mean ± SEM (n =4). Values in each column with different alphabet superscript are significantly different (P < 0.05). ISG: Iron Sufficient Feed, IDG: Iron Deficient Feed, CF: Commercial Feed, NH:N-hexane cocoa, EA: ethyl acetate cocoa, ET: ethanol cocoa fractions.

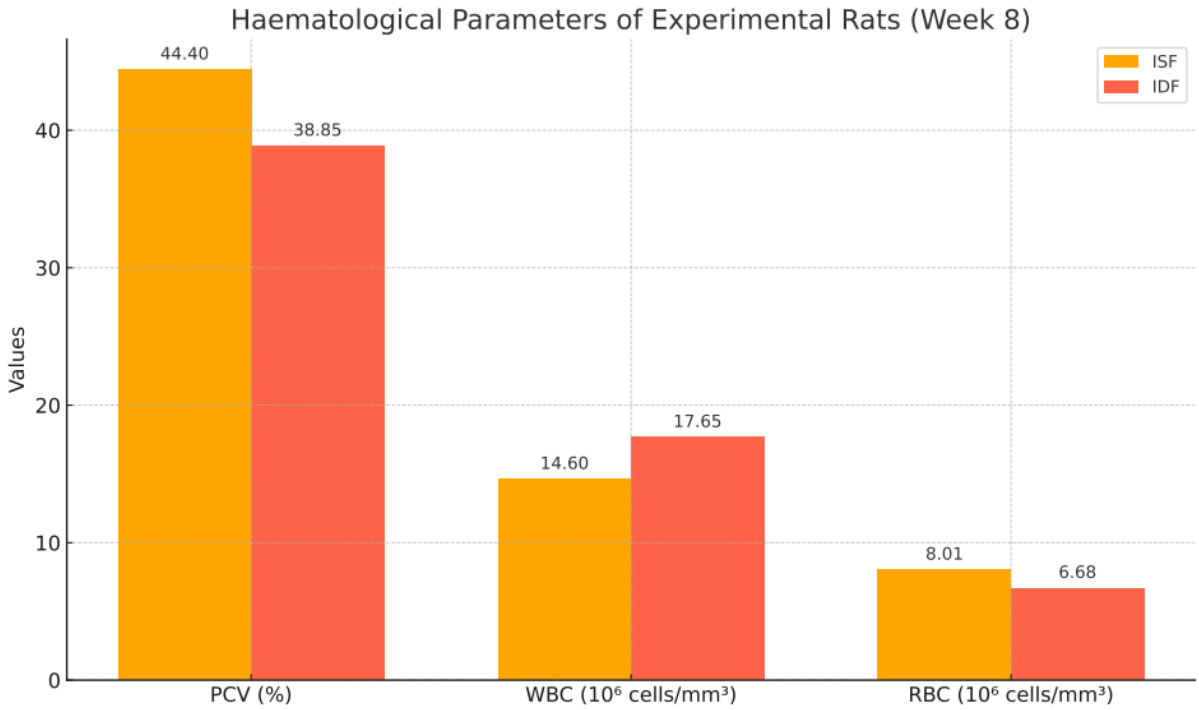

Figure 2: Haematological Parameters of Experimental Rats as At Week 8

Values carrying different superscripts(a-b) different from the control and down the group for each feed type and for each parameter are significantly different (P< 0.05), n=4 replicates + S.D, the extract was administered for 14 days ;PCV ,packed cell volume; WBC ,white blood cell ;RBC, red blood cell; ISF: iron sufficient feed fed rats ; ID :iron deficient feed fed rats.

Subsequent upon the establishment of anaemia in IDF groups, treatment with 25 mg/kg body weight of SEFTC for two (2) weeks revealed a progressive increase in the weight of the animals (Figure 3) accompanied by significant surge in erythrocytic indexes with corresponding reduction in WBC count compared with the IDF group (Table 3).

The recuperation from the weight and haematological assaults of IDF by the *T. cacao* fractions of the extract particularly its ethanol and ethyl acetate fractions, suggests effective amelioration of iron deficiency-induced growth-

limiting and infection susceptibility effects of iron deficiency, through phytochemicals such as phlobatannins, tannins, and saponins that promote hematopoiesis and enhance the biological utilization of iron. This aligns with the report of the previous works including that of Modupe and Oladiji (2015), Modupe *et al.* (2018), Nyirenda *et al.* (2022), Oladiji *et al.* (2007), Yembeau *et al.* (2021) that supported the use of plant as alternative therapy to curb anaemia in low-resource settings.

Values carrying different superscripts(a-d) different from the control and down the group for each feed type and for each parameter are significantly different ($p< 0.05$), n=4 replicates + S.D, the extract was administered for 14 days ;PCV ,packed cell volume; WBC ,white blood cell ;RBC, red blood cell; ISF: iron sufficient feed fed rats ; IDF :iron deficient feed fed rats: EAcocoa (ethyl acetate extract fraction), NHcocoa (n-hexane extract fraction) and ETcocoa (ethanolic extract).

Ferritin, a notorious iron storage protein and a critical biomarker for the appraisal of iron level in the system. This critical protein not only reflect the amount of bioavailable iron stored in tissues but also plays essential roles in maintaining normal erythropoiesis (Correnti *et al.*, 2024). Prior to clinical manifestation of anaemia, a low serum ferritin levels is indicative of iron depletion that signifies iron deficiency anaemia while its elevated levels suggest adequate iron reserve and metabolism (Camaschella and Hershko, 2025). In the presented study, reduced serum ferritin levels of the IDF group corroborate the classical manifestation of iron deficiency anaemia early established and pointed to depletion in iron storage. Both the iron-sufficient and commercial feed fed groups maintained higher ferritin levels, indicating adequate iron homeostasis. Administration of 25 mg of the SEFTC produced a significant improvement in ferritin level (Figure 4). Although, the three fractions of the extracts produced significant increase in ferritin level, the outstanding increase in ferritin levels of the rats treated with the ethyl acetate and ethanol fractions of *T. cacao* which approached or surpassed that of the controls, suggests a restorative effect on iron storage. This agrees with the work of Lotfi *et al.* (2018) where similar increase in ferritin level was recorded following a 6 weeks consumption of beetroot juice.

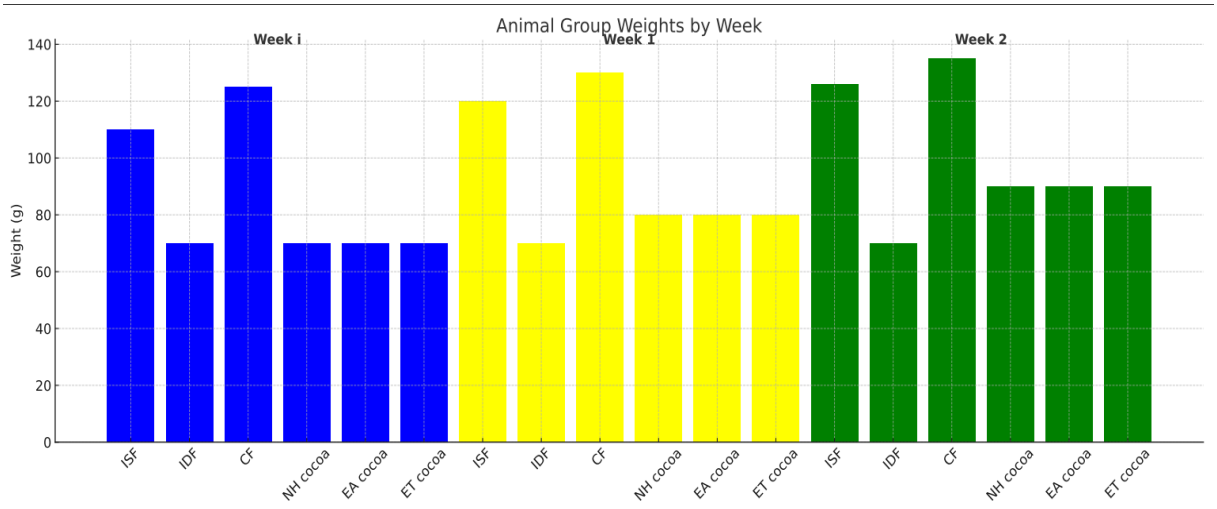

Figure 3: Weight Progress in Rats as At 10th Week (Administration Weeks)

Values are expressed as Mean ± SEM (n =4). Values in each column with different alphabet superscript are significantly different ($P < 0.05$). ISG: Iron Sufficient Feed, IDG: Iron Deficient Feed, CF: Commercial Feed, NH: N-hexane cocoa, EA: ethyl acetate cocoa, ET: ethanol cocoa fractions

Table 3: Effect of Oral Administration of Solvent-Extracted Fractions of *Theobroma Cacao* Stem Bark on Haematological Parameters of Feed-Induced Iron Deficient Rats

Groups	PCV (%)	RBC (10^6 cells/mm$^{3)}$	WBC (10^6cells/mm^3)
ISF	47.93 ± 1.07^c	7.89 ± 0.45^c	14.26 ± 1.60^a
IDF	17.73 ± 0.73^a	2.26 ± 0.46^a	34.10 ± 7.00^b
CF	45.74 ± 0.92^b	7.54 ± 0.26^c	15.64 ± 2.42^a
EAcocoa	48.80 ± 0.00^c	6.69 ± 0.25^b	15.80 ± 0.31^a
NHcocoa	43.10 ± 0.30^b	7.90 ± 0.00^c	17.03 ± 6.43^a
ETcocoa	45.10 ± 1.10^b	$7.19 \pm 0.49^{b,c}$	13.60 ± 2.75^a

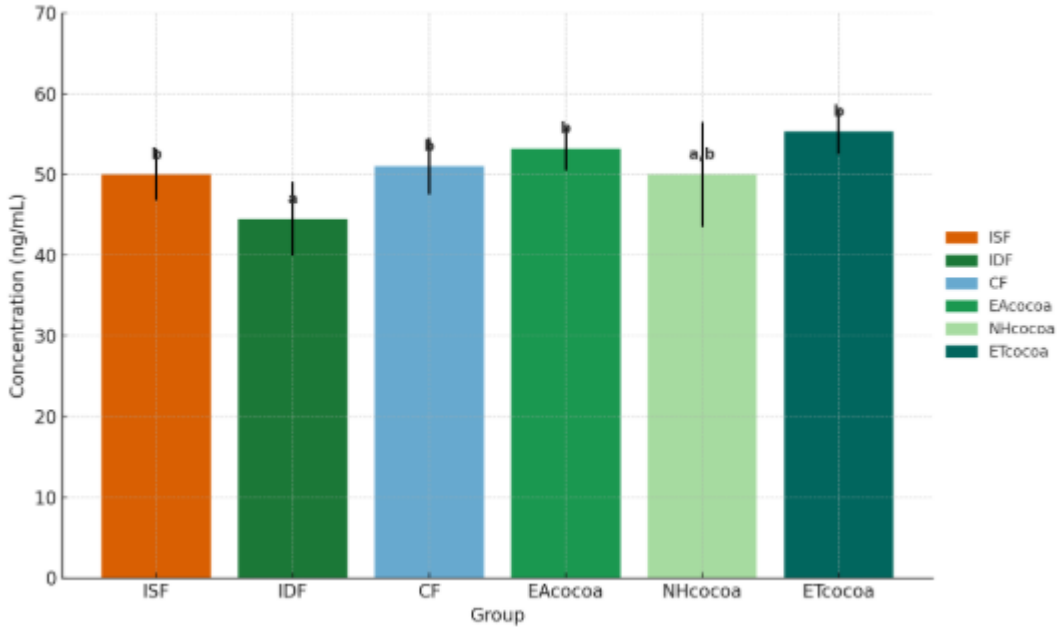

Figure 4: Effect of oral administration of solvent-extracted fractions of *Theobroma cacao* stem bark on serum ferritin level of feed-induced iron deficient rats at 10th week

NB: Values carrying different superscripts(a-d) different from the control and down the group for each feed type and for each parameter are significantly different ($p < 0.05$), n=4 replicates + S.D, the extract was administered for 14 days ;PCV, packed cell volume; WBC ,white blood cell ;RBC, red blood cell; ISF: iron sufficient feed fed rats ; IDF :iron deficient feed fed rats: EAcocoa(ethyl acetate extract fraction), NHccocoa(n-hexane extract fraction) and ETcocoa (ethanolic extract).

CONCLUSION

In all, this research has established ethanol and ethyl acetate as a moderately polar solvents both of which could be used to extract hematinic agents of *T. cacao* that can potentially improve iron-induced anaemic conditions including improved haematological indices and restoration of ferritin levels, possibly due to their rich composition of phytochemicals such as saponins, glycosides, and tannins. The present research validates not only the traditional use of *T. cacao* in treating iron-related ailments but also positions it as a potential therapeutic alternative that can support erythropoiesis and iron homeostasis. A detail research into the mechanism of action of the implicated phytochemicals against iron-induced anaemia is desired in future research.

Conflicts of Interest

The authors declare no conflict of interest.

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