CHAPTER III

Influence of Nag bhasma and Lead nitrate on blood haemoglobin content and its distribution in erythrocytes.

Toxic effects of lead on the hemopoietic system were studied and reviewed extensively (de Bruin, 1971; Arena, 1974; Skerfring, 1987). Anaemia is not of serious consequence of lead toxicity and can be reversed. The toxic effects of lead on hemopoiesis are (1) abnormal circulating erythrocyte (2) impairment of the production of haemoglobin, owing to lead activity in the bone marrow and (3) stimulation of erythropoiesis in the bone marrow. Lomola and Yamna (1974) have suggested a screening test based upon measurement of blood fluorescence from the zinc protoporphyrin in erythrocytes of non-anaemic subjects.

Lead binds readily to SH^-, PO_4^{3-} and $COOH^-$ containing ligands of the erythrocyte membrane to increase the mechanical pressure, but decreased osmotic fragility of erythrocytes. In *in vitro* human erythrocytes exhibit osmotic fragility on exposure to less than 200 μg lead/100 ml with even exceeding 900 μg/ 100 ml the cells develop a

mild resistant to lysis (Lesser and Walters, 1973; Lavender *et al*, 1975). Lead alters membrane permeability (Bhishop and Surgerer, 1964) and binds to active sites involved in transport permeability and inhibits active transport by blocking Na^+ - K^+ transporter ATPase. Lead seems to liberate normally inactive carrier molecules (Pass *et al*, 1961). Although the general metabolism within the erythrocyte cells is not very much disturbed, the cells shrink from efflux of K^+ and water and their survival is shortened. Lead initiates stimulation of erythropoiesis in bone marrow to increase the production of RBCs, basophilic cells with nuclear abnormal and inadequate haemoglobin (de Bruin, 1971) and enhances haem synthesis too. Iron metabolism is disturbed in lead deficiency (Kerchgessuer and Reichlmayo, 1981). Oral dose of 44 mg/dl per kg body weight of rat was given for 9, 15 and 30 days. Nine days after of treatment few RBCs showed spiny surface. Treatment for 15 days showed appearance of irregular shaped RBCs in addition to spiny surfaces those appeared earlier. Thirty days treatment resulted in the appearance of spherocytes and crenate shaped RBCs with numerous spiny processes (Karmarkar *et al* 1990).

The data shows that RBCs and their synthesis are affected primarily. In present project also to test and compare the toxicity of lead

the alterations in haemoglobin content and its cytological localisation in erythrocytes was studied.

MATERIAL AND METHODS

The experimental schedule was as described in Chapter II (Material and Methods). The rats were killed at specified times and blood samples were aspirated and used for the assay of haemoglobin and cytological preparations. The methods used for assay and cytological staining are described in detail in Chapter II.

RESULTS

The data on the variations in haemoglobin contents are presented in Table 1 and Figure 1.

Seven animals each from five groups of EXPERIMENTAL ANIMALS were sacrificed on day 8, 15 and 22nd day, which received the treatment of lead nitrate/different doses of Nag bhasma for 7, 14 and 21 days. The normal rats of Group I showed 14.54 ± 0.78, 14.57 ± 0.81 and 14.38 ± 0.79 g hemoglobin/100 ml of blood.

Lead nitrate (20 mg/kg body wt) treated rats :

The rats that received 20 mg $Pb(NO_3)_2$/kg body weight daily for 7, 14 and 21 days (Group II) showed 19.87, 28.69 and % 16.41

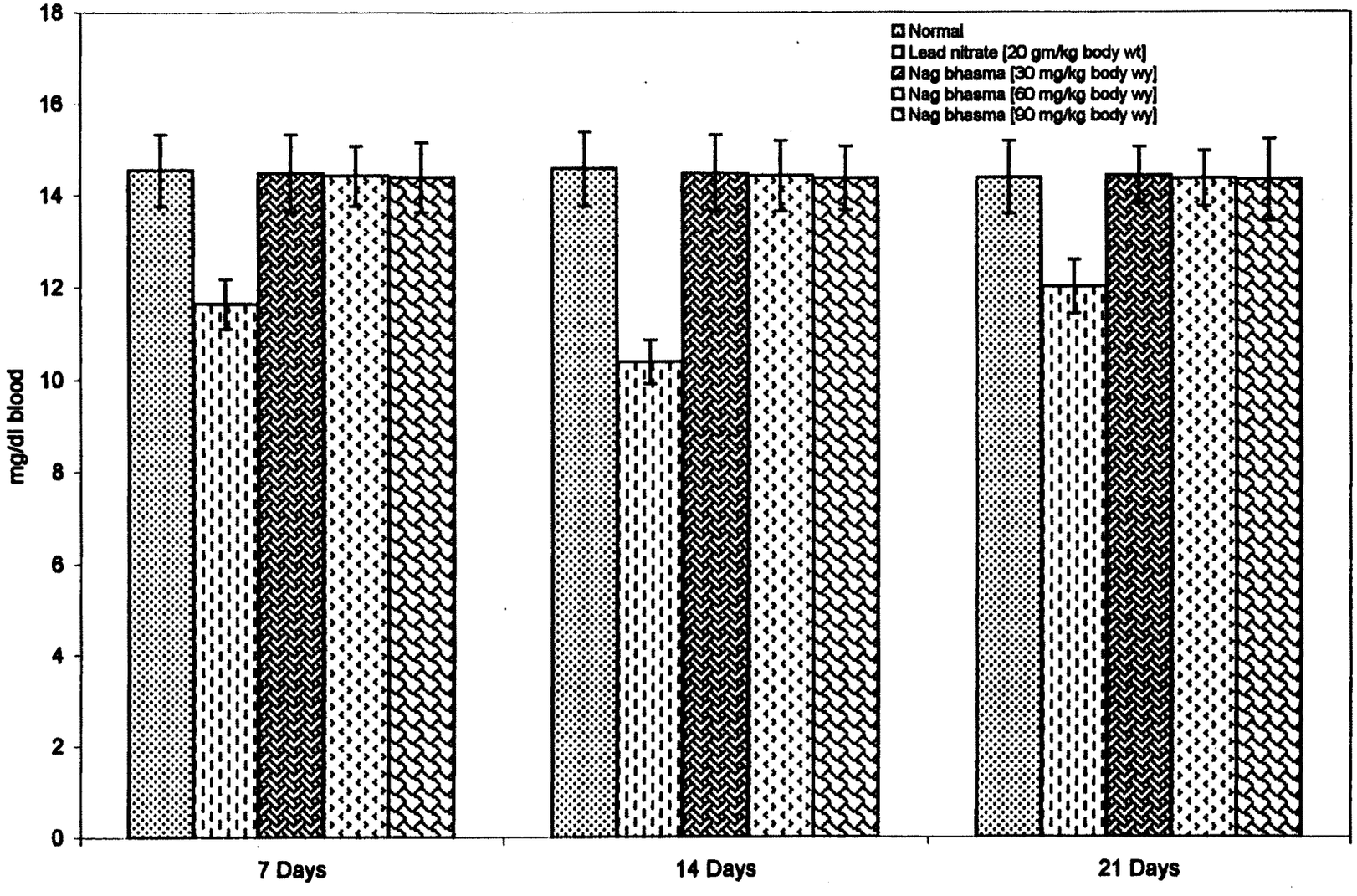
Table 1:- Haemoglobin content of rat blood after the treatments of different doses of Nag bhasma to male albino rats.

Values expressed as mg/dl serum
Values are mean ± SE of 7 animals

Group	Duration of treatment		
	7 Days	14 Days	21 Days
Normal	14.54 ± 0.78	14.57 ± 0.81	14.38 ± 0.79
Lead nitrate [20 mg/kg body wt]	11.65 ± 0.54[b]	10.39 ± 0.47[c]	12.02 ± 0.58[a]
Nag bhasma [30 mg/kg body wt]	14.48 ± 0.84[d]	14.48 ± 0.83[d]	14.43 ± 0.62[d]
Nag bhasma [60 mg/kg body wt]	14.42 ± 0.65[d]	14.42 ± 0.77[d]	14.36 ± 0.60[d]
Nag bhasma [90 mg/kg body wt]	14.39 ± 0.76[d]	14.37 ± 0.69[d]	14.33 ± 0.88[d]

P values are as in Table 1.

Figure 1 - Influence of lead nitrate and Nag bhasma on haemoglobin content of rat blood



reductions in haemoglobin contents as compared to haemoglobin contents of in respective normal rats.

While the rats that belong to Groups III, IV and V and received 30 mg, 60 mg and 90 mg Nag bhasma doses per kilogram body weight of the rats respectively did not show any alteration in the content of haemoglobin.

30 mg Nag bhasma/kg body wt treated rats :

The rats treated with 30 mg lead nitrate/g body weight/day for 7 days showed marginal reduction of 4.13 % haemoglobin content as compared to that of adult normal rats of Group I. Daily doses of 30 mg, for 14 days (rats of Group III, IV and V) resulted in 6.18 % fall in haemoglobin level, which was insignificant. On the contrary, haemoglobin content of blood of rat treated for 21 days showed insignificant marginal rise in haemoglobin content.

60 mg Nag bhasma/kg body wt treated rats :

Haemoglobin value was reduced by 8.26 and 10.29 % due to the administration of Nag bhasma for 7 and 14 days, whereas it was brought nearer to the corresponding normal value, which was lesser by 1.39 % only.

90 mg Nag bhasma/kg body wt treated rats :

The alterations in rat blood haemoglobin caused by 90 mg Nag bhasma/kg body wt were parallel to the observations made in group IV. rats. Losses of 10.32 and 11.67 % were noted in haemoglobin after the administrations of Nag bgasma for 7 and 14 days. While it was brought towards the normal level after the administration of Nag bhasma for 21 days, where only reduction of 3.48 % was observed.

Distribution of haemoglobin :

The cytological alterations in blood smears stained with benzidine method are given in Figures 1 - 6.

1.Normal rats:

The blood smears stained for haemoglobin showed normal appearance and stained haemoglobin. (Fig. 1).

20 mg/kg body wt Lead Nitrate treated rats :

The rats that received daily 20mg per kg body weight for 7 days showed weakly stained RBCs but very few morphologically altered cells. But they became more common and evident in the blood smears of rats that were treated with daily 20 mg per kg body weight for 14 days (Fig 2). They continued to show the spiny appearance, crenate shape, altered shapes and spherocytes in blood smears of rats that were treated with daily 20 mg per kg body weight for 21 days (Fig. 3).

Captions to Figs. 1 - 6

Fig. 1: Normal rat: Blood Smears stained for Haemoglobin. Note normal RBCs and stained haemoglobin.Note less number of broken cells. x150

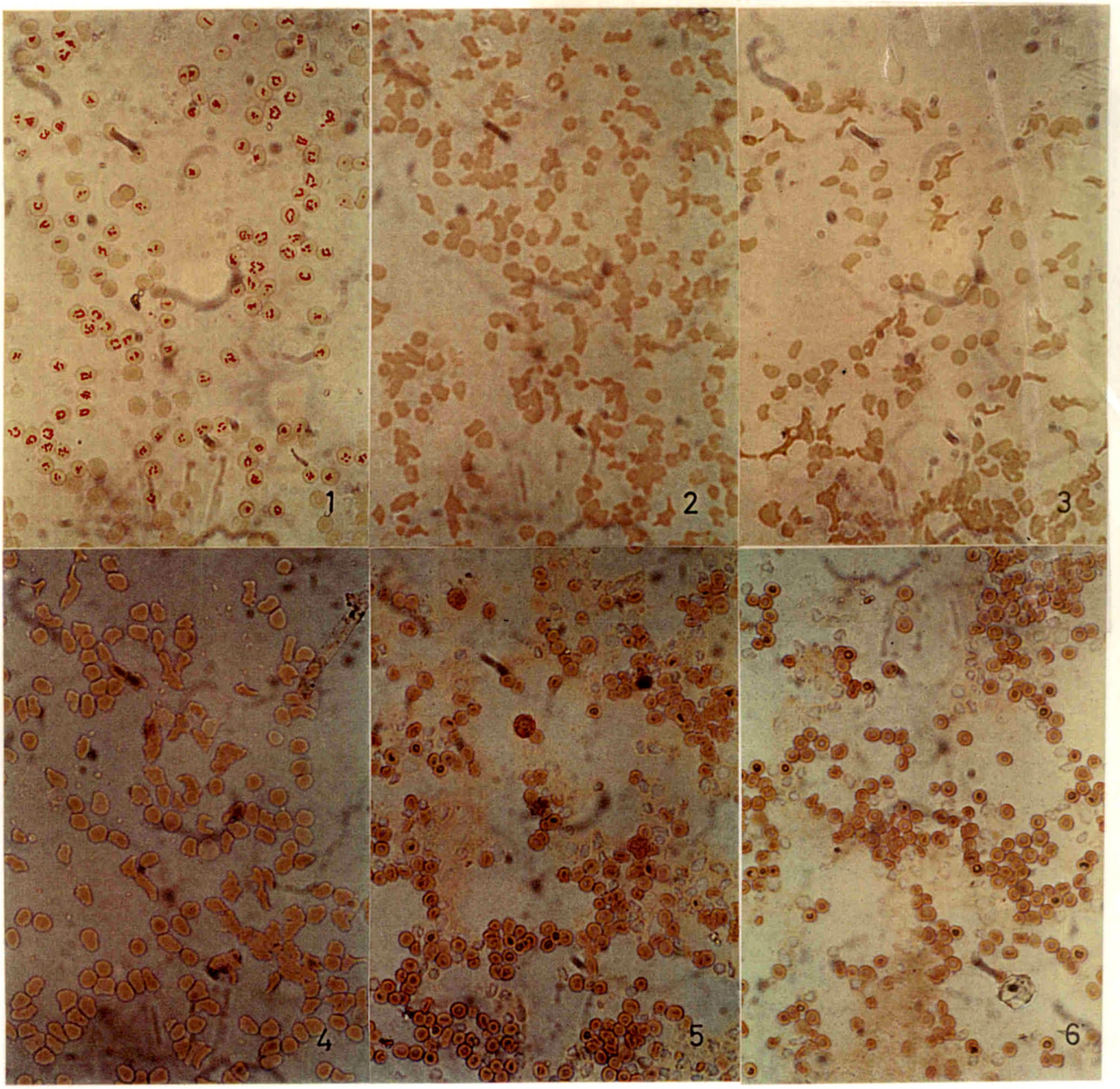
Fig. 2: Lead nitrate treated rat (20 mg/kg body wt daily for 21 days): Blood Smears stained for Haemoglobin. Note altered RBCs and not properly stained haemoglobin. Observe broken cells. X 150

Fig. 3: Lead nitrate treated rat (20 mg/kg body wt daily for 21 days): Blood Smears stained for Haemoglobin. Note altered sperocyte shaped, crenate shaped RBCs and not properly stained haemoglobin. Note more broken cells that showed sensitivity to mechanical stress X 150

Fig. 4: Nag bhasma treated rat (30 mg/kg body wt daily dose for 21 days): Blood Smears stained for Haemoglobin. Note unaltered normal shaped, RBCs well stained for haemoglobin. Note small number of broken cells. X150

Fig. 5: Nag bhasma treated rat (60mg/kg body wt daily for 21 days): Blood Smears stained for Haemoglobin. Note unaltered normal shaped, RBCs well stained for haemoglobin. Note small number of broken cells. X150

Fig. 6: Nag bhasma treated rat (90mg/kg body wt daily for 21 days): Blood Smears stained for Haemoglobin. Note unaltered normal shaped, RBCs well stained for haemoglobin. Note small number of broken cells. X150


1
2
3
4
5
6

Nag bhasma treated rats:

The blood smears that were stained for haemoglobin from the rats that were orally fed with 30 mg per kg body weight, 60 mg per kg body weight and 90 mg per kg body weight; each of which was given for days 7, 14 and 21. Since results being similar the microphotographs of the stained blood smears of the rats treated with 30 mg per kg body weight, 60 mg per kg body weight and 90 mg per kg body weight daily for 21 days (i.e. longest duration of all the doses used (Figs 4, 5 and 6). The stained RBCs were normal in all the doses and durations and showed normal staining of haemoglobin.

DISCUSSION

The lowered levels of haemoglobin were noted in $Pb(NO_3)_2$ treated rats. Although similar observations were noted by Karmarkar et al (1990) by the oral treatment of 44 mg lead acetate per kg body wt. of rats for 9, 15 and 30 days resulted in haemoglobin levels which were below sub toxic levels (60 µg/100ml). The decreases observed at various intervals in present studies are not below the sub toxic levels. The maximum percentage decrease of 28.69 % was reported after 14 doses of lead nitrate. The lowest of percentage decrease was noted after 21 doses of lead nitrate. With the results obtained after only three intervals makes the data restricted and it becomes difficult to draw conclusion/s

about long term alterations. Still within the three successive weeks, cyclic alterations were noted in haemoglobin levels peak being after 2 weeks. The peak may be the result of new recruit of RBCs since although the general metabolism within the erythrocyte cells is not very much disturbed. The cells shrink from efflux of K^+ and water and their survival is shortened. Lead initiates stimulation of erythropoiesis in bone marrow to increase the production of RBCs, basophilic cells with nuclear abnormal and inadequate haemoglobin (Gibson and Goldberg, 1970; de Bruin, 1971). In present results haemoglobin results never dropped to sub toxic levels. This difference may have been the results of use of organic lead compounds and higher dose.

The cytological demonstration of haemoglobin in RBCs illustrated normal distribution but the lead nitrate treatment altered morphological features. These can be clearly noted at all the intervals of lead nitrate treatment in present results. The dilated cells, spiny, crenated and spherocyte type of cells became more abundant with increasing intervals of treatment. Similar comparable shapes of RBCs were noted in rat by Karamarkar et al (1990) with oral treatment of lead acetate by scanning electron microscopic observations.

All the results discussed so for were of the lead nitrate treated rats that are used for comparison. The nag bhasma treatment with 30

mg, 60 mg and 90mg given for three intervals 7, 14 and 21 days in treatment of each of the doses did not showed any alteration in haemoglobin levels. The cytological demonstrations of haemoglobin did not show any morphologically difference in RBCs comparable to those observed in lead nitrate treated rats indicating no toxic effects as demonstrated by inorganic lead toxicity or any other type different than normal.

The reason may be the different types of shodhan and maran treatments that are given to crude Nag/lead. The treatments include mostly use of herbs as described in chapter II. It is stated in Ayurvedic text that these treatments are used to loose its toxic properties and improve the effectiveness in therapy (ayurved Sarsangrah, 1964; Bodas, 1982).