IJPSR (2026), Volume 17, Issue 2 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 21 August 2025; received in revised form, 24 September 2025; accepted, 16 January 2026; published 01 February 2026

ANTI-ANAEMIC ACTIVITY AND POTENTIAL TOXICITY OF HYDROALCOHOLIC EXTRACT OF *ASTERACANTHA LONGIFOLIA* AND MORINGA OLEIFERA IN 2, 4-DIPHENYLHYDRAZINE INDUCED ANAEMIC RATS MODEL

Nazneen Azad and Chumkijini Chhatria *

Department of Biotechnology and Bioinformatics, Sambalpur University, Jyoti Vihar - 768019, Odisha, India.

Keywords:

Anaemia, *Moringa oleifera*, *Asteracantha longifolia*, 2,4-Diphenylhydrazine, Hematological parameters, Hydroalcoholic extract

Correspondence to Author:
Dr. Chumkijini Chhatria

Assistant Professor,
Department of Biotechnology and Bioinformatics, Sambalpur University, Jyoti Vihar - 768019, Odisha, India.

E-mail: chumkijini@gmail.com

ABSTRACT: Anaemia remains a widespread health issue, particularly in developing countries, due to nutritional deficiencies and oxidative stress. The use of plant-based remedies offers a safer alternative to conventional treatments. This study aimed to evaluate the anti-anaemic activity of hydroalcoholic extracts of *Asteracantha longifolia* and *Moringa oleifera* in 2, 4-diphenylhydrazine (DPH)-induced anaemic rats. Anaemia was induced in Wistar albino rats using 2, 4-diphenylhydrazine, a known hemolytic agent that generates oxidative stress. Animals were divided into five groups: normal control, anaemic control, standard treated (ferrous sulfate), and two test groups treated with hydroalcoholic extracts of *Asteracantha longifolia* and *Moringa oleifera*, respectively. Extracts were administered orally for 14 days. Hematological (RBC count, hemoglobin, hematocrit), biochemical (serum iron, total iron-binding capacity), and histopathological evaluations of the liver and bone marrow were conducted to assess the efficacy. Both plant extracts significantly improved hemoglobin levels, RBC counts, and serum iron parameters compared to the anaemic control. *Moringa oleifera* showed slightly superior hematinic activity compared to *Asteracantha longifolia*, which was also effective but to a lesser extent. Histopathological studies supported the restoration of hematopoietic tissue structure in treated groups. The hydroalcoholic extracts of *Moringa oleifera* and *Asteracantha longifolia* demonstrated potent anti-anaemic activity in DPH-induced anaemic rats, with *Moringa oleifera* being comparatively more effective. These findings validate their traditional use and highlight their potential as natural hematinic agents for anaemia management.

INTRODUCTION: Anaemia is a global public health concern affecting approximately one-third of the world's population, particularly in developing countries, where nutritional deficiencies, infections, and chronic diseases are prevalent [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.17(2).688-96

This article can be accessed online on www.ijpsr.com

DOI link: https://doi.org/10.13040/IJPSR.0975-8232.17(2).688-96

Characterized by a reduction in red blood cell (RBC) count, hemoglobin concentration, or hematocrit, anaemia leads to impaired oxygen transport and can result in fatigue, weakness, compromised immunity, and reduced quality of life [2].

Iron deficiency remains the most common etiology, though other causes such as vitamin B12/folate deficiencies, hemolytic processes, and chronic inflammation also contribute to the pathogenesis. Conventional anti-anaemic therapies often include

iron supplements and hematopoietic growth factors. However, these treatments are sometimes associated with gastrointestinal side effects, oxidative stress, and poor patient compliance [3]. Consequently, there is growing interest in plant-based remedies that may offer safer and more holistic alternatives [4].

Phytochemicals from medicinal plants with hematopoietic potential can stimulate erythropoiesis, improve iron bioavailability, and modulate oxidative stress involved in anaemia pathogenesis. *Moringa oleifera*, commonly known as the drumstick tree, is a nutrient-rich [5]. Plant with high concentrations of iron, calcium, vitamins A and C, and essential amino acids it has been traditionally used to treat anaemia, malnutrition, and inflammation. Several studies have demonstrated its erythropoietic, antioxidant, and immunomodulatory properties, supporting its use in anaemia management [6]. *Asteracantha longifolia* (syn. *Hygrophila auriculata*), known for its rejuvenating and restorative properties in Ayurveda, is another plant with a history of use in treating hematologic and hepatic disorders [7]. It is rich in flavonoids, saponins, alkaloids, and tannins phytochemicals known to enhance iron absorption and stimulate red blood cell synthesis. However, limited comparative studies exist evaluating its anti-anaemic efficacy alongside other potent hematinic herbs like *Asteracantha longifolia*. 2,4-Diphenylhydrazine (DPH)-induced anaemia in rats is a well-established model that mimics oxidative stress-mediated hemolytic anaemia, allowing the assessment of hematological and antioxidant parameters in response to therapeutic interventions [8]. This model is particularly valuable for evaluating plant extracts with potential erythropoietic and free radical-scavenging activity. Given the individual reports of hematinic activity in both *Asteracantha longifolia* and *Moringa oleifera*, the present study aims to comparatively evaluate their anti-anaemic efficacy using hydroalcoholic extracts in a 2,4-DPH-induced anaemic rat model. This investigation could provide a scientific basis for their traditional use and potentially identify a more effective botanical agent for managing anaemia.

MATERIALS AND METHODS:

Chemicals and Solvents: The materials used in this study included 2,4-diphenylhydrazine (Sigma-Aldrich, India) for anaemia induction. Hydroalcoholic extracts of *Asteracantha longifolia* and *Moringa oleifera* were prepared using 70% ethanol (Merck India Ltd., Mumbai). Biochemical assay kits for serum iron and TIBC were obtained from Erba Diagnostics, India. Hematological analyses were performed using a Mindray BC-2800 hematology analyzer (Mindray Medical International Ltd.). All other chemicals and solvents were of analytical grade from Merck, and animals were maintained on standard diet from Nutrivet Life Sciences, Pune.

Sample Collection: The plant parts, such as the aerial parts and roots of *Asteracantha longifolia* and the stem and leaves of *Moringa oleifera* were collected from nearby areas of Sambalpur. Dust particles were removed by washing with tap water followed by distilled water and then the plant materials were air-dried. The dried plant material was ground to a fine powder using a mechanical grinder, kept in airtight polybags and labelled respectively.

Soxhlet Extraction: The powder samples were taken for Soxlet extraction. 60g powder of aerial parts of *Asteracantha longifolia* and 54g of *Moringa oleifera* stem and leaves were used for Soxhlet extraction [9]. A hydro-alcoholic solution (ethanol: distilled water, 70:30) was used as solvent for extraction. To guarantee the highest phytochemical output, the extraction procedure was conducted for 48 hours and included continuous cycles of solvent reflux and condensation. After passing through the Whatman filter paper, the extracts were concentrated at 50°C in a rotary evaporator.

Determination of Percentage Yield of Plant Extract: Percentage yield measures the effectiveness of the entire extraction process. % yield was calculated using the formula given below:

$$\text{Percentage yield} = (\text{Weight of extract}) / (\text{Weight of powder sample taken}) \times 100$$

***In-vivo* Study:** The animal study using wistar rats was approved by animal ethical committee. Wistar rats (180-200g) 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 hr light/dark cycle.

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

Acute Toxicity: Single doses of different concentrations such as, 250, 500, 1000, 2000 and 5000 mg/kg of the *M. oleifera* and *A. longifolia* (15-18 gm) were administered orally to six groups, with six animals in each group (n=6) [10]. They were placed under observation for 14 days after which the number of dead rats was recorded [11]. The results of toxicity study showed no signs of toxicity or death in the animals even at highest concentration of 5000 mg/kg.

Subacute Toxicity: Twenty wistar rats were randomly divided into two groups with ten animals in each group (n=6). *M. oleifera* and *A. longifolia* (15-18 gm) were administrated orally at a daily dose of 250 mg/kg body weight for 28 days following the repeated oral toxicity test. The animals were observed daily during the experiment to detect death or abnormal clinical signs.

The body weight, water intake and food intake were recorded. At the end of the day number 29th animal were sacrificed and collected the blood by cardiac heart puncture, collected the all vital organ for the toxicological studies.

Induction of Anaemia: The anaemic model was developed by administrating 2, 4-diphenylhydrazine to the rats *via* Oral Gavageat a concentration of 40mg/kg for 2 consecutive days. When the haemoglobin is reduced to 30%, the animals were considered to be anaemic [12].

Experimental Design: Rats were divided into five groups containing six rats in each group (n = 6). Group-I rats were treated as normal control. The rats of Group-II were treated as anaemic control induced with 2, 4-diphenylhydrazine consider as a negative group. Group-III was treated as positive control where Livogen XT was given orally. Group-IV & V were the treatment groups where *M. oleifera* and *A. longifolia* were administered to the animals by oral cavage method.

Hematological Analysis: For the acute and sub-acute toxicity, the animals were anaesthetised by mild anaesthesia (isoflurane) followed by cervical dislocation and blood was collected by cardiac puncture. Blood cell parameters were analysed using CBC analyser [13].

The collected blood was centrifuged at 5000 rpm for 10 min and for the anaemic the blood samples were collected from the retro-orbital on days 0- before the induction of anaemia and on 3rd, 7th, 10th and 15th day after the induction of anaemia. Blood samples were examined for red blood cells (RBCs), hemoglobin (Hb), and hematocrit (HCT) levels.

Serum Biochemistry Analysis: For the biochemistry part of the *in-vivo* study, 1 ml of blood was taken from the rat and was subjected to centrifugation for 10 min at 10000 rpm so that the cells would accumulate in the palette and the supernatant was collected which contained the serum [14].

This serum was taken in a biochemistry analysis to identify and quantify different types of biomolecules such as Glucose (GLU), Albumin (AB), Urea, Creatinine (CREA), Cholesterol (CHOL), Triglycerides (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), Low-density lipoprotein(LDL).

Histopathology: For acute and sub-acute toxicity, animals were sacrificed to isolate the liver, lung, kidney, heart, spleen, and femur bone for bone marrow examination [15].

The collected tissues were processed, stained by Hematoxylin and Eosin staining methods, and examined under the light microscope for histological changes. For the anaemic model, on the last day i.e. 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 by Hematoxylin and Eosin and studied under the microscope for any histological changes.

RESULTS AND DISCUSSION:

Percentage Yield of Plant Extract: The result of plant extract yield potential was 9.46% in *Moringa oleifera* followed by *Asteracantha longifolia* aerial part which is represented in the **Table 1**. Plant extract yield potential differ because of the solvent used and the extraction techniques.

TABLE 1: YIELD PERCENTAGE OF PLANT EXTRACT

Plant material	Extract	Weight of plant material (g)	Weight of extract (g)	% Yield (W/W)
Asteracantha longifolia (Aerial parts)	Hydro-alcoholic	60	2.70	4.5%
Moringa oleifera (Stem and leaves)	Hydro-alcoholic	54	5.11	9.46%

***In-vivo* Study:** This study compare the anti-anaemic potential of hydroalcoholic extracts of *Asteracantha longifolia* (aerial parts) and *Moringa oleifera* (stem and leaves) using a 2,4-diphenylhydrazine-induced anaemic rat model. 2,4-Diphenylhydrazine, a known hemolytic agent, induces anaemia by generating oxidative stress that damages red blood cells, resulting in significant reductions in hemoglobin (Hb), red blood cell (RBC) count, and hematocrit (HCT).

These hematological disturbances are evident in the anaemic control group showed in the **Table 3** and **Fig. 1**. Following extract administration, the blood biochemistry parameters of rats treated with both plant extracts significantly improved hematological indices compared to the anaemic control. *Moringa oleifera* extract demonstrated superior efficacy, showing a marked increase in hemoglobin, RBC count, and HCT levels represented in the **Table 3** and **Fig. 1.**

This effect may be attributed to its high content of iron, vitamin C, flavonoids, and polyphenols, which are essential for erythropoiesis and the stabilization of red blood cells. In contrast, *Asteracantha longifolia* also improved these parameters but to a lesser extent, possibly due to a lower concentration of hematinic constituents. There was no significant difference in body weight, food intake, or water intake between untreated and treated groups **Table 2**. Moreover, animals treated with the extracts showed improved body weight and enhanced food and water intake over the 28-day study period which was presented in the **Table 2** indicating a general improvement in health and metabolism. Body Weight (23 days days experiment) in experimental groups (Anaemic Model) was given in the **Table 5.**

In case of normal control body weight increases, in negative control it was decreases. However in positive control, *M. oleifera* and *A. longifolia* treated it was increases. The enhanced nutritional status may have contributed to improved hematological outcomes. Organ functions show undistinguishable profiles among the control and treated groups. No significant differences could be detected in the liver function test, kidney function test, lipid profile, serum glucose, and different salts between the treated and untreated groups.

After the administration of 2,4-Diphenylhydrazine, there was significant decrease in RBC count in the animals ($p<0.001$)., as given in the **Table 7**. Haemoglobin level was increased by the treatment of plant extracts and Livogen XT. The plant extract were found to be more effective as compared to positive control (Livogen XT). The presence of minimal grade of adipose tissue was observed in anaemia- induced rats which revealed the depletion of bone marrow cellular population.

TABLE 2: EVALUATION OF BODY WEIGHT, FOOD INTAKE, AND WATER INTAKE AMONG CONTROL AND TREATED GROUPS

No. of Days	Normal Control	*M. oleifera*	*A. longifolia*
		Body Weight (g)	
01	178.833±9.154	171.5±5.439	168.5±7.041
07	190.333±7.608	181±5.597	179.166±7.403
14	203±7.505	196.333±4.714	198±9.398
21	211.666±13.046	204.166±14.746	212.833±9.973
28	224.166±10.221	223.333±9.655	213.666±6.342

Food Intake (g)			
01	18.316±0.371	12.783±0.233	13.7±2.076
07	18.416±0.389	14.083±0.766	13.833±2.009
14	18.4±0.365	15.35±1.642	13.733±2.053
21	18.4±0.294	16.55±2.483	14.3±1.957
28	18.483±0.291	17.733±3.352	14.883±2.282
Water Intake (ml)			
01	15.6±3.083	20.183±3.638	16.483±3.925
07	16.183±2.274	20.216±1.446	17.4±2.997
14	17.483±4.653	21.466±1.216	18.8±4.684
21	18.916±5.517	22.016±1.171	19.183±4.753
28	19.933±6.143	21.133±1.894	19.5±5.448

TABLE 3: BIOCHEMICAL PARAMETERS AFTER 28 DAYS OF TREATMENT (250 MG/KG)

Parameter	Control	*M. oleifera*	*A. longifolia*
Glucose (GLU)	128.95±3.86	96.32±4.34	87.82±6.66
Albumin (ALB)	2.41±0.39	3.18±0.82	1.723±0.42
Urea (UREA)	44.27±2.67	33.003±2.25	23.1±14.11
Creatinine (CREA)	1.346±0.08	0.595±0.04	1.3±0.22
Cholestrol (CHOL)	119.93±3.65	123.01±2.77	122.18±2.27
Triglycerides (TG)	134.16±12.89	108.16±7.57	115.416±10.92
Alanine Transaminase (ALT)	80.56±3.74	65.65±2.70	107.8±6.51
Aspartate Aminotransferase (AST)	21.45±3.12	21.26±1.40	34.016±2.27
Total Protein (TP)	6.1±0.95	5.5±1.62	4.993±0.07
Magnesium (MG)	2.28±0.36	1.55±0.22	1.63±0.18
Phosphorus (PHOS)	4.26±0.45	3.65±0.23	4.16±0.32
Calcium (CA)	8.83±0.22	11.03±0.64	75.875±4.16
Direct Bilirubin (DBIL)	0.891±1.74	4.325±0.22	0.2286±0.14
Total Bilirubin (TBIL)	0.085±0.09	0.2±0.10	0.183±0.03
High-density Lipoprotein (HDL)	61.66±2.12	53.47±158	43.55±1.76
Gamma-glutamyl Transferase (GGT)	1.88±0.27	2.73±0.31	11.343±18.79
Alkaline Phosphatase (ALP)	17.82±0.88	4.97±0.26	53.06±13.66

TABLE 4: HEMATOLOGICAL PARAMETERS AFTER 28 DAYS OF TREATMENT

Parameter	Control	*M. oleifera*	*A .longifolia*
White blood cell count (WBC (10^3/L)	5.466±0.249	5.4 ±0.163	8.8 ±0.374
Neutrophils (Neu# (10^3/L)	4.466±0.286	6.433±0.249	7.066±0.205
Lymphocytes (Lym# (10^3/L)	3.466±0.249	3.333±0.205	4.066±0.124
Monocytes (Mon# (10^3/L)	0.433±0.124	0.6±0.081	0.5±0.081
Eosinophils (Eos# (10^3/L)	0.123±0.028	0.027±0.001	0.23±0.043
Basophil (Bas# (10^3/L)	0.05±0.024	0.063±0.012	0.036±0.004
NLR	1.466±0.169	1.7±0.081	1.413±0.347
PLR	0.019±0.001	0.023±0	0.017±0.004
red blood cell count (RBC (10^12/L)	5.866±0.286	5.566±0.205	5.236±0.338
Hemoglobin (HGB (g/dL)	13.5±0.294	13.766±0.286	14.123±0.033
HCT	43.633±0.169	44.3±0.163	45.566±0.805
MCV (fL)	84.433±0.124	87.633±0.205	88.566±0.329
MCH (pg)	30.133±0.249	30.6±0.163	30.233±0.939
MCHC (g/L)	327±1.632	342.333±2.054	354±5.099
RDW-CV	12.7±0.326	14.066±0.124	13.566±0.205
RDW-SD (fL)	37.5±0.244	42.766±0.124	42.366±0.249
platelet count (PLT (10^3/L)	2.733±0.124	3.6±0.163	3.566±0.205
PCT (mL/L)	10.5±0.244	11.5±0.163	11.363±0.312

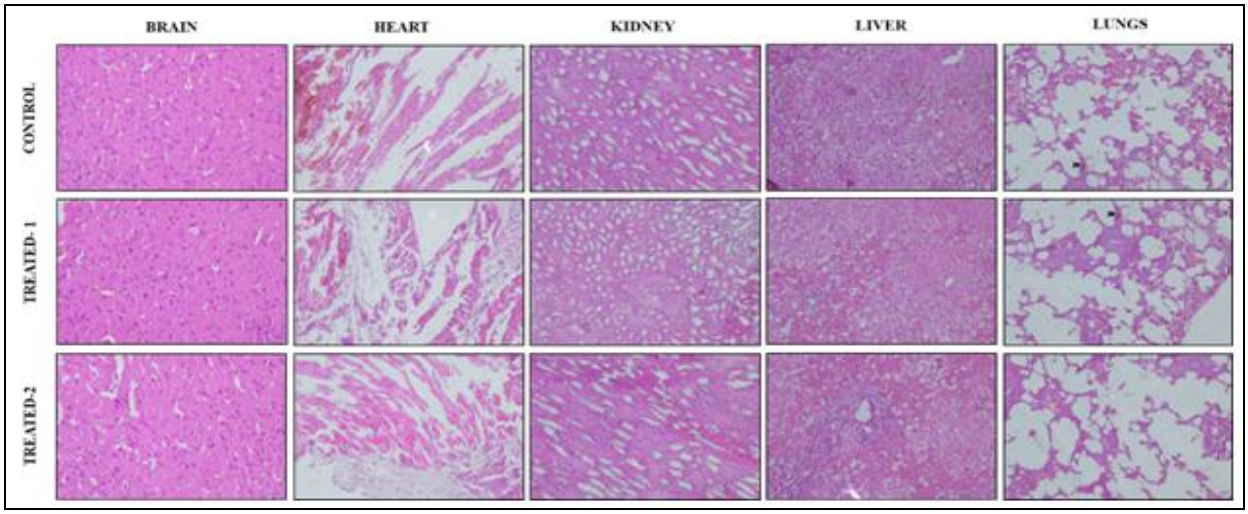

FIG. 1: PHOTOMICROGRAPHS OF ORGANS BRAIN, LUNGS, HEART, LIVER, AND KIDNEYS SHOWED NO SIGNIFICANT HISTOPATHOLOGICAL CHANGES AFTER SUBACUTE TOXICITY TESTING OF *M. OLEIFERA* AND *A. LONGIFOLIA* AT 250 MG/KG FOR 28 DAYS

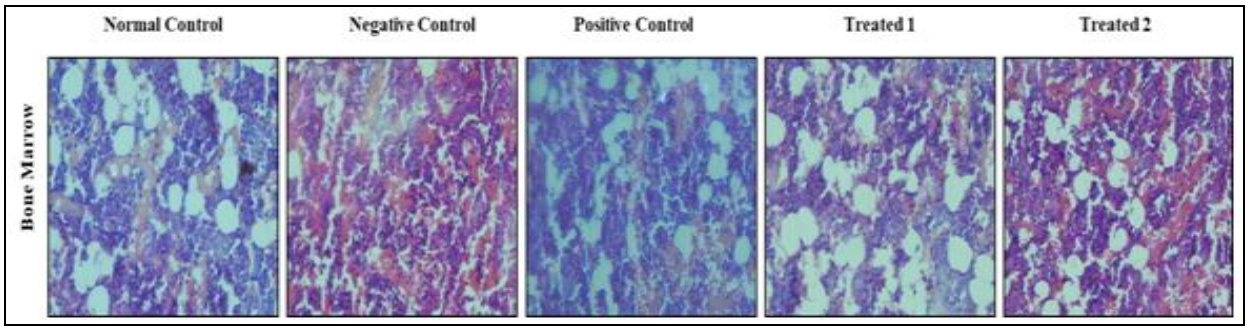

FIG. 2: REPRESENTATIVE IMAGES OF BONE MARROW SECTIONS FROM ANIMALS OF (A) NORMAL CONTROL SHOWING PRESENCE OF ADEQUATE NUMBER OF BONE MARROW CELLS; (B) ANAEMIC CONTROL INDICATING PRESENCE OF CERTAIN FOCI OF MINIMAL GRADE IN BONE MARROW CONTAINING ADIPOSE TISSUE; (C) LIVOGEN XT AND (D) TREATED-I: *M. OLEIFERA*(E) TREATED-II: *A. LONGIFOLIA*. (H AND E X40)

The biochemical parameters, particularly serum ALT, AST, and total bilirubin, were elevated in the anaemic group due to hepatic stress induced by oxidative damage. Treatment with both extracts significantly normalized these biochemical markers showed in the **Table 4**, indicating hepatoprotective effects in addition to hematopoietic support. The hepatoprotective action may be linked to the antioxidant and anti-inflammatory activities of the phytoconstituents present in both extracts. Histopathological examination of the bone marrow further corroborated the biochemical and hematological results. The treated groups, particularly those receiving *Moringa oleifera*, showed restored architecture of hematopoietic tissues, reduced congestion, and increased erythropoietic activity compared to the anaemic control group was shown in the **Fig. 2.** The regenerative changes observed reflect stimulation of hematopoiesis, likely mediated by the antioxidant and micronutrient content of the extracts.

TABLE 5: BODY WEIGHT IN EXPERIMENTAL GROUPS (ANAEMIC MODEL)

	Body Weight (g)				
Days	**Normal control**	**Negative control**	**Positive Control**	***M. oleifera***	***A. longifolia***
0	217.22±4.14	235±5.93	209.96±7.06	177.2±7.67	174.4±7.6
4	217.94±4.5	233±6.09	208.08±6.58	176.76±9.46	173.76±10.3
7	218.86±5.1	229.6±6.08	211±8.23	182.2±7.85	179.2±7.93
11	219.6±5.49	226±5.96	211.52±8.81	183.2±6.67	180.8±6.67
15	220.54±5.91	222.8±5.7	212.04±9.39	186.6±6.24	189.2±6.17
19	221.3±6.32	220±5.96	212.56±9.98	188.6±6.65	185.4±5.71
23	222.08±6.7	216.2±5.45	213.08±10.56	191.2±6.93	191.8±5.49

TABLE 6: BLOOD BIOCHEMISTRY IN ANAEMIC MODEL

Days	Normal Control	Negative Control	Positive Control	*M. oleifera*	*A. longifolia*
Alanine Transaminase (U/L), (Range -10.00 - 125.00)					
Before induction 0	88.98±28.12	107.46±5	117.04±2.05	111.77±4.56	111.17±4.23
4	106.66±5.15	126±1.06	107.88±0.87	107.88±0.87	107.81±1.01
7	106.23±7.9	126.08±0.72	114.23±5.12	108.23±0.79	108.43±1.59
11	102.72±4.97	126.58±1	116.72±3.6	108.32±1.28	108.42±1.97
15	105.1±6.96	127.24±0.7	111.5±4.69	106.7±1.76	109.9±2.63
19	107.98±6.31	127±0.4	115.98±3.77	106.78±1.82	108.98±2
23	110.56±7.33	128.52±0.6	112.56±4.32	110.56±4.12	110.56±4.12
Aspartate Aminotransferase (U/L), (Range- 0 - 50.0)					
Before induction 0	27.7±4.07	31.12±2.36	33.16±3.12	35.1±4.47	35.04±4.52
4	27.06±3.54	54.66±0.62	40.84±4.49	34.2±0.52	34.66±0.62
7	31.72±4.44	55.72±1.39	36.78±5.84	34.9±1.11	35.72±1.39
11	33.66±4.37	55.66±0.96	35.56±2.09	41±5.01	37.35±2.82
15	33.28±4.34	55.26±0.25	33.66±4.15	36.66±3.32	35.94±4.57
19	33.92±2.22	55.02±0.97	41.54±1.81	34.18±1.71	35.86±4.03
23	35.32±0.74	55.32±0.74	40.62±1.67	39.48±4.73	39.04±3.22
Alkaline Phosphatase (U/L), (Range- 0.1 - 212.0)					
Before induction 0	152.8±7.65	151.4±2.87	165±32.14	162.8±22.05	162.8±22.05
4	165.4±8.3	258.8±17.78	162.6±20.33	151.6±9.41	151.6±9.41
7	151.6±11.05	255.6±20.91	155.6±20.91	156.2±20.74	156.2±20.74
11	162.8±6.91	262±25.08	162±25.08	154.6±23.49	154.6±23.49
15	147.6±9.35	236.2±14	136.4±14	143.2±6.27	146.6±10.22
19	150±8.64	242.6±23.09	142.6±23.09	140.6±7.73	141.8±7.11
23	148.6±9.72	256.4±15.22	156.2±15.24	156.8±14.4	154.6±15.71
Total Bilirubin (mg/dl), (Range- 0 - 0.90)					
Before induction 0	0.44±0.03	0.4±0.08	0.54±0.19	0.46±0.03	0.45±0.04
4	0.53±0.02	0.05±0.02	0.53±0.02	0.53±0.02	0.53±0.02
7	0.6±0.19	0.06±0.01	0.53±0.02	0.53±0.02	0.55±0.01
11	0.4±0.01	0.17±0.19	0.61±0.21	0.44±0.03	0.42±0.03
15	0.53±0.03	0.29±0.32	0.49±0.07	0.52±0.01	0.52±0.02
19	0.49±0.04	0.03±0.01	0.41±0.16	0.5±0.05	0.48±0.08
23	0.49±0.08	0.02±0	0.51±0.02	0.53±0.02	0.51±0.02
High-density Lipoprotein (mg/dl), (Range- 35.0 - 88.0)					
Before induction 0	59.08±5.26	50.04±7.28	60.52±7.85	64.74±5	64.74±5
4	48.9±7.06	30.42±2.07	54.44±5.4	53.96±5.03	53.96±5.03
7	50.16±3.44	30.22±3.11	59.38±3.74	57.7±5.72	57.7±5.72
11	53.48±6.42	29.96±3.07	64.84±5.02	56.68±4.56	56.68±4.56
15	51.86±1.73	30.48±3.65	70.98±1.3	59.44±4.72	55.8±3.81
19	59.7±2.28	30.28±2.49	70.98±2.04	55.96±5.05	58.74±3.48
23	61.66±7.8	31.78±2.37	67.68±3.25	56.44±2.61	57.32±6.95
Phosphorus (mg/dl), (Range- 3.00-6.20)					
Before induction 0	4.25±1.31	3.6±0.63	4.21±0.26	3.88±0.47	3.88±0.47
4	4.72±1.18	2.44±0.15	4.21±0.68	3.84±0.41	3.84±0.41
7	4.16±0.62	2.42±0.14	4.65±0.25	4.17±0.7	4.17±0.7
11	4.2±0.84	1.88±0.37	4.99±0.47	4.37±0.66	4.65±0.41
15	4.2±0.5	1.76±0.55	4.77±0.56	4.65±0.41	3.98±0.56
19	3.98±0.56	2.1±0.5	5.36±0.78	3.49±0.69	4.14±0.72
23	4.37±0.78	1.72±0.44	4.43±0.76	4.12±0.71	4.28±0.85

TABLE 7: EFFECT OF *M. OLEIFERA* AND *A. LONGIFOLIA* ON BLOOD LEVELS OF RBCS, HEMOGLOBIN (HB) AND HEMOCRAIT (HCT) IN ANAEMIA INDUCED RAT MODEL

Days	Normal Control	Negative Control	Positive Control	*M. oleifera*	*A. longifolia*
RBC (10^12/L), (Range- 4.20-6.0)					
Before induction (0 day)	7.77±0.94	7.96±0.25	8.08±0.21	8.38±0.44	8.36±0.45
4	7.88±0.87	7.36±0.44	8.48±0.22	8±0.44	7.92±0.49
7	8.23±0.79	6.74±0.4	9.18±0.19	7.56±0.29	7.5±0.34
11	8.72±0.81	6.08±0.28	9.84±0.29	7.8±0.28	7.76±0.31
15	7.5±0.3	5.46±0.44	10.42±0.32	8.2±0.23	8.18±0.25

19	7.98±0.84	4.98±0.44	11.16±0.32	8.52±0.22	8.54±0.19
23	8.56±0.64	4.42±0.33	11.62±0.41	9.02±0.22	9.04±0.19
HGB (g/dL), (Range- 13-17)					
Before induction (0 day)	13.1±0.91	12.26±0.35	12.02±0.34	12.02±0.33	12.08±0.41
4	14.2±0.52	11.74±0.37	12.3±0.38	11.44±0.31	11.5±0.39
7	14.96±1.05	11.14±0.51	12.78±0.44	11.06±0.32	11.14±0.41
11	15±0.61	10.22±0.63	13.34±0.44	11.4±0.3	11.3±0.3
15	15.2±0.44	9.28±0.73	13.98±0.61	11.84±0.29	11.76±0.31
19	15.26±0.46	8.5±0.61	14.42±0.48	12.28±0.22	12.16±0.23
23	15.48±0.51	7.34±0.34	14.8±0.49	12.76±0.3	12.56±0.38
HCT count (%), (Range- 39-52)					
Before induction (0 day)	0.44±0.03	0.47±0.11	0.43±0.01	0.42±0.03	0.4±0.04
4	0.53±0.02	1.41±0.16	0.43±0.01	0.42±0.03	0.42±0.03
7	0.54±0.02	1.88±0.19	0.43±0.02	0.43±0.02	0.43±0.02
11	0.42±0.02	2.31±0.15	0.43±0.02	0.43±0.01	0.43±0.01
15	0.52±0.02	2.76±0.16	0.44±0.03	0.44±0	0.44±0.01
19	0.51±0.04	3.12±0.1	0.44±0.03	0.46±0.01	0.45±0.02
23	0.53±0.02	3.57±0.26	0.44±0.04	0.47±0.02	0.46±0.03

CONCLUSION: In summary, the findings suggest that both *Asteracantha longifolia* and *Moringa oleifera* possess significant anti-anaemic activity, with *Moringa oleifera* demonstrating comparatively greater efficacy. This may be due to its richer nutritional profile and stronger antioxidant capacity. These results justify the traditional use of these plants in anaemia management and suggest their potential in developing plant-based hematinic formulations. Further studies involving isolation of active constituents and molecular mechanistic evaluations are recommended to explore their full therapeutic potential.

ACKNOWLEDGEMENTS: Authors remain thankful to Head, Department of Biotechnology and Bioinformatics, Sambalpur University and COE, NPT of P.G. Department of Biotechnology and Bioinformatics, Sambalpur University for necessary facilities. Also, authors are thankful to Institutional Animal Ethical committee (IAEC) SU/BTBI/IAEC/2025/01 for the animal model research work.

CONFLICTS OF INTEREST: Nil

REFERENCES:

1. Brittenham GM, Moir-Meyer G, Abuga KM, Datta-Mitra A, Cerami C, Green R, Pasricha SR and Atkinson SH: Biology of anaemia: A public health perspective. Journal of Nutrition 2023; 153 (1): 7-28.
2. Pasricha SRS, Flecknoe-Brown, SC, Allen, KJ, Gibson, PR, McMahon, LP, Olynyk, JK, Roger, SD, Savoia, HF, Tampi, R, Thomson, AR, Wood, EM & Robinson KL: Diagnosis and management of iron deficiency anaemia: A clinical update. Medical J. of Australia 2010; 193: 525-532.
3. Crawley J: Reducing the burden of anaemia in infants and young children in malaria-endemic countries of Africa: From evidence to action. American Journal of Tropical Medicine and Hygiene 2004; 71 (Supplement): 25-34.
4. Anacletus FC, Monanu MO and Ugwu GM: Anti-anaemic Potentials of folic acid, vitamin B_{12} and Aqueous Extract of *Limonia acidissima* Leaves on Phenylhydrazine-Induced Anaemic Wistar rats. Int. Journal of Recent Research in Life Sciences 2018; 5: 51-60.
5. Gopalakrishnan L, Doriya K and Kumar DS: *Moringa oleifera*: A review on nutritive importance and its medicinal application. Food Sc. and Human Wellness 2016; 5: 49-56.
6. Posmontier B: The medicinal qualities of *Moringa oleifera*. Holistic Nursing Practice 2011; 25: 80-87.
7. Dash AK, Dutta GK, Sahoo GA, Sardar KK and Mishra SK: Nutri-biochemical composition of *Asteracantha longifolia*. *Medicinal Plants* 2015; 7: 233-236.
8. El-Shehry MSEF, Amrymi RA, Atia T, Lotfy BMM, Ahmed SHA, Qutb SA, Ali SB, Mohamed AS, Mousa MR, Damanhory AA, Metawee ME and Sakr HI: Hematopoietic effect of echinochrome on phenylhydrazine-induced hemolytic anaemia in rats. Peer J 2023; 11: 16576.
9. Alara OR, Abdurahman NH and Ukaegbu CI: Soxhlet extraction of phenolic compounds from *Vernonia cinerea* leaves and its antioxidant activity. J. of Applied Res. on Medicinal and Aromatic Plants 2018; 11: 12-17.
10. Frimat M, Boudhabhay I and Roumenina LT: Hemolysis derived products toxicity and endothelium: Model of the second hit. Toxins 2019; 11: 660.
11. OECD 2022. Test Guideline 425: Acute Oral Toxicity – Up-And-Down Procedure. Adopted 16 October 2008. Corrected 30 June 2022.
12. Mohammadi T: Effect of quercetin and mirtazapine on spermatogenesis and testis structure in phenylhydrazine-induced hemolytic anaemia mice: An experimental study. *Food and Chem.* Toxicol 2024; 189: 114732.
13. Onyeabo C, Achi NK, Ekeleme-Egedigwe CA, Ebere CU and Okoro CK: Haematological and biochemical studies on *Justicia carnea* leaves extract in phenylhydrazine induced-anemia in albino rats. Acta Scientiarum Polonorum. Technologia Alimentaria 2017; 16: 217-230.
14. Gupta D, Kushwah C, Joshi A, Malviya S and Kharia A: Anti-anemic activity of hydro-alcoholic extract of fruit of

Allium tricoccum in phenylhydrazine induced anemic rats. Pharma Tutor 2018; 6: 5-7.

15. Nwachukwu KC, Ibe C, Nwachukwu NC and Ugbogu OC: Hematological, biochemical and histopathological assessment of the toxicity potential of seed kernel extracts of *Mangifera indica* Linn. varieties in mice. Discovery 2022; 58: 1117-1127.

How to cite this article:
Azad N and Chhatria C: Anti-anaemic activity and potential toxicity of hydroalcoholic extract of *Asteracantha longifolia* and *Moringa oleifera* in 2, 4-diphenylhydrazine induced anaemic rats model. Int J Pharm Sci & Res 2026; 17(2): 688-96. doi: 10.13040/IJPSR.0975-8232.17(2).688-96.