

function. This could be of high adaptive value for the fish under pesticide stress.

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## EFFECT OF LIV-52 ON BLOOD SUGAR IN BERYLLIUM NITRATE-EXPOSED RATS

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THE toxic effects of beryllium on laboratory animals and humans are well known<sup>1-4</sup>. Aldridge and coworkers<sup>5</sup> studied the mode of its toxic action in rats and rabbits and found that the immediate cause of the death was lowering of blood sugars and liver damage. An Ayurvedic drug, Liv-52 (Himalaya Drug Company, Bombay, India) which is used clinically in various liver disorders<sup>6-9</sup> has also been reported to increase the protective index in beryllium-treated rats<sup>10</sup>. The present investigation, therefore, deals with the effect of oral administration of Liv-52 on the

blood sugar and histopathology of liver in beryllium nitrate-exposed rats.

Adult albino rats ( $150 \pm 10$  g) of Sprague Dawley strain were selected from the rat colony of the department. All the rats were maintained under uniform husbandry condition of light, temperature and were given pelleted diet (Hindustan Levers, Bombay) and water *ad libitum*. Beryllium nitrate was dissolved at a concentration of 0.316 mg/ml in pyrogen-free distilled water and was injected to the experimental animals intravenously once only at a dose of 0.316 mg/kg body weight ( $1/10$ th of  $LD_{50}$ )<sup>11</sup>. This dose of beryllium nitrate was toxic in pregnant rats<sup>12</sup> and therefore, selected for further studies.

Liv-52 syrup (obtained from Himalaya Drug Company, Bombay) contained the extracts of *Capparis spinosa*, *Cichorium intybus*, *Solanum nigrum*, *Cassia occidentalis*, *Terminalis arjuna*, *Achilla milleplium* and *Tamarix gallica*.

The selected animals were divided into four groups of ten each and were treated as follows:

Group 1. Animals were given vehicle only; Group 2. Animals were primed with Liv-52 for 10 days prior to the experiment and thereafter received Liv-

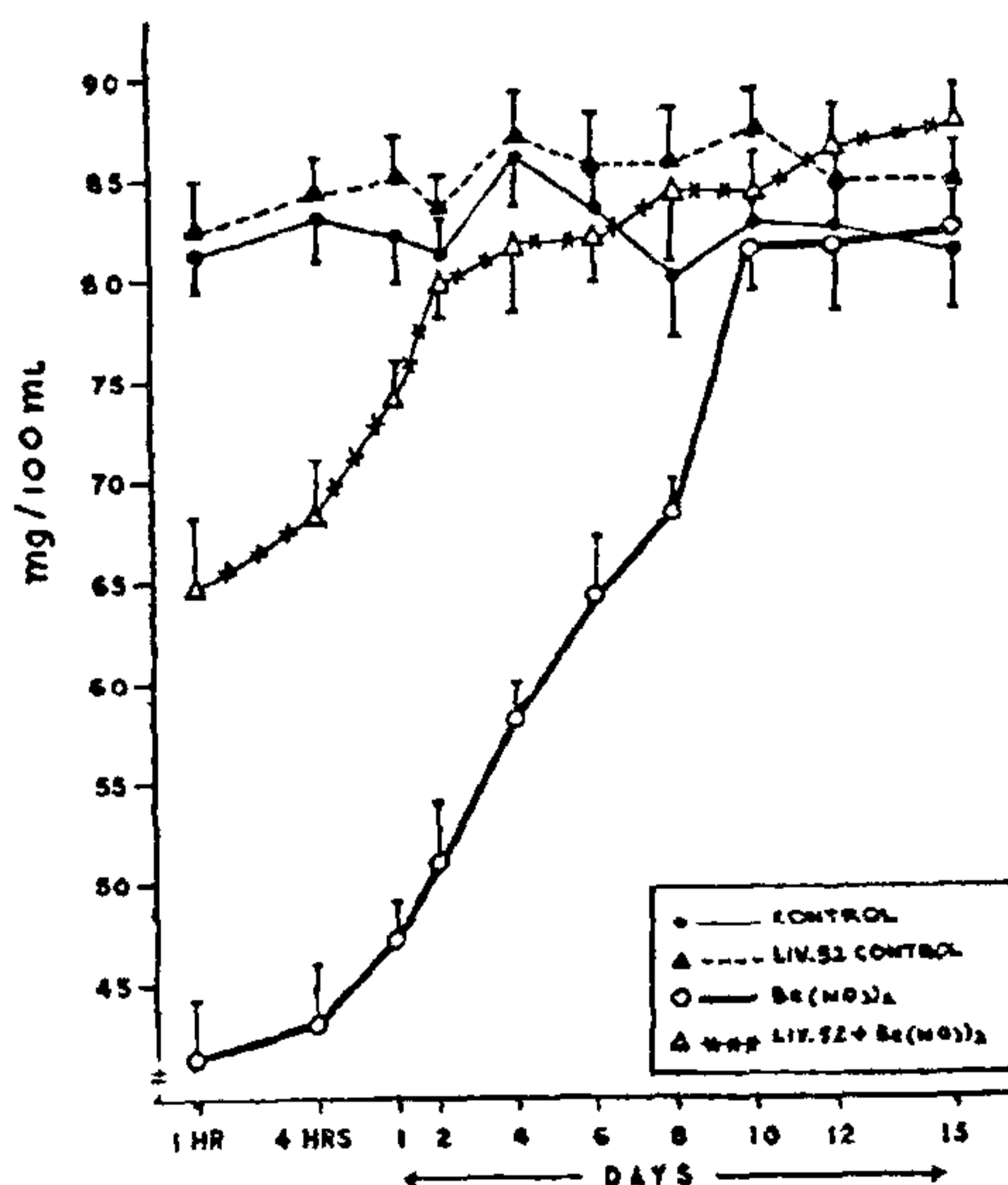
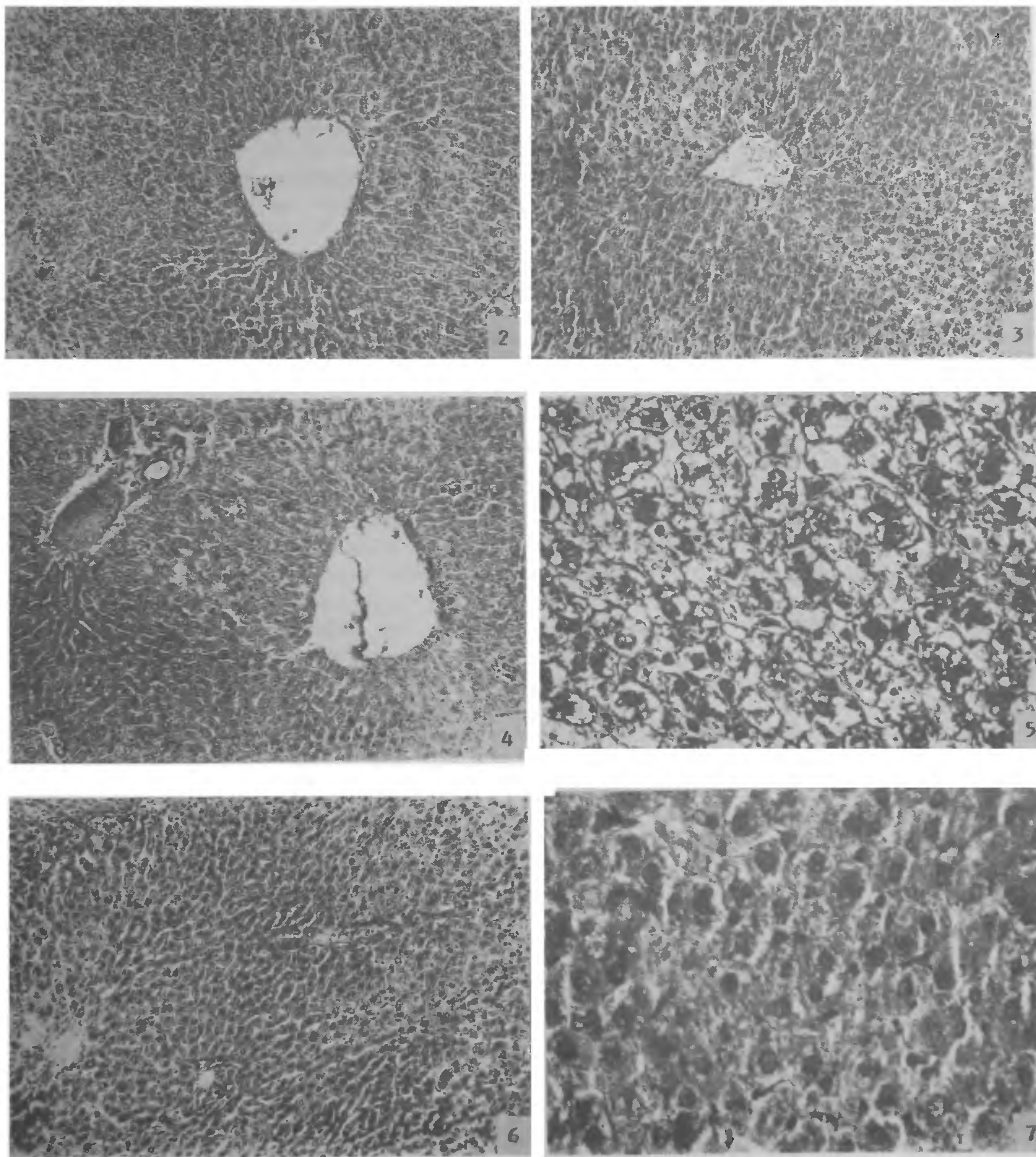


Figure 1. Effect of beryllium nitrate on the blood sugar level in adult rats primed with Liv-52 syrup.



**Figures 2–7.** 2. Photomicrograph of liver at 2 days after beryllium exposure showing pre-necrotic changes at distant places from the hepatic vein ( $\times 120$ ). 3. Liver of rat showing normal histoarchitecture with Liv-52 treatment after 2 days of beryllium exposure ( $\times 120$ ). 4. Liver of rat after 10 days of beryllium nitrate administration showing congestion and damage in hepatic artery, hepatocytes and nuclei ( $\times 120$ ). 5. Cytoplasmic vacuolation and severe damage in liver histoarchitecture after 10 days of beryllium administration ( $\times 400$ ). 6. Liver of rat showing improvement with Liv-52 treatment after 10 days of beryllium administration. 7. Figure of rat liver showing slight damage and granulation in hepatocytes after Liv-52 and beryllium nitrate treatment.

52 daily till 15th day of experimentation; Group 3. Animals were first primed with Liv-52 for 10 days as in group 2 and then were exposed with beryllium nitrate once at 0.316 mg/kg dose (intravenously). This time was designated as zero hr. Simultaneously these animals received daily dose of Liv-52 till the 15th day of experimentation; Group 4. Animals were administered beryllium nitrate intravenously once at a dose of 0.316 mg/kg and the time was designated as zero hr.

The blood samples were collected from each rat at different time intervals ranging from 1 hr to 15 days by puncture of the retro-orbital venous sinus<sup>13</sup> and each sample was processed for the estimation of sugars using the method of Asattor and King<sup>14</sup>. The results were analyzed statistically. Simultaneously, liver pieces were taken out, and fixed in Bouin's fluid for histopathological Studies.

The blood sugar level of the control rats remained almost static during 15 days (figure 1). Liv-52 *per se*, when given to group 2 rats, showed no significant change in blood sugar level as compared to the control rats. The administration of beryllium nitrate at a dose of 0.316 mg/kg body wt intravenously decreased the level of blood sugar significantly and maximum decrease was observed after one hour of injection and then the level recouped gradually and became equal to group 2 after 10 days. When beryllium nitrate is administered to Liv-52 primed animals (group 4), the sugar level remained significantly high even after 1 hr of its administration when compared to group 3; however, these values are significantly lower when compared to rats of group 2 of Liv-52 primed. Within 2 days, the blood sugar level of group 4 became almost equal to that of group 2.

Histopathological examination of the liver of beryllium-treated rats revealed severe pathological changes, which were overcome by the Liv-52 treatment. Figure 2 revealed that after 2 days of beryllium nitrate administration, although the chord arrangements of hepatocytes were maintained, they showed preneurotic changes at places distant from the hepatic vein. On the contrary, its parallel control which received Liv-52 also showed completely normal histological structures in the liver (figure 3). After 10-15 days of beryllium administration the liver was still in a damaged condition. The arteries showed damage and congestion, severe cytoplasmic vacuolation and pyknotic nuclei were also present (figures 4 and 5). In contrast to this the liver of Liv-52-treated rats was free of such manifestation (figures 6 and 7) and showed significant regeneration through-

out the section, though minor granulation and damage was present.

Lowering of blood sugar after the administration of beryllium salts, reported earlier<sup>5</sup>, has been described due to the disturbances in carbohydrate metabolism accompanied by liver damage<sup>15,16</sup>. Beryllium administration also inhibits hexokinase in the liver<sup>17</sup>. Liv-52 is known to correct liver dysfunctions and ailments in many chronic liver diseases<sup>6-9</sup>. It is interesting to note from the present findings that the administration of Liv-52 syrup to beryllium-exposed rats tended to maintain blood sugar at levels considerably higher than in untreated animals. Administration of beryllium nitrate *per se* showed severe liver damage, including increase in the periphery parenchyma, disturbed nuclei, hepatocytes and hepatic vascularity. With the Liv-52 treatment, the liver damage was reduced and it showed significant improvement. Although the exact mode of protective action of Liv-52 against beryllium toxicity is yet to be elucidated, it seems worthwhile to search out and define those mechanisms that may be involved.

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# EFFECT OF HUMAN CHORIONIC GONADOTROPHIN ON METHALLIBURE INHIBITED SPERMATOGENESIS IN *RANA TIGRINA* DURING THE PREPARATORY PERIOD

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THE antigonadotrophic property of methallibure (MB) is well known in mammals<sup>1-3</sup>. The present work was undertaken to determine the gonadotrophin-dependent/sensitive spermatogenic stages in the Indian bullfrog *Rana tigrina* during the preparatory period when rapid spermatogenic activity occurs<sup>4</sup>. Since human chorionic gonadotrophin (hCG) is known to induce spermatogenesis in *R. tigrina*<sup>5</sup>, it was administered to MB-treated frogs to examine its effect on spermatogenesis in the frogs deprived of endogenous gonadotrophins.

Adult male frogs were obtained from Karwar in the first week of April. They were acclimated to the laboratory conditions for 5 days. Five frogs were killed to serve as start controls. The remaining frogs were kept in cement tanks containing little water. They were fed with minced beef on alternate day. The treatment groups were as follows:

- Group 1. 0.25 ml distilled water (controls)
- 2. 1 mg MB in 0.25 ml distilled water
- 3. 1 mg MB in 0.25 ml distilled water + 20 IU hCG in 0.25 ml distilled water

Injectons (ip) were given on alternate days for 30 days. The frogs were autopsied a day after the last

injection. Five frogs from each group were used. The testes were fixed in Bouin's fluid and processed for histological observations. The parameters used for the evaluation of spermatogenic activity are the cell nest countings and frequency distribution of cells in the sectioned spermatocysts as described previously<sup>4-6</sup>.

The seminiferous tubules of start control frogs contained cell nests of stages 0 to V (table 1). Cell nests of stages II to V in distilled water injected frogs increased significantly over the start controls (table 1). However, cell nests of stages 0 and I were numerically reduced.

MB treatment significantly reduced the mean number of cell nests of stages II, IV and V compared to controls. There was a corresponding increase in the cell nests of stage 0 (table 1). Cell nests of stages 0 to V in MB+hCG-treated frogs did not differ from those of the controls (table 1). However, when compared to MB-treated frogs, there was a significant reduction in the cell nests of stage 0 and corresponding increase in stage II. Further, there was a numerical increase in the mean number of cell nests of stages III to V.

Frequency distribution studies revealed that the secondary spermatogonial cell nests containing 13-18 cells were the highest in start controls, MB and MB+hCG groups. In controls the peak was in the cell nests that contained 19-24 cells (table 2A).

With regard to primary spermatocytic cell nests, start controls, MB and MB+hCG groups showed the peaks in the cell nests containing 19-24 cells. In controls the peak occurred in the cell nests with 31-36 cells (table 2B).

Secondary spermatocytic cell nests containing 37-48 cells were the highest in start controls (table 2C). In MB-treated frogs the peak occurred in the cell nests that contained 49-60 cells while cell nests having 61-72 cells were the highest in both controls and MB+hCG groups (table 2C).

The antigonadotrophic effect of MB has been reported in some amphibians<sup>7-10</sup>. In *R. tigrina* MB inhibited the spermatogenic activity as revealed by the quantitative analysis of spermatogenic stages. In intact *Rana esculenta*, Rastogi *et al*<sup>7</sup> observed an increase in the spermatogonial cysts and a notable decrease in the primary spermatocytic cysts due to MB while secondary spermatocytes and spermatids were hardly affected. In *R. tigrina* also MB increased the primary spermatogonia and reduced the primary spermatocytic cysts but the number of spermatids and sperm bundles decreased significantly.

It has been suggested that the action of MB is at