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Similar Effects *In Vivo* of Two Aluminum Salts on the Liver, Kidney, Bone, and Brain of *Rattus norvegicus*

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The widespread distribution of aluminum (Al) compounds in nature and their use have stimulated considerable interest in the toxicity of this metal. Aluminum accumulation has been suggested to be an associated phenomenon in various human diseases such as renal dialysis dementia, senile dementia, dialysis osteomalacia, microcytic hypochromic anaemia, gastrointestinal toxicity and Alzheimer's disease (for detail see Ganrot 1986, Krishnan et al 1988).

The daily intake of Al has been estimated to be 9-14 mg (Pennington 1988). Pharmacological dose of Al as antacids is estimated to be 1-3g daily (Greger and Baier 1983). Following absorption, the metal is widely distributed throughout human body (Venugopal and Luckey 1978, Skalsky and Carchman 1983, Ganrot 1986) and accumulates in different tissues.

The present work was undertaken to observe the effects of different concentrations of aluminum following oral ingestion for various durations on various organs of rats and also to compare two different Al salts at doses having the same amount of Al. The findings can be of relevance owing to the widespread use of aluminum compounds by oral route either as medicines or unintentionally through utensils and cookwares.

MATERIALS AND METHODS

One hundered thirty five laboratory bred, healthy albino male rats Rattus norvegicus about 2 months old, weighing 120-150g were used for the experiment. The animals were maintained on standard pellet diet (Hindustan Lever, India) and given water ad libitum.

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five animals were kept in one cage under standard laboratory conditions.

Two salts of aluminum [aluminum sulphate, $Al_2(SO_4)_3.18 H_2O$ and potassium aluminum sulphate $KAl(SO_4)_2. 12H_2O$] were used and dissolved in deionized water.

The following doses (Table 1) were administered daily up to 21 days to the animals by gavage.

Table 1 showing different doeses of test chemical

Salt	Dose in terms of LD_{50}	Amount of salt/100g body wt.	Amount of metal/100g body wt.
Aluminum sulphate	1/5th LD_{50}	212.0 mg	17.18 mg
	1/10th "	106.0 mg	8.59 mg
	1/20th "	53.0 mg	4.29 mg
	1/30th "	35.5 mg	2.86 mg
	1/40th "	26.5 mg	2.16 mg
	1/50th "	21.2 mg	1.72 mg
Potassium aluminum sulphate		76.5 mg	4.29 mg
		50.3 mg	2.86 mg

Control sets were maintained by feeding the animals with deionized water. For each concentration in each experiment and for controls, 15 rats were used. Five rats were sacrificed at the end of each week.

The tissues namely, heart, liver, kidney, brain, testes, stomach and femur bone were dissected out and fixed in phosphate-buffered formalin. Fixed tissues were processed for microtomy. sections were processed and stained following the haematoxylin-eosin y double staining schedule (Pearse 1968).

RESULTS AND DISCUSSION

The effects of the two salts were similar at the comparable doses. The general effects are described below.

Dose dependent cytotoxic effects were observed in the liver. Control sets showed normal structure (Fig. 1A). The lower doses 1.72,2.16 and 2.86 mg of $AlSO_4$ and 2.86 mg of $KAlSO_4$ affected the periphery of the lobule

showing cytoplasmic degeneration and the nucleus as seen by hyperchromacia. With increasing doses multifocal degeneration of the entire liver tissue followed by the fibrous tissue proliferation was observed (Fig. 1B). There was also overall increase in congestion and dilatation of the sinusoids. The effects of both salts were similar at comparable doses.

Treatment with the lowest dose (1.72 mg) of $AlSO_4$ showed a slight swelling of the tubules of the cortex,but otherwise tissue structure was normal.With increasing dose (2.16 mg) increased swelling and degeneration of the cortical tubules were seen (Fig. 1C) .Treatment with 2.86 mg of both salts caused contraction of glomeruli and degeneration of distal tubules.Subcapsular necrosis in some areas with dilatation and degeneration of medulla was also noticed with increasing period of treatment.A higher dose (4.29 mg) of both salts induced more marked change in the cortical tissue affecting glomeruli as well as tubules.Higher dose (8.59 mg) induced haemorrhage and dilatation of tubules following prolonged exposure.Highest dose (17.18 mg) caused marked degeneration of tubules with loss of structure (Fig. 1D).Thus kidney also showed dose dependent degeneration primarily of the tubules followed by necrosis mostly observed in the cortex.In higher doses the medulla showed marked degeneration and cystic dilatation of the tubules.

Treatment with 1.72 mg for 21 days and 2.16 mg for 7 days did not show any appreciable change.After 14 and 21 days there was degeneration of the nerve cells with dilatation of blood cells (figs. 2A,2B).Treatment with 2.86 and 4.29 mg of either salt caused neuronal degeneration of the cerebral cortex with dilatation of blood vessels which increased with duration of exposure.With higher doses (8.59 mg and 17.18 mg) the damage of the nerve fibre was marked in the subcortical region and in the base of the brain.This degeneration was multifocal (Figs.2C,2D) and visible without specific nerve stain. The effects were dose and duration dependent and similar in both salts at the doses compared.

Testes did not show any histological damage up to 4.29 mg set of either salt.There was some evidence of spermatogonial cell decrease after 21 days of treatment with 8.59 mg and 14 days with 17.18 mg aluminum sulphate.

Heart did not show any histological effect in all the doses tried in this study.

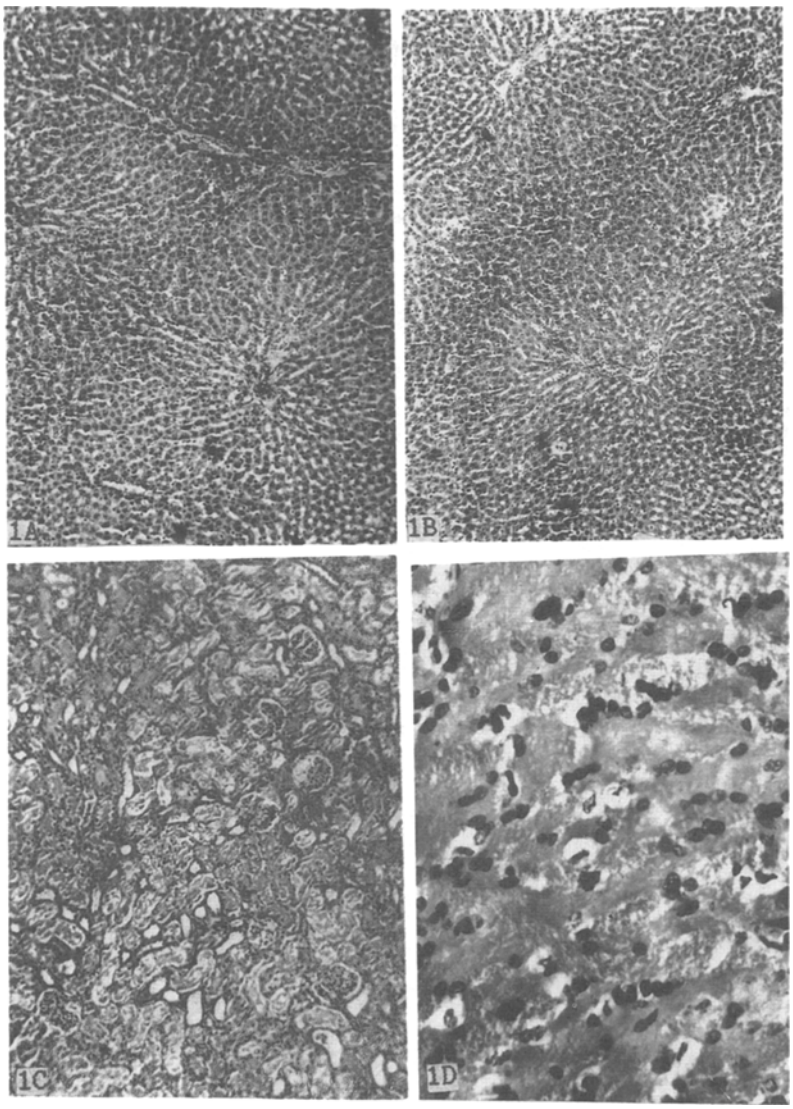

PLATE 1 : Microphotographs of section through liver (figs.1A-B),kidney (figs.1C-D) showing.

Fig. 1A : normal structure in control rats (Cax100)

Fig. 1B : multifocal degeneration of liver cells localized around the portal vessel following treatment with aluminum sulphate (8.59mg/100g body wt.) for 21 days (Ca *100)

Fig. 1C : swelling and degeneration of cortical tubules following treatment with aluminum sulphate (2.86 mg/100g body wt.)for 21 days (Ca*100)

Fig. 1D : marked degeneration of tubules with loss of structure following treatment with aluminum sulphate (17.18 mg/100g body wt.) for 14 days (Ca*600)

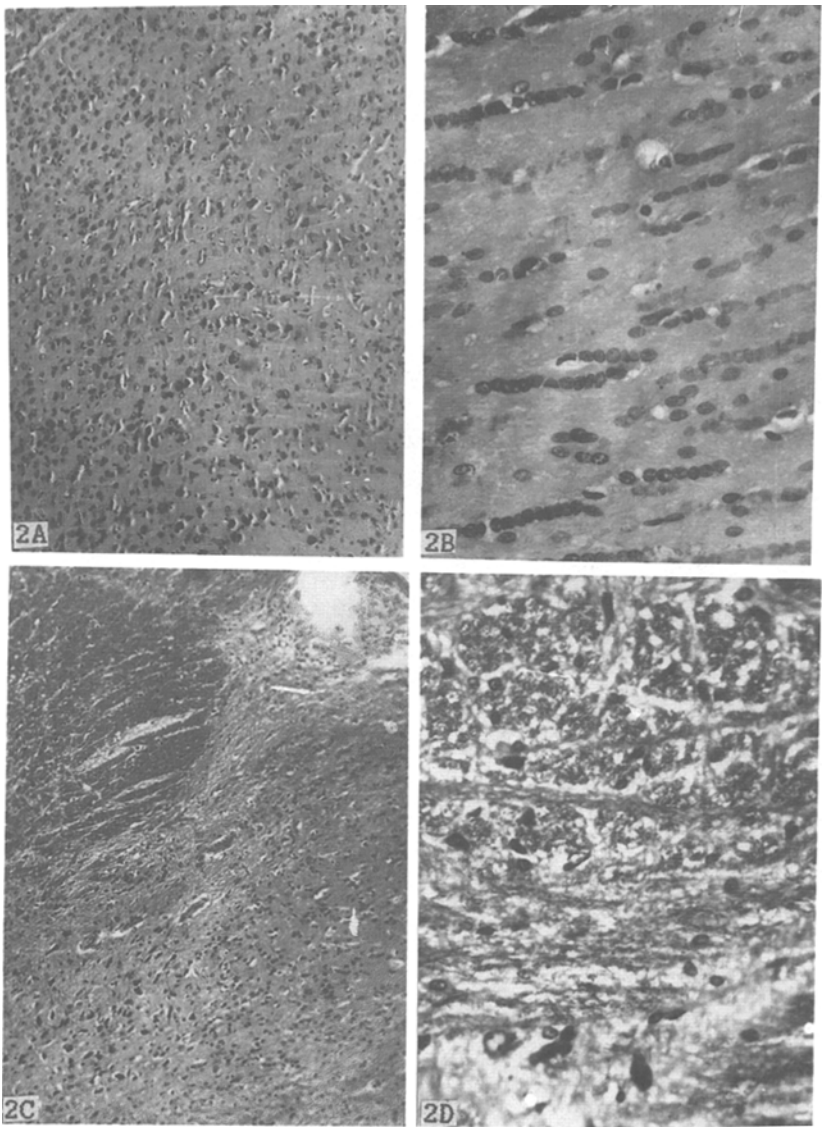

PLATE 2 : Microphotographs of section through brain (figs. 2A-D)showing :

Fig. 2A : slight degeneration of nerve cells and dilatation of blood vessels following treatment with aluminum sulphate (2.86 mg/100g body wt.) for 14 days (Ca *100)

Fig. 2B : Fig. 2A magnified (Ca *600)

Fig. 2C : multifocal degeneration and marked fibrosis following treatment with aluminum sulphate (17.18 mg/100g body wt.)for 21 days (Ca *100)

Fig. 2D : Fig.2C magnified (Ca *600)

Bone was not affected in control and up to 2.86 mg treated sets of both salts. In the 4.29 mg treated set of both salts there was multifocal degeneration and decalcification which increased with increasing duration,however,the obteoblast cells remained apparently normal.Degeneration of calcified bone and irregularity of osteoblasts was observed markedly in animals treated with 8.59 and 17.18 mg of aluminum sulphate.

Mucosal layer was thickened with some alteration of superficial layer. Hyperplasia and ulceration of stomach were recorded in some regions in 8.59 mg and 17.18 mg treated sets. Lower doses of both salts up to 4.29 mg were not toxic.

Aluminum has been found to be neurotoxic (See Roy 1987,Krishnan et al. 1988.Lipman et al.1988).It induces neurofibrillary degeneration and neuronal loss. The present observations indicate that the effects of Al on brain tissues depend both on the dose administered and the duration of treatment.

Liver possibly plays a significant role in the metabolism of Al.Previous in vivo and in vitro studies have indicated the toxic responses of the hepatic tissue to Al (Ebina et al.1984) as well as its accumulation in liver (Yokel 1983,Alfrey et al.1985,Klein et al 1988).Rats fed with 257 and 1075 ug Al/g diet for 67 days accumulated significantly greater amount of Al in their tibias,kidney and liver (Greger et al. 1986)while rats injected intraperitoneally for 10 days with 2.7 mg showed significant Al accumulation in brain, liver, spleen,bone and heart (Burnatowska-Hledin and Mayor 1984).

The observations made here confirm previous reports of damage induced by treatment with Al on brain and liver.These effects increase with increasing doses and longer periods of exposure.Similar damage was induced in kidney as well.The degenerative effect on the liver is followed by fibrosis observed after prolonged treatment in two highest doses of $AlSO_4$.Similarly the kidney cortex also shows tubular degeneration.The effects are suggestive of functional damage on a prolonged treatment.The deposition of Al in the brain neuronal fibers has been reported in Alzheimer's disease or pre-senile dementia(Perl and Brody 1980) although the correlation is not yet conclusively established.The observation that prolonged use causes increase of fibrosis of the cerebral cortex following neuronal degeneration can be correlated with the toxic effect of Al.

The decrease of osteoid cells and degeneration of calcified bones is suggestive of osteomalacic changes as observed in dialysis syndrome (Hodsman et al.1982, Goodman 1986).

However,despite the earlier records of accumulation of Al in skeleton,testes (Venugopal and Luckey 1978)and heart and other muscles (Skalsky and Carchman 1983) the observations made here do not show any appreciable histological damage in these organs. The gastro-intestinal tract in particular,is not affected to any significant extent,despite prolonged oral administration.

There is no significant difference in the effects of the two salts, when given in concentrations which would release equivalent amount of the metal per 100g body weight (see Table 1).This suggests that the two salts are metabolized equally effectively in the animal system.

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