Applied Biological Research 15 (1): 62-65; 2013 **Short Communication**

HAEMATINIC EFFECT OF *Hygrophila spinosa* ON INDUCED ANAEMIC RATS

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ABSTRACT

The present study was conducted to assess the haematinic property of *Hygrophila spinosa* extract in induced anaemic albino rats with phenylhydrazine. The haematinic activity of orally administered *H. spinosa* extract (pre-flowering extract @ 40 mg kg^{-1} body weight) and powdered aerial part (@ 40 mg extract kg^{-1} body weight) was assessed by treating the induced anaemic rats for 30 days. Treatments ameliorate the anaemic condition by improving haematological parameters and RBC indices. On 30th day post-treatment, the rats treated with plant extract showed more improvement in anaemic condition and increase in body weight in comparison to the rats treated with crude leaf.

Key words: Anaemia, haematinic, *Hygrophila spinosa*, pre-flowering extract

INTRODUCTION

Anaemia is the most substantial disorder of blood characterized by decreased levels of circulating haemoglobin and tissue iron contents leads to several functional abnormalities with adverse health consequences (Pennix, *et al*., 2004). In rural parts of India, aqueous infusion of succulent part is used to cure anaemia. *H. spinosa* (Common name Talmakhana, Kullkhara, Family: Acanthaceae) is a wild herb widely used in 'Ayurveda' as 'Rasayana' (Annonymous, 2002) for treatment of various disorders. This is a semi-woody herb inhabitant of damp and swampy area is found throughout the planes of India (Oudhia, 2003). Different parts of the *H. spinosa* are used in several patho-physiological conditions (Biswas and Ghosh, 2002; Kirtikar, and Basu, 2005) and ethanolic extract of the aerial part reportedly has positive effect on haemopoietic parameters (Dasgupta *et al*., 2001).This herbal remedy is devoid of any side effects with proven effectiveness thus popularity of this herb is increasing day by day. However, there is paucity of information about the effect of this herb on haematological profile. The present investigation was aimed to explore the haematological profile on experimental rodents with induced anaemia treated with aerial parts of *H. spinosa* extract.

MATERIALS AND METHODS

Twenty four adult 'Wistar' rats of either sex weighing 150±50 g were housed under standard conditions of temperature (35±2^{0}C) and natural photoperiod in cages and fed with standard feed and clean drinking water *ad-libitum*. Deworming was done by using praziplus @ 7.5 mg kg^{-1} body weight orally to individual rats. After 30 days acclimatization, anaemia was induced by giving single intra-peritoneal (i/p) dose of phenyl-hydrazine hydrochloride @ 90 mg kg^{-1} body weight in 18 rats. Another

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6 rats received in the form of distilled water. Anaemic rats with haemoglobin concentration between 6 to 8 gm% were divided into 3 groups. Group II (gr. II) rats (n = 6) were given equal volume of distilled water and served as anaemic control. Group III (gr. III) rats (n = 6) were given crude leaf (@ 40 mg plant extract kg^{-1} body weight) orally daily for 30 days and group IV (gr. IV) rats (n = 6) were given plant extract @ 40 mg kg^{-1} body weight orally daily for 30 days. To ensure accurate dosing the extract, crude leaf suspension or distilled water was placed below the pharynx with help of a specially made intra-gastric needle with maximum volume administered to each rat was 2 ml. Individual body weights of each rat was recorded at 0, 7, 15 and 30th day of experiment and body weight gain per 100 g calculated.

Blood samples were collected by retro-orbital venous plexus method inducing mild anesthesia with diethyl-ether either with heparin (0.2 mg ml^{-1} blood) or without any anti-coagulant. Red blood cell (RBC) count, Hb concentration, packed cell volume (PCV) and red blood corpuscle indices (MCV, MCH and MCHC) were determined (Jain, 1990) at days 0, 7, 15 and 30. Cobalt, copper, serum iron, total iron binding capacity (TIBC) and percent transferrin saturation were analyzed using kit Ferrozine method (Viollier E., 2009) supplied by Crest Biosystems, India. Data was statistical analyzed by using complete randomized block design-single factor analysis of variance to assess impact within and between the treatments groups at 5% level (Snedecor and Cochran, 1967).

RESULTS AND DISCUSSION

The RBC count, Hb gm%, PCV, cobalt and copper showed decrease on day 0 (3rd day after phenyl-hydrazine administration) and increase in MCH, MCV, TIBC, serum iron value; but insignificant change in MCHC value (Table 1). An increase in MCV due to highest increase from zero value in count of erythrocytes with heinz bodies and normoblasts, as well as a 60% increase in MCH on 3rd day after anaemia induction due to increase plasma haemoglobin is in conformity with Berger (2007). The osmotic fragility increased over 3 days after anaemia induction may possibly be due to the consequences of reticulosis (Berger 2007; Redondo *et al.*, 1995).

On 7th day, increase in Hb gm% from the base value (group I) approximated in anaemic rats untreated (gr. II), treated with crude leaf (gr. III) and treated with extract (gr. IV) were 68, 110 and 118%, respectively; whereas increase in RBC count were 6, 13 and 18%; respectively. Similarly increases in MCV from the base value were 81, 62 and 47%; increase in MCH 46, 69 and 84% and increase in MCHC value 6, 34, and 10%, respectively. Treated anaemic rats and untreated anaemic rats showed increase in PCV from 78 to 99% with a varied degree of RBC count which might be due to hypercellularity with erythroid hyperplasia; leucocytosis with neutrophilia and lymphocytosis (Berger, 2007). Rats of gr. III and IV showed significant decrease in serum iron concentration as compared to the normal rats. The group III and IV rats did not show any significant difference in TIBC concentration as compared to normal rats. On 15th day higher value of Hb, PCV, MCV was observed in rats of gr. II, III and IV in comparison to gr. I. Group II rats showed significantly higher MCH than gr. I rats whereas gr. III and IV rats showed lower MCHC, non-significant higher than rats of gr II. On 30th day group III and IV rats revealed more macrocytosis than group II rats. However, haematological parameters, TIBC and percentage transferrin saturation was maximum in group IV.

On 7th and 15th day of treatment the *Hygrophila* extract and crude leaf showed haematinic property, this might be due to haemopoetic stimulation (Dasgupta *et al.*, 2001). On 15th day of treatment gr.-III and IV rats had mean serum iron value similar to normal rats whereas anemic rats without treatment showed significant highest value after a significant decrease in level. Circulating iron concentration in gr. III and IV rats showed better circulating iron, TIBC and percent transferrin saturation indicating that the treatment with *Hygrophila spinosa* extract and crude leaf increases the bioavailability of iron from day 7 which was also evident by serum, copper and cobalt concentration. This indicates that treatment with *H. spinosa* extract lead to haemopoietic stimulation (Dasgupta *et al.*, 2001) by activating growth factor like cytokines along with elevated availability of iron in diet (Burkhard *et al.*, 2001). Phenyl-hydrazine treatment decreased body weight gain in Group II, III and

Table 1: Effect of *Hygrophila spinnosa* treatments on hematological parameters in rats (mean and SE)

Parameters	Day	Treatments				$CD_{0.05}$
		Normal (I)	Anaemic (II)	Crude leaf treated (III)	Extract treated (IV)	
RBC count (x 10^6 $Mm^{-3)}$	0	6.79±0.47[a]	3.06±0.24[a]	3.86±0.28[a]	3.97±0.42[a]	1.04
	7	6.98±0.40[a]	3.25±0.20[a]	4.36±0.15[a]	4.47±0.20[a]	0.79
	15	6.86±0.20[a]	6.54±0.57[b]	5.44±0.34[b]	6.86±0.44[b]	0.87
	30	6.54±0.60[a]	6.86±0.44[c]	6.58±0.40[b]	6.26±0.30[b]	1.29
Hb concentration (gm %)	0	10.09±0.22[a]	6.45±0.51[a]	6.97±0.47[a]	7.22±0.43[a]	1.26
	7	10.44±0.62[b]	10.85±0.76[b]	14.65±0.34[c]	15.78±0.67[c]	1.83
	15	10.15±0.29[a]	11.62±0.32[b]	11.55±0.21[b]	11.64±0.47[b]	1.00
	30	10.55±0.22[c]	10.85±0.76[b]	14.89±0.91[c]	15.97±0.93[c]	2.26
PCV (%)	0	41.00±1.31[a]	23.50±0.76[a]	26.83±1.20[a]	25.83±1.00[a]	3.24
	7	42.50±1.40[a]	45.67±0.80[b]	45.33±1.40[b]	46.33±0.50[b]	3.29
	15	42.67±0.88[a]	49.50±1.40[c]	49.67±0.61[c]	51.16±2.00[c]	3.97
	30	41.16±2.74[a]	45.00±1.30[b]	44.50±0.84[b]	46.00±1.00[b]	4.92
MCV (fl)	0	68.69±5.97[a]	75.22±9.13[a]	71.35±2.15[a]	69.25±3.54[a]	17.22
	7	67.28±3.82[a]	142.83±10.0[b]	104.69±6.00[b]	99.17±3.18[c]	18.80
	15	68.47±2.95[a]	85.93±2.73[a]	79.66±2.33[a]	80.48±4.33[b]	9.50
	30	66.16±3.63[a]	65.52±5.34[a]	70.00±4.00[a]	69.71±3.28[ab]	12.34
MCH (pg)	0	15.75±1.00[a]	23.40±2.00[a]	20.00±2.00[a]	18.35±3.54[a]	5.16
	7	14.99±0.70[a]	34.13±3.00[b]	33.87±2.00[b]	33.77±1.00[c]	6.10
	15	15.28±0.80[a]	21.81±2.00[a]	18.29±0.70[a]	18.00±1.00[a]	3.98
	30	15.56±1.00[a]	66.39±5.00[c]	22.97± 0.40[a]	24.00±1.00[b]	14.98
MCHC (%)	0	24.89±1.00[a]	25.35±2.00[a]	24.18±2.00[a]	30.98±2.00[b]	5.75
	7	24.71±2.05[a]	23.72±1.00[a]	32.45±1.00[b]	34.00±1.00[b]	3.76
	15	24.63±1.04[a]	23.50±0.47[a]	22.94±0.40[a]	22.40±0.50[a]	1.60
	30	25.57±2.03[a]	35.79±1.00[b]	32.99±2.00[b]	34.55±2.00[b]	5.66
TIBC (µg dl^{-1})	0	790.0±52.53[a]	1675±187.86[c]	1129.9±177.1[b]	1611.7±219[b]	505.35
	7	753.3±129.0[a]	1241.7±115.8[b]	663.9±76.46[a]	716.8±75.2[b]	277.35
	15	700.0±102.3[a]	1137±118.82[a]	479.5±83.99[a]	478.5±36.9[a]	248.79
	30	670.5±102.8[a]	1109.7±115.8[b]	413.3±24.72[a]	384.9±20.0[a]	260.87
Tranferrin saturation (%)	0	5.77±0.39[a]	6.56±0.83[a]	10.15±1.58[a]	6.86±1.14[a]	3.17
	7	7.42±1.44[a]	8.80±0.95[a]	13.49±1.71[a]	11.55±1.00[a]	4.29
	15	7.34±1.55[a]	16.79±2.86[b]	10.96±0.78[a]	9.89±0.62[a]	5.01
	30	7.90±1.35[ab]	4.86±0.42[a]	11.47±1.41[a]	11.29±1.12[a]	3.40
Serum iron (µg dl^{-1})	0	44.69±1.22[b]	102.88±3.44[bc]	101.49±2.26[c]	101.49±2.00[c]	8.20
	7	45.28±1.04[a]	104.04±2.87[c]	83.73±3.29[b]	76.86±2.79[b]	7.80
	15	45.85±1.03[a]	95.34±1.71[b]	44.42±1.00[a]	47.69±0.91[a]	3.63
	30	46.25±0.99[a]	58.19±2.35[a]	49.42±2.00[a]	47.86±1.75[a]	5.39
Copper (ppm)	0	6.69±0.70[a]	2.27±0.30[a]	2.33±0.29[a]	2.82±0.42[a]	1.35
	7	7.24±0.33[a]	5.19±0.62[b]	5.02±0.83[b]	5.84±0.36[b]	1.70
	15	7.13±0.49[a]	4.92±0.42[b]	6.73±0.43[b]	10.02±0.71[c]	1.60
	30	7.20±0.50[a]	5.89±0.61[b]	9.53±1.26[c]	12.68±0.62[c]	2.38
Cobalt (ppm)	0	81.84±6.51[a]	31.55±3.35[a]	28.87±3.00[a]	30.41±3.37[a]	12.71
	7	81.08±6.50[a]	39.00±2.45[b]	39.76±0.94[b]	40.68±0.53[b]	10.19
	15	80.24±5.57[a]	42.97±2.13[b]	68.14±4.60[d]	73.98±4.42[d]	12.92
	30	84.47±4.86[a]	50.13±0.87[c]	53.31±2.40[cd]	61.74±2.00[c]	8.73

The values in each column with same superscript do not differ from each other at $P_{0.05}$. CD is given for significant of mean values in rows at 5% level ($P_{0.05}$).

Table 2: Body weight gain (%) in rats by *Hygrophila spinnosa* treatment

Days after treatment	Groups				$CD_{0.05}$
	Normal (I)	Anaemic (II)	Crude leaf treated (III)	Extract treated (IV)	
0	8.33 ± 1.44^{a}	-7.55 ± 1.17^{a}	-5.97 ± 0.65^{a}	-6.96 ± 0.72^{a}	2.79
7	7.51 ± 0.48^{b}	2.54 ± 0.27^{b}	2.66 ± 0.59^{b}	3.52 ± 0.71^{b}	1.57
15	7.44 ± 0.82^{b}	4.92 ± 0.52^{c}	5.94 ± 0.81^{b}	6.56 ± 0.94^{b}	2.34
30	7.16 ± 1.46^{c}	6.73 ± 1.58^{c}	11.67 ± 2.42^{c}	15.76 ± 1.58^{c}	4.87

The values in each column with same superscript do not differ from each other at $P_{0.05}$. CD is given for significant of mean values in rows at 5% level ($P_{0.05}$).

IV (Table 2). On 7, 15 and 30th day gr. III and IV rats showed increased body weight; but significant value was observed in gr. III. The observations indicate that that treatment with *H. spinosa* extract and crude leaf powder showed almost similar effect in terms of haematinic property in induced anaemia. However, more study is needed on the mechanism of action in relation to haemopoiesis and on the active principle in the *H spinosa* involved in the activity.

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