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Toxicity of Dichloroacetic Acid (DCA) in the testis of Albino rats

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ABSTRACT

Dichlroacetic acid (DCA) is a haloacetic acid compound. It is a disinfection by-product (DBPs) and also used as a therapeutic agent. It is also know as dichloroacetate and dichloroethanoic acid. Male albino rats were orally administered with 125mg/kg-body weight of Dichloroacetate for 30 days, 60 days and 90 days. The animals were sacrificed and testis was quickly dissected out and fix in 10% formalin for rutine histological techniques. The 30 days treated rats, histology showed minimum impact on the process of spermatogenesis and few sperms were present in lumen (LU), disorganization of the epithelium (EP) and leydig cell (LC) hypertrophy at 60 days and the germinal epithelium (GE) degeneration and the seminiferous tubules filled with giant cell (GC) at 90 days. These findings indicate that DCA is reproductive toxicant and cause deleterious effect on cells of germinal epithelium and leydig cells of the testis and its probable adverse effects on spermatogenesis which may cause male infertility is discussed.

Key words : Dichloroacetic acid, Disinfection byproduct, Seminiferous tubules, Giant cell, Leydig cells.

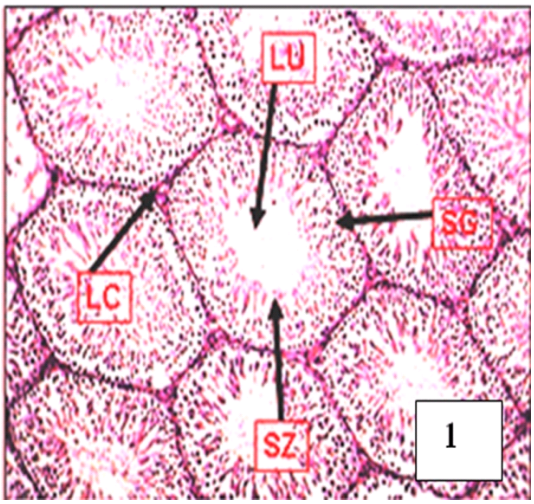
Dichloroacetic acid (DCA) is a colourless slightly yellow liquid with a pungent odour[7,14,22]. It is used as a topical astringent, fungicide and medicinal disinfection a test reagent for analytical measurements, to treat lactic acidosis and in the synthesis of organic materials, including pharmaceuticals[7,12,17,21]. Dichloroacetic acid has also been detected in swimming pool water. In a German study of 15 indoor and 3 outdoor swimming pools[4,9,23,25]. Dichloroacetic acid is likely to be found as a disinfection by-product in meat and other food products[25]. DCA is a

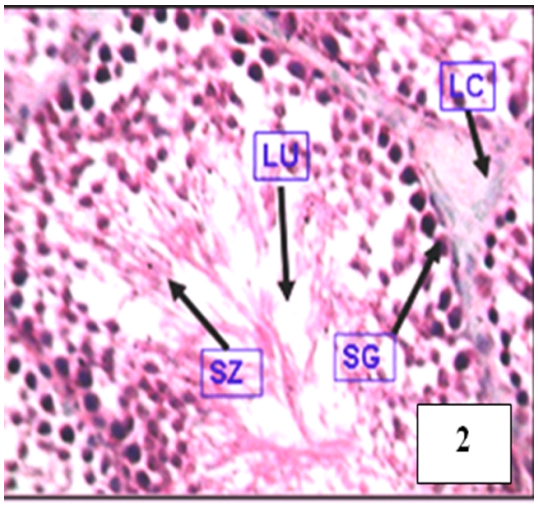
probable carcinogen to humans and other animals[11]. DCA is carcinogenic and a reproductive toxicant in rats[3,10,19]. DCA produce reproductive toxicity and neurotoxicity at high doses. Adverse effects of DCA include polyneuropathy and testicular degeneration. Dibromoacetic acid (DBA), dichloroacetic acid (DCA) and bromochloroacetic acid (BCA) produce delayed spermiation, mis-shapen spermatids and fusion of spermatids and sperm[13]. This study therefore was undertaken to further examine the effects of DCA on the histology of the testis in albino rats, in view of the fact that the effect of DCA on the morphology of the testis has already been determined[5,16,19,24].

Twenty four adult 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 contol. 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.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 days, 60 days and 90 days. The rats were sacrificed after 30 days, 60 days and 90 days of the experiment. The testis were quickly dissected out and fix in 10% formelin for rutine histological techniques. The tissues were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 5 micron thick were obtained using a rotatory microtome. The deparaffinized sections were stained routinely with H & E. microphotographs of the desired sections were made for further observations.

The control sections of the testis showed normal histological features with the cross section of the convoluted seminiferous tubules displayed a perfect picture of normal spermatogenesis. The interstitial tissue comprising of leydig cells occurring in the inter tubular space also bears a normal appearance **(Fig-1&2)**. The 30 days treated rats DCA (125mg/kg b.w.) histology showed minimum impact on the process of spermatogenesis and few sperms were present in lumen (LU) than control rats **(Fig-3&4)**. After 60 days of treatment seminiferous tubules showed disorganization of the epithelium. Hypertrophic of leydig cells were seen between the seminiferous tubules **(Fig-5&6).** After 90 days of treatment the germinal epithelium degenerated and the tubules were filled with giant cells **(Fig-7&8).**

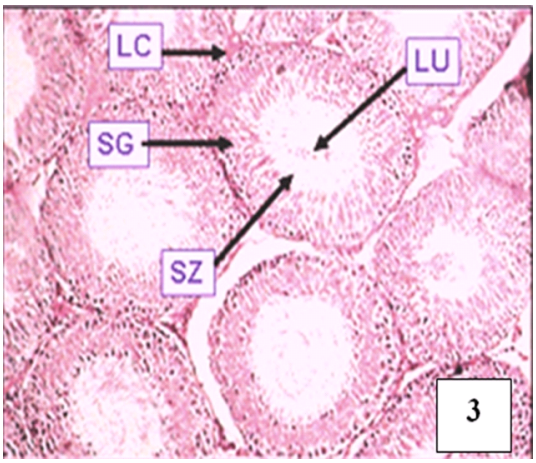
The results (H & E) reactions showed cystic degenerative changes with giant cells and leydig cells showed arteriole form when compared to the control sections. The toxic effects of DCA on the testis observed in this experiment may underline the possible effects already reported[5,16,19]. The actual mechanism by which DCA induced cellular degenerative and hypertrophic changes observed in this experiment needs further investigation. Some workers[4,11,12] observed that sodium dichloro-acetate (NaDCA) was given by gavage daily for 3 months with dose range 0, 125, 500 or 2000 mg/kg-day to rats. Testicular germinal epithelial degeneration was seen in 40% of males at 500mg/kg-day and the testis appeared spermatogenic and contained syncytial giant

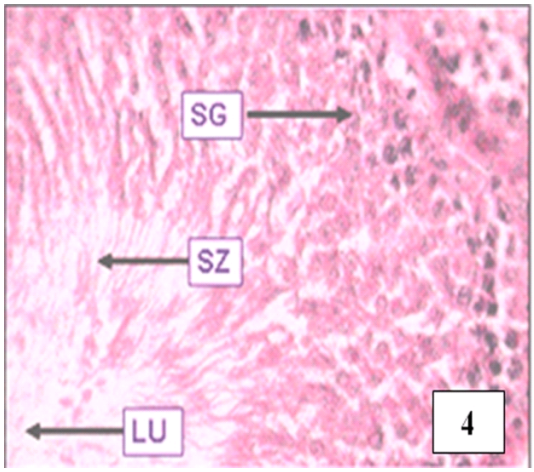




100X H & E CONTROL TESTIS **400X H & E CONTROL TESTIS**

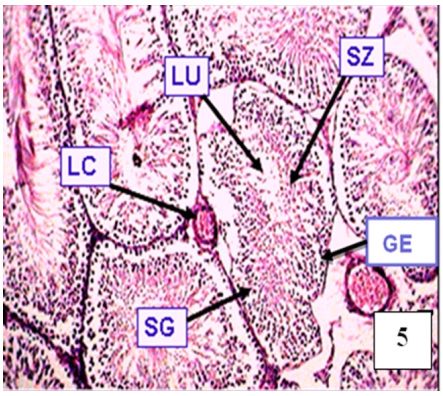
Fig-1,2 -The photograph is showing the transverse section of testis of albino rat **(Control Group). (100X & 400X)**



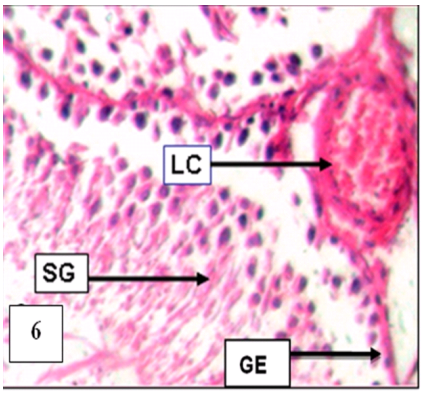


100X H & E 30 DAYS TESTIS **400X H & E 30 DAYS TESTIS**

Fig-3,4 -The photograph is showing the transverse section of testis of albino rat after dosing 125 mg/kg-bw DCA treated for **30 days.(100X & 400X).**
[LU-Lumen, LC-Leyding cells, SZ- Spermatozoa, SG-Spermatogonia]

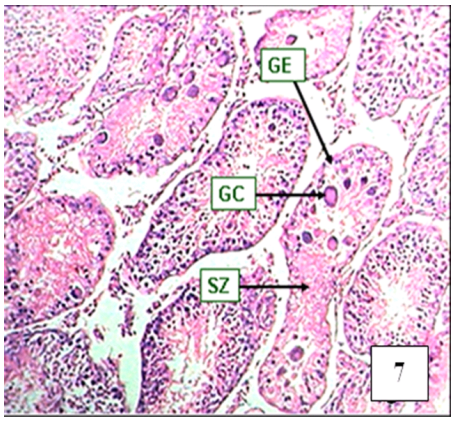


100X H & E 60 DAYS TESTIS

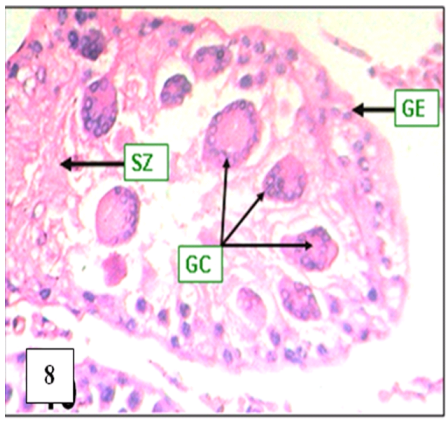


400 X H & E 60 DAYS TESTIS

Fig-5,6 -The photograph is showing the transverse section of testis of albino rat after dosing 125 mg/ kg- bw DCA treated for **60 days. (100X & 400X).**



100X H & E 90 DAYS TESTIS



400X H & E 90 DAYS TESTIS

Fig-7,8- The photograph is showing the transverse section of testis of albino rat after dosing 125 mg/ kg-bw DCA treated for **90 days.(100X & 400X).**
[LU-Lumen, LC-Leyding cells, SZ- Spermatozoa, SG-Spermatogonia, GE-Germinal Epithelium, GC-Giant Cell]

cells in the germinal epithelium was seen at 2000 mg/kg-day. Similar results were reported in our study.

In a sub chronic toxicity study Katz *et al*.,[16] dosed rats (10 to 15/sex/dosage group) with 0, 125, 500 or 2,000 mg/kg-day of sodium dichloroacetate given daily for 3 months. Testicular germinal epithelial degeneration was seen in 40% of males at 500 mg/kg-days and in all males at 2000mg/kg-day, the testis appeared aspermatogenic and contained syncytial giant cells in the germinal epithelium were seen in 20% of the male rats dosed at 500mg/kg-day.

Reproductive toxicity was reported by Bhat *et al*.,[5] following the sub chronic oral

dosing of DCA in male rats. Groups of male Sprague-Dawley rats (5/group) were administered 0 or 1,100 mg/kg-day DCA in drinking water for 90 days. Dichloroacetic acid exposure decreased testis weight ($p<0.01$) and was associated with signs of tissue atrophy. In addition, the seminiferous tubules contained very few spermatocytes and no mature spermatozoa. Some researchers[2,3,18] have reported that short term aluminium chloride exposures to rats and guinea pigs caused gonadal toxicity whereas in chronic exposure the sperm density and motility were affected in agreement with the work of Chinoy *et. al.*,[8] who showed that the administration of sodium fluoride (NaF, 10mg/kg, body weight) together with aluminum chloride ($AlCl_2$ 200mg/kg body weight) to mice for 30 days, caused degenerative in structure of spermatogenesis and formation of giant cells. These results agree with our results obtained after the treatment with DCA.

Mishra and Bhiwgade[20] studied the effect of doxorubicin (DOX) on testis. Examination of the testis in his study showed that the seminiferous tubules of DOX treated rats showed pronounced shrinkage and their epithelium was thoroughly disorganized resulting in an increased intertubular space and the numbers of leydig cells were reduced and the cells were atrophied. Their cytoplasm was reduced and the presence of pycnotic nuclei in these cells suggested their loss of functional integrity. Jewo *et al.*,[15] investigated that artesunate administration was given to rat. They obtained degenerative changes in the testis.

Ait *et al.*,[1] observed in his study that 500mg/l Lead was given to rats for 90 days. The result obtained that the presence of interstitial exudates, degeneration and necrosis of spermatogenic and interstitial (Leydig) cells with focal areas of vacuolar degenerative changes appeared in the cytoplasm of the spermatogenic epithelium and abnormal distribution of spermatozoa showed in Lumina of the seminiferous tubules.

Bhatia *et al.*,[6] investigated that 500mg/kg dose of adiantum lunulatum burm was given to albino rats for 30 and 60 days. The result obtained after 30 days that the seminiferous tubules presented significant degenerative changes, the damage of the germinal epithelium and degeneration of spermatozoa, spermatogonia, spermatocytes and spermatids. The interstitium was highly reduced and packed with atrophied leydig's cells. After 60 days the seminiferous tubules showed degenerative changes. The lumen of the seminiferous tubules was filled with oedematous fluid and cellular debris.

In summary, it is concluded from present study that DCA at the dose of 125mg/kg bw/day introduced by gavage is responsible for irreversible damage and disturbances in metabolism of male reproductive organs. The results clearly show that DCA has a deleterious impact on the quality of spermatozoa, resulting in a decrease in spermatozoal number, an increase in morphologically abnormal spermatozoa in testis. In inference these confirm earlier report that chlorinated disinfection byproduct DCA may be a cause of male infertility.

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