

Protection of Mice Liver with Liv.52 Against Cadmium Intoxication

Rathore, H.S. and Rita Verma

School of Studies in Zoology, Vikram University, Ujjain, India.

ABSTRACT

20 ppm of cadmium chloride (CdCl_2) 10 ml solution mixed in food was fed to one group of mice for 6 to 30 days. The second group of mice CdCl_2 and 5 ml of Liv.52 syrup. The third group acted as control. There was a significant rise in liver enzymes in the CdCl_2 treated group as compared with the control on days 15 and 31. Histology of the liver revealed dead hepatocytes and damaged blood vessels as compared to the normals. Liv.52 protected the liver from the toxic effects of CdCl_2 . Mild damage caused by CdCl_2 on day 5 was reverted to normal with Liv.52 on days 15 and 31. The results suggest that Liv.52 protects mice liver against CdCl_2 toxicity.

INTRODUCTION

The mammalian liver has been shown to be the organ most susceptible to the toxic effects of Cd following exposure to it (Meek, 1959; Friberg *et al.*, 1974; Hoffmann *et al.*, 1975). Dudley *et al.* (1982) suggests that Cd-induced lethality is due, in part, to hepatic failure and hepatic necrosis. Recently, it has been reported that Zn pretreatment could reduce Cd-induced hepatotoxicity (Goering and Klaassen, 1984 a and b). Thus the aim of this investigation was to determine if administration of Liv.52 along with CdCl_2 in food can prevent liver damage so that Liv.52 can be used to cure Cd-induced liver dysfunction in man professionally or accidentally exposed to it.

MATERIALS AND METHODS

Four months old Swiss albino male mice, obtained from the Biological Products Division, Veterinary College, Mhow (M.P.) were used. The mice were placed in propylene cages, and were given water and food *ad libitum* 2.5 ml syrup of Liv.52 contains extracts of the following plants:

<i>Capparis spinosa</i>	17 mg
<i>Cichorium intybus</i>	17 mg
<i>Solanum nigrum</i>	8 mg
<i>Cassia occidentalis</i>	4 mg
<i>Terminalia arjuna</i>	8 mg
<i>Achillea millefolium</i>	4 mg
<i>Tamarix gallica</i>	4 mg

Treatment:

The mice were divided into three large groups:

- A. Liv.52 syrup 5 ml + cadmium chloride 10 ml of 20 ppm solution mixed with 50 gm of food
- B. Cadmium chloride 10 ml of 20 ppm solution mixed with 50 gm of food
- C. Standard food 50 gm

Each group of mice was further divided into three subgroups of 8 mice each. Three groups of 8 mice received treatment A, three groups of 8 mice received treatment B and three groups of 8 mice received standard food alone.

All mice from each group receiving treatments A, B and C were sacrificed on day 6, day 15 and day 31 from the date of starting treatment. Their livers were removed and divided into two parts; one part was fixed in Bouine's fluid or aceto-alcohol for histological examination and the second part was homogenized in chilled 0.9% saline, and the supernatant was used for estimation of GOT, GPT, alkaline phosphatase and bilirubin at a private pathological laboratory. The sections from fixed livers were stained with haematoxylin and eosin, while the acetoalcohol fixed sections were stained with methyl green pyronin.

Camera lucida drawings were made on brown paper using tube type camera lucid with oculometer and stage micrometer. Figures measuring microns were corrected for magnification to obtain actual dimensions.

RESULTS AND DISCUSSIONS

Histological Observations

The livers of control mice showed typical hepatocytes arranged around a central vein which had smooth margins. No inclusion, infiltration or necrosis was observed (Figs.1 and 2). After 6 and 15 days of CdCl₂ feeding the livers showed infiltration of acute inflammatory cells, necrosis, pyknosis and karyorrhexis of the nuclei (Figs.3 to 6). Rough margins of blood vessels indicated injury. Following the 30th day of CdCl₂ feeding the livers showed all the same pathological signs observed after 15 days of Cd feeding but with greater intensity, as well as small-sized hepatocytes at a few places indicating regeneration (Figs.7 and 8). Livers from Liv.52 + CdCl₂ fed mice showed pathological changes (Figs. 9 and 10) on day 6; but following 15 days administration of Liv.52 with CdCl₂ the livers showed distinct nuclei at certain places and almost smooth margins of blood vessels (Figs.11 and 12). 31 days after Liv.52 + CdCl₂ feeding, the livers showed normal structures like the controls, as no pathological signs were present except scanty infiltration (Figs.13 and 14).

Table 1 shows the mean cellular and mean nuclear diameters of liver cells in the three treatment groups. For each section twenty hepatocytes were examined and the mean diameters estimated for each animal. It is seen that at 31 days the mean diameters of group B (cadmium alone) were significantly smaller than those of group C (control). But group A (Liv.52 + cadmium) mice were no different from controls. On days 6 and 15 the nuclear diameters in group A were not significantly different from those in controls.

Table 1: Showing mean cellular diameters and nuclear diameters of hepatocytes from animals of the three groups sacrificed on days 6, 15 and 31

Group	No. of animals	Duration of treatment	Cellular diameter (microns) mean \pm SE	Nuclear diameter (microns) mean \pm SE
C – Control	8	31	20.472 \pm 1.06	6.563 \pm 0.323
B-Cadmium chloride	8	6	Cell membranes distorted	4.763 \pm 0.178*
	8	15	Cell membranes distorted	4.818 \pm 0.232*
	8	31	16.727 \pm 0.590	5.327 \pm 0.254*
A– Cadmium chloride + Liv.52	8	6	Cell membranes distorted	5.927 \pm 0.136
	8	15	17.563 \pm 0.778*	5.8 \pm 0.247
	8	31	22.581 \pm 0.343	7.2 \pm 0.150

*Significant based on 't' test at 5% level of significance. In each case 8 slides were observed and about 20 hepatocytes in each case were measured for nuclear and cytoplasmic diameters.

PHOTOMICROGRAPHS OF MICE LIVERS (T.S.)

Fixed in Bouine's Fluid and Stained with Haematoxylin and Eosin

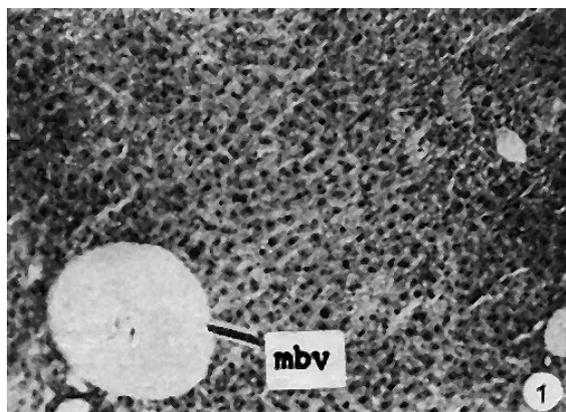


Fig. 1: Control: Regular pattern of normal hepatocytes. Distinct rounded nuclei. Rounded blood vessel with smooth margin. No inclusion and no infiltration (100 x)

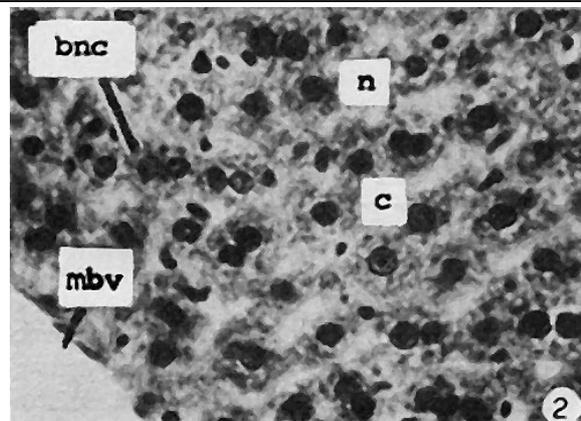


Fig. 2: Magnified view of Fig. 1 to show smooth margin of blood vessel, typical hepatocytes and a few bi-nucleated cells (400 x)

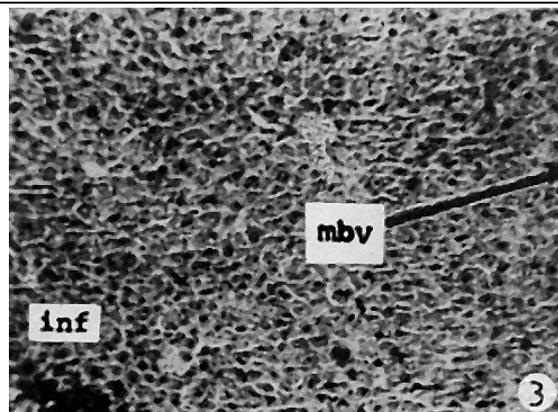


Fig. 3: 6 days after CdCl₂ treatment. Nuclei and cell walls of hepatocytes are damaged. Blood vessel collapsed with roughened margins. Infiltration of blood cells (100 x)

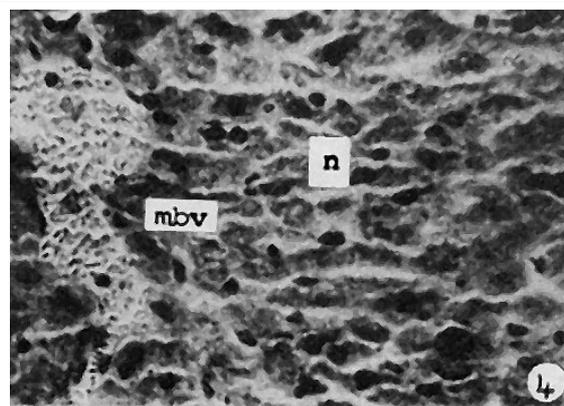


Fig. 4: Magnified view of Fig. 3 to show damaged nuclei; blood vessel and infiltration of blood cells (400 x)

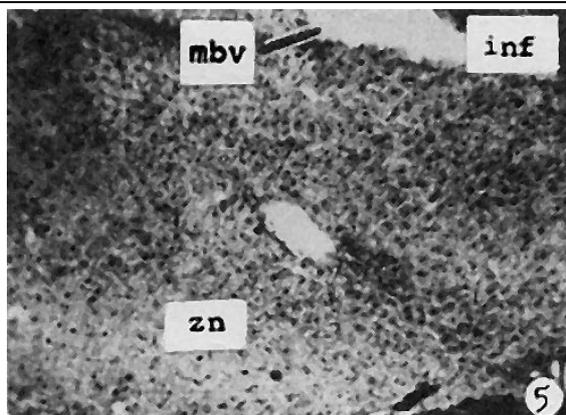


Fig. 5: 15 days after CdCl₂ treatment. advanced necrosis; cell wall and nuclei both are damaged. Roughened margins of blood vessel, infiltration of blood cells and dark stained particles are seen (100 x)

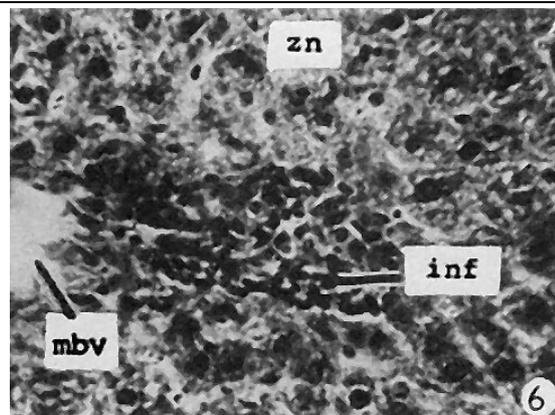


Fig. 6: Magnified view of Fig. 5 to show badly damaged hepatocytes and blood vessel. Infiltration of blood cells (400 x)

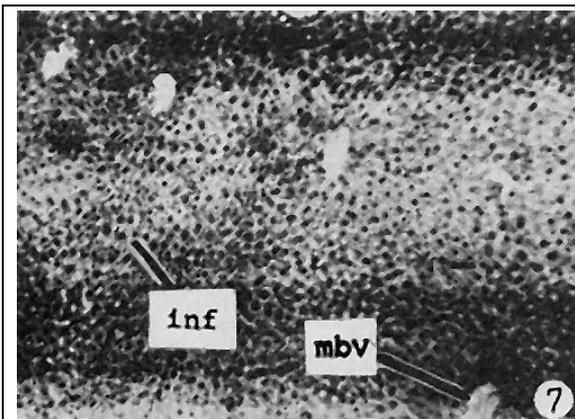


Fig. 7: 31 days after CdCl₂ treatment. Small hepatocytes with small nuclei suggest regeneration. Still blood vessels do not show smooth margins and infiltration is evident. Dark stained particles are seen (100 x)

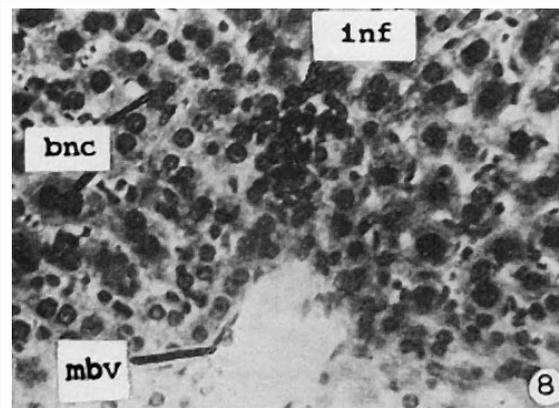


Fig. 8: Magnified view of Fig. 7 to show that regeneration has started but blood vessels are damaged. Size of nuclei is smaller when compared with Fig. 2. Infiltration is evident (400 x)

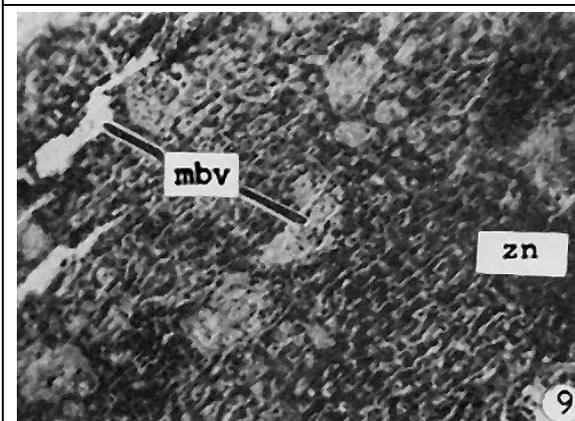


Fig. 9: 6 days after Liv.52 therapy. Necrosis evident. Infiltration and dark stained particles are seen. Blood vessels are collapsed and do not show smooth margins (100 x)

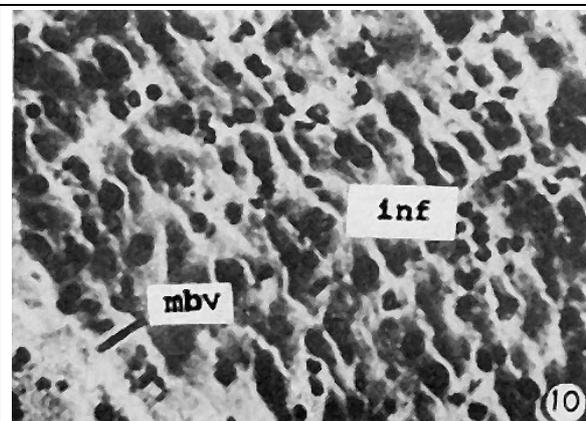


Fig. 10: Magnified view of Fig. 9 to show damaged liver (400 x)

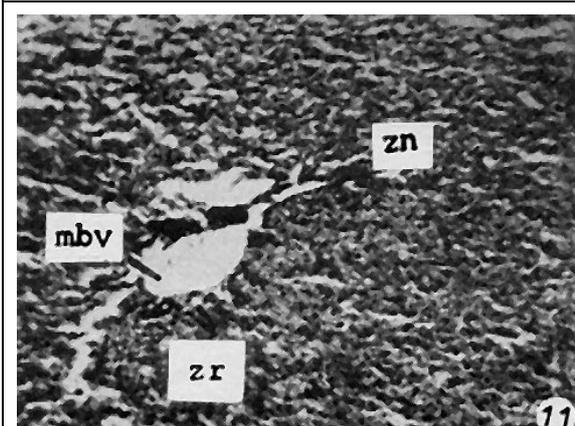


Fig. 11: 15 days after Liv.52 therapy. Zones of necrosis and zones of regeneration are seen. Roughened margins of blood vessel and infiltration are seen (100 x)

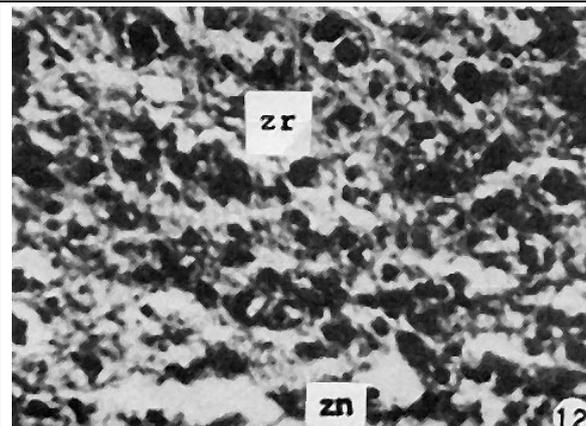


Fig. 12: Magnified view of Fig. 11 to show regenerated cells near the damaged ones (400 x)

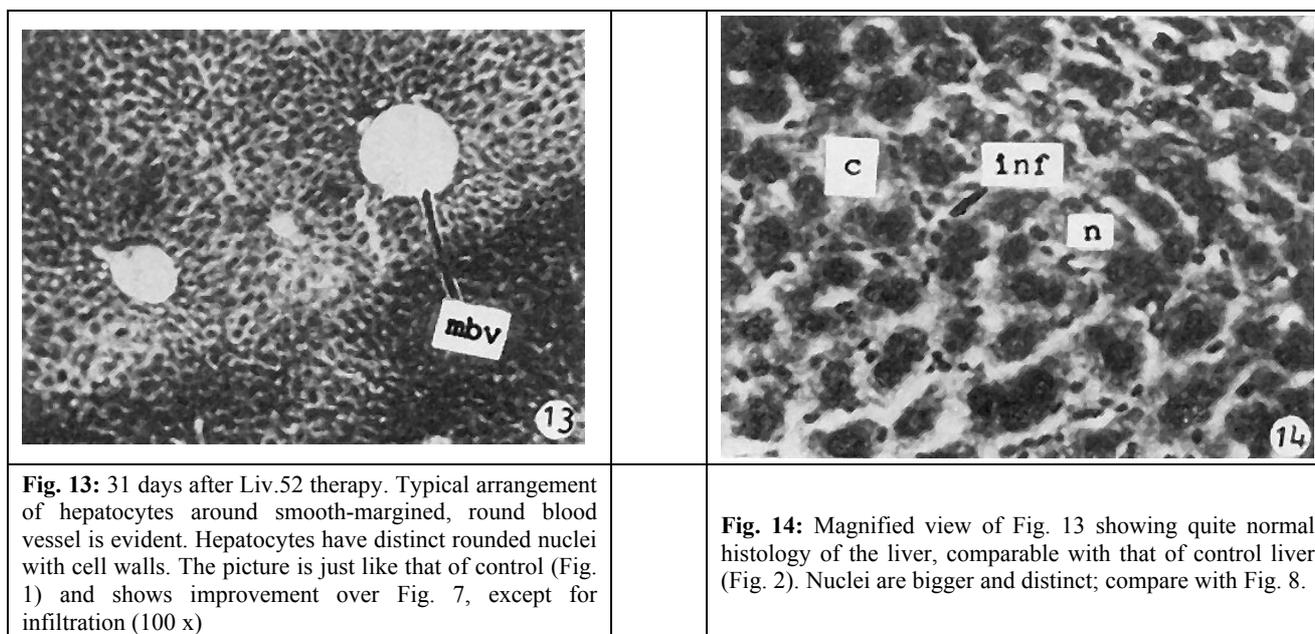


Table 2 shows the mean changes in GOT, GPT, alkaline phosphatase (AP) and bilirubin in liver homogenates. Cadmium chloride treatment (Group B) caused significant elevations in these parameters. But simultaneous treatment (Liv.52 + Cadmium chloride) in Group A prevented these changes.

Table 2: Results of biochemical estimations from liver homogenates from mice of the three groups sacrificed on the 31st day (mean \pm SEM)

Group	GOT (Units/ml)	GPT (Units/ml)	Alk. phosphatase (KA Units/ml)	Bilirubin (mg/100 ml homogenate)
C – Control (8)	191.45 \pm 6.925	187.27 \pm 17.129	1.327 \pm 0.042	0.454 \pm 0.072
B – Cadmium chloride (8)	266.18 \pm 31.690*	303.63 \pm 37.290	9.963 \pm 2.836	0.727 \pm 0.114
A – Cadmium chloride + Liv.52	163.63 \pm 36.78	155.00 \pm 21.709	1.381 \pm 0.334	0.272 \pm 0.11

*Significantly high, based on 't' test at 5% level of significance, when compared with controls. Numbers in parentheses indicate number of animals used.

At the end of 31 days, biochemical studies showed increased activities of GOT, GPT and AP following CdCl₂ feeding. These returned to normal following Liv.52 addition with CdCl₂ feeding.

DISCUSSION

The results suggest that CdCl₂ causes pathological changes in mice liver, which include infiltration with acute inflammatory cells and cytoplasmic eosinophilia, congestion and diffuse necrosis (Figs.3 to 8). These results are in complete conformity with previous reports (Dudley *et al.*, 1982; Goering and Klaassen, 1984 a and b). The morphological evidence of liver injury is consistent with the biochemical observation as CdCl₂ caused a sharp rise in the activity of GOT, GPT and AP: and this is also a known effect of Cd (Prodan, 1932; Piscator, 1966; Nogawa *et al.*, 1980). Enhanced activities of these enzymes might have been a result of enzyme induction by Cd as suggested by Rees and Sinha (1966). A toxin can stimulate increased production of certain enzymes. Indeed

Shakoori *et al.* (1982) and Krample *et al.* (1975) have found a sharp rise in the activities of GOT, GPT and AP in rat liver homogenates following dieldrin feeding.

Normal hepatic architecture following Liv.52 + CdCl₂ feeding is possibly due to the protective effect of Liv.52 which is known to protect the liver from necrosis induced by alcohol, carbon tetrachloride paracetamol, thioacetamide and radiation (Joglekar *et al.*, 1963; Joglekar and Balwani, 1967; Joglekar and Leevy, 1972; Prasad, 1976; Saini and Saini, 1985). Liv.52 also maintains normal activities of GOT, GPT and AP in serum as it protects as well as promotes liver regeneration (Qazi, 1965; Majumdar and Kulkarni, 1977; Subbarao and Gupta, 1976; Prasad, 1975).

Liv.52 possesses some additional properties, which can probably nullify the toxicity of cadmium. Liv.52 corrects nucleic acid and protein synthesis (Subbarao and Gupta, 1978), which is impaired by cadmium (Furnuta, 1978). Liv.52 enhances the activity of the microsomal drug metabolizing enzymes (Saxena and Garg, 1979; Thabrew *et al.*, 1982; Bardhan *et al.*, 1985) which is lowered by cadmium (Taere *et al.*, 1976). Liv.52 protects cellular membranes (Saxena *et al.*, 1980) from toxic agents, while cadmium binds with membranes and damages them (Taere *et al.*, 1976). Cadmium can damage blood vessels (Gunn *et al.*, 1963) while Liv.52 can protect them at least against radiation-induced damage (Saini and Saini, 1984).

According to Goering and Klaassen (1984 a and b) zinc pretreatment reduces Cd-induced hepatotoxicity by altering the hepatic subcellular distribution of Cd. The mode of action of Liv.52 needs to be further studied.

ACKNOWLEDGEMENTS

The authors thank Prof. G.N. Johri, D.Sc. for providing departmental facilities and The Himalaya Drug Company, Bombay for the research grants.

REFERENCES

1. Bardhan, P., Sharma, S.K. and Garg, N.K., *Ind. J. Med. Res.* (1985): 82, 359
2. Dudley, R.E., Svoboda, D.J. and Klaassen, C.D., *Toxicol. Appl. Pharm.* (1982): 65, 302
3. Friberg, L., Piscator, M., Nordberg, G. and Kyllstrom, T., "Cadmium in the environment", 2nd Ed., CRC Press, Cleveland, Ohio (1974).
4. Furuta, H., *Experimentia* (1978): 34, 1317,
5. Goering, P.L. and Klaassen, C.D., *Toxicol. Appl. Pharm.* (1984 a): 74, 299
6. Goering P.L. and Klaassen, C.D., *Toxicol. Appl. Pharm.* (1984 b): 74, 308
7. Gunn, S.A., Gould, T.C. and Anderson, W.A.D., *Am. J. Pathol.* (1963): 42, 685
8. Hoffmann, E.D., Cook, J.A., Diluzio, N.R. and Coover, J.A., *Lab. Invest.* (1975): 37, 655
9. Joglekar, G.V. and Balwani, J.H., *J. Exp. Med. Sci.* (1967): 11, 7
10. Joglekar, G.V. and Leevy, C.M., *Probe* (1972): 2, 81

11. Joglekar, G.V., Chitale, G.K. and Balwani, J.H, *Acta Pharmacol. Et Toxicol.* (1963): 20, 73
12. Krample, V. and Hladka, A.M., *Bull. Environ. Contem. Toxicol.* (1975): 14, 571
13. Mujumdar, S.M. and Kulkarni R.D., *The Ind. Practit.* (1977): 11 and 12, 479
14. Neek, E.S., *Brit. J. Exp. Pathol.* (1959): 40, 503
15. Nogawa, Koji, "Itai-Itai disease and follow-up studies", in "Cadmium in the environment, Part II", (Ed Nriagu J.O.), Wiley Interscience, N.Y. and Toronto, 1980, pp. 1-34.
16. Piscator, M., "Proteinuria in chronic cadmium poisoning –"Thesis", K.L. Beckmans Tryckerier A.B. Stockholm (1966): 1.
17. Prasad, G.C., *J. Res. Ind. Med.* (1975): 4, 15.
18. Prasad, G.C. *J. Res. Ind. Med. Yoga & Homeo.* (1976): 4, 38.
19. Prodan, L., *J. Indust. Hyg.* (1932): 14, 132.
20. Qazi, I.H., *Probe* (1965): 1, 1.
21. Rees, K.R. and Sinha, K.P., *J. Pathol. Bacteriol.* (1960): 80, 297.
22. Saini, M.R. and Saini, N., *Radiobiol. Radiother.* (1985): 26, 379.
23. Saini, M.R., Kumar, S., Jagetia, G.C. and Saini, N., *The Ind. Practit.* (1984): 12, 1133.
24. Saxena, A. and Garg, N.K., *Ind. J. Exp. Biol.* (1979): 17, 662.
25. Saxena, A., Sharma, S.K. and Garg, N.K., *Ind. J. Exp. Biol.* (1980): 18, 1330.
26. Shakoori, A.R., Rasul, Y.G. and Ali, S.S. *Pak. J. Zool.* (1982): 14, 191
27. Subbarao, V.V. and Gupta, M.L., *Yugoslav. Physiol. Pharma. Acta* (1976): 12
28. Subbarao, V.V. and Gupta, M.L., *The Ind. Practit.* (1978): 11, 831.
29. Taere, F.W., Jasansky, P. and Reneaud, P.R., *Toxicol. Appl. Pharm.* (1976): 41, 57
30. Thabrew, M.L., Godwin, O.E. and subbarao, V.V., *Toxicol. Letters* (1982): 14, 183.