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ANTIANEMIC ACTIVITY OF MAHUA FLOWER CONCENTRATE AND MAHUA LADDOO AGAINST 2,4-DINITROPHENYLHYDRAZINE INDUCED ANEMIA IN A RAT MODEL

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Keywords:

Mahua flower concentrate, Mahua laddoo, 2,4-Dinitrophenylhydrazine-induced anaemia, Antianemic activity, Proximate parameters

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ABSTRACT: Anaemia is a widespread problem that can be characterized by a decreased number of red blood cells and decreased haemoglobin levels. The aim of this study was to explore the antianaemic efficacy of mahua flower concentrate and mahua laddoo in comparison to the clinically prescribed iron tablet, Livogen XT, in an anaemic rat model. The anaemic rat model was developed by the administration of 2,4-dinitrophenylhydrazine. The antianaemic effects of mahua flower concentrate and mahua laddoo were evaluated by quantifying haematological limits, including haemoglobin contents, red blood cell count, and haematocrit. Additionally, proximate analysis was performed for the mahua flower concentrate and mahua laddoo. The proximate content of mahua concentrate and mahua laddoo exhibited good equilibrium of macronutrients, including carbohydrates and proteins, along with fat content, which contributes to their energy-providing properties. Orally, mahua concentrate and mahua laddoo were administered for a period of 23 days. The results revealed a significant increase in the haematological parameters in the treated groups compared with those in the negative control group. The use of mahua concentrate and mahua laddoo significantly increased the RBC count, haemoglobin level and haematocrit level. It is concluded that mahua flower concentrate and mahua laddoo are beneficial in the management of anaemia. Further studies are necessary to discover the fundamental molecular mechanisms involved and to confirm their clinical importance. The presence of mahua flower-based food products in the diet may suggest a promising alternative method to battling nutritional and drug-induced anaemia.

INTRODUCTION: In Greek, the term anaemia means "without blood". The WHO has recognized anaemia as a global problem in developing countries such as India, where the percentage varies from 10--20% on the basis of a recent survey [1]. In anaemic conditions, there is a reduction in haemoglobin concentrations and red blood cells [2]. It is not considered a disease but rather an underlying condition that is based upon biological mechanisms [3].

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Iron deficiency anaemia is the most widespread nutritional deficiency syndrome and an important cause of anaemia in children. The prolonged effect of iron deficiency anaemia leads to neuro developmental and mental disorders, which may not be fully reversible even with the treatment of iron deficiency anaemia [4].

Currently, the risk of anaemia is relatively low because of the availability of food Supplemented with iron. For the formation of RBCs, several minerals, including iron, magnesium, cobalt, and vitamin A and B group vitamins, are needed. However, iron plays a key role in the delivery of oxygen by Hb in RBCs [5]. Owing to this growing health problem, iron supplements or iron-rich diets are preferred by nutritionists for the management of

anaemic patients. The long-term use of iron supplements has certain disadvantages. Oral therapy leads to insufficient absorption of iron, and the overuse of iron supplements leads to several health issues, such as cancer and neurogenic disorders [6]. These findings clearly indicate that there is a need to find a safe and effective substitute for controlling anaemia. Nature has gifted us with varieties of plants that have numerous medicinal and pharmacological properties that can overcome several diseases, including anaemia. It is necessary to maintain a healthy lifestyle for both pregnant and lactating women, as they require more nutrients than other women do [7]. The daily required dose of iron for these women is 35 mg/day [8]. One of the essential plants is *Madhuca indica* (Mahua), which has all the essential nutrients required for pregnant and lactating women. The plant is widely present throughout the country. The flower of a plant contains significant amounts of calcium, phosphorus, iron and vitamins, which are responsible for overcoming several diseases. According to Ahirwar *et al.,* 2020, dried mahua flowers contain 1412.3 ppm iron, which is essential for Hemoglobin [9]. The 2009 standard limit of MICR is that Mahua flowers are the best source of iron ions. Therefore, on the basis of the above data, we can overcome anaemia via the consumption of dry mahua flowers and several food products from dry mahua flowers, such asmahua concentrate andmahua Laddoo.

MATERIALS AND METHODS:

Preparation of Mahua Juice Concentrate: The juice concentrate was prepared *via* soaking (overnight) the dried mahua flower in drinking water. The following day, the flowers were ground to make a thick paste, and the juice was subsequently extracted by hand-pressing method in an 8-layer muslin cloth. The extracted juice was transferred to a sterile container followed by gentle heating at a controlled temperature (40°C) until a thick consistency was achieved. The mahua juice concentrate was maintained at 65°C. The prepared juice extract was stored in a glass container for further use.

Preparation of Mahua Laddoo: To prepare a base for the mahua laddoo, dried mahua flowers (approximately 200 g) were finely ground. The dry fruits (cashew, almond, and raisin) (50 g) were roasted until golden brown. In the same pan, the wheat flour was dry roasted to release the nutty aroma. jaggery was used as a binding agent, which was melted at a low temperature to create a smooth syrup. In a large bowl, all the above prepared ingredients were mixed until a dough-like consistency was achieved. Finally, ghee is incorporated to increase its richness. The dough is then given the shape of laddoo, and it is then allowed to cool. Furthermore, they are stored in airtight containers for future use.

Proximate Analysis:

Protein Estimation: Protein estimation was Performed by the Lowry method with bovine serum albumin (BSA) used as a standard. Briefly, the samples, along with the standard, were incubated with alkaline copper solution, and Folin-Ciocalteu reagent was subsequently added. After an incubation period of 30 minutes, the absorbance was measured at 750 nm. The protein content was quantified via a standard curve generated from the standard [10].

Carbohydrate Estimation: The carbohydrate content of the food product was estimated *via* anthrone method. The anthrone reagent reacts under acidic conditions to obtain a green color complex, which is measured at 620 nm. The carbohydrate content was calculated by comparing the absorbance of the sample with a standard curve with glucose as the standard [11].

Reducing Sugar Content: The reducing sugars present in the food samples were quantified *via* Dinitrosalicylic acid (DNS) method. In this method, DNS was reduced in an alkaline medium, which subsequently formed an orange–red complex. The intensity of the Color were measured spectrophotometrically at 540 nm. The reducing sugars were further quantified *via* comparison with the calibration curve, in which glucose was used as a standard [12].

Fat Estimation: The fats from the sample was extracted by the Soxhlet method using hexane. The solvent comprising the extracted fat was gathered in the flask, and the solvent was evaporated in the rotary evaporator, leaving behind fat [13].

The weight of the extracted fat was then estimated using the following formula:

Fat content (%) = (weight of sample (g) / weight of fat extracted (g)) × 100

Determination of the Calorific Value: The calorific value of the prepared food products was estimated using a bomb calorimeter, where a defined mass of the sample was combusted in the calorimeter and the released heat increased the temperature of the surrounding water [14]. It is calculated involving the following formula:

$$\text{Calorific value} = Q_{total}/m_{food}$$

Where $Q_{total} = m_w.c_w.\ \Delta T + C_{bomb}\cdot\Delta T$

- m_w: mass of water (g),
- c_w: specific heat capacity of water (4.18 J/g°C),
- ΔT: temperature change of the water (°C),
- C_{bomb}: heat capacity of the bomb (J/°C),
- m_{food}: mass of the food sample (g).

***In-vivo* Study:** The animal study using Wistar albino rats was approved by the animal ethical committee. Wistar albino rats (180–200 g) of either sex were used for the study (n=6). The animals were acclimatized for seven days under standard housing conditions in an animal facility. They were housed in polypropylene cages and maintained at 25 ± 2°C and 50 ± 10% relative humidity under a 12 h light/dark cycle.

Chemical: The tablet Livogen-XT (P&G Health India) was procured from Merck Ltd., India, for research purposes, and 2,4-dinitrophenylhydrazine was procured from SRL Ltd., India, for research purposes.

Acute Toxicity: Single doses of different concentrations, such as 250, 500, 1000, 2000 and 2500 mg/kg mahua concentrate and mahua laddoo (15–18 g), were administered orally to six groups, with six animals in each group (n=6). The rats were placed under observation for 14 days, after which the number of dead rats was recorded (OECD Test No. 425, 2022). The results revealed no signs of toxicity or death in the animals, even at the highest concentration of 5000 mg/kg [15].

Subacute Toxicity: TwelveWistar rats were randomly divided into two groups with six animals in each group (n=10). Mahua concentrate and mahua laddoo (15–18 g) were administered orally at a daily dose of 500 mg/kg body weight for 28 days following the repeated oral toxicity test (OECD, 2008). The animals were observed daily during the experiment to detect death or abnormal clinical signs. Body weight, water intake and food intake were recorded. At the end of 29 days the animals were sacrificed, blood was collected via cardiac heart puncture, and all vital organs were collected for toxicological studies [16].

Induction of Anaemia: The anaemia model was developed by administering 2,4-diphenylhydrazine to the rats via oral gavage at a concentration of 40 mg/kg for 2 consecutive days. When haemoglobin is reduced to 30%, the animals are considered anaemic [6].

Experimental Design: The rats were divided into five groups with six rats in each group (n = 6). The Group-I rats were treated as normal controls. The rats in Group II were treated with 2,4-diphenylhydrazine as a negative control. Group III was treated as a positive control, where Livogen XT was given orally. Groups IV and V were the treatment groups in which Mahua concentrate and Mahua laddoo were administered to the animals via oral cavage and the chewing method.

Hematological Analysis: After the 29th day of treatment for the subacute toxicity test the animals were anaesthetized by mild anaesthesia (isoflurane) followed by cervical dislocation, and blood was collected *via* cardiac puncture. Blood cell parameters were analyzed *via* a CBC analyser (BYOVET). Blood samples were collected retro-orbitally on day 0 day before anemia induction and on the 4th, 7th, 11th, 15th, 19th, and 23rd days after anemia induction. Blood samples were examined for red blood cell (RBC), haemoglobin (Hb), and haematocrit (HCT) levels [17].

Serum Biochemistry Analysis: Biochemical analysis of the serum was analysed using a biochemical analyser. Briefly, 1 ml of blood was taken from the rat and subjected to centrifugation for 10 min at 10000 rpm so that the cells were accumulated in the palette, and the supernatant, which contained the serum, was collected. This serum was subjected to biochemical analysis to identify and quantify different types of biomolecules, such as glucose (GLU), albumin (AB), urea, creatinine (CREA), cholesterol

(CHOL), triglyceride (TGL), alanine transaminase (ALT), aspartate aminotransferase (AST), total protein (TP), magnesium (MG), phosphorus (PHOS), calcium (CA), direct bilirubin (DBIL), total bilirubin (TBIL), high-density lipoprotein (HDL), gamma glutamine transpeptidase (GGT), alkaline phosphatise (ALP), and low-density lipoprotein (LDL).

Histopathology: The animals were sacrificed to isolate different organs such as the liver, lung, kidney, and heart for toxicity studies if any *via* the animals from the subacute group. For anemic study, the femur bone marrow was examined.

The collected tissues were processed, stained using hematoxylin and eosin staining methods, and examined under a light microscope for histological changes. For the anemic model, on the last day, i.e., the 23rd day, the animals were sacrificed to segregate the femur bone for bone marrow investigation. The organs were preserved in formalin, and the tissues were further processed, stained with hematoxylin and eosin and studied under a microscope for any histological changes.

RESULTS:

Proximate Analysis: The proximate analysis of the mahua concentrate and mahua laddoo revealed high content of fat, protein, carbohydrate, and reducing sugar contents and caloric value. The protein content was 3.84 ± 0.10 g/100 g and 3.92 ± 0.28 g/100 g in the mahua concentrate and mahua laddoo, respectively.

The fat content of laddoo was the highest, i.e., 9.48%, due to the presence of clarified butter and dry fruits, but the fat content of the mahua concentrate was lower, i.e., 0.10%. Carbohydrates are the most abundant macronutrients found in mahua concentrate and mahua laddoo.

The concentrate presented the highest carbohydrate content, i.e., 91.17 ± 2.29 g/100 g, compared with that of mahua laddoo, i.e., 76.33 ± 0.74 g/100 g. These differences may be attributed to the changing levels of starches and fibres present in each sample. The reducing sugar content also varied significantly, with 43.25 ± 1.80 g/100g in mahua concentrate and 43.25 ± 1.80 g/compared to 40.09 ± 4.05 g/100 g in the mahua laddoo. The difference in reducing sugars probably imitates the existence of simple digestible carbohydrates, which may affect the sweetness and nutritional shape of samples.

These results reveal prominent alterations in the macronutrient content of food samples, which may provide insight into their nutritional value and possible health benefits. The calorific value of the food products depends on the composition, i.e. macronutrients. Mahua concentrate exhibited 220.5 Kcal/100g, whereas the mahua laddoo exhibited 180.0522 Kcal/100g. This value represents the amount of energy it provides to the body after the metabolism.

***In-vivo* Study:** The rats were treated with food samples (both mahua concentrate and mahua laddoo) at single doses of different concentrations, such as 250, 500, 1000, 2000 and 2500 mg/kg body weight for acute toxicity studies and daily doses of 50 mg/kg, 200 mg/kg and 500 mg/kg body weight for 28 days For subacute studies, as per the OECD guidelines. No signs of mortality were observed.

The estimated LD_{50} value of the mahua concentrate and mahua laddoo was estimated to be 5000 mg/kg body weight. There was no significant difference in body weight, food intake, or water intake between the untreated and treated groups of animals for the entire duration of the experiment **Table 1**.

At the end of the experiments, all the rats exhibited normal behaviour and were healthy. After 28 days of treatment, the rats were anaesthetized and sacrificed. Blood was collected for biochemical analysis, and vital organs were collected for histopathology.

The blood biochemistry data and complete blood counts **Table 2** and **Table 3** revealed no significant differences ($p>0.05$) between the control and treated rats. The vital organs, such as the kidney, liver, heart, spleen and brain, of the animals treated with the mahua extract and mahua laddoo did not differ from those of the untreated control group **Fig. 1**.

The *in-vivo* study confirmed that both the mahua concent rate and the mahua laddoo have no side effects or toxicity and can be considered safe products.

TABLE 1: EVALUATION OF BODY WEIGHT, FOOD INTAKE, AND WATER INTAKE AMONG CONTROL AND TREATED ANIMALS. THE CONTROL AND TREATMENT GROUPS WERE MONITORED FOR BODY WEIGHT, FOOD INTAKE, AND WATER INTAKE AT REGULAR INTERVALS THROUGHOUT THE ENTIRE DURATION OF THE EXPERIMENT

No. of Days	Normal Control	Mahua concentrates	Mahua Laddoo
Body Weight (g)			
01	175.5 ± 6.28	176.3 ± 5.19	180.1 ± 9.65
07	186.4 ± 5.98	186.1 ± 4.32	188.4 ± 3.60
14	201.2 ± 6.20	203.7 ± 5.60	203.9 ± 7.46
21	207.6 ± 11.64	220.2 ± 6.77	219 ± 6.84
28	223.6 ± 10.92	229 ± 12.71	230.7 ± 11.52
Food Intake (g)			
01	17.13 ± 2.33	13.7 ± 1.61	14.58 ± 3.06
07	17.18 ± 2.31	13.82 ± 1.56	15.38 ± 3.15
14	17.07 ± 2.50	13.68 ± 1.59	16.09 ± 3.09
21	17.42 ± 1.97	14.86 ± 1.67	16.96 ± 3.11
28	17.72 ± 1.43	16.05 ± 2.27	17.82 ± 3.21
Water Intake (ml)			
01	17.53 ± 3.53	16.79 ± 3.37	18.54 ± 4.38
07	18.4 ± 3.33	17.97 ± 2.48	18.86 ± 3.81
14	18.76 ± 3.97	19.84 ± 3.87	21.8 ± 1.91
21	20.18 ± 4.62	20.83 ± 4.35	20.19 ± 3.98
28	20.65 ± 5.01	21.7 ± 5.50	20.19 ± 3.91

TABLE 2: BLOOD BIOCHEMISTRY PARAMETERS OF RATS TREATED WITH FOOD SAMPLES AT A DAILY DOSE OF 200 MG/KG BODY WEIGHT FOR 28 DAYS. ORGAN FUNCTIONS PRESENTED UNDISTINGUISHABLE PROFILES BETWEEN THE CONTROL AND TREATED GROUPS. NO SIGNIFICANT DIFFERENCES IN LIVER FUNCTION, KIDNEY FUNCTION, LIPID PROFILE, SERUM GLUCOSE, OR DIFFERENT SALTS WERE DETECTED BETWEEN THE TREATED AND UNTREATED GROUPS

Parameter	Control	Mahua concentrates	Mahua Laddoo
Glucose (GLU)	122.83 ± 4.94	110.11 ± 2.28	103.58 ± 4.29
Albumin (ALB)	2.67 ± 0.21	2.92 ± 0.36	2.56 ± 0.32
Urea (UREA)	32.09 ± 2.32	28.33 ± 4.83	29.42 ± 7.62
Creatinine (CREA)	1.4 ± 0.32	1.15 ± 0.24	1.17 ± 0.16
Cholestrol (CHOL)	144.98 ± 11.32	153.08 ± 10.85	143.51 ± 8.61
Triglycerides (TG)	102.66 ± 7.08	127.33 ± 13.18	91.26 ± 23.64
Alanine Transaminase (ALT)	85.99 ± 2.22	102.4 ± 11.13	65.77 ± 29.31
Aspartate Aminotransferase (AST)	34.22 ± 4.6	31.96 ± 0.83	20.67 ± 8.92
Total Protein (TP)	6.92 ± 0.2	6.2 ± 0.4	4.65 ± 1.42
Magnesium (MG)	2.08 ± 0.29	1.78 ± 0.14	1.45 ± 0.31
Phosphorus (PHOS)	4.8 ± 0.65	4.57 ± 0.7	4.34 ± 0.86
Calcium (CA)	9.26 ± 0.26	9.3 ± 0.68	7.58 ± 0.68
Direct Bilirubin (DBIL)	0.37 ± 0.14	0.33 ± 0.14	0.33 ± 0.05
Total Bilirubin (TBIL)	0.37 ± 0.16	0.17 ± 0.12	0.44 ± 0.24
High-density Lipoprotein (HDL)	56.51 ± 4.9	54.51 ± 4.71	61.48 ± 6.68
Gamma-glutamyl Transferase (GGT)	4.41 ± 0.9	4.35 ± 1.56	4.46 ± 1.83
Alkaline Phosphatase (ALP)	77.73 ± 19.26	95.34 ± 13.07	103.67 ± 14.97

TABLE 3: BLOOD PARAMETERSOF RATS TREATED WITH FOOD SAMPLES AT A DAILY DOSE OF 500 MG/KG BODY WEIGHT FOR 28 DAYS. NO SIGNIFICANT DIFFERENCES WERE DETECTED IN THE WBC COUNT (WBC), MONOCYTE COUNT (MON), EOSINOPHIL COUNT (EOS), NEUTROPHIL COUNT (NEU), LYMPHOCYTE COUNT (LYM), BASOPHIL COUNT (BAS), RBC COUNT (RBC), HAEMOGLOBIN CONCENTRATION (HB), HAEMATOCRIT (HCT), MEAN CORPUSCULAR VOLUME (MCV), MEAN CORPUSCULAR HAEMOGLOBIN (MCH), MEAN CORPUSCULAR HAEMOGLOBIN CONCENTRATION (MCHC) OR PLATELET COUNT BETWEEN THE TREATED AND UNTREATED GROUPS

Parameter	Control	Mahua concentrates	Mahua Laddoo
White blood cell count (WBC (10^3/L)	5.21 ± 0.98	5.1 ± 1.22	8.01 ± 1.03
Neutrophils (Neu# (10^3/L))	4.83 ± 1.36	4.4 ± 1.1	5.69 ± 0.53
Lymphocytes (Lym# (10^3/L)	2.93 ± 0.42	3.01 ± 0.95	3.12 ± 0.9
Monocytes (Mon# (10^3/L)	0.38 ± 0.07	0.51 ± 0.05	0.48 ± 0.12

Eosinophils (Eos# (10^3/L)	0.28 ± 0.05	0.25 ± 0.18	0.23 ± 0.05
Basophil (Bas# (10^3/L)	0.32 ± 0.37	0.4 ± 0.3	0.07 ± 0.03
NLR	1.5 ± 0.23	1.5 ± 0.15	1.89 ± 0.3
PLR	0.01 ± 0	0.02 ± 0	0.04 ± 0.06
red blood cell count (RBC (10^12/L)	4.93 ± 0.5	5.43 ± 0.32	5.28 ± 0.41
Hemoglobin (HGB (g/dL)	13.98 ± 0.5	13.19 ± 0.5	14.65 ± 0.52
HCT	42.91 ± 1.58	41.92 ± 2.55	46.17 ± 1.51
MCV (fL)	82.63 ± 3.23	82.9 ± 4.69	84.68 ± 4.62
MCH (pg)	30.18 ± 1.2	29.58 ± 1.89	30.56 ± 1.19
MCHC (g/L)	32.87 ± 0.49	32.8 ± 1.72	34.05 ± 0.91
RDW-CV	13.15 ± 0.48	12.99 ± 0.64	13.82 ± 1.02
RDW-SD (fL)	40.4 ± 2.43	43.26 ± 1.76	46.31 ± 4.24
platelet count (PLT (10^3/L)	237.66 ± 40.86	264.83 ± 62.53	227.83 ± 34.75
PCT (mL/L)	0.2 ± 0.04	0.25 ± 0.07	0.3 ± 0.07

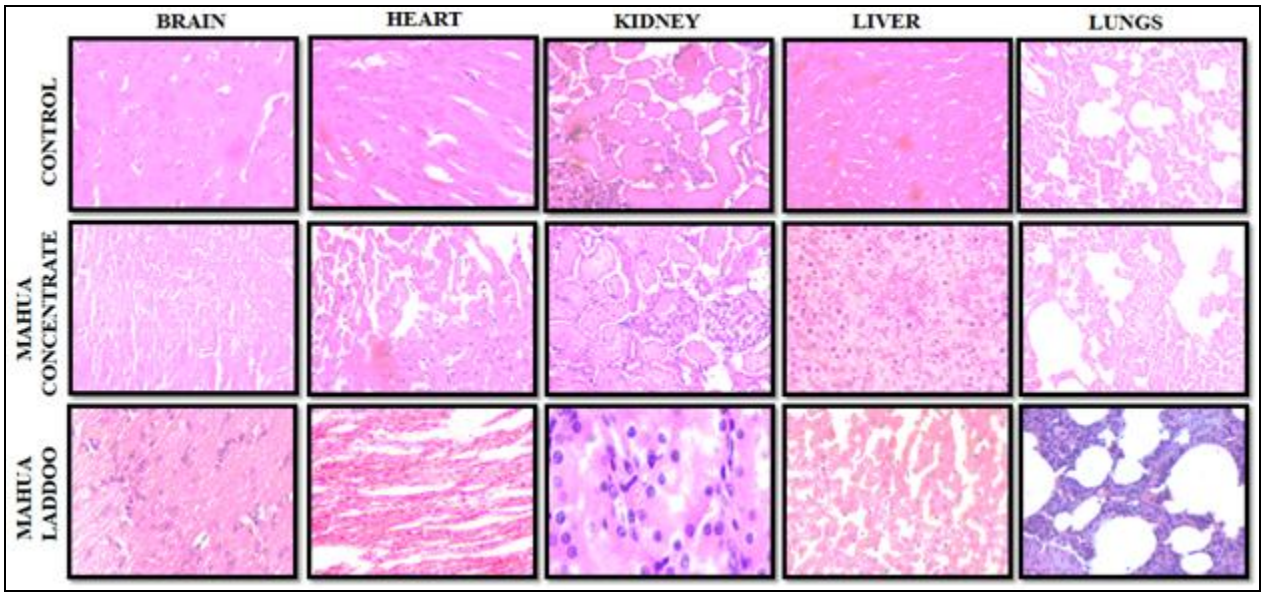

FIG. 1: PHOTOMICROGRAPHS OF DIFFERENT VITAL ORGANS, I.E., THE BRAIN, LUNGS, HEART, LIVER AND KIDNEY, OF CONTROL AND TREATED RATS AT A DOSE OF 200 MG/KG BODY WEIGHT OF MAHUA CONCENTRATE AND MAHUA LADOO FOR 28 DAYS. THERE WAS NO SIGNIFICANT DIFFERENCE BETWEEN THE CONTROL AND TREATED GROUPS OF ANIMALS WITH RESPECT TO CELLULAR ARCHITECTURE

The blood biochemistry parameters of rats treated with mahua concentrate or mahua laddoo at a daily dose of 200 mg/kg body weight for 28 days in an anaemia-induced model were investigated. Organ functions presented undistinguishable profiles between the control and treated groups. No significant differences were detected in the liver function test, kidney function test, lipid profile, serum glucose, or different salts between the treated and untreated groups **Table 4**. There was no significant difference in body weight, food intake, or water intake between the untreated and treated groups of animals for the entire duration of the experiment **Fig. 2**. After the administration of 2,4-dinitro-phenylhydrazine, there was a significant decrease in the RBC count in the animals ($p<0.001$), as shown in **Table 5.** There was an increase in the RBC count following treatment with the liveogens XT, mahua concentrate and mahua laddoo. The food products were found to be more effective than the positive control, i.e., liveogen XT. Similarly, after induction with 2,4-dinitro - phenylhydrazine, the Hemoglobin levels were content decreased significantly ($p<0.001$). Haemoglobin level was increased by treatment with the livogens XT, mahua concentrate and mahua laddoo. The food products were found to be more effective than the positive control, i.e., liveogen XT. Similarly, there was a decrease in the haematocrit (HCT) count ($p<0.001$) due to the induction of 2,4-dinitro-phenylhydrazine in the rats compared with the normal rats. The decrease in the HCT count was reversed by treatment with the food products as well as the liveogen XT. The food

products were found to be more effective than the positive control, i.e., liveogen XT. The presence of minimal-grade central foci containing adipose tissue was observed in anaemia-induced rats, which revealed the depletion of the bone marrow cellular population. However, treatment with food products (mahua concentrate and mahua laddoo) and the carcinogen XT prevented these histological changes, and the bone marrow cellular population increased **Fig. 3**.

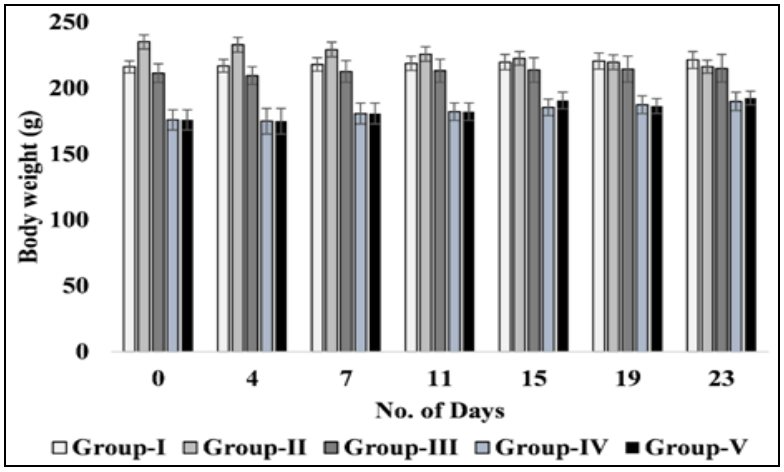

FIG. 2: EVALUATION OF BODY WEIGHT AMONG CONTROL AND TREATED ANIMALS. THE BODY WEIGHTS OF THE CONTROL AND TREATED GROUPS WERE MONITORED AT REGULAR INTERVALS THROUGHOUT THE ENTIRE DURATION OF THE EXPERIMENT. (GROUP-I: CONTROL GROUP; GROUP-II: NEGATIVE CONTROL; GROUP-III: LIVOGEN XT; GROUP-IV: MAHUA CONCENTRATE; GROUP-V: MAHUA LADDOO)

TABLE 4: BLOOD BIOCHEMISTRY PARAMETERS OF RATS TREATED WITH MAHUA CONCENTRATE OR MAHUA LADDOO AT A DAILY DOSE OF 200 MG/KG BODY WEIGHT FOR 28 DAYS IN AN ANEMIA-INDUCED RAT MODEL. ORGAN FUNCTIONS PRESENTED UNDISTINGUISHABLE PROFILES BETWEEN. THE CONTROL AND TREATED GROUPS. NO SIGNIFICANT DIFFERENCES WERE DETECTED IN THE LIVER FUNCTION TEST, KIDNEY FUNCTION TEST, LIPID PROFILE, OR DIFFERENT SALTS BETWEEN THE TREATED AND UNTREATED GROUPS. HERE, DAY 0 IS CONSIDERED BEFORE INDUCTION, WHERE ALL THE PARAMETERS ARE NORMAL, AND DAYS 1 TO 23 ARE CONSIDERED ANEMIC MODELS

Days	Normal Control	Negative Control	Positive Control	Mahua Concentrate	Mahua Laddoo
Alanine Transaminase (U/L), (Range -10.00 - 125.00)					
Before induction					
0	93.7±27.75	107.9±4.67	117.15±1.89	111.09±4.43	110.59±4.07
After induction					
4	105.28±5.62	125.98±0.97	107.96±0.82	107.96±0.82	107.92±0.95
7	106.79±7.32	126.26±0.78	113.45±4.98	108.45±0.88	109.62±3.04
11	105.72±8.09	126.46±0.94	115.55±4.2	108.38±1.18	275.97±374.65
15	105.72±6.5	126.83±1.11	112.72±5.07	107.05±1.79	110.05±2.42
19	108.05±5.76	126.98±0.37	114.72±4.45	107.05±1.76	109.38±2.03
23	112.31±7.76	128.65±0.62	113.65±4.64	110.35±3.79	110.35±3.79
Aspartate Aminotransferase (U/L), (Range- 0 - 50.0)					
Before induction					
0	26.65±4.39	30.88±2.22	33.81±3.2	34.43±4.35	34.43±4.35
			After induction		
4	28.11±4	54.45±0.73	41.35±4.25	34.45±0.73	34.45±0.73
7	32.45±4.36	55.46±1.39	36.18±5.5	35.38±1.48	35.38±1.48
11	33.73±4	55.4±1.05	36.6±3.01	42.06±5.16	36.71±2.94
15	32.35±4.47	55.16±0.31	34.93±4.74	36.5±3.05	34.84±4.84
19	34.06±2.05	54.98±0.88	41.16±1.85	35.68±3.7	35.68±3.7
23	35.25±0.69	55.25±0.69	39.38±3.15	38.58±4.76	39.4±3.05
Alkaline Phosphatase (U/L), (Range- 0.1 - 212.0)					
Before induction					
0	152.5±7.01	153±4.43	161±30.67	157.5±23.36	3.79±0.47
After induction					

4	167.66±9.12	254.33±19.05	157.5±21.78	148.33±11.27	4.07±0.63
7	148.83±11.83	255.5±19.09	155.5±19.09	156±18.93	4.31±0.71
11	161±7.48	266±24.58	166±24.58	159.83±24.43	4.72±0.41
15	149±9.09	244±21.62	144.16±21.56	149.83±15.89	3.89±0.55
19	151±8.2	202.58±91.92	144.16±21.37	142.5±8.24	3.95±0.78
23	149±8.92	255.83±13.95	155.66±13.97	156.16±13.22	4.46±0.88
Total Bilirubin (mg/dl), (Range- 0 - 0.90)					
Before induction					
0	0.44±0.02	0.38±0.08	0.52±0.18	0.45±0.03	0.45±0.03
After induction					
4	0.52±0.02	0.04±0.02	0.53±0.02	0.53±0.02	0.53±0.02
7	0.59±0.18	0.05±0.02	0.54±0.02	0.54±0.02	0.54±0.02
11	0.4±0.01	0.16±0.18	0.65±0.21	0.43±0.03	0.43±0.03
15	0.53±0.02	0.25±0.31	0.49±0.06	0.52±0.02	0.52±0.02
19	0.49±0.04	0.03±0.01	0.43±0.15	0.5±0.04	0.5±0.08
23	0.5±0.08	0.03±0.01	0.52±0.02	0.53±0.02	0.51±0.02
High-density Lipoprotein (mg/dl), (Range- 35.0 - 88.0)					
Before induction					
0	58.11±5.26	49.85±6.66	61.03±7.26	63.66±5.16	63.66±5.16
After induction					
4	49.61±6.64	29.53±2.74	55.58±5.56	55.21±5.38	55.21±5.38
7	52.35±5.81	29.86±2.95	60.05±3.73	57.51±5.24	57.51±5.24
11	52.6±6.18	30.48±3.04	63.73±5.2	57.08±4.26	57.08±4.26
15	51.48±1.79	31.11±3.62	70.53±1.55	60.11±4.56	55.13±3.78
19	60.28±2.46	30.13±2.3	70.55±2.09	57.25±5.44	58.68±3.18
23	61.1±7.23	32.35±2.51	67.75±2.97	55.25±3.57	59.61±8.16
Phosphorus (mg/dl), (Range- 3.00-6.20)					
Before induction					
0	4.3±1.21	3.78±0.67	4.33±0.36	3.79±0.47	157.5±23.36
After induction					
4	4.65±1.09	2.22±0.52	4.03±0.75	4.07±0.63	148.33±11.27
7	4.15±0.56	2.43±0.13	4.72±0.28	4.31±0.71	156±18.93
11	3.57±1.6	1.87±0.34	4.85±0.54	4.49±0.66	159.83±24.43
15	4.41±0.67	1.65±0.56	4.66±0.57	4.72±0.41	145.66±9.56
19	3.89±0.55	2.15±0.47	4.99±1.08	3.41±0.65	142±6.5
23	4.17±0.85	1.86±0.5	4.41±0.7	4.39±0.88	154.5±14.34

TABLE 5: EFFECTS OF MAHUA CONCENTRATE AND MAHUA LADDOO ON THE BLOOD LEVELS OF RED BLOOD CELLS (RBCS), HAEMOGLOBIN (HB) AND HEMOCYANINS (HCTS) IN AN ANAEMIA-INDUCED RAT MODEL

Days	Normal Control	Negative Control	Positive Control	Mahua Concentrate	Mahua Laddoo
RBC (10^12/L), (Range- 4.20-6.0)					
			Before induction		
0	4.41±0.26	4.28±0.07	4.26±0.03	4.51±0.24	4.51±0.24
After induction					
4	4.66±0.38	4.13±0.07	4.49±0.06	4.76±0.19	4.76±0.19
7	4.91±0.37	4.01±0.08	4.66±0.11	4.95±0.19	4.95±0.19
11	5.21±0.35	3.91±0.1	5.02±0.2	5.12±0.19	5.12±0.19
15	5.44±0.28	3.78±0.09	5.23±0.23	5.25±0.17	5.25±0.2
19	5.69±0.15	3.68±0.09	5.46±0.24	5.45±0.19	5.57±0.17
23	5.88±0.12	3.45±0.17	5.67±0.26	5.61±0.2	5.78±0.18
HGB (g/dL), (Range- 13-17)					
Before induction					
0	13.31±0.43	13.36±0.34	13.36±0.22	12.8±0.58	12.8±0.58
After induction					
4	13.55±0.45	12.06±0.46	13.71±0.15	12.25±0.64	12.25±0.64
7	13.8±0.38	11.3±0.53	14.13±0.12	12.16±0.84	12.16±0.84
11	14.18±0.34	10.3±0.45	14.46±0.26	12.58±0.73	12.58±0.73
15	14.41±0.34	9.4±0.46	14.78±0.29	13.06±0.69	13.06±0.69

19	14.6±0.34	8.58±0.43	15.05±0.29	13.51±0.66	13.72±0.93
23	14.78±0.28	7.53±0.22	15.35±0.31	13.93±0.54	14.23±0.62
HCT count (%), (Range- 39-52)					
Before induction					
0	41.5±1.7	41.5±0.95	41.5±1.38	40±0.75	40±0.75
After induction					
4	43.16±1.21	39.5±0.95	43.33±1.69	38±0.75	38±0.75
7	45.16±0.89	37.66±1.1	45.16±1.21	37.83±1.45	37.83±1.45
11	813.5±1714.39	35.5±1.25	46.5±1.38	39.5±1.38	39.5±1.38
15	41.66±15.95	33.5±2.06	47.83±1.34	42±1.19	42±1.19
19	50±1.52	31.83±2.03	49.33±1.24	43.66±1.57	48.83±2.58
23	51.16±1.46	30.33±2.28	51.33±0.94	45.33±1.38	50±2.67

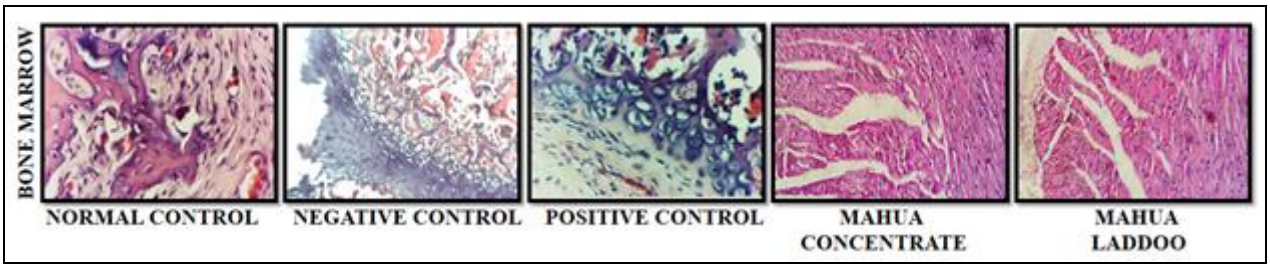

FIG. 3: REPRESENTATIVE IMAGES OF FEMUR BONE MARROW SECTIONS FROM (A) NORMAL CONTROL ANIMALS SHOWING THE PRESENCE OF AN ADEQUATE NUMBER OF BONE MARROW CELLS; (B) ANEMIC CONTROL INDICATING THE PRESENCE OF CERTAIN FOCI OF MINIMAL GRADE IN BONE MARROW CONTAINING ADIPOSE TISSUE; (C) LIVOGEN XT AND (D) TREATED-I: MAHUA CONCENTRATE; (E) TREATED-II: MAHUA LADDOO. *(H AND E X40)*

DISCUSSION: Anaemia is a major threat to global health and affects almost 24.8% of the total population worldwide. In the context of India, approximately 58.5% the population is affected, specifically women. In contrast, a greater percentage of the Odisha population, i.e., 60.2% of the total female population and 71.3% of children are affected [18].

Anemic conditions refer to a decrease in red blood cells or hemoglobin, which in turn decreases the oxygen-carrying capacity. This is caused by a deficiency of iron, folic acid and certain vitamins [19]. However, there are certain conventional medications for the treatment of anaemia, such as iron supplements. However, interest lies in natural remedies for the management of anaemia. The mahua tree is abundant in India, and its flower is well known for its sweetness index [20]; hence, it is used for the preparation of several value-added food products. The flowers are well known for their nutritional content, especially iron, which can be used to combat anaemia among women and children. The presence of iron increases red blood cell formation and prevents birth defects [21]. The Anti-anemic potential of "mahua concentrate and mahua laddoo" prepared from dried mahua flowers has been explored. The iron content found in mahua flowers is 35 mg/100 g. A high iron content enhances the nutritional value of mahua, especially for women and children who are susceptible to iron deficiency [22]. Although similar studies related to the Antianemic properties of mahua concentrate and mahua laddoo have not been carried out, the nutritional composition may be the reason for its Antianemic activity. Proximate analyses of mahua flowers [22], mahua concentrate and mahua laddoo have shown that they contain several essential nutrients, such as carbohydrates, proteins, fats, minerals, and vitamins, which are important for combating anaemia. The minerals found in mahua flowers, such as iron, copper, and zinc, could be responsible for the production and maturation of RBCs [23]. Proteins can also act as oxygen carriers in the blood stream. Although these studies have encouraging potential, further work is needed to elucidate the mechanisms related to its Antianemic activity and ensure its safety in human subjects. The currently available data indicate, however, that mahua concentrate and mahua laddoo may be valuable traditional foods of major importance for treating anaemia, with potential advantages in combating anaemia.

CONCLUSION: In conclusion, The mahua flower concentrate and mahua laddoo exhibited anti-

anemic activity in a rat model induced with 2,4-dinitrophenylhydrazine. This study also highlights the therapeutic potential of value-added food products. Both products display substantial effects in ameliorating anaemia, as demonstrated by enhancements in red blood cell count, haemoglobin levels, and overall haematological parameters. These findings indicate that mahua-based preparations may possess valuable properties, which could contribute to their efficacy in treating anaemia. Further studies are necessary to discover the mechanisms of action, optimal dosages, and potential for clinical application in human populations. However, this investigation offers insights into the use of mahua flowers as a natural medicine for the management of anaemia.

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