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We- together to save yourself society e-ISSN 0976-7614

Volume 3, Issue 2, April 2012 Research Article

Histopathological Study of Subacute Toxic Effects of Chloroacetic Acid on Albino Rats and its Correlation with Serum Levels of Malondialdehyde

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Abstract: Human beings are increasingly being exposed to chloroacetic acid (CAA), a type of halo acetic acid. It would not be an exaggeration to say that almost the whole humankind today is affected by it or its metabolites. The concern over the carcinogenicity of haloacetic acids led the United States Environmental Protection Agency to regulate the allowable concentration of haloacetic acids in drinking water as part of the Disinfectants and Disinfection Byproducts Rule promulgated in 1998. Keeping this view in mind, the present study on histolopathological evaluation of different types of tissues viz., brain, kidney, liver, spleen and testes of *Rattus norvegicus* was performed, to find out the subacute toxicity of chloroacetic acid and correlation between CAA administration and changes in malondialdehyde (MDA) level in blood.

Keywords: Chloroacetic acid, Histopathology, *Rattus norvegicus*, MDA.

1. Introduction

Haloacetic acids are probable human carcinogenic compounds[1,2]. Haloacetic acids are naturally formed in the atmosphere during the photochemical degradation of chlorinated solvents[3]. In addition, they have been found as disinfection byproducts that result from the addition of a chlorine compound, such as hypochlorous acid, hypochlorite or dichlorine of water or wastewater for disinfection purposes.[4]

Chloroacetic acid (CAA) is used in research and development laboratories, in medical and surgical hospitals and in chemical and pharmaceutical preparations. It is also used in fabric mills, in communication equipment (television, radio, telegraphs) and in automotive stamping[5]. Because of their widespread occurrence, toxicity to plants and aquatic organisms, and most importantly their suspected human carcinogenicity, there is a great need to find treatment methods for haloacetic acids.

CAA a type of haloacetic acid has been chosen in the present study because human beings are increasingly being exposed to this chemical. It would not be an exaggeration to say that almost the whole humankind today is affected by it or its metabolites. Also, CAA has been nominated by the Environmental Protection Agency for toxicology and carcinogenicity testing because of its large production volume, its presence in drinking water supplies, and the lack of information on its carcinogenic potential.

The present study aimed at histolopathological evaluation to find out the subacute toxicity of CAA on different types of tissues viz., brain, kidney, liver, spleen and testes of *Rattus norvegicus* and correlation between CAA administration and changes in serum malondialdehyde level.

2. Material and Methods

The present study was carried out on adult male albino rats, in the departments of Forensic Medicine & Toxicology and Pathology, J.N. Medical College, A.M.U., Aligarh. The animals were provided with a standard pellet laboratory diet (Lipton India Limited) and water *ad libitum*. They were housed under identical diurnal conditions and temperature for seven days to get

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acclimatized. The animals were divided into two groups: Control and Study group. A freshly prepared solution of Chloroacetic acid in distilled water was prepared and injected intraperitoneally daily in a dosage of 0.24mg/100g body weight, for seven days to the study group. The healthy male adult albino rats were taken as a control group.

2.1 Procurement of tissues and blood

Kidney, spleen, liver, brain and testes were collected after seven days of sacrificing the test animal for histological examination. To maintain the comparative analysis control rats was also sacrificed at the same time. Blood was collected by heart puncture for the evaluation of malondialdehyde (MDA) estimation. The tissues were fixed in 10% formalin, embedded in paraffin, cut into 3-5µ thick sections and stained with haematoxylin & eosin.

2.2 MDA estimation

For assay, 1.0ml of serum was mixed with 2.5ml of 20 percent trichloroacetic acid and 1.0ml of 0.67 percent of an aqueous solution of thiobarbituric acid. The mixture was heated in boiling water bath for 30 minutes; the pink pigment Formen was extracted with 2ml of N-butanol and its observance was read spectrophotometrically, at 532nm against N-butanol as blank.

3. Result

At the end of the seven-day period during which the rats were injected with a freshly prepared solution of CAA, all the rats showed markedly reduced general activity.

The histopathological findings of subacute exposure of chloroacetic acid, on albino rat brain tissue, resulted in generalized edema (spongiosis) of brain parenchymal tissue and focal neuronal degeneration (Fig. 1). Another positive finding was the congestion of blood vessels in the chloroacetic acid treated study group.

The liver showed marked portal and septal fibrosis along with sinusoidal dilatation and the presence of mild mononuclear cells infiltrate (Fig. 2). Central vein congestion was also seen along with individual hepatic cell necrosis.

The red pulp of the spleen showed marked expansion along with congestion and large number of megakaryocytes. Pigment-laden macrophages could also be noted in the red pulp with slight to marked atrophy of white pulp.

Focal tubular necrosis and parenchymal (interstitial) haemorrhage of the kidney tissue were noted in the study group. Marked congestion of blood vessels and glomerular capillaries was also seen (Fig. 3).

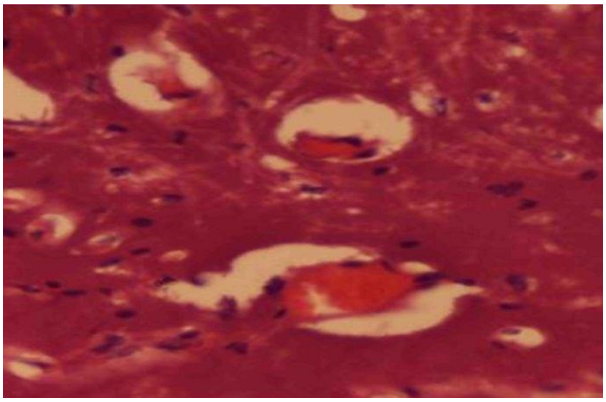

Fig. 1. BRAIN: Photomicrograph shows spongiosis, marked congestion of blood vessels and focal neuronal degeneration. Hematoxylin & Eosin X 40.

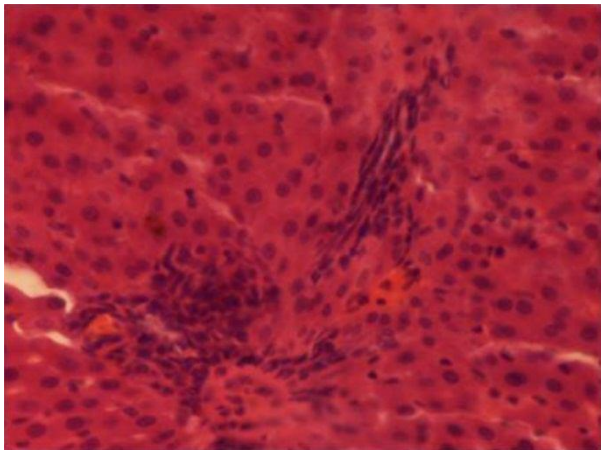

Fig. 2. LIVER: Photomicrograph shows septal and portal fibrosis, sinusoidal dilatation and individual cell necrosis with mild mononuclear cell infiltrate. Hematoxylin & Eosin X 40.

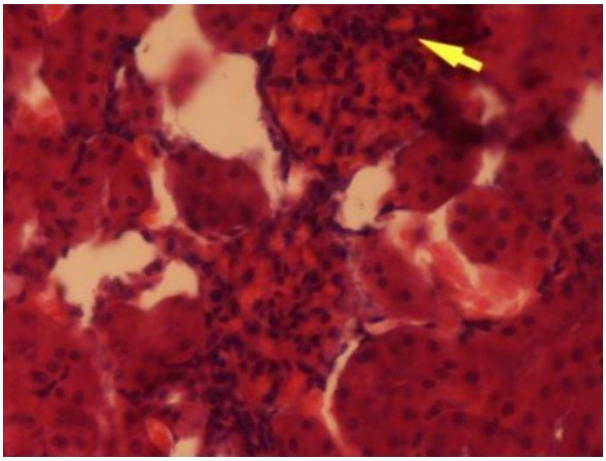

Fig. 3. KIDNEY: Photomicrograph shows congested glomerular capillaries and blood vessels. Hematoxylin & Eosin X 40.

Histopathology of the testes showed marked tubular atrophy and fibrosis along with testicular degeneration (Fig. 4).

The different organs i.e., brain, liver, spleen, kidney and testis, in the control group, on gross examination, appeared to be normal in size and consistency and the histological examination of these showed the normal microscopic appearance.

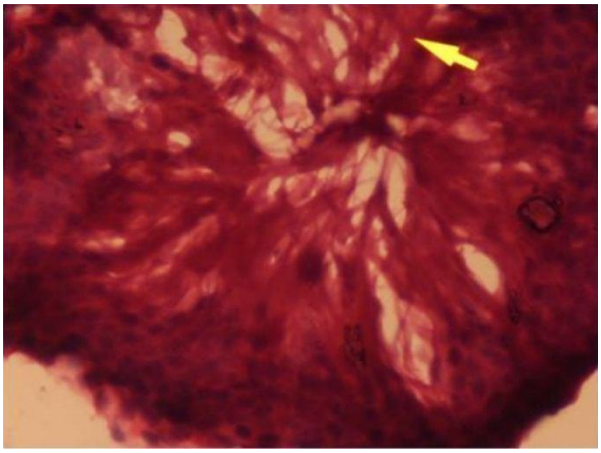

Fig. 4. TESTIS: Photomicrograph shows marked testicular degeneration and tubular fibrosis. Hematoxylin & Eosin X 40.

The serum level of MDA in the study group before administration of the drug was found to be 1.27 ± 0.35nmol/ml. The level after exposure was found to be 6.04 ± 0.89nmol/ml (p-value< 0.001) which was highly significant. The serum level of MDA in the control group was found to be 1.03 ± 0.01nmol/ml.

4. Discussions

This study deals with subacute toxic effects of chloroacetic acid (CAA) on different organs of rats. CAA is used as preservative, herbicide and for treatment of plantar warts. As for hazards posed to human beings, at least two deaths have been reported due to exposure to CAA[5]. In view of the harmful effects of pesticides and food additives, which are being consistently recorded, it is essential that toxic effects of this chemical should be evaluated under a variety of conditions.

Though new and sophisticated techniques are being developed, several conventional techniques still provide useful information on the toxicity of these compounds. In the present study, we have evaluated the toxic potential of CAA in blood, kidney, liver, testes, spleen and brain tissues of *Rattus norvegicus* employing these conventional techniques; *i.e*. LPO assays to find out changes in malondialdehyde (MDA) level in the blood and histopathological changes produced by the CAA in the above tissues.

Pereira[6] studied in groups of 90 females B6C3F1 mice, 7 – 8 weeks of age, when they were administered dichloroacetic acid (a type of haloacetic acid) continuously in the drinking-water at concentrations of 2.0, 6.67 and 20.0mmol/L [236, 854 or 2350mg/L], adjusted to pH 6.5–7.5 with sodium hydroxide, and a control group of 134 animals who received 20.0mmol/L sodium chloride. Mice were killed after 360 days of exposure and their livers evaluated for foci of altered hepatocytes (eosinophilic and basophilic), adenomas and carcinomas. No such histological findings were found in our study in albino rats after 7 days of CAA exposure.

Dote *et al*.,[7] have demonstrated features of hepatocellular degeneration in their study on fatal occupational accidents due to CAA. Toshina *et al*.,[8] have also shown that CAA causes hepatocellular degeneration. Halit-Demir[9] has demonstrated that TCA, which is also a haloacetic acid, causes liver toxicity and leads to deranged liver function test. Our study showed marked portal and septal fibrosis, congestion and sinusoidal dilation along with mild mononuclear cells infiltrate in the liver parenchyma. Mild individual hepatic necrosis was also observed in our study, which indicated clearly that lower doses of chloroacetic acid can induce hepatic damages.

Our study revealed widespread and generalized edema (spongiosis) of brain parenchymal tissue after subacute peritoneal exposure of CCA on albino rats. Quite similarly Kulling *et al*.,[10] have reported cerebral edema after 80% exposure of CCA through the skin surface.

We also noticed focal tubular necrosis of the kidney tissues along with parenchymal (interstitial) hemorrhage in the study group. Quite similarly Kulling *et al*.,[10] and Toshina *et al*.,[8] have demonstrated mild tubular degeneration and renal insufficiency after CAA exposure in their experimental models. Dote *et al*.,[7] have further potentiated the findings of renal dysfunction in their study on fatal occupational accidents of monochloroacetic acid.

Our histopathological study on albino rats after CCA exposure for 7 days showed marked testicular degeneration and tubular fibrosis but no literature is available for correlation on this aspect of our work.

The red pulp of the spleen showed the presence of pigment-laden macrophages and marked expansion due to congestion. We also noticed slight to marked atrophy of the white pulp. No literature is available to potentiate our findings in this regard.

The level of MDA in the control group was found to be 1.03 ± 0.01 in our study. The level of MDA in the study group before and after administration of chloroacetic acid was found out to be 1.27 ± 0.35 and 6.04 ± 0.89 respectively with p-value of < 0.001, which is highly significant. There is still a lack of information about CAA exposure and its correlation with levels of MDA in blood. Our study showed a significant positive correlation between the drug chloroacetic acid and MDA levels in blood level of study group of rats exposed to the drug (r-value == 0.932, p = 0.01).

5. Conclusion

There is a positive correlation between CAA administration and serum malondialdehyde levels in a study group of rats exposed to the drug. Considering the susceptibility to *R. norvegicus* to CCA, possible exposure of other mammals including humans to this compound should be studied to know their toxic effects on different body tissues.

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