CHAPTER VI

Nag bhasma mediated changes in lysosomal enzymes of liver and kidney of albino rats

In reviews on hepatotoxicity and nepherotoxicity (Hinton and Grasso, 1995; Lock, 1995) toxic effects in general on lysosomes are discussed. Compounds may affect lysosomes in two ways. The indigestible material may reach lysosomes causing accumulation enlargement and interference in cellular functioning. The compounds may cross the lysosomal membrane and may be trapped as a result of the difference in pH. Due to acidic pH, non ionizable, diffusible weak bases slowly accumulate in lysosomes and as they concentrate water enters the lysosomes to maintain an osmotic balance resulting in a marked distention.

Lysosomal enzymes *viz.* Acid phosphatase, β-glucuronidase, and Cathepsin D, were studied in liver and kidney of rat during present study. The changes occurred in the enzyme activities of the rats treated with lead nitrate and Nag bhasma are compared with respect to the respective enzyme activities of normal animals.

MATERIAL AND MATHODS

The experiments were carried out as described in Chapter II. The assays of lysosomal enzymes were done as described in Chapter II.

RESULTS

Lysosomal enzymes studied during present work were Acid phosphatase, Cathepsin D and β-glucuronidase. The enzymes were studied in liver and kidneys of experimental rat of groups I to V.

Liver -

Acid phosphatase :-

The alterations in acid phosphatase activity of rat liver after the treatments of lead nitrate and different doses of Nag bhasma are shown in Table 1 and Figure 1 and 2.

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

Normal rat liver exhibited 2842.41 ± 132.25, 2568.14 ± 126.22 and 2523.54 ± 118.57 units/g tissue and 17.20 ± 1.06, 14.66 ± 0.68 and 14.38 ± 0.69 units/mg protein. Administration of 20 mg lead nitrate/kg body wt to the rats for 7 days did not alter liver acid phosphatase activity per g tissue (1.03 fold). However marginal loss in this enzyme activity

Table 1 :- The influence of different doses of Nag bhasma on acid phosphatase activity of rat liver liver

Values expressed as Units/g tissue
A – units/g tissue; B Units/mg protein

Group	Duration of treatment					
	7 Days		14 Days		21 Days	
	A	B	A	B	A	B
Normal	2842.41 ± 132.25	17.20 ± 1.06	2568.14 ± 126.22	14.66 ± 0.68	2523.54 ± 118.57	14.38 ± 0.69
Lead nitrate [20 mg/kg body wt]	2926.00 ± 156.49^{d}	16.21 ± 0.93^{d}	898.37 ± 36.15^{c}	3.73 ± 0.14^{c}	2183.62 ± 98.15^{b}	18.48 ± 0.77^{a}
Nag bhasma [30 mg/kg body wt]	2547.36 ± 117.28^{b}	14.80 ± 0.72^{d}	1680.00 ± 113.64^{c}	13.39 ± 0.75^{d}	4241.36 ± 210.76^{c}	23.09 ± 1.32^{c}
Nag bhasma [60 mg/kg body wt]	3214.72 ± 167.31^{c}	13.07 ± 0.59^{d}	3269.75 ± 182.93^{c}	14.46 ± 0.82^{d}	5366.67 ± 287.25^{c}	32.01 ± 1.47^{c}
Nag bhasma [90 mg/kg body wt]	3028.20 ± 158.04^{a}	18.09 ± 0.97^{d}	3046.23 ± 167.19^{c}	17.08 ± 0.89^{a}	2588.14 ± 225.19^{d}	14.75 ± 0.67^{d}

Values are mean ± SE of 7 animals

Values are mean ± SE of 7 animals

Figure 1 - Effects of lead nitrate and Nag bhasma on acid phosphatase/g tissue activity of rat liver

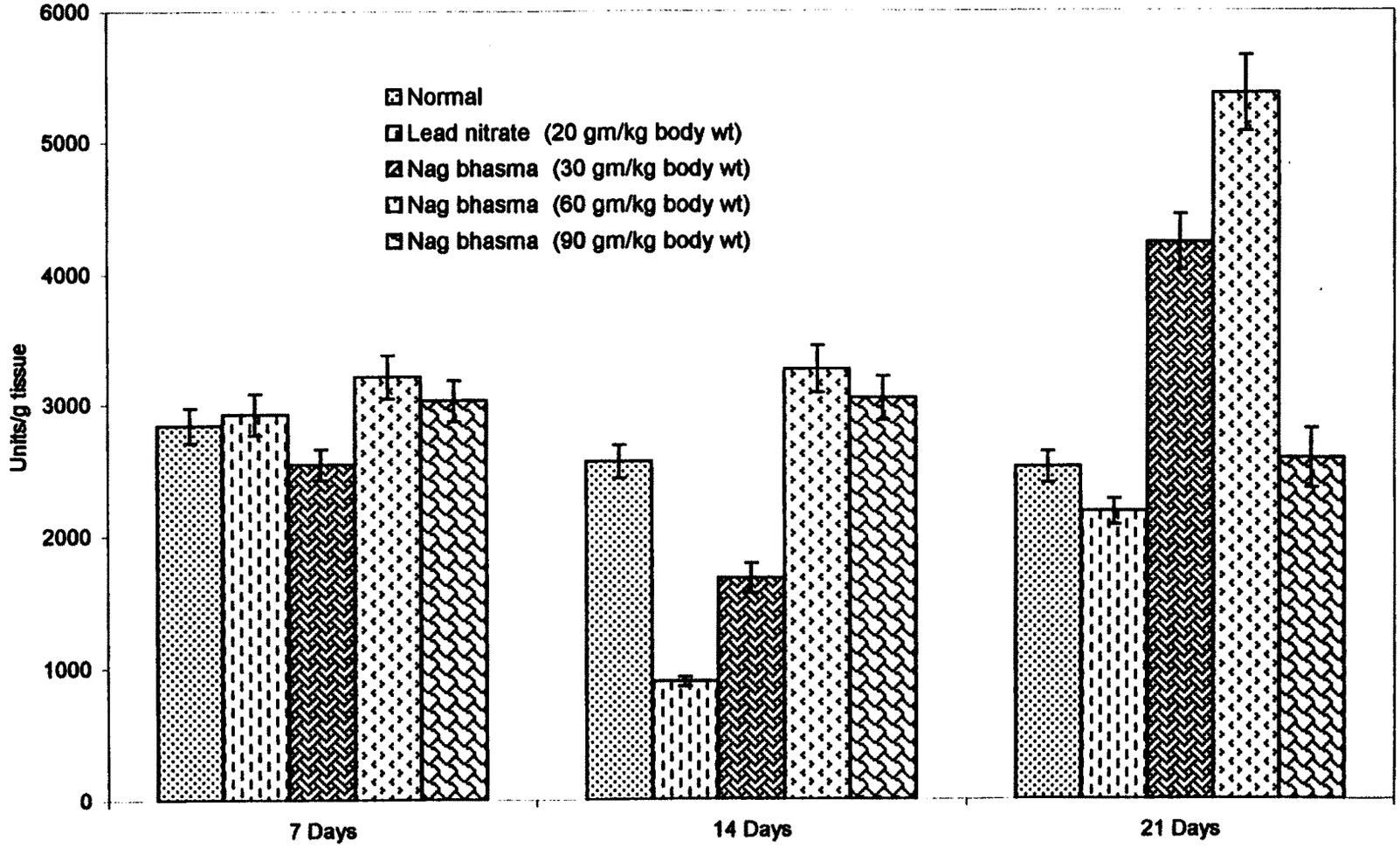
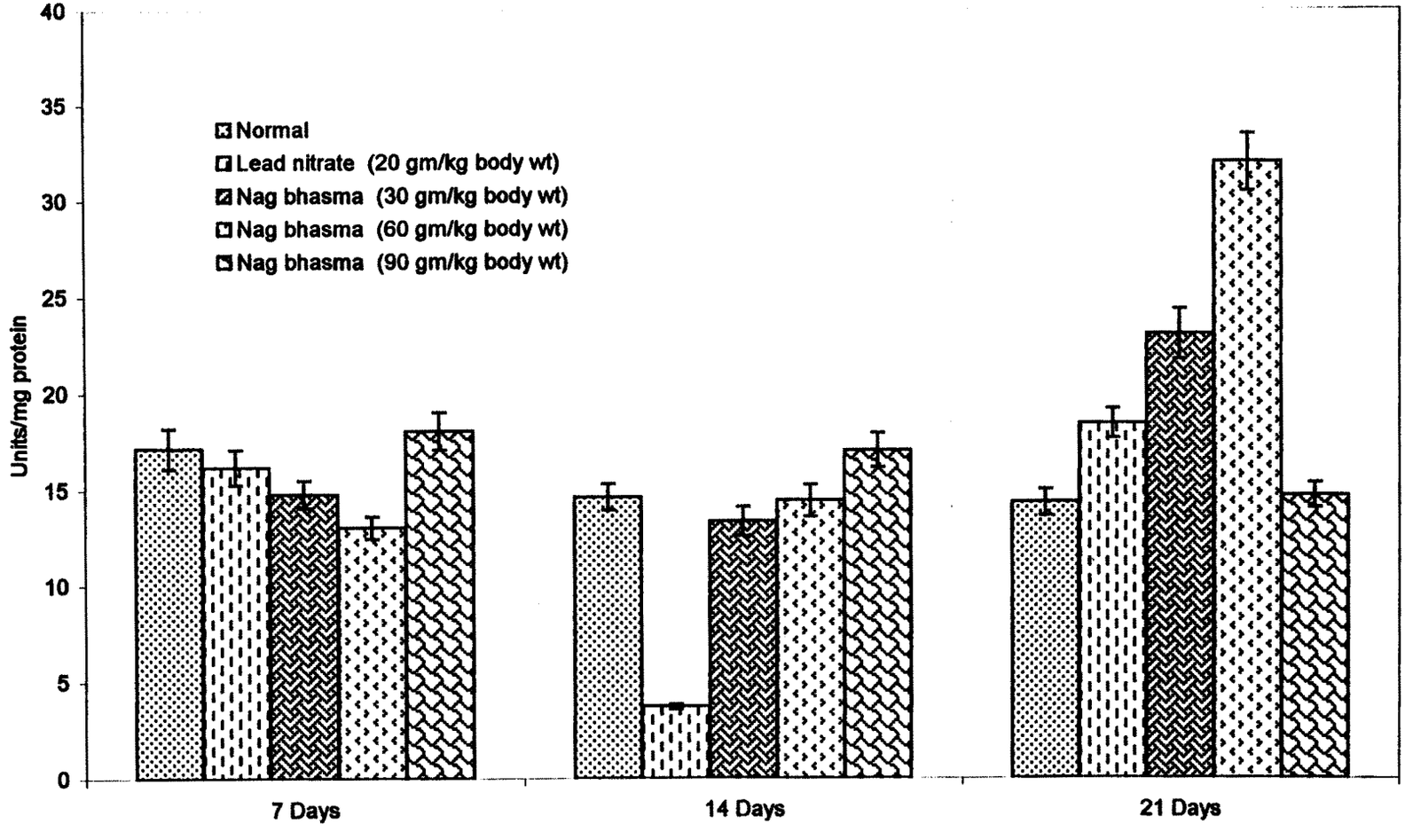


Figure 2 - Influences of laed nitrate and Nag bhasma on acid phosphatase activity/mg protein of rat liver



(5.76 %) was noticed after expressing per mg protein. Oral treatment of 20 mg lead nitrate/kg body wt for 14 days resulted in 65.02 and 74.56 % reductions in acid phosphatase activity on expressing per g tissue and per mg protein respectively. Acid phosphatase activity/g tissue was reduced by 13.47 % after the treatment of lead nitrate for 21 days. This activity exhibited 1.29 fold rise on expression per mg protein.

30 mg Nag bhasma/kg body wt treated rat :

Seven days treatment of Nag bhasma caused 10.38 % reduction in rat liver acid phosphatase activity/g tissue. The reduction of 13.10 % was also noticed when activity was expressed per mg protein. Further treatment of Nag bhasma for 14 days reduced rat liver acid phosphatase activity/g tissue by 34.58 % (8.66 % reduction was noted on expressing per mg protein). However oral administration of Nag bhasma for 21 days resulted in the elevation of acid phosphatase activity by 1.68 fold/g tissue and 1.61 fold/mg protein.

60 mg Nag bhasma/kg body wt treated rat :

Oral administration of 60 mg Nag bhasma/kg body wt for 7 days resulted in 1.13 fold rise in liver acid phosphatase activity/g tissue. On the contrary it exhibited 24.01 % fall after presenting per mg protein. Similarly administration of 60 mg Nag bhasma/kg body wt for 14 days

caused 1.27 fold rise in acid phosphatase activity/g tissue and showed 1.36 % fall after presenting per mg protein. While further treatment of Nag bhasma for 21 days enhanced liver acid phosphatase activity by 2.13 and 2.23 folds after expressing per g tissue and per mg protein.

90 mg Nag bhasma/kg body wt treated rat :

Administration of 90 mg Nag bhasma/kg body wt for 7 days caused the marginal elevation in rat liver acid phosphatase activity either expressing as per g tissue (1.07 fold) or per mg protein (1.05 fold). The enzyme activity was further enhanced by 1.19 fold/g tissue and 1/17 fold/mg protein. Again the enzyme activity declined and brought towards normal level by further administration of 90 mg Nag bhasma for 21 days. When compared to the normal values the increments of 1.03 fold either expressing the enzyme activity per g tissue or per mg protein.

β - Glucuronidase :-

Normal rat :

β-Glucuronidase exhibited 660.00 ± 33.33, 682.19 ± 37.11, 700.25 ± 32.00 units and 3.99 ± 0.14, 2.18 ± 0.11 and 3.99 ± 0.16 units on expression per mg protein in normal rat liver (Table 2 and Figure 3 and 4).

Table 2 :- influences of different doses of Nag bhasma on β-glucuronidase activity of rat liver

Values expressed as Units/g tissue
A – units/g tissue; B Units/mg protein

Group	Duration of treatment					
	7 Days		14 Days		21 Days	
	A	B	A	B	A	B
Normal	660.00 ± 33.33	3.99 ± 0.14	682.19 ± 37.11	2.18 ± 0.11	700.25 ± 32.00	3.99 ± 0.16
Lead nitrate [20 mg/kg body wt]	745.32 ± 42.93^{c}	4.13 ± 0.22^{d}	864.08 ± 44.69^{c}	3.59 ± 0.28^{c}	960.28 ± 62.07^{b}	8.13 ± 0.41^{c}
Nag bhasma [30 mg/kg body wt]	1266.67 ± 54.16^{c}	7.36 ± 0.39^{c}	964.82 ± 56.28^{c}	5.03 ± 0.27^{c}	720.57 ± 45.82^{d}	3.92 ± 0.17^{d}
Nag bhasma [60 mg/kg body wt]	958.47 ± 47.36^{c}	4.79 ± 0.43^{a}	1174.57 ± 62.13^{c}	5.19 ± 0.33^{c}	1382.76 ± 84.71^{c}	8.27 ± 0.44^{c}
Nag bhasma [90 mg/kg body wt]	764.45 ± 57.41^{a}	4.57 ± 0.25^{a}	920.63 ± 46.90^{c}	5.16 ± 0.42^{c}	765.41 ± 40.32^{a}	4.36 ± 0.23^{d}

Values are mean ± SE of 7 animals

Values are mean ± SE of 7 animals

Figure 3 - Effects of lead nitrate and Nag bhasma on B-glucuronidase activity/g tissue of rat liver

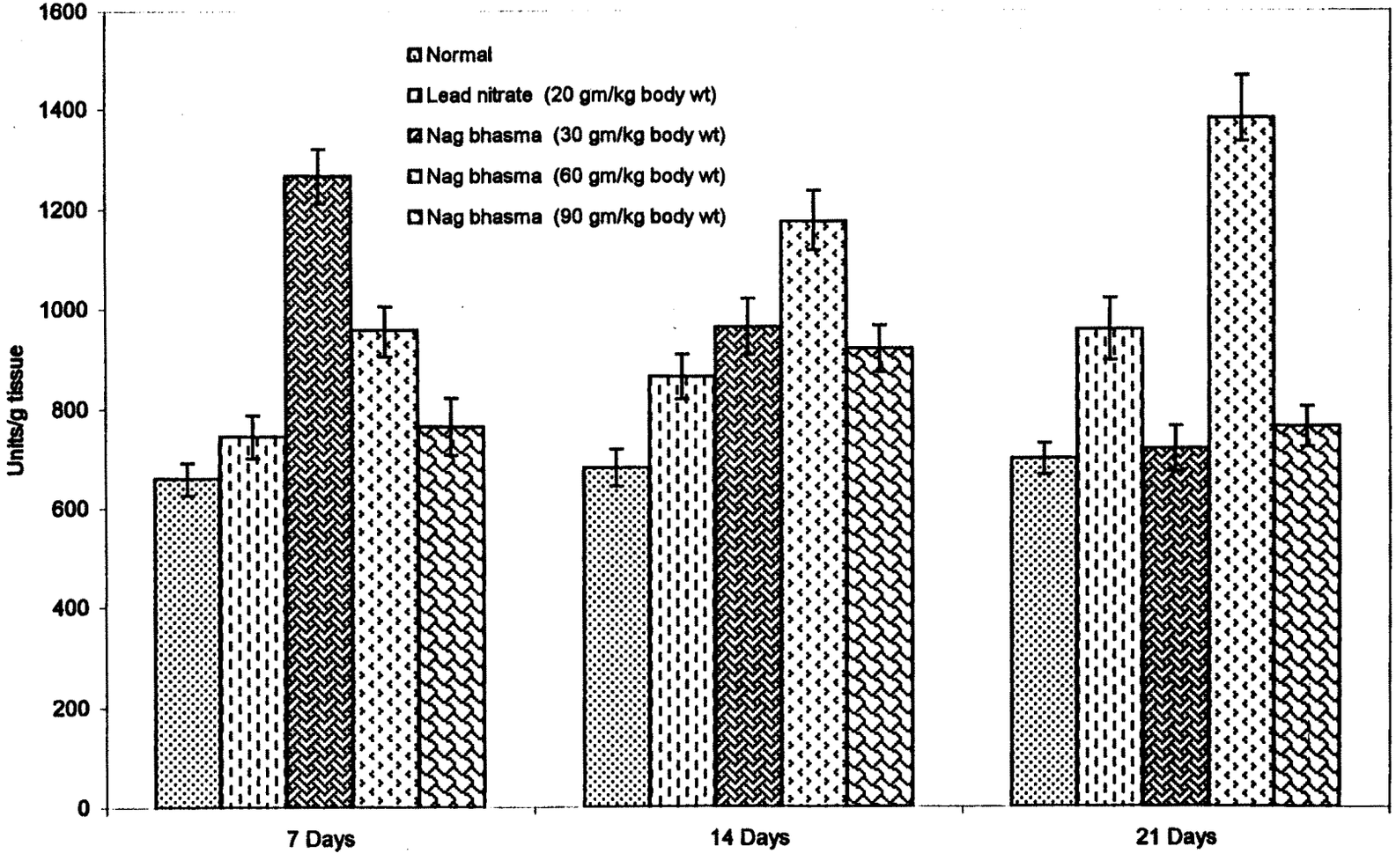
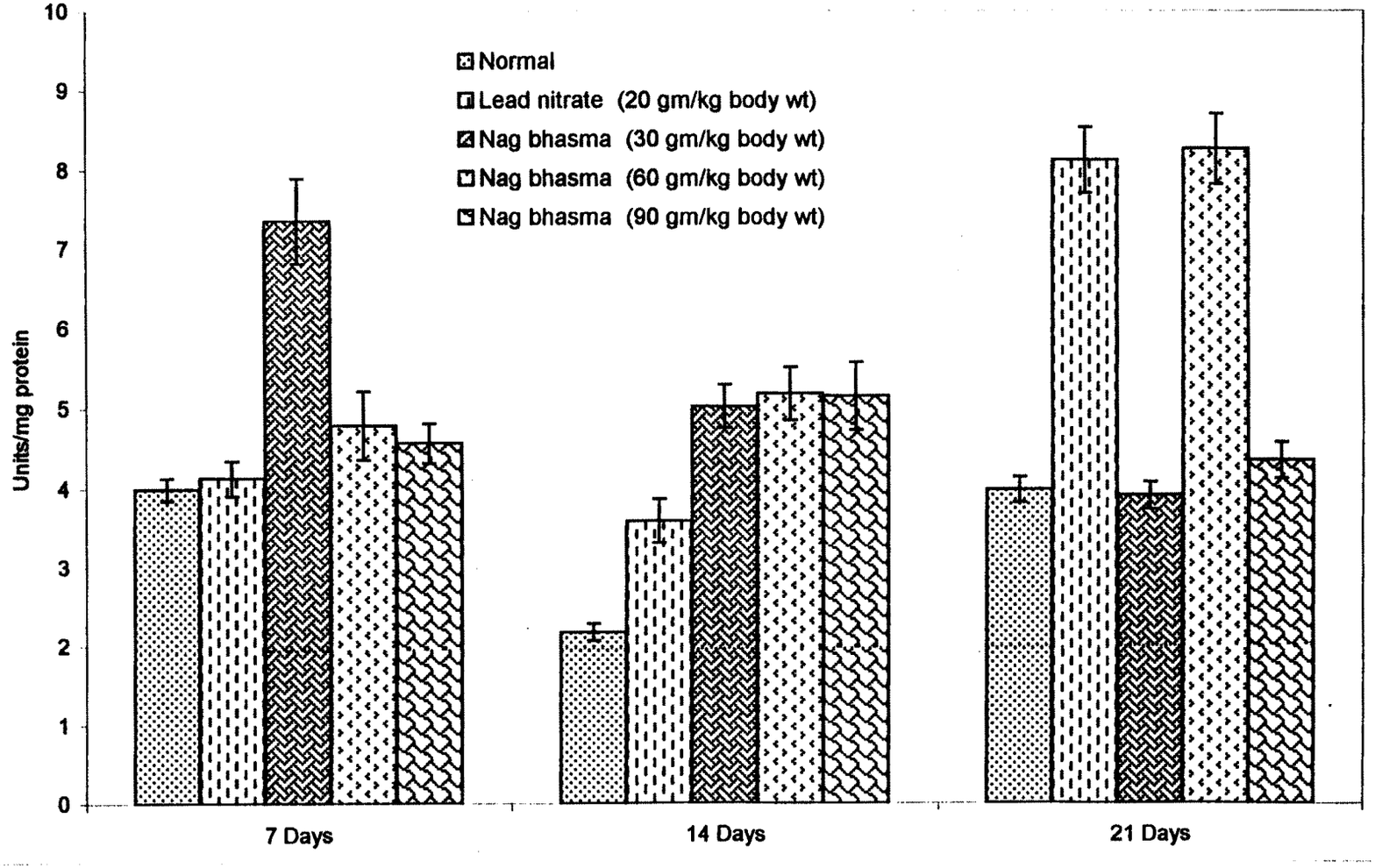


Figure 4 - Influences of lead nitrate and Nag bhasma on B-glucuronidase activity/mg protein of rat liver



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

Elevation in β-glucuronidase activity (1.13 fold/g tissue and 1.27 fold/mg protein) was noticed after the administration of lead nitrate for 7 days. Further treatment of lead nitrate for 14 and 21 days caused progressive elevations in liver β-glucuronidase activities/tissue (1.37 and 1.65 folds respectively). When the enzyme activity was expressed per mg protein showed reduction as compared to the activity noted after 7 days treatment of lead nitrate, although it was marginally increased (1.04 fold) over normal value. The enzyme activity per mg protein was higher (2.04) than the value noted in normal rat liver and that noted after 7 days treatment of lead nitrate.

30 mg Nag bhasma/kg body wt treated rat :

β-Glucuronidase activity of rat liver was elevated by 1.92 fold/g tissue and 1.41 fold/mg protein after the administration of 30 mg Nag bhasma/kg body wt for seven days. Treatment of Nag bhasma for 14 days reduced the activity/g tissue when compared to the value noted after 7 days treatment, but this activity was significantly higher when expressed per mg protein. The activity/g tissue was 1.03 fold higher and was 1.84 fold greater than normal value when expressed per mg protein.

Adminstration of Nag bhasma for 21 days too resulted in enhancement of β-glucuronidase activity by 2.31 fold/g tissue and 1.75 fold/mg protein.

60 mg Nag bhasma/kg body wt treated rat :

Administration of 60 mg Nag bhasma/kg body wt for 7 days enhanced β-glucuronidase activity by 1.45 fold/g tissue and 1.72 fold/mg protein. It was also higher by 1.97 fold/g tissue and 1.20 fold/mg protein after 14 days treatment of Nag bhasma. The highest activity was observed by 21 days treatment of Nag bhasma. The activity was higher by 2.38 fold/g tissue and 2.07 fold/mg protein than the respective normal value.

90 mg Nag bhasma/kg body wt treated rat :

β-Glucuronidase activity was elevated by the administrations of 90 mg Nag bhasma/kg body wt for 7, 14 and 21 days. The activity/g tissue was 1.16 fold grater than normal value and was higher by 1.35 fold/mg protein after 7 days treatment. Administration of Nag bhasma for 14 days caused 1.09 fold/g tissue and 1.15 fold/mg protein rises in β-glucuronidase activity. Similarly the enzyme activity was elevated 2.37 fold/g tissue and 1.09 fold/mg protein.

Cathepsin D :-

Normal rat liver exhibited 155.42 ± 58.04, 148.79 ± 7.46 and 144.56 ± 6.67 units/g tissue (0.94 ± 0.06, 0.85 ± 0.04 and 0.82 ± 0.05 per mg protein) on 7^{th}, 15^{th} and 22^{nd} day (Table3 and Figure 5 and 6).

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

Inhibitions of 10.77 % per g tissue and 18.09 % per mg protein were observed in Cathepsin D activity of rat liver due to the administration of 20 mg lead nitrate/kg body weight for 7 days. Progressive loss in Cathepsin D activity was noticed after the treatments of Lead nitrate for 14 and 21 days. On comparison with normal values the declinations of 34.94 % and 70.90 % were noticed in Cathepsin D activities/g tissue after the administrations of lead nitrate for 14 and 21 days respectively. Similarly the reductions of 52.94 % and 56.10% were observed on presenting the enzyme activity per mg protein respectively due to the administrations of Nag bhasma for 14 and 21 days.

30 mg Nag bhasma/kg body wt treated rat :

Treatment of Nag bhasma (30 mg/kg body wt) for 7 days enhanced Cathepsin D activity over normal value, when expressed both either per g tissue (1.60 fold) or per mg protein (1.54 fold). Further

Table 3 :- Effect of Nag bhasma on Cathepsin D activity of albino rat liver

Values expressed as Units/g tissue
A – units/g tissue; B Units/mg protein

Group	Duration of treatment					
	7 Days		14 Days		21 Days	
Normal	155.42 ± 5.84	0.94 ± 0.06	148.79 ± 7.46	0.85 ± 0.04	144.56 ± 6.67	0.82 ± 0.05
Lead nitrate [20 mg/kg body wt]	138.68 ± 6.92[a]	0.77 ± 0.04[a]	97.22 ± 4.83[c]	0.40 ± 0.02[c]	42.07 ± 2.24[c]	0.36 ± 0.02[c]
Nag bhasma [30 mg/kg body wt]	249.15 ± 13.46[c]	1.45 ± 0.07[c]	133.65 ± 6.75[d]	0.70 ± 0.05[a]	147.31 ± 8.76[d]	0.80 ± 0.04[d]
Nag bhasma [60 mg/kg body wt]	196.32 ± 8.91[b]	0.98 ± 0.06[d]	163.25 ± 8.79[d]	0.72 ± 0.04[a]	138.57 ± 6.90[d]	0.83 ± 0.05[d]
Nag bhasma [90 mg/kg body wt]	187.67 ± 9.77[c]	1.12 ± 0.10[c]	139.42 ± 5.94[d]	0.78 ± 0.05[d]	133.33 ± 5.89[d]	0.76 ± 0.03[d]

Values are mean ± SE of 7 animals

Values are mean ± SE of 7 animals

Figure 6 - Infuences of lead nitrate and nag bhasma on Cathepsin D/mg protein activity/mg protein of rat liver

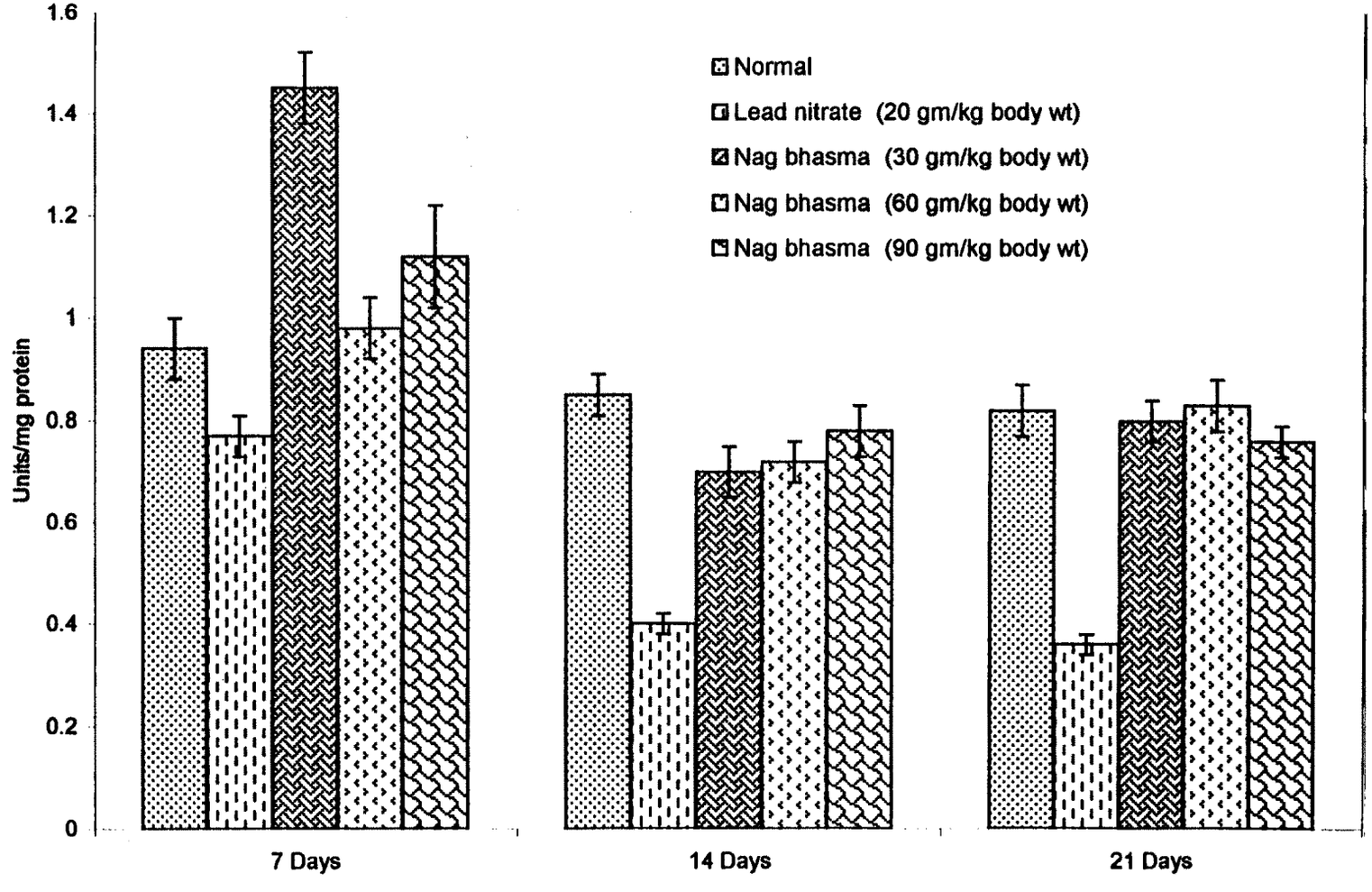
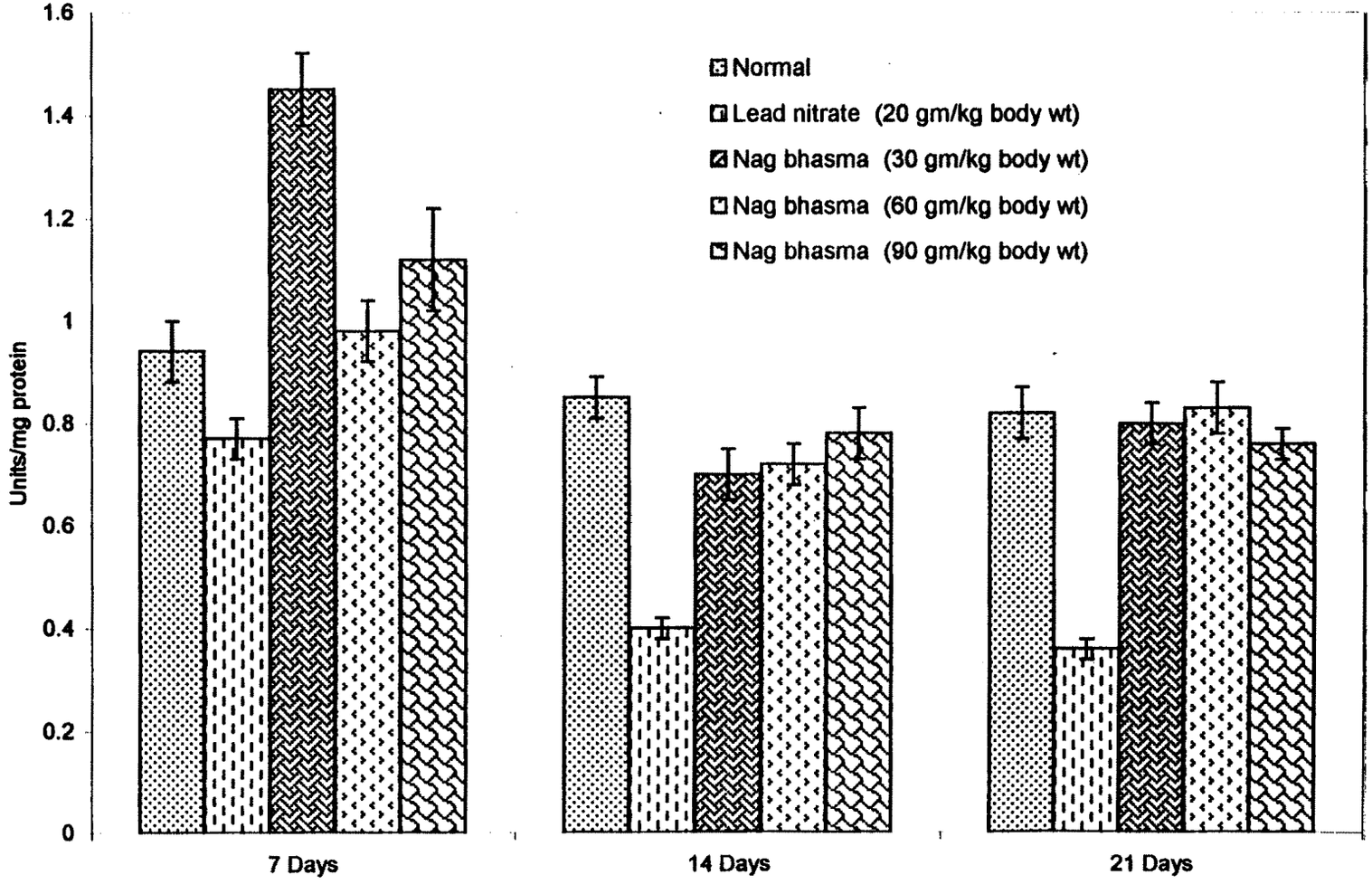


Figure 6 - Infuences of lead nitrate and nag bhasma on Cathepsin D/mg protein activity/mg protein of rat liver



administration of Nag bhasma for 14 days reduced the enzyme activity by 10.18 %/g tissue and 17.65 %/mg protein. On the contrary continuation of Nag bhasma treatment unto 21 days caused insignificant increase in Cathepsin D activity/g tissue, similarly marginal fall in the enzyme activity was observed on expressing per mg protein.

60 mg Nag bhasma/kg body wt treated rat :

Nag bhasma (60 mg/kg body wt) treatment for 7 days elevate Cathepsin D activity/g tissue by 1.26 fold, showed 4.26 % fall on presenting as per mg protein. Further continuation of Nag bhasma administration until 14 days resulted in 1.10 fold rise in the enzyme activity, but exhibited 15.29 % fall after expressing per mg protein. Cathepsin activity/g tissue was declined marginally (4.14 %) after the administration of Nag bgasma for 21 days, which did not alter (1.01) when, presented per mg protein.

90 mg Nag bhasma/kg body wt treated rat :

Enhancements of 1.21 fold/g tissue and 1.19 fold/mg protein were noted in Cathepsin activity due to the treatment of Nag bhasma for 7 days. However the losses of 6.30 % /g tissue and 8.24 %/mg protein were observed due to the administration of Nag bhasma for 14 days.

Similarly treatment of Nag bhasma for 21 days too caused 7.77 % loss/g tissue and 7.32 % loss/mg protein in Cathepsin D activity.

Kidney –

Acid phosphatase :-

Normal rat showed 3112.63 ± 153.78, 4782.55 ± 213.08 and 2192.16 ± 105.37 units/g tissue and 20.10 ± 0.97, 31.10 ± 1.45 and 15.10 ± 0.89 units/mg protein of acid phosphatase activity in rat kidney during present experiment on 8^{th}, 15^{th} and 22^{nd} day (Table 4 and Figure 7 and 8).

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

Oral administration of 20 mg lead nitrate/kg body wt for 7 days resulted in the reduction of acid phodphatase activity of rat kidney by 55.65 %/g tissue and 34.63 %/mg protein. Treatment of lead nitrate for 14 and 21 days also caused reduction in the enzyme activity. Loss of 40.09 %/g tissue (58.13 %/mg protein) was noted. Similarly acid phosphatase activity was decreased by 13.13 %/g tissue, but when the activity was expressed per mg protein increase of 1.21 fold was observed.

Table 4 :- The effects of different doses of Nag bhasma on acid phosphatase activity of albino rat kidney kidney

Values expressed as Units/g tissue
A – units/g tissue; B Units/mg protein

Group	Duration of treatment					
	7 Days		14 Days		21 Days	
	A	B	A	B	A	B
Normal	3112.63 ± 153.78	20.10 ± 0.97	4782.55 ± 213.08	31.10 ± 1.45	2192.16 ± 105.37	15.10 ± 0.89
Lead nitrate [20 mg/kg body wt]	1380.45 ± 59.82^{c}	13.14 ± 0.63^{b}	2865.17 ± 149.30^{c}	12.98 ± 0.57^{c}	2480.00 ± 164.29^{d}	18.28 ± 0.83^{d}
Nag bhasma [30 mg/kg body wt]	1185.44 ± 52.37^{c}	7.18 ± 0.39^{c}	3563.08 ± 178.49^{c}	28.44 ± 1.39^{d}	6728.22 ± 341.68^{c}	44.43 ± 2.04^{c}
Nag bhasma [60 mg/kg body wt]	2448.88 ± 125.00^{b}	15.05 ± 0.81^{b}	4134.15 ± 237.80^{c}	37.46 ± 1.54^{a}	3786.04 ± 178.43^{a}	25.94 ± 0.95^{a}
Nag bhasma [90 mg/kg body wt]	2080.00 ± 104.32^{c}	13.94 ± 0.64^{b}	4521.69 ± 193.42^{b}	32.21 ± 1.78^{d}	2369.33 ± 132.60^{d}	15.68 ± 0.76^{d}

Values are mean ± SE of 7 animals

Values are mean ± SE of 7 animals

Table 7 - Effects of lead nitrate and Nag bhasma on acid phosphatase activity/g tissue of rat kidney

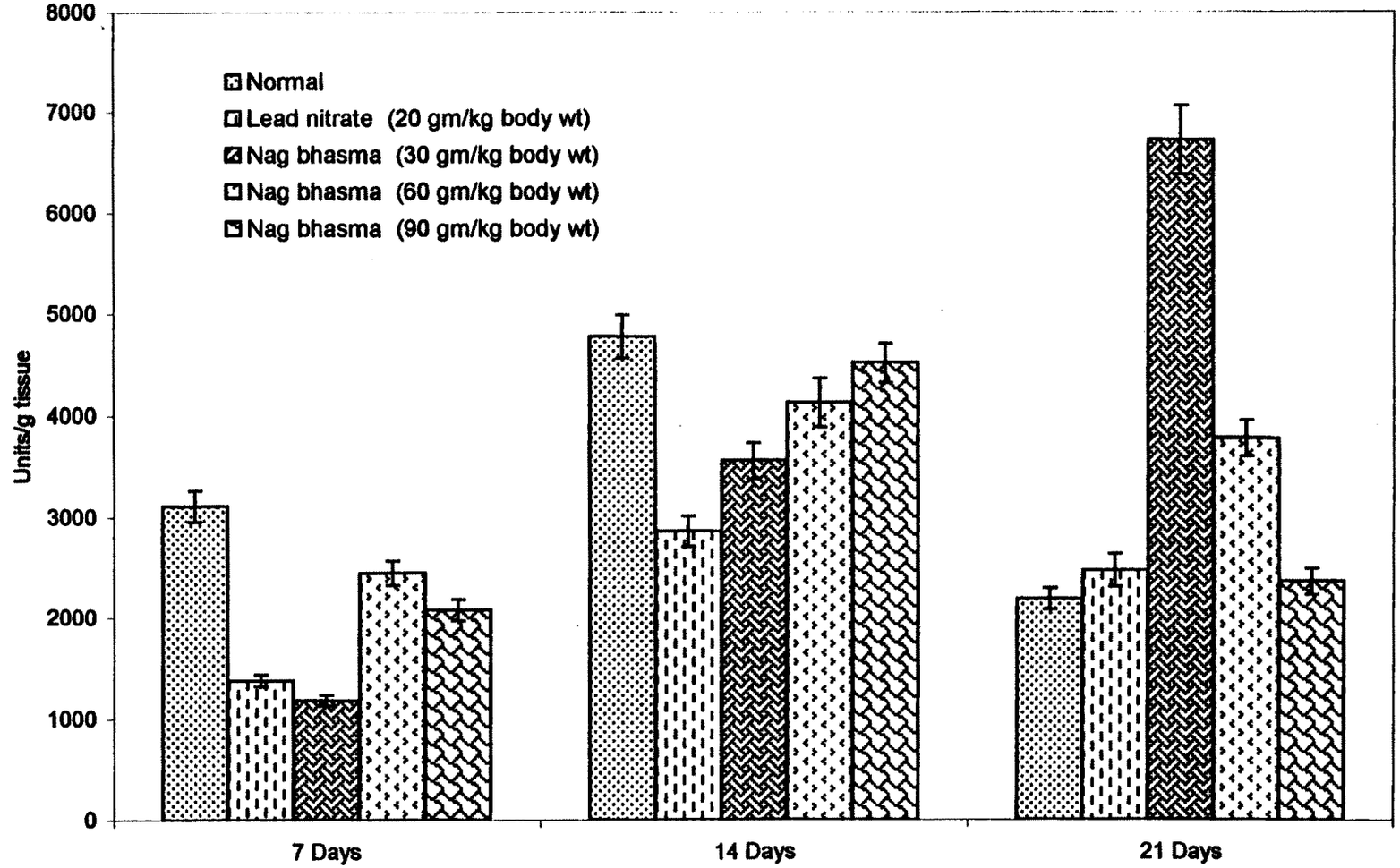
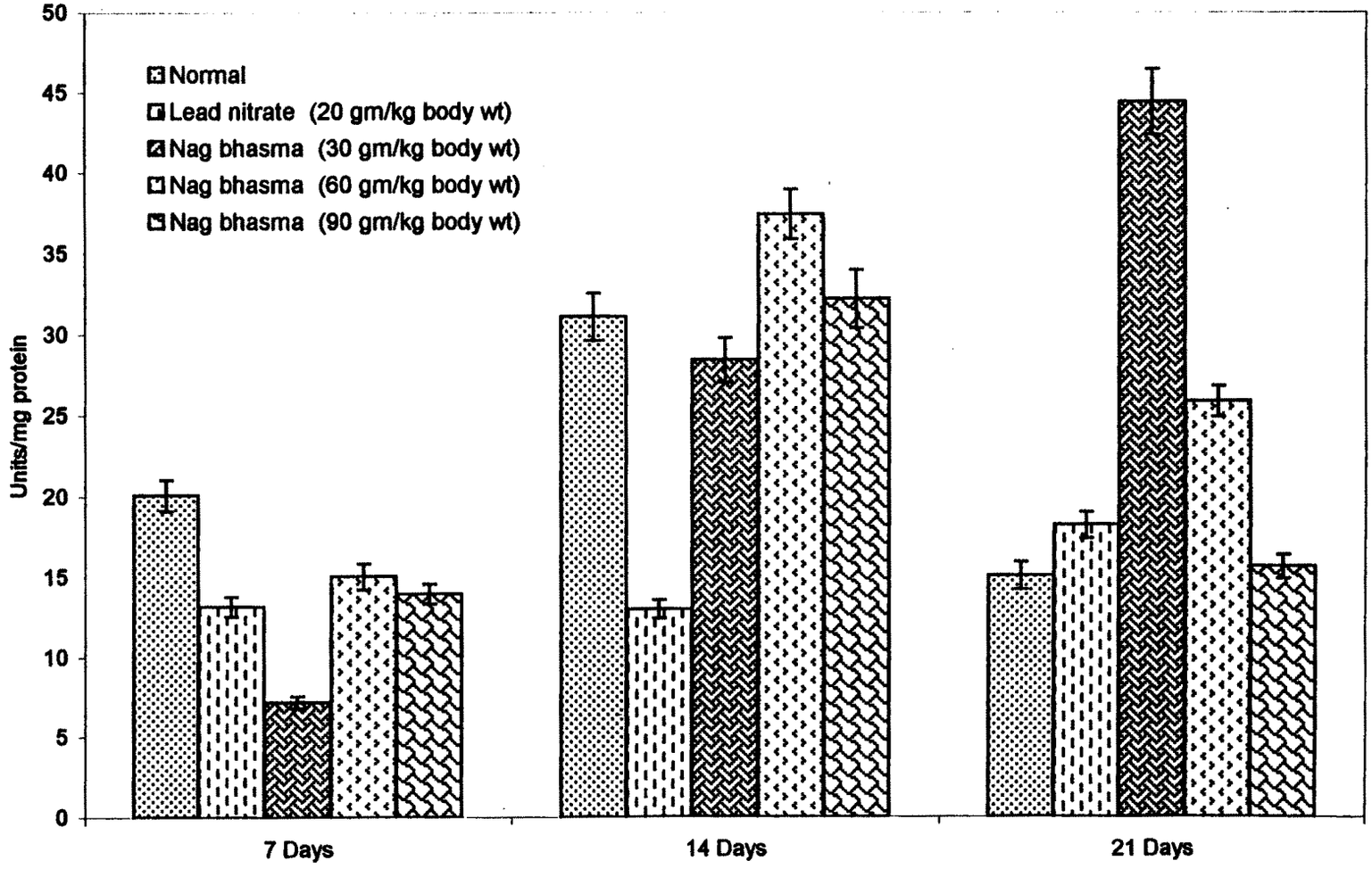


Figure 8 - Effects of lead nitrate and Nag bhasma on acid phosphatase activity/mg protein of rat kidney



30 mg Nag bhasma/kg body wt treated rat :

Acid phosphatase activity of rat kidney was conspicuously declined due to 7 days treatment of 30 mg Nag bhasma/kg body wt. Fall of 61.91 % in the enzyme activity/g tissue (64.28 %/mg protein) was noticed. Compared to acid phosphatase activity on 7 days, the reduction in this enzyme activity was less after 14 days treatment of 30 mg Nag bhasm/kg body wt. This enzyme activity was reduced by 13.56 %/g tissue and 8.55 %/mg protein when compared with corresponding normal value. However administration of Nag bhasma for 21 days raised acid phosphatase activity by 3.07 fold/g tissue and 2.94 fold/mg protein.

60 mg Nag bhasma/kg body wt treated rat :

Acid phosphatase activity/g tissue was reduced by 21.32 % (25.12 %/mg protein) due to the administration of 60 mg Nag bhasma for 7 days. Treatment of nag bhasma for 14 days exhibited 13.56 5 loss in acid phosphatase activity/g tissue, while it was increased by 1.20 fold on presenting per mg protein. The activity was raised by 1.73 and 1.72 folds when presented per g tissue and mg protein respectively after the administration of Nag bhasma for 21 days.

90 mg Nag bhasma/kg body wt treated rat :

Reductions of 33.18 % and 30.65 % were observed in acid phopshatase activity per g tissue and per mg protein respectively by the treatment of 90 mg Nag bhasma/kg body wt for 7 days. When compared to the activity of rat kidney of 7 days treated rat, acid phosphatase activity of 14 days Nag bhasma treated rat exhibited less reduction. The activity showed the loss of 5.45 %/g tissue and 3.57 %/mg protein as compared to the corresponding normal value. While acid phosphatase activity was elevated by 1.08 fold and 1.04 fold on presenting per g tissue and per mg protein.

β-Glucuronidase :

Normal rat kidney showed 382.16 ± 18.90, 340.00 ± 16.24 and 424.08 ± 23.19 units/g tissue and 2.54 ± 0.13, 2.21 ± 0.11 and 2.92 ± 0.08 units/mg protein of β-glucuronidase activity (Table 4 and Figures 9 and 10).

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

Lead nitrate (20 mg/kg body wt) administration for 7 says suppressed β-glucuronidase activity by 40.04 % and 14.17 % when presented per g tissue and per mg protein. Similarly treatment of Lead

Table 5 :- Nag bhasma mediated changes in β-glucuronidase activity of rat kidney

Values expressed as Units/g tissue
A – units/g tissue; B Units/mg protein

Group	Duration of treatment					
	7 Days		14 Days		21 Days	
Normal	382.16 ± 18.90	2.54 ± 0.13	340.00 ± 16.24	2.21 ± 0.11	424.08 ± 23.19	2.92 ± 0.08
Lead nitrate [20 mg/kg body wt]	229.13 ± 10.57[b]	2.18 ± 0.11[d]	208.34 ± 10.12[c]	0.94 ± 0.05[c]	785.67 ± 39.47[c]	5.79 ± 0.29[c]
Nag bhasma [30 mg/kg body wt]	300.00 ± 13.33[b]	1.82 ± 0.09[b]	234.71 ± 12.46[c]	1.87 ± 0.08[d]	217.53 ± 13.05[c]	1.44 ± 0.07[c]
Nag bhasma [60 mg/kg body wt]	308.24 ± 13.67[b]	1.89 ± 0.08[b]	357.32 ± 16.38[d]	3.24 ± 0.18[d]	620.68 ± 38.72[c]	4.25 ± 0.25[c]
Nag bhasma [90 mg/kg body wt]	325.47 ± 3.92[b]	2.18 ± 0.14[d]	336.14 ± 14.52[d]	2.39 ± 0.15[d]	314.21 ± 16.21[c]	2.00 ± 0.07[c]

Values are mean ± SE of 7 animals

Values are mean ± SE of 7 animals

Figure 9 - Influences of lead nitrate and Nag bhasma on B-glucuronidase activity/g tissue of rat kidney

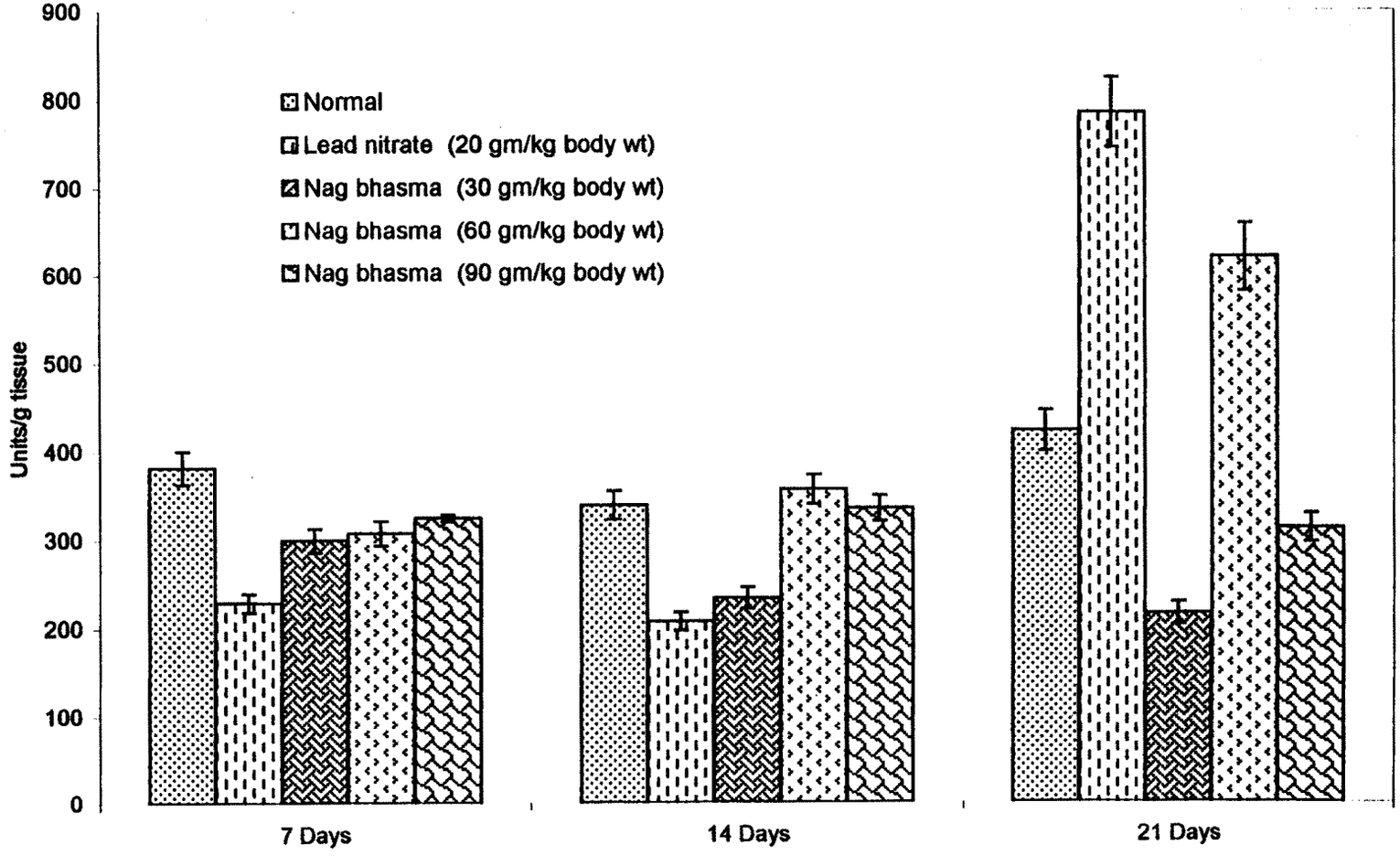


Figure 10 - Effects of lead nitrate and Nag bhasma on B-glucuronidase activity/mg protein of rat kidney

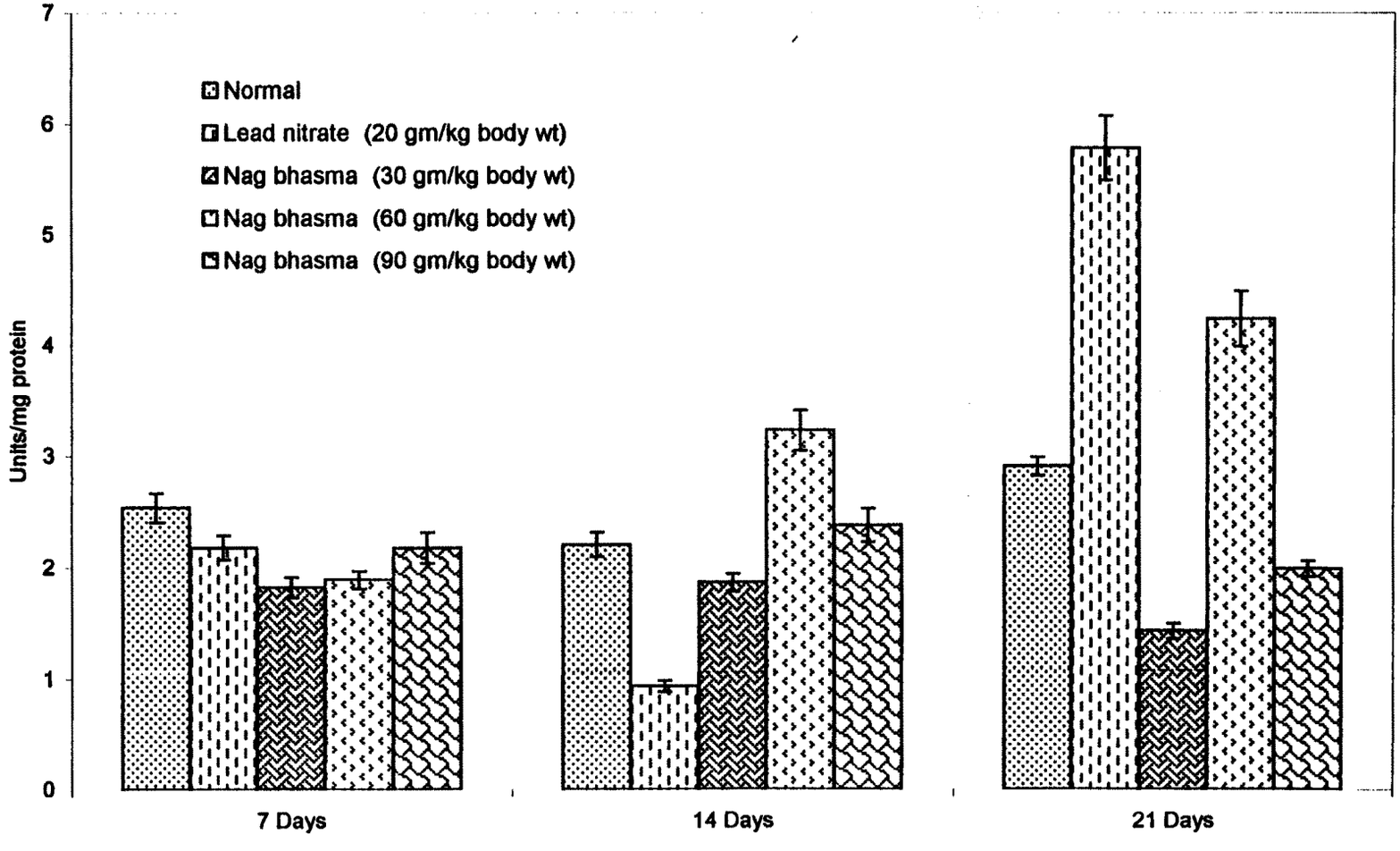


Table 6 :- Variations in Cathepsin D activty of kidney due to Nag bhasma treatment of albino rat

Values expressed as Units/g tissue
A – units/g tissue; B Units/mg protein

Group	Duration of treatment					
	7 Days		14 Days		21 Days	
Normal	206.19 ± 9.38	1.37 ± 0.06	187.36 ± 10.54	1.22 ± 0.09	193.22 ± 10.50	1.33 ± 0.05
Lead nitrate [20 mg/kg body wt]	175.76 ± 11.04[a]	1.67 ± 0.09[a]	135.87 ± 6.91[b]	0.62 ± 0.03[c]	157.33 ± 8.79[c]	1.16 ± 0.06d
Nag bhasma [30 mg/kg body wt]	225.31 ± 13.26[d]	1.36 ± 0.07[d]	153.22 ± 10.06[a]	1.22 ± 0.05[d]	184.67 ± 9.13[d]	1.22 ± 0.07[d]
Nag bhasma [60 mg/kg body wt]	280.00 ± 14.57[c]	2.54 ± 0.12[c]	274.50 ± 16.39[c]	2.49 ± 0.14[c]	268.52 ± 11.48[c]	1.84 ± 0.11[c]
Nag bhasma [90 mg/kg body wt]	278.45 ± 16.67[c]	1.98 ± 0.10[c]	361.42 ± 16.89[c]	2.57 ± 0.16[c]	306.00 ± 14.67[c]	2.02 ± 0.09[c]

Values are mean ± SE of 7 animals

Values are mean ± SE of 7 animals

Figure 11 - Effects of lead nitrate and Nag bhasma on Cathepsin D activity/g tissue of albino rat kidney

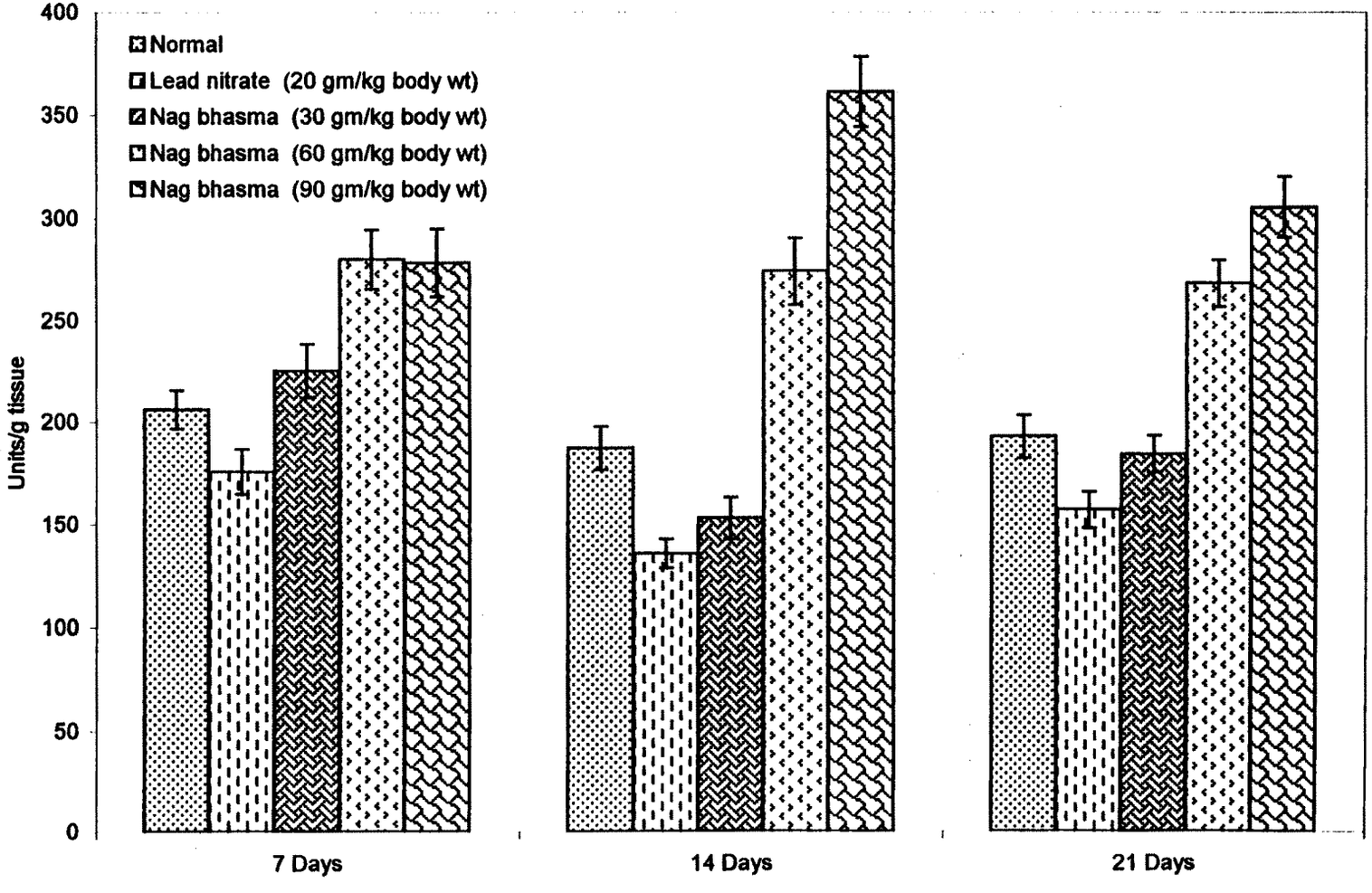
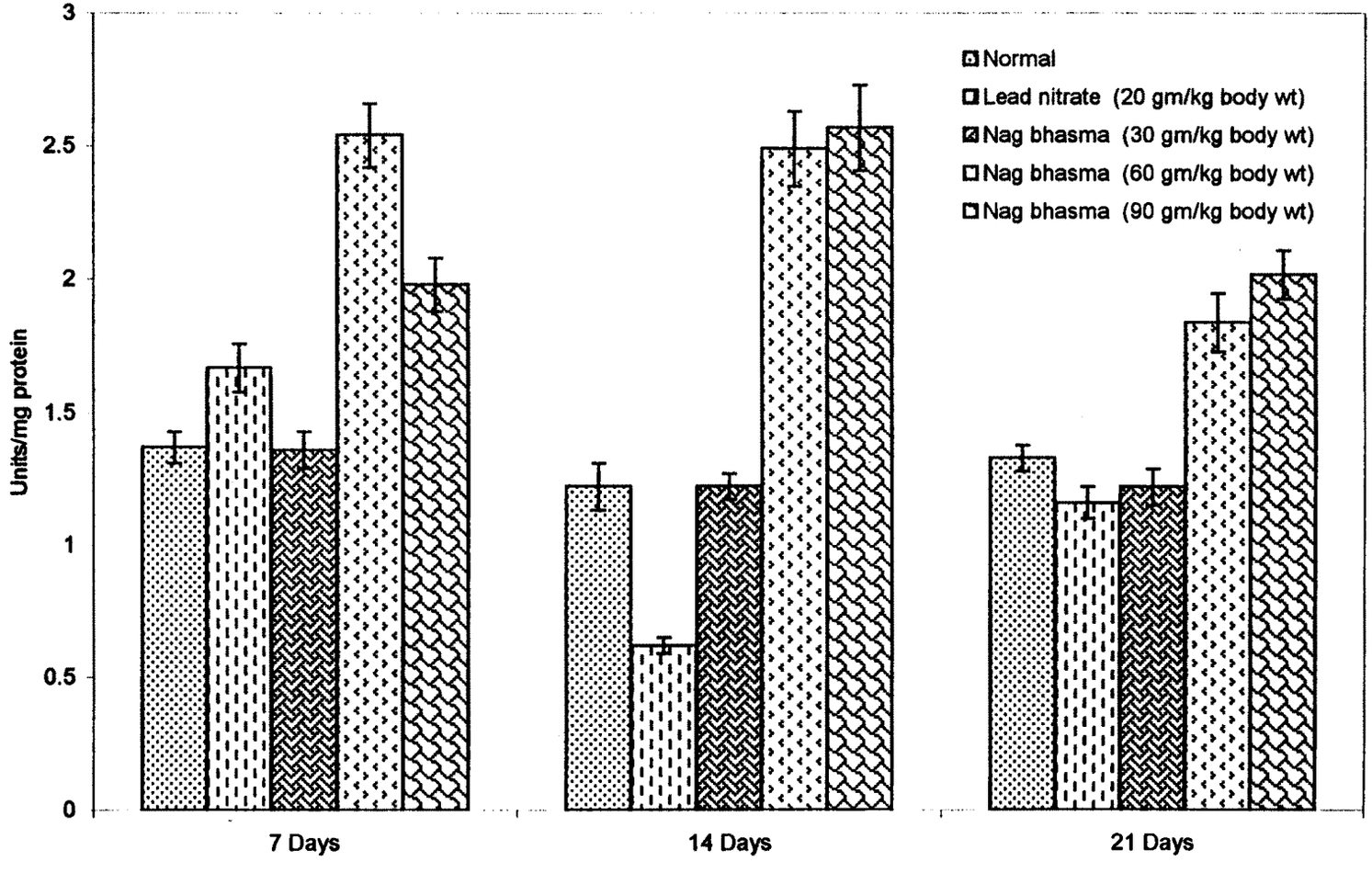


Figure 12 - Variations in Cathepsin D activity/mg protein of rat kidney due to lead nitrate and Nag bhasma



nitrate for 14 days reduced β-glucuronidase activity by 38.72 %/g tissue and 57.47/mg protein. On the contrary lead nitrate administration of 21 days caused 1.85 fold/g tissue and 1.98 fold/mg protein.

30 mg Nag bhasma/kg body wt treated rat :

Administration of 3o mg Nag bhasma/kg body wt for 7, 14 and 21 dyas caused progressive loss in β glucuronidase activity due to the administration of 30 mg Nag bhasma/kg body wt except the activity per mg protein of 14 days treated rat kidney, which was lesser then the corresponding value of 7days treated rat. The losses of 21.50, 30.97 and 48.71 % in β-glucuronidase activities/g tissue (28.35, 15.38 and 50.68 %/mg protein.) were noted due to the treatments of Nag bhasma for 7, 14 and 21 days.

60 mg Nag bhasma/kg body wt treated rat :

β-Glucuronidase activity of rat kidney was reduced by 19.34 % and 25.35% on presenting per g tissue and per mg protein after the administration of 60 mg Nag bhasma/kg body wt to the rats for 7 days. Further treatment of Nag bhasma for 14 days also caused 5.09 % reduction in β-glucuronidase activity/g tissue, while it sowed activation of 1.47 fold after presenting per mg protein. On the contrary administration of 60 mg Nag bhasma/kg body wt for 21 days activated β-

glucuronidase activity by 1.47 fold/g tissue and 1.45 fold/mg protein. The observations show the progressive rise in β-glucuronidase activity as the treatment of 60 mg Nag bhasma progresses unto 21 days.

90 mg Nag bhasma/kg body wt treated rat :

β-Glucuronidase activity was declined by 14.83 % and 14.17 % when presented per g tissue and per mg protein respectively after the adminstration of 90 mg Nag bhasma for 7 days. Nag bhasma treatment for 14 days resulted in non-significant marginal reduction in β-glucuronidase activity/g tissue, but rise of 1.08 fold was seen on presenting per mg protein. Further treatment of Nag bhasma unto 21 days resulted in 25.91 % loss in β-glucuronidase activity/g tissue (31.51 % loss per mg protein).

Cathepsin D –

Normal rat kidney exhibited 206.19 ± 9.38, 187.36 ± 10.54 and 193.22 ± 10.50 units/g tissue (1.37 ± 0.06, 1.22 ± 0.09 and 1.33 ± 0.05 units/mg protein) of Cathepsin D on 8^{th}, 15^{th} and 22^{nd} days of the experiments (Table 6 and Figures 11 and 12).

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

Administration of lead nitrate for 7 days resulted in 14.76 % loss in Cathepsin D activity/g tissue of rat kidney. However enhancement of 1.22 fold was noticed after expressing the enzyme activity per mg protein. Losses of 27.48% and 49.18 % were observed in Cathepsin D activity on presenting per g tissue and per mg protein respectively due to the administration of Lead nitrate for 14 days. Similarly administration of Lead nitrate for 21 days caused 18.57 % reduction in the Cathepdin D activity/g tissue (12.78 %/mg protein) of rat kidney.

30 mg Nag bhasma/kg body wt treated rat :

Cathepsin D activity/g tissue of rat kidney was enhanced by 1.09 fold due to the treatment of 30 mg Nag bhasma/kg body wt for 7 days, but activity did not show the change (0.73 % loss) after expressing per mg protein. Similarly this enzyme activity per g tissue was reduced by 18.22 % after the treatment of Nag bhasma for 14 days, but no change was observed when the activity was presented per mg protein. Further administration of Nag bhasma for 21 days caused the loss of 4.43 % in Cathepsin D activity/g tissue (and loss of 8.27 % per mg protein).

60 mg Nag bhasma/kg body wt treated rat :

Nag bhasma (60 mg/kg body wt) treatment for 7 days caused the elevation in rat kidney Cathepsin D activity per g tissue (1.36 fold) and per mg protein (1.85 fold). Administration of Nag bhasma for 14 days also caused the rise 1.47 fold in the enzyme activity/g tissue (and rise of 2.24 fold/ mg protein). Similarly administration of Nag bhasma for 21 days caused enhancement of 1.39 fold in Cathepsin D activity/g tissue, and 1.38 fold rise after presenting per mg protein.

90 mg Nag bhasma/kg body wt treated rat :

Administration of 90 mg Nag bhasma/kg body wt for 7, 14 and 21 days caused the elevations in Cathepsin D activities when expressed either per g tissue or per mg protein. Elevations 0f 1.35, 1.93 and 1.58 folds were noticed in rat kidney Cathepsin D activity per g tissue (and the elevations of 1.45, 2.11 and 1.52 folds) were noticed after the administrations of Nag bhasma for 7, 14 and 21 days.

DISCUSSION

Effect of daily dose of 20 mg lead nitrate per kg body weight given for 7days did not alter the levels of enzymatic activity. 14 doses of lead nitrate showed significant decrease in enzyme activity and 21 days treatment resulted in 21% increase in activity. The activity showed 4 fold

increase as compared to the activity noted on 14 days of treatments. 30 mg nag bhasma daily /kg body weight treatment reduced the activities marginally; while on day 15 of treatment the activities remained unaltered. After 21 doses of treatment 1.64 folds increase in the activities was observed. Similar trend was followed by the acid phosphatase activities noted in rats that received 14 doses of nag bhasma.90mg nag bhasma/kg body weight daily given for 7 and 14 days indicated no alteration while 21 days treatment showed marginal increase. In kidney of rats' 20 mg-lead nitrate treatment daily given for 7 and 14 days resulted in decreased acid phosphatase activities and on day 21 they were increased marginally. Thirty mg Nag bhasma given daily per kg body weight to rats continued to increase renal acid phosphatase activities through 7, 14 and 21 days respectively; but as compared to renal acid phosphatase activities in normal rat the activities were significantly reduced on day 7 of treatment while marginal decrease was noted on day 14th of treatment and were significantly very high on day 21st of treatment. Sixty mg Nag bhasma/per kg body weight given daily for 7, 14 and 21 days to rats showed marginal decrease, marginal increase and significantly high increase in acid phosphphatase activities as compared to the enzyme activities noted on respective days in normal rat. Similar trend of renal acid phosphatase activities was noted in rats that received 90 mg Nag bhasma/kg body weight. As compared to

corresponding normal rat the activities are not altered significantly. Lead nitrate treatment decreased the β-glucuronidase activities in liver at all the intervals studied as compared to the corresponding intervals in normal rats. The increase was significant only in rats that received 21 doses of lead nitrate. In rats that received 30 mg of Nag bhasma treatment the hepatic β-glucuronidase activities were increased with 7 and 14 days of treatments; while treatment of 21 days did not alter the activities. The activities within the 30 mg Nag bhasma treated rats the enzyme activities decreased with the increasing intervals lowest being on day 22. In 60 mg Nag bhasma treated rats β-glucuronidase activities also showed decrease as compared to activities noted in livers of normal rat. Within the same dose (60 mg Nag bhasma) treated groups of rats the enzyme activities increased with the increased intervals. In 90 mg Nag bhasma treated rats, the enzyme activities were significantly high only on day 14th of the treatment. Within the same group of rats that received 90 mg Nag bhasma the activities were in the same range. The renal β-glucuronidase activities were decreased on 20 mg lead nitrate treatment on day 8th and 15th days lowest being on day 15th. The enzyme activity showed significant increase on day 22nd of the treatment. The enzyme activities of renal β-glucuronidase were nearly half in rats that received 30 mg Nag bhasma dose; but in the rats that received 90 mg Nag

bhasma treatment the enzyme activities were nearly same. In 60 mg Nag bhasma treated rats within the same group the activities increased with the increasing intervals; but as compared to normal rats decrease was observed only on day 5th of treatment and remaining intervals the activities were increased. Lead nitrate (20 mg) treatment to rats showed that Cathepsin D activity was practically similar on days 8 and 15; but significantly low on 22nd day. Thirty, 60 and 90 mg Nag bhasma treatment kept the enzyme activities in the same range; as compared to normal nearly same in 30 mg Nag bhasma treated rats while marginally high incase of 60 and 90 mg Nag bhasma treated rats.

The lysosomal enzymes hydrolyse specific type of bonds at defined pH. The lysosomal metabolism is well-reviewed (Holtzman, 1975; Alberts, 1995). The material to be digested is obtained through endocytosis as the external material is received but with amino acid sequence specific entry is given to the materials that enter autophagosomes. In liver nondigestable material is excluded in bile (Godfrey *et al* 1981) while in other cells the lysosomes may eliminate their waste in intracellular place from where the trafficking cells remove the debris which is also true incase of liver cells where Kupffer cells do the function. All these activities indicate heterogeneity in lysosomes, which need dynamic membranes, which can fuse rapidly and exchange the material rapidly

for clearance (Tessitor *et al* 1979). They are recruited time to time from Golgi apparatus as prelysosomes, which further mature to become functional lysosomes (Holtzman, 1975; Alberts, 1995). There fore the alterations in lysosomes will be considered with relation to histological picture of the organ/s and role of enzyme/s in the respective organ. Lead nitrate treatment did not alter the acid phosphatase activity. Histochemically the alterations were not significant and hence acid phosphatase may not be affected. Fourteen days treatment may have started accumulation of lead in a concentration which may not have altered the histology significantly and which may not be detected histochemically as the deposition of lead is known to occur with smallest of the dose received. Besides the RBCs in heamoglobin preparation studies have shown crenate shape. The deposition may have inhibited the enzyme activities. The highest activities on 22nd day may of the newly recruited lysosomes against the lead mediated load on lysosomes. In Nag bhasma treated rats (30 and 60 doses) in liver in early intervals the activity was not changed but in last interval it was changed since the liver showed no stress on normal metabolism also and the rescheduling may have required the acid phosphatase dependent activities. With largest of the dose this phase may have been in the early stages, which we have not noted, as at any of the intervals activity has not altered significantly. In kidney, lead nitrate treatment had inhibited the activities

at all intervals. Even though histochemically identifiable lead is not noted in kidney, the other works have indicated impairment of proximal tubule functions that include glucose, amino acid and phosphate reabsorption (Goyer, 1982), which may have inhibited enzyme activities. The lowest dose of Nag bhasma have shown duration dependent increase in enzyme activities and at the longest duration normal kidney architecture which was not altered significantly at earlier intervals also. The fluctuation in activities indicates functioning of lysosomes, but within the physiological stress that is not reflected in kidney structure. This is also true in case of 60 mg Nag bhasma dose. Here dose dependent increased initial start is noted over earlier dose and it had also shown duration dependent increase in enzyme activities highest being at longest interval. However at highest dose the activities were lowest at shortest duration in normal histological architecture. The lysosomal acid phosphatase dependent activities may have carried in early few days that we have not studied here. The median and highest duration doses have remained in normal range. Since in kidneys major lead toxicity alterations are observed in proximal tubules (that also contain large number of lysosomes also in normal kidney and the activities may depict the proximal tubular changes directly. In liver many wastes are expelled as glucuronoid conjugates the β-glucuronidase activity may have related distantly. Lead nitrate treatment increased the enzyme activities marginally (except on

last day) duration dependent. But the enzyme activities behave antagonistic to trend that was shown in acid phosphatase activity; though both the enzymes are lysosomal. Similar difference in the behaviour of these two enzymes has been reported earlier (Kanase *et al* 1998). Lowest dose of Nag bhasma has shown duration related decrease; median dose showed duration related increase in activity while largest dose showed reasonably steady activity. The results differ from acid phosphatase activity both being lysosomal enzyme. The lysosomes are heterogenous in behaviour (Tessitor *et al,* 1979) and enzymes alter independently. In kidney, β-glucuronidase activities showed alterations in steady range except at highest duration of lead nitrate treatment when kidney showed foggy necrosis the enzyme activities were high. The alterations clearly indicate that β-glucuronidase activities are less involved in kidney metabolisms than liver metabolisms. Cathepsin D activities in liver were reduce on lead nitrate treatment and decreased with increasing duration. In Nag bhasma treated rats shortest duration of all the 3 doses sowed increased activities. In both the further durations and all the doses activity altered hardly in insignificant range. Thus the drug mediated Cathepsin D activities are altered in first 7 days of the treatment and not influenced further. In kidney the activities of Cathepsin D were marginally high after first 7 days of treatment but further the activities were lowered may be as consequences of lead

toxicity and in first 7days the lysosomes may not have affected harmfully the increased activity at longest duration showed significant increase as compared to median interval the results are concurrent with the foggy necrosis which may have initiated partial degradation of accumulated material which is an indication that the dose of lead nitrate is not totally inhibiting the lysosomal activities. In Nag bhasma treated rats the lowest dose has not influenced the cathepsin D activities at any duration. The median and largest doses in general showed same range of medially high activities at any of the intervals. With their unaltered histology it appears median turn over of metabolisms that involved Cathepsin D activities; which may be influenced by Nag bhasma. The Cathepsin D activities remained in normal range indicate that there may not be any of the reason like amino acid starvation, insulin deficiency, glucagon increase or thyroid hormone increase, because they are known to cause lysosomal protein degradation (Mortimore and Schworer, 1977; Neely *et al*, 1977). Some inhibitors internally may inhibit lysosomal degradation (Knowels and Ballard, 1976). Thus the alterations in normal range indicate that they are not any of the internal activators or inhibitors supporting the view that Nag bhasma acts as general tonic and carries out normal functioning without any physiological stress even within the tolerable limit.

CHAPTER VII

General Discussion

As mentioned in Introduction the aim of the project is to test the toxicity of the Ayurvedic drug Nag bhasma. Nag bhasma is prescribed against diabetes, urinary tract diseases, anaemia, liver and spleen disorders, general weakness, rheumatoid arthritis etc. Ayurvedic drugs are known to be developed using many lengthy procedures that involve herbal or other natural products' treatment. Since the crude herbs or natural products are used the organocomplex formed can't be explained in modern forms. Since the modern therapy is more popular people apply the same tests for their evaluation even if the ayurvedic testing methods are available. The importance of these and other ethnic drugs has been realised. Ayurvedic drugs are time proved drugs and hence needed to be convinced for their use in modern physiological, pathological, pharmacological basis so that they can be used as independent and integrated medicine. In the modern therapy toxicity testing in animal models is essential.

Nag bhasma is prepared from Nag means lead and hence most of the modern practitioners avoid to use such an important drug. Therefore it was decided to test the toxicity of Nag bhasma. The preparation of Nag bhasma being important it was prepared in the laboratory by the method described in Material and Methods. Its' purity was tested by the methods described in Ayurveda.

Using highest therapeutic dose in use did the toxicity testing. It was split in three different doses and different intervals. Since lead toxicity is probability and hence lead toxicity was tested as control.

The results showed that the decrease in haemoglobin and abnormal RBCs were noted in lead treated rats, while such changes were not observed in Nag bhasma treated rats. In clinical liver and kidney function tests Nag bhasma was not hepatotoxic or nepherotoxic.

Under toxicity testing schedule histological alterations in liver and kidney were included. The three doses of nag bhasma studied with three different variations in treatment in albino rats showed that the liver and kidney structures were free of any of the toxicological effects described under pathology let aside the lead toxicity induced pathology. In liver, healthy lobules with well-distributed cords of cells were observed. In kidney also all the components of nepheron were free of any of the physiological stress, and the architecture was normal. The liver

and kidney structural details showed no normal dietary or excretory load instead ideal lobular and hepatic cell structure and nepheron structure was evident.

The results support the claim of the Ayurveda that it can be used as general tonic that strengthens the normal structure and function of any organ in present case that of liver and kidney.

The alterations in lysosomal enzyme activities of acid phosphatase, β-glucuronidase and Cathepsin D showed that the acid phosphatase activities vary with different patterns, especially acid phosphatase and β-glucuronidase behave antagonistic. Similar results were obtained earlier (Kanase *et al*, 1998) Its homeostatic activity also altered in nag bhasma treated rats both in liver and kidney. But β-glucuronidase activity seems to change only in liver and not in kidney. The alterations remained within the range. Lysosomal Cathepsin D activities are influenced by many internal activators *viz.* Insulin, glucagon, thyroid hormone or inhibitors (Mortimore and Schworer, 1977; Mortimore *et al*, 1973; Dean, 1975). The present results indicate that nag bhasma is not mediating through any of the activitors including hormones or inhibitors.

In the present project following points were reported- i) Nag bhasma is not toxic. ii) It is free of any of the pathological activities in addition to lead induced pathology. iii) RBCs are not altered. iv) The liver

and kidney function tests were normal. v) Histology was normal and had healthy appearance and strengthens the claim of general tonic. vi) Lysosomal enzymes altered within the homeostatic range a) acid phosphatase activities altered in both liver and kidney b) β-glucuronidase activities altered in liver and not in kidney c) Cathepsin D activities showed that they altered within the normal range and were not influenced by activators or inhibitors. These are significant results from the point of view of Nag bhasma as a drug; since it is not mediating through any of the hormonal pathway. Vii) The lysosomal changes indicate the normal turnover of the lysosomes and assure normal functioning of lysosomal system.