Histological Studies of the Effects Of Dichloroacetic Acid (DCA) Exposure on The Hippocampus of Male Albino Rats

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ABSTRACT
Dichloroacetic acid (DCA) is commonly found in drinking water as a by-product of chlorination disinfection. It is formed from organic material during water chlorination and is considered as a neurotoxins in rats, dogs, and humans. The objective of the present study is to investigate the histopathological changes in the hippocampus area of the brain after the administration of DCA (125mg/kg body weight) in male albino rats for 30, 60 & 90 days. Normal histology of the hippocampus in control rats have shown distinct & intact layers as stratum oriens, stratum pyramidale and stratum radiatum. Pyramidal layer through CA1 to CA3. Dentate gyrus (DG) is also well stained & intact. Various subfields of hippocampus containing granule cell layer (GCL) & Hilus (H) are found to be intact & well marked. The cells of pyramidal layer are showing destruction and vacuolization of the molecular layer (ML) of dentate gyrus of hippocampus is seen after 30 days of DCA treatment. Where as after 60 days, there is higher destruction of cells in the CA1 & CA2 regions than the CA3 region of the pyramidal cell layer (PCL). After 90 days chronic treatment of DCA extensive vacuolization in the cells of molecular layer & destruction in the pyramidal cell layer has been seen. The changes in the cellular structure with DCA administration has been correlated to its possible neurotoxic effects with reference to alterations in the memory, learning & behavior of the animal.

Key words:- Dichloroacetic acid (DCA), Pyramidal cell layer (PCL), Granule cell layer (GCL), Molecular cell layer (ML), Hilus (H), Dentate gyrus (DG), Hippocampus.

INTRODUCTION
DCA is a major disinfection by-product (DBPs). It is used as a topical astringent, fungicide and medicinal disinfectant a test reagent for analytical measurements, to treat lactic acidosis and in the synthesis of organic materials, including pharmaceuticals [2,11,13]. It is a colorless to slightly yellow liquid with a pungent odour [2,7]. Use of chlorine as a disinfectant in the treatment of drinking water has virtually eliminated water borne diseases, because chlorine can kill or inactive most microorganisms commonly found in water. Disinfection is essential to safeguard drinking water; the health risks from disinfection by-products are much less than the risks from consuming water that has not been disinfected. DCA is formed from organic material during water chlorination. The health effects associated with exposure to halo acetic acids vary with the specific compound. It has been used as a therapeutic agent. [10] studied the effect of DCA in the treatment of MELAS (mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes) and found peripheral nerve toxicity which overshadows any potential benefit of the DCA in the treatment of lactic acidosis.[18] demonstrated that DCA induces a reversible inhibition of myelin-related proteins for its peripheral neuropathy effects. There are reports of neurotoxicity in humans, dogs and rats. Peripheral neuropathy in humans found [12,16,19]. [4,9] reported that there is hind limb paralysis and peripheral neuropathy in rats. [1] concluded that focal vacuolation and gliosis were present in the forebrain and brain stem of rats. Partial paralysis of the hind limbs in beagal dogs due to DCA is seen by [3,9].

Effects of DCA treatment have been limited to transient central neuropathy, periphery neuropathy in humans [17]. [12] reported that DCA produced neurological effects including sedation and peripheral
neuropathy. [15] investigate degenerative changes in scattered neurons, and vacuolar demyelination in spinal cord, as well as gray matter vacuolation. DCA developed partial paralysis of the hind limbs in beagle dogs was demonstrated [9]. Hind limb weakness, nerve conduction and abnormal gait were observed [20] in rats. [17] in his studies concluded that DCA can cause a reversible peripheral neuropathy that may be related to thiamine deficiency. Neurological complications were supported in studies using thiamine supplementation to reverse the effects of DCA found [18] in rats and humans.

Neuromuscular toxicity of DCA was suggested in rats. DCA produce neurotoxicity in both laboratory animals and humans [1]. [3] report vacuolization in myelinated tracts in the brains of both dogs and rats. [4] demonstrate that hind limb paralysis and peripheral neuropathy in rats. Several cases of mild peripheral neuropathy following dichloroacetic acid have been examined [19]. Peripheral neuropathy of DCA was studied in rats, dogs and humans. Periphery neuropathy has been noted [16] in humans and dogs.

**MATERIAL AND METHODS**

**Animals:** - 24 adult’s male albino rats with average weight of 150-250g were randomly assigned into four groups A, B, C and D of (n=6) in each groups. Groups A, B, C and D (n=6) serves as treatment groups while group D (n=6) is the control. The animals will be maintained in hygienic environment and fed with commercially available pellets of rat chow and water *ad libitum*. They will be kept in humidity and temperature controlled rooms and exposed to 12-h dark light cycles.

**Dichloroacetic acid administration:** - The rats in the treatment group A, B and C was given 125mg/kg-bodyweight dose of DCA, with orally. The control D group was fed with normally rat chow and pellets without DCA for 30, 60 and 90 days. The rats were sacrificed after 30, 60 and 90 days of the experiment. The hippocampus area of the brain was quickly dissected out and fixed in 10% formalin for routine histological techniques.

**Histological study:** - They were fixed in 10% formalin (pH 7.1), for twenty-four hours for histological procedures. Brains were routinely processed for paraffin wax embedding. Then, 8 μm thick paraffin cross sections containing hippocampal tissue were mounted on slides and stained using routine haematoxylin and eosin (Drury and Wallington, 1980) method. Microscopy was conducted on an Olympus microscope (Tokyo, Japan) and images were captured and processed by an attached eyepiece camera.

**RESULTS**

The normal histology of the hippocampus for the control group showed three distinct layers as shown in Figure 1 & 2. They are stratum oriens, stratum pyramidale and stratum radiatum. In the control group the cells were intact with well stained nuclei. The regions of the principal cell layer of the hippocampus proper (pyramidal layer) were also intact through CA1 to CA4 and well demonstrated in this group. The dentate gyrus (DG) was intact and well stained. Its principal cell layer, the granular layer was well demonstrated. The treatment sections of the hippocampus of treated rats showed some histological changes that were at varied with those obtained in the control. The cells of pyramidal layer are showing destruction and vacuolization of the molecular layer (ML) of dentate gyrus of hippocampus is seen after 30 days of DCA treatment (Figure 3 & 4). After 60 days, there is higher destruction of cells in the CA1 & CA2 regions than the CA3 region of the pyramidal cell layer (PCL) (Figure 5 & 6). After 90 days chronic treatment of DCA extensive vacuolization in the cells of molecular layer & destruction in the pyramidal cell layer has been seen (Figure 7 & 8).

**DISCUSSION**

[8] reported that Methamphetamine 10mg/kg, i.p. was administered to adult Wister rats for three rat. They results obtained that extensive vacuolations was observed in the pyramidal layer, neuronal cell death was more in the CA1 and CA2 region and CA3 region the hilus of DG and granular cell layer of DG, were least affected in the hippocampus.

[5] investigated that ethanol and acetaminophen were administered to adult Wister rats with chronic simultaneous 100mg/kg-1 b.wt acetaminophen and 25% ethanol in 2% sucrose solution was given to T1 treated group, 25% ethanol in 2% sucrose solution was given to T2 treated group and 100mg/kg b.wt acetaminophen was given to T3 treated group for six weeks. They reported that degeneration of neurons with loss of nissel substance in the CA1,CA2 and CA3 layers with severe degeneration of pyramidal neurons more noticeable in the CA1 and CA3 layers in treated section T1, treated group T2 showed vacuolations severe degeneration of pyramidal neurons around the dentate gyrus (DG) and CA3 layer. Treated section T3 showed vacuolations many normal cells with few degenerated neurons around the CA3 layer.
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40X H & E CONTROL HIPPOCAMPUS

100X H & E CONTROL HIPPOCAMPUS

40X H & E 30-DAYS HIPPOCAMPUS

100X H & E 30-DAYS HIPPOCAMPUS

40X H & E 60-DAYS HIPPOCAMPUS

100X H & E 60-DAYS HIPPOCAMPUS
[6] found histological and Tau-immunohistochemical features of the dentate gyrus in aged male albino rats. They reported that treated group-II, showed the dentate gyrus granule cell layer with few mature granule cells and increased number of immature neurons. Group-III showed thin dentate gyrus granule cell layer that contained numerous apoptotic cells with darkly stained pyknotic nuclei surrounded by numerous astrocytes.

[14] found that curcumin and simvastatin were administered to albino rats 30mg/kg/day for 4 weeks and 20mg/kg/day for 4 weeks respectively. The animals were divided into 6 groups. Group-I (control group), group II (sham-operated group), group III (ischemic induced group), group IV (received curcumin for 4 weeks before induction of ischemia), group V (received simvastin for weeks before induction of ischemia). Group-III showed many degenerated pyramidal cells. The cells appeared shrunken, irregular in shape with pyknotic nuclei. Group-IV showed some degenerated pyramidal cells appeared irregular and shrunken with pyknotic nuclei. Group-V showed few shrunken pyramidal cells with pyknotic nuclei.

REFERENCES

CITATION OF THIS ARTICLE