CHAPTER V

Nag bhasma mediated changes in the histological architecture of the liver and kidney of albino rats

In earlier chapters effects of nag bhasma on liver function tests and kidney function tests in Chapter IV and blood haemoglobin content and haemoglobin distribution in RBCs in Chapter III were described. Therefore to evaluate detailed changes in the histolological architectures of liver and kidney the histopathological studies were carried out after the administrations of lead nitrate and the different doses of Nag bhasma for 7, 14 and 21 days.

The histology is the basis of function of any organ. Therefore alterations in the biochemical parameters are explained on the basis of histological architecture. As described in introduction liver and kidney are the main organs studied and their histology is given in detail.

MATERIAL AND METHODS

Detailed experimental protocol and the methods for the histopathologial studies are given in Chapter II. At the end of the experiments the rats were killed by giving deep ether anaesthesia and liver and kidney were dissected and processed for histopathological studies.

RESULTS

It is already mentioned in the Introduction that for probable toxicological effects for comparison, the dose of 20 mg lead nitrate/kg body weight of rats was given for days 7, 14 and 21. The alterations in liver are given in figs 1 - 4

Liver

Normal rat :

Histochemical demonstration of lead -

The sections of normal rat liver were stained with phloxin and Sodium Rhodizonate for the demonstration of lead in the sections. No staining for the lead was observed in normal.

Twenty one normal rats were maintained without any treatment and killed on days 8, 15 and 22 along with the rats that were given treatments of lead nitrate (of 20 mg lead nitrate/kg body wt of rats) and Nag bhasma (30 mg, 60 mg and 90 mg per kg body wt of rats). Since they have shown the similar architecture only one figure each of the periarterial and centrolobular regions is presented in the data.

Periarterial region (Figs 1) -

The periarterial region was normal with normal hepatic cords, clear bile canaliculi, and healthy hepatic cells.

Centrolobular region (Figs 2) -

The centrolobular region of normal rat showed well-organised hepatic cords, bile canaliculi well marked and normal hepatoparenchymal cells.

Lead nitrate treated rats :

Histochemical demonstration of lead –

P*eriarterial region (Fig. 3) –*

Captions to Figs.1-6

Fig. 1: Normal rat: Periarterial region shows normal hepatic cords, hepatocytes, sinusoids, bile canaliculi, Kupffer cells X 250

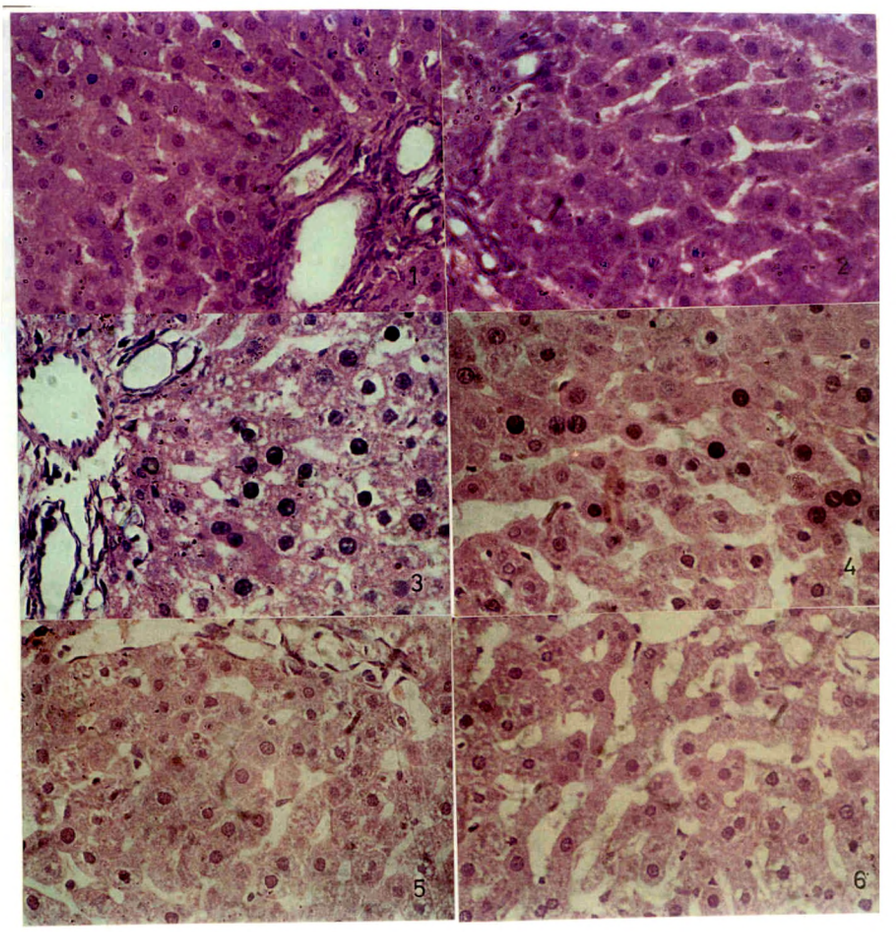
Fig. 2: Normal rat: centrolobular region shows normal hepatic cords, hepatocytes, sinusoids, bile canaliculi, Kupffer cells X 250

Fig. 3: 20 mg lead nitrate (20 mg/kg body wt/day for 21 days) treated rat : Periarterial region. stained for lead by Phloxin and counterstained by Eosin. Note lead loaded nuclei intensly stained. Nearby nuclei are weakly stained X 250.

Fig. 4: 20 mg lead nitrate (20 mg/kg body wt/day for 21 days) treated rat: Centrobular region. stained for lead by Phloxin and counterstained by Eosin. Note lead loaded nuclei intensely stained and distributed in islands. Nearby nuclei are weakly stained or unstained. X 250.

Fig. 5: 20 mg lead nitrate (20 mg/kg body wt.daily dose for 21 days) treated rat : Periarterial region. stained for Toludine Blue pH 3.00 and counterstained by Eosin. Note reduction in normal area and numerous empty spaces altered hepatic cord arrangement, increased number of Necrotic hepatocytes with vacuoles. Bile canaliculi Collapsed. Nuclear staining was comparatively weak X 250

Fig. 6: 20 mg lead nitrate (20 mg/kg body wt/day for 21 days) treated rat : Centrolobular region. stained for Toludine Blue pH 3.00 and counterstained by Eosin. Note enlarged empty areas and hepatic cord arrangement distorted. Increased number of Necrotic hepatocytes, and dead cells. Bile canaliculi Collapsed. Nuclear staining was comparatively weak X 250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Deposition of lead was noted in nuclei in-groups and not uniform. Islands of lead stained nuclei were large and distributed throughout the liver in periarterial region.

The centrolobular region (Fig. 4) –

Islands of the cells containing lead loaded nuclei were visible in centrolobular region also.

Lead nitrate (20 mg/kg body wt/day) treatment was given to the rats 7, 14 and 21 days and were killed after the treatment. The histology of the periarterial and centrolobular regions of liver was not altered by 7 and 14 days treatments. But the liver parenchymal cells showed reduction in nuclear staining by Toludine Blue B pH 3.00 counter stained with eosin in the rats killed on 22nd day after the treatment.

Periarterial region (Fig. 5) -

The hepatic cords in this region was not sharply well organised. Numerous hepatic cells showed vacuolar necrotic appearance. The specificity of vacuolar cells was that the vacuoles were predominantly surrounding the nuclei and not comparable to CCl_4 induced necrosis earlier where a cytoplasmic rim surrounds the nucleus (Kanase et al, 1994; 1998; Buwa *et al,* 2000) and weakly stained nuclei with poor distribution of Kupffer cells. The boundaries of the hepatic cells were not

Captions to Figs.1-6

Fig. 7: 30 mg Nag bhasma (20mg/kg body wt/day for 21 days) treated rat : Periarterial region. stained for Toludine Blue pH 3.00 and counterstained by Eosin. Note normal areas and hepatic cord arrangement normal hepatocytes Bile canaliculi cleared Normal distribution of Kupffer and sinusoidal cells.X 250

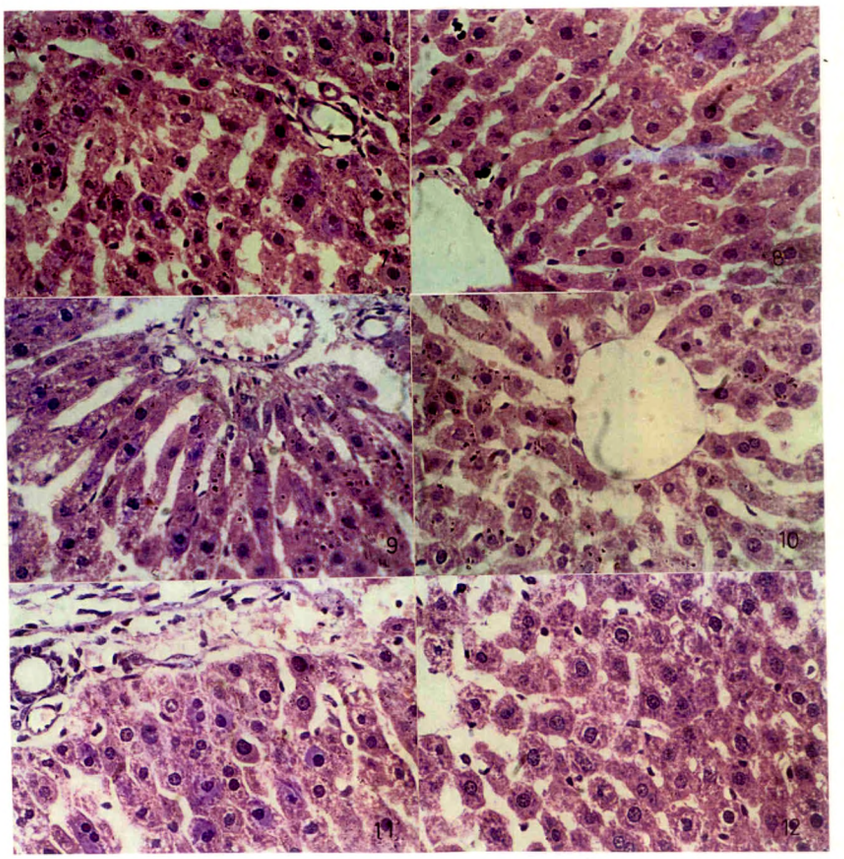
Fig. 8: 30 mg Nag bhasma (30 mg/kg body wt/day for 21 days) treated rat : Centrolobular region. Stained for Toludine Blue pH 3.00 and counterstained by Eosin. Note normal areas and hepatic cord arrangement normal hepatocytes Bile canaliculi cleared Normal distribution of Kupffer and sinusoidal cells.X 250

Fig. 9: 60 mg Nag bhasma (60 mg/kg body wt. daily dose for 21 days) treated rat :Periarterial region. stained for Toludine Blue pH 3.00 and counterstained by Eosin. Note normal areas and hepatic cord arrangement normal hepatocytes Bile canaliculi cleared Normal distribution of Kupffer and sinusoidal cells.X 250

Fig. 10: 60 mg Nag bhasma (60 mg/kg body wt/day for 21 days) treated rat :Centrolobular region. stained for Toludine Blue pH 3.00 and counterstained by Eosin. Note normal areas and hepatic cord arrangement normal hepatocytes Bile canaliculi cleared Normal distribution of Kupffer and sinusoidal cells.X 250

Fig. 11: 90 mg Nag bhasma (90 mg/kg body wt. daily dose for 21 days) treated rat :Periarterial region. stained for Toludine Blue pH 3.00 and counterstained by Eosin. Note normal areas and hepatic cord arrangement normal hepatocytes Bile canaliculi cleared Normal distribution of Kupffer and sinusoidal cells.X 250

Fig. 12: 90 mg Nag bhasma (90 mg/kg body wt. daily dose for 21 days) treated rat :Centrolobular region. stained for Toludine Blue pH 3.00 and counterstained by Eosin. Note normal areas and hepatic cord arrangement of normal hepatocytes that show recognisable basophilia. Bile canaliculi cleared. Normal distribution of Kupffer and sinusoidal cells. X 250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defined in spite of void spaces in some regions and Obliterated bile canaliculi. The cellular association appeared as if clumped and not natural association.

The centrolobular region (Fig. 6) -

In centrolobular region hepatic cords were better organised, although noted numbers of cells were vacuolated. Numerous hepatic cells showed vacuolar necrotic appearance similar to periarterial region. The specificity of vacuolar cells was that the vacuoles predominantly surrounding the nuclei and not comparable to CCl_4 induced necrosis earlier in which a cytoplasmic rim surrounds the nucleus and traverses as strands through the vacuoles (Kanase et al, 1994; 1998). Equal number of cells was with normal appearance also present. Many void spaces with necrotic cells distributed in them were observed. In this organisation also most of the cells did not show clear boundaries. The weak nuclear staining was similar to the periarterial region. Obliterated bile canaliculi were observed.

Nag bhasma treated rats :

Three doses of Nag bhasma 30, 60 and 90 mg/kg body wt/day were given to rats 7, 14 and 21 days and animals were killed on the next days of last doses of treatments. Since the architectures of the liver were

not altered significantly, the histological architectures of livers of rats that were given the longest intervals (21 days) of each of the doses studied are presented through the microphotographs.

30 mg Nag bhasma/kg body wt treated rat for 21 days :

Periarterial region (Fig. 7) -

The hepatic cells were normal loosely arranged in cords cytoplasm was eosinophilic. Normal distribution of Kupffer cells was also noticed.

Centrolobular region (Fig. 8) -

Well organised architecture of healthy hepatic cells in cords, cleared bile canaliculi and normal distribution of Kupffer cells.

Daily dose of 60 mg per kg body weight of rat treated for 21 days :

Periarterial region (Fig. 9) -

The histological appearance was normal.

Centrolobular region (Fig. 10) -

The architecture was normal. Many of the cells from the hepatic cords showed defined boundaries and healthy appearance.

Daily dose of 90 mg per kg body weight of rat treated for 21 days :

Periarterial region (Fig. 11) -

The architecture was normal. In healthy well-defined hepatic cells faint cytoplasmic basophilia was noted.

Centrolobular region (Fig. 12)

The architecture was normal. In centrolobular region the hepatic cells were typically pentagonal with well-defined boundaries. The cytoplasmic basophilia in the cells was well defined.

Kidney

Cortex (Fig.13-25) :

Normal rat (Fig. 1)

Twenty one normal rats each containing five animals were maintained and killed on days 8, 15 and 22 along with the rats of groups II to V. Since they have shown the similar architecture only one microphotograph of the longest treatment is used for description (Fig. 13). In cortical region Malphighian bodies were distributed in outer as well as inner cortex. Glomeruli were normal. Bowman's capsules

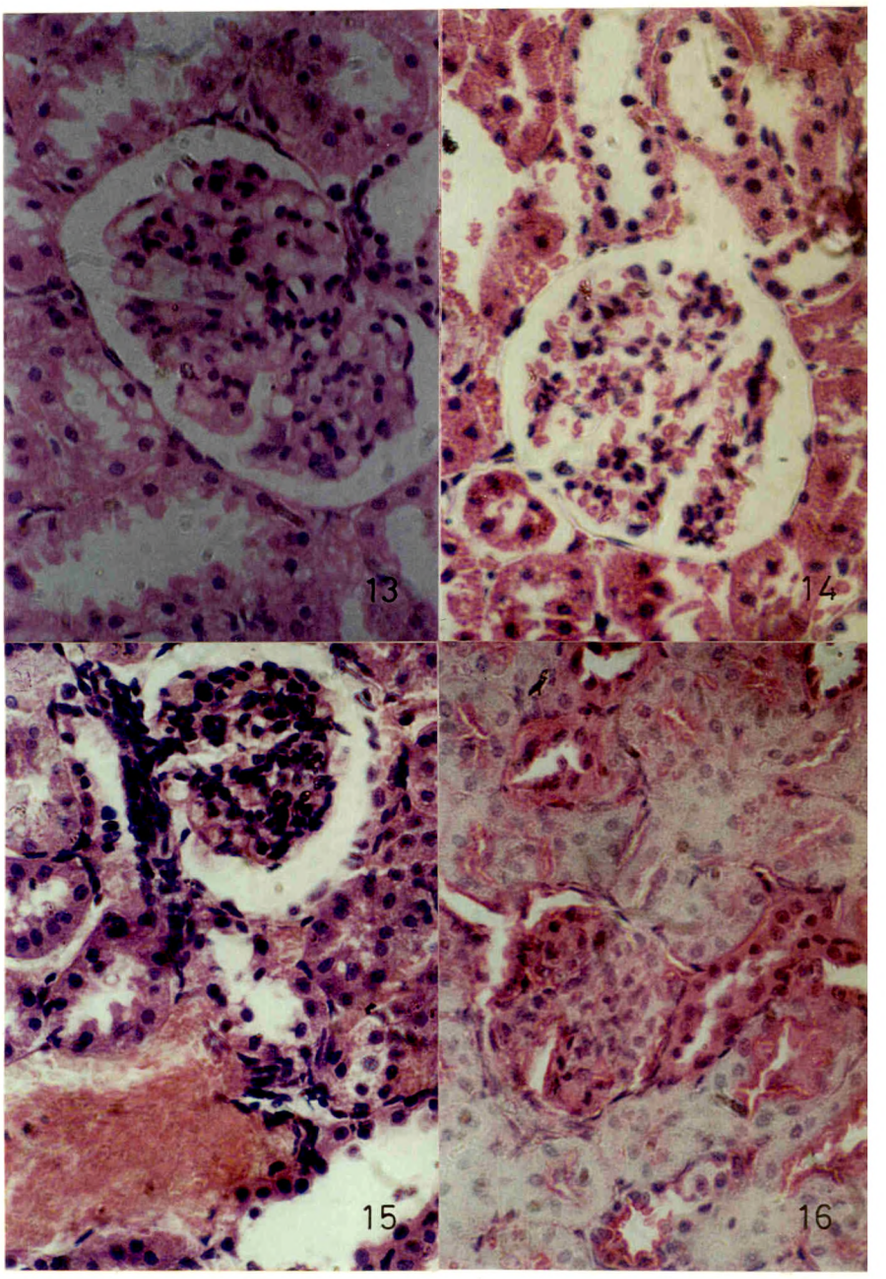
Captions to figs 13-16

Fig 13: Normal rat: Cortex region of Kidney: Glomeruli normal Bowman's capsule normal X 250

Fig 14: Lead nitrate treated rat (daily 20mg/kg body wt. given for 7 days): Cortex region of Kidney: Glomerular area reduced and appears blood bathed. Bowman's capsule appears normal. X 250

Fig 15: Lead nitrate treated rat (daily 20mg/kg body wt. given for 14 days): Cortex region of Kidney: Glomerular area shrinked. Blood reduced from glomerulus or withdrawn. Bowman's capsule appears partially swollen. Note the blood pool associated with glomerulus. X 250

Fig 16: Lead nitrate treated rat (daily 20mg/kg body wt. given for 21 days): Cortex region of Kidney: Glomerular area shrinked. Blood reduced from glomerulus or withdrawn. Bowman's capsule collapsed completely. Note the foggy glomerulus and surrounding tubules.X 250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were normal and were surrounded by proximal and distal tubules and the other parts of the uriniferous tubules.

20 mg lead nitrate treated rats :

The treatments of 20 mg lead nitrate/kg body wt/day were given for 7, 14 and 21 days. The alterations in kidney cortex were noted and presented in microphotographs.

The kidney showed normal appearance of glomerulus and Bowman's capsule after seven doses of lead nitrate (Fig. 14), but as compared to normal glomerulus the most of the glomeruli in kidney of lead nitrate treated rats showed flushed blood where RBCS are clearly visible. Bowman's capsules were normal surrounded by apparently normal tubules in kidney.

The treatment of lead nitrate for 14 successive days resulted in partially dilated Bowman's capsules and most of the glomeruli without any rushed blood (Fig. 15). The significant observation noted was the appearance of blood pools, wherever malphighian bodies were observed. This feature was the marking of 14^{th} day toxicity.

The treatment of 20 mg lead nitrate per kg body weight daily given for 21 days resulted in completely collapsed Bowman's capsules and most of the glomeruli showed thickly packed appearance with cloudy

basophilic material all over the body of the glomerulus (Fig. 16). The tubules surrounding the malphighian body were clouded heavily. Some of them were also free of the cloudy appearance. This feature was specific of the longest duration of treatment.

Nag bhasma treated rats :

Daily treatment of 30 mg, 60 mg and 90 mg Nag bhasma per kg body weight, was given to 9 groups of rats each group of which was killed on day 8, 15 and 22 of the treatment respectively. The alterations in kidney cortex were noted and presented in microphotographs.

30 mg Nag bhasma/kg body wt/day treated rats :

The treatment of seven doses of nag bhasma showed normal appearance of glomerulus and Bowman's capsule were normal surrounded by normal tubules in kidney (Fig. 17).

The treatment of Nag bhasma for 14 successive days resulted in normal Bowman's capsules and normal organisation of Malphighian bodies was observed (Fig. 18).

The normal Bowman's capsules normal glomeruli were noticed after the treatment of 30 mg Nag bhasma/kg body wt/ day given for 21 (Fig. 19). The tubules surrounding the Malphighian body were normal.

Captions to Figs. 17 -25

Fig 17: Nag bhasma treated rat (daily 30 mg/kg body wt. given for 7 days): Cortex region of Kidney.Most normal appearance of glomerulus and Bowmans capsule. X 250

Fig 18: Nag bhasma treated rat (daily 30 mg/kg body wt. given for 14 days): Cortex region of Kidney.Most normal appearance of glomerulus and Bowmans capsule. X 250

Fig 19: Nag bhasma treated rat (daily 30 mg/kg body wt. given for 21 days): Cortex region of Kidney. Most normal appearance of glomerulus and Bowmans capsule. X 250

Fig 20: Nag bhasma treated rat (daily 60 mg/kg body wt. given for 7 days): Cortex region of Kidney. Normal appearance of glomerulus and Bowmans capsule. X 250

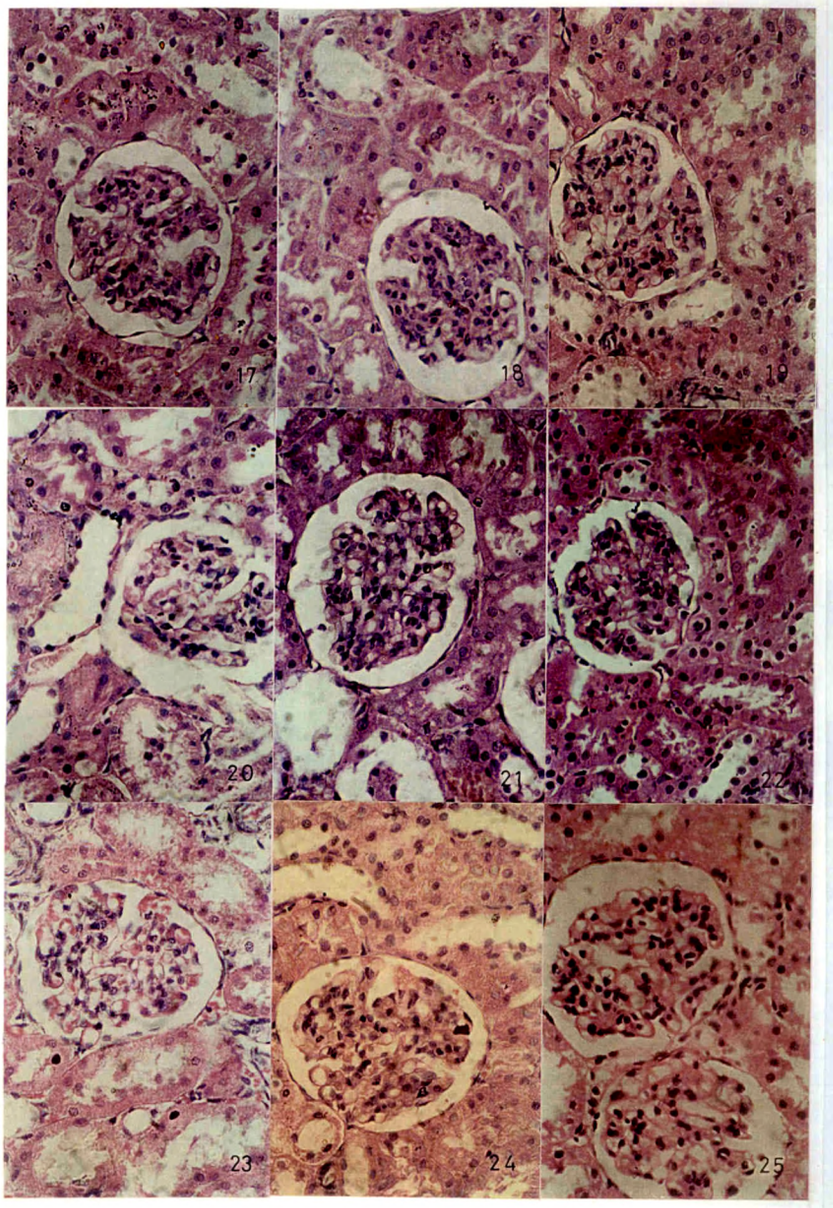
Fig 21: Nag bhasma treated rat (daily 60 mg/kg body wt. given for 14 days): Cortex region of Kidney. Normal appearance of glomerulus and Bowmans capsule. X 250

Fig 22: Nag bhasma treated rat (daily 60 mg/kg body wt. given for 21 days): Cortex region of Kidney. Normal appearance of glomerulus and Bowmans capsule. X 250

Fig 23: Nag bhasma treated rat (daily 90 mg/kg body wt. given for 7 days): Cortex region of Kidney. Ideal normal appearance of glomerulus and Bowmans capsule. X 250

Fig 24: Nag bhasma treated rat (daily 90 mg/kg body wt. given for 14 days): Cortex region of Kidney. Ideal normal appearance of glomerulus and Bowmans capsule. X 250

Fig 25: Nag bhasma treated rat (daily 90 mg/kg body wt. given for 21 days): Cortex region of Kidney. Ideal normal appearance of glomerulus and Bowmans capsule. X 250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60 mg Nag bhasma /kg body wt/day treated rats :

The treatment of Nag bhasma for 7 successive days resulted in normal organisation of Malphighian bodies was observed (Fig. 20).

The Bowman's capsules and glomeruli of rat kidney showed normal appearance after the administration of 60 mg nag bhasma per kg body weight daily given for 14 days (Fig. 21).

The treatment of 60 mg nag bhasma per kg body weight daily given for 21 successive days resulted in normal Bowman's capsules with normal glomeruli (Fig. 22).

90 mg Nag bhasma /kg body wt/day treated rats :

The treatment of seven doses of nag bhasma showed normal appearance of glomerulus and Bowman's capsule were normal surrounded by normal tubules in kidney (Fig. 23).

The treatment of Nag bhasma for 14 successive days did not affect the kidney and showed normal Bowman's capsules and normal organisation of glomeruli were observed (Fig. 24).

The treatment of 90 mg Nag bhasma per kg body weight daily given for 21 successive days resulted in normal Bowman's capsules

normal glomeruli showed The tubules surrounding the Malphighian body were normal (Fig. 25).

Proximal Tubules :

Normal rat –

The 3 groups of normal rats each containing 7 animals were maintained and killed on days 8, 15 and 22 along with the rats that were given treatments of lead nitrate (the dose of 20 mg lead nitrate /kg body weight of rats) and Nag bhasma (30 mg, 60 mg and 90 mg per kg body weight of rats). Since they have shown the similar architecture only one microphotgraph is used in description.

In cortical region proximal tubules were distributed in outer as well as inner cortex (Fig. 26). They were normal and were surrounded by other parts of the uriniferous tubules.

20 mg Lead nitrate/kg body wt treated rats :

The lead nitrate treatment of 20 mg per kg body weight daily was given to rats. Every time 7 rats were killed after the treatment of lead nitrate for 7, 14 and 21 days. The alterations in kidney cortex were noted and presented in microphotographs.

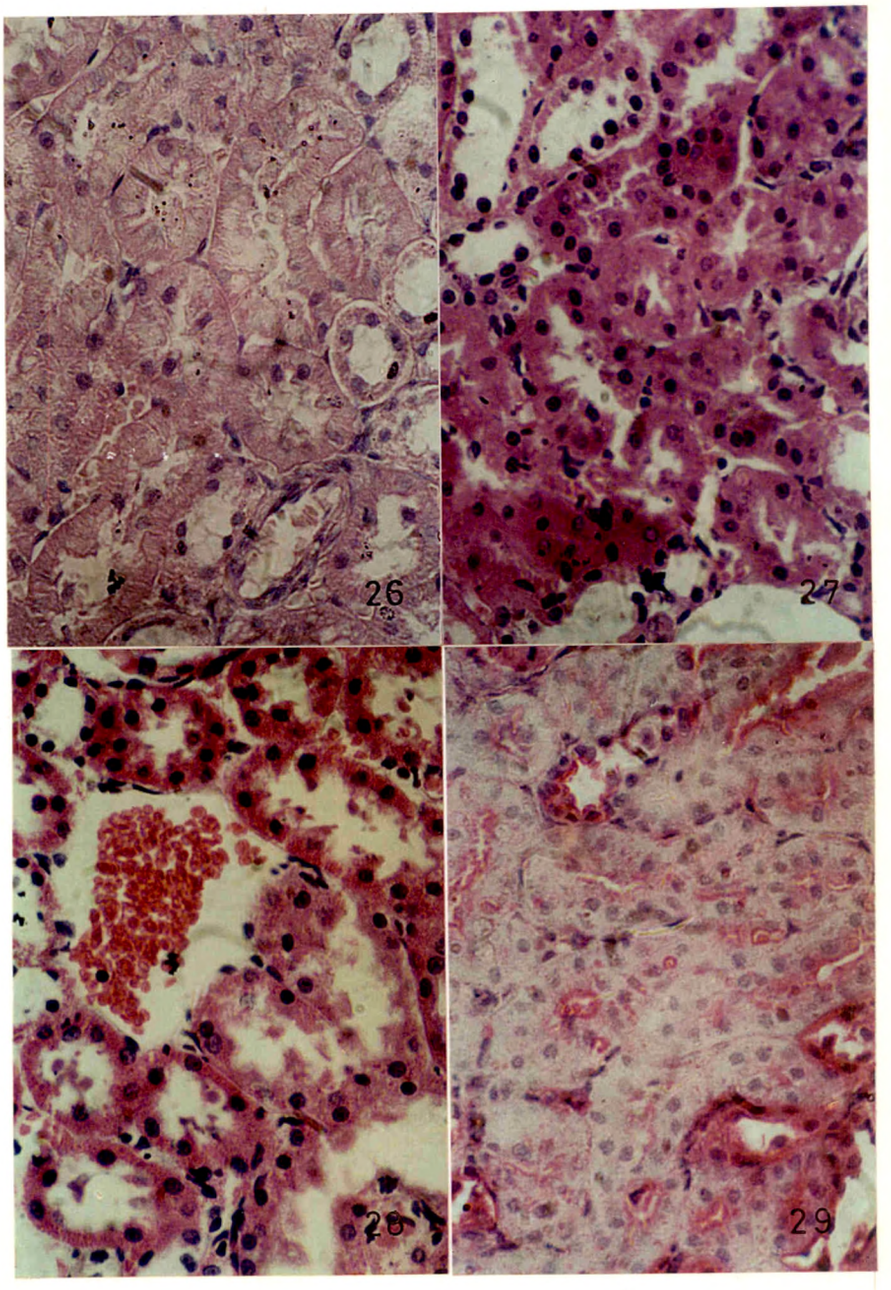
Captions to Figs. 26 - 29

Fig 26: Normal rat: Cortex region of Kidney: Proximal tubules normal X 250.

Fig 27: Lead nitrate treated rat (daily 20 mg/kg body wt. given for 7 days): Cortex region of Kidney: Swollen proximal tubules. Reduced lumina of tubules.X 250.

Fig 28: Lead nitrate treated rat (daily 20 mg/kg body wt. given for 14 days): Cortex region of Kidney: Proximal tubular lumina increased and tubular cell's area reduced. X 250.

Fig 29: Lead nitrate treated rat (daily 20 mg/kg body wt. given for 21 days): Cortex region of Kidney: Foggy proximal tubules with collapsed lumina. X 250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Proximal tubules of the rats treated with lead nitrate for 7 days showed normal appearance with notable eosinophilia (Fig. 27). In few cells of the tubules vacuolated cytoplasm was observed with reduced lumina.

Proximal tubules showed notable eosinophilia along with dilated lumina as a result of 14 days treatment of lead nitrate (Fig. 28). The blood pools markedly identified the kidney. Such pools were observed within the tubular parts of the cortex region.

Proximal tubules showed notable by their cloudy appearance by the administration of lead nitrate foe 21 days (Fig. 29). The kidney was markedly identified by foggy tubules densely packed with collapsed lumina.

Nag bhasma treated rats:

The alterations in kidney cortex caused by the treatments of 30, 60 and 90 mg Nag bhasma/g body wt/day were presented here in microphotographs.

30 mg Nag bhasma /kg body wt/day treated rats :

The normal histological appearance of proximal tubules was not altered by seven doses of nag bhasma (Fig.30). these proximal tubules were surrounded by other normal tubules of kidney.

Normal organisation of proximal tubules and other tubules of rat kidney was evident after the treatment of Nag bhasma for 14 successive days (Fig. 31).

The treatment of 30 mg nag bhasma per kg body weight daily given for 21 successive days did not change the normal histology of the tubules including proximal tubules (Fig. 32).

60 mg Nag bhasma/kg body wt/day treated rats :

The treatment of seven doses of Nag bhasma (60 mg/kg body wt/day) showed normal appearance of proximal tubules without any change (Fig. 33). Those were surrounded by other normal tubules of kidney.

The normal organisation of proximal tubules and other kidney tubules was not altered by the treatment of Nag bhasma for 14 successive days resulted in (Fig. 34).

The treatment of 60 mg nag bhasma per kg body weight daily given for 21 successive days exhibited normal histology of the tubules including proximal tubules (Fig. 35).

90 mg Nag bhasma/kg body wt/day treated rats :

The treatment of seven doses of nag bhasma showed normal appearance of proximal tubules those were surrounded by other normal tubules of kidney (Fig. 36).

The treatment of Nag bhasma for 14 successive days resulted in normal organisation of proximal tubules (Fig. 37).

The treatment of 90 mg nag bhasma per kg body weight daily given for 21 successive days showed normal tubules including proximal tubules without any change in histology (Fig. 38).

Distal tubules

Normal rat –

In cortical region distal tubules were distributed in outer as well as inner cortex. They were normal; and were surrounded by other parts of the uriniferous tubules (Fig. 39).

Lead nitrate treated rats :

The lead nitrate treatment (20 mg per kg body weight daily) was given to the rats for 7, 14 and 21 days. The alterations in kidney cortex were noted and presented in microphotographs.

Captions to Figs. 30-38

Fig30: Nag bhasma treated rat (daily 30mg/kg body wt. given for 7 days): Cortex region of Kidney. Normal appearance of proximal tubules. X 250

Fig 31: Nag bhasma treated rat (daily 30mg/kg body wt. given for 14 days): Cortex region of Kidney. Normal appearance of proximal tubules. X 250

Fig 32: Nag bhasma treated rat (daily 30mg/kg body wt. given for 21 days): Cortex region of Kidney. Normal appearance of proximal tubules. X 250

Fig 33: Nag bhasma treated rat (daily 60mg/kg body wt. given for 7 days): Cortex region of Kidney. Normal appearance of proximal tubules. X 250

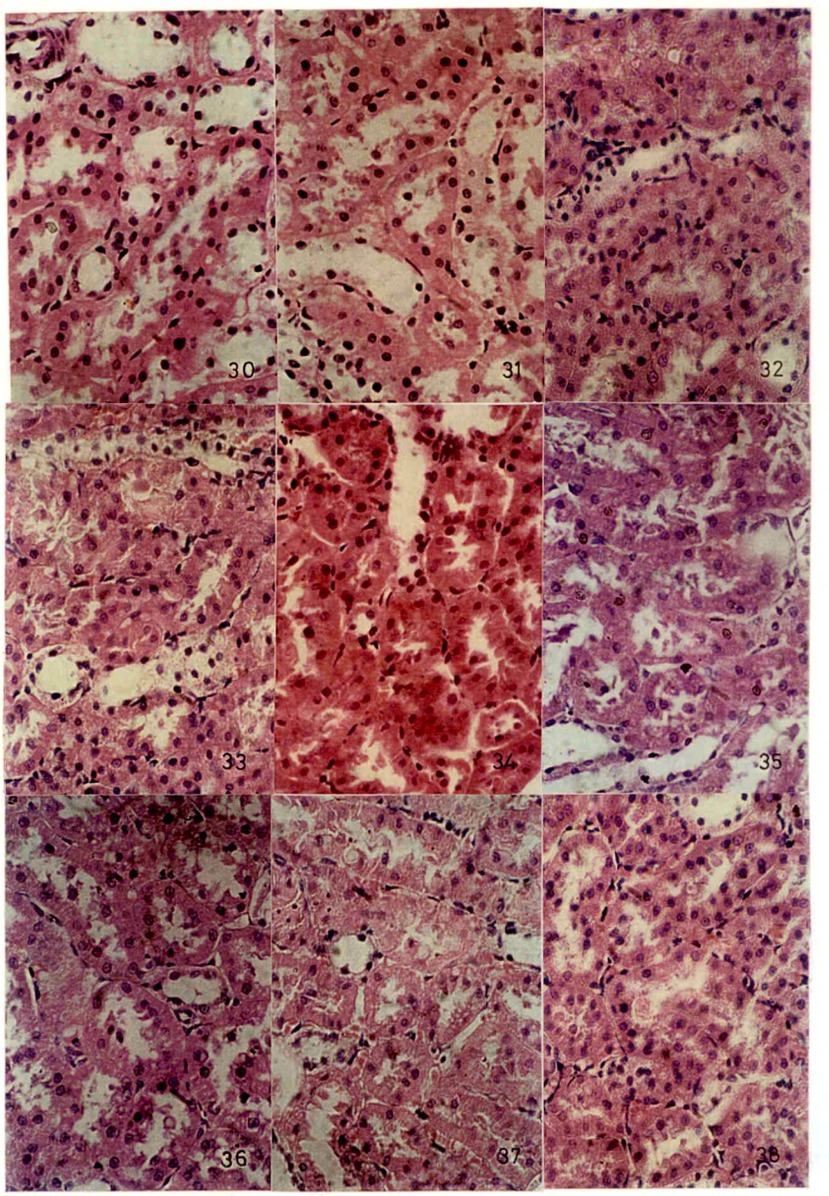
Fig 34: Nag bhasma treated rat (daily 60mg/kg body wt. given for 14 days): Cortex region of Kidney. Normal appearance of proximal tubules. X 250

Fig 35: Nag bhasma treated rat (daily 60mg/kg body wt. given for 21 days): Cortex region of Kidney. Normal appearance of proximal tubules. X 250

Fig 36: Nag bhasma treated rat (daily 90mg/kg body wt. given for 7 days): Cortex region of Kidney. Ideal normal appearance of proximal tubules.x 250

Fig 37: Nag bhasma treated rat (daily 90mg/kg body wt. given for 14 days): Cortex region of Kidney. Ideal normal appearance of proximal tubules. X 250

Fig 38: Nag bhasma treated rat (daily 90mg/kg body wt. given for 21 days): Cortex region of Kidney. Ideal normal appearance of proximal tubules.x 250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Distal tubules showed normal appearance with notable eosinophilia without any deviation by lead nitrate (Fig. 40). In few cells of the tubules vacuolated cytoplasm was observed. Small blood pools were noted distributed in cortex.

Distal tubules showed notable eosinophilia by 14 days treatments of lead nitrate (Fig. 41). The blood pools markedly identified the kidney. In addition to such pools fluid filled areas were observed within the tubular parts of the cortex region.

Distal tubules showed notable cloudy appearance due to the administration of lead nitrate for 21 days (Fig. 42). They differed from the proximal tubules, as they were distinct by their intensely stained eosinophilic apical domains against cloudy basal part of cells. The kidney was markedly identified by foggy tubules.

Nag bhasma day treated rats :

Daily treatments of 30, 60 and 90 mg Nag bhasma per kg body weight, was given the rats III, IV and V respectively. The alterations in kidney cortex were noted and presented in microphotographs.

30 mg Nag bhasma/kg body wt/day treated rats :

After the treatment of seven doses of Nag bhasma rat kidney showed normal appearance of distal tubules (Fig. 43) and was surrounded by other normal tubules of kidney.

The treatment of Nag bhasma for 14 successive days resulted in normal organisation of distal tubules (Fig. 44).

The treatment of 30 mg Nag bhasma per kg body weight daily given for 21 successive days resulted in normal tubules including distal tubules (Fig. 45).

60 mg Nag bhasma/kg body wt/day treated rats :

The treatment of seven doses of Nag bhasma showed normal appearance of distal tubules (Fig. 46) and was surrounded by other normal tubules of kidney.

Normal organisation of distal tubules was noticed after the treatment of Nag bhasma for 14 successive days (Fig. 47).

The treatment of 60 mg nag bhasma per kg body weight daily given for 21 successive days did not alter the normal histology of the tubules including distal tubules (Fig. 48).

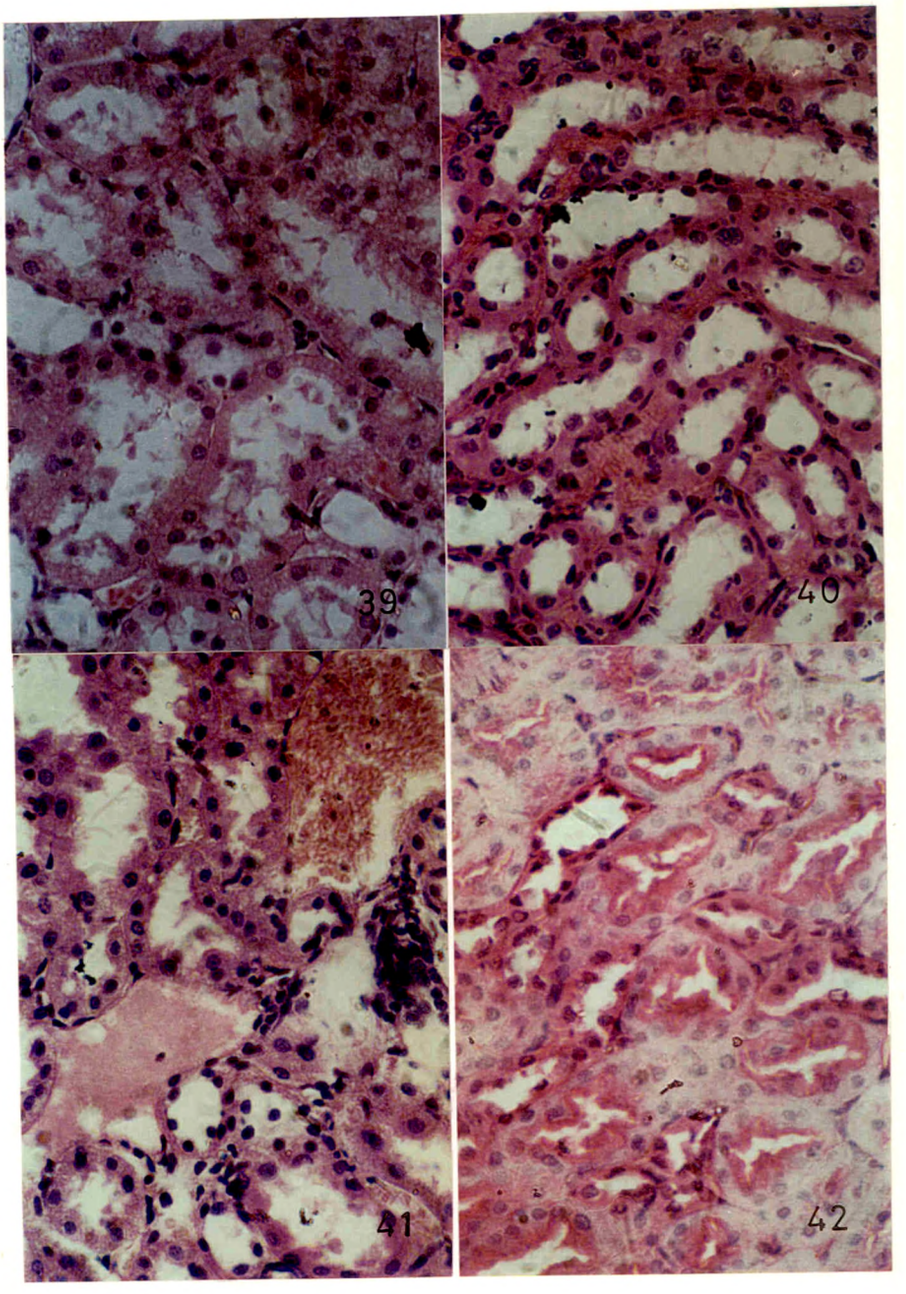
Captions to Figs. 39 - 42

Fig 39: Normal rat: Inner Cortex region of Kidney: Distal tubules normal X 250.

Fig40: Lead nitrate treated rat (daily 20 mg/kg body wt. given for 7 days):Inner Cortex region of Kidney:. Reduced lumina of distal tubules. Note blood accumulated areas.X 250.

Fig 41: Lead nitrate treated rat (daily 20 mg/kg body wt. given for 14 days):Inner Cortex region of Kidney: Distal tubular lumina increased and tubular areas were full of blood pools or fluid filled areas. X 250.

Fig 42: Lead nitrate treated rat (daily 20 mg/kg body wt. given for 21 days):Inner Cortex region of Kidney: Foggy Distal tubules with lumina frilled with eosin stained border.X 250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Captions to Figs. 43 - 51

Fig 43: Nag bhasma treated rat (daily 30 mg/kg body wt. given for 7 days): Cortex region of Kidney. Normal appearance of distal tubules. X 250

Fig 44: Nag bhasma treated rat (daily 30 mg/kg body wt. given for 14 days): Cortex region of Kidney. Normal appearance of Distal tubules. X 250

Fig 45: Nag bhasma treated rat (daily 30 mg/kg body wt. given for 21 days): Cortex region of Kidney. Normal appearance of distal tubules. X 250

Fig 46: Nag bhasma treated rat (daily 60 mg/kg body wt. given for 7 days): Cortex region of Kidney. Normal appearance of distal tubules. X 250

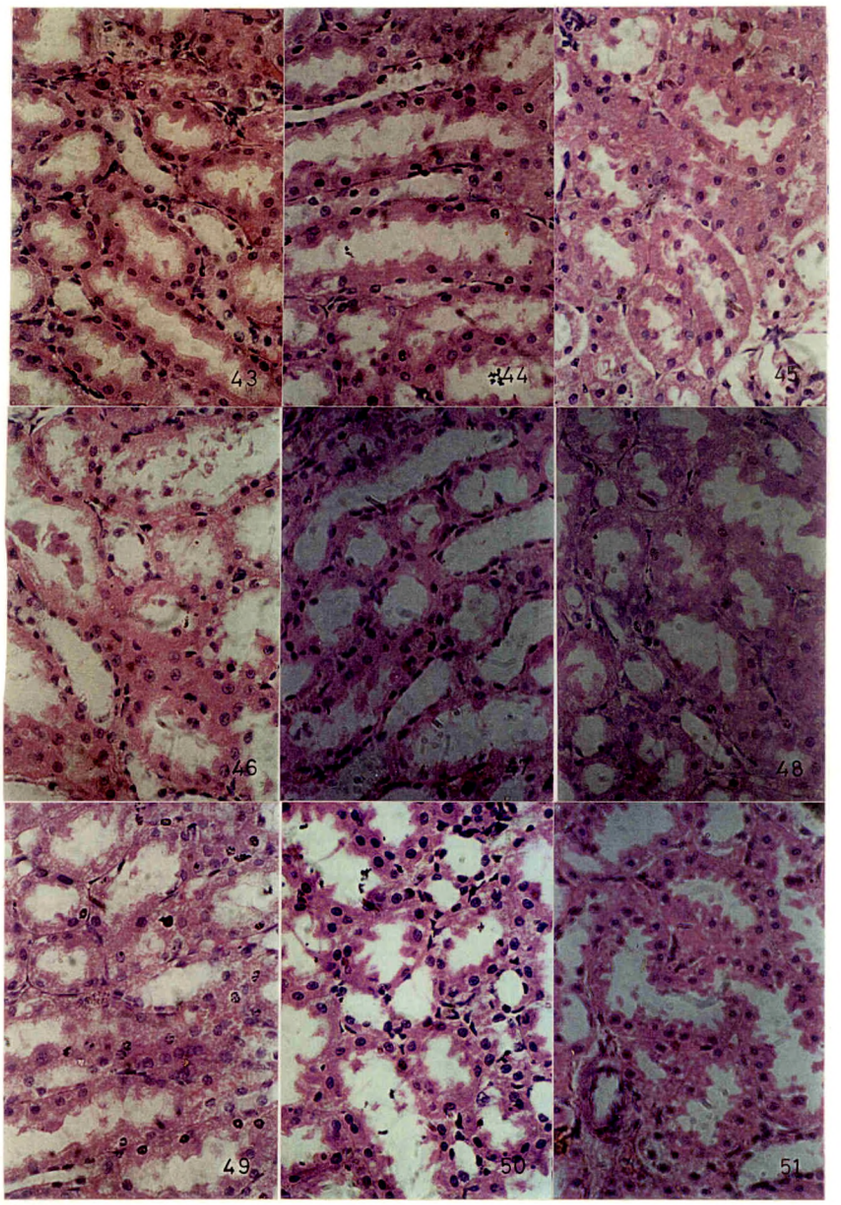
Fig 47: Nag bhasma treated rat (daily 60 mg/kg body wt. given for 14 days): Cortex region of Kidney. Normal appearance of distal tubules. X 250

Fig 48: Nag bhasma treated rat (daily 60 mg/kg body wt. given for 21 days): Cortex region of Kidney. Normal appearance of distal tubules. X 250

Fig 49: Nag bhasma treated rat (daily 90 mg/kg body wt. given for 7 days): Cortex region of Kidney. Ideal normal appearance of distal tubules.x250

Fig 50: Nag bhasma treated rat (daily 90 mg/kg body wt. given for 14 days): Cortex region of Kidney. Ideal normal appearance of distal tubules. X 250

Fig 51: Nag bhasma treated rat (daily 90 mg/kg body wt. given for 21 days): Cortex region of Kidney. Ideal normal appearance of distal tubules.x250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90 mg Nag bhasma/kg body wt/day treated rats :

The treatment of seven doses of Nag bhasma showed normal appearance of distal tubules those were surrounded by other normal tubules of kidney (Fig. 49).

The treatment of Nag bhasma for 14 successive days did not change the normal organisation of distal tubules (Fig 50).

90 mg Nag bhasma/kg body wt/day treated rats :

The treatment of 90 mg nag bhasma per kg body weight daily given for 21 successive days showed the normal tubules including distal tubules without any change in histology of distal and other kidney tubules (Fig. 51).

Medulla:

Collecting tubules:

Normal rat –

Collecting tubules:

In medullary region, collecting tubules showed normal distribution of tubules (Fig. 52).

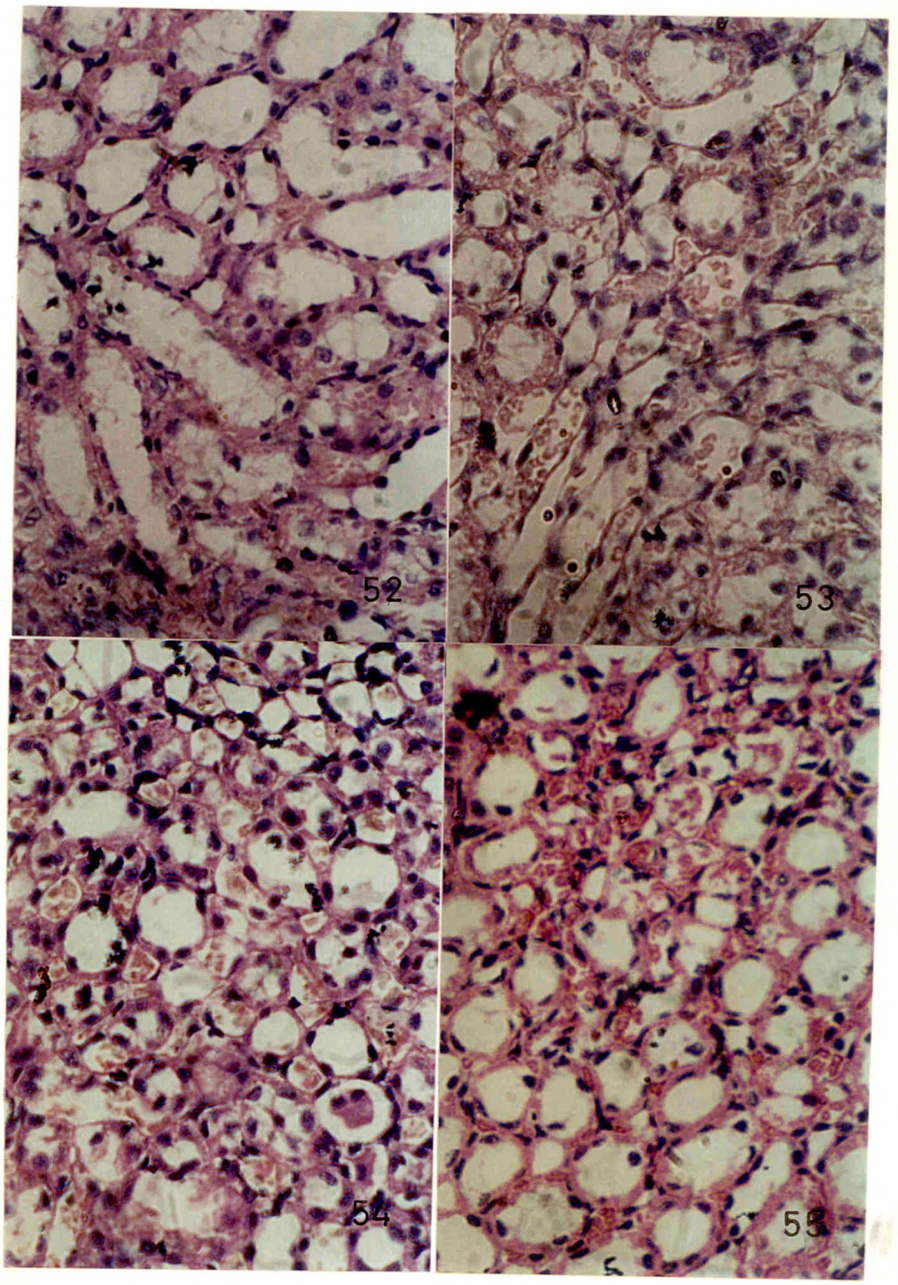
Captions to Figs. 52 - 55

Fig 52: Normal rat: medulla region of Kidney: X 250

Fig 53: Lead nitrate treated rat (daily 20 mg/kg body wt. given for 7 days): Medulla region of Kidney: Collecting tubules were filled with blood accumulated areas. X 250

Fig 54:Lead nitrate treated rat (daily 20 mg/kg body wt. given for 14 days): Medulla region of Kidney: Collecting tubules with blood areas distributed all medullary region.X 250

Fig 55:Lead nitrate treated rat (daily 20 mg/kg body wt. given for 21 days): Medulla region of Kidney: Numerous areas with blood filled pools.X 250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20 mg Lead nitrate/kg body wt/day treated rats :

The treatments lead nitrate (20 mg per kg body weight daily) was given to the rats of group II for 7, 14 and 21 days respectively. The alterations in kidney medulla were noted and presented in microphotographs.

Collecting tubules showed normal appearance with notable eosinophilia due to 7 doses of lead nitrate (Fig. 53). The medullary tubules were intermingled with blood pools.

Collecting tubules showed notable eosinophilia due to 14 days treatment of lead nitrate (Fig. 54). The blood pools markedly identified the kidney. In addition to such pools some cloudy areas were observed within the tubular parts of the medulla region.

Collecting tubules showed much blood containing areas distributed in medullar region by the administration of lead nitrate for 21 days (fig. 55).

Nag bhasma treated rats:

The alterations in kidney medulla were noted and presented in microphotographs.

Captions to Figs.56 - 64

Fig 56: Nag bhasma treated rat (daily 30 mg/kg body wt. given for 7 days): Medulla region of Kidney. Most normal appearance of collecting tubules.X 250

Fig 57: Nag bhasma treated rat (daily 30 mg/kg body wt. given for 14 days): Medulla region of Kidney. Most normal appearance of collecting duct. X 250

Fig 58: Nag bhasma treated rat (daily 30 mg/kg body wt. given for 21 days): Medulla region of Kidney. Most normal appearance of collecting tubules. X 250

Fig 59: Nag bhasma treated rat (daily 60 mg/kg body wt. given for 7 days): Medulla region of Kidney. Normal appearance of Collecting tubules.X 250

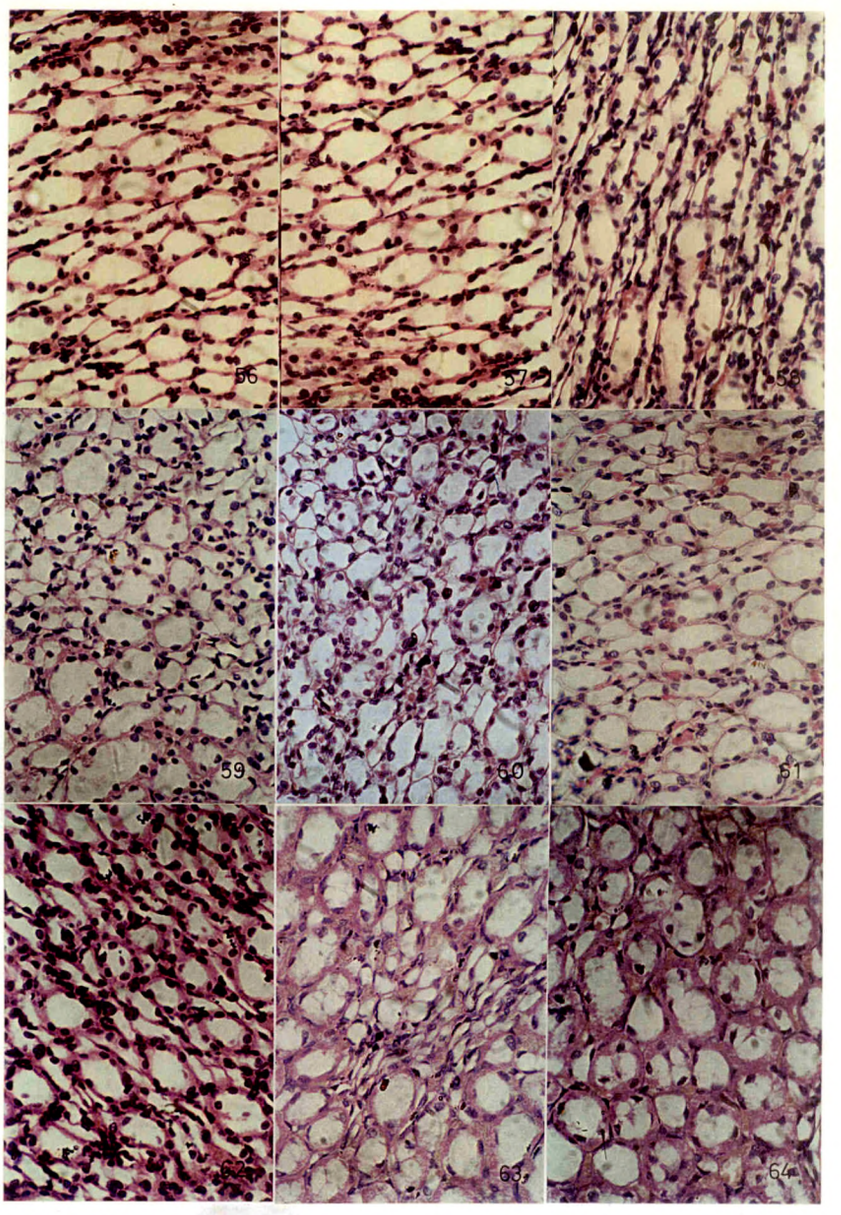
Fig 60: Nag bhasma treated rat (daily 60 mg/kg body wt. given for 14 days): Medulla region of Kidney. Normal appearance of Collecting tubules.X 250

Fig 61: Nag bhasma treated rat (daily 60 mg/kg body wt. given for 21 days): Medulla region of Kidney. Normal appearance of collecting tubules. X 250

Fig 62: Nag bhasma treated rat (daily 90 mg/kg body wt. given for 7 days): Medullary region (from transitory region) of Kidney. Normal appearance of Collecting tubules. X 250

Fig 63: Nag bhasma treated rat (daily 90 mg/kg body wt. given for 14 days): Medullary region (from transitory region) region of Kidney. Ideal normal appearance of Collecting tubules. X 250

Fig 64: Nag bhasma treated rat (daily 90 mg/kg body wt. given for 21 days): Medullary region (from transitory region) region of Kidney. Ideal normal appearance of Collecting tubules. X 250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30 mg Nag bhasma/kg body wt/day treated rats :

By the treatment of seven doses of Nag bhasma (30 mg/kg body wt/day) to the rats, the histology of kidney medulla was not altered and showed normal appearance of collecting tubules (Fig. 56).

The treatment of Nag bhasma for 14 successive days did no alter the normal histology of the kidney medulla and organisation of collecting tubules (Fig. 57).

After the treatment of 30 mg nag bhasma per kg body weight daily given for 21 successive days showed in normal tubules including collecting tubules (Fig. 58).

60 mg Nag bhasma/kg body wt/day treated rats :

The treatment of seven doses of Nag bhasma showed normal appearance of collecting tubules in medulla (Fig.59).

The treatment of Nag bhasma for 14 successive days did not change the normal histological organisation of collecting tubules (Fig. 60).

After the treatment of 60 mg nag bhasma per kg body weight daily given for 21 successive days showed in normal collecting tubules (Fig. 61).

60 mg Nag bhasma/kg body wt/day treated rats :

Normal appearance of collecting tubules was observed by the treatment of 90 mg Nag bhasma for 7 days (Fig. 62).

The treatment of Nag bhasma for 14 successive days did not change the normal organisation of collecting tubules (Fig. 63)

90 mg Nag bhasma/kg body wt/day treated rats :

The treatment of 90 mg Nag bhasma per kg body weight daily given for 21 successive days did not alter the normal tubules including collecting tubules (Fig. 64).

DISCUSSION

The normal architecture of periarterial region was altered by the administration of lead nitrate. Many spaces were observed in lobular area. Distribution of Kupffer cells was not significantly altered. Bile canaliculi were obliterated. Hepatocytes were necrotic and few were normal, but all of them were not defined in their boundaries. Though similar structure was noted in centrolobular region, the lobular spaces were less and necrotic cells were also less in number.

The results indicate that the periarterial region showed more toxic effects as evidenced by lobular spaces (due to dead cells), necrotic

cells (effect of lead nitrate), obliterated bile canaliculi (Failure to clear bile and hence increased blood bile pigments), not defined cell boundaries (failure to secrete bile and lipoproteins possibly). The high toxicity in periarterial region may be due to non-clearance of urea, which is produced in this region (increased blood urea).

For studies on nuclei the sections of liver and kidneys were stained by Toludine Blue (pH 3.00) + Eosin as described in Chapter II (Material and Methods) the results were confirmed using Methylene blue and Hematoxylin. Similarly histochemical localisation of lead was also studied by two methods. Lead was noted only in livers of 21 days treated rats. Some of the cells in groups in periarterial and centrolobular region showed lead deposits in nuclei. The detailed observations of toludine blue B-eosin stained slides showed heavy reduction in nuclear staining. The literature on intra nuclear inclusions (Robert and Goyer, 1968; Mahaffey et al, 1981; Fowler, 1998), give their ultra structure, staining properties in detail; but it neglects the light microscopic or electron microscopic observations concerned with nuclei. It may be because of the large doses that were used and excessive interests in nuclear inclusions since they occupy the major nuclear space. In present work lead depositions were stained histochemicaly. These results indicated that in early intervals of 7 and 14 days of treatment histochemicaly

demonstrable lead was not detected in liver either in nuclei or any other part of the cytoplasm. But cytologicaly detectable lead was observed only on 21 days of treatments and that also in nuclei of some hepatocytes. The islands of heavily lead-stained cells were noted in periarterial and centrolobular region. But nearby cells did not show affinity to lead stains used. When the nuclear staining and distribution of lead that was reported predominantly in nuclei are compared, irrespective of the nuclear inclusions the nuclei showed reduction in nuclear staining by Toludine blue. Thus the nuclear lead deposits in the nuclei do not affect Toludine blue positive nuclear material is reduced by lead nitrate treatment. Numerous nuclei were enlarged in their volume may be as a result of accumulation of material. In liver since polyploid nuclei also show increased nuclear volume, possibility of such cells can not be avoided but as compared to normal liver their occurrence was more detectable and hence the dilation of nuclei due to altered physiological conditions of cells may be possible. Increased volume of nuclei may be in case of some accumulation of lead in some polyploid cells and may be in some cells infiltration of material with response to lead toxicity.

Thus results indicate that some of the cells preferentially accumulate histochemicaly detectable lead indicating differential

response of cells in accumulation of lead may be physiological status of the cells is the influential factor in it.

The appearance of cells in the liver of 7th and 14th days of treatment did not show any apparent change either in periarterial or centrolobular region except that their occurrence was more in periarterial region. But the hepatic cells of the rat that received 21 doses of lead nitrate showed more vacuolar cells in the periarterial region than noted in the centrolobular region. The necrotic cells showed vacuolar appearance different from the vacuolated necrotic cells that were noted earlier in CCl_4 induced centrolobular necrosis, where a thin layer of cytoplasm surrounds the nuclei from where cytoplasmic strands extended through vacuolar region to the rim of cytoplasm at periphery of the cell (Kanase et al, 1994; 1997). In present observations the vacuolated cells in periarterial and centrolobular regions showed vacuolar area more concentrated in perinuclear region than and nuclei appear in the hollow space around it. These observations indicated that the type of vacuolar necrosis induced, as lead toxicological effects is different from the CCl4 induced necrosis.

Seven, fourteen and twenty-one doses of 30, 60 and 90 mg Nag bhasma per kg body wt treatments had hardly altered the normal light microscopic picture of the liver. In all the slides observed the hepatocytes

were more ideal in appearance than even that are observed in the normal liver. Considering the fact only microphotographs of last interval used in studies are exhibited.

The picture of periarterial region showed well organised architecture with clear hepatic cords, well defined cellular boundaries, clear bile canaliculi, normal distribution of Kupffer and sinusoidal cells and basophilia in cytoplasm of the few of the cells. Similar picture of the centrolobular region was observed with many cells of pentagonal appearance. Similar architecture of centrolobular region was also observed in the livers of the rats that received 60mg-nag bhasma per kg body weight for 21 days. Even the centrolobular region of liver also showed earlier described architecture. The daily dose of 90mg per kg body weight administered for 21 days resulted in healthy appearance of liver architecture both in periarterial region and centrolobular region. The centrolobular region included well-organized cords that contained the cells with defined boundaries, clear bile canliculi, normally distributed Kupffer and sinusoidal cells. It differed from periarterial region as well as liver architecture of any of the nag bhasma treated animals in having the recognisable basophilia in the hepatocytes.

These features more prominently exhibited the normal picture of liver indicating no hepatic toxicity. But it should be noted here that other

form of effects that results in hyperactivity of liver (Schwarz, 1974; Luckey, 1975b) was not noted in case of nag bhasma by any of the doses used for studies. Both the regions of the liver showed cleared bile canaliculi and more ideal appearance of hepatic cords and cells with clear boundaries; showing no stress of even of the normal metabolism. The distribution of Kupffer and sinusoidal cells indicate normal trafficking of the cells in lobular regions. The results are continuous with the claims of Ayrveda (Ayurveda Sarsangrah, 1971) where the nag bhasma is used as the general tonic. There remains a need to study the other claims of the nag bhasma in appropriate physiological models.

The normal histology of kidney was changed by the dose of 20mg-lead nitrate per kg body weight of the animal for 7,14 and 21days.On administration of 7 doses in majority of the Mapighian bodies, the Bowmans' capsule was dilated, and the glomeruli were bathing in blood. The proximal tubules and distal tubules appeared swollen and in some areas accumulation of blood was observed. The blood occupied small areas were also noted in medullary regions. When the lead nitrate treatment was continued for 14 days, the glomeruli showed shrunken appearance while Bowmans' capsules showed dilation. The proximal and distal tubules showed widen lumina. In the surrounding area many blood pools were visible. In the medullar tubular lumina showed

numerous contents with stagnant blood in the region. The toxic effects observed on day 22nd of the treatment of lead nitrate included collapsed Bowmans' capsules, glomeruli with foggy material. The proximal and distilled tubules were foggy so that nuclear staining appeared very faint. The proximal tubules showed obliterated lumina while luminal surface of the distal tubules was thick and intensly stained by eosin. In the cortex many large pools of blood or eosinophilic fluid were observed. The medullar tubules continue to remain filled with the material and blood accumulated areas.

The observations indicate that with advancing intervals of lead nitrate treatments, the accumulation of blood increased glomerular filtration affected proximal and distal tubules were swollen, foggy, followed by collapse of lumina which indicates failure of proximal and distal tubular functions.

But these type of effects were not observed in the kidney of rats that were treated with daily doses of 30mg, 60mg and 90mg nag bhasma per kg body weight for 7, 14 and 21 days. The appearance of glomeruli, Bowmans' capsules, proximal and distal tubules and medullar tubules after all the intervals studied was free of any type of foggy appearance or blood pools. The architecture of kidney was normal as observed in the microphotographs of various regions of kidney. The

results indicate that nag bhasma in the studied doses and intervals is not either hepatotoxic or nepherotoxic.

In low concentration lead is capable of stimulating liver (Schwarz, 1974; Luckey, 1975b) and the lead withdrawal followed by involution of liver occurs through apoptosis (Columbano *et al*, 1985). Lead stimulates kidney also (Cho and Richter, 1974); while the toxic capacity of lead depends on the bioavailability of lead which in turn depends on the solubility of the lead compound (Dieter *et al*, 1993). The toxic capacity can be monitored by different treatments *viz.* by simultaneous treatment of Zinc (Satija and vij, 1995); by decreasing the led absorption in intestine by rhamnogalacturonan II dimer (Tahiri *et al*, 2000); elimination of lead by chelating compounds in garlic (Hanafy *et al* 1994). All of these observations indicate lead is capable of improving biological capacities of liver and kidney when available in proper proportion and proper form. Nag bhasma is prepared by the numerous herbal treatments (described in Material and Methods) to lead. By these treatments lead may have been converted into biologically acceptable nontoxic form may be a organometallocomplex and hence its toxicity or hyperactivity may not be observed while the cellular metabolisms may be kept in equilibrium and this state may be the condition when side effects are not observed. In Ayurveda Sarsangrah (1971) or in Rasa shastra

(Bodes, 1981) the authors have alerted in preparation of nag bhasma about free lead and methods are also described to detect it. Thus it appears that Writers of Ayurveda were aware of the lead toxicity; and they knew how to eliminate it. To test the other effects and also to test the purity of bhasma there is a need of bioassay development since the crude herbs/products are being used. To test them on the basis of modern drug pharmacology is injustice to the drugs and their Ayurvedic pharmacology.