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Effect of Mercuric Chloride on the Survival, Food-intake, Body Weight, Histological and Haematological Changes in Mice and their Prevention with Liv.52

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ABSTRACT

Six month old male Swiss albino mice were given mercuric chloride ($HgCl_2$) in drinking water (1 mM and 5 mM) for 100 days and 30 days respectively. Liv.52 was also given simultaneously (0.5 ml/day/mouse). The results revealed that Hg-exposure at 5 mM resulted in high mortality, while at 1 mM (and 5 mM also) there was loss of body weight and appetite, histopathological changes in the liver and kidney, haematological disturbances and increased serum alkaline phosphatase (A.P.) activity. When Liv.52 was administered alone, with 5 mM and 1 mM $HgCl_2$, it reduced the mortality and prevented Hg-induced toxic effects. Recovery was better in the post-Liv.52 therapy' group than in the 'natural recovery' group (without Liv.52). Liv.52 alone enhanced weight gain and appetite but did not adversely affect histology of the organs and haematological parameters. The probable mode of action of this multiherbal, hepatotonic remedy is discussed.

INTRODUCTION

Mercury pollution is still a worldwide problem ever since the outbreak of mercury poisoning in Minamata, Japan way back in the 1950s and in Iraq in 1971-72 (WHO Report EHC-118,[1]). About 100 tonnes of organomercurials are produced in India every year (Annon[2]). Moreover, recently certain common Indian food items like fish, prawn, cabbage and amaranthus have been found to contain high levels of Hg (Ghoshdastidar and Chakrabarti[3]; Lenka[4] *et al.*; Panda[5] *et al*.), Mercury accumulates in mammalian target organs and damages them (Macgregor and Clarkson[6]). Only a few substances can reduce its toxicity (Vitamins D & E, thiol compounds, Se, Zn and Cu), and costly chelators like BAL and DMSA (dimercaptosuccinic acid) can mobilize it from the body (Megos and Webb[7]).

A multiherbal hepatotonic remedy Liv.52 has been found to protect mammalian target organs against damage due to alcohol (Chauhan and Kulkarni[8]), carbon tetrachloride (Joglekar[9] *et al.)* beryllium (Mathur[10] *et al.*), cadmium (Rathore[11], Rathore and Verma[12], and Rathore and Rawat[13]) and radiations (Saini[14]). It, therefore, appeared worthwhile to test this economic, yet effective, herbal remedy against mercuric chloride intoxication in mice.

MATERIALS AND METHODS

Six month old male Swiss albino mice, obtained from the Biological Production Division, Veterinary College, Mhow, Madhya Pradesh were used. They were divided into 6 groups of 10 mice each and placed in propylene cages. Drinking water was supplied through a bottle

fitted with a tube in cork. Standard food was given. Details of groupings and treatments follow:

Group I: **Controls:** Mice on standard food and distilled de-ionized drinking water *ad libitum.*

Group II: **Mercuric chloride treated:** $HgCl_2$ (Ranbaxy 99.9% pure) dissolved in distilled de-ionized water to prepare solutions of 1 mM and 5 mM concentration. These solutions were given as drinking water for 100 days and 30 days respectively, with standard food.

Group III: **Mercuric chloride treatment + drug:** Mice received 1 mM or 5 mM mercuric chloride solution; each mouse was also given 0.5 ml Liv.52 syrup/day for 100 days and 30 days respectively.

Group IV: **Post-therapy:** After mercuric chloride exposure as in Group II, each mouse was given 0.5 ml Liv.52 syrup/day for the next 15 days.

Group V: **Natural recovery:** After mercuric chloride exposure as in Group II, the mice were shifted to Hg-free water and allowed to recover naturally for the next 15 days.

Group VI: The mice received only 0.5 ml Liv.52 syrup daily.

During the trial, survival, body weight and food consumption were recorded. At the end of the experimentation, i.e. on Day 31 and Day 101, blood was collected directly from the heart for serum assays and alkaline phosphatase activity. Bouin's-fixed tissues were sectioned and stained in Delafied's haematoxyline-eosine. Photomicrographs were taken for detailed analysis of results. Data was subjected to statistical analysis.

RESULTS

For convenience the results are described under separate headings:

1. **Survival:** No mortality was seen among controls and those drinking 1 mM $HgCl_2$ solution (100% survival): 50% and 20% mortality (50% and 80% survival) was recorded in Group II (drinking 5 mM $HgCl_2$ solution alone) and Group III animals ($HgCl_2$ + Liv.52) respectively.

2. **Body weight:** (Table 1) Group I mice (controls) and Group VI mice (Liv.52 alone) showed significant weight gain after 30 days, while those of Group II (drinking 5 mM $HgCl_2$ solution) showed significant weight loss. When Liv.52 was administered to Group III ($HgCl_2$ + Liv.52) or Group IV (Hg and Liv.52, later), significant weight loss was recorded.

But in both cases, the mean weight was significantly higher than in Group II ($HgCl_2$-treated). No significant change in body weight was noted among the different groups in another experiment done with 1 mM $HgCl_2$.

Table 1: Effect of mercuric chloride and Liv.52 on animal body weight and food consumption
Exp: 1 5mm – 30 days Mean (Gm ± SE)

Body weight after 30 days at 5 mM							Food consumption after 30 days at 5 mM						
Initial weight	Group I (Controls)	Group II ($HgCl_2$)	Group III ($HgCl_2$ + Liv.52)	Group IV (Liv.52 after $HgCl_2$)	Group V (Natural recovery)	Group VI (Liv.52 alone)	Initial value	Group I (Controls)	Group II ($HgCl_2$)	Group III ($HgCl_2$ + Liv.52)	Group IV (Liv.52 after $HgCl_2$)	Group V (Natural recovery)	Group VI (Liv.52 alone)
16.60 ±0.57	17.50 ±0.44	14.00[a] ±0.23	15.50[ab] ±0.31	15.00[ab] ±0.21	–	19.00[ab] ±0.51	5.64 ±0.26	6.75 ±0.40	3.14[a] ±0.29	5.18[ab] ±0.38	4.18[ab] ±0.28	–	7.40 ±0.56

Food consumption at various intervals in different groups at 1 mM
Exp. II: 1 mm – 100 days

Days	Group I (Controls)	Group II ($HgCl_2$)	Group III ($HgCl_2$ + Liv.52)	Group IV (Liv.52 + $HgCl_2$)	Group V (Natural recovery)	Group VI (Liv.52 alone)
25	6.40[a] ±0.20	3.68[a] ±0.19	3.64[a] ±0.13	–	–	7.60[a] ±0.26
50	6.65[a] ±0.11	4.51[a] ±0.11	5.38[ab] ±0.27	–	–	8.11[a] ±0.42
75	6.67[a] ±0.10	5.01[a] ±0.14	5.89[a] ±0.17	–	–	9.08[a] ±0.26
100	6.73[a] ±0.12	5.57[a] ±0.11	6.63[a] ±0.20	–	–	9.16[a] ±0.28
115	–	–	–	6.50 ±0.18	6.35 ±0.20	–

Initial food consumption at 0 day, i.e. before starting the trial, was 5.64 ± 0.26 gm/Mouse. Statistically significant at 5% level of significance a = Groups I vs. II or III or IV or V or VI were compared
b = Groups II vs. III or IV or V were compared, and c = Groups IV vs. V were compared.

3. **Food consumption:** (Table 1) During the 30-day trial period, food consumption increased in Group I mice (controls) and reached still higher values in Group VI mice (only Liv.52). Group II animals given 5 mM $HgCl_2$ solution showed significant reduction in food intake. But Group III ($HgCl_2$ solution showed significant reduction in food intake. But Group III ($HgCl_2$ + Liv.52) and Group IV (Liv.52 after $HgCl_2$ exposure) animals showed significantly higher food intake than Group II mice (only $HgCl_2$).

   During the 100-day trial, the control mice (Group I) showed gradual increase in food consumption from Day 25 onwards, while those receiving only Liv.52 (Group VI) displayed very high food consumption throughout the trial period. Group II mice receiving 5 mM $HgCl_2$ solution experienced significant loss in food intake upto the 25th day; but gradual rise was seen during Days 25-50, 50-75 and 75-100 respectively. However, on the 100th day, the values remained significantly lower than those in controls. In Group IV (Liv.52 after $HgCl_2$ exposure), the mice displayed lowered food

intake till the 25th day, but there was a sharp rise during the next 25 days and a further gradual increase during 50-75 and 75-100 days. On the 100th day, food consumption levels reached close to those of controls.

4. **Histology:** (Figs. 1 to 16 and Table 2) The liver was badly damaged in Group II mice (drinking 5 mM $HgCl_2$), but when Liv.52 was also given to mice drinking $HgCl_2$ (Group III) their livers also showed disorganisation but the damage was less severe. In Group IV (Liv.52 after $HgCl_2$ exposure), quite normal histology was seen. Mice drinking 1 mM $HgCl_2$ solution (Group II) also showed toxic effects such as swollen and dead hepatocytes, but when Liv.52 was given simultaneously (Group III), quite normal structure was seen. Group IV (Liv.52 after $HgCl_2$) fared better as compared to Group II mice. Group V animals (natural recovery) did not show any improvement, Liv.52 alone does not affect liver histology.

   In Group II mice (5 mM $HgCl_2$ solution) the kidneys showed shrinkage and death of tubules as 'casts' were visible; the glomeruli were not distinct. In Group III ($HgCl_2$ + Liv.52) mice better histology was evident, i.e. the tubules and glomeruli were distinct. Mice in Group IV (Liv.52 after $HgCl_2$) showed better histology, but those in Group V (natural recovery) died.

   In Group II mice receiving 1 mM $HgCl_2$ there was renal disorganisation, namely hyperplasia of tubules and indistinct glomeruli. But in Group III ($HgCl_2$ + Liv.52) the damage was less severe, i.e. dilatation of tubules and distinct glomeruli and few casts were seen. Group IV (Liv.52 after $HgCl_2$) also showed better histology, i.e. no hyperplasia. Group V (Natural recovery) did not improve as the damage persisted Liv.52 alone did not affect kidney histology.

Table 2: Distribution of hepatocytes per microscopic field following mercuric chloride poisoning and Liv.52 therapy
Expressed as percentage from actual count

Type of cells	Exp. 1 5 mM – 30 days					Exp. 2 1 mM – 100 days				
	Group I (Controls)	Group II ($HgCl_2$)	Group III ($HgCl_2$ + Liv.52)	Group IV (Liv.52 after $HgCl_2$)	Group V (Natural recovery)	Group I (Controls)	Group II ($HgCl_2$)	Group III ($HgCl_2$ + Liv.52)	Group IV (Liv.52 after $HgCl_2$)	Group V (Natural recovery)
% Healthy cells	93.00 ±3.70	18.85^{a} ±2.80	73.68^{ab} ±2.11	62.42^{ab} ±3.95	–	93.00 ±3.70	22.85^{a} ±2.50	79.24^{ab} ±2.81	55.55^{abc} ±0.00	30.00^{a} ±2.67
% Affected cells	Nil	76.00^{a} ±1.11	21.57^{ab} ±1.02	36.36^{ab} ±1.33	–	Nil	77.28^{a} ±2.01	14.33^{ab} ±0.87	39.11^{abc} ±1.12	67.27^{ab} ±1.87
% Binucleate cells	6.25 ±0.41	Nil	5.26 ±0.68	3.03^{ab} ±1.02	–	6.25 ±0.41	2.38 ±0.61	6.41 ±0.57	4.45 ±0.87	2.27^{a} ±0.61

– Affected includes both mild to severely damaged ones. – Statistically significant at 5% level of significance.
[a] = 1 vs. II or III or IV were compared; [b] = II vs. III or IV were compared and [c] = IV vs V were compared.

5. **Haematology and serum alkaline phosphatase activity:** (Table 3) It has been observed that the administration of Liv.52 alone does not affect these parameters. Group II mice (on 5 mM $HgCl_2$) became anaemic, but in Group III animals ($HgCl_2$ + Liv.52) normal Hb values were recorded. Other parameters did not measure up to those in controls but showed significantly better ones than those in Group II ($HgCl_2$ only). Group IV mice (Liv.52 after $HgCl_2$) fared better.

Also Group II mice (on 1 mM $HgCl_2$) showed disturbances in these parameters. The addition of Liv.52 to $HgCl_2$ (Group III) could restore normal Hb% and MCH; all other parameters showed significant improvement. Similar results were found in Groups IV and V (Liv.52 after $HgCl_2$ and natural recovery respectively).

Group II mice (on 5 mM $HgCl_2$) showed high serum alkaline phosphatase (A.P.) activity. In Groups III ($HgCl_2$ s + Liv.52) and IV (Liv. 52 after $HgCl_2$) better values were recorded as compared to Group II ($HgCl_2$ only). In the 1 mM $HgCl_2$ category also, high A.P. activity was noticed, but in Groups III ($HgCl_2$ + Liv.52), IV (Liv.52 after $HgCl_2$) and V (natural recovery) normalcy was restored.

MOUSE LIVER-HEMATOXYLIN-EOSINE-PREPARATION
C.S. 105 X
(Plate I - Figs. 1 to 8)

Fig. 1: Control, distinct hepatocytes around blood vessel, no sign of pathology.

Fig. 2: $HgCl_2$ (5 mM – 30 days) Cytoplasmic membrane mostly damaged, zones of necrosis are visible.

Fig. 3: $HgCl_2$ + Liv.52, better histology, few cells show cytoplasmic vacuolization and nuclear hypertrophy.

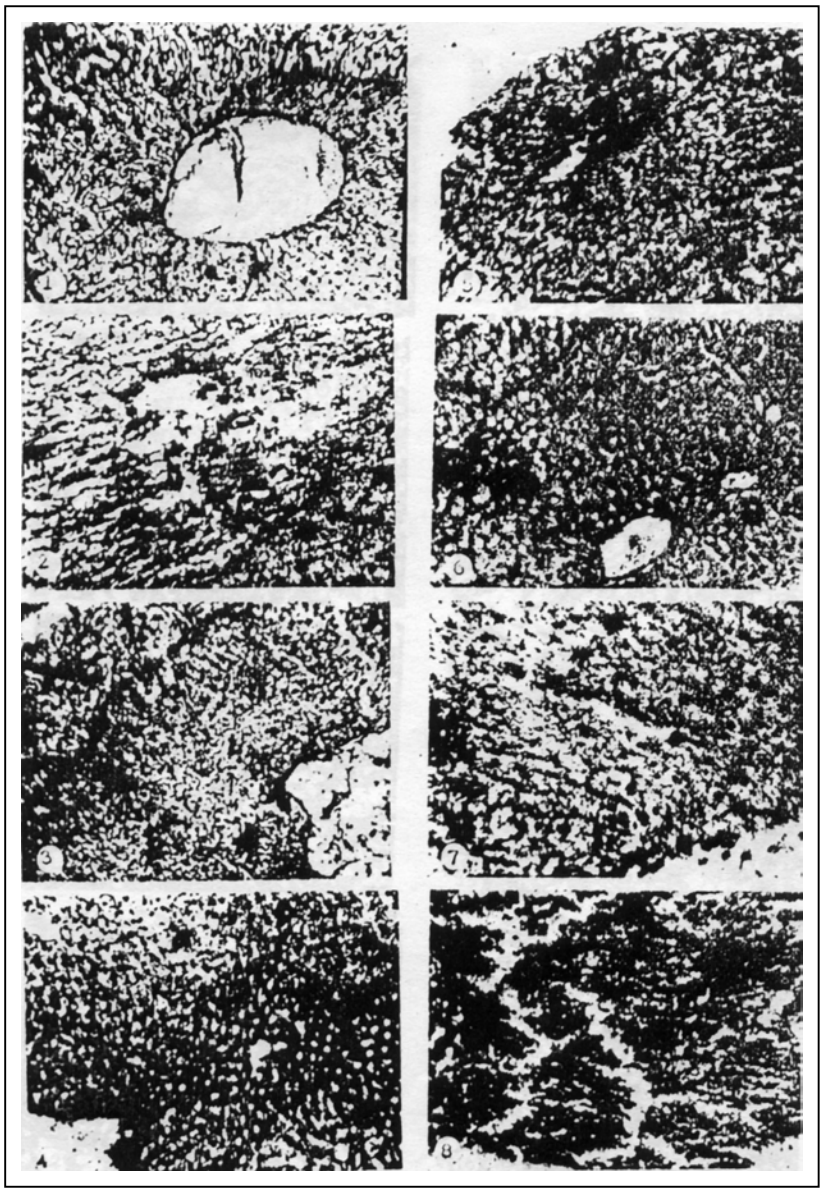
Fig. 4: Post-therapy (15 days Liv.52 therapy to 30 days Hg-poisoned mice), quite normal histology.

Fig. 5: $HgCl_2$ (1 mM – 100 days) damage to cell and nucleus, few swollen calls show early sign of death.

Fig. 6: $HgCl_2$ + Liv.52, quite O.K. like controls.

Fig. 7: Post-therapy (15 days Liv.52 therapy to 100 days Hg-poisoned mice), better histology than what is seen in Fig. 5 (less damaged cells).

Fig. 8: Natural recovery-following 100 days Hg exposure, disorganisation seen (No improvement).



MOUSE KIDNEY – HEMATOXYLIN-EOSINE-PREPARATION
C.S. 105 x
(PLATE – II Figs. 9 to 16)

Fig. 9: Control, distinct glomeruli and tubules.

Fig. 10: $HgCl_2$ (5 mM 30 days), severe shrinkage of tubules and even their death (C-CAST); and glomeruli are not distinct.

Fig. 11: $HgCl_2$ + Liv.52, better picture, tubules show less shrinkage as lumen and are organised. Glomeruli are clear.

Fig. 12: Post-therapy (15 days Liv.52 administration to 30 days – Hg-poisoned mice), better than Hg-exposed (Fig. 10), glomeruli, distinct, most of the tubules are dilated, only few show death (CAST).

Fig. 13: $HgCl_2$ (1 mM – 100 days) disorganisation, hyperplasia of tubules and glomeruli are affected.

Fig. 14: $HgCl_2$ + Liv.52, better picture, glomeruli distinct; tubules show only dilatation, few 'CAST' seen.

Fig. 15: Better picture than Hg-exposed (Fig. 13), glomeruli distinct.

Fig. 16: Natural recovery following 100 days Hg-exposure, disorganisation of tubules and glomeruli (no improvement).

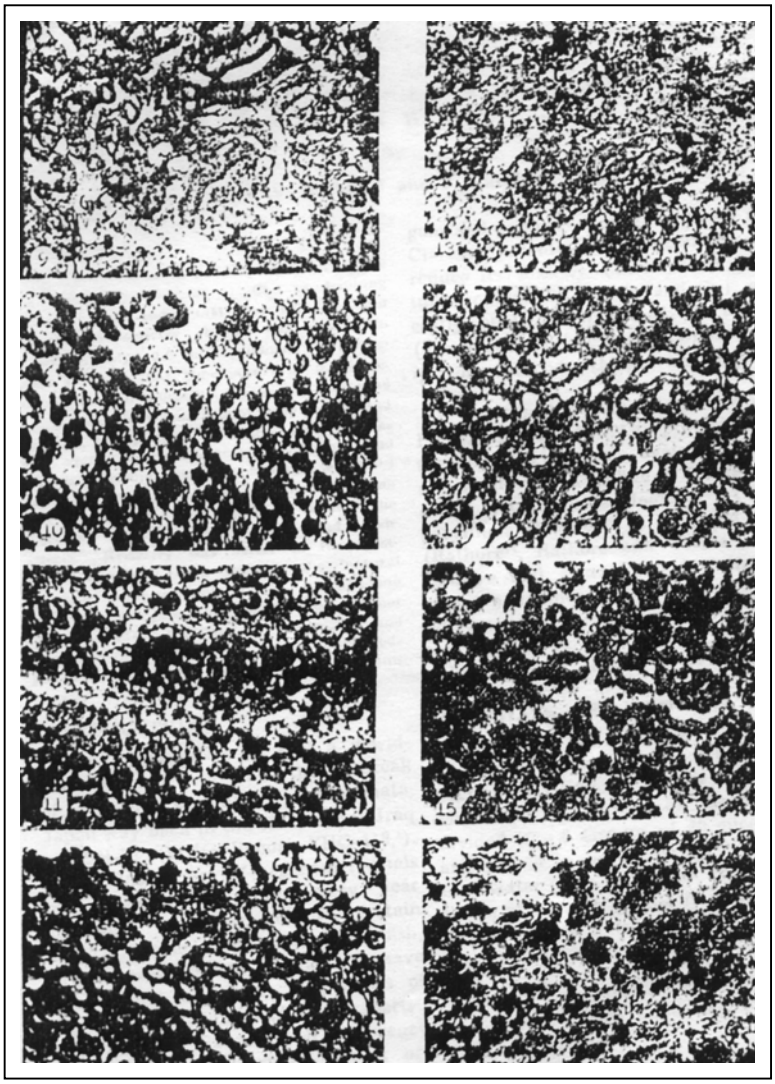


Table 3: Effect of $HgCl_2$ alone and in combination with Liv.52 on haematological parameters and serum A.P activity

Parameters	Exp. 1 5 mM – 30 days					Exp. 2 1 mM – 100 days			
	Group I (Controls)	Group II ($HgCl_2$)	Group III ($HgCl_2$ + Liv.52)	Group IV (Liv.52 after $HgCl_2$)	Group V (Natural recovery)	Group II ($HgCl_2$)	Group III ($HgCl_2$ + Liv.52)	Group IV (Liv.52 after $HgCl_2$)	Group V (Natural recovery)
HB%	14.40 ±0.18	10.73[a] ±0.25	13.37 ±0.47	11.90[ab] ±0.19	–	11.50[a] ±0.15	14.20 ±0.12	14.25 ±0.19	14.00 ±0.22
PVC	73.00 ±0.31	35.60[a] ±1.86	53.00[ab] ±1.54	48.60[ab] ±1.94	–	53.60[a] ±0.87	68.60[ab] ±0.74	67.87[ab] ±0.37	67.25[ab] ±1.10
TRBC	7.12 ±0.11	4.75[a] ±0.17	6.48[ab] ±0.13	5.50[a] ±0.58	–	5.79[a] ±0.06	6.40[ab] ±0.14	6.90 ±0.30	6.70 ±0.28
MCHC	24.78 ±0.12	19.49[a] ±0.15	22.10[ab] ±0.29	21.20[ab] ±0.27	–	21.50[a] ±0.15	23.80[ab] ±0.12	24.37 ±0.31	24.25 ±0.14
MCH	21.40 ±0.24	17.22[a] ±0.23	19.40[ab] ±0.48	17.23[a] ±0.24	–	18.36[a] ±0.22	21.14 ±0.79	21.20 ±.33	19.65[ab] ±0.39
MCV	109.00 ±1.77	83.00[a] ±1.22	98.38[ab] ±2.67	86.70[a] ±1.62	–	86.95[a] ±2.19	99.25[ab] ±1.25	108.75 ±1.37	107.00 ±0.43
A.P.	11.60 ±0.39	19.40[a] ±0.50	14.80[ab] ±0.25	16.25[ab] ±0.32	–	16.00[a] ±0.40	11.37 ±0.23	11.62 ±0.24	13.00 ±0.73

Statistically significant at 5% level of significance
a = 1 vs. II or III or IV were compared; b = II vs. III or IV were compared and c = IV vs V were compared.

DISCUSSION

In the present trial 5 mM $HgCl_2$ was quite a high concentration. LD_{50} for mice is 10 mg/kg body weight; hence death is not an unexpected finding. If each mouse consumed 1 ml of 5 mM solution (1035 μg per ml) per day, this is to be expected.

The results indicate Hg-induced weight loss. Similar observations have been made in rats by Chang and Hertmann[15] and Gasner and Kirschner[16] after administering 0.8 mg Hg/kg body wt./day for 11 weeks and 3 mg Hg/kg body wt./day for 100 days respectively. Earlier workers have also noted brain lesions but later ones have not done so. The present results also showed Hg-induced loss in food intake, i.e. hypophagia; such findings do not exist in the literature. Of course, in one report (Berthound[17] *et al.*), 1 mg/kg body wt./day of methyl mercury has been found to reduce the mean food intake and body weight, and bring about brain lesions. According to Grossman[18], lesions in the areas involved in the regulation of food intake can cause hypophagia. Hence the present results can be so explained that at 5 mM $HgCl_2$, degenerative changes in the brain were observed (unpublished), but not at 1 mM $HgCl_2$. So loss of appetite at this dose might have been due to degenerative changes in the liver, kidney and gut (unpublished), discussed later.

The liver shows Hg-induced pathological changes. Ashe[19] *et al.,* had reported severe hepatic effects in rabbits exposed to metallic Hg-vapors. Accidental, fatal Hg-vapor inhalation exposure in a young child caused hepatocellular damage and biochemical alterations (Jafee[20] *et al.*).

The kidney is badly damaged by Hg exposure. Fitzhuge[21] *et al.*, studied Hg-acetate (25 ppm)-induced changes in the kidney of rats and reported a dose-related change in its structure and function. Among human beings, inorganic Hg salt ingestion results in anuria and uraemia from acute tubular necrosis (Kazantzis[22] *et al.*).

Before explaining the possible protective role of Liv.52, it seems essential to describe the mechanism of action of Hg. Hg ions bind with – SH groups in the bio-membranes, and damage them via lipid peroxidation (Clarkson[23], Hughes[24]). Hg also binds with lysosomal membranes and renders them labile (Verity and Reith[25], Lauwerys and Buchet[20]). It inhibits protein synthesis (Nakada[27] *et al.*), alters the tertiary structure of RNA and DNA (Eichhorn and Clark[28], Gruenwedel and Davidson[29]) and affects their synthesis. Hg disturbs the structure and function of inner mitochondrial (Humus and Weinberg[30]). All these effects can be held responsible for the inorganic Hg-induced cellular damage (EHC-118[1]).

On the other hand, this multiherbal remedy Liv.52 has been found to stabilise lysosomes and to inhibit the activities of acid-phosphatase, cathepsin-B and acid-deoxyribonuclease, (Saxena and Garg[31]). It lowers lipid peroxidation and enhances the activities of cytochrome P-450, ATPase, Cytochrome-C-oxidase and SDH (Saxena[32] *et al.,* Saxena and Garg[33], Goel and Dhawan[34], Bardhan[35] *et al.*).

Hg induces anaemia in human beings (Campbell[36]). On the other hand, Liv.52 is known to cure anaemia (Mathur[37] *et al.*) and to restore normal levels of transaminases (Subbarao and Gupta[38]).

Liv.52 has also been reported to prevent carbon tetrachloride-induced loss in the RNA, DNA, total and microsomal protein contents (Subbarao and Gupta[39]).

Hg affects SH enzymes like alcohol dehydrogenase (Waku and Nakazawa[40]). Quite recently the unique action of this remedy in lowering the accumulation of acetaldehyde by its rapid removal and reducing the injurious effect of ethanol on the liver has been reported by Chauhan and Kulkarni[7].

Hg causes chromosomal breaks (Zasukhina[41] *et al.*), while Liv.52 has been found to reduce radiation-induced chromosomal damage in bone marrow (Jagetia and Ganapathi[42]). It has also been found to enhance tissue GSH contents (Sarkar[43] *et al.*). All these properties of Liv.52 might have been responsible for reducing or nullifying the injurious effects of $HgCl_2$ in mice in the present trial. In the near future, our nearly finished work with Liv.52 in relation to uptake, retention and excretion of Hg using atomic absorption spectroscopy shall hopefully throw more light on its protective action.

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