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Effect of methomyl on fertility, embryotoxicity and physiological parameters in female rats

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ABSTRACT

The widespread use of pesticide leads to severe environmental pollution and health hazards. In the present study sexually mature female rats were administered methomyl at three different doses (2 mg/kg b.wt, 1 mg/kg b.wt and 0.67 mg/kg b.wt corresponding to 1/10 and 1/20 and 1/30 LD_{50} methomyl, respectively) daily by oral gavage for 28 consecutive days. Some biochemical parameters (lipid profiles, total proteins), levels of sex hormones in addition to fertility index and reproductive outcomes were determined. The levels of MDA and the activities of ovarian antioxidant enzymes (CAT, SOD, Gpx) were also estimated. Methomyl exposure increased maternal levels of cholesterol, triglyceride, total lipids, and levels of estradiol; and reduced the levels of progesterone in the 1/10 and 1/20 LD_{50} groups whereas no changes in total proteins were observed. In addition, methomyl treatment significantly reduced the number of implantation sites, the number of live fetuses and increased the incidence of dead embryos and resorption sites at the same dose levels. The antioxidative status in the ovary at the higher dose of methomyl was markedly depleted. Histopathological examination of the intoxicated ovaries revealed variable degrees of degenerative changes in the 1/10 and 1/20 LD_{50} groups. In conclusion, exposure of female rats to methomyl induced maternal and developmental toxicity at 1/10 and 1/20 LD_{50} groups. However, 1/30 LD_{50} methomyl dose produced no evidence of maternal toxicity. Therefore, its application should be limited to a designed program with special care in handling to minimize its hazards.

INTRODUCTION

During the last several decades, there have been widespread uses of potent substances that, although effective in their intended use, have also been suspected of being harmful to health (FAO, 2003). This mixture of environmental contaminants that may adversely affect human fertility includes pesticides (Cooper *et al.*, 2010).

The use of pesticides to manage pests in land and water has posed health hazards to live stock and wildlife. Problems and outbreaks have been reported to occur among animals and human from insecticide exposure (Salih and Jaafar, 2013). Prolonged exposure to insecticides is known to cause chronic neurological syndrome, malignant tumors, immunosuppressive action, teratogenic effect, abortion and decreased fertility in experimental animals (Meeker *et al*., 2006). Pesticides may cause reproductive toxicity through direct damage to cells, interference with biochemical processes necessary for normal cell function and biotransformation resulting in toxic metabolites (Sangha *et al*., 2013). Carbamates are among the most extensively used insecticides comprising the third major group of synthetic insecticides being utilized worldwide for agriculture (West and Marnett, 2006). They have frequently been used because of their relatively short life in the environment and fast action on the target pest (Kaur.and Sandhir, 2006).

It is possible that carbamates may be involved in oxidative stress through the generation of free radicals and changes in antioxidant enzymes. Lipid peroxidation is known to be one of the molecular mechanisms of carbamate-induced toxicity (Ott *et al*., 2007). Methomyl is an oxime carbamate insecticide that controls a broad spectrum of arthropods (Kidd and James 1991).

It is classified as a pesticide of category-1 toxicity and has been accused of causing short-term adverse health effects (WHO, 1996). Methomyl is an endocrine disruptor and also potent genotoxic, capable of inducing structural and numerical chromosomal aberration in mammalian cells (Andersen *et al*., 2002).

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It induces acute cholinergic poisoning by inhibiting acetylcholinesterase activity reversibly with a subsequent accumulation of acetylcholine at peripheral and central nervous systems (Fikes, 1990). Some pesticides may interfere with the female hormonal function, which may lead to negative effects on the reproductive system through disruption of the hormonal balance necessary for proper functioning (Bretveld *et al*., 2006). Pesticides may cause reproductive toxicity through several different mechanisms: direct damage to the structure of cells, interference with biochemical processes necessary for normal cell function, and biotransformation resulting in toxic metabolites (Sangha *et al.*, 2013).

Several pesticides may interfere with the female hormonal function and thereby cause negative effects on the reproductive system. Most previous studies focused on interference with the estrogen and/or androgen receptor, but the hormonal function can be disrupted in many more ways through pesticide exposure (Stamati *et al.*, 2007). Reproductive effects that have been associated with pesticide exposure in females are decreased fertility, spontaneous abortions, stillbirth, premature birth, low birth weight, developmental abnormalities, ovarian disorders, and disruption of the hormonal function (Sharpe and Irvine, 2004). The impact of adverse effects on reproductive health also includes impaired gametogenesis, ovulation and menstrual disturbances, infertility, and developmental anomalies. The effects can be reversible, permanent or even transgenerational, take place in the offspring (Dohle *et al.*, 2005). Exposure to pesticides has been implicated in the aetiologies of miscarriage and other reproductive disorders (Garry, 2004), genital deformities (Baskin *et al*., 2001), other birth defects (Schreinemachers, 2003); behavioural abnormalities (Zala and Penn, 2004) and skewed offspring sex ratios (Mackenzie and Constanze, 2005). Since the female function can be compromised by exposure to toxic chemicals, the present study was conducted to assess the effect of exposure to methomyl at three different doses on reproductive function, antioxidative status in ovaries and biochemical parameters in female rats with estimation of histopathological changes in the ovary of female rats.

MATERIALS AND METHODS

Chemicals

Methomyl (Methomex®, *S*-methyl *N*-(methylcarbamoyloxy) thioacetimidate) was obtained from the Central Laboratories of Agricultural Pesticides, Dokki, Egypt in the form of a pure white crystal powder. It was dissolved directly in saline and freshly prepared every week.

Animals

Adult virgin female albino Sprgue-dawely rats (4-5 months old, 190-200 g body weight), obtained from the animal house of the High Institute of Public Health, Alexandria University, Alexandria, Egypt. Animals were housed in stainless steel cages (5 rats/cage) under controlled hygienic conditions at room temperature (23 ± 2 ^{o}C), relative humidity (50 ± 10 %), and a photoperiod of 12h D/12h L. The animals were maintained on the standard laboratory pelleted rodent food and drinking water, *ad libitum*, throughout the period of experimentation.

Experimental design and dose levels

After two weeks of acclimatization period, female rats were divided into four groups (35 rats/each). Females in group 1 served as normal controls and were administered orally 0.5 ml of saline solution, which was used as a vehicle for methomyl. Group 2 (methomyl treated) females were given methomyl orally in saline at a dose level of 0.67 mg/kg bw equivalent to 1/30 LD_{50} methomyl. Female rats in group 3 were administered 1 mg/kg bw equivalent to 1/20 LD_{50} methomyl. Females of group 4 were given 2 mg/kg bw equivalent to 1/10 LD_{50} methomyl. Methomyl and saline were administered daily by oral gavage for 28 consecutive days.

Body and organs weight

Initial and final body weights were recorded for the calculation of body weight gain. After the experimental duration, rats were dissected and some organs were removed and stripped from fatty tissues and blood vessels, blotted, and their absolute weights were determined. To normalize the data, organ weights were expressed per 100 gram body weight.

Blood sampling (n=10)

After 28 days of treatment with methomyl, under light anesthesia with diethyl ether, blood samples from the dorsal aorta were collected from females (10 female rats from each group) into heparinized tubes. The collected blood was then centrifuged at 4000 rpm for 15 minutes; the obtained plasma was stored at -20ºC for biochemical and hormonal analyses.

Biochemical assays (n=10)

Total Lipids were assayed according to the method of Knight *et al.* (1972). Total cholesterol was determined after enzymatic hydrolysis and oxidation according to the method of Richmond (1973). Triglycerides were measured using the method of Fossati and Principle (1982).

High density lipoprotein-cholesterol (HDL-c) and low density lipoprotein-cholesterol (LDL-c) were determined according to the methods of Warnick *et al.* (1983) and Bergmenyer (1985), respectively. Very low-density lipoprotein (VLDL) was calculated mathematically by dividing the values of TG by a factor of 5 according to Friedewald *et al.* (1972). Total protein was assayed in plasma and liver homogenate according to Lowry *et al.* (1951). The concentration of albumin was determined according to the method of Doumas *et al*. (1971).

Determination of estradiol and progesterone hormones (n=10)

The estradiol and progesterone levels in plasma of female rats were analyzed by using immulte/immulite1000, and chemiluminescent enzyme immunoassay. Assay Kit (Catalog

Number LKE21, DPC) of estradiol and assay Kit (Catalog Number LKPG1, DPC) of progesterone were used for assessment process (Bergquist *et al.,* 1983).

Preparation of ovarian homogenate

The ovaries were excised and washed in ice cold isotonic saline solution containing 1 mM EDTA. Homogenization for 1 ovary from each animal was performed in 8 mL of cold buffer (50 mM potassium phosphate buffer, pH 7.4, containing 1 mM EDTA) using a Potter-Elvejham homogenizer at 4°C. The crude tissue homogenate was then centrifuged at 8000 rpm for 15 minutes at 4°C and the supernatant was removed and kept at -20°C for estimation of malondialdehyde (MDA) and the activity of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). The other ovary was used for histological preparation.

Ovarian oxidative stress

MDA, as a marker for lipid peroxidation (LPO) was measured colorimetrically in ovarian homogenate according to the method of Ohkawa *et al.* (1979). The activity of GPx was determined as described by Paglia and Valentine (1967). According to the method of Nishikimi *et al.* (1972), the activity of SOD was assayed. The CAT activity in the ovarian homogenates was assayed according to the method of Aebi (1984).

Mating and fertility assessment

On post-treatment day (PTD) 29, the remainder treated female rats (n= 25 from each group) were cohabitated with proven fertile adult males (2:1) to evaluate their mating and fertility status. Vaginal smears were collected every day and females showing sperm-positive vaginal smears were considered to be copulated. The time taken for observed vaginal smears was recorded as zero day of pregnancy (GD 0).

Litter assessment

After fertility assessment, all copulated females (n= 25) were weighed and sacrificed on gestation day (GD) 18. The uterine horns were dissected, removed and examined and the following data were recorded: number of implantation sites, number of live and dead fetuses, and number of resorption sites, fetal sex ratio, fetal body weight, and gross external fetal alterations. The obtained data was analyzed to calculate the copulation index, preimplantation loss % and post-implantation loss (Goyal et al., 2003). The uteri of apparently nonpregnant mice were stained with 10% sodium sulfide (Salewski, 1967) and examined for evidence of implantation sites. One-half of the fetuses were fixed in Bouin's solution and examined by a serial sectioning technique (Wilson and Wurkany, 1965; Staples, 1974). The remaining fetuses were preserved in 95% ethanol, eviscerated, subsequently cleared, and stained with Alizarin Red S for detection of skeletal malformations (Wilson and Wurkany, 1965; Hayes, 1994).

Histological Examination

For histopathological examinations, the ovary and uterus were quickly removed and fixed in 10% neutral buffered formalin solution. Following fixation, specimens were dehydrated in graded ethanol, embedded in wax, sectioned to 5 microns thickness. The sections were stained with Haematoxylin and Eosin (Banchroft *et al.,* 1996) and examined using light microscope.

Statistical Analysis

Data are expressed as mean ± standard error (SE). Data were analyzed using Statistical Package for Social Science (SPSS/ Version 17.0) software. Significance between experimental groups was determined using one-way analysis of variance (ANOVA) followed by least significant difference (LSD) test for comparison between two groups. P values less than 0.05 were considered statistically significant.

RESULTS AND DISCUSSION

Maternal Observations

Maternal clinical signs of toxicity

There were no deaths or abortions during the experimental course (28 days) of the present study. Signs of cholinergic toxicity including salivation, lacrimation, piloerection, straub tail, flat body appearance, nasal bleeding, huddling, mild muscular tremor abdominal cramps, sweating, muscle incoordination and irregular respiration and heat rate and diarrhea were noted in dams at 1/10 LD_{50} methomyl group compared with the control group. These signs appeared on day 9^{th} of treatment in 60–80% of treated dams with the high dose groupsn and progressed in the same animals throughout the period of the treatment (28 days). However, no toxic effects were noted in the general appearance of animals in 1/20 LD_{50} or 1/30 LD_{50} groups.

Maternal body weight gain

The effect of repeated doses of methomyl insecticide on body weight gain of the female rats was recorded in **table (1)**. The findings indicated that female rats treated with 1/10 LD_{50} methomyl showed significant decrease in their final body weights after administration for 28 days as compared with control group while the body weight was not obviously changed on the other tested groups after 28 days of treatments.

Maternal organs weights

The effects of methomyl administration for 28 days on organs weight (liver, kidney, brain, spleen, heart, ovary, placenta, gravid uterus) are shown in table (1). According to this table, the administration of methomyl at the high dose (1/10 LD_{50}) was found to be associated with a significant decrease in the weight of liver, kidney, brain, heart, ovary and gravid uterus comparable to the control group. The observed recorded decrease in the weight of these organs was dose-dependent.

Table. 1: Effect of oral administration of methomyl at three different doses (1/10 LD_{50}, 1/20 LD_{50} and 1/30 LD_{50}) for 28 days on the body and relative reproductive organs weights of female rats.

Parameters	Methomyl LD_{50} dose				*p*
	Control	**1/30**	**1/20**	**1/10**	
No. of assigned females	**35**	**35**	**35**	**35**	-
Dams with toxicological death	0	0	0	0	-
Body weights (g)					
Initial body weight (g)	197.7±16.6	199.4±18.9	200.2±24.5	196.7±20.3	> 0.05
Final weights (g)	245.3±26.5	241.4±25.6	236.4±22.65	210.9±20.5*	< 0.05*
Total body weight gain/100 g b.w.[a]	24.08±3.23	21.06±5.22	18.08±2.07	7.22±1.31*	<0.05
Net organ weights (g)					
Liver	6.49±0.85	6.23±0.45	5.43±0.52	3.14±0.365*	<0.05*
Kidney	1.34±0.107	1.37±0.052	1.17±0.106	1.01±0.11*	<0.05*
Brain	2.11±0.13	2.05±0.25	1.84±0.16	1.18±0.107*	<0.05*
Spleen	0.17± 0.13	0.17± 0.014	0.17±0.013	0.16±0.012	>0.05
Heart	1.08±0.011	1.00±0.069	0.91±0.085	0.59±0.036*	<0.05*
Ovary	0.343±0.025	0.334±0.025	0.203±0.016	0.165±0.011*	<0.05*
Placenta	0.22±0.021	0.20±0.013	0.21±0.016	0.21±0.011	>0.05
Gravid uterus	73.12±7.36	72.65±6.85	70.15±6.98	55.55±5.69*	<0.05*
Relative organ weights/100 g b.w.[b]					
Liver	2.645±0.25	2.954±0.36	2.296±0.25	1.30±0.16*	<0.05*
Kidney	0.546±0.052	0.649±0.052*	0.494±0.036	0.418±0.041	>0.05
Brain	0.860±0.069	0.972±0.065	0.778±0.066	0.488±0.042*	<0.05*
Spleen	0.069±0.001	0.80±0.016*	0.071±0.003	0.066±0.001	>0.05
Heart	0.440±0.013	0.474±0.041	0.384±0.052	0.244±0.025*	<0.05*
Ovary	0.139±0.013	0.158±0.011	0.085±0.01	0.068±0.016*	<0.05*
Placenta	0.089±0.013	0.082±0.001	0.088±0.003	0.099±0.001	>0.05
Gravid uterus	29.8±1.98	30.0±2.85	29.6±2.36	26.3 ±2.41	>0.05

The data are given as the mean ± standard deviation. (n=8)
a: Final body weight-initial body weight/initial body weight x 100
b: Net organ weight/ final body weight x 100
*: Significantly different from control at p<0.05.
**: Significantly different from control at p<0.001.
P: probability

Table. 2: Implantation data after the oral administration of methomyl at three different doses (1/10 LD_{50}, 1/20 LD_{50} and 1/30 LD_{50}) for 28 days in female rats.

	Methomyl LD_{50} doses				p
	Control	1/30	1/20	1/10	
Dams	25	25	25	25	
No. of copulated dams	25	25	25	25	
No. of pregnant dams (%)	24 (96)	23 (92)	23(92)	22(88)	
Preterm delivery	0	0	0	0	
Implantations/litter	16.1±1.06	15.3±1.036	16.9±1.25	15.9±1.08	>0.05
Litters (fetal number)	24(370)	23(329)	23(359)	22(312)	
Live featured/ litter	15.36±1.68	14.27±1.65	15.65±1.62	13.71±1.36*	<0.05*
Dead fetuses /litter[a]	0	0	0	0	
Early resorptions %[b]	3.4±0.32	4.4±0.42	5.3±0.51*	10.3±1.09**	<0.05*
Late resorptions %[c]	1.2±0.85	2.3±0.26*	2.1±0.31*	3.9±0.25*	<0.05*
Postimplantation loss %d	4.6±0.45	6.7±0.75	7.4±0.79*	14.2±1.02*	<0.05*
Total number of live fetuses	368.64±33.9	328.21±35.6	359.95±34.6	301.62±30.2*	<0.05*
Fetal body weight (g)					
Male	4.65±0.98	4.62±0.46	4.51±0.52	4.29±0.52	>0.05
Female	4.26±0.46	4.25±0.51	4.27±0.51	3.98±0.41	>0.05
Crown rump length (CRL) (mm)					
Male	35.59±0.36	34.43±0.41	33.85±0.36	29.33±0.28*	>0.05
Female	33.13±0.36	32.24±0.41	32.62±0.36	26.92±3.11*	>0.05
Sex ratio					
Male litter	9.5±0.98	7.8±0.78	6.9±0.71	7.3±0.69	>0.05
Female	5.9±0.69	6.5±0.58	8.8±0.78*	6.4±0.61	<0.05*
Total males	228.0±20.1	179.4±16.5*	158.7±14.6*	160.6±16.07*	<0.05*
Total females	141.6±12.65	149.5±13.55	202.4±18.98*	140.8±14.69	<0.05*
Male: Female	228.0:141.6	179.4:149.5*	158.7:202.4*	160.6:140.8*	<0.05*
% Male	52.0	53.0	52.1	53.5	
% Female	48.0	34.1	47.9	46.5	

The data are given as the mean ± standard deviation.
a: Dead fetuses did not breathe spontaneously after the uterus was opened.
b: Early resorption: only decidual or placental tissues visible or implant site only visible after staining.
c: late resorption: embryonic or fetal tissue visible in addition to placental tissue.
d: Post-implantation loss (%)= (no. of implantation sites – no. of live fetuses/no. of implants)x100.
*: Significantly different from control at p<0.05.

Table. 3: Fetal anomalies in female rats after the oral administration of methomyl at three different doses (1/10 LD_{50}, 1/20 LD_{50} and 1/30 LD_{50}) for 28 days.

	Methomyl LD_{50} doses			
	Control	**1/30**	**1/20**	**1/10**
Viable litters	24	23	23	22
No. of examined fetuses	331	313	311	273
Morphological anomalies/ examined [a]	0/83	0/76	0/78	22/91
Microsomia (stunting)	0	0	0	7
Visceral hernia	0	0	0	6
Ophthalmological anomalies	0	0	0	1
Cleft lip	0	0	0	3
Limb anomalies	0	0	0	2
Tail defects (short or tailless)	0	0	0	2
Dermal edema	0	0	0	1
Number of litters (%)	0 (0%)	**0 (0%)**	0 (0%)	20 (91.0)
Number of features (%)	0 (0%)	0 (0%)	0 (0%)	22 (8.1)
Skeletal anomalies/examined [a]	5/133	5/135	6/121	22/113
Short or absent ribs	2	2	3	7
Reduced skull bones	1	1	1	4
Long bones anomalies	2	2	2	8
Reduction of vertebral body	0	0	0	3
Number of litters (%)	4 (17.7%)	4 (17.4%)	6 (26.1%)	19 (86.4%)
Number of features (%)	5 (1.5%)	5 (1.6%)	6 (1.9%)	22 (8.1%)
Visceral anomalies/examined [a]	0/115	0/102	4/112	14/69
Heart edema	0	0	1	3
Ureter distended	0	0	0	4
Visceral enlargement	0	0	2	4
Pulmonary edema	0	0	1	3

The data are given as the mean ± standard deviation.
a: Fetus may be represented more than once in listing of malformations.

Effects of methomyl on biochemical parameters

Acetyl cholinesterase

Effects of methomyl on the activity of acetyl cholinesterase are illustrated in figure (1). According to this figure, a pronounced inhibition in the activity of acetyl cholinesterase was observed after 28 days of methomyl administration for doses of 1/10 LD_{50}, 1/20 LD_{50} compared to the control group.

Lipid profiles

Figure (2) exhibits the plasma concentration of total lipids (TL), triglycerides (TG) and total cholesterol (TC) after 28 days of the oral administration of methomyl at three different dose levels (1/10 LD_{50}, 1/20 LD_{50} and 1/30 LD_{50}). According to these results, significant and dose-dependent increases were observed in the concentration of total lipids, triglycerides and total cholesterol at the mid and high doses (1/20 and 1/10 LD_{50}) compared to control group value. **Figure (2)** also revealed a significant increase in the level of low density lipoproteins cholesterol (LDL-C) and high density lipoproteins in cholesterol (HDL-C) in female rats treated with 1/10 LD_{50} and 1/20 LD_{50} methomyl in the experimental duration of the present study (28 days) compared to the control group. On the other hand, the level of very low density lipoprotein in cholesterol (VLDL-C) was found to be significantly deceased after methomyl administration at the 1/10 LD_{50} dose only.

Levels of total protein, albumin and globulin

The concentration of total protein, albumin and globulin in plasma showed no tendency towards any significant alterations from control value at the different doses of methomyl groups (1/10 LD_{50}, 1/20 LD_{50} and 1/30 LD_{50}) after 28 days experimental durations as shown in **figure (3).**

Levels of steroid hormones

Progesterone

There was a significant decrease in the level of progesterone in response to administrating high 1/10 LD_{50} dose of methomyl for 28 days of experiment. Values were found to be 11.2±3.22 for doss of 1/10 LD_{50} methomyl, compared to 21.9±2.11for the control group. However, an insignificant increase with 1/20 LD_{50} dose was evident (Figure, 4).

Estrogen

After 28 days of methomyl administration, the levels of estrogen continue to show a dose-dependent increase at 1/10 LD_{50}, 1/20 LD_{50} and1/30 LD_{50} doses when compared to the control group (Figure, 4).

Effects of methomyl on lipid peroxidation and antioxidative parameters

methomyl treatment after 28 days of oral administration exhibited a significant increase in the level of MDA (marker of lipid peroxidation) at 1/10 LD_{50}, 1/20 LD_{50} and 1/30 LD_{50} doses as compared to the control group (Figure, 5). The antioxidative status of female rats was affected by the administration of methomyl at the three different dose levels in the present study. Significant decrease in the activity of the antoxidant enzymes namely superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (Gpx) was observed in female rats administered methomyl at dose level of 1/10 LD_{50}, 1/20 LD_{50} and 1/30 LD_{50} after 28 consecutive days (Fig. 5).

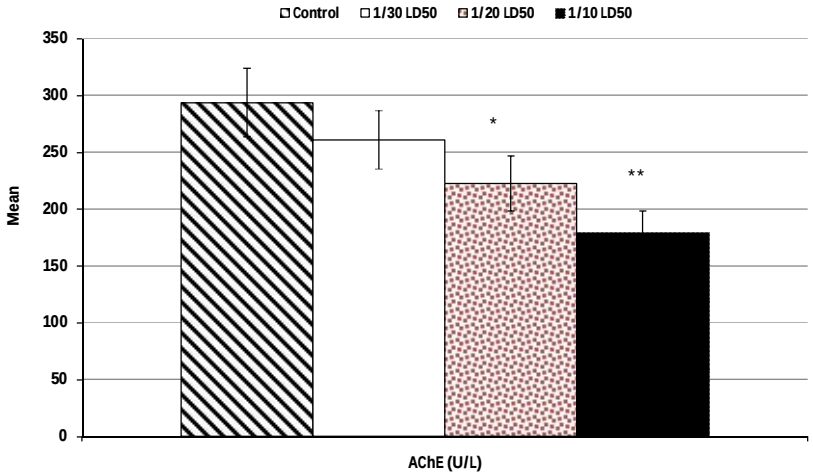

Fig. 1: Effect of oral administration of methomyl at three different doses (1/10 LD_{50}, 1/20 LD_{50} and 1/30 LD_{50}) for 28 days on the activity of acetylcholinesterase activity in the plasma of female rats.

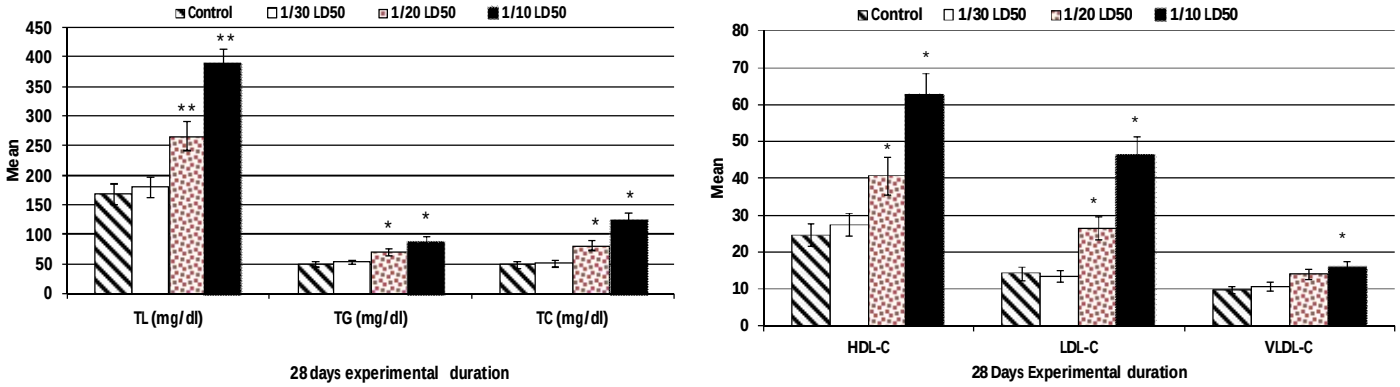

Fig. 2: Effect of oral administration of methomyl at three different doses (1/10 LD_{50}, 1/20 LD_{50} and 1/30 LD_{50}) on the lipid profile in the plasma of female rats after 28 days.

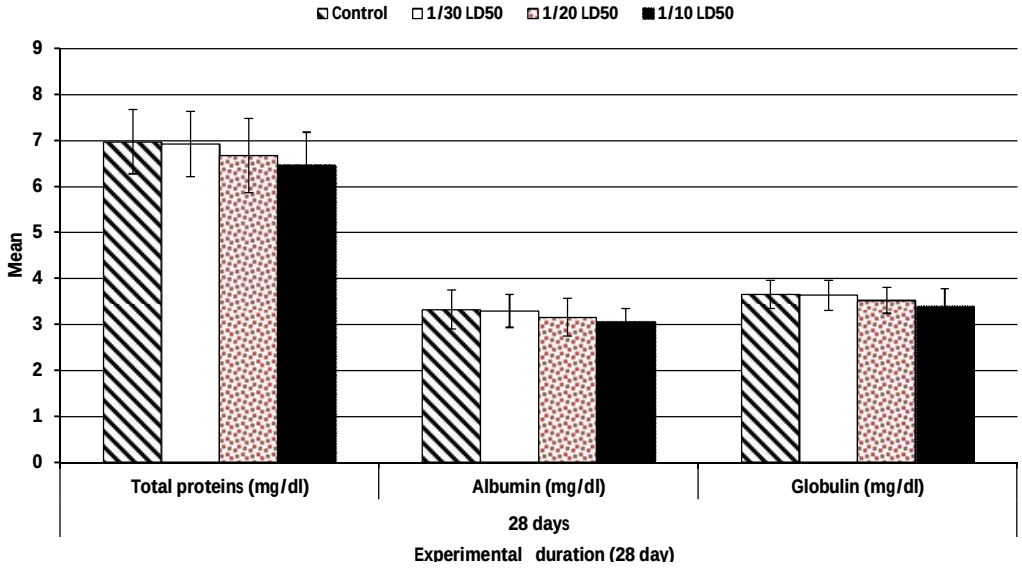

Fig. 3: Effect of oral administration of methomyl at three different doses (1/10 LD_{50}, 1/20 LD_{50} and 1/30 LD_{50}) for 28 days on the concentration of total proteins, albumin and globulin in plasma female rats.

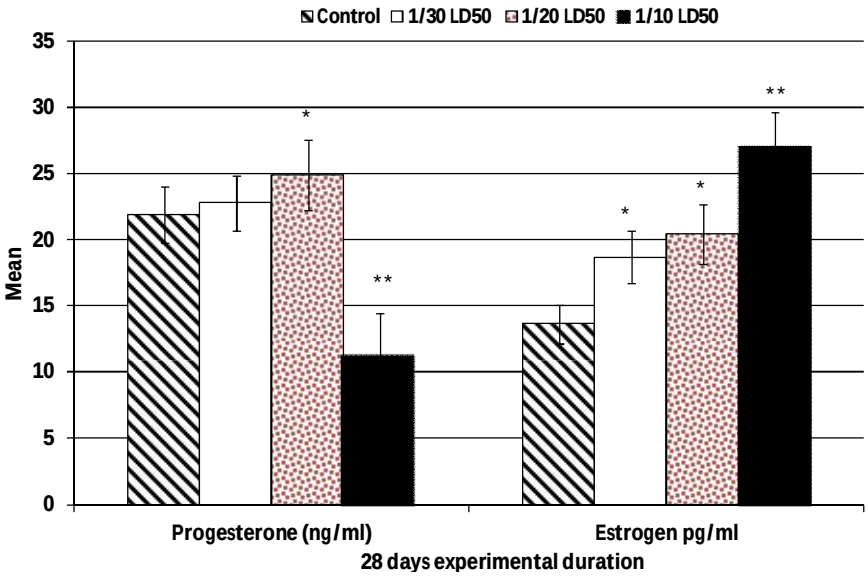

Fig. 4: Effect of oral administration of methomyl at three different doses (1/10 LD_{50}, 1/20 LD_{50} and 1/30 LD_{50}) for 28 days on the levels of progesterone and estrogen in plasma of female rats.

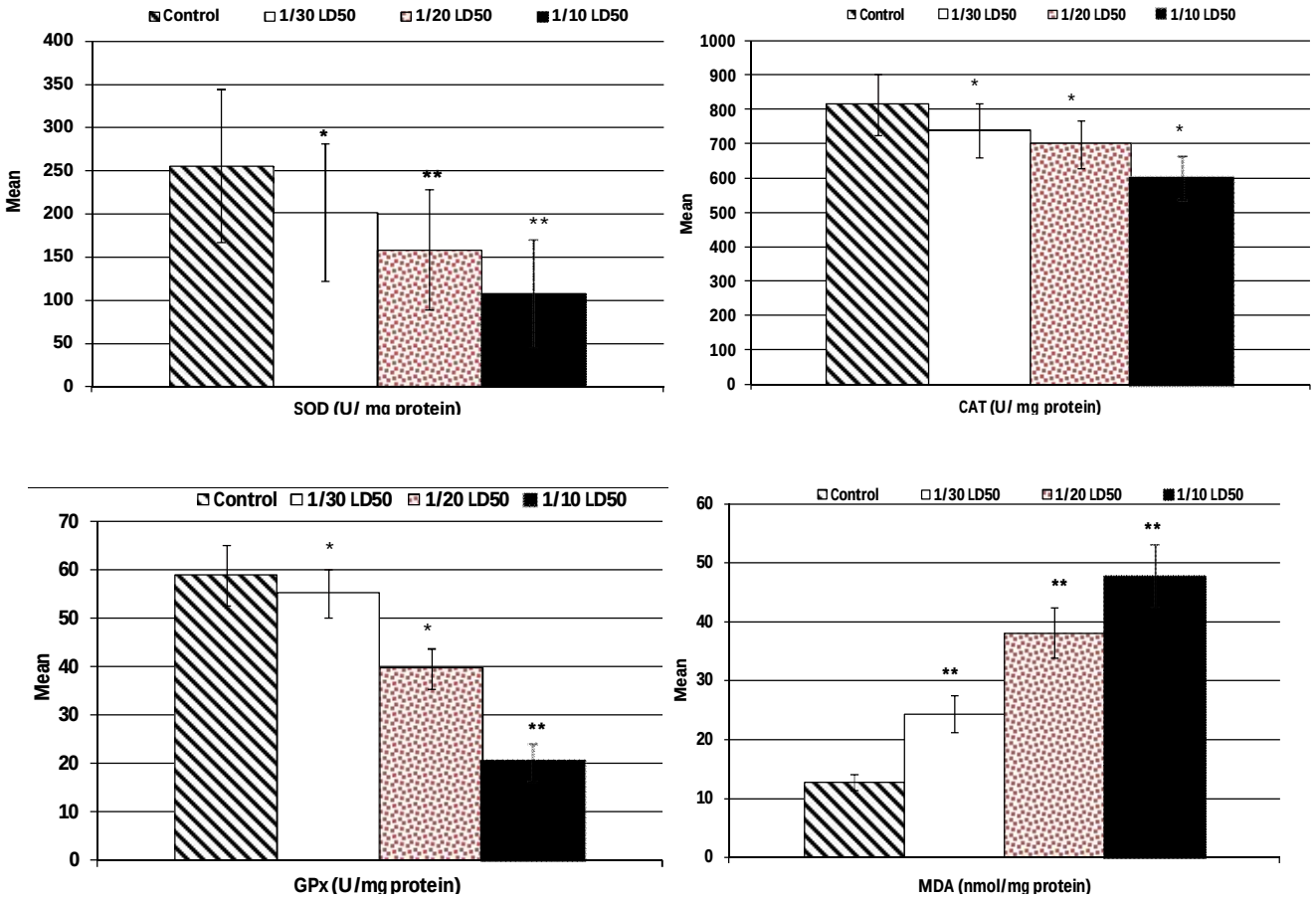

Fig. 5: Effect of oral administration of methomyl at three different doses 1/10 LD_{50}, 1/20 LD_{50} and 1/30 LD_{50} for 28 days on the activity of the antioxidative enzymes and level of MDA in ovarian homogenate of female rats.

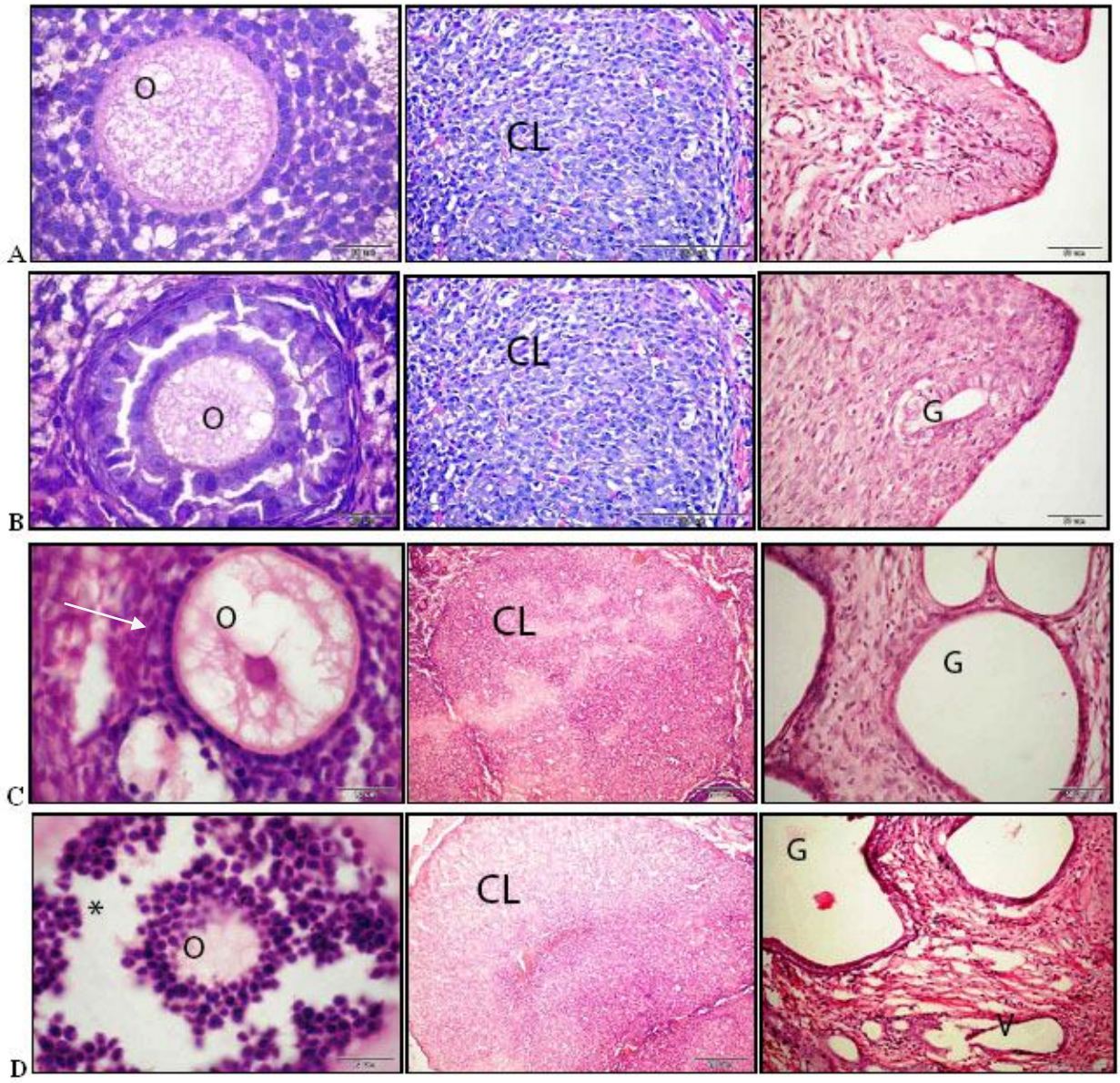

Fig **6:** Reprsesentive photopmicrographs show histopathology of ovarian sections of mature rats, corpus luteum and uterus of control and methomyl treated female rats for 28 day. Controls (panel A), methomyl 1/30 LD_{50}, (panel B), methomyl 1/20 LD_{50}, (panel C), 1/10 LD_{50} (panel D). Notice: CL= corpus, endometrial glands (G), oocyte (O), arrow (zona granulosa), asterisk (antrum).

Pregnancy and Implantation endpoints

Table (2) summarizes the uterine implantation data. Uterotoxicity was mainly indicated in pregnancy endpoint of dams treated with 1/10 LD_{50} methomyl. There were no significant differences between the controls and the 1/20 LD_{50} or 1/30 LD_{50} dose groups for any of the developmental parameters assessed. Overall 0% of implants were dead. The results indicate that the coupled rats became pregnant showing no significant alterations in the implantation sites in 1/20 LD_{50} or 1/30 LD_{50} methomyl groups. However, there were a number of statistically significant findings in the high (1/10 LD_{50}) dose group (Table, 2), fetal viability was reduced in the high (1/10 LD_{50}) dose group as indicated by a significant reduction in the number of viable fetuses/dam. Also, in the 1/10 LD_{50} group the mean gravid uterine weight was lowered than controls (Table, 1). In this group, the number of postimplantation loss was increased for the mid and high groups (1/20 LD_{50} and 1/30 LD_{50}). Similarly, there was evidence of fetal toxicity in the high dose group as shown by a significant reduction in mean fetal weights for males and females compared to control mean (Table, 2). Reduction in fetal weight of mid (1/20 LD_{50})

group did not significantly affect. Fetal sex ratio was randomly affected (Table, 2). In 1/30 LD_{50} dose, there were no test methomyl-related effects on the dams, embryos or fetuses. The observed-adverse-effect level (OAEL) in this study was 1/10 LD_{50} for embryos and fetuses and also for dams.

Developmental toxicity outcomes

No developmental toxicities observed in maternally toxic females of 1/20 LD_{50} or 1/30 LD_{50} groups. Fetotoxic and teratogenic effects were manifested by the presence of increased resorptions, skeletal malformations and loss of fetal weight by high 1/10 LD_{50} dose treatment. There was a significant increase in morphological, visceral and skeletal malformations in the 1/10 LD_{50} group (Table, 3). Morphological malformations were seen only in the 1/10 LD_{50} group in twenty two fetuses from twenty different litters (22/20). The principal external observations in the high dose group included stunting (less than 4 g) and visceral hernia (7 and 6 fetuses in 20 litters), respectively. Visceral malformations were also detected in the 1/20 LD_{50} and 1/10 LD_{50} groups. The visceral observations have included distended ureter (4 fetuses in 12 litters), abdominal defects (visceral enlargement and abdominal hemorrhage) (4 fetuses in 12 litters) and heart edema (3 fetuses in 12 litters). The incidence of visceral anomalies was statistically significant in 1/20 LD_{50} or 1/10 LD_{50} groups compared to control. The incidence of skeletal malformations, singly or combined, was observed scattered throughout the experimental groups. high dose-treatment possessed the numerous skeletal variations and malformations characterized by. reduced ossification of skull bones, short or absent ribs, reduction of vertebral body and long bones (Table, 3).

Histological findings

Histopathology of ovaries, corpus lutea and uteri are shown in the photomicrographic panels in Figure 6. The ovaries of the control group showed normal histological features, illustrating a well defined zonal granulosa surrounding the oocyte and compact theca folliculi and the presence of some primordial follicles (Figure, 6A). Normal growing and antral ovarian follicles in the 1/30 LD_{50} group were observed. No significant changes were found in the overian histology (Figure, 6B). They contained numerous well developed corpora lutea(CL, panels A and B Figure, 6). In the 1/20 LD_{50} group, Histopathological examination of the ovary was almost normal, but there are pyknotic granulosa cells of antral follicle (Figure, 6C). Ovaries from 1/20 LD_{50} and 1/10 LD_{50} treated groups distinguished by vacuoles and hemorrhage present in the luteinized cells of corpora lutea (CL, panels C and D Figure, 6). Ovarian lesions were observed in group of 1/10 LD_{50} after 28^{th} day of experimental period. The histopathologic examination revealed widespread ovarian follicle atresia, degenerating antral follicle with desquamation of pyknotic granulosa cells and degenerated oocyte (O, panel D, Figure 6). In addition, the ovaries of this treated group showed some hypocellularity of the Theca folliculi, complete distortion/destruction of the basement membrane separating the theca folliculi from the zona granulosa and atrophic changes were observed in the oocyte and zona granulosa (arrow, panel D, Figure 6). Degeneration of uterus with occasional vaculations (V, panels C and D, Figure 6), increased and dilation of endometrial glands was also evident in 1/10 LD_{50} mathomyl treated females (G, panels C and D, Figure 6). Administration of mathomyl at a dose level of 1/20 LD_{50} revealed similar but less pronounced endometrial abnormalities in uterine histology in comparison with that of 1/30 LD_{50} dose group (G, panels B and C, Figure 6).

DISCUSSION

The present study was planned to evaluate the adverse effects of methomyl on fertility, developmental toxicity and various biochemical parameters of female rats. In the present findings, chronic exposure to methomyl resulted in decrease in body weight gain of the treated female rats which was significant at 1/10 LD_{50} of methomyl as compared with control. These findings could be attributed to the decreased in the food intake by disturbance in hormonal balance and/or direct cytotoxic effect of mehtomyl insecticide (Al-Shinnawy (2008). Loss of appetite and/or metabolic disturbance due to methomyl may also explain the observed decrease in body weight gain (Abd El-Ghaney, 2002). 1/10 LD_{50} dose of methomyl caused a significant decrease relative organs weights (ovary, uterus and Placenta) whereas 1/30 and 1/20 LD_{50} doses did not significantly differ from control. Several possible mechanisms for anti-gonadal actions of carbamates have been postulated. Ferguson *et al*. (1984) have suggested that the treatment with carbamate pesticide carbofuran inhibits acetylcholinesterase, resulting in alterations in the pituitary gonadotropins and could influence on gonadal function directly through the effect on the pituitary acetylcholinesterase in rats. This may be due to imbalance in gonadal steroids which are essential for normal functioning of the gonads (Carter *et al*., 1984). The data in the present study indicates that administration of methomyl at different doses for 28 days provoked statistically significant dose-dependent increase in the concentration of total lipids, triglycerides, total cholesterol, low density lipoproteins and very low density lipoproteins. The level of high density lipoproteins was significantly decreased in experimental durations. These changes in lipid profile may be attributed to the increased fat catabolism in response to methomyl administration (Ibrahim and El-Gamal, 2003). Methomyl may lead to increased concentrations of catecholamines which in turn stimulate lipolysis and fatty acid formation in the blood which in turn have number of effects on the metabolism of lipoproteins (Mansour *et al*., 2009).

Non significant decrease in the level of total protein content, albumin and globulin were observed in the present study following exposure to different doses of methomyl. Decrease in total protein and albumin levels may be indicative for the development of a disorder in protein synthesis and metabolism (Eraslan *et al*., 2009). Prolonged exposure to the carbamate carbosulfan was found to cause a significant decrease in total protein level in mice which might be due to catabolism of protein and/ or malfunction of liver (Ksheerasagar and Kaliwal, 2006).

In the present study, methomyl administration to rats resulted in a marked dose-dependent increase in the lipid peroxidation (LPO) as indicated by the increase in the level of malondialdehyde (MDA) in ovaries of methomyl-treated rats. These results are consistent with the previous studies as reported for other carbamates, e.g. propoxur in erythrocytes (Seth *et al*., 2001), propoxur in serum (Suke *et al*., 2006), aldicarb in plasma (Yarsan *et al*., 1999), carbofuran in brain (Rai and Sharma, 2007), carbofuran in liver (Kaur and Sandhir, 2006), benomyl in liver (Banks and Soliman, 1997) and carbendazim in leydig cells of rats (Rajeswary *et al*., 2007). The methomyl-induced increase in MDA level might be due to the conjugation of methomyl or its degradation products to the polyunsaturated fatty acids (Gutteridge and Halliwell, 2000). Methomyl have shown to cause overproduction of reactive oxygen species (ROS) (Garg *et al*., 2009). The anticholinesterase activity of methomyl may be involved in the production of free radicals in target tissues (El-Khawaga, 2005). Whereas its first toxicological effects are attributed to acetylcholinesterase inhibition, oxidative stress is also attributed to methomyl toxicity (El-Shenawy *et al*., 2010).

Normal cellular functioning depends on a balance between ROS produced and antioxidant defense mechanisms present in the cell. Antioxidant enzymes such as SOD and CAT are considered to be a primary defense that prevents biological macromolecules from oxidative damage. According to the present data, the activities of CAT, SOD and GPx in ovaries of methomyl-treated rats were significantly decreased. These results strongly suggested that methomyl has the capability to induce free radicals and oxidative damage as evidenced by perturbations in various antioxidant enzymes (Salama *et al.,* 2005). Depletion of antioxidant enzyme activity could be due to the direct effect on the enzymes by methomyl-induced ROS generation, direct inhibition of the enzymes by methomyl, depletion of the enzymes substrates and down-regulation of transcription and translation processes (Garg *et al.* (2008).

Pesticides are endocrine disruptors where they mimic, enhance (agonists), or inhibit (antagonists) the action of endogenous hormones (Crisp *et al*., 1998). Results of the present investigation indicated an elevation in estrogen level at the three different dose levels whereas 1/10 LD50 significantly decrease the level of progesterone. Hormonal balance is important to preserve female reproduction and maintain fertility and can be disturbed by estrogen/progesterone imbalance. Disruption can occur in all stages of hormonal regulation, hormone synthesis, hormone release and storage, hormone transport and clearance, hormone receptor recognition and binding (Bretveld *et al*., 2006).

The developmental process is particularly vulnerable to adverse environmental conditions including chemical pollutants such as insecticides which have been ubiquitous because of its widespread manufacture, and disposal all over the world. They have direct effects resulting in impaired fertility, high rates of abortions, and abnormal pregnancies (Sanin *et al.,* 2009). In fact, every developmental stage is vulnerable to any environmental insult which encompasses a spectrum of possible effects which includes malformation of fertilized egg or zygote, of the embryo during organogenesis, the fetus in the post embryonic period of gestation and the postnatal until sexual maturity of offspring (Schettler *et al.,* 2003). Female reproductive function can be compromised by exposure to toxic chemicals at a variety of sites, including the hypothalamus, pituitary gland, ovary and reproductive tract. Disruption of any of these sites can ultimately manifest as a disruption of ovarian function, resulting in infertility (Schreinemachers, 2003).

CONCLUSION

We can conclude that chronic exposure to the methomyl has a direct toxic effect on female reproductive system as manifested by implantation sites, fertility index, fetal weights reproductive hormonal disruption. This reproductive toxicity may be linked to methomyl-induced oxidative stress which becomes an important issue in human and animal reproduction. Therefore, the application of the methomyl should be limited to a designed program with special care in handling to limit or minimize its hazards.

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