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Reproductive toxicity of methomyl insecticide in male rats and protective effect of folic acid

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ABSTRACT

The acute toxicity (LD_{50}) of insecticide methomyl and its effects on male reproduction in rats were carried out. Methomyl was given orally to male rats daily for 65 successive days at two doses (0.5 and 1.0 mg kg^{-1} b.wt., corresponding to 1/40 and 1/20 LD_{50}) alone and in combination with folic acid (1.1 mg kg^{-1} b.wt., corresponding to acceptable daily intake, ADI). Fertility index, weight of sexual organs, semen picture, serum testosterone level and histopathology of testes were the parameters used to evaluate the reproductive efficiency of treated rats. The reversibility of methomyl effects was also studied after 65 days post-administration. The oral LD_{50} of methomyl was 20.0 mg kg^{-1} b.wt. in male rats. Methomyl significantly decreased the fertility index, weight of testes and accessory male sexual glands, serum testosterone level and sperm motility and count, but increased sperm cell abnormality. It induced testicular lesions characterized by moderate to severe degenerative changes of seminiferous tubules and incomplete arrest of spermatogenesis. These toxic effects were not persistent (reversible). Coadministration of folic acid with methomyl decreased its reproductive toxicity. A great attention should be taken during field application of methomyl to avoid its deleterious effects in farm animals and occupationally exposed humans.

1. Introduction

Pesticides have brought about the green revolution in the world and are being widely used to control agricultural pests and insects causing public health hazards. Problems and/or outbreaks are reported to occur among animals and human from insecticide toxicity, which usually occurs either from direct exposure to insecticides or indirectly from contaminated feeds or water by such chemicals. Prolonged exposure to insecticides causes chronic neurological syndrome, malignant tumors, immunosuppressive action, teratogenic effect, abortion and decreased male fertility in experimental animals (Nafstad et al., 1983; El-Rahman, 1988; Al-Qwari et al., 1999; Meeker et al., 2006; Youssef, 2010).

Carbamates, derivatives of carbamic acid, represent a large variety of compounds which have some field applications as insecticides, herbicides and fungicides. Many of these chemicals are potential neurotoxicants, particularly following occupational, accidental or intentional exposure. These compounds cause reversible carbamaylation of the acetylcholinesterase enzyme, allowing accumulation of acetylcholine, the neuromediator at parasympathetic neuro-effector junctions (muscarinic effect) and autonomic ganglia (nicotinic effect) and in the brain (CNS effect). Methomyl acts as an insecticide against lepiodopterous, suppresses coleopterous and some hemipterous insects. It acts as an ovicide against cotton bollworms and tobacco budworms (Mahgoub and Mednay, 2001). In Egypt, the use of insecticides represent a great risk because of the huge amounts of insecticides used in the field and the lack of proper protective measures against pollution by these chemicals (El-Rahman, 1988).

Folic acid is necessary for the production of red blood cells and DNA synthesis. Folate is necessary for normal fertility in men and women as it is important during cell division and growth periods such as infancy and pregnancy. In males, folate is necessary for spermatogenesis (Kamen, 1997).

The present work aimed to determine the acute oral LD_{50} of methomyl insecticide and to examine its toxic effects, alone and in combination with folic acid, on male fertility in rats. The reversibility of methomyl effects was also studied 65 days post-administration.

2. Material and methods

2.1. Insecticide

Methomyl (Methomex®) is *S*-methyl *N*-(methylcarbamoyloxy) thioacetimidate with molecular weight 162.2 and molecular formula $C_5H_{10}N_2O_2S$. It was obtained from the Central Laboratories of Agricultural Pesticides, Dokki, Egypt in the form of a pure white crystal powder.

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2.2. Folic acid

Folic acid (Pteroylglutamic acid, Vit. B-9 or Vit. M) is a water soluble vitamin B member. It was obtained in the form of sodium folate solution; equivalent of folic acid 5 mg/ml, from El Gomhoryia Company, Egypt.

2.3. Animals

Male and female rats of Sprague Dawley strain, weighing 160 ± 20 g and 16–18 weeks of age, were selected from inbred colony maintained in the Animal House of Pharmacology Department, Faculty of Veterinary Medicine, Cairo University. The animals were kept under controlled hygienic conditions and maintained at a temperature of 25 ± 2 °C, relative humidity of 50 ± 5% and photoperiod at 12-h dark/light. Rats were fed on rat pellets which composed of wheat bran 10%, soy bean powder 44%, net protein 22%, fats 4.7%, fibers 3.3%, fish meal, molasses, salts (sodium chloride, calcium carbonate, calcium phosphate) and methionine. These pellets are manufactured by Cairo Agriculture Development Company, 6 October City, Giza, Egypt. The diet was offered and water was allowed *ad libitum* during the experiment period. Rats were allowed to acclimatize to the laboratory environment for 7 days before start of the experiment.

2.4. Acute toxicity experiment

For estimating the LD_{50} of methomyl, 50 male Sprague Dawley rats were distributed into five groups each containing 10 animals. Rats were given orally, by stomach tube, the tested insecticide in graded doses. Toxic symptoms and the number of rats that died in each group after 48 h observation were recorded. The LD_{50} of methomyl was then calculated according to the method described in Gad and Weil (1982).

2.5. Fertility index

Effects of methomyl at two dose levels (1/20 and 1/40 of the LD_{50}) and folic acid at acceptable daily intake (1.1 mg kg^{-1} b.wt.) and both of them on the fertilizing capacity of male rats were determined by serial mating of the treated males with normal (untreated) females of regular oestrous cycle. After serial mating, the fertility index for each male rat was determined by the number of females which become pregnant in relation to the mated females, i.e., pregnancy rate.

2.6. Serial mating technique

Sixty mature male rats were used in the experiment where each male was paired with two oestrous females for a period of 5 days. The process was repeated for four successive times at 10-days intervals with new females in oestrous cycle. Successful mating was confirmed by presence of sperms in the vaginal smears in morning of the next day. The pregnancy percentages were recorded at each time and calculated at 15, 30, 45 and 55 days during the experimental period. At day 66, half of these males (30 rats) were sacrificed for evaluating their reproductive efficiency. The other half of males (30 rats) was left for other 65 days after stopping of methomyl administration and then the same parameters were carried out for studying the reversibility of the effects.

2.7. Fertility experiment

For estimating the effect of methomyl, folic acid and their combination on male fertility, 30 mature male rats were allocated into six equal groups. The 1st group was given orally 1.0 ml distilled water/day (vehicle) and kept as normal control. The 2nd and 3rd groups were given orally 1/20 (1.0 mg kg^{-1} b.wt.) and 1/40 (0.5 mg kg^{-1} b.wt.) of the LD_{50} of methomyl, respectively. The 4th group of male rats was orally given 1.1 mg kg^{-1} b.wt. of folic acid calculated from acceptable daily intake (ADI) in man according to Paget and Barnes (1964). The 5th and 6th groups were given 1/20 and 1/40 of the LD_{50} of methomyl, respectively, and after 2–4 h rats were given ADI of folic acid. Oral administration of the tested compounds continued for 65 consecutive days to cover the spermatogenic cycle according to Amann (1982). At the end of experiment, blood samples were collected for estimating total testosterone in the serum. The rats were sacrificed and the testes, seminal vesicle and prostate glands were removed and weighed. Semen samples were collected from cuda epididymis by cutting the tail of epididymis and squeezing it gently on clean slide. The semen was used for estimating the epididymal sperm characters (motility and count) according to the method adopted by Bearden and Fluquary (1980) and the sperm cell abnormality was determined microscopically. The testes were preserved in 10% neutral formalin solution till processed for histopathological examination.

2.8. Semen picture

Epididymal contents of the treated rats were obtained after cutting the tail of epididymis, squeezing it gently in clean slide and the progressive motility and sperm cell count were recorded according to the method described by Bearden and Fluquary (1980). Seminal smears were also prepared for the microscopic examination of sperm cells abnormality.

2.9. Testosterone determination

Serum samples of the treated male rats were used for estimating testosterone concentration using radioimmunoassay (RIA) method (kit catalog #1119). This method is based on the competitive binding principal where the unknown or standards samples were incubated with radioactive iodine^{125} labeled testosterone in antibody-coated tubes. After incubation, the liquid contents in the tubes were withdrawn and the bound radioactivity was determined using gamma counter according to method described by Wilke and Utley (1987).

2.10. Histopathological examination

Testes of the treated rats were taken and fixed in 10% neutral formalin solution. The fixed specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. These specimens were cleared in xylene, embedded in paraffin, sectioned at 4–6 μm thickness and stained with Hematoxylen and Eosin (H&E) then examined microscopically according to Luna (1968).

2.11. Statistical analysis

All data were expressed as mean ± S.E. and statistical analysis was carried using Student '*t*' test according to Snedecor and Cochran (1986).

3. Results

The results of acute toxicity study revealed that oral LD_{50} of methomyl insecticide in male rats was 20.0 mg kg^{-1} b.wt. The toxic symptoms were muscular tremors, abdominal cramps, sweating, muscle incoordination and irregular respiration and heart rate. These symptoms were seen during the first 24 h post-administration.

Fertility indexes of the male rats given methomyl at doses of 1.0 and 0.5 mg kg^{-1} b.wt. (Corresponding to 1/20 and 1/40 of the calculated LD_{50}) for 65 consecutive days were 55.56% and 77.78%, respectively, compared to 100% in the control normal group. Rats given folic acid at a dose of 1.1 mg kg^{-1} b.wt. (Corresponding to ADI) have fertility index of 100%. In male rats given combination of folic acid and methomyl at the two tested doses, the fertility indexes were 66.67% and 88.89%, respectively, as shown in Table 1.

As recorded in Table 2, oral administration of methomyl at 1.0 and 0.5 mg kg^{-1} b.wt. for 65 successive days caused significant decreases in the weight of testes, seminal vesicles and prostate glands, as compared to the normal control group. Coadministration of folic acid at acceptable daily intake (1.1 mg kg^{-1} b.wt.) with methomyl (1.0 and 0.5 mg kg^{-1} b.wt./day) significantly increased the weight of testes, seminal vesicles and prostate gland, as compared to the normal control group. The decrease in the weight of

Table 1
Effect of oral administration of methomyl at doses 1/20 and 1/40 of LD_{50}, folic acid (ADI) and their combination for 65 successive days on the fertility index in male rats.

Groups	Doses	No. of pregnant/no. of mated females	Fertility index (%)
Control (D. water)	1.0 ml kg b.wt.	9/9	100
Methomyl (1/20 LD_{50})	1.0 mg kg^{-1} b.wt.	5/9	55.56
Methomyl (1/40 LD_{50})	0.5 mg kg^{-1} b.wt.	7/9	77.78
Folic acid (ADI)	1.1 mg kg^{-1} b.wt.	9/9	100
Methomyl (1/20 LD_{50}) + folic acid (ADI)	1.0 mg kg^{-1} b.wt. + 1.1 mg kg^{-1} b.wt.	6/9	66.67
Methomyl (1/40 LD_{50}) + folic acid (ADI)	0.5 mg kg^{-1} b.wt. + 1.1 mg kg^{-1} b.wt.	8/9	88.89