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LIVER AND KIDNEY FUNCTION IN MOLYBDENUM AND COPPER POISONING

S. V. S. RANA and A. KUMAR

School of Environmental Contamination and Toxicology, Department of Zoology, D. A. V. (P. G.) College, Muzaffarnagar-251001, India

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The present study reports on the liver and kidney function tests i. e. total bilirubin, hippuric acid, glucose-6-phosphatase, cholinesterase, serum urea, urine albumin and urine specific gravity, and the dilution test performed to detect hepato-renal damage in the rates fed with molybdenum and copper. Observations on bilirubin indicated haemolysis in copper fed rats, are presented. Low levels of hippuric acid also reflected liver injury. Increased urine albumin in molybdenum and copper treated rats was found to be a valuable indicator of renal injury. However, the dilution test was found less valuable in determining long-term injury by these elements.

Environmental contamination by metal particulates including lead, cadmium, mercury, copper and molybdenum has risen considerably with their increasing use in diverse agricultural, chemical, technological and industrial processes (1, 2). Molybdenum and copper are important in animal and plant nutrition (3, 4) as well as in industry (5). Although data are available on the pathological effects of these elements (6—14), relevant information on liver and kidney function is lacking. Since many of the metals have a tendency to accumulate in the liver and kidney, we have analysed the results of some liver and kidneys function tests after twenty days of treatment with molybdenum and copper.

MATERIAL AND METHODS

Forty male albino rats (*Rattus rattus* albino), 90 days old, weighing 100 ± 10 g were selected from own laboratory stock. Animals were divided at random in four groups of ten rats each. Each rat was

housed separately in an unpainted wooden cage. The animals were fed on standard laboratory diet (Hindustan Lever Ltd., Bombay) and drank tap water ad libitum. They were maintained at 25 ± 5 °C (humidity 60 ± 10%) in laboratory conditions. The rats in group A received ammonium molybdate $(NH_4)_2MoO_47H_2O$ at the dosage of 1.0 g/kg body weight by gavage in addition to laboratory diet, daily for a total period of twenty days. The rats in group B received 0.1 g/kg body weight copper sulphate ($CuSO_45H_2O$) and the rats in group C were fed on a mixture of ammonium molybdate and copper sulphate in the ratio of 1 : 1 at the total dosage of 2.0 g/kg body weight (1.0 g/kg body weight of ammonium molybdate and 1.0 g/kg body weight of copper sulphate) in addition to laboratory diet for the same duration. The rats in group D receiving laboratory diet and tap water alone served as controls. The applied dose levels were determined after making preliminary toxicological test such as oral LD_{50}. After the scheduled treatments, the rats were starved for 24 hours and serum urea and serum bilirubin were measured. In urine hippuric acid, albumin, and specific gravity were determined and dilution test was made using the prescribed methods.

A sample of 1.6 ml of blood was collected from the caudal vein of each rat under ether anaesthesia. It was mixed with 1.6 ml of 0.9% sodium chloride containing 2% potassium oxalate and centrifuged. Urea (15) and bilirubin contents were determined in plasma (16). Six rats from each group thus starved were made to empty their bladder and were transferred to urine collecting cages (17) where drinking water was freely available. Urine was collected for 24 hours and its specific gravity (SG) was determined using a pycnometer (approximate capacity 0.5 ml) (18). Hippuric acid (19) and albumin (20) were determined in all urine samples. For the dilution test, the remaining rats were given, by gastric intubation, the tap water corresponding to their body weight and urine was collected at 30 minute intervals for 120 minutes (18). The volume and SG of each sample were determined. The urine volume collected in 120 minutes expressed as a percentage of the water given and SG of the largest volume collected in a 120-minute period were recorded.

After the above tests, all the animals were anaesthetized by ether, and slices of the liver were quickly excised and frozen. The 10% (w/v) homogenates of the liver were prepared in 0.25 M ice-cold sucrose solution. The temperature was maintained near 0 °C throughout the period of homogenization. The homogenates were centrifuged for 120 minutes at 1500 g and clear supernatant fluids were used for estimating glucose-6-phosphatase (21) and cholinesterase (22). The protein content in the homogenates was determined using bovine serum albumin (BSA) as the standard (23). The Fisher's »t« test (24) was used to calculate the statistical significance.

RESULTS AND DISCUSSION

The results of the liver function test in copper fed rats indicate a significant deviation from control rats except for cholinesterase. The concentration of plasma bilirubin decreased insignificantly in molybdenum treated rats, whereas the hippuric acid declined after molybdenum and copper treatments. Molybdenum alone and in combination with copper did not stimulate the enzyme glucose-6-phosphatase, but an inhibition was noted in the activity of glucose-6-phosphatase and cholinesterase after the copper treatment (Table 1).

Table 1

Liver function test in molybdenum and copper fed rats

Test	Control	Treatment		
		Mo	Cu	Mo+Cu
Plasma bilirubin (mg/100 ml)	0.32 ± 0.02	0.26 ± 0.02	0.53 ± 0.06*	0.46 ± 0.06*
Urine hippuric acid (% in 2 hr)	45.0 ± 2.0	30.0 ± 3.0**	25.0 ± 2.5**	40.0 ± 2.0
Liver glucose-6-phosphatase	0.72 ± 0.26	0.82 ± 0.029	0.57 ± 0.019**	0.78 ± 0.015
Liver cholinesterase (Units)	42.0 ± 5.23	45.0 ± 5.89	28.0 ± 1.05	39.0 ± 3.68

Activity is expressed in mg of inorganic phosphate liberated/mg protein/h at 37 °C. Each value represents the mean ± standard error (3 observations). The values in the same row bearing an asterix differ significantly from control values — $*P < 0.05$; $**P < 0.01$ (Fisher's »t« test) (24).

Renal function tests (Table 2) showed a change in the percentage of serum urea and urine albumin. The specific gravity of urine significantly increased after the treatment with molybdenum and copper given either separately or in combination, whereas the dilution test indicated a change in the volume percentage of urine. Specific gravity increased in rats fed on molybdenum and copper individually and simultaneously as compared with control animals.

The most widely used criteria of the toxic action of a metal in animals are the reduction in the rate of body weight gain, the detection of gross histological lesions in the organs, the change in the organ weight and increased mortality rate (25). However, functional tests are also helpful in determining chronic injury to the liver and kidneys. It is reasonable to assume that the present observation of increased bilirubin level in copper fed rats reflects haemolysis, since bilirubin is the breakdown product of haemoglobin. Data in molybdenum treated rats

Table 2

Kidney function tests in molybdenum and copper fed rats

Test	Control	Treatment		
		Mo	Cu	Mo+Cu
Serum urea (mg %)	32.0±0.92	56.2±2.05***	36.0±1.06*	40.0±1.13**
Urine albumin (mg %)	30.0±1.60	115.6±8.02***	58.4±1.69***	86.0±2.72***
Urine specific gravity	1.041±0.002	1.086±0.002***	1.062±0.003**	1.051±0.001**
Urine dilution test (volume %)	97.1±1.60	86.2±1.98**	90.0±2.10*	95.1±1.86
Urine dilution test (Specific gravity)	1.0017±9x10—4	1.0048±8x10—4*	1.0030±9x10—4	1.0022±6x10—4

Each value represents the mean ± standard error (3 observations). The values in the same row bearing an asterix differ significantly from control values — *P < 0.05; **P < 0.01; ***P < 0.001 (Fisher's »t« test) (24).

are in good agreement with the percentage of haemoglobin as reported earlier (12), but bilirubinaemia not accompanied with a fall in haemoglobin (12) in rats fed both molybdenum and copper remained unexplained. Similarly, the hippuric acid test helped in estimating the functional capacity of liver parenchyma. A reduced value for hippuric acid corresponded to the severity of the liver damage reported elsewhere (12, 13). A correlation between the liver damage, renal impairment and hippuric acid concentration was established for many hepatotoxins whereas enzymological changes broadly reflected necrosis (25, 26).

Although a series of renal function tests have been developed, the most useful are those that constitute the standard urine analysis. Observations on serum urea probably indicate the effects of molybdenum and copper on the initial reaction in the catabolism of many amino acids involving the removal of the amino group which was converted to urea. The increase of urine albumin in molybdenum and copper fed rats was also supported by an earlier histopathologic observation on renal damage (12, 13). Thus only the presence of albumin in urine was found to be a valuable indicator of renal injury.

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Sažetak

FUNKCIJA JETRE I BUBREGA PRI TROVANJU MOLIBDENOM I BAKROM

Autori iznose rezultate testiranja funkcije jetre i bubrega (ukupni bilirubin, hipurna kiselina, glukoza-6-fosfataza, kolinesteraza, urea u serumu, albumin u mokraći, specifična težina mokraće i dilucijski test) koje su proveli da bi otkrili eventualna oštećenja jetre i bubrega štakora koji su primali hranu s dodatkom molibdena i bakra. Vrijednosti bilirubina u štakora hranjenih hranom s dodatkom bakra upućivali su na hemolizu. Niske razine hipurne kiseline također su ukazivale na oštećenje jetre. Povišeni albumin u mokraći štakora tretiranih molibdenom i bakrom pokazao se vrijednim indikatorom bubrežnog oštećenja. Međutim, dilucijski test pokazao se manje vrijednim u određivanju dugotrajnog oštećenja uzrokovanog tim elementima.

Škola za onečišćenje okoliša i toksikologiju,
Odjel za zoologiju,
Muzaffarnagar, Indija

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