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Effect of methomyl on developmental toxicity and oxidative stress in male mice

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Abstract:

The intensive use of pesticide leads to severe environmental pollution and health hazards. The present study was carried out to investigate the alterations in free radicals and enzyme activities induced by methomyl in testicular tissue of male mice and general reproductive performance. Animals were assigned at random to one of the following treatments: group 1 served as control, while groups 2, 3 and 4 were treated with 1mg methomyl/kg body weight for 10, 20, and 30 days, respectively. Methomyl significantly increased testicular Thiobarbituric Acid Reactive Substances (TBARS), while decreased glutathione level (GSH) and the activities of glutathione S-transferase (GST), superoxide dismutase (SOD), catalase (CAT), transaminases (AST, ALT) and acid phosphatase. Methomyl significantly decreased the mating and fertility index, body and testis weights and relative testis weight, serum testosterone level and sperm motility and count, but increased sperm abnormality. In conclusion, the exposure to methomyl can produce enhanced toxic effects on enzyme defense system and reproductive function on male mice and the effect was more evident in 30 days treated group.

Introduction

Pollutants in the environment cause various hazards on living organisms. 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 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 (Shalaby *et al*., 2010). The issue of testicular toxicity is of growing concern as many pesticides adversely affect the testicular functions in experimental animals (Shalaby *et al*., 2010 and Joshi *et al*., 2007). Prolonged exposures to pesticides could diminish or destroy the fertility of workers sparked a concern about the effects of hazardous substances on male reproductive health (Joshi *et al*., 2007).

Also, insecticides cause oxidative stress, leading to generation of free radicals and alteration in antioxidants enzymes, chronic neurological syndrome, malignant

tumors, immunosuppressive action, teratogenic effect, abortion and decreased male fertility in experimental animals (Shalaby *et al*., 2010; Joshi *et al*., 2007; Nafstad *et al*., 1983; Meeker *et al*., 2006 ; El-Demerdash, 2007 and Aziz *et al*., 2008) .

Carbamates 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. Methomyl, S-methyl N-(methylcarbamoyloxy) thioacetimidate, is a carbamate insecticide with anticholinesterase activity. As a broad-spectrum insecticide, it is one of the most frequently used pesticides in agriculture (Sinhaseni *et al*., 1995). It is classified as a highly hazardous compound by World Health Organization (Mansour *et al*., 2009) .

Therefore, the present study was undertaken to investigate the toxicity of methomyl on mouse testis. Testicular biochemistry, oxidative stress, sperm characteristics, testosterone level and histopathological changes were the criteria used to evaluate the reproductive efficacy of treated mice.

Materials and methods

1.Chemicals:

Methomyl (HUAYANG, 90 %) is S-methyl N-(methylcarbamoyloxy) thioacetimidate with molecular weight 162.2 and molecular formula $C_5H_{10}N_2O_2S$. It was obtained from Shandong Huayang Technology Ltd. Company, China. All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other reagents used were of analytical grade.

2.Experimental design:

Eighty male CD-1 mice (weighing 35- 40 g) were obtained from the animal house of the Faculty of Medicine, Alexandria University, Alexandria, Egypt. The local committee approved the design of the experiments, and the protocol conforms to the guidelines of the National Institutes of Health (NIH). Animals were caged in groups of twenty and given food and water *ad libitum*. The animal room was maintained at 21–24°C and 40–60% relative humidity with 12-h light–dark cycles, the light cycle coinciding with the day light hours. After 2 weeks of acclimation, the groups were assigned at random to one of the following treatments: group 1 served as control, while groups 2, 3 and 4 were treated daily with (1/10 LD_{50}) one mg methomyl/kg body weight (BW) orally for 10, 20 and 30 days, respectively. The LD_{50} of methomyl when administered orally to mice was reported to be 10 mg/kg BW (Baron, 1991) .

3. Serum and tissue preparation:

Blood samples were collected from the retro orbital plexus using glass capillary tubes. Serum was obtained by centrifugation of the samples at 860 g for 20 min, and was stored at -60°C. After decapitation, testis was immediately removed, weighed, minced and homogenized (10% w/v) separately in ice-cold 1.15% KCl-0.01M sodium, potassium phosphate buffer (pH 7.4) in a Potter-Elvehjem type homogenizer. The homogenate was centrifuged at 18,000 Xg for 20 min at 4°C, and the resultant supernatant was used for the determination of different enzyme assays, TBARS and glutathione content.

4. Biochemical parameters:

Serum samples were used for testosterone determination using commercial kits (RIA). Thiobarbituric Acid Reactive Substances (TBARS) were measured in testis using the method of Ohkawa *et al.* (1979). Glutathione contents (GSH) were measured in testis homogenate after reaction with 5, 5-dithiobis-(2-nitrobenzoic acid) using the method of Ellman (1959). Testis glutathione S-transferase (GST; EC 2.5.1.18) activity was determined according to Habig *et al.* (1974), using para-nitrobenzylchloride as a substrate. Superoxide dismutase (SOD; EC 1.15.1.1) was assayed according to Misra and

Fridovich (1972). The assay procedure involves the inhibition of epinephrine auto-oxidation in an alkaline medium (pH 10.2) to adrenochrome, which is markedly inhibited by the presence of SOD. The enzyme catalase (CAT; EC 1.11.1.6) converts H_2O_2 into water. The CAT activity in tissue supernatant was measured spectrophotometrically at 240 nm by calculating the rate of degradation of H_2O_2, the substrate of the enzyme (Aebi, 1984) . Testis alanine aminotransferase (ALT; EC 2.6.1.2) and aspartate aminotransferase (AST; EC 2.6.1.1) activities were assayed by the method of Reitman and Frankel (1957) . For assaying acid phosphatase (AcP; EC 3.1.3.2) activity, the method of Moss (1984) was used . The protein content was determined by following the method described by Lowry *et al.* (1951) using bovine serum albumin as a standard.

5. Reproductive performance indices:

5.1. Mating:

It is the assessment of the ability of treated male mice for copulation. After daily dose exposure, each male mouse was co-housed with unexposed proven fertility, sexually receptive, female mice (one to one/box) for at most five days (regular estrous cycle) following which they were separated (Fox and Laird, 1970) . The females were examined daily for the presence of the vaginal plug as a criterion of successful insemination that was considered the day zero of gestation.

5.2. Fertility:

Each plug-positive female was caged individually. The number of inseminated and/or pregnant non-pregnant females was recorded.

5.3. Reproductive outcomes:

On the 20^{th} day of gestation, the females were anesthetized with diethyl ether and killed by decapitation. After collection of the uterus, the numbers of implantation sites, resorptions, dead and live fetuses were determined. And then reproductive indices were calculated according to the method of Adilaxmamma *et al.* (1994).

6.Evaluation of sperm characteristics:

6.1. Sperm collection:

After the sexual cohabitation and fecundity test period (five days), the epididymdes were carefully separated from the testis. The sperm count was assessed from right cauda epididymdes while sperm motility and morphology were analyzed from the left one. Epididymis was excised and minced in 1ml of phosphate buffered saline (pH 7.2) to obtain sperm suspension (Narayana *et al.*, 2002).

6.2. Sperm motility:

In each semen sample, at least 10 microscopic fields were examined with at least 100 sperm/field was counted. The number of motile sperm cells in each field was divided by the total number, and the average of the fields was assayed. The percentage of motile spermatozoa was determined (Linder *et al*., 1995 and Lobet *et al*., 1995).

6.3. Sperm count:

The epididymal spermatozoa count was conducted in the filtrate using a Neubauer hemocytometric chamber (Narayana *et al.*, 2002) and examined under microscope at 400X. The total sperm count in squares of $1mm^2$ each was determined to express the number of spermatozoa/epididymis. To minimize the error, the count was repeated three times on each sample.

6.4. Sperm viability:

Sperm viability was assessed using the eosin–nigrosin stain (Tardif *et al*., 1999) . The staining was performed with one drop of freshly collected semen (10µL) were placed on a slide and stained with two drops of freshly prepared staining solution (20µl) of eosin–nigrosin (1 g eosin + 5 g nigrosin/100 ml deionized water). The live unstained and dead stained spermatozoa were analyzed under the microscope at 400X. The dye exclusion was evaluated in 100 spermatozoa.

Sperm viability was defined as the percentage of dead sperm cells. Viability was evaluated according to WHO guidelines (WHO , 2001) .

7. Statistical analysis:

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

Results and discussion

1. Body and organs weight:

Male body and organ weights are presented in Table (1). Male body weight and weight gain were significantly reduced after 20 and 30 days of methomyl treatment. No statistically significant differences in relative epididymides weights were noted in any of methomyl treated groups compared to the control group. Absolute and relative testis weights were reduced dramatically in the groups treated with methomyl after 20 and 30 days compared to the 10 days treated group and control.

Table (1): Effect of methomyl on body weight, testis and epidedimus weights of male mice after 10, 20 and 30 days of exposure.

Period of exposure / Groups	Body weight			Absolute testis weight (g)	Relative testis weight% (g/100g B.Wt)	Absolute Epididymis weight (g)	Relative Epididymis Weight% (g/100g B.Wt)
	Initial (g)	Final (g)	% Change				
10-days							
Control	37.4±2.65	38.5 ±3.11	2.9±0.69	0.25 ±0.03	0.65±0.041	0.07 ±0.008	0.18 ±0.046
Treated	38.7±3.65	39.7 ±4.01	2.6±0.51	0.24 ±0.05	0.60±0.033	0.07 ±0.012	0.18 ±0.051
20-days							
Control	36.2±4.22	38.4±3.98	6.1±0.68	0.26±0.013	0.68±0.078	0.07±0.0045	0.18 ±0.021
Treated	37.6±4.09	33.8 ±3.25*	10.1±1.06**	0.21±0.036**	0.62±0.06*	0.06±0.012	0.18± 0.013
30-days							
Control	38.8±4.08	41.6±4.58	7.2±1.03	0.25±0.042	0.60±0.035	0.08±0.028	0.19±0.052
Treated	36.5±3.98	31.2±3.58**	14.5±1.20**	0.20±0.046*	0.64±0.044*	0.06±0.018	0.19±0.063

Values are means ± SD for 20 mice in each group.
***Significantly different at $P < 0.05$.**
****Highly significant at $P < 0.01$.**

2. Biochemical parameters:

Results indicated that TBARS concentration was significantly increased, while GST, SOD, CAT activities and GSH level were significantly ($p < 0.05$) decreased in testicular tissue of mice treated with methomyl for all periods of exposure (Table, 2). The highest inhibition value was observed after 30 days of exposure. In addition, a significant decrease in testicular AST (30 %, 41% and 52%), ALT (38%, 56%, 69%) and AcP (17%, 25% and 35%) activities was observed after treatment with methomyl for 10, 20 and 30 days, respectively. Data showed that oral administration of methomyl for 10, 20 and 30 days induced a significant ($P < 0.01$) decrease in serum testosterone levels in a time dependent manner as compared to the control group (Figure, 1).

Table (2): Effect of methomyl on thiobarbituric acid reactive substances (TBARS) levels, glutathione content (GSH) and some enzymatic activities in mice testes.

Parameters	Control	10 days	20 days	30 days
TBARS[!]	19.3±0.81	24.0±0.53*	25.7±1.92*	27.5±0.70**
GSH[!!]	2.42±0.071	1.91±0.076*	1.62 ±0.060*	1.42 ±0.080**
GST[+]	0.70±0.056	0.53±0.032*	0.47±0.027**	0.37±0.020**
SOD[++]	77±1.14	57±3.98*	47±2.89**	41±3.42**
CAT[++]	113±4.32	95±3.21[*]	77±2.27**	60±1.65**
AcP[#]	13.00±0.87	10.85±0.42*	9.74±0.46*	8.51±0.39**
AST[#]	109±4.90	76±1.82*	64±3.70**	53±2.65**
ALT[#]	97.6±2.29	60.2±5.12*	42.7±2.93**	30.6±2.28**

Values are expressed as means±SD; n = 20 for each treatment group.
***Significantly different at $P < 0.05$. **Highly significant at $P < 0.01$**
[!] **TBARS is expressed as nmol/g tissue**
[!!] **Glutathione is expressed as mmole**
[+] **GST is expressed as specific activity, µmol/h/mg protein)**
[++] **SOD or CAT is expressed as units/mg protein**
[#] **AcP, AST and ALT is expressed as IU/mg; international unit, the amount of the enzyme that under defined assay conditions will catalyze one mol of substrate/min/mg protein.**

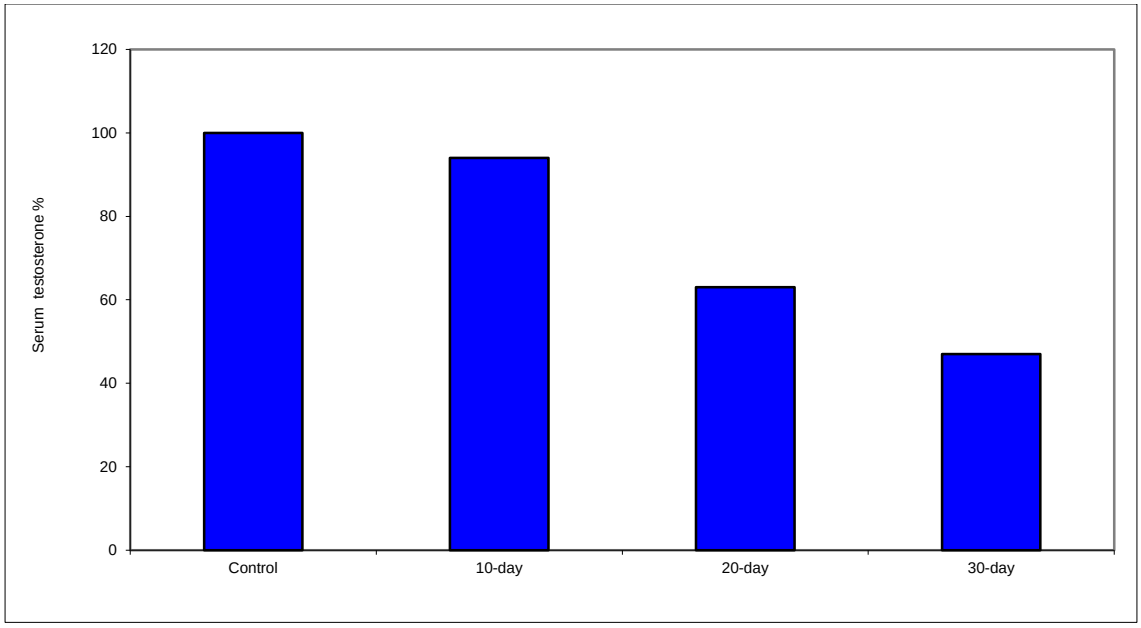

Figure (1): Serum testosterone percent after exposure to 1/10 LD_{50} of methomyl during different periods.

3. Reproductive toxicity:

3.1. Sperm counts and motility:

Regarding sperm quantity and quality after oral administration of methomyl at 1/10 LD_{50} for 20 and 30 successive days to male mice produced significant decreases in sperm count, viability and progressive motility in addition to an increase in the percentage of total abnormalities of sperm morphology (Table, 3). Although the sperm quantity and quality in 10-day group were lower than the control group, there are no statistical differences between the two groups (Table, 3).

Table (3): Effects of methomyl on sperm characteristics in male mice after 10, 20 and 30 days of exposure.

Sperm Parameters	Control	10-days	20-days	30-days
Count ($\times 10^6$)	6.2 ± 0.88	5.1 ± 0.96	2.4 ± 0.45**	1.3 ± 0.33**
Motility (%)				
Progressive	69.4±6.25	65.1±5.98	25.4±4.25**	12.8 ±2.58**
Non-Progressive	10.9±2.65	14.4±3.01	18.9±3.27**	24.6 ±4.25**
Immotile	19.7±3.21	20.5±3.58	55.7 ±8.98**	62.6 ±10.2**
Morphology (%)				
Normal	83.7 ±15.6	79.3 ±14.2	68.8±10.8*	62.4 ±11.09*
Abnormal head	7.6 ±1.03	8.9 ±1.22	15.2±1.98*	19.0 ±2.11*
Abnormal tail	8.1 ±1.06	11.1 ±1.89	14.9 ±2.07*	17.1 ±2.08*
Other abnormalities	0.6 ±0.036	0.7 ±0.052	1.1 ±0.98*	1.5 ±0.79*
Viability (%)	88.5±15.6	86.5 ±18.9	73.13 ±9.89**	56.45±6.78**

Values are means ± SD for 20 mice in each group.
***Significantly different at $P < 0.05$. **Highly significant at $P < 0.01$**

3.2. Mating index and fertility index:

As shown in Table (4) mice treated with methomyl showed a significant decrease in mating and fertility indexes after 20 and 30 days of exposure as compared to control. While, the mice group treated with methomyl for 10 days did not show any significant change.

Table (4): Reproductive efficiency of male mice after 10, 20 and 30 days of methomyl exposure.

Period of exposure/ Groups	Number of males[a]	Mating index (%)[b]	Fertility index (%)[c]
10-days			
Control	20	100 (20/20)	100 (20/20)
Treated	20	95 (19/20)	94.7 (18/19)
20-days			
Control	20	100 (20/20)	100 (20/20)
Treated	20	80 (16/20)*	75 (12/16)*
30-days			
Control	20	100 (20/20)	20/20 (100)
Treated	20	60 (12/20)*	50 (6/12)**

[a]; Number of males which used for mating.
[b]: Number of females with vaginal plug /number of females cohabited×100.
[c]; Number of cohabited females becoming pregnant/number of non-pregnant with evidence of vaginal plug×100.
***Significantly different at $P < 0.05$. **Highly significant at $P < 0.01$**

3.3. Reproductive outcomes:

A statistically significant decrease in the number of live offsprings in mice groups treated with methomyl for 20 and 30 days was observed. However, the number of implantation sites and the number of late resorptions were not significantly changed in any of the treated groups. On the other hand, a significant increase in the number of early resorptions in the 20 and 30 days treated groups was detected (Table, 5).

Table (5): Reproductive outcomes of untreated females after cohabitation with mice exposed to methomyl for 10, 20 and 30 days.

Exposure periods				
	Control	**10 days**	**20 days**	**30 days**
Number of pregnant females	20	18	12	6
Implants/litter	10.88±1.85	10.84±1.42	10.42±1.33	10.31±1.52
Live (%)	10.34±1.74 (95.03)	10.28±1.68 (94.83)	8.60±1.99* (82.53)	6.95±1.52* (67.41)
Dead (%)	0.00	0.00	0.00	0.00
Early resorption / litter (%)	0.32 ±0.106 (2.94)	0.33 ±0.189 (3.04)	1.58 ± 0.054** (15.16)	3.11 ±0.13** (30.16)
Late resorption / litter (%)	0.22 ±0.098 (2.02)	0.23 ±0.045 (2.12)	0.24 ±0.026 (2.30)	0.25 ±0.021 (2.42)
Post implantation loss % [a]	4.96±0.98	5.16±2.06	17.66±4.06**	35.11±5.22**

Values are means ± SD for 20 mice in each group
[a]; [(number of implants – live fetuses)/number of implants] x100
***Significantly different at $P < 0.05$. **Highly significant at $P < 0.01$**

The toxic symptoms observed in methomyl toxicity were muscular tremors, abdominal cramps, sweating, muscle incoordination, irregular respiration and heart rate (Kidd *et al*., 1991). Reduction in male body weight can be attributed to the reduction in feed consumption and systemic toxicity of methomyl in male mice. The administration of methomyl brought about marked alteration in the weight of testes accompanied by necrotic changes and this simply reflects regressive changes in seminiferous tubules (Udoh and Kehinde , 1999). The change in testicular weight has also corresponded to the presence or absence of postmeiotic germ cell in addition to reduction in the number of spermatogenic elements and spermatozoa (Takihara *et al.*, 1987 and Sinha *et al.* , 1995) .

In the present study, an increase in TBARS levels in testicular tissue in mice treated with methomyl for different periods of time was observed. The products of lipid peroxidation (LPO) formed by free-radical mediated attack on membrane lipids can propagate a chain of reactions of LPO processes in the presence of oxygen, possibly leading to membrane destruction. In consistence with the present results, El-Khawaga, 2005 and Lohitnavy and Sinhaseni,1998, reported that methomyl induced LPO and oxidative stress in experimental animals . The significant accumulation of TBARS may be caused by the oxidation process that takes place in testicular cell membranes of mice exposed to methomyl. The resultant lipid peroxides may also react with GSH and lead to a decrease in GSH and the activity of glutathione related enzymes (Maran *et al*., 2010). Oxidative damage, therefore, may be attributed to the consequence of insufficient cellular antioxidant potential. Carbamates, and their degradation products act on the membranes, oxidizing its lipid components and enhancing TBARS production during their exposure. Mammalian cells have developed several defense mechanisms against reactive oxygen species (ROS). Glutathione is particularly important because it provides a first line of defense against lipid peroxidation. It also serves as the substrate for the two major antioxidant enzyme systems, glutathione peroxidase and glutathione-S-transferase (Grosicka-Maciag *et al.*, 2008). It has been reported that the toxicity of some carbamates is correlated with the decrease in GSH content, and that the GSH redox cycle is the major pathway to provide the reducing equivalents for carbamate reduction

(Grosicka-Maciag *et al.*, 2008 and Kanno *et al*., 2003). Because glutathione is the most abundant soluble cellular redox buffer, it is predictable that its deficit can induce or facilitate oxidative stress. The antioxidative enzymes were significantly reduced in methomyl treated mice. GST, a family of isoenzymes serving a major role in the biotransformation of many reactive compounds, could also catalyze the conjugation of GSH with a wide variety of organic peroxides to form more water-soluble compounds (Vos and Van Bladeren, 1990). Consistent with the present results, Lohitnavy and Sinhaseni (1998) reported a significant decrease in the GST activity after a single intraperitoneal dose of methomyl to mice. Superoxide dismutase protects oxygen-metabolizing cells against harmful effects of superoxide free radicals through destruction. SOD protects hyaluronate against depolymerization by the action of free radicals, which indicates that exogenous SOD might possess an anti-inflammatory effect. In agreement with the present results, methomyl decreased the activity of SOD in mice through induction of oxidative stress in addition to its anticholinesterase potency (El-Khawaga, 2005). Catalase is ubiquitously enzyme present in a wide range of aerobic cell types with the highest activities in mammals. Some studies have indicated that superoxide radicals can inhibit CAT activity, and the increased H_2O_2 resulting from CAT inhibition could finally inhibit SOD activity. This is an indicative of the rate of formation of free radicals (Mansour and Mossa , 2009) .

Results showed that the increase in the duration of exposure of methomyl caused a significant decrease in the activity of AcP, AST and ALT in testes of male mice. The inhibition might be due to cellular damage or increased permeability of plasma membrane (El-Demerdash, 2004 and Ksheerasagar and Kaliwal, 2006). The AST and ALT enzymes are involved in amino acid metabolism and any change in these enzymes indicates tissue damage due to the toxic effect of methomyl in testis. The change in AcP activity may be because of methomyl on absorptive or secretory surface of the cell membrane causing cellular leakage as an adaptive response in enzyme activity to the persistent stress (Ksheerasagar and Kaliwal, 2006). Concerning serum testosterone level, data showed that mice treated with methomyl induced a significant decrease in serum testosterone levels in a time dependent manner (Figure, 1). This finding agreed with that reported by Shalaby *et al*. (2010) and Mahgoub and Mednay (2001) who recorded a significant decrease in the level of testosterone in serum of methomyl intoxicated rats.

The spermatogenesis in mammals depends on testosterone production by Leydig cells in response to stimulation by follicle stimulating and luteinizing hormones. Follicle stimulating hormone increases Sertloi cell synthesis of an androgen binding protein needed to maintain high concentrations of testosterone. The hormonal changes produced by carbofuran compounds favour direct toxic effect of insecticide or possibly through a change in the neuroendocrine environment resulting into acetylcholinestase inhibition (Aziz *et al*., 2008) .

In consistence with the present results, methomyl adversely affect male reproductive organs and semen characteristics leading to significant decreases in epididymal sperm count and sperm motility, with an increase in sperm abnormal morphology. This can be attributed to the hormonal changes and testicular damage caused by methomyl (Shalaby *et al*., 2010; Mahgoub and Mednay, 2001 and Pant *et al*., 1997) . Other investigation was studied by Berger *et al.* (2000) who found that thiocarbamate insecticide molinate reduced the sperm fertilizing capacity of male rats concurrently with a reduction in sperm motility and

viability. In addition, Akbarsha *et al.* (2001) reported that carbendazim decreased sperm cell count; caused inhibition of sperm motility and increased incidence of sperm abnormalities.

Sperm motility is an important functional measurement to predict sperm fertilizing capacity (Aitken *et al*., 1984). Any negative impact on motility would seriously affect fertilizing ability (Murugavel *et al*., 1989). Marked inhibition of sperm motility may be because of low level of ATP content. Sperm motility may be affected by altered enzymatic activities of oxidative phosphorolytic process that required for ATP production, a source of energy for the forward movement of spermatozoa. Full ATP pool is crucial for normal spermatozoal movement and a slight deprivation of ATP leads to reduction in motility, which may cause infertility (Tso and Lee , 1981 and Bedford,1983) . Sperm count is one of the important factors that affect fertility (Bett *et al*., 1996). Suppression of gonadotrophins might have caused decrease in sperm density in testes (Sinha *et al*., 1995) . Also, toxicants have direct effect on Sertoli cell function, which appears to be involved in the control of spermiation, and when disturbed caused epithelial disorganization and subsequent tubular atrophy (Bardin *et al*., 1998) . The negative fertility test may be attributed to lack of forward progression and reduction in density of spermatozoa and altered biochemical mileu of cauda epididymis.

Reduction in mating and fertility indices in 20 and 30 days methomyl treated groups may simply represent the effects of methomyl exposure on sperm parameters and testis histopathologic changes with different period of treatments. Mating index reduction could be due to adverse effects on libido in relation to a possible decrease of testosterone level in addition to the other reproductive organs such as seminal vesicles or prostate have been affected (IPCS/WHO, 2001). Spermatogenesis and fertility are critically dependent upon the maintenance of adequate levels of testosterone (Kidd and James, 1991) . Therefore, the effects of methomyl on the fertility in this study can be attributed to its ability to reduce serum testosterone levels and sperm counts. Pregnancy outcomes were affected by exposure of male mice to methomyl for 20 and 30 days. Number of live fetuses was decreased while early resorption number was increased in the same groups. Therefore, the male-mediated effects of methomyl on pregnancy outcome may be attributed to the testicular toxicity.

In conclusion, the insecticide methomyl is a highly toxic compound. It has the capability to induce oxidative damage as evidenced by increasing LPO and perturbations in antioxidant enzymes. Methomyl induces reproductive toxicity in male mice manifested by decreases in the fertility index, weights of the sexual organs, semen characteristics and serum testosterone level. So, a great attention should be taken during field application of methomyl to avoid its deleterious effects on the male reproductive system of farm animals and occupationally exposed human.

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