[***Probe*** (1990): (XXIX), 4, 280]

# Recovery from Beryllium-induced Lesions after Liv.52 Treatment

**Prakash, A.O., Mathur, S.** and **Mathur, R.**
School of Studies in Zoology, Jiwaji Universsity, Gwalior, India.

[Abstracted from: "Environmental and Occupational Health Hazards"
Proceedings – Asia-Pacific Symposium on Environmental and Occupational Toxicology,
held at Singapore, No. 8, 1988, p. 293]

## ABSTRACT

*An Ayurvedic remedy, Liv.52 (The Himalaya Drug Company) has been investigated as a prophylactic agent against beryllium-induced toxicity in rats. Liv.52-primed adult rats were exposed to beryllium nitrate intravenously and were killed after 2, 10 and 30 days of exposure. Various organs were studied for histopathological changes. Most of the organs revealed severe necrotic changes and maximum damage was observed at the 10 day schedule. In the remarkable changes, the liver cells were rounded in appearance and the cytoplasm showed globular spaces of variable sizes accompanied with pyknotic nuclei. The kidney showed degeneration of Bowman's capsule and increase in the number of glomerular nuclei. Lung histoarchitecture revealed emphysema and thickening in the interalveolar space. Splenic white and red pulp showed atrophying features with hemosiderin pigmentation. However, none of these changes was observed after Liv.52 treatment as they reverted to normalcy.*

## INTRODUCTION

Beryllium is a rare earth metal, which has found wide application in the manufacture of atomic reactors, space crafts, defence equipment and tank sheets[1]. Starting from its extraction from the ores to the metallic transformation, workers both in mines and industries come in contact with beryllium chiefly through skin contact and inhalation[2]. The toxic effects of beryllium salts in laboratory animals have been reviewed by a number of investigators[3-4]. Furthermore, in order to overcome these toxic effects the use of chelating agents like ATA, sodium citrate, sodium salicylate, EDTA etc., has been studied but even the most effective agent, ATA itself causes toxicity and hence could not be used frequently[5-7]. An Ayurvedic remedy, Liv.52, which is known to protect the liver from various toxic substances[8,9] has been used in the present investigation against beryllium-induced toxicity in rats through histopathological lesions.

## MATERIALS AND METHODS

Adult female albino rats (150 ± 10 g) of Sprague Dawley strain were selected from the animal colony of the department. All the rats were maintained under uniform husbandry conditions of light and temperature and were given a pelleted diet (Hindustan Lever, Bombay) and water *ad libitum*. Beryllium nitrate was dissolved at a concentration of 0.316 mg/ml in pyrogen-free distilled water and injected to the experimental animals intravenously once only at a dose of 1 ml/kg body weight ($1/10^{th}$ of $LD_{50}$). The animals were divided into 4 groups and were treated as follows:

*Group 1* – Animals were given vehicle only. *Group 2* – Animals were first primed with Liv.52 for 10 days and then received Liv.52 daily (1 ml/rat/d) till the last day of the experiment. *Group 3* – Animals were administered beryllium nitrate intravenously once only at a dose of 0.316 mg/kg and the time of administration was designated as zero hour. *Group 4* – Animals were first primed with Liv.52 for 10 days and then exposed to beryllium nitrate once only at 0.316 mg/kg dose (IV). Simultaneously these animals received daily doses of Liv.52 till the 30$^{th}$ day of experiment.

After 24 hours of the last treatment the animals were sacrificed using light ether anaesthesia. The vital organs viz., liver, kidney, lungs and spleen were excised, freed from adhering tissue, and were fixed in alcohol-Bouin's fluid for 7-8 hours. The material was processed for the preparation of paraffin blocks through the alcoholic series using the methyl benzoate method. Later, haematoxylin-eosin stained slides were examined for changes in the cellular organization.

**RESULTS**

The histopathology of the various organs with different treatments is described below.

*Liver*

After 2 days of beryllium exposure, the hepatic vein showed dilation and prenecrotic changes were observed in most of the hepatocytes (Fig. 1). Cytoplasmic granulation and vacuolations was remarkable in the periphery. The liver showed severe necrotic changes after 10 days of beryllium exposure. In addition to the changes observed earlier the hepatic blood vessels also showed congestion, fibrosis and infiltration of red blood cells (Fig. 2). After 30 days of administration similar histological alterations were observed, but these changes were less conspicuous in comparison to previous durations. Additionally the peripheral hepatocytes showed hypertrophy, granulation and vacuolation (Fig. 3).

With conjoint treatment of Liv.52 in beryllium-exposed rats the liver histoarchitecture showed less

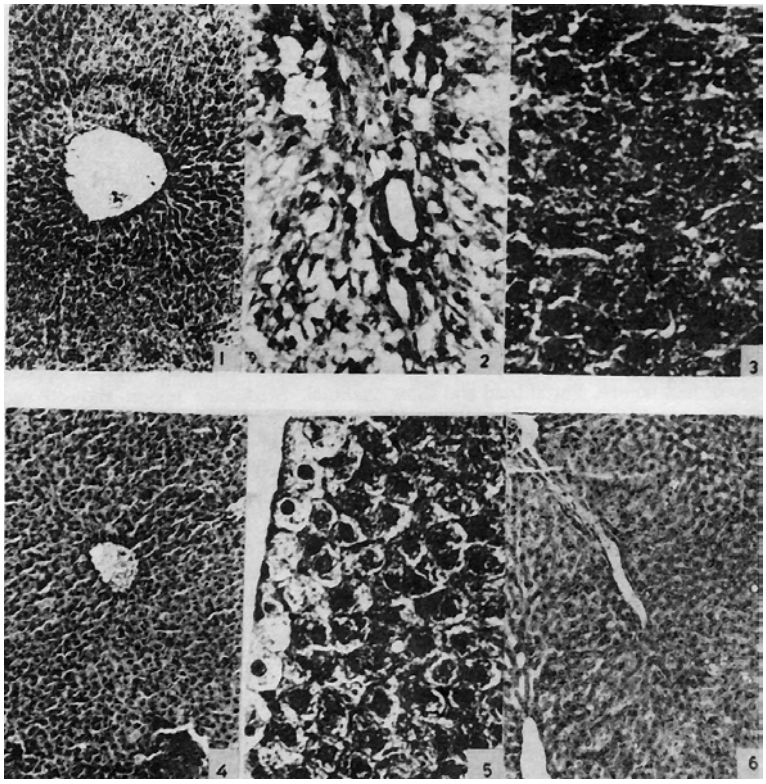


**Plate 1: Figs. 1-6:** Photomicrographs of rat liver exposed to beryllium nitrate.
**Fig. 1:** After 2 days of treatment. Note dilation in central vein and necrotic changes in peripheral region (X 120). **Fig. 2:** After 10 days of treatment. Hepatic blood vessels are fibrotic and show congestion (X 400). **Fig. 3:** After 30 days of exposure. Note hypertrophy and damage in the peripheral region (X 400). **Fig. 4:** After 2 days with Liv.52. Hepatocytes are normal but congestion is present in the sinus (X 400). **Fig. 5:** After 2 days with Liv.52. Note the cellular hypertrophy and vacuolations at the periphery (X 400). **Fig. 6:** After 30 days with Liv.52. Note well maintained portal triads (X 400).

damaged in comparison to beryllium nitrate-treated animals. At the 2-day schedule some binucleate hepatocyes were observed and congestion was present in the sinusoids (Fig. 4). The Kupffer cells were normal and towards the periphery the liver picture showed damage with considerable increase in the hepatic parenchyma (Fig. 5). After 10 days of beryllium and Liv.52 treatment, the liver picture showed less damage as the hepatic cells were cuboidal and chord arrangement was maintained. After 30 days of beryllium exposure and Liv.52 treatment the liver picture was more or less normal. Portal triad and sinus showed normal structure and moderate number of Kupffer cells (Fig. 6).

*Kidney*

Numerous pathological changes were observed in the kidney after I.V. exposure to beryllium nitrate at various durations. After 2 days of beryllium exposure the glomeruli occupied the whole Bowman's capsule (Fig. 7) and cortical tubules showed exfoliation of the nuclei in the lumen. Some of the tubules showed degeneration with darkly stained nuclei and vacuolations in the cytoplasm. After 10 days of beryllium administration, similar pathological changes were observed; however, degeneration was increased in the Bowman's capsule and cortical tubules (Fig. 8). The cuboidal epithelial lining of the tubules was completely damaged and most of the nuclei had undergone degeneration. Some of the medullary tubules showed complete liquefaction (Fig. 9). After 30 days' beryllium exposure the capsular wall became thick and fibrotic at some places. The number of degenerated tubules had also increased. The peripheral glomeruli occupied the whole Bowman's capsule (Fig. 10), while those towards the medulla showed a shrunken appearance. Anastomosis of medullary tubules and scanty

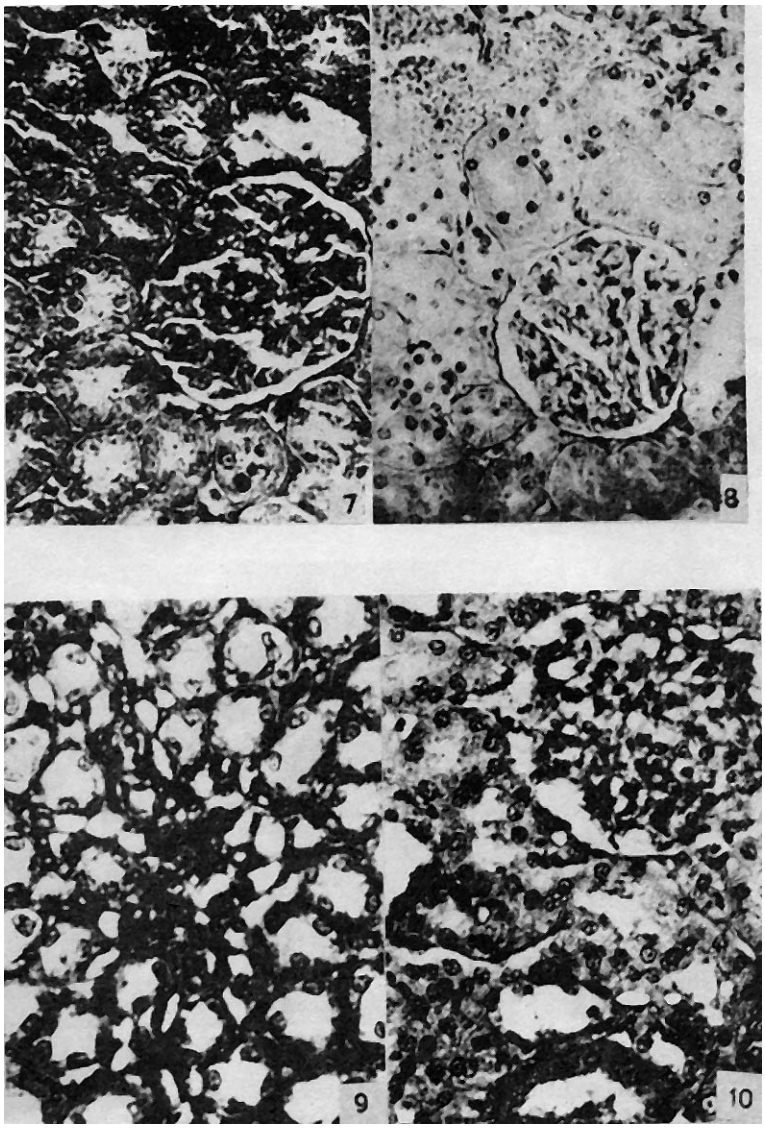


**Plate 2: Figs. 7-10:** Photomicrographs of rat kidney exposed to beryllium nitrate.
**Fig. 7:** After 2 days of treatment. Note degeneration in the Bowman's capsule (X 400). **Fig. 8:** After 10 days of treatment. Note increased degeneration in Bowman's capsule and tubules (X 400). **Fig. 9:** After 10 days of treatment. Note complete liquefaction of some medullary tubules (X 400). **Fig. 10:** After 30 days of exposure. Note degeneration in glomeruli which occupy the whole Bowman's capsule (X 400).

stroma were observed. Liv.52 treatment prior to and after beryllium administration had improved the kidney histoarchitecture significantly. At the 2-day schedule the renal corpuscles appeared fairly normal (Fig. 11); however, in some cortical tubules, slight hypertrophy still persisted. The nuclei of both cortical and medullary regions appeared normal. Medullary region showed accumulation of debris and exfoliated cells (Fig. 12). At the 10-day schedule the kidney revealed some improvement. Majority of the tubules showed regeneration. Normal pattern of the tubules was partially restored in the medulla. At the 30-day schedule some of the cortical tubules showed disturbed cuboidal epithelium with dark nuclei. Obliteration of the lumen due to slight hypertrophy of the epithelial cells was observed. In the medullary region the general appearance was more or less normal (Fig. 13); however, in some regions focal degeneration was observed.

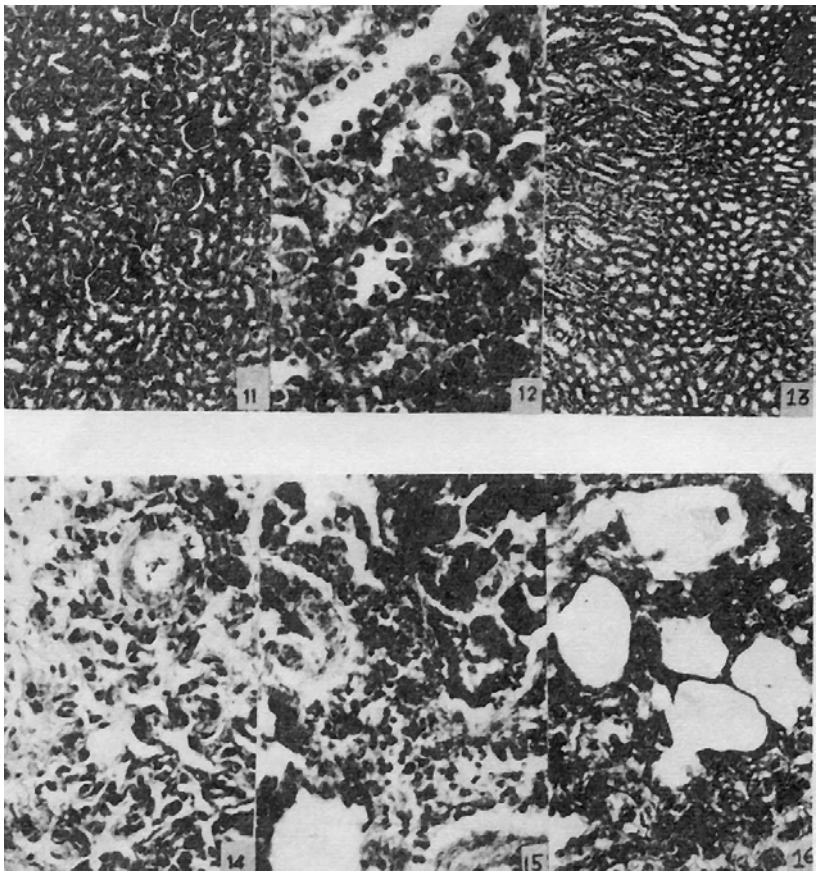


**Plate 3:**
**Figs. 11-13:** Photomicrographs of rat kidney exposed to beryllium nitrate. **Fig. 11:** After 2 days with Liv.52. Note significant improvement in renal corpuscles and tubules (X 120). **Fig. 12:** After 2 days with Liv.52. Medullary tubules show accumulation of debris and exfoliated cells (X400). **Fig. 13:** After 30 days with Liv.52. Medullary tubules are more or less normal.

**Figs. 14-16:** Photomicrographs of rat lung exposed to beryllium nitrate. **Fig. 14:** After 2 days of exposure. Note the presence of fibroblasts and considerable increase in pyknotic nuclei (X 400). **Fig. 15:** After 10 days of treatment. Note increased number of phagocytes, blood cells and encapsulated masses of particles (X 400). **Fig. 16:** After 10 days of exposure. Note large alveoli and accumulation of various cells in alveolar parenchyma (X 400).

*Lungs*

After 2 days of beryllium treatment the pulmonary alveoli showed a tendency to emphysema, which increased gradually towards the distal region. The cuboidal epithelial lining was disrupted and ducts terminated in variable number of alveolar sacs. Interalveolar walls were mildly to moderately thickened by oedema and infiltration of plasma cells and lymphocytes. Pulmonary parenchyma showed the presence of fibroblasts and considerable increase in the number of pyknotic nuclei (Fig. 14). After 10 days of beryllium exposure, the alveoli showed persistent emphysema. The interalveolar septum was thickened due to increase in connective tissue. It showed increased

number of phagocytes, blood cells and encapsulated masses of particles (Fig. 15). At the longest duration of 30 days after beryllium exposure, the lung structure showed emphysema along with regional distribution of connective tissue between the alveoli. Numerous cells were present in the alveolar walls and the alveoli were of different sizes and shapes (Fig. 16). After 2 days of conjoint Liv.52 and beryllium treatment, the alveoli resumed their original cup shaped structure and were lined with normal epithelial cells. Maximum improvement was observed after 30 days of conjoint treatment of beryllium and Liv.52. The alveoli were few in number proximally but became more numerous distally (Fig. 17). Respiratory bronchioles were generally lined with ciliated cuboidal epithelium (Fig. 17). Alveolar ducts were long and branched repeatedly. Their walls were formed by pulmonary sacs and alveoli without intervening patches of cuboidal epithelium. Pulmonary alveoli were normal and thin-walled. The inter-alveolar septum consisted of normal endothelial cells, connective tissue, septal cells and phagocytic cells (Fig. 18).

*Spleen*

After 2 days of I.V. exposure the splenic histoarchitecture showed severe damage. In the germinal centres small and large cells were observed with different staining properties. In some cells the mitotic stages were clearly visible and heterochromatic differentiation was also observed (Fig. 19). In some regions intense polymorphonuclear infiltration was observed (Fig. 20). The number of blood cells and phagocytes was considerably increased. At the 10-day schedule the capsule wall and trabeculae were mainly thickened and showed fibrosis. At the 30-day duration, splenic sinuses were dilated and contained small cells. Disorganization was observed in the splenic pulp and the number of pyknotic nuclei increased (Fig. 21). Splenic histoarchitecture showed improvement with conjoint

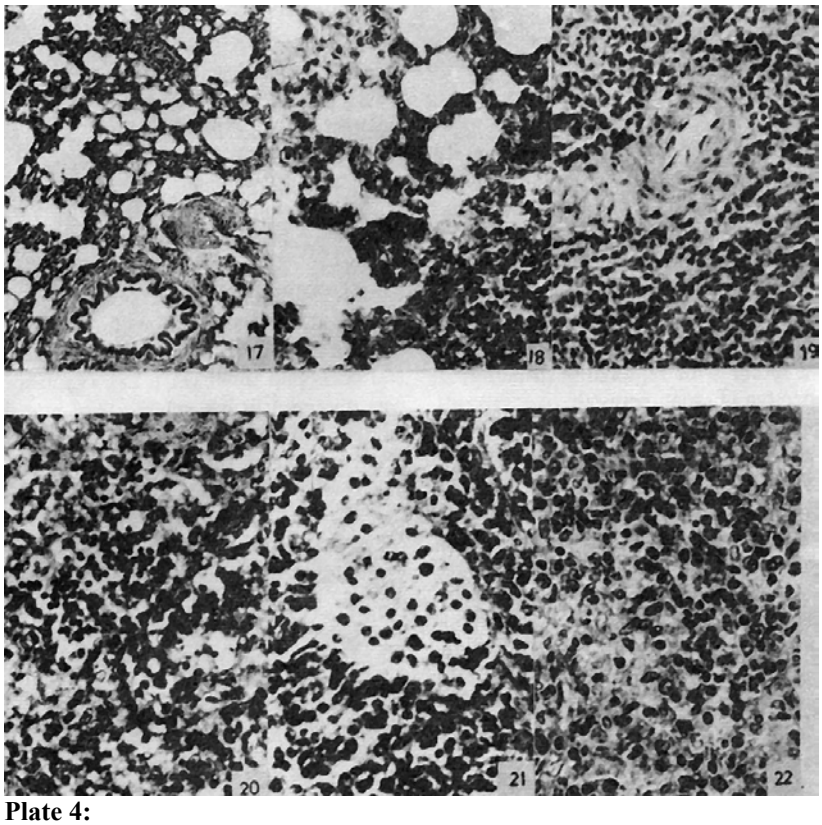


**Plate 4:**

**Figs. 17-18:** Photomicrographs of rat lung exposed to beryllium nitrate. **Fig. 17:** After 30 days with Liv.52. Note normal respiratory bronchiole and alveoli lined with ciliated cuboidal epithelium (X 120). **Fig. 18:** After 30 days with Liv.52. Interalveolar septum consists of normal endothelial cells and phagocytes (X 400).

**Figs. 19-22:** Photomicrographs of rat spleen exposed to beryllium nitrate. **Fig. 19:** After 2 days of exposure. Note mitotic stages in splenic pulp and heterochromatic appearance of nuclei (X 400). **Fig. 20:** After 2 days of treatmement. Note polymorphonuclear infiltration in splenic pulp (X 400). **Fig. 21:** After 30 days of exposure. Note disorganization in splenic pulp and increase in number of pyknotic nuclei (X 400). **Fig. 22:** After 30 days with Liv.52. Note significant improvement in the splenic pulp.

treatment with Liv.52. After 30 days the structure of the spleen was more or less normal (Fig. 22). The white pulp constituted normal Malpighian corpuscles filled with lymphoid tissue and lymphocytes. The red pulp consisted of numerous leucocytes and phagocytes.

**DISCUSSION**

Intravenous administration of an aqueous solution of beryllium sulphate causes necrotizing of the liver, spleen, kidney and bone-marrow[10,11]. Aldridge and his associates[4] confirmed the toxic effects of beryllium and also described a progressive fall in blood sugar level until hypoglycaemic convulsions ensued immediately before death. Central necrosis in the liver due to beryllium toxicity was evident in the hepatocytes and portal triads[12]. In the present study intravenous administration of beryllium nitrate has also caused necrotic changes in the liver. Initial symptoms include granulation and vacuolation of the cytoplasm and degeneration of nuclei; however, at later durations hepatocytes showed various degrees of necrosis which were most predominant at the periphery of the hepatic lobules. Similar findings have been reported by several other authors[11,13].

A number of workers have reported necrotic lesions in the kidney[14,15]. Lisco and White[5] have reported mild degeneration in the renal tubular epithelium and glomeruli. One possible mechanism of renal damage following beryllium intoxication may be the excretion of absorbed beryllium through urine. However, plasma beryllium has not been reported to pass through the glomerulus with damage inflicted upon the tubular epithelium in the course of its secretion[1]. In the present study intravenous administration of beryllium nitrate has revealed shrinkage and degeneration of the glomeruli. Glomeruli revealed severe hypertrophy and increase in the number of nuclei. Interestingly maximum alterations were observed after 10 days of treatment followed by less degenerative changes at later durations. Histological changes cannot be detected in the kidney during the first 24 hours of exposure. However, at 48 hours, changes occurred in the proximal convoluted tubules. At 72 hours the damaged tubules became more conspicuous owing to the formation of more casts, but the number remained unaltered[11].

Exposures to dust and fumes containing beryllium have resulted in a number of pulmonary diseases[16,17]. Others have also reported chronic lesions in the lungs due to some beryllium salts, represented by inflammatory infiltration composed of lymphocytes, less numerous plasma cells and oedematous fluid[18]. Lung cancer can be induced in rats and monkeys by the inhalation of beryllium compounds[19,20]. In the present study when beryllium nitrate was administered intravenously, it caused pathological changes in the lungs. The main lesions included exudation in interalveolar spaces due to infiltration of phagocytes, the tendency to emphysema in the alveoli and bronchiolytic congestion.

Intravenous administration of beryllium sulphate resulted in damage to the splenic red pulp and white pulp[15]. Alteration occurred in the red pulp, whereas the white pulp remained normal at a dose of 1 mg/kg body weight of beryllium lactate[11]. The present investigation also revealed marked histological changes in the spleen after 2, 10 and 30 days of beryllium exposure.

Mathur *et al.,*[21] have reported that the $LD_{50}$ of beryllium salts increases many fold with Liv.52 treatment. Similarly, various haematological parameters reverted to normal with the prophylactic use of Liv.52[22,23]. Liv.52 effectively controls and prevents the degenerative changes induced by carbon tetrachloride[9]. Similarly, Liv.52 is known to reduce hepatic toxicity caused by many chemical toxicants[24,25]. In the present investigation the administration of Liv.52 has significantly reduced the toxic lesions in all the vital organs induced by beryllium exposure.

Therefore, on the basis of our present findings, it is concluded that the administration of beryllium nitrate induces severe toxic lesions in all the vital organs especially the liver. Our findings also reveal that the combined administration of Liv.52 and its administration prior to beryllium exposure certainly reduces the toxic effects and also protects the liver from intoxication. Although biochemical and physiological reports are essential to strengthen these findings, yet it can be safely said that Liv.52 definitely acts as a shield and can be used as a potent prophylactic agent against beryllium toxicity.

**ACKNOWLEDGEMENTS**

The authors are thankful to Dr. P.K. Ramachandran, Director, Defence Research Laboratory, Gwalior for his valuable suggestions. They are also thankful to The Ministry of Defence, New Delhi for providing grants. They are very much indebted to Dr. R.M. Captain, The Himalaya Drug Co., Bombay, India for providing financial support and encouragement.

**REFERENCES**

1. Reeves, A.L., Beryllium. In: Handbook on the toxicology of metals. (Eds.) L. Priberg, *et al.,* Elsevier North-Holland Biomedical Press, 1979, p. 329.
2. Stokinger, H.E., Experimental Toxicology. In: The toxicology of beryllium. (Ed.) J. Tabershaw, P.H.S. Chap. III, Pub. No. 2173, 1972, U.S.P.H.S., p. 17.
3. Reiner, E., Binding of beryllium to proteins. In: Symposium on mechanism of toxicity (Ed.) W.W. Aldridge, MacMillian, London, 1971, p. 111.
4. Aldridge, W.N., Barnes, J.M. and Denz, F.A., *Brit. J. Exp. Pathol.* (1951): 31, 473.
5. Lisco, H. and White, M.R., *Brit. J. Exp. Pathol.* (1955): 36, 27.
6. Schubert, J. and Rosenthal, M.W., *A.M.A. Arch. Industr. Hlth.* (1959): 19, 169.
7. Lindenbaum, A., White, M.R. and Schubert, J., *J. Biol. Chem.* (1952): 196, 273.
8. Mujumdar, S.M. and Kulkarni, R.D., *Probe* (1978): 2, 110.
9. Joglekar, G.V., Chitale, G.K., and Balwani, J.H., *Acta Pharmacol. et Toxicol.*, (1963): 20, 73.
10. Scott, J.K., *Arch. Pathol.* (1948): 45, 354.
11. Aldridge, W.N., Barnes, J.M., and Denz, F.A., *Brit. J. Exp. Pathol.* (1949): 30, 375.
12. Hazard, J.B., *A.M.A. Arch. Industr. Hlth.,* (1959): 19, 179.

13. Skilleter, D.N. and Price, R.J., *Chem. Biol. Interact.,* (1978): 20, 383.

14. Aldridge, W.N., Barnes, J.M. and Denz, F.A., *Nature,* (1950): 165, 772.

15. Browning, E., Beryllium in toxicity of industrial metals, Butterworth and Co., Publishers, London, 2nd edition, 1969, p. 67.

16. Van Ordstrand, H.S., Hughes, R., De Nardi, J.M. and Carmody, M.G., *J. Am. Med. Assoc.* (1945): 129, 1080.

17. Gardner, L.U. and Heslington, H.F., *Fed. Proc.,* (1946): 5, 221.

18. Dudley, H.R. and Boston, M.D., *A.M.A. Arch. Industr. Hlth.*, (1959): 19, 184.

19. Groth, D.H., *Environ. Res.* (1980) : 21, 56.

20. Groth, D.H., Kommineni, C. and Mackay, G.R., *Environ. Res.* (1980): 21, 63.

21. Mathur, S., Prakash, A.O. and Mathur, R., *Curr. Sci.* (1986): 55, 899.

22. Mathur, S., Prakash, A.O. and Mathur, R., *Curr. Sci.* (1987): 56, 322.

23. Mathur, R., Mathur, S. and Prakash, A.O., *Industr. Hlth*. (1987): 25, 131.

24. Sheth, S.C., Northover, B.J., Tibrewala, N.S., Warekar, U.C., Karande, V.S. and Nair, B.Y.L., *Ind. J. Ped.* (1960): 27, 204.

25. Prasad, G.C., *J. Res. Ind. Med.* (1974): 2, 60.