



The Toxicopathological Effect of Median Lethal dose of Methomyl on Male's Reproductive organs of Albino Rats In Iraq

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Abstract

Methomyl was severing toxic if ingested orally & mild- moderate toxic if the inhaled. It is effect on the Acetylcholinesterase (AChE) enzyme by inhibition of this enzyme in humans & animals at high enough doses; in the later, lead to over stimulate of the nervous system. This our research aimed to study toxicopathological changes associated with methomyl toxicity in testes & epididymis of a rats. A total of twenty-four of a rats were, they were divided evenly into three groups after adaption of animals (n=8 rats) as the followings; C control negative group, G1 administered methomyl 0.1 ml/100 gm b.w (1/10 LD50) for 30 days and G2 administered methomyl 0.1 ml/100 gm b.w (1/20 LD50) for 30 days. All animals group sacrificed post 30 days and 1 cmm³ autopsies from testes and epididymis which kept in buffered neutral formalin(10%)for histological observation. Results revealed hypospermatogenesis with absence of sperm in lumens of the seminiferous tubules, also mild - moderate inflammatory cells infiltration. The epididymus sections revealed reduced in the length of the epithelial lining; in addition to absent of microvillus and abnormal shaped of the sperm inside the lumen. **Conclusion:** From our data, there were toxicopathological lesions of methomyl of the testes and epididymus of rats when administered different LD50 doses (1\10 LD50, 1\20LD50), respectively.

Keywords: methomyl, male rat, pathology, reproductive system

Introduction

A pesticide recognized as any material and /or mixture of materials purposed to destroying, preventing, & controlling for any pest (1). Pesticides were ubiquitous that contaminants & pollutants of our environment such as soil, air and water, also present in animal tissue specimens from whole over the world, Pesticides include a wide range of components which were using in the pest prevention & control, involving rodenticides fungicides, insecticides, molluscicides & herbicides, (2). Furthermore the pesticides were applied to controlling the pests in water & lands which produced numerous health hazards & problems for the live stocks, human, wildlife, and animals (3). After prolonged exposure of the experimental animals to the pesticides, they are many problem were reported such as cancer,

immunosuppressive action, teratogenic change , chronic neurological syndrome, finally decreased fertility & abortion (4,5). Methomyl it is a wide ranging-carbamates pesticide, marketed as and present in solid form & an aqueous solution, The toxicity of methomyl in lab animals revealed that this carbamate was severe toxic activity on testes, liver, kidney, and the lungs of mice, rats, & rabbits tissues (5,6,7). Also Methomyl was used widely in animal shelters and in the agricultural applications to prevent insects all around the world (8,9). The methomyl absorption may be from gastrointestinal tract, skin, and respiratory system ,It was also severe toxic to mammals & birds when administrated via mouth which appears its effect on inhibition of AChE so the hydrolytic deactivation of (ACh) will be decline



and that would continues to stimulate the postsynaptic receptors to eventually cause nerve and/or tissue failure (10). In a study of rats which observed by Radad et al.(11) when taken methomyl (2 mg/kg) for period about 3 months and showed significant histopathological lesions liver, testis, kidney, lung, & spleen, In another research which performed in the brain of rats, there were sever degenerative changes in this organ of the methomyl-given rats (10 mg/kg, 2 mg/kg) in comparison to the untreated rats (12). The toxicopathological studies which related to methomyl toxicity were very restricted in the veterinary medicine field & were no published researches in Iraq, Therefore, this experiment aimed to investigate toxicopathological effects of methomyl administration on the testes and epididymis of rats .

Materials and Methods:

Lab animals

A total of twenty-four adult male albino rats with averaged aged about three months and weighed ranged (150-200 gm) were used to perform this experiment. The experimental animals are housed & bred in the animals house of Veterinary Medicine college/ Baghdad university under typical condition (14:10 hr light:dark cycle & $20 \pm 2^\circ\text{C}$). Rats are kept in metal cages with dimensions (20X30X50) cm³, additionally drinking water & commercial feed pellets are given during the period of experiment (13).

Experimental design

After one month of acclimatization, the experimental rats are divided equally into three groups. Daily administered methomyl in dose of (1/10 LD₅₀ and 1/20 LD₅₀) (14). As follows:

Group-C: (n=8) left untrated & considered as control negative

Group-G1: (n=8) administered orally of methymol 1/10 of LD₅₀ (0.1 mg/kg bw) for 30 days

Group-G2: (n=8) administered orally of methymol 1/20 of LD₅₀ (0.1 mg/kgbw) for 30 days

After 30 days of the experiment, Rats were anesthetized by injection of ketamine/xylazine intraperitoneally (IP), then animals were sacrificed and the testes & epididymis specimens were taken & directly preserved in 10% neutral buffered formalin for observed any abnormal gross & microscopic changes in this organs .

Toxicological study:

Acute toxicity study:

Up and down method (**Dixon, 1980**) was used for determination of median lethal dose (LD₅₀) of methomyl given orally to rats. Total eight male rats weighed (150 - 200 gm) were used in this study in which methomyl was prepared at concentration (25mg/ml) and drawl 50mg of methomy completed to 10 mg/ml with distal water. The volume of doses was calculated according to the animal weight methomyl given orally to the animals at range of doses (21-24)mg/kg.B.W. LD₅₀ was calculated after (24) hours observation of lethality in dosed animal. The LD₅₀ is calculated by using the following equation

Dixon method:

This test calls for dosing individual animals in sequence singly at 12-hour intervals, with the initial dose set at "the toxicologist's best estimate of the LD₅₀."Following each survival, it was increased, according to a prespecified dose progression factor. If a death followed an initial direction of increasing doses, of 10-20% or a survival followed an initial direction of decreasing dose with the same ratio, three additional animals were tested following the same dose adjustment pattern and then testing was ended. The LD₅₀ was calculated by using the following equation

$$LD\ 50 = xf + kd$$

Xf = last dose administrated

K= value from



d = difference between dose levels

Histopathological preparation:

Tissue specimens from testes & epididymis about 1 cmm³ were preserved & fixed by 10%

neutral buffered formalin to make paraffin blocks wax, later hematoxylin and eosin staining for observing any abnormal microscopic changes (15).

Results:

1.1.Acute toxicity study

This study revealed that LD50 of methomyl to Dixon method was (25 mg/kg. B.W) with toxicity rate 3 which considered as moderately toxic, (as in table 1). The acute toxicity

symptoms which were observed after dosing animals include, severe depression, shallow respiration, frequent urination, slight convulsion then death (the severity of symptoms was positively proportional to the dose).

Table 1: This table shows the LD50 of Methomyl in rats

Initial dose mg/kg B.W	Final dose mg/kg B.W	Number of animal	Result after 24 hours	LD50 Mg/kg B.W
21	24	6	OOOXOX	25

O= survival of animal , X=dead animal

$LD50 = xf + kd$

$LD 50 = 24 + (1.288 \times 1)$

$LD50 = 25.288 \approx (25) \text{ mg/kg. B.W}$

Xf= last dose administrated

K= value from appendix d= difference between dose levels (Dixon,1980).

1.2.Pathology study :

1.2.1.Gross changes:

The treated groups (G1) were showed paleness carcasses (liver and intestine)as at

(fig.1-a) also in other rats were showed congested blood vessels of and testis (fig.1-b).

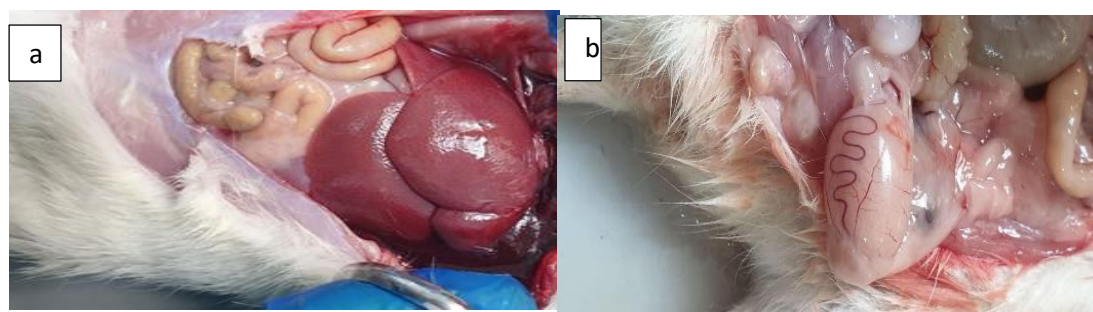


Figure (1): gross appearance of the G1 group rat showed paleness of liver and intestine (a) while (b) showed congested testis.

1.2.3.Histopathological finding:

Variable microscopic findings were observed in males' reproductive system of treated groups compared to the control negative group.

1.2.3.1 Testes

Rats received Methomyl 1/10 LD50 (G1): Their histopathological lesions of testis at 30 days revealed thickening of capsule,



hypospermatogenesis; increase rounded cells inside the lumen of the some seminiferous tubules (Figure-2a), necrosis in others with subcapsular congested blood vessels (Figure-2a&b). In (G2) were rats received 1/20 LD50 showed some seminiferous tubules with depletion & degeneration of elongating spermatid; also hyperchromatic germinal layer of spermatogonia, abnormal space between adjacent steroli cells, additionally regression in leydig cells in the interstitial tissue and hypo spermatogenesis (Figure-2c&d) seminiferous tubules due to severe degeneration and necrosis of spermatogonia, spermatids and presence of rounded cells which represent degenerated sperm, hyperchromatic germinal cells also noted that agreed with (16) who showed decreased fertility index and testicular changes which revealed moderately to severely degenerative effections in the seminiferous tubules with arrest of spermatogenesis incompletely ; which are used this parameters to estimate the fertility of the treated rats, but increased sperm cell abnormality, at acute toxicity when given methomyl via oral to male rats daily for 65days at two doses (0.5 and 1.0

mg kg (-1) b.wt corresponding to 1/40 and 1/20 LD50. Half-life of methomyl is about in soil 50 days (17), and about 262 days in the pure water (18). In addition the increasing the uses of the methomyl in the fields of agriculture with the recurrent obsevation in the environment especially water environment ,with highly concern & the interest which have been raised concerning the environmental behavior with degradation mechanism of methomyl. Although methomyl was used in wide range widely used in agriculture and treat field crops with contain highly water solubility, it observed & detected infrequently as a pollutants& contaminant of water bodies in the world especially USA, methomyl classified as a limited-use pesticide due to its severe toxicity to different non target species, Furthermore to preventing severe toxicity in non-target species or the associated with possibility of the contamination and pollution with this pesticide , in conclusion when used all insecticides , with great caution &care could be taken when using products containing methomyl for, residential, agricultural or for other uses (19,20).

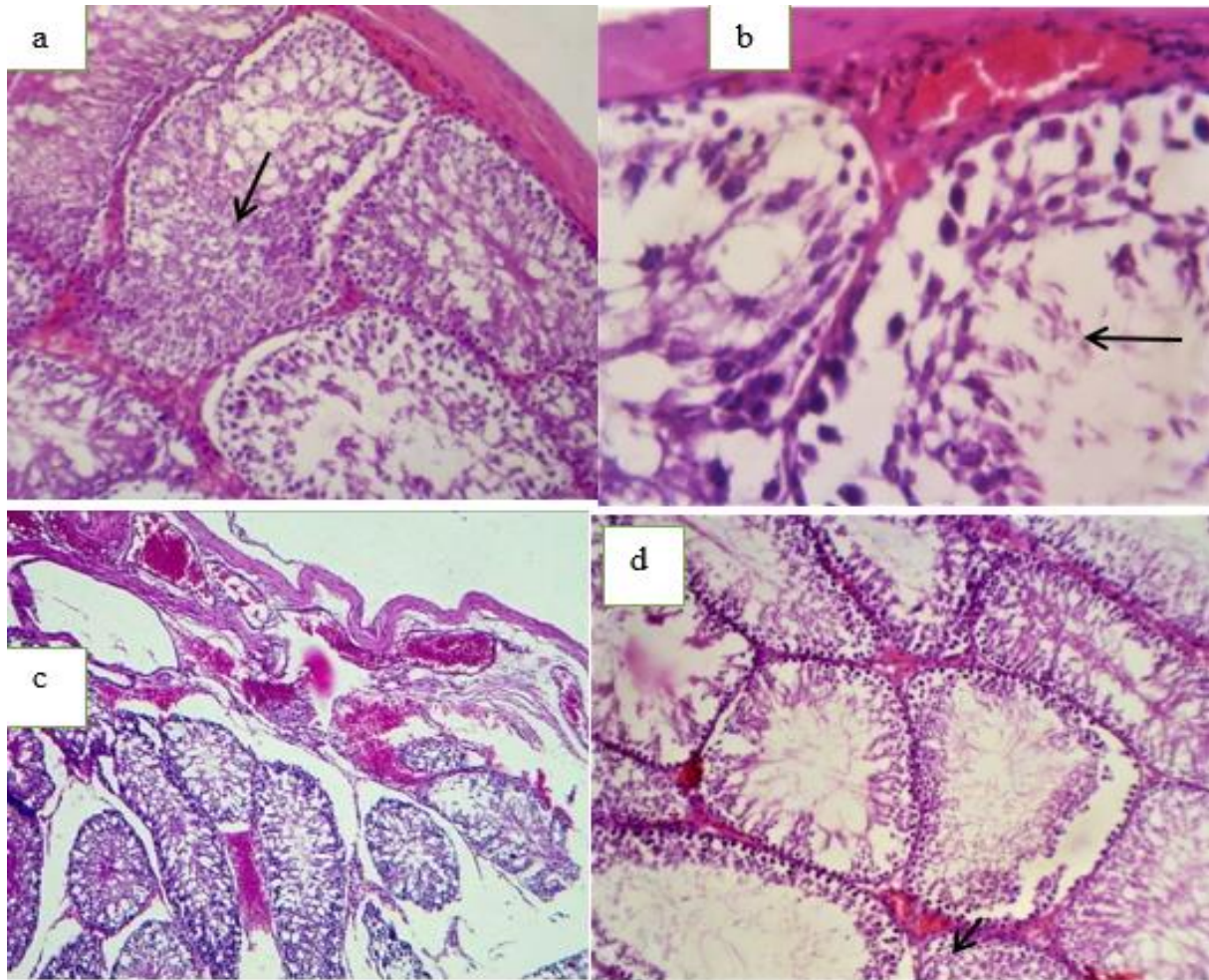


Figure-2: Histopathological section of testis of G1, G2groups; shows (a&b) thickening of fibrous capsule, increase rounded cells (arrow) inside in lumens of the some seminiferous tubules. In (c&d): necrosis of spermatogonia (arrow) with congested blood vessels, few numbers of Sertoli cells and leydig cells and inflammatory cells in interstitium. (H&E stain 100X400X).

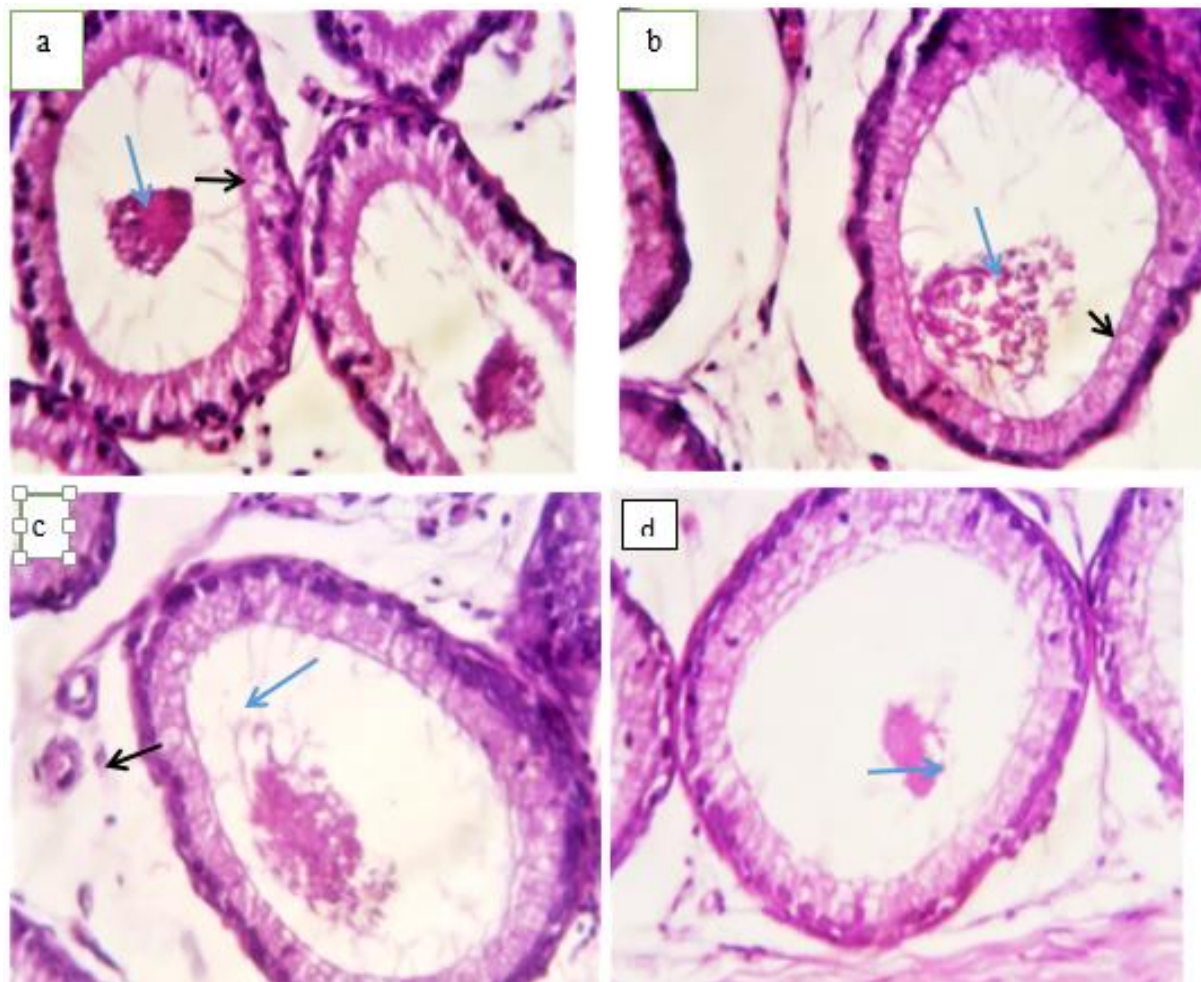


Figure-3: Histopathological section of epididymis of G1&G2 groups; shows marked swollen with microvacuolar and macrovacuolar degeneration (arrow) of the lining epithelial cells with necrosis and loss of cilia with stasis of sperms and eosinophilic material at the lumen (blue arrow). (H&E stain, 400X)

Ethics

This experiment was approved & confirmed by the veterinary medicine college council / Baghdad university , Baghdad , Iraq

Recommendation: extreme caution and attention by farmers when using this pesticide, to prevent accumulation and contamination widely.

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Conclusion:

The environment exposure to chemical toxins that effect the health of living organisms comes from studies on animals so the results of Methomyl toxicity had tissue degenerative effects in testes and epididymis of rats orally administered it proved at this study .

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