

THE EFFECT OF METHYLENE CHLORIDE ON THE TESTIS HISTOLOGY OF ALBINO WISTAR RATSTarfa M. P.^{*1}, Mshelia P. P.², Mahdi O.¹ and Leonard, M. P.³¹Department of Human Anatomy, College of Medical Sciences, Gombe State University, Gombe, Nigeria.²Department of Human Physiology, College of Medical Sciences, Abubakar Tafawa Balewa University, Bauchi, Nigeria.³Department of Human Physiology, College of Medical Sciences, Gombe State University, Nigeria.***Corresponding Author: Tarfa M. P.**

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ABSTRACT

Randomly bred normal Forty (40) male albino rats of wistar strain obtained from the wistar rat colony of the Department of Pharmacology, University of Jos, were used for this study. All the animals weighing between 90 – 230 grams and 10 – 20 weeks old were maintained under uniform conditions across ventilated room (temperature 25 – 28°C, and 12h light/dark cycle), with free access to standard diet of Growers Mash (Vital Feed Nig PLC, Maiduguri) and portable water *ad Libitum*. The rats were divided into groups I, II, III and IV with 10 rats each. However, the rats in groups II, III and IV received oral administration of the solution of Methylene Chloride at 420mg/kg, 840mg/kg and 1260mg/kg respectively for 5 weeks via oro-gastric intubation. Routine histological sections of the testes obtained from rats treated with methylene chloride at all the dose levels (420mg/kg, 840mg/kg and 1260mg/kg) for 5 weeks were characterized by relative slit decrease in the size of the seminiferous tubules and interstitial connective tissues. The seminiferous tubule epithelium showed the presence of distorted Sertoli and germ cells and also epithelial necrosis after a while, the effects became more pronounced with greatly increased seminiferous tubular luminal diameter from 57µm to 62µm, the interstitial cells of Leydig were not greatly affected. From the result, it was seen that methylene chloride has a degenerative effect on the cyto-architecture of the testis which and this could result to infertility and be detrimental to reproductive health.

KEYWORDS: Methylene chloride; testis; wistar rats.**INTRODUCTION**

Methylene Chloride which is also known as dichloromethane, is a chemical compound widely used as a solvent for organic materials. It is a colourless volatile liquid with a moderately strong aroma, which is sweet in some sense but which makes some people feel very uncomfortable. Methylene chloride is the least toxic of the simple chlorohydrocarbons, but it is not without its health risks as its high volatility makes it an acute inhalation hazard.^[1] Methylene Chloride is produced by treating either methyl chloride or methane with chlorine gas at 400–500 °C. At these temperatures, both methane and methyl chloride undergo a series of reactions producing progressively more chlorinated products. Some people take it as substance of abuse which can lead to alteration of their mood, emotion or state of consciousness, while others use it as a solvent with other drugs of addiction.^[1]

Dichloromethane is also metabolized by the body to carbon monoxide potentially leading to carbon monoxide poisoning.^[2] Acute exposure by inhalation has resulted in

optic neuropathy.^[3] Prolonged skin contact can result in the dichloromethane dissolving some of the fatty tissues in skin, resulting in skin irritation or chemical burns.^[4]

It may be carcinogenic, as it has been linked to cancer of the lungs, liver, and pancreas in laboratory animals. Dichloromethane crosses the placenta. Fetal toxicity in women who are exposed to it during pregnancy, however, has not been proven. In animal experiments, it was fetotoxic at doses that were maternally toxic but no teratogenic effects were seen.^[5]

Volatile substance abuse such as glue sniffing, inhalant abuse, solvent abuse is the deliberate inhalation of volatile substances in order to achieve intoxication. It has now been reported from most parts of the world, mainly among adolescents, individuals living in remote communities and those whose occupations give ready access to abusable substances.^[6] Methylene chloride is a constituent of many substances abused in Northern Nigeria today. The concern about its effects on the reproductive life of the populace and with paucity of

published work as at present on the effect of methylene chloride on the male reproductive system especially the testis form the basis of this research. This study therefore, attempts to determine any histological changes in the testis of experimental rats due to methylene chloride exposure.

MATERIALS AND METHOD

Materials

Hot plate, beaker, syringe, intubation tube, measuring cylinders, oven, electric sensitive balance, beam balance, rats, ocular graticle, filter paper, cotton wool, Magnifying lens, cage, tissue and sample bottles, water bottles, forcep, slides, chemicals, water, rotary microtome, razor blade, light microscope, Vanox Olympus Trinoculra Research Microscope (Analog), hand gloves, wooden chuck, water bath and Microscope Warming Table.

Preparation of Dose

1ml of Methylene Chloride which was equal to 1.33g was dissolved in 9mls of distilled water by vigorous shaking to give 10% of the solution and was kept in the refrigerator for overnight before administration.

Experimental Protocol or Experimental Groups

The rats were divided into four (4) groups as follows; groups I, II, III and IV of 10 rats per group. The rats were marked, weighed and put into separate cages.

Group I (control group) did not receive any administration but had access to feed and water freely. However, the rats in group II, III and IV received oral administration of the solution of Methylene Chloride at 420 mg/kg, 840 mg/kg and 1260 mg/kg respectively for 5 weeks. This was administered once daily via orogastric intubation method. Two rats from all the groups were sacrificed at the end of every week for the period of five (5) weeks their testes removed for histological study. The pituitary glands were removed for histological study and were also weighed using electrical beam balance.

The experiment was conducted in accordance to the principle of laboratory animal care of the University of Maiduguri, Nigeria.

Histopathological Examination

From Bouin's fixative, the tissues were transferred to 70% alcohol for dehydration through higher grades of alcohol (95% and 100%). The tissues were then transferred to two changes of xylene for clearing, followed by infiltration in three changes of paraffin and lastly embedded in paraffin. Paraffin sections were cut at 8µm using rotary microtome (Leitz 1512 model) and stained in Harris Heamatoxylin and Eosine (H&E). The procedures as outline by^[7,8] was followed. Light microscopic study was wholly done at the Histology laboratory of the Department of Human Anatomy, University of Maiduguri. Ocular graticle was used to measure the length of each seminiferous tubule and the

diameter of the same seminiferous tubule to see if there was decrease or increase in the size of the tubules.

RESULT AND DISCUSSION

The Testis

Light micrographs of paraffin sections of the testis from control rats show normal spermatogenic features with the Leydig cells in the interstitial spaces and normal Sertoli cells and the germ cells together with the spermatozoa in the lumen of the seminiferous tubule, (figure I).

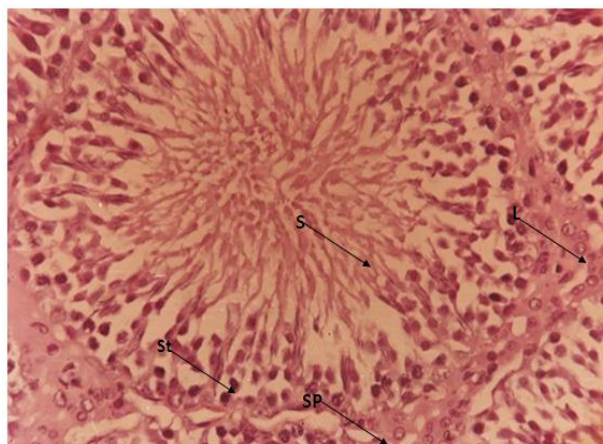


Figure I: Micrograph of a control rat testis S: Spermatozoa in the lumen of seminiferous tubule, L: Leydig cell and St: Sertoli cells and Sp: Spermatocyte ×400 magnification.

Routine histological sections of the testes obtained from rats treated with methylene chloride at all the dose levels (420 mg/kg, 840 mg/kg and 1260 mg/kg) for 5 weeks were characterized by relative slit decrease in the size of the seminiferous tubules, tubular basement membrane, interstitial connective tissues and the seminiferous epithelium showed the presence of distorted Sertoli and germ cells and also epithelial necrosis, (figure II).

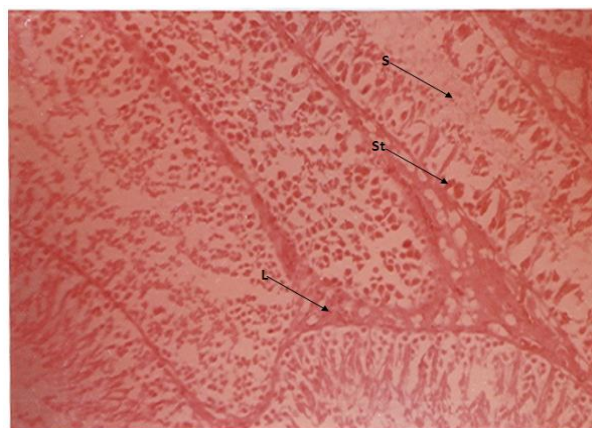


Figure II: Micrograph of a rat testis treated with 420mg/kg of methylene chloride S: Few Spermatozoa in the lumen of seminiferous tubule, L: Leydig cell and St: Sertoli cells × 400 magnification.

After a while, the effects became more pronounced with greatly increased seminiferous tubular luminal diameter from 57 μ m to 62 μ m, but it maintained normal Leydig cells, (figure III).

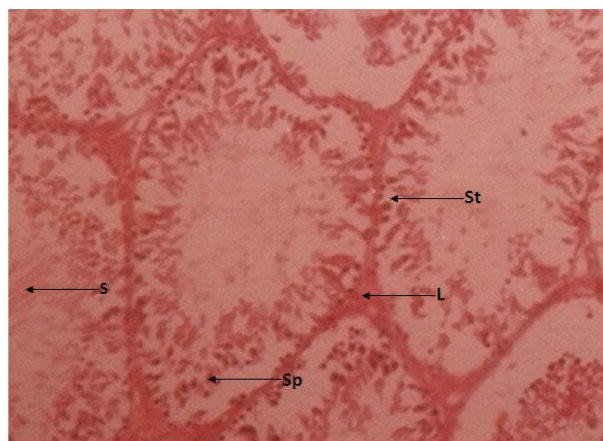


Figure III: Micrograph of a rat testis treated with 840mg/kg of methylene chloride S: Few spermatozoa in the lumen of seminiferous tubule, L: Leydig cell and St: Sertoli cells and Sp: Spermatocyte $\times 400$ magnification.

The interstitial cells of Leydig were not greatly affected while the seminiferous epithelium showed little decrease of germ cells at various stages of spermatogenesis, (figure IV).

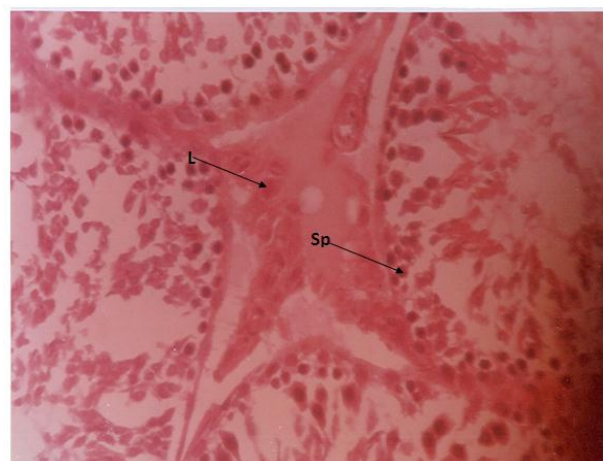


Figure IV: Micrograph of a rat testis treated with 1260mg/kg of methylene chloride L: Leydig cell and Sp: Spermatocyte $\times 400$ magnification.

Table 1: Measurement of the Test is using Ocular Graticle.

Group	DST (μ m)	LD (μ m)	SLC (μ m)
I	81	57	15
II	78	59	14
III	73	62	15
IV	65	60	16

DST: Diameter of Seminiferous Tubule, **LD:** Luminal Diameter and **SLC:** Size of Leydig Cell.

Tissue activities such as decrease in the size of seminiferous tubule, tubular basement membrane, interstitial tissue, interstitial cells, number of germ cells etc, as shown in this study were clear evidence of under activity. The effects were shown to be dose and duration dependent in the lowest dose of the treatment. However, the intermediate and the high dose treated rats respectively showed some degree of degenerative changes ranging from decrease in the size of seminiferous tubules and interstitial cells diameter to reduction in the number of Sertoli and germ cells. The degenerative changes were pronounced in the intermediate dose treated group.

The effect of methylene chloride showed seminiferous tubules with much larger luminal diameter, as the adluminal germ cells gradually degenerated and were adsorbed within the tubules. The process of involution continued until only the basal layer of spermatogonia remained with a very wide luminal diameter, mainly occupied by reticular fibers. The Sertoli and Leydig cells show similar transformations, increasing in size during growth processes and diminishing in periods of degeneration. There may be a decrease in serum testosterone level, an indication that the interstitial cells were partially stimulated, and this could account for the observed decrease in the size of Leydig cells, which was necessitated by the decrease intracellular activity required for testosterone synthesis.^[9,10]s

The gonadotrophs themselves are responsible for the secretion of gonadotropins; luteinizing hormone (LH) and Follicle stimulating hormone (FSH).^[11,12] LH, in turn, is involved in the positive feedback loop regulating testosterone synthesis by the Leydig cells.^[13] Methylene chloride has a degenerative effect on the cells and size of the seminiferous tubule and this explain the cessation of fertility observed by.^[14]

CONCLUSION

From this research, methylene chloride possesses antifertility effect. Therefore, its intake should be banned and appropriate sanction be met out for those found culpable.

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