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Comparative study of haematinic and iron utilization property of pre and flowering plant leaf extracts of *Asteracantha longifolia* (L.) Nees

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The study was conducted to validate and compare the haematinic property of pre-flowering and flowering *Asteracantha longifolia* (L.) Nees leaf extract. A phenylhydrazine induced anaemic rat model was used for this study. Concentrate hot water extract of succulent aerial part of pre-flowering and flowering leaf was orally administered @ 40 mg kg-1 body weight for 30 days, and equivalent weight of crude leaf was also administered to observe as well as to compare the amelioration and iron utilization. Pre- flowering extract effectively improved the concentration of membrane bound haemoglobin, RBC indices, and concentration of serum copper and cobalt; and normalized free haemoglobin concentration, percent of haematocrit, serum cobalt concentration, and lipid peroxidation. Pre-flowering extract also normalized the serum iron concentration. This observation could justify the indusion of pre-flowering extract to ameliorate anaemic condition caused by haemolysis due to the reduction of serum iron and free haemoglobin concentration and improvement of RBC bound haemoglobin concentration. The plant extract contains anti-oxidants and other constituents like, Cu, Co, Fe.

Keywords: *Hygrophila auriculata* (Schumach.) Heine, Anaemia, Free haemoglobin, Serum iron, Lipid peroxidation

IPC Int.Cl.[8]: A61K 36/00, A61, A01G 20/00, A61K 35/16, B44F 9/10, C01B 13/14, G06M 11/02, G01N 33/48

Asteracantha longifolia Nees (Common Bengali name "*Kullakhara*"; Hindi name "*Tal-Makhana*", *Gokulakanta*; Sanskrit name "*Kokilaksha*") is a semi-woody herb of Acanthaceae family, an inhabitant of damp or swampy area found throughout the planes of India[1]. Different parts (leaves, roots, seeds) of the herb are used in several patho-physiological conditions such as jaundice, rheumatism, renal stone, gonorrhoea, hepatic disorders, and anti-tumor activity[2]. Ethanolic extract of the aerial part is reported to increase hemopoietic parameters[3]. In the rural area of West Bengal, it is a common practice to feed the hot water infusion of succulent aerial parts of pre-flowering and flowering plant to prevent anaemia indiscriminately. Most of the work has been done to evaluate the effect of this plant material in iron deficiency anaemia but reports on the effect of this plant on anaemia due to haemolysis are very few. The treatment of anaemia due to haemolysis is a difficult task as it causes the iron deposition in different organs and free haemoglobin which causes further haemolysis and aggravates the condition. Recently at the University of California, Los Angeles, has designed hepcidin agonists, which they call minihepcidins that mimic the ability of natural hepcidin to lower the levels of iron in blood of mice may be a useful new approach for treating individuals with iron overload disorders[4]. A group of scientists of Division of Molecular Medicine, Bose Institute Kolkata, India found that extract of *Caesalpinia crista* ameliorate liver injury in iron overloaded mice[5]. Consumption of wheat grass juice decreased in the requirement of packed red cells (measured as gm/kg body weight/year) by 25% or more; Blood transfusion requirement fell by >25% in 8 (50%) patients with a decrease of >40% documented without any adverse effect[6].

Due to paucity of reports and in order to depict the effect of traditionally used extracting methods, as well as to document potentiality of this natural gift on iron utilization and amelioration of haemolytic anaemia, present research was conducted.

Materials and methods

Experiment was designed to compare the haematinic effect and iron utilization in experimentally induced haemolytic anaemic rodents

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exposed to concentrate extract of the aerial parts of *Asteracantha longifolia* at its pre-flowering (PF) and flowering (Fl) stages and crude leaf (Cl).

Plant materials

The plant was identified as *Astercantha longifolia* Nees (Acanthaceae) by Dr Smt Ranjana Shrivastava, Professor and Head, Department of Botany and Microbiology. Govt. VYT PG Autonomous College, Durg (CG) having specimen No. 04/EVM/Durg and a voucher specimen was preserved for further reference. The genus *Astercantha* is an angiospermic plant belonging to the family Acanthaceae. The family is composed of a number of genous and species having medicinal value and thery are usually perennial herbs or shrubs; the plant Astercanthae are distinguished by a number of characters, notably the presence of cystolith in vegetative organs, the presence and development of floral bracts and bracteoles, usually bilabiate corollas associated with the bilocular ovary , generally bivalvate elastically dehiscing capsules and usually by the curved retinacula supporting the seeds. It is useful in the treatment[7] of anasaraca, disease of urinogenitla tract, dropsy of chronic Bright's disease, hyperdipsia, vesical calculi, flatulence, diarrhea, dysentery, leucorrhea, gonorrhoea, asthama, blood disease, gastric diseases, painful maturation menorrhagea, etc. Fresh leaf and aerial parts of identified *A. longifolia* at its PF and Fl stages were collected in bulk from low land area around the girl's hostel of Anjora Veterinary College campus, Durg (CG), during the month of August-September 2008. The leaves and aerial parts of plants were cleaned, shade dried and pulverized.

Preparation of extracts

Pulverized plant materials (80 gm) were dispersed in 1000 ml boiled distilled water and cooled at room temperature in a closed glass container. The content was filtered using double layered muslin cloth, followed by filtration through Whatman's filter paper No-1 to remove solid particles. Filtrate was concentrated in the rotary vacuum evaporator (MAC Rotary Vacuum Evaporator, BUCHI Type; MSW-191) at 60 ^{0}C and low pressure and was completely dried at 60 ^{0}C temperature in hot air oven. The recovery was measured by weighing the dried extract. The extracts were kept in airtight container and preserved in a refrigerator for further use.

Screening of phytochemicals

The total antioxidant capacity of plant extracts was evaluated[8] , Fe^{2+} concentration was measured using an atomic absorption spectrophotometer (ECIL-Elements AAS 4141) after digesting 1 gm samples with 10 ml tri acids (Nitric acid: sulphuric acid: perchloric acid; 9:2:1).

Experimental animals

Thirty adult Wistar rats of either sex weighing 100-125 gm procured from a registered laboratory animal breeder were housed in standard condition, acclimatized, de-wormed with praziplus @ 7.5 mg /kg b wt and maintained for 15 days with standard feed as per AOAC[9] and clean drinking water ad-libitum under natural temperature and photoperiod.

Induction of haemlytic anaemia

Haemolytic anaemia was induced in 24 rats by administering single intra-peritoneal (i/p) dose of phenylhydrazine (PHZ) @ 90 mg kg^{-1} body weight (b wt) and 6 rats received distilled water as placebo. Rats containing total Hb% of 6-8 gm/dl were considered as anaemic and used for the experiment[10].

Experimental protocol

The normal rats served as negative control (NC), which received equal volume of distilled water instead of extract. The anaemic rats were divided into 4 groups randomly on the basis of body weight and the anaemic rats received orally extracts for 30 days as follows:

Positive control (PC): the anaemic rats received equal volume of distilled water.

Crude leaf treated (Clt): received crude leaf powder @ equivalent to 40 mg of extract kg^{-1} b wt

Pre flowering extract treated (PFt): received pre-flowering extract @ 40 mg kg^{-1} b wt

Flowering extract treated (Flt): received flowering extract @ 40 mg kg^{-1} b wt

All the doses were calculated on the basis of previously estimated ED_{50} of pre-flowering extract on induced anaemic rats.

The research was conducted in accordance with the internationally accepted principles for laboratory animal use and care and the experimental protocol was approved by institutional animal ethical committee Vide No. 445/CPCSEA/2008/04 on 05.07.2008.

Haematological study

Blood samples were collected from retro-orbital vein by inducing mild anesthesia using diethyl ether.

1.5 ml of blood was collected in two fresh and sterilized tubes containing heparin (0.2 mg ml^{-1} of blood) as an anti-coagulant or without anticoagulant and the following parameters was estimated to detect the haematinic properties of the extract. Total erythrocyte count (TEC), haematocrit (Hct) percentage (%), mean corpuscular volume (MCV), mean corpuscular haemoglobin calculated from membrane bound haemoglobin (rMCH), mean corpuscular haemoglobin concentration calculated from membrane bound haemoglobin (rMCHC) were measured using the method stated by Jain[11]. Total haemoglobin (Hbt)[12] and membrane bound haemoglobin (rHb) were considered in order to evaluate the concentration of physiologically active haemoglobin and free haemoglobin (fHb). To evaluate rHb, RBCs were separated from 0.25ml of blood by centrifuging the blood at 2,500 rpm for 30 minutes at 25 ^{0}C using REMI centrifuge machine (C24). Supernatant was discarded and RBCs were washed with phosphate buffer saline solution and centrifuged again. Same procedure was followed thrice and rHb was estimated[12]. rHb concentration in blood was calculated as follows:

rHb = (Hb estimated in RBC x %Hct /100) gm dl^{-1}.

fHb: fHb was considered in this experiment as it react with hydrogen peroxide to form a highly reactive ferryl haem species, that also damage lipids of RBC membrane and causes further haemolysis and decreases membrane bound Hb concentration. fHb was calculated from the difference of tHb and rHb.

fHb= (tHb-rHb) gm dl^{-1}.

Micro elements

0.1 ml serum sample from each animal was digested using 5ml tri acid (Nitric acid: sulphuric acid: perchloric acid- 9:2:1) to estimate copper (Cu), cobalt (Co) and iron (Fe) using an atomic absorption spectrophotometer (ECIL-Elements AAS 4141) to detect the iron concentration and concentration of micro element aid in haemopoisis.

Biochemical constituents to determine the availability of iron for reticulosys

Total serum iron binding capacity (TIBC) [13], per cent transferrin saturation (%TS; calculated from serum Fe and TIBC) were measured in serum on 0 day and 30th day of experiment for detection of iron available to haempoitic system. RBC lipid per oxidation (LPO)[14] was measured in blood.

Statistical analysis

Statistical analysis was done by using complete randomized block design (CRD)-single factor analysis of variance of the effect within and between the treatment groups at 5% level[15]. The results were expressed as Mean±SE.

Results

Phytological screening

Phytological screening revealed antioxidant concentration in PF extract and Fl extract as 317.5 and 283.33 µmole of ascorbic acid equivalent mg^{-1} of extract, respectively; Fe concentrations were 0.41, 0.43 and 0.31 µg mg^{-1} of Cl, PF and Fl, respectively. The observed Fe concentration in PF extract, Fl extract and Cl was much lower compared to methanolic extract[16].

Haematological study

Induction of anaemia significantly reduced TEC, tHb, Hct%, and rMCHC (Table 1) rHb (Fig. 1), Cu concentration and Co concentration; increased fHb (Fig. 2) in rats (PC, Clt, PFt and Flt) compared to NC.

Treatment for 15 days with PF, Fl and Cl normalized the TEC; PC showed a significant lower count compared to NC (Table 1). rHb and fHb concentrations were found normal in Flt, PFt, Clt and PC (Fig. 1, Fig. 2). On the same day of experiment a non significant lower HCt% was found in PC alone amongst all the groups. rMCH value increased significantly compared to 0day in the rats either received treatment (Clt, PFt and Flt) or kept for natural recovery (PC) and the value obtained were statistically similar to NC. The highest value rMCH was observed in PFt amongst the groups. All most similar trend was observed in rMCHC % and was similar to the mean value observed in NC except the rats received treatment with Fl.

Further increase in tHb was observed on 30th day of experiment in all the anaemic rats either received treatment (Clt, PFt, and Flt) or not (PC) and the concentrations were found higher than NC. A higher rHb concentration compared to NC was observed in PFt, Flt and Clt; PC showed lowest concentration (Fig. 1). On the same day of experiment a significant increase in fHb compared to 15th day of experiment was observed in Flt, Clt and PC, except PFt (Fig. 2). The fHb concentration found in rats treated with PFt was similar to that of NC and all other groups (Pc, Clt and Flt) showed significant higher

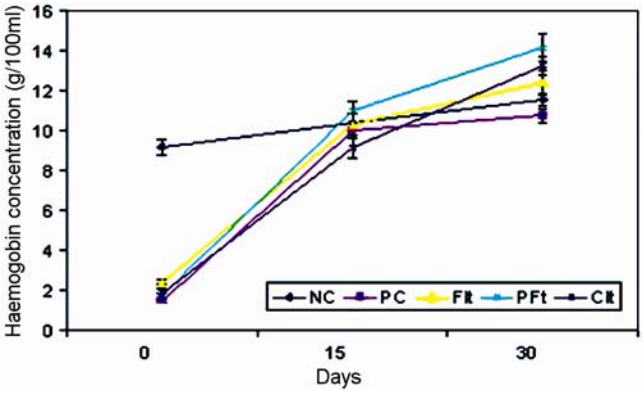

Fig. 1—Showing RBC bound Hb concentration in blood of different groups on different days of experiment (mean ± SE)

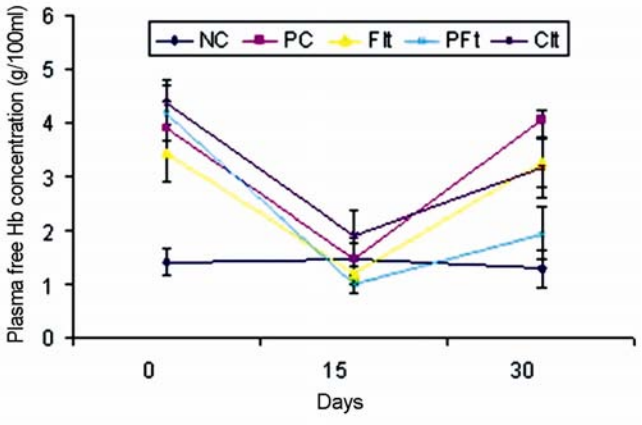

Fig. 2—Showing plasma free Hb concentration of different groups on different days of experiment (mean ± SE)

Table 1—Haematological parameters of different groups on different days of treatment [Values are mean of determinations ±SEM (Standard error of the mean)]

Haemato-logical parameters	Days of treatment	NC	PC	Clt	PFt	Flt
Hbt (g/100ml)	0 day	10.56 ± 0.45^{bp}	5.38 ± 0.48^{ap}	5.88 ± 0.42^{ap}	5.96 ± 0.42^{ap}	6.27 ± 0.33^{ap}
	15 day	11.87 ± 0.19^{q}	11.45 ± 0.30^{q}	11.47 ± 0.22^{q}	11.94 ± 0.47^{q}	11.04 ± 0.32^{q}
	30 day	12.84 ± 0.24^{ar}	14.77 ± 0.49^{br}	15.64 ± 0.49^{br}	16.07 ± 0.98^{br}	16.40 ± 0.43^{br}
TEC	0day	$7.27\pm0.62^{b\,p}$	3.05 ± 0.24^{ap}	3.78 ± 0.21^{ap}	3.55 ± 0.46^{p}	3.97 ± 0.32^{p}
($x10^6$ cu.mm)	15 day	$6.86\pm0.23^{b\,q}$	5.45 ± 0.35^{aq}	6.30 ± 0.21^{abq}	5.73 ± 0.48^{abq}	6.23 ± 0.54^{abq}
	30 day	7.09 ± 0.60^{r}	6.69 ± 0.45^{q}	6.56 ± 0.38^{q}	6.68 ± 0.39^{q}	6.68 ± 0.44^{q}
Hct (%)	0day	39.00 ± 1.32^{p}	21.50 ± 0.76^{ap}	24.83 ± 1.22^{ap}	23.33 ± 1.17^{ap}	22.83 ± 1.17^{ap}
	15 day	44.67 ± 0.88^{abq}	42.83 ± 0.83^{aq}	47.17 ± 0.40^{abr}	47.00 ± 1.59^{abq}	47.17 ± 1.01^{abq}
	30 day	45.00 ± 1.00^{cq}	41.17 ± 1.08^{aq}	43.33 ± 0.92^{abq}	44.83 ± 1.05^{bcq}	44.00 ± 0.68^{abcq}
MCV (fl)	0 day	55.92±5.71	72.41±5.71	65.99 ± 1.99^{p}	70.68±7.89	58.54 ± 2.91^{p}
	15 day	65.56±2.85	81.23±8.41	75.26 ± 2.61^{q}	87.29±12.61	78.63 ± 6.63^{q}
	30 day	65.97±6.28	63.34±5.48	67.08 ± 3.86^{pq}	67.83±2.46	67.22 ± 4.25^{pq}
MCHr (pf)	0day	13.10 ± 1.35^{bp}	4.92 ± 0.44^{ap}	6.55 ± 0.46^{ap}	5.75 ± 1.17^{ap}	4.83 ± 0.57^{ap}
	15 day	15.24 ± 0.79^{abpq}	18.74 ± 1.42^{abq}	16.39 ± 0.98^{abq}	20.22 ± 2.82^{bq}	15.02 ± 1.15^{aq}
	30 day	16.92 ± 0.77^{aq}	20.25 ± 1.86^{abq}	19.81 ± 1.36^{ar}	25.57 ± 2.44^{bq}	21.99 ± 1.85^{abr}
MCHCr (%)	0day	23.54 ± 0.95^{c}	6.90 ± 0.58^{ap}	9.94 ± 0.69^{bp}	$7.76\pm0.80^{ab\,p}$	$8.29\pm0.90^{ab\,p}$
	15 day	23.39 ± 1.26^{b}	23.42 ± 1.18^{bq}	21.81 ± 1.20^{abq}	23.25 ± 0.82^{bq}	19.39 ± 1.19^{aq}
	30 day	25.67 ± 0.75^{a}	26.17 ± 1.11^{abq}	28.65 ± 1.53^{bcr}	31.42 ± 1.09^{cr}	30.07 ± 0.80^{bcr}

Superscript a< b<c<d showing difference row wise and p<q<r showing difference column wise at P<0.05 significantly different

concentrations. No significant difference in TEC was observed between the groups on 30th day of experiment. Statistically similar Hct% was observed in rats those received treatment with extracts (PFt and Flt) and NC. A lower Hct% compared to NC was observed in Clt and PC. Statistically similar rMCH value was observed in rats of all the groups except PFt, which showed a higher value compared to other groups on 30th day of experiment. Higher than normal (NC) rMCHC % were observed in all the treated groups and highest value was observed in rats treated with PFt on the same day of experiment.

Microelements

Induction of anaemia decreased serum Co and Cu concentration and an increased serum Fe concentration (Table 2). On 15th day, treatment improved the serum Co concentration, however, were lower than normal except in the rats treated with Pf

Table 2—Micro element in serum of different groups on different days of treatment [Values are mean of determinations ±SEM (Standard error of the mean)]

Micro elements	Days of treatment	NC	PC	Clt	PFt	Flt
Co ppm	0 day	81.85 ±6.51^{b}	31.55 ±3.36^{ap}	29.32 ±0.72^{ap}	30.27 ±0.38^{ap}	31.72 ±2.74^{ap}
	15 day	80.25 ±5.58^{d}	42.98 ±2.13^{aq}	67.20±1.13 ^{bcr}	74.83±1.46 ^{cdr}	59.74±2.60 ^{bq}
	30 day	86.48±4.86 ^{c}	50.19±0.87 ^{ar}	67.80±4.49 ^{bq}	61.50±0.97 ^{bq}	67.80±4.49 ^{bq}
Cu ppm	0 day	6.64±0.70 ^{b}	2.27±0.29 ^{ap}	2.34±0.29 ^{ap}	2.79±0.40 ^{ap}	3.02±0.32 ^{ap}
	15 day	7.13±0.50 ^{b}	4.93±0.49 ^{aq}	6.74±0.82 ^{bq}	9.30±0.40 ^{cq}	10.03±0.75 ^{cq}
	30 day	7.20±0.50 ^{a}	5.90±0.61 ^{aq}	10.02±0.61 ^{br}	12.00±0.73 ^{cr}	13.32±0.72 ^{cr}
Fe ppm	0 day	44.69±1.22 ^{a}	102.88±3.44 ^{bq}	101.49±2.27 ^{bq}	102.02±3.53 ^{bq}	101.02±2.07 ^{br}
	15 day	45.85±1.03 ^{a}	95.34±1.71 ^{bq}	44.42±1.10 ^{ap}	47.70±0.92 ^{ap}	47.03±0.72 ^{ap}
	30 day	46.25±0.99 ^{a}	58.19±2.35 ^{cp}	49.42±1.95 ^{abp}	47.87±1.76 ^{abp}	52.52±1.36 ^{bq}

Superscript a< b<c<d showing difference row wise and p<q<r showing difference column wise at P<0.05 significantly different

extract. A lower serum Co concentration was observed on 15th day of experiment in PC though the concentration improved non-significantly compared to 0 days. Treatment for 15 days with extracts (PF and Fl) increased the serum Cu concentration and found higher compared to PC as well as Clt and was even higher than NC. On the same day of experiment serum Fe concentration decreased to normal in response to the treatment and found lower than PC and similar to NC.

On 30th day of experiment rats of treated groups still carried a lower serum Co concentration compared to that of NC, but were higher than PC. Serum Cu concentrations were increased in response to treatment and higher concentrations were observed compared to PC and even NC. Serum Fe concentrations were decreased back to the normal level in rats of Clt and PFt; rats of Flt and PC showed higher and highest concentration respectively.

Biochemicals

On 0 day of experiment increased TIBC (Table 3) values were observed in rats (PC, Clt, PFt and Flt) compared to NC. On the same day the result of %TS was not in a conclusive manner.

On 15th day of treatment serum TIBC values decreased significantly compared to 0day in PCt, Clt, PFt and Flt. The concentrations were maintained in all the treated rats (Clt, PFt and Flt) on 30th day, however, increased abruptly in PC. No definite trend was observed in serum %TS throughout the experimental period; however, treated groups (Clt, PFt and Flt) maintained the concentration on

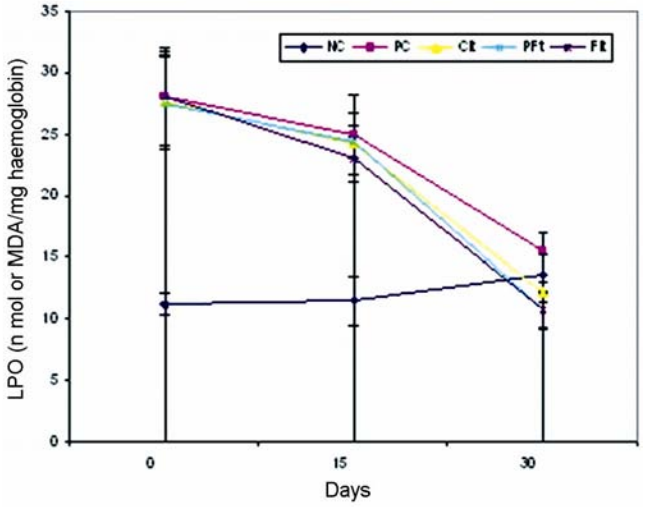

Fig. 3—Showing lipidperoxidation in RBC of different groups on different days of experiment (mean ± SE)

30th day as the concentration observed on 15th day of treatment but a significant lower %TS was observed in rats of PC compared to 15th day of experiment as well as to NC. The %TS saturation observed in anaemic rats those received treatment was significantly higher than NC also.

Induction of anaemia increased LPO activity (Fig. 3) in RBC on 0 day of experiment followed by a decreasing trend was observed in all the anaemic rats. LPO activity in haemolysate was reduced back to normal in rats treated with PF extract, other treatment groups (Clt and Flt)) on 30th day of experiment and were similar to NC, however, the values were higher. LPO in rats of PC was still higher than NC at the end of experiment.

Table 3—TIBC and %TS element in serum of different groups on different days of treatment [Values are mean of determinations ±SEM (Standard error of the mean)]

Biochemical parameters	Days of treatment	NC	PC	Clt	PFt	Flt
TIBC (µg/dl)	0 day	790.00±52.54 ^{a}	1675.00±187.86^{bq}	1098.33±96.62^{aq}	1618.33±147.84^{bq}	1091.67±195.11^{aq}
	15 day	753.33±129.27^{c}	645.00±98.82 ^{bcp}	418.33±31.56 ^{abp}	480.00±32.15 ^{abp}	375.00±26.55 ^{ap}
	30 day	670.56±102.08^{c}	1241.67±115.77^{dr}	483.33±46.67 ^{abp}	386.67±32.52 ^{ap}	444.94±85.38 ^{abp}
%transferrin	0day	5.77±0.39 ^{ap}	6.56±0.83 ^{a}	10.16±0.48 ^{b}	6.69±0.56 ^{ap}	10.98±1.95 ^{b}
	15 day	7.34±1.55 ^{aq}	16.79±2.86 ^{c}	10.95±0.34 ^{ab}	11.57±0.66 ^{abq}	12.91±1.06 ^{b}
	30 day	7.90±1.35 ^{ap}	4.86±0.42 ^{a}	11.43±0.62^{b}	11.31±1.32^{bq}	13.37 ±1.72^{b}

Superscript a< b<c<d showing difference row wise and p<q<r showing difference column wise at P<0.05 significantly different

Discussion

Increased haemolysis and lipid peroxidation on 0 day of experiment were due to PHZ induced oxidative stress on the red cell membrane[17,18,19,20]. PHZ and other oxygen generating systems cause elevation of LPO level (Fig. 3) in RBC[21]. PHZ in presence of Hb auto oxidized, which leads to haemolysis, resulting in severe haemolytic anaemia might be responsible for all the haematological changes (Table 1) on 0day of experiment in all the anaemic rats. Before onset of treatment serum Fe concentration (Table 2) increased due to leakage of Fe in diffusible form as a consequence of heam oxidation in RBC exposed to variety of oxidizing agents like PHZ[22,23].

Lowering of the circulating Hb concentration stimulates bone marrow to an increased erythropoietic effort resulted in increased TIBC (Table 3) and decreased serum Co and Cu concentration (Table 2) on the same day of treatment.

fHb (Fig. 2) react with hydrogen peroxide to form a highly reactive feryl heam species also damaged lipids of RBC membrane[24] causes further haemolysis and decreased circulating Hb concentration, that stimulated bone marrow to an increased erythropoietic effort with an average cell life of 15 days[25] is responsible for further higher haemolysis (Fig. 2) in Pc and moderate haemolysis in Clt and PFt, and lowest in PFt on 30th day of experiment. A free radical containing one or more unpaired electron[26] induced oxidative stress and increased LPO level[17,18,19,20]. Presence of Fe, betulin[27], antioxidant, Cu, Co[28] and other phytological component like phenolic compounds[29] lupeol and terpenoids[29], might be responsible for ameliorating property of *A. longifolia*. But moderate concentration of antioxidant and Fe present in PF extract might cause better amelioration of haemolytic anaemia.

Treatment with Cl, PF and Fl ameliorate the PHZ induced haemolytic anaemia in rats and better amelioration was observed in PHZ induced haemolytic rats received treatment with PF extract. Amelioration was due to presence of Fe, Cu, Co, antioxidant, phenolic compounds, lupeol and terpenoids in *A. longifolia*, presence of higher concentration of Fe and antioxidant are responsible for better ameliorating property of PF extract of *A. longifolia*.

Traditional significance

1 Tradiotionally plant is used indiscriminately for the preparation of leaf extract to treat anaemia in rural areas. Our observations suggest that the haematinic property of the plant is better in pre-flowering stages than the flowering stages.

2 With the present experiment though there is non significant difference in all the treatment groups in terms of haematological parameters (TEC, tHb, Hct%, and rMCHC), but the RBC bound haemoglobin concentration (rHb) was higher and lipid peroxidation (LPO) of RBC and free haemoglobin (fHb) concentration was lower in the animals treated with preflowering extract showing the lower haemolysis. So, it could be conclude that the preflowring *Astarcantha longifolia* extract shows better haematinic property compared to flowering plant extract and crude leaf extract.

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