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BIOCHEMICAL AND HISTOPATHOLOGICAL OBSERVATIONS IN THE KIDNEY OF RAT AFTER METHANOL TREATMENT

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Methanol which is an important industrial intermediate used in manufacturing of varnishes, paints, and as alternative fuel in motor vehicle. It has slow metabolism and remains in the blood for a long time. Methanol exerts its effect through its metabolite formaldehyde and cause blood acidosis. Kidney function test alongwith histopathological and ultrastructural changes in the kidney after methanol exposure show that methanol cause increase in blood urea and decrease in urine specific gravity, pH, creatinine, hippuric acid, blood pH and serum albumin. Increased vascular permeability and damage to basement membrane lead to changes in glomerular structure and function. Methanol cause lipid peroxidation enlargement of glomerulus and ultrastructural changes in kidney.

INTRODUCTION

Methanol (MeOH) has gained attention recently as a potential alternative fuel. It is also an industrial intermediate in formaldehyde manufacture and is used in varnishes, paints, thinners, stain removers and as solvent. Methanol is volatile substance and exposure occur mostly through breathing chemical in the work environment *i.e.* during glue sniffing, solvent abuse, synthesis, transport and use.

According to Economic Survey of India Report (1992) 20 lakhs industrial workers are employed in chemical industries. Amongst the aliphatic solvents, methanol is used for several industrial purposes. Methanol exposure affects on eye through conversion of methanol into formaldehyde and blood acidosis. Methanol has long been recognized as intoxicant due to its abuse as substitute for ethyl alcohol in alcoholic beverages (Roe, 1948). Wood & Buller (1904) reported 235 cases of blindness and death due to exposure to methanol. Acute poisoning, accidental poisoning or suicidal effect are most often caused by the oral administration of methanol. Methanol poisoning associated with severe neurotoxicity is still a problem in United States and worldover (Mc Martin *et al.,* 1980; Sejersted *et al.,* 1983). Neurological sequel of methanol poisoning have also been studied by Angerer & Bauman (1979). Developmental toxicity and post natal behavioural abnormalities in rats have been observed by Cumming (1993).

Methanol is selectively concentrated in areas with highest water content like intraoccular, cerebrospinal fluid and blood. Although methanol is metabolized in the organism but the rate of metabolism is slow and remains in the blood for a long time. There is every possibility of renal toxicity after methanol exposure. So we undertook a study on renal toxicity of methanol poisoning among laboratory rats.

MATERIALS AND METHODS

10 male Charles Foster rats *Rattus rattus* (albino) were selected from laboratory stock weighing (160 ± 10 gm) and maintained under laboratory conditions (room temp. 25 ± 5°C relative humidty 60 ± 10%). They were divided into two groups. Each rat was housed individually and provided commercial pellets (Lipton India, Calcutta) and tap water *ad libitum.* Rats of group (a) were injected with sublethal dose (0.2 ml) of 5 mM extra pure methyl alcohol (S. Merck, Bombay) on each alternate day for thirty days. The rats of group (b) were injected with 0.2 ml of normal saline on each alternate day to serve as control.

On thirty first day urine was collected from each group of rats through metabolic cages and sacrificed by light ether anesthesia. Blood was collected from heart through heparinized syringe for

blood analysis. Small pieces of the kidney of rats were quickly removed and processed for estimation of malondialdehyde by thiobarbituric acid (Wako, Japan) method of Placer *et al.* (1966) 1, 2, 3 tetramethoxy propane was used as standard. Reduced glutathione (GSH) was estimated in Kidney through Ellman (1959) method.

Specific gravity of the urine was estimated through a urinometer. Creatinine in the urine was determined using Alkaline Picrate method of Toro & Ackerman (1975). Urinary hippuric acid was estimated by direct colorimetric method of Ogata & Hobara (1979).

Blood urea was estimated by the urease neslerization method using span diagnostic Kit (Surat India). Serum proteins were estimated by Lowry Method (1951) using Bovine Serum Albumin (Sigma) as standard. Urine and blood pH was estimated through pH meter. Serum Na^+ was estimated by Flame Photometry (Systronics, India).

Student's "t" test was applied for statistical analysis (Fisher, 1950).

For histological studies small pieces of kidney from solvent treated rats were quickly removed and fixed in 10% buffered formalin. Paraffin sections thus prepared were stained with hematoxylin-eosin stain. For ultrastructural studies 1-2 mm pieces of kidney were fixed for 4 hours in Karnovsky's fixative at 4°C and washed in 0.1 M Phosphate buffer. Post fixation was carried out in osmium tetraoxide and processed further. Ultra thin sections were cut on a Reichert Jung Ultracut E. ultramicrotome and stained with uranyl acetate and lead citrate. Sections were examined on a T.E.M. (Philips C.M.-10) at Regional Electron Microscope Facility, All India Institute of Medical Sciences, New Delhi.

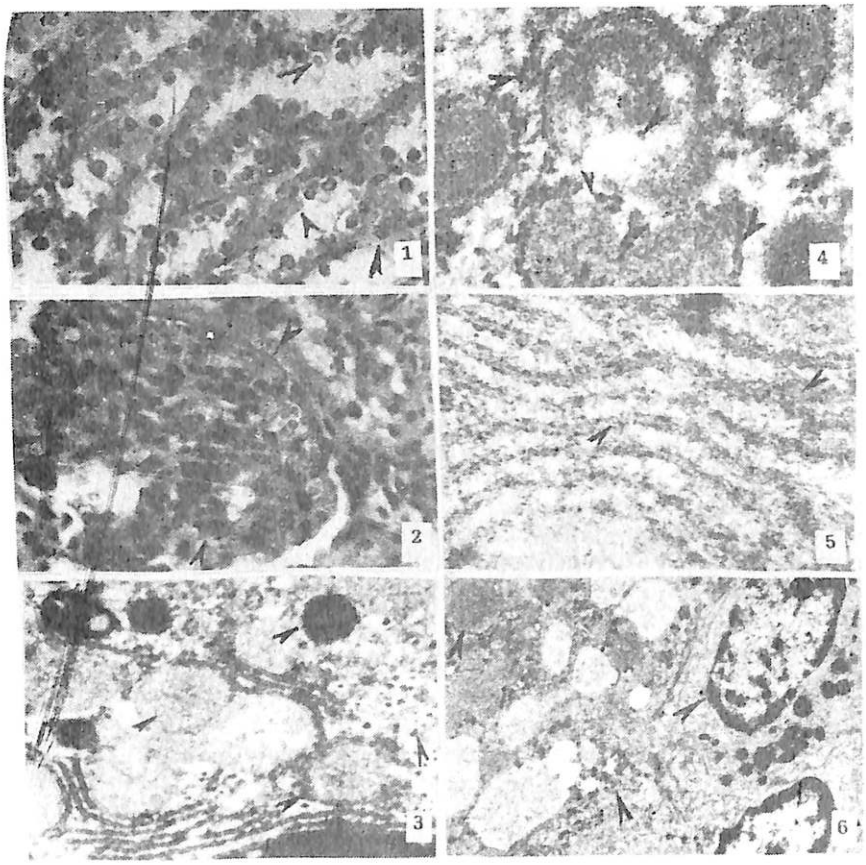
RESULTS AND DISCUSSION

Renosomatic index and specific gravity of urine declined non significantly after methanol treatment, however, creatinine and hippuric acid declined significantly in comparision to control rats. Methanol treatment cause blood acidosis as indicated by the decrease in blood and urine pH (Table I). These results are supported by Shahangian *et al.* (1986), Heinrich *et al.* (1982) that formic acid, a product of methanol metabolism is a major contributor to the acidosis of methanol poisoning. However accumulated formic acid rapidly metabolized via a tetrahydrofolate dependent pathway

Table I : Biochemical changes in urine, blood and renal tissue of methanol treated rats.

S. No.	Parameters	Control (B)	Treatment (A)
1.	Renosomatic Index	0.823 ± 0.03	0.798 ± 0.13 NS
2.	Malondialdehyde (n mole/mg protein)	0.139 ± 0.009	0.217 ± 0.05 **
3.	Glutathione (µg/g tissue)	800 ± 4.95	780 ± 2.01 **
4.	Specific gravity of urine	1.020 ± 0.002	1.018 ± 0.001 NS
5.	Creatinine (mg/litre)	750 ± 2.10	690 ± 2.95 **
6.	Hippuric acid (mg/litre)	772 ± 1.84	759 ± 2.16 *
7.	Urine pH	6.60 ± 0.08	6.05 ± 0.02 *
8	Blood pH	7.45 ± 0.04	6.68 ± 0.09 **
9.	Blood Urea (m Eq/litre)	26.5 ± 1.08	24.1 ± 0.96 NS
10.	Serum albumin (gm/100 ml)	4.22 ± 0.26	3.21 ± 0.21 *
11.	Serum Protein (gm/100 ml)	7.60 ± 0.51	5.12 ± 0.35 *
12.	Serum Na^+ (m Eg/litre)	147.0 ± 1.60	169 ± 2.80 ***

Results are mean ± S.E. of 5 observations in each group of rats; p = * < 0.05, ** < 0.02, *** < 0.01, (between control and experimental group of rats).



Figs. 1-6. 1. T.S. of kidney from methanol treated rat caused damaged to tubular epithelium with displaced nuclei × 300; **2.** T.S. of kidney from methanol treated rat showing enlargement of glomerulus × 300; **3.** Kidney of methanol treated rat with lipid droplets, accumulation of ribosomes and collagen fibres × 16300; **4.** Electron micrograph of methanol treated rat shows thickened basement membrane, ballooning in cytoplasm, collagen fibres and obliterated capillary-lumen × 18000; **5.** Electromicrograph of kidney showing enlargement and proliferation of endoplasmic reticulum × 22000; **6.** Electron micrograph of kidney from methanol treated rat shows degenerative changes evidenced by lipid droplets, cytosomes and macrophage surrounded by collagen fibres × 12000.

(Tephly *et al.*, 1984). Methanol treatment cause hypernatermia decrease in blood urea and serum ablumin. Methanol treatment cause significant increase in kidney malondialdehyde and decreases kidney glutathione level.

Histology of methanol treated rats kidney shows damage to tubular epithelium, nuclear proliferation, enlargement of glomerulus, clumping of nuclei, glomerulonephritis, dilated glomeruli and tubules. Degenerative necrosis was also observed (Figs. 1 & 2). Electronmicrograph of methanol treated rat shows presence of collagen fibres, cytosomes, microbodies, swelling of endothelial cells and large amount of amorphous material. Ballooning of cells, presence of lipid droplets and

enlargement of endoplasmic reticulum were also observed. Increase in number of mitochondria, thicknening of basement membrane, increased lysosomal activity were clear (Figs. 3-6).

Our results suggest that methanol cause both pathological and functional changes in the kidney of rats. Observation on serum protein suggest that methanol cause hypoproteinimia and decrease the serum albumin level. Hypoalbuminia is a common finding in acute renal disease such as glomerulonephritis and protein lossing diarrhoea, where, albumin molecules escape from the vascular system (Reynolds, 1971). Kidney malondialdehyde level was found elevated after treatment. Methanol generate oxidative radicals which, cause lipid peroxidation. Methanol affect the glutathione cycle as reduced glutathione (G.S.H.) level declined after treatment. Tephly *et al.* (1991) suggested that in the intact rat, peroxidase catalase system plays a dominant role in the initial oxidation of methanol. Results are supported by histological and ultra structural studies on kidney. These observation can be extrapolated on the humans, who are supposed to be more susceptible to methanol poisoning than lower species (Tephly, 1991). The results are further supported by our own findings on antiperoxidative enzymes reported earlier (Rana & Kumar, 1995). Activated oxygen species produced are the source of oxidative stress, responsible for the induction of lipid peroxidation and reduction of glutathione level.

Methanol treatment reduces the specific gravity of urine and blood urea. The end product of metabolism urea, creatinine, hippuric acid are not absorbed but are allowed to pass through urine. While 99% of water of glomerular filterate is conserved. Only 4% of urea and no creatinine is reabsorbed. It indicate that kidney fails to remove creatinine from the blood due to disturbances caused by solvents in the excretory capacity of tubule. The amount of these substances can also be related to the total number of nephrons particularly in filteration processes.

Damage to kidney function was also observed in solvent exposed subjects (Askergen, 1982). Glomeruli appears to be particularly vulnerable to damage for several reasons. Injured basement membrane of the filtering apparatus may lead to proteinuria causing deleterious effects. Increase vascular permeability in acute inflammation may produce glomerular changes.

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