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Toxicopathological Experimental Assessments of Median lethal dose (LD50) of Dimethyl formamide on male reproductive system of albino rats (epididymis)

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Abstract:

This experiment aimed to investigate the Toxicopathological effect of N, N-Dimethyl formamide (DMF) (LD50) on the adult male rats reproductive system (epididymis) in acute and sub-acute exposure in male rats. Twelve male rats used in the study, six rats used to estimate the acute toxicity (LD50), While the other group Normal diet and water for twenty-four hours. The experiment has lasted for twenty-four hours at the end of the experiment all animals killed, epididymis was kept in 10% formalin used for histological examinations. results revealed thinking of the capsule of the epididymis absent of sperm from the lumen with mild infiltration of inflammatory cells, other sections showed decreased in the high of the epithelia covered the epididymis tubules with the absence of microvillus with stasis of abnormally shaped sperm inside the lumen. conclusion: there was the histopathological effect of DMF on the male reproductive system of rats (epididymis) with its different LD50 dose.

Keywords:N,N-Dimethylformamide,histopathology,epididymis,rat**.**

Introduction:

N, N-Dimethylformamide (DMF) is an important chemical substance that is commonly used in various industries, especially in the production of synthetic fibers, inorganic chemicals, pharmaceutical products, as well as synthetic leather, and synthetic organic materials and is also used as a pesticide. In addition, it is an excellent general solvent (Liu*et al* ., 2012; Hu *et al* ., 2020). N, N-dimethylformamide (DMF) is a colorless to slightly yellow liquid with a faint amine-like odor and is termed the universal organic solvent because of its extensive miscibility with water and most common organic solvents, and thus is globally used in a wide variety of industrial applications (Kim & Kim,2011; Li & Zeng, 2019). By entering into several areas in the industry, DMF is released into the environment, affecting air quality and human health. China consumes the most DMF, producing approximately45 % of the world's DMF each year. China consumes two-thirds of the global total of DMF (Zhang *et al.*, 2014).With the increase in the use of DMF annually, so has a concern about its potentially toxic effects. DMF has been shown in animal experiments and epidemiological studies to have a negative impact on the liver, kidneys, and reproductive system in humans and animals (Luo*et al* .,2001; Hu *et al* ., 2020). As is well known, the toxicity of an industrial solvent may differ according to species, strain, age, and sex of the experimental animals as well as the route of administration (Tanaka, 1971; Mustafa & Jawad, 2019).Dimethylformamide is embryotoxic in animals; Reduced implantation efficiency, decreased mean fetal weight, and increased abortions have been reported in rats exposed by inhalation.In rabbits exposed to dimethylformamide via dosing

(delivery of the chemical into the stomach), at high doses the average fetal weight was very low as well as a high percentage of deformed fetuses and a high percentage of birth defects. (EPA, 1999).Environmental toxins can cause oxidative stress, reproductive abnormalities, and infertility.As a toxic pollutant for the environment, dimethylformamide is well-known to have negative effects on a variety of living organisms (Al-sabaawy andAl-Kaisie , 2020). Some prevalent disorders found in the workers exposed to DMF were alcohol intolerance, possible embryotoxicity, and teratogenicity (Feely, 1989; Kim &Seoh, 1998). Due to little studies on DMF in Iraq, we designed this study to investigate the possible toxic effect in rats.

Materials and methods

Chemical :

All chemicals **purchase** from (Alpha Chemika) India.

Animal:

twelve male Albino rats, weighing about 300-350 gm. Their age ranged from 10 to 12 weeks. Animals were obtained from the Baghdad University College of Veterinary Medicine animal house, acclimatized to laboratory conditions, and housed in plastic cages for two weeks of acclimatization in the animal house at ambient temperatures of 25°C and 45-55 percent of comparative humidity, with 12h of dark and light. Animals have been treated with distilled water, Laboratory Animal Care Principles. Bedding changed every five days to ensure a clean environment(Hafez,1970).

Experimental design:

twelve adult male rats were used in this experiment. After acclimatization for two weeks they were divided equally into two groups as follows:

1. Six rats were used for the up and down method (LD50) study .
2. Group two control (C): six rats were received distilled water orally daily for twenty-four hours.

Acute toxicity up and down method:

The median lethal dose LD50 of DMF measured by the "up-and-down" method (Dixon, 1980). Total six albino adult male rats weighed (300-350 gm) were used in this study for determination of median lethal dose (LD50) of DMF given orally with following doses(5.5mg/kg.BW,6.05mg/kg.BW,6.655mg/kg.BW,6.05mg/kg.BW,6.655mg/kg.BW and 6.05 mg/kg.BW).Animals observed for any clinical signs of toxicity and lethality within 24hr.This test summed up by dosing singular creatures in succession independently at 24-hour stretches, with the underlying portion set at " the toxicologist's best gauge of the LD50. " Following every passing, the portion was brought down; following every endurance, it was expanded, as per a pre-indicated portion movement factor. The LD50 was calculated by using the following equation (LD50):

LD50 = xf + kd

Xf = last dose administrated

K = value from appendix

D= difference between dose levels.

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Histopathology Changes:

epididymis obtained after twenty-four hours of exposed and fixed in 10 % formalin. Paraffin sections of the thickness of 3 - 4 μm were prepared and stained with hematoxylin and eosin (H and E) stain for histopathological examination under light microscopy(Bancroft *et al.*, 2018).

Results :

This study revealed that the LD50 of Dimethyl formamide according to the Dixon method is 6455mg/kg.BW (Table1) with toxicity rats of 4 which considered as slightly toxic. The acute toxicity symptoms which were observed after dosing the animals include, rapid abdominal respiration, dullness, salivation, urination, after one to twelve hours unbalance, ataxia, incoordination, rolling, and after 20 hours convulsion, coma and death. (the severity of symptoms was positively proportional to the dose).

Table (4-1): Oral LD50 of Dimethylformamide in rats according to up and down method (Dixon,1980).

Initial dose Mg/kg B.W	Final dose Mg/kg B.W	Number of animals	Results after 24 hours	Different between doses	LD50 Mg/kg B.W
5500	6050	6	OOXOXO	550	6455

LD50= 6050 + (0.737) * 550

= 6050+405=6455 mg / kg B.W orally Dimethylformamide in rats.

Histopathological change

The epididymis from the (LD50) group :

Treated rats at this group showed (fig-1) the epididymis tubules revealed stasis of sperm in lumen and absent in others. while other sections showed thinking of the capsule of the epididymis, the tubules revealed stasis of the sperm in the lumen of it, hyperplasia of some epithelia cover the tubules (fig-2). microvacuolar and microvacuolar degeneration of the epithelia covered the epididymis tubules, abnormal sperm inside the lumen with rounded cells.. fig-3. this section showed moderate hyperplasia of the epithelia with microvaculation of the epithelial cells and stasis of abnormal sperm in the lumen Fig-4. Other sections of epididymis showed a decrease in the high of the epithelia covered the epididymis tubules with the absence of microvillus Fig-5.

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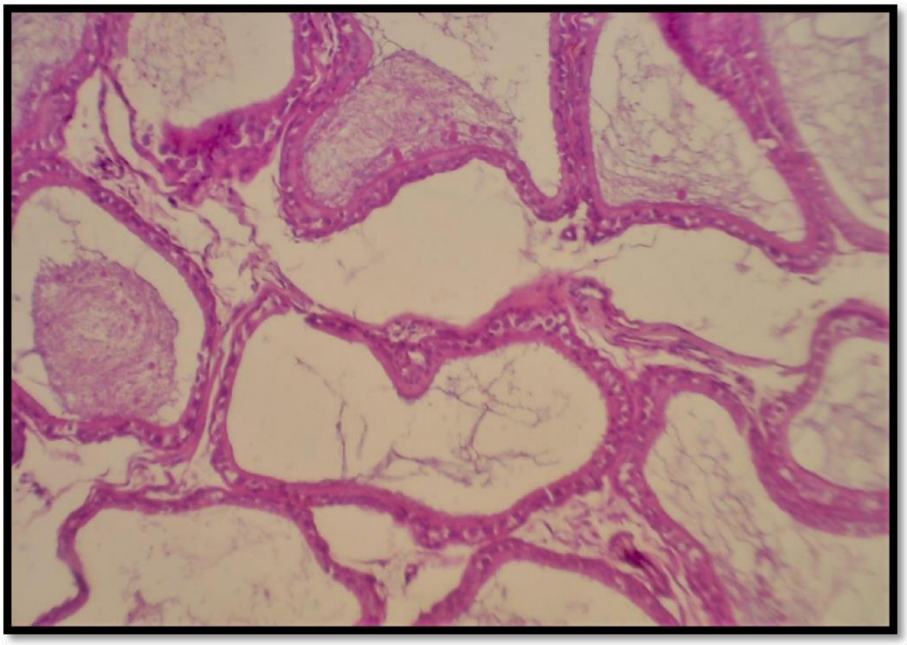

Fig-1 histopathological section of epididymis (LD_{50}) showed epididymis tubules revealed stasis of sperm in lumen and absent in others.(H&E 100X).

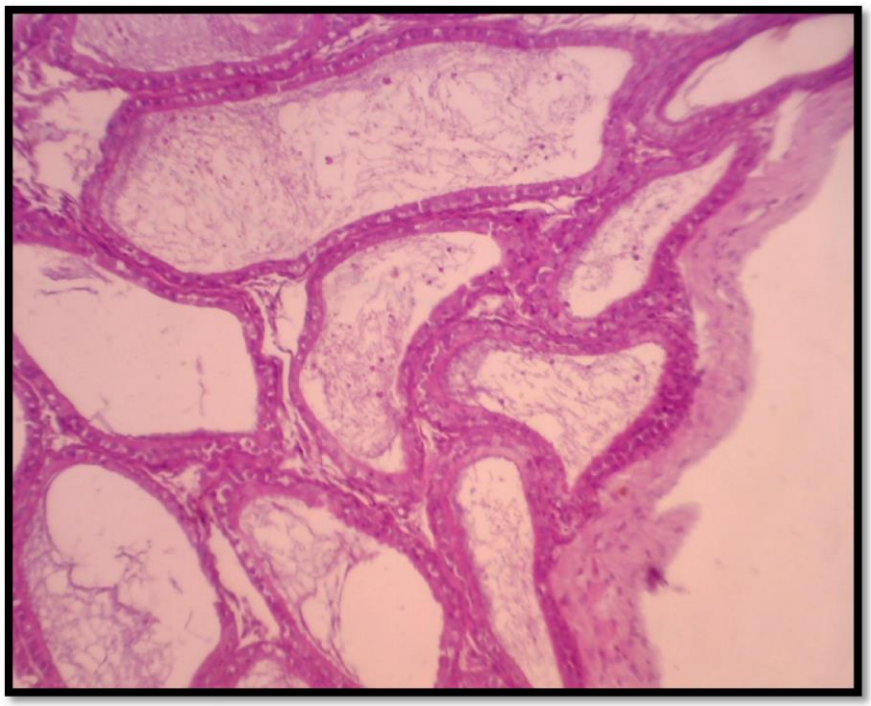

Fig-2 histopthological section of epididymis (LD_{50}) showed epididymis thickening of the capsule of the epididymus , tubules revealed stasis of the sperm in the lumen of it , hyperplasia of some epithelia cover the tubules.(H&E100X).

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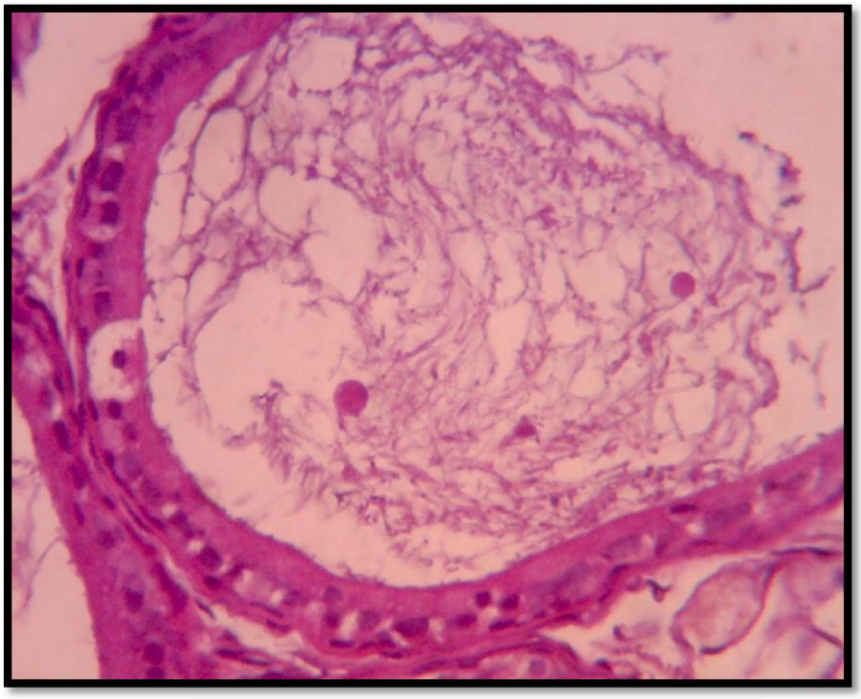

Fig-3 histopathological section of epididymis (LD_{50}) showed epididymis microvacuolar and microvacuolar degeneration of the epithelia covered the epididymis tubules,abnormal sperm inside the lumenwith rounded cells.(H&E400X).

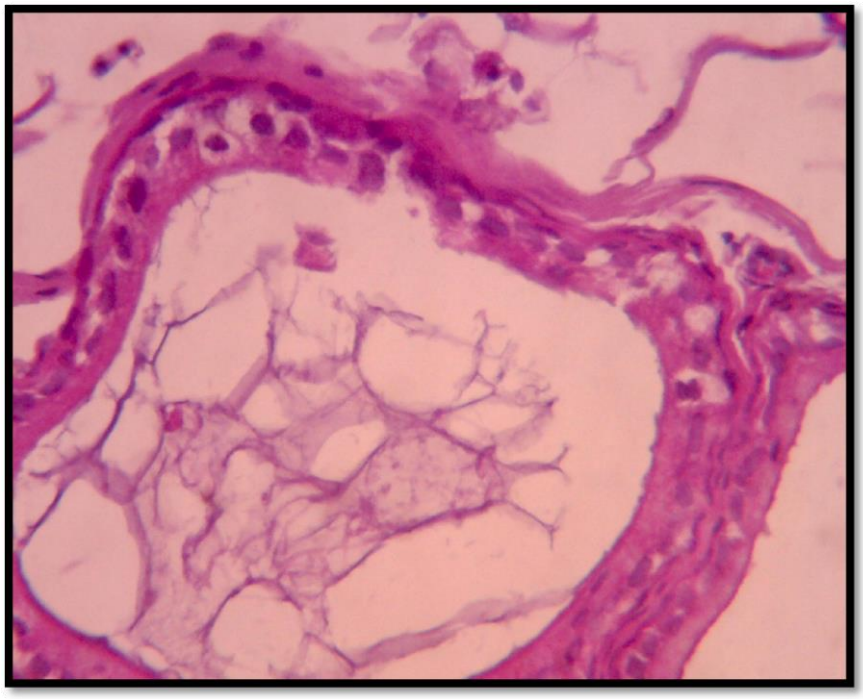

Fig-4 histopathological section of epididymis (LD_{50})showed epididymis with moderate hyperplasia of the epithelia with microvaculation of the epithelial cells and stasis of abnormal sperm in the lumen (H&E400X).

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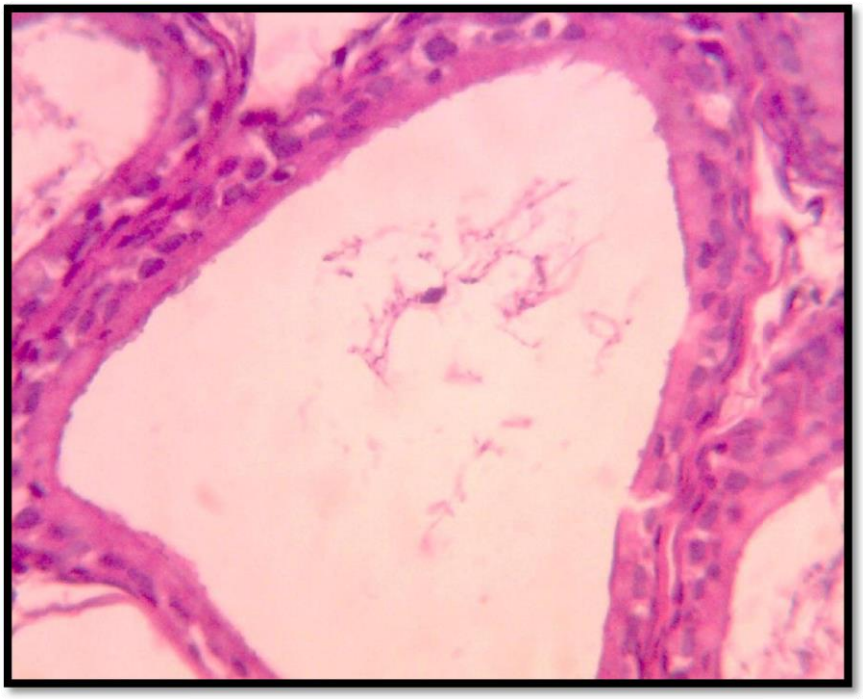

Fig-5 histopathological section of epididymis(LD_{50})showed decreased in the high of the epithelia covered the epididymis tubules with absent of microvillus (H&E400X).

Discussion:

The results of the acute toxicity of the solvent DMF were evident some toxic signs include, rapid abdominal respiration, dullness, salivation, urination, after 12 hours then unbalance, ataxia, incoordination, rolling, and after 20 hours' convulsion, coma and death, as it was found that DMF has a slight toxicity as shown in table (1). The metabolic pathway of DMF induce toxicity extremely discussed in previous studies especially their easily absorption orally, dermally and inhalation (Lynch *et al.,* 2003; Kim &Kim ,2011). Furthermore, the toxic metabolites that liberated in the liver after metabolized by CYP2E1 which various endogenous and exogenous substrates to breaks to reactive metabolites, and thereby produces reactive oxygen species (ROS) (Caro &Cederbaum, 2004; Wang *et al.,* 2016).The imbalance in the production of antioxidants and the production of free radicals and arising the ROS then elevated of lipid peroxidation inside the liver have the main role in causing most of the toxicities, as well as some mutations at the level of DNA, which in turn cause cancer. (Senoh*et al.,* 2004 ; Jyothi*et al.,* 2012). As well as the result of acute toxicity LD50 was confirmed by histopathological sections of epididymis fig (1,2,3,4,5). The most lesions that observed in epididymis of rat received acute doses of DMF was thickening of the capsule with mild hyperplasia of the epithelia and microvacuolar degeneration covered the epididymis tubules, abnormal sperm inside the lumen with rounded cells. Anotherslides showed there were many abnormal sperms stasis at the lumen.It is known that DMF metabolized in the liver hepatocyte to more toxic metabolite monomethylformamide(MMF) followed by formammide formation in animals(Itoh, 1988). we thought the highly concentrations of free radicals liberated in extravascular fluid in the tissue caused many

deleterious effect in the srtolie cells, then caused damage in the epididymis. Furthermore, the ability of DMF to penetrate the bodies barrier confirmed by (Kennedy, 2012) who reported that DMF caused decrease in count and motility of sperm in mice.

Conclusion: DimethylformamideLD50 dose reveled histopathological changes effect on male reproductive system significant lesions of albino rat epididymis.

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