BULLETIN
OF
JAWAHARLAL INSTITUTE
OF POSTGRADUATE MEDICAL EDUCATION AND RESEARCH
PONDICHERRY - INDIA.

FILE COPY
VOLUME - II - 1977

NATIONAL LIBRARY
NOV 13 1975
OF MEDICINE
METHANOL TOXICITY – AN EXPERIMENTAL STUDY

K. Ramesha Rao, A. L. Aurora, S. Muthaiyan and S. Ramakrishnan
Departments of Pathology and Biochemistry

Methanol poisoning is a well-known hazard among workers employed in shellac and varnish industries. Despite its well known toxicity, it has been often used in bootleg liquors and as a substitute for ethanol by poor populations. Many pathological studies in humans as well as in animals have shown changes chiefly confined to brain, eyes and lungs (Scott, Hetz and McCord, 1933; Menne, 1938; McGregor, 1943; Bennet et al, 1953; Erlanson et al, 1965). Changes observed in the brain include congestion, oedema, neuronal degeneration and haemorrhages in pons, medulla, and basal ganglia. But the nature of the oedema and biochemical changes associated with it remain an enigma.

In the present study acute and chronic experiments with methanol were carried out in rabbits and monkeys to find out the nature of oedema, and damage to myelin, if any. The protective effect of pyrazole in methanol toxicity also has been assessed in these experiments. Pyridine was included in the present study as it is considered to be a contaminant in certain methanol preparations responsible for methanol poisoning. (Dr. Bhide, personal communication).

Materials and Methods

Thirty one randomly bred male rabbits and 8 male monkeys, free of any signs of disease were selected. All animals were marked and housed in appropriate cages. The rabbits weighed from 1.2 to 1.5 Kgs and monkeys weighed from 4.5 to 5.5 Kgs. The rabbits were maintained on a laboratory diet consisting of 60 g Bengal gram per day, seasonal leafy vegetables and water ad libitum. Each monkey received two plantains and 30 g per Kg body weight of Bengal gram per day. In a pilot study carried out earlier on 14 rabbits and 7 monkeys, it was found that 6 ml/day/Kg body weight of absolute methanol given as 30% solution was fatal to rabbits in 5 to 7 days. The monkeys survived only 3 days on a daily dosage of absolute methanol of 3 ml/Kg body weight given as a 30% solution. On this basis a desirable dosage was chosen. Methanol G. R. grade (Sarabhai M Chemicals), pyridine AR grade (BDH) and pyrazole (E. Merck) were used in the experiments.

The details of the groups, subgroups, the type of experiments carried out on rabbits and monkeys, chemicals used, mode and dosage of administration are given in Tables I and II respectively.

Laboratory Investigations in Rabbits

Blood: Methanol level in the blood was estimated at weekly intervals in
animals of chronic experiments. In acute experiments, methanol estimation was done twice, once two hours after the initial dose and again at the time of sacrifice. Methanol was estimated by type "B" procedure of Blanke (1970).

Brain tissue: Small pieces of unfixed brain were utilized for the estimation of water, electrolytes and protein (Ames III, Isom and Nesbett 1965). Electrophoretic studies using polyacrylamide gel also were carried out on brain extract (Raymond, 1964).

Colloidal carbon studies: Biological Ink (Pelikan) C11/1431 a of particle size 250-400 A° (colloidal carbon) was given to 7 animals of group II. Three of these animals belonged to subgroup "a" and two each to subgroups "b" and "c". Carbon was rendered sterile by autoclaving and was given by intracardiac route in a dosage of 100 mg/Kg exactly half an hour before sacrifice. One animal, which belonged to subgroup "a" of group III was also given carbon.

Autopsy studies: Group I animals were killed under pentothal anaesthesia when they appeared moribund. Two animals expired after 7 days. The other animals survived from 7 to 72 days. Group II animals were killed after 48 hours of the start of experiment irrespective of their general condition, except the animals of subgroup "a" who were allowed to live for 5 to 7 days. Control animals were maintained on laboratory diet and were sacrificed after 15 days. On all these animals, autopsy was done. A small piece of tissue from the frontal lobe of the brain was fixed in formal calcium and sectioned in cryostat (–20°C) at 10-15μ. The sections were stained by gold hydroxamate method for myelin (Adams, 1965). Rest of the brain was fixed in buffered formalin. Sections from following areas were taken for histology: frontal lobe, through optic chiasma, through mamillary body and hippocampus, inferior colliculi and cerebellum including pons. Spinal cord was studied in all animals of group II and III. All these pieces of tissue and samples from all other organs were processed for paraffin sectioning. Sections were stained with haematoxylin and eosin stain (H & E stain). Special stains such as periodic acid Schiff's (PAS) stain. Alcian blue and Masson's trichrome were used wherever indicated according to standard procedure (Luna, 1968). The eye balls were studied grossly and microscopically in all rabbits.

Investigation in Monkeys: Investigations similar to those performed in rabbits were also carried out in monkeys except in animals of group I. The colloidal carbon was given to group II and III only. All the animals were sacrificed 48-72 hours after the start of experiments except for one animal which expired. Animals of group I were studied routinely for morphological studies.

Results:

Methanol levels in blood

Rabbits on chronic experiments (Group I): Methanol levels at the time of sacrifice/death varied from 37 mg percent to 117 mg percent in animals fed methanol alone. Values of 37 mg percent and 45 mg percent were obtained in the animals that survived for 7 days each. In the animals who survived for 72 days, the increase in methanol con-
centration was gradual until values of 111 mg percent and 117 mg percent were obtained at the time of sacrifice. Methanol concentration in methanol-pyridine fed animals varied from 37 mg percent to 94 mg percent. The former value was obtained in an animal which survived for 7 days. The other animal showed a gradual increase in methanol concentration until values of 47.69 and 94 mg percent were obtained after 25, 42 and 37 days respectively.

Rabbits on acute experiments (Group II): The animals fed with methanol alone recorded a final methanol concentration which varied from 232 mg to 273 mg percent. In the animals given methanol and pyrazole (subgroups “b” and “c”) values were on an average 247 mg percent. One animal which was given methanol pyrazole (subgroup “b”) showed a final methanol concentration of 294 mg percent.

Methanol levels in monkeys: The final methanol concentration in the two animals which were given methanol-pyrazole combination were 194 mg and 207 mg percent.

Brain water and electrolyte content:

Rabbits: Brain water and electrolyte contents of rabbits are given in Table III. The experimental animals showed slightly higher levels of brain water as compared to normal animals. The increase was more marked in methanol and methanol-pyridine fed (subgroups “a” and “b”) animals of group I. It was observed that when compared to controls all animals except pyridine treated group (subgroup “c” of group I) showed increase in the concentration of sodium with decrease in potassium. Maximum increase was observed in methanol fed rabbits of subgroup “a” of group II. The changes in electrolyte content were statistically significant (P < 0.05).

Monkeys: The levels of water, sodium and potassium in the brain are given in Table III. As in rabbits, there was slight increase in water content and sodium concentration with decrease in potassium in experimental animals compared to controls.

Studies with carbon:

Rabbits: Histological examination of the brain revealed that in methanol fed animals of group II there was evidence of stasis and many vessels contained plugs of carbon compared to minimal amounts in an occasional vessel in the control animals. There was no leakage of carbon from vessels. In animals which were given methanol and pyrazole together, though the amount of carbon within the vessels was considerably less than that observed in methanol fed animals, it was slightly more than in the controls. Even in these there was no evidence of leakage of carbon from vessels. Examination of liver, lung and adrenals similarly revealed greater amounts of carbon within the blood vessels and in Kupffer cells in methanol fed rabbits than in controls (Fig. 1).

Monkeys: Two animals each of methanol-pyrazole fed and pyrazole fed groups were given colloidal carbon. There was only marginal difference in the amount of carbon contained in the vessels of the animals of the two groups. The vessels of the animals of group II, who received methanol pyrazole combination, contained slightly greater amount of carbon than group III animals.
Morphologic changes: In Brain:

Rabbits on chronic experiments: (Group I)

Compared to control animals, all methanol fed animals (subgroup "a") showed mild to moderate oedema and mild congestion. In some areas neurons showed vacuolation of cytoplasm and nucleus. There was perineuronal oedema. The loss of myelin was noticeably shown by the loss of tinctorial affinity. Myelin was still present about some axons.

In methanol-pyridine fed animal (subgroup "b") brain showed mild to moderate oedema and congestion. One animal of this subgroup showed extensive neuronal damage and satellitosis (Fig. 2). The marked increase in oligodendroglial cells was obvious when compared to similar areas in control animals. Myelin studies revealed changes similar to methanol fed animals.

Pyridine fed animals (subgroup "c") showed only minimal oedema and congestion. There was only slight decrease of myelin when compared to normal.

Rabbits on acute experiments: (Group II)

The animals in this group showed only mild oedema and congestion though it was a little more marked in methanol fed animals (subgroup "a"). The stains for myelin in methanol fed animals revealed greater sponginess of the brain (Fig. 3). The well delineated appearance of myelin in the region of centrum semiovale seen in control animals was not obvious. At some places myelin appeared thinned out with swelling of the axons.

Methanol pyrazole fed animals (subgroups "b" and "c") showed mild oedema and congestion. The myelin was relatively well preserved. However, focal fragmentation of myelin was present.

Pyrazole fed animals (subgroups "d" and "e") showed minimal oedema and congestion. Myelin was well preserved in most areas though focal fragmentation of myelin was observed in some areas.

In control animals (Group III) the myelin fibres were well stained, traceable and formed fine network (Fig. 4).

Changes in the Eye

Rabbits: Oedema of the nerve fibre layer and chronic cell change of the ganglion cells characterized by shrinkage of the cytoplasm and nucleus were observed in methanol fed animals of chronic experiments (subgroup "a" of group I). These changes were more pronounced in two animals who survived for 72 days. All the animals showed oedema of the inner nuclear and inner plexiform layers. One methanol pyridine fed animal (subgroup "b" of group I) which survived for 42 days showed marked oedema which rendered Muller's fibres prominent. Mild degree of oedema was also noted in methanol-pyrazole fed animals (subgroups a, b and c of acute experiments (group II). The animals that received only pyridine (subgroup "c" of group I) did not show significant alterations.

Monkeys: Changes in the eyeballs were similar to those observed in rabbits.

Discussion

Methanol levels in chronically fed rabbits showed a cumulative effect. The increase was small in the first two weeks. During this period metabolism of methanol was obviously geared
FIG. 1. Photomicrograph of a section from liver of a methanol fed rabbit of acute experiments (group II) showing abundant carbon in Kupffer cells (H & E x 100).

FIG. 2. Photomicrograph of a section from brain of a methanol pyridine fed animal of chronic experiments (group I) showing neuronal damage, satalitis and increase in the number of oligodendrocytes (H & E; x 400).
FIG. 3. Photomicrograph of a section taken from the frontal lobe of brain of a methanol fed rabbit of acute experiments (grec. p II), showing thinning and focal loss of myelin (Gul'd hydroxamate x 400).

FIG. 4. Photomicrograph of a section taken from the frontal lobe of the brain of a control animal stained by gold hydroxamate method to show normal myelin network (x 400).
up with the rate of methanol administration, though unsuccessfully. From third week onwards, there was a greater increase in the concentration of methanol in blood than before, indicating a partial inhibition of degradation of methanol as the dose of methanol administered remained the same. After about 8 weeks, the rise in concentration became relatively less. One of the animals achieved equilibrium by 9th week, after which no further increase in methanol concentration was observed till the date of sacrifice. These findings suggest that rabbits can adapt their metabolism on continuous exposure to methanol.

The addition of pyridine did not interfere in any way with the cumulative effect of methanol. In acute experiments on rabbits (group II) methanol levels similarly showed cumulative effect. Despite high values obtained, except for one animal which expired, others remained active until the time of sacrifice. Addition of pyrazole did not significantly alter levels of methanol in blood.

The total amount of methanol given to an individual rabbit of chronic experiments varied from 28 ml in the animal that survived for 7 days to 288 ml in the animal which survived for 72 days. This wide variation in fatal dose of methanol has been reported earlier by several workers both in humans as well as in the experimental animals (Scott, Hetz and McCord, 1933; Roe, 1943; Chew et al, 1946; Leaf and Zatman, 1952; Bennet et al, 1953.

The addition of pyridine did not significantly alter the survival. The duration varied from 7 to 42 days in methanol-pyridine fed subgroup of animals. The data do not suggest enhanced toxicity.

Pyrazole has been shown to be a potent inhibitor of liver alcoholic dehydrogenase. In the present study it was used to find out whether it could effectively counter the toxic manifestations of methanol in rabbits. The results indicate that pyrazole is only marginally effective, but when given alone, it does cause damage to the brain in the rabbits. Recent studies, by Lieber et al (1970) have shown that pyrazole is capable of inhibiting the liver enzymes other than alcoholic dehydrogenase and produce swelling of the mitochondria and smooth endoplasmic reticulum. Magnusson et al (1972) reported significant toxic effect of pyrazole on testes which became atrophic and on thyroid which showed adenomatous hyperplasia. Blum et al (1971), Traiger and Plaa (1972) and Goldberg et al (1972) have shown that pyrazole synergizes the toxic effect of ethanol by interfering with its elimination, thus producing high ethanol values in the blood. They demonstrated that pyrazole can independently cause neuromuscular incoordination. These observations suggest that pyrazole may act in a similar way to enhance the toxicity of methanol. Though in the present study, the animals fed pyrazole alone did not suffer from obvious toxic effects, they did however, show focal fragmentation of myelin on histological examination.

In chronic experiments on rabbits with methanol, there was a slight increase
in the water content when compared with control animals. This was associated with marked increase in the sodium content and decrease in potassium content. The changes in the electrolytes were statistically significant. The changes in electrolytes and water content of the brain were not associated with significant increase in the protein content of the brain extract. The increase in vascular permeability was not, therefore, enough to allow the escape of proteins. It is known that seepage of water and electrolytes into cerebrospinal fluid equilibrates with increase in sodium content of brain without affecting potassium levels (Katzman and Pappius, 1973). The gel electrophoresis also failed to reveal any variation in the pattern of protein between the control and experimental animals. These findings suggest that there is accumulation of a fluid which is rich in sodium but free of protein. Colloidal carbon studies in acute experiments corroborated this finding. Large amounts of carbon were seen in many vessels in all organs, especially brain, liver, adrenals and lungs in animals given methanol, with or without pyrazole. This indicated clearly that an element of stasis was a prominent feature of methanol toxicity. Stasis would not only deprive the tissues of adequate amounts of nutrients and oxygen but would also expose them to toxic metabolites, viz. methanol and its breakdown products. The element of stasis leading to anoxia over a unit time would favour increase in vascular permeability. However, as there is no escape of colloidal carbon or protein from the vessels, it is to be assumed, that increased permeability results only in the formation of plasma ultrafiltrate. Low values obtained for potassium can be explained on the basis of dilution caused by the escape of an ultrafiltrate poor in potassium.

Several workers (Scott, Hetz and McCorrd, 1933; Menne, 1938; Bennet et al, 1953,) observed congestion and oedema of the brain both in humans and in experimental animals. Erlanson et al (1965) described haemorrhage into the brain with formation of haemorrhagic cysts. In a recent study conducted on victims of illicit liquor consumption, Saras Bharathi, Ramamurthi and Ganapathy (1976) were not able to demonstrate myelin damage. However, in an earlier study, again on human beings, Sheidegger (1972) (quoted by Saras Bharathi et al) was able to demonstrate damage in only one case. In the present study, the animals of chronic experiment showed oedema which was obvious from increased sponginess of the brain tissue. This was associated with mild congestion and definite damage to neurons. Special stains for myelin revealed decreased tinctorial affinity for myelin due to its obvious thinning or even loss. Apart from mild oedema and congestion, changes in the myelin could also be demonstrated in the animals of acute experiments. However, changes were less severe compared to those of chronic experiments. Many of the axons still retained myelin sheaths that appeared thinned out. It appears that changes in myelin are related to duration of methanol administration. Since all the animals used were adults, the decrease in the amount of myelin appears to be due to loss than to decreased formation.

The changes in the brain were not significantly altered by pyridine. The
mild to moderate oedema, congestion and myelin damage were similar to those seen with methanol alone.

The changes observed in the eye in the present study, such as oedema of the nerve fibre layer and chronic cell change have been recorded earlier by Scott et al. (1933), Menne (1938) and Bennet et al (1953). Pyrazole not only failed in the present study to prevent the lesions produced by methanol but it independently produced mild oedema of the nerve fibre layer.

The present study thus highlights the hitherto unsuspected damage to myelin, which along with oedema of the brain could explain the symptom complex of methanol toxicity.

Acknowledgement

The authors are grateful to the Indian Council of Medical Research for financial support for this project. The authors are also grateful to the Principal, Jawaharlal Institute of Postgraduate Medical Education and Research, Pondicherry-6, for facilities provided for this study.

References


6. Bhid, N. K. Professor of Pharmacology- All India Institute of Medical Science, New Delhi-110016 (Personal Communication).


<table>
<thead>
<tr>
<th>Group and type of experiments (Group I)</th>
<th>Number of animals</th>
<th>Subgroups with number of animals in each</th>
<th>Dosage of chemicals (per kg. of body weight)</th>
<th>Route of administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic experiments</td>
<td>12</td>
<td>b (4)</td>
<td>13.3 ml./kg. of 30% methanol in distilled water</td>
<td>Gastric intubation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c (4)</td>
<td>13.3 ml./kg. of 2.5% pyridine in 30% methanol in distilled water</td>
<td>-do-</td>
</tr>
<tr>
<td>Acute experiments (Group II)</td>
<td>15</td>
<td>a (4)</td>
<td>20 ml./kg. of 30% methanol in distilled water</td>
<td>-do-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b (5)</td>
<td>(i) 20 ml./kg. 30% methanol in distilled water</td>
<td>(i) Gastric intubation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(ii) 300 mg./kg. of aqueous pyrazole solution (540 mg./ml, 0.6 ml = 300 mg)</td>
<td>(ii) i.p. given half an hour before methanol administration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>c (2)</td>
<td>(i) 20 ml/kg. of 30% methanol</td>
<td>(i) Gastric intubation</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>(ii) 300 mg./kg. aqueous pyrazole solution (100 mg./ml.)</td>
<td>(ii) i.v. half an hour before methanol administration</td>
</tr>
<tr>
<td></td>
<td></td>
<td>d (2)</td>
<td>300 mg./kg. aqueous pyrazole solution (540 mg./ml.) once daily (0.6 ml = 300 mg.)</td>
<td>i.p.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>e (2)</td>
<td>300 mg./kg. aqueous pyrazole solution (540 mg./ml.) twice daily (0.6 ml = 300 mg.)</td>
<td>-do-</td>
</tr>
<tr>
<td>Controls (Group III)</td>
<td>4</td>
<td>a (2)</td>
<td>0.6 ml. of normal saline</td>
<td>-do-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>b (2)</td>
<td>Nil</td>
<td>Nil</td>
</tr>
<tr>
<td>Route of administration</td>
<td>Dose of chemicals (per kg body weight)</td>
<td>Number of animals</td>
<td>Group</td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------------------------</td>
<td>-------------------</td>
<td>-------</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>300 mg/kg aqueous pyrazole...</td>
<td>1</td>
<td>I</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>300 mg/kg aqueous pyrazole...</td>
<td>2</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>10 ml/kg 30% methanol...</td>
<td>2</td>
<td>II</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>10 ml/kg 30% methanol in distilled water...</td>
<td>3</td>
<td>I</td>
<td></td>
</tr>
</tbody>
</table>
### TABLE III

**BIOCHEMICAL STUDIES IN THE BRAIN OF RABBITS**

<table>
<thead>
<tr>
<th>Group</th>
<th>Subgroup</th>
<th>Water content percent of wet weight</th>
<th>Na g./kg wet weight</th>
<th>K g./kg wet weight</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>RABBITS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group I</td>
<td>Methanol</td>
<td>80.3 ± 1.158</td>
<td>1.76 ± 0.56</td>
<td>1.91 ± 0.56</td>
</tr>
<tr>
<td>(Chronic)</td>
<td>Group (4) *</td>
<td></td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Methanol pyridine</td>
<td>80.3 ± 0.71</td>
<td>1.96 ± 0.47</td>
<td>1.96 ± 0.64</td>
</tr>
<tr>
<td></td>
<td>Group (4)</td>
<td></td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Pyridine</td>
<td>79 ± 0.816</td>
<td>1.93 ± 1.01</td>
<td>2.13 ± 0.59</td>
</tr>
<tr>
<td></td>
<td>Group (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Group II</td>
<td>Methanol</td>
<td>79.8 ± 1.55</td>
<td>2.69 ± 0.66</td>
<td>2.16 ± 0.56</td>
</tr>
<tr>
<td>(Acute)</td>
<td>Group (4)</td>
<td></td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Methanol pyrazole</td>
<td>79.1 ± 0.73</td>
<td>1.98 ± 0.16</td>
<td>2.23 ± 0.44</td>
</tr>
<tr>
<td></td>
<td>Group (7)</td>
<td></td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
</tr>
<tr>
<td></td>
<td>Pyrazole</td>
<td>79.1 ± 0.3</td>
<td>1.69 ± 0.011</td>
<td>1.57 ± 0.57</td>
</tr>
<tr>
<td></td>
<td>Group (4)</td>
<td></td>
<td>P &lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Group III</td>
<td>(Control)</td>
<td>78.4 ± 0.2</td>
<td>1.33 ± 0.12</td>
<td>3.29 ± 0.5</td>
</tr>
<tr>
<td>(4)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>MONKEYS</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>(1)</td>
<td>78.4</td>
<td>1.44</td>
<td>3.42</td>
</tr>
<tr>
<td>Methanol</td>
<td>(2)</td>
<td>80.5</td>
<td>2.52</td>
<td>1.92</td>
</tr>
<tr>
<td>Pyrazole</td>
<td>(2)</td>
<td>78.9</td>
<td>1.92</td>
<td>2.37</td>
</tr>
</tbody>
</table>

* Figures in the parenthesis indicate number of animals in each subgroup.
804) Rao KR, Aurora AL, Muthaiyan S, Ramakrishnan S.
Methanol toxicity - an experimental study.
MYELIN /EDEMA /EYE
BRAIN /CHRONIC /MONKEY
RABBITS /METHANOL
Myelin thinning or even loss was observed in the MeOH treated
animals in both the acute and chronic studies in both monkeys and
Effects of detergent formula chelating agents on the metabolism and toxicity of cadmium in mice. Engstrom, Birgitta; Ruston, J.; Berglund, G.; Gustafsson, E. Karolinska Institute, Stockholm, Sweden. Acta. Vet. Pharmacal. Toxicol. 1978, 43(5), 387-97 (Eng). Chelating agents (NTA [119-13-9], STPP [Na5P3O10] [7756-29-4], and EDTA [50-56-4]) were useful as a combination of the effects observed in a short-term study, mice were exposed to CdCl2 (3.2 mg Cd/kg) and STPP (32 mg/kg) and demonstrated a higher mortality compared to animals given CdCl2 alone. This increase in mortality was similar to the response in CdCl2 (3.2 mg Cd/kg) combined with NTA (500 mg/kg). Animals exposed to CdCl2 + STPP or CME + NTA showed histological evidence of liver necrosis 24 h after exposure, and no animals given the combination of 500 mg CdCl2 and 32 mg STPP survived the treatment. The results suggest that the combination of chelating agents may be ineffective in only one of the three chelating agents tested, but the combination of all three chelating agents resulted in a significant increase in the toxicity of CdCl2 compared to the other two chelating agents.