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BIOCHEMICAL AND PHYSIOLOGICAL CHANGES IN SOME ORGANS OF ALBINO RATS TREATED WITH THE BIOPESTICIDE "ABAMECTIN"

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ABSTRACT

The aim of this study was to examine the effects of the biopesticide abamectin on the thyroid function , body weight , testosterone hormone and male fertility in albino rats through acute and subchronic oral toxicity .

The investigation results revealed that the abamectin biopesticide significantly increased the thyroxine (T4) after 60 days at 1.5 and 6 ppm of the tested compound . While tri-iodothyronine (T3) level was significantly decreased at abamectin concentrations 1.5 and 3 ppm after 90 days .

Feed consumption and body weight gain decreased significantly in treated rats after 15 , 60 and 90 days at abamectin concentrations 1.5 and 3 ppm . Plasma testosterone level was increased significantly after 60 and 90 days at abamectin concentration 1.5 and 6 ppm. A remarkable decrease in both sperm motility and sperm concentration were observed . At the end of recovery period , the values of the above parameters returned to the normal levels as in the untreated animals.
Keyword: thyroxine, tri-iodothyronine, feed consumption, testosterone.

INTRODUCTION

Abamectin is a natural fermentation product of the soil bacteria *Streptomyces avermitilis*. It acts as an insecticide by affecting the nervous system and paralyzing insects (Ray, 1991). Abamectin is used to control insect and mite pests of citrus, pear and nut tree crops, and used by homeowners in the control of fire ants.

There is a lake of information; about the biological effects of abamectin in animals. The symptoms of acute toxicity of abamectin observed in laboratory animals include pupil dilation, vomiting convulsions and /or tremors, and coma (Lankas and Gordon, 1989 and EPA, 1990).

Walmsley and white (1994) found that the primary hormones produced by the thyroid gland are 3, 5, 3'- tri-iodo thyronine, (T3) and thyroxine 3, 5, 3', 5'- tetra-iodo thyronine , (T4). Also, it secretes calcitonin, a hormone that participates in the regulation of plasma Ca++ concentration by inhibiting bone resorption , the nuclear receptors of thyroid hormones which have higher affinity to T3 than T4 (Hood, 1980) .

Treatment of female rats with nitrofen (herbicide) during gestation period led to a decrease in the binding of T3 to (alpha-1) and (beta-1) forms of the thyroid hormone receptor in non-competitive way (Brandsma *et al.*, 1994).

Sub chronic toxicity (90 days) of Zineb in diet at 0, 0.3, and 0.6% levels into rabbits caused depression in serum T3 concentration (Nebbia *et al.*, 1995 and Hotz *et al.*, 1997). Investigating the effects of long – term (90

days) feeding of 3000 ppm thiazopyr to rats on thyroid gland metabolism indicate that the compound increased deiodination and biliary excretion of thyroid hormone (T4) which led to an increase in the rate of (T4) elimination from blood.

Plasma testosterone level was significantly reduced in male rats administered with buprofezin at three tested rates particularly after 65 and 90 days. Testosterone had a normal levels after stopping the treatment . 30 : days (Farid 1997). Application of dimethyl methyl phosphate (DMMP), an organophosphorus compound , impaired fertility in male rodents. Male rats treated with DMMP showed a decrease in sperm motility and count (Chapin *et al.*, 1984).

On the other hand avermectin had no effect on speramatagensis or reproductive performance (Dauiro *et al.*, 1987) . Exposure of male rats to the fungicide carbendazim depressed the fertility after one week of exposure. The fungicide caused failure to produce a pregnant female (Carter *et al.*, 1987).

Higher percentage of abnormal sperm heads was observed in male Swiss albino mice, with 0.5 ml diluted abamectin (Salama *et al.*, 1995).

The effect of avermectin on the quality of semen in boars was also studied by Wrona and Krzyranowski (1995) Three boars were injected with avermectin at 1 ml /33 kg body weigh. Their semen motility, spermatozoa morphology and the percentage of dead spermatozoa were not affected by avermectin.

Also Srivastava *et al.*, (1995) stated that carbaryl oral administration to male albino rats at dose levels of 50 mg or 100 mg / kg body weigh for 90 days led to nonsignificant change in the weight of testes, regarding to the sperm analysis. Carbaryl caused loss in sperm motility and an increase of abnormal sperm morphology.

The aim of the current work to investigate the effect of different doses of biopesticide, abamectin,on certain biological functions and biochemical contents in rats.

MATERIALS AND METHODS

Materials:

Male albino rats 2 – 2.5 months old each weighing (130g ± 10) were purchased from General Organization of Serum and Vaccine (Helwan Farm). The animals were allowed to acclimatize to laboratory condition for 2- weeks prior to the experiment . Animals were fed on balanced diet throughout the experimental period . Feed and water were available at all times during the treatment.

Pesticide :

Abamectin (Vertimec) is a mixture of avermectin B1a, $C_{48}H_{72}O_{14}$ (80%), and avermectin B1b, $C_{47}H_{70}O_{14}$ (20%). It was obtained from Pesticide Central Laborotory (Giza).

Methods:

Acute oral toxicity (LD50):

The medium lethal dose (LD50) was determined according to the method of Weil (1952) as follows: sixteen male albino rats were divided into four groups, each of them were intubated orally by four different doses. The dosage levels were spaced in a geometric progression, the chosen geometric factor (R) was (1.2).

Subchronic toxicity (90 days):

The rats were divided into four groups each contains 10 animals. Three groups were supplied with drinking water contains abamectin at concentrations of 1.5 , 3 and 6 ppm during the experimental period (90 days); then the animals were provided with clean water free from the pesticide for 30 days (the recovery period). The fourth groups was kept untreated and serve as control.

Blood samples were collected from orbital sinus veins by heparinized capillary tubes at 0 (pre- treatment), 15 , 30 , 60 , 90 and 120 days into clean dry and labeled eppendorf tubes (1.5ml). The tubes contained heparin as anticoagulant (7.5 I.U/ml blood) according to Schalm (1986). Blood samples were centrifuged to collect plasma which was deep frozen at (-20°C) for chemical analysis.

Biochemical determination :

Thyroxine (T4) and tri-iodothyronine (T3) determination was performed in plasma according to Britton *et al.* (1975)

Testosterone determination was performed in plasma according to Jaffe and Behrman method (1974)

The progressive motility of sperms was examind according to the method described by Bearden and Fuquay (1980)

Statistical analysis:

Student t-test was performed to test the significance level at $p<0.05$ (*),$p<0.01$ (**) and $p<0.001$ (***) according to Snedecor and Cochran (1967).

RESULTS AND DISCUSSION

Data in Table (1) show that the biopesticide,abamectin, at the low and high concentrations (1.5 and 6 ppm) caused significant increase in the thyroxine level T4 (hyperthyroidism) after 60 days. These results are in agreement with those obtained by Porter *et al.*, (1993), who mentioned that thyroxine (T4) was increased due to carbamate insecticides (oldicarb, methomyl) and triazine herbicide (metribuzin) in rats.The elevation in thyroxine reported in the present study may be due to the decrease utilization in different cells or due to decrease conversion of T4 into T3 by monodeiodination (Kaneko, 1989).

Moreover, Van Leeuwen *et al.*, (1995) suggested that hyperthyroidism could be attributed to changes in the enzymatic reactions that play a role in the biosynthesis of the thyroid hormones and / or in the breakdown of thyroid-binding-protein (TBP) into the thyroid hormones (T3,T4) and their secretion into the circulation blood.

Table (1): Effect of treating with different concentrations of the biopesticide; Abamectin on thyroxine (T4) concentration (ng / 100 ml) in plasma of male albino rats.

Abamectin (concentration)	Thyroxine (T4) concentration (ng / 100 ml)				
	Treatment period (days)				
	Pre-treatment (0)	60	%	90	%
Control(0 ppm)	7.32 ± 0.41	4.67 ± 0.21		4.84 ± 0.22	
1.5 ppm	7.32 ± 0.41	6.59** ± 0.42	(141.11**)	5.13 ± 0.41	(105..99)
3 ppm	7.32 ± 0.41	6.31 ± 0.72	(135.12)	5.22 ± 0.18	(107.85)
6 ppm	7.32 ± 0.41	6.04** ± 0.25	(129.34**)	5.30 ± 0.23	(109.50)

Each value represents mean + S.E.
*** Significant differences versus control at p < 0.05**
**** Significant differences versus control at p < 0.01**
(% from the control)

Data in Table (2) revealed that abamectin at concentrations of 1.5 and 3ppm caused significant decrease of tri-iodothyronine (T3) level (hypothyroidism) after 90 days which are in accordance with the results reported by Nebbia *et al.* (1995), who observed a marked decrease in tri-iodothyronine (T3) level after treatment with zineb pesticde in rats. In addition Farid (1997) reported that buprofezin pesticide induced a marked decrease in (T3) concentration. Van leeuwen *et al.,* (1995) also found that the hypothyroidism may be caused by impaired liver for the conjugation of thyroid hormones with glucouroinc acid, so that the resulting conjugate can be excreted and/or due to long-acting thyroid stimulators(LATS).

Table (2): Effect of treating with different concentrations of the biopesticide; Abamectin on tri-iodothyronine (T3) concentration (ng / 100 ml) in plasma of male albino rats.

Abamectin (concentration)	Tri-iodothyronine (T3) concentration (ng / 100 ml)				
	Treatment period (days)				
	Pre-treatment (0)	60	%	90	%
Control (0 ppm)	128.40 ± 4.86	107.40 ± 3.93		101.20 ± 6.90	
1.5 ppm	128.40 ± 4.86	110.60 ± 4.77	(102.98)	75.00* ± 6.41	(74.11*)
3 ppm	128.40 ± 4.86	109.00 ± 3.20	(101.49)	69.60** ± 2.71	(68.77**)
6 ppm	128.40 ± 4.86	96.60 ± 6.44	(89.94)	81.00 ± 5.70	(80.04)

Each value represents mean + S.E.
*** Significant differences versus control at p < 0.05**
**** Significant differences versus control at p < 0.01**
(% from the control)

Table (3) indicated that the biopesticide abamectin administered to male albino rats at low concentration (1.5 ppm) caused significant decreases in feed consumption after 15 and 60 days periods. At the end of the recovery period , 30 days the values of feed consumption returned to the normal value.Data in Table (4) showed that a decrease in body weight gain was significant after 60 days of treatment for the low and medium concentrations (1.5 and 3 ppm respectivly).

Table (3): Effect of treating with different concentrations of the biopesticide; Abamectin on feed consumption (gm) to male albino rats.

Abamectin (concentration)	Feed consumption (gm) Treatment period (days)								Recovery period (days)	
	15	%	30	%	60	%	90	%	30	%
Control(0 ppm)	12.58 ± 0.27		12.87 ± 0.77		13.93 ± 0.46		15.63 ± 0.38		17.33 ± 0.23	
1.5 ppm	10.87***±0.28	(86.41***)	12.0±0.80	(93.94)	12.26* ± 0.53	(88.01*)	15.23±0.46	(97.44)	17.04 ± 0.21	(98.33)
3 ppm	12.06 ± 0.41	(95.87)	13.36±1.22	(103.81)	13.89 ± 0.43	(99.71)	15.79±0.37	(101.02)	17.46 ± 0.24	(100.75)
6 ppm	12.65 ± 0.36	(100.56)	13.02±0.45	(101.17)	12.78 ± 0.35	(91.74)	15.32±0.46	(98.02)	17.10 ± 0.26	(98.67)

Table (4): Effect of treating with different concentrations of the biopesticide; Abamectin on body weight gain (gm) to male albino rats.

Abamectin (concentration)	Body weight gain (gm) Treatment period (days)								Recovery period (days)	
	15	%	30	%	60	%	90	%	30	%
Control (0 ppm)	21.50 ± 1.80		28.20 ± 1.66		72.60 ± 1.78		55.80 + 4.60		44.833 + 0.95	
1.5 ppm	20.10±0.43	(93.49)	27.90±0.38	(98.94)	39.30**±2.43	(54.13**)	53.50 + 0.83	(95.88)	42.333 + 1.31	(94.42)
3 ppm	19.40±1.52	(90.23)	26.70±0.70	(94.86)	54.60***± 3.15	(75.21***)	64.50 + 5.63	(115.59)	47.333+0.92	(105.58)
6 ppm	17.30 + 2.55	(80.46)	27.00+2.76	(95.74)	68.40 + 5.50	(94.21)	53.80 + 4.74	(96.420	43.00 + 1.48	(95.91)

Each value represents mean + S.E.
*** Significant differences versus control at p < 0.05**
**** Significant differences versus control at p < 0.01**
***** Significant differences versus control at p < 0.001**
(% from the control)

Decreasing body weight gain may be attributed to the interference of abamactin with metabolic process or could be due to pathological lesions in the gastrointestinal tract, (Bruss, 1989).It could be noted that after recovery period, 30 days after treatment, the body weight gain was returned to the normal range.

Data presented in Table (5) showed that the level of testosterone was significantly increased at abamactin concentrations 1.5, 3 and 6 ppm after 60 and 90 days. The medium concentration treatment (3 ppm) seems to have more effect on this parameter than the low and the high concentrations (1.5 and 6 ppm). In this connection Krause (1977) found that a marked elevation in testosterone level was due to disturbance in the hormonal control to produce testosterone by administration with abamectin.

Table (5): Effect of treating with different concentrations of the biopesticide; Abamectin on testosterone level (ng / 100 ml) in plasma of male albino rats.

	Testosterone level (ng / 100 ml)				
Abamectin (concentration)	Treatment period (days)				
	Pre-treatment (0)	60	%	90	%
Control (0 ppm)	22.92 ± 3.88	72.60 ± 4.66		29.87 ± 6.39	
1.5 ppm	22.92 ± 3.88	214.40** ± 37.42	295.32**	145.60*** ± 20.1	487.45***
3 ppm	22.92 ± 3.88	152.60*** ± 6.38	210.19***	102.40* ± 20.95	342.82*
6 ppm	22.92 ± 3.88	134.40** ± 13.36	185.12**	90.40* ± 24.75	302.65*

Each value represents mean + S.E.
*** Significant differences versus control at p < 0.05**
**** Significant differences versus control at p < 0.01**
***** Significant differences versus control at p < 0.001**
(% from the control)

Results presented in Table (6) indicated that administration of abamectin to male rats for 90 days led to significant decrease in sperm motility and sperm concentrations. At the end of recovery period, the values of sperm motility and sperm concentrations were returned to their normal values. The present results are in consistent with those obtained by Farid (1997), who mentioned that buprofezin pesticide caused significant decrease in sperm motility and concentration in treated animals. Dixon and Lee (1973) stated that a reduction in sperm concentration may be attributed to reduction in meiotic index of the testicular cells as a result of the passage of the insecticides across the blood testis barrier (BTB) and gain access to the germ cells in seminiferous tubules. Shanker *et al.*, (1995) suggested that the reduction in sperm count in the cauda epididymis might be attributed to the lower spermatid count in the testis.

Table (6): Effect of treating with different concentrations of the biopesticide; Abamectin on sperm motility (%)and sperm concentration (10^6 / ml) in male albino rats.

Abamectin (concentration)	Sperm motility %				Sperm concentration (10^6 / ml)			
	Treatment period (days)		Recovery period (days)		Treatment period (days)		Recovery period (days)	
	90	%	30	%	90	%	30	%
Control (0 ppm)	59.43 ± 2.89		60.06 ± 1.36		40.00 ± 1.47		36.00 ± 3.08	
1.5 ppm	48.67* ± 3.25	(81.90*)	59.26 ± 1.59	(98.66)	27.00* ± 3.24	(67.50*)	36.75 ± 0.63	(102.08)
3 ppm	49.39* ± 2.82	(83.10*)	60.06 ± 1.36	(100.00)	21.25** ± 4.1	(53.12**)	31.25 ± 2.46	(86.81)
6 ppm	54.06 ± 5.19	(90.97)	55.51 ± 3.84	(92.49)	26.25*** ± 1.65	(65.62***)	36.25 ± 0.48	(100.69)

Each value represents mean ± S.E.
*** Significant differences versus control at $p < 0.05$**
**** Significant differences versus control at $p < 0.01$**
***** Significant differences versus control at $p < 0.001$**
(% from the control)

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تغيرات كيميائية وفسيولوجية في بعض أعضاء الفئران البيضاء المعاملة بالمبيد الحيوي أبامكتين

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الهدف من الدراسة هو دراسة تأثير المبيد الحيوي أبامكتين علي كل من وظائف الغدة الدرقية ووزن الجسم وهرمون التستوستيرون وخصوبة ذكور الفئران من خلال جرعات سمية حادة وأخري تحت مزمنة.

وقد أظهرت النتائج المتحصل عليها أن المبيد تحت الدراسة يؤدي إلي زيادة معنوية في هرمون الثيروكسين بعد ٦٠ يوم بجرعات ١,٥ ، ٦ ، جزء في المليون، بينما كان هناك نقص في هرمون تراي أيودوثيرونين معنويا بعد ٩٠ يوم بجرعات ١,٥ ، ٣ جزء في المليون .

أدي استخدام المبيد إلي حدوث نقص في كل من معدل استهلاك الغذاء ومعدل زيادة وزن الفئران المعاملة بعد ١٥ ، ٦٠ ، ٩٠ يوم وبجرعات ١,٥ ، ٣ جزء في المليون .

ومن الدراسة وجد أن مستوي التستوستيرون زاد زيادة واضحة بعد ٦٠ و ٩٠ يوم وبجرعات ١,٥ و ٦ جزء في المليون من المبيد الحيوى ابامكتين.

أدت معاملة ذكور الفئران لمدة ٩٠ يوم بمركب الأبامكتين إلي نقص واضح في كل من تركيز وحيوية الحيوانات المنوية وفي نهاية مدة الاستشفاء وجد أن كل القياسات السابقة قد عادت إلي مستواها الطبيعي كما في المجموعة الضابطة.