Haematological effects of aqueous extract of Ornamental plants in male Swiss albino mice

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Abstract

Treatment of mice with crude extract of *Hibiscus rosa sinensis* flowers (500 mg/kg BW) and *Bougainvillea spectabilis* leaves (800 mg/kg BW) for a period of 30 days indicates a significant increase in the level of hemoglobin and count of RBC but a significant decline in the level of MCH and MCV in the former case. On the other hand, in *B. spectabilis* treated animals, the level of hemoglobin, RBC count & PCV declined significantly. Hence, it is concluded that the use of *H. rosa sinensis* whereas may not cause any adverse effect on animals, *B. spectabilis* is to be used with care as its chronic use may cause anemia.
Keywords: Crude extract, *H. rosa sinensis*, *B. spectabilis*, hemoglobin, MCH, MCV, PCV

Abbreviations: BW (Body Weight), Hb (Haemoglobin), MCV (Mean Corpuscular Volume), MCH (Mean Corpuscular haemoglobin), MCHC (Mean Corpuscular Haemoglobin Content), PCV (Packed Cell Volume), PLT(Platelets), RBC (Red Blood Cells), WBC (White Blood Cells)

Introduction

For thousands of years, people have looked to natural means of healing. In developing countries of the world, most the people depend on herbal medical care (Ekpe *et al*., 1990). The popularity of traditional medicine is due to the belief that some diseases only respond to traditional treatment (Bannerman *et al*., 1975). Extracts of root, stem, bark, leaves and flowers of some medicinal plants have been shown to have activities against most dreaded pathogenic organisms like the bacteria, fungi etc (Bannerman *et al*., 1975; Khan *et al*.,1980; Madunagu *et al*., 1990; Singh and pattak, 1994), while some others are cytotoxic (Russel *et al*., 1997; Prohp and Alaiya, 2003; Prohp and Maduduemezia, 2004; Prohp *et al*., 2004, Prohp *et al*., 2006a, Prohp *et al*., 2006b). Besides, ingestion of some plant materials (either in the raw form or their extracts) having useful medicinal properties may cause anaemia resulting from the sequestration of RBC in spleen, impaired red blood cell production or primary bone marrow dysfunction (Watt and Breyer, 1962; Cheeke, 1998).

In the present study, an attempt is made to study the therapeutic value of the crude extract of two most common locally available ornamental plants flourishing throughout the year, namely *Hibiscus rosa sinensis* and *Bougainvillea spectabilis*. Keeping in mind the report of Williamsons (2001), who has emphasized that the whole or partially purified extract of plant offer advantages over a single isolated ingredient.

In this investigation, we have sought to verify the effect of the administration of aqueous extract of *H. rosa sinensis* flowers and *B. spectabilis* leaves on few hematological parameters.

Materials and Methods

Animals: Adult male and female Swiss albino mice procured from CCS Haryana Agricultural University, Hisar were mated and resulting progeny was maintained in a well ventilated animal house with 12:12 light/dark cycle. After quarantine and acclimization period of 1 week mice were used for this study.

Test animals were kept in polyproline cages

with iron bar tops and maintained on standard pellet diet (Hindustan Levers). Tap water was made available *ad libitum.* As far as possible, necessary sterile conditions were provided and cleanliness was maintained in the animal cages as well as in the room. Prior approval for experiments was taken from Institutional Animal Ethics Committee as per CPCSEA (Govt. of India) norms.

Preparation of dose formulations: Extraction of aqueous extract of *H. rosa sinensis* and *B. spectabilis* the Flowers and leaves collected from Banasthali University campus were oven dried and powdered. The powdered material was extracted using aqueous by soxhlet method (Sharma and Grag, 2009). It was further dried, which was used for the experimentation.

Experimental Protocol: The animals were randomly selected into 3 groups and each group contained 12 animals each:

Ist Group: Treated orally with aqueous *H. rosa sinensis* flowers extract per day at the dose of 500mg/kg BW for 30 days.

IInd Group: Treated orally with aqueous *B. spectabilis* leaves extract per day at the dose of 800mg/kg BW 30 days.

IIIrd Group: Control group.

Blood was withdrawn from eye orbit at regular interval of 10th, 20th, 30th day and after withdrawal of the treatment for 60 days.

Collection of samples**:** For haematological parameter blood sample was collected from retro – orbital plexus using micro – capillary technique (Sorg and Buckner, 1964). Heparin was used as an anticoagulant.

Determination of Haematological Parameters: Blood was collected into EDTA-containing sample bottles on day 10th, 20th, 30th and 90th from experimental animals for hematological analysis as recommended by Malomo *et al*., (2002). RBC and WBC counting was done with the help of Neubaur's chamber. Packed cell Volume (PCV) with Wintrobe hematocrit tubes, Haemoglobin (Hb) by Sahli's method (Sharma, 2007). Other haematological indices were calculated with the aid of formulas.

MCV (Mean Corpuscular Volume) - The average volume of RBC. Expressed in femtoliters (cubic micrometers).

MCH (Mean Corpuscular haemoglobin) - The average content (mass) of Hb in a given volume of packed RBCs, expressed in picograms.

MCHC (Mean Corpuscular Haemoglobin Content) is the average concentration of Hb in a given volume of packed RBCs, expressed in grams per deciliter or in percent.

Statistical Analysis: Results are expressed as mean ± Standard Deviation (S.D.). Statistical significance between the different groups was determined by one way Analysis of Variance (ANOVA) using the SPSS (Ver. 16). Post hoc testing was performed for inter-group comparisons using the Tukey multiple comparison test at $P<0.05$. Whenever sphericity was significant, degree of freedom and F-value are corrected by Huynh Feldt epsilon.

Results

Effect on Haematological parameters: The effect of aqueous extract of *H. rosa sinensis* flowers and *B. spectabilis* leaves on Swiss albino mice on day 10th, 20th and 30th along with a recovery period are presented in Table 1. Administration of *H. rosa sinensis* aqueous extract produced significant increase ($P<0.05$) in the count of the RBC other along with Hb content and decline in Platelet count however, no effect could be observed on MCHC level. On the contrary in case of *B.spectabilis* treated animals, the count of RBC declined significantly along with Hb level whereas increase in WBC. However the level of PCV, MCH, MCHC and platelet count remained unaffected.

Discussion

The administration of any chemical compound for long duration may bring about significant changes in the structure, function, metabolic transformation and concentration of biomedical enzymes and even metabolic pathways. These alterations may be rapid or slow and may lead to different biochemical mechanisms, producing a pathological state (Murray *et al*., 2000).

Table-1. Effect of aqueous extract of *H. rosa sinensis* flower and *B. spectabilis* leaves on some (a) haematological parameters and (b) haematological Indices in male mice.

(a) Haematological Parameters

Parameters	Treatment	10 days	20days	30days	90days
WBC(x103 µl)	Control	20.13±0.110	20.3±0.374	20.28±0.291	20.15±0.135
	H.rosa sinensis	19.97±0.309	19.8±0.676	19.63±0.727	20.47±0.782
	B.spectabilis	21.6±1.365*	22.77±1.126*	23.27±0.228*	22.6±1.537*
RBC (x106µl)	Control	9.38±0.31	9.43±0.27	9.33±0.34	9.41±0.28
	H.rosa sinensis	9.96±0.47*	10.01±0.14	10.18±0.39*	10.09±0.38*
	B.spectabilis	9.28±0.36	9.23±0.41	9.26±0.34	9.36±0.31
Hb(g/dl)	Control	13.42±0.375	13.18±0.563	13.35±0.369	13.28±0.480
	H.rosa sinensis	13.53±0.984	13.87±0.634	14.2±0.216	13.83±0.541
	B.spectabilis	12.32±0.315*	12.33±0.589	10.72±0.978*	12.16±0.637*
PCV (%)	Control	47.20±1.330	47.03±0.970	47.04±1.497	46.87±1.730
	H.rosa sinensis	46.77±0.552	47.93±1.659	48.27±3.369	46.43±1.019
	B.spectabilis	42.24±1.394*	40.49±3.561*	39.66±2.761*	42.32±1.312*
PLC(µl)	Control	1253.17±41.862	1254.83±40.321	1254.8±39.486	1254.3±44.187
	H.rosa sinensis	905.67±41.624*	879±17.426*	855.66±10.609*	870±22.211*
	B.spectabilis	1251.5±43.668	1249.67±45.923	1236.5±35.780	1246.5±44.761

(b) Haematological Indices

Parameters	Treatment	10 days	20days	30days	90days
MCH(pg)	Control	14.32±0.45	13.99±0.62	14.33±0.46	14.13±0.56
	H.rosa sinensis	13.32±0.48	13.48±1.05	11.56±0.86*	13.01±0.42*
	B.spectabilis	13.62±1.33	13.89±0.92	14.05±0.33	13.84±0.92
MCHC(g/dl)	Control	28.44±0.89	28.01±1.07	28.41±1.02	28.37±1.09
	H.rosa sinensis	29.22±1.55	30.66±2.84	27.14±2.89	28.76±1.68
	B.spectabilis	28.77±2.07	28.95±1.40	29.62±2.00	29.82±1.55
MCV(fl)	Control	50.36±1.96	49.91±1.63	50.48±2.47	49.82±2.08
	H.rosa sinensis	45.61±2.72*	43.86±3.42*	42.85±2.80*	45.31±1.93*
	B.spectabilis	47.05±1.83	47.95±1.96	47.46±3.22	46.12±2.28*

The values are expressed as mean ± SD for 10 animals (n=10) per group.
Treatment was discontinued after 30 days, and animals were sacrificed 60 days after withdrawal of treatment.
* indicates statistically significant with Control at $p < 0.05$.

In the present study, aqueous extract of *H. rosa sinensis* and *B. spectabilis* has investigated on hematological parameters on day 10, 20 and 30 of treatment. The results obtained are summarized in table 1a & b. The results indicate, that the treatment with *H. rosa sinensis* for 30 days significantly increases the count of RBC and WBC from day 10 onward but causes a significant decline in the count of blood platelets. Besides, through Hb, PCV, MCHC remain unaffected, MCH decreased significantly.

However, in the case of *B. spectabilis* extract treated animals for the same duration, though WBC count, MCH, MCHC, MCV and PLT remain unaffected, a significant decline in RBC and PCV is indicated.

Actually, assessments of haematological parameters are used to determine the extent of deleterious effect of the extracts on blood of an animal. Straus, (1998), Adedapo *et al*., (2007), Onyeyilli *et al*., (1998) reported, that reduction in RBC, Hb and PCV is an indication of either the destruction of RBC or their decreased production, which may lead to anemia.

On the contrary an increase in the count of RBC, Hb and PCV is suggestive of polycythemia and positive erythopoisis (Iranloye, 2002; Mansi and Lahham, 2008; Kuppast *et al*., 2009; Okpuzor *et al*., 2009). Hence, a significant increase in RBC with no alteration in Hb and PCV in *H. rosa sinensisi* treated animals indicates that the extract causes no toxic effect on RBC. However, a significant reduction of RBC and Hb from day 10 in *B. spectabilis* treated animals is suggestive of anaemia.

Reports about WBC counts have pointed out that whereas increased count of WBC is supposed to be helpful in boosting immune system (Adedapo *et al*., 2007; Mohajeri, 2007), a decreased count of WBC shows the suppression of leucocytes and their production from bone marrow (Odesanmi *et al*., 2010; Jimoh *et al*., 2008; Osuigwe *et al*., 2007; Adedapo *et al*., 2004).

Therefore, an increased count of WBC in *B.*

spectabilis treated animals, as observed in the present study, suggests that *B. spectabilis* might be having a good potentiality to boost immune system. However, this report in not agreement with Adebayo *et al*., (2005), where they have reported that ethanolic extract of *B. spectabilis*, even at the dose of 200mg/kg, decreases the count of WBC.

Blatti, (1997), Adedapo *et al*., (2008), Adeniyi *et al*., (2010)have pointed out, that reduced blood platelets affect the viscosity of blood, which is corelated positively to blood pressure. As shown in the table, *H. rosa sinensis* treatment for 30 days adversely affects the count of blood platelets which may produce negative effect on the viscosity of blood. Probably the duration of the treatment may prove toxic to animals.

The hematological parameters like MCH, MCHC and MCV are related to individual RBC, while Hb and PCV are associated with total population of RBCs. Therefore, if MCH, MCHC and MCV are not affected by the treatment with extracts, it means that neither the incorporation of Hb into RBC, nor the morphology and osmotic fragile of RBCs is altered (Adebayo *et al*., 2005; Ashafa *et al*., 2009).

It is thereby concluded, that both the extracts are selectively toxic to hematological parameters. Chronic use of the extracts is not advisable, despite of their popularity having antidiabetic and antilipidemic properties.

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Conflict of interest

Authors declare that they have no conflict of interest.

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