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COMPARATIVE STUDY ON THE EFFECT OF ORAL ADMINISTRATION OF SOME INSECTICIDES ON SERUM GLUCOSE, SOME LIPIDS, AND NON-PROTEIN NITROGEN CONSTITUENTS OF RABBIT.

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ABSTRACT

The present study was conducted to investigate and compare the intoxication effects of daily oral administration of 1/10 LD_{50}/kg body wt. of Tamaron, Parathion and Confidor for 10 days on serum non-protein nitrogen constituents e.g. urea, uric acid and creatinine, glucose, cholesterol and triglycerides of rabbit. The daily oral administration of any of the three insecticides for 10 days caused a general increase of urea concentration in rabbits blood serum compared to the control level. In general, significant increase of urea content was observed from the third day of inoculation. Insecticides administration also raised up the concentration of uric acid and the highest serum content of uric acid was noticed in the tenth day of insecticides treatment. However, creatinine content did not show any significant changes in response to the treatments by Tamaron, Parathion or Confidor during all the time intervals studied.

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Keywords : Tamaron, Parathion, Confidor, serum non-protein nitrogen constituents - urea –uric acid - creatinine - glucose - cholesterol - triglycerides in blood serum of rabbits

Glucose content of rabbits blood serum showed different fluctuations in response to insecticides administration. On the other hand, Parathion and Confidor, in general, showed significant increase in serum cholesterol content at the different time intervals studied whereas Tamaron produced significant increase in serum cholesterol level at the fifth day of inoculation.

Triglycerides content of rabbits blood serum responded differently to the treatments by the tested insecticides. It is possible to conclude that insecticides administration for 10 days, even at low doses i.e. 1/10 LD_{50}/kg body wt., had harmful effects on kidney, liver, carbohydrate and lipid metabolisms.

INTRODUCTION

Insecticides are synthetic chemicals of potential toxicity not only to insect pests but also to all animals including farmers working with these insecticides. However, the use of insecticides is unavoidable, in fact it is necessary in certain conditions to increase agricultural production. Insecticides enter the body through different routs including, inhalation, absorption via the skin, or ingestion of food or contaminated water (Lippman, 1987; Health & Safety Commission, 1988; British Medical Association, 1992 and Health & Safety Executive, 1993). In Gaza Strip, the most over populated area in the world and is one of the areas suffering from many environmental contaminants, farmers used insecticides excessively and in an irresponsible way (Abd Rabou & Al-Agha, 1998). More than 250 metric tons of formulated pesticides (mainly insecticides) are used annualy in the Gaza Strip (Safi, 1995). This could result in a leakage of insecticides into underground water (U.S. EPA, 1990) and therefore put the whole exposed population at a risk, particularly in the Gaza Strip which depends totally on ground water for human

consumption and the water quality is already poor (Policy Research Incorporated, 1992 and UNRWA, 1993).

The present study deals with three of the common used insecticides in the Gaza Strip which are Tamaron, Parathion and Confidor (Safi *et al.* 1993 and Abd Rabou & Al-Agha, 1998), although Parathion is internationally banned. Tamaron and Parathione are synthetic organophosphorus insecticides whereas Confidor is a synthetic chloronicotinyle insecticide.

Most organophosphorus insecticides including Tamaron and Parathion exert their toxicity to target and non-target species through inhibition of acetylcholinesterase in nerve and muscle tissues (El-Sebae *et al.* 1981; Sheets *et al.* 1997 and Mileson *et al.* 1998). The resulting persistence of acetylcholine in cholinergic synapses leads to repetitive stimulation of muscarinic and nicotinic receptors in target tissues, producing clinical signs of cholinergic poisoning that are manifested in tremors and even death (Meinzingen, 1993 and Ecobichon, 1996). However, the degree of toxicity varies with the type of organophosphorus insecticide used (Abd EL-Aziz, 1999). On the other hand, Confidor is a new insecticide with selective toxicity for insects over vertebrates and has both multiple agonist and antagonist effects on the neuronal nicotinic acetylcholine receptor-channels (Matsuda *et al.* 1998 and Nagata *et al.* 1998).

Although extensive research has been carried out on the action of different insecticides on the nervous system, little attention has been paid to their biochemical effects on non-target organisms. However, the biochemical effects of many pesticides were investigated. Matsumura (1995) noticed that the biochemical systems particularly affected by some pesticides are lipoprotein lipases, glucose transporter proteins, carbohydrate metabolism and insulin secretion. On the other hand, Rigon *et al.* 1994 found that Carbaryl significantly elevated blood glucose levels in treated rats. Ammonia and urea levels increased in the crabs exposed to the sublethal concentrations of some pesticides

(Radhakishnaiah *et al.* 1995). In addition, administration of Parathion to rats at increasing dose level caused an increase in urea concentration relative to controls but did not induce alterations in creatinine levels

(Guilihermino *et al.* 1998). Six month feeding of Fenvalerate in dogs resulted in an increase of serum cholesterol level (Parker *et al.* 1984).

The aim of the present study is to evaluate and compare the effects of oral administration of 1/10 LD_{50} of the three common used insecticides in the Gaza Strip, Tamaron, Parathion and Confidor, on serum glucose, some lipids and non-protein nitrogen constituents e.g. urea, uric acid and creatinine of rabbit after 1,3,5 and 10 days. The findings can then be extrapolated to human beings to asses the potential hazards in the human populations due to Tamaron, Parathion and Confidor exposure.

MATERIALS AND METHODS

i-Examined Insecticides:

The examined insecticides, Tamaron (O,S - Dimethyl Phosphoramidothioate, Methamidophos), Parathion (O,O-diethyl-O-(4-nitrophenyl) phosphorothioate) and Confidor (1- { (6-chloro-3-pyridinyl) methyl }-N-nitro-2- imidazolidinimine, Imidacloprid) were produced by Payer AG Chemical Company. They were obtained in the form of a commercial product from the local markets in sealed bottels.

ii- Experimental Animals:

A group of male rabbits (800-1000 g) were maintained in the animal house under the ambient conditions. They were fed on a commercial balanced diet prepared specially for rabbits (590). The diet and tab water were offered and libitum all over the experimental period. Rabbits were divided into four groups of 24 rabbits each, except for the control group which was consisted of 8 rabbits. The first group (control) was force fed with 3 ml of distilled water,

orally, by means of a stomach tube. The second, third and fourth groups received orally 3 ml solution containing 1/10 of the LD_{50} (oral LD_{50} of rat) of Tamaron (2.1 mg/kg body wt./day), Parathion (0.2 mg/kg body wt./day) and confidor (45 mg/kg body wt./day) for 10 days, respectively.

iii-Blood sampling:

Six rabbits were taken randomly of each group after 1,3,5 and 10 days of administration. At each sampling date, rabbits were decapitated and blood samples were collected directly from jugular vein. Clear serum samples were separated by centrifugation at 3000 r.p.m for 20 min and then collected and stored in a deep freeze at (-20 C) for different biochemical analysis.

iv-Chemical analysis:

Serum samples were analyzed for glucose , triglycerides and total cholesterol by the methods described by Trinder (1969) , Fossati and Prencipe (1982) and Allain (1974) ,respectively. Non-protein nitrogen constituents were determined by the methods of Mackay and Mackay (1927) for urea , Fossati *et al.*. (1980) for uric acid and Bartels and Bohmer (1972) for creatinine.

v- The statistical analysis:

The statistical analysis for T- test was performed by using SPSS.

RESULTS AND DISCUSSION

As indicated in Tables 1,2 and 3, daily oral administration of 1/10 LD_{50} of Tamaron, Parathion and Confidor, respectively caused a general increase in urea concentration in the blood serum of treated rabbits compared with the control level. In general, significant increment of urea contents were observed from the third day of the insecticides treatment (Fig. 1). Urea is the principal end product of protein

catabolism. Enhanced protein catabolism and accelerated amino acid deamination for gluconeogenesis is probably an acceptable postulate to interpret the elevated levels of urea. The elevated serum urea levels observed in the present study may be due to the destruction of red blood cells during inoculation.

Table (1) : The effect of the oral administration of Tamaron (1/10 of LD_{50}) on some of the chemical constituents of rabbit's serum after 1,3,5 and 10 days of administration.

Parameters	Experimental Periods				
	Control n=9	1st day n=6	3rd day n=6	5th day n=6	10th day n=6
Urea (mg/dl)	36.44 ±1.48	39.50 ±2.32	* 44.00 ±2.67	* 42.50 ±0.71	* 45 60 ±2 74
Uric acid (mg/dl)	0.52 ±0.06	0.47 ±0.03	0.62 ±0.14	* 0.72 ±0 09	* 0.85 ±0.07
Creatinine (mg/dl)	0.90 ±0.03	0.87 ±0.05	0.91 ±0.06	0.90 ±0 08	0.95 ±0.06
Glucose (mg/dl)	132.21 ±4.97	* 158.5 ±7.18	116.33 ±10.04	125.0 ±4.96	145.00 ±16.47
Cholesterol (mg/dl)	62.50 ±2.53	75.0 ±9.14	70.25 ±8.23	* 81.00 ±8.07	60.13 ±2.05
Triglycerides (mg/dl)	138.0 ±7.53	* 252.33 ±16.03	* 253.66 ±11.61	* 192.0 ±15 0	* 168.25 ±10.23

All values expressed as mean ± SE

* Significant differences at $p < 0.05$

A significant decrease in red blood cell count (RBC) and hemoglobin content (Hb) were demonstrated following daily oral administration of 1/10 LD_{50} of Tamaron, Parathion or Confidor (Kerrit *et al.* 1999). The presence of some toxic compounds might increase blood urea and decrease plasma protein (Varely, 1976 and Ashour, 1999). In addition, the elevation of blood urea is a good indicator for kidney

diseases. On the other hand, an increase in urea suggested that animals experienced hemoconcentration due to a mild dehydration (Guilhermino *et al.* 1998).

Table (2) : The effect of the oral administration of Parathion (1/10 of LD_{50}) on some of the chemical constituents of rabbit's serum after 1,3,5 and 10 days of administration.

Parameters	Experimental Periods				
	Control n=6	1st day n=6	3rd day n=6	5th day n=6	10th day n=6
Urea (mg/dl)	36.44 ±1.48	38.50 ±2.01	*47.33 ±0.88	*42.25 ±1.70	*44.33 ±3.67
Uric acid (mg/dl)	0.52 ±0.06	0.55 ±0.13	0.65 ±0.12	*0.87 ±0.07	*0.88 ±0.08
Creatinine (mg/dl)	0.90 ±0.03	0.96 ±0.08	0.88 ±0.06	0.87 ±0.05	0.90 ±0.04
Glucose (mg/dl)	132.21 ±4.97	*149.86 ±4.53	*97.67 ±3.93	*146.4 ±2.46	*150.83 ±1.22
Cholesterol (mg/dl)	62.50 ±2.53	*94.75 ±8.89	*73.33 ±3.84	*86.33 ±4.33	*96.50 ±4.70
Triglycerides (mg/dl)	138.0 ±7.53	*98.0 ±2.67	*84.25 ±5.07	*192.0 ±24.16	144.0 ±6.13

All values expressed as mean ± SE

- Significant differences at $p < 0.05$

In general, uric acid content in rabbit's blood serum increased in response to the daily oral administration of Tamaron, Parathion and Confidor (Tables 1,2 and 3).

The highest serum concentration of uric acid was observed in the tenth day of the treatments by Tamaron, Parathion and Confidor, where uric acid concentration in rabbits serum treated by Tamaron, Parathion and Confidor were 1.63, 1.69 and 1.58 times greater, respectively, than the control level (Fig. 1). Uric Acid is the end product of the catabolism

of tissue nucleic acid, i.e. purine and pyrimidine bases metabolism (Wolf *et al.* 1972). In the present work, the serum uric acid levels exhibited significant increment in the inoculated rabbits for 10 days. This may be due to degradation of purines and pyrimidines or to an increase of uric acid level by either overproduction or inability of excretion (Wolf *et al.* 1972).

Table (3) : The effect of the oral administration of Confidor (1/10 of LD_{50}) on some of the chemical constituents of rabbit's serum after 1,3,5 and 10 days of administration.

Parameters	Experimental Periods				
	Control n=8	1st day n=6	3rd day n=6	5th day n=6	10th day n=6
Urea (mg/dl)	36.44 ±1.48	39.20 ±3.12	*40.33 ±1.45	*48.40 ±2.99	*45.00 ±2.00
Uric acid (mg/dl)	0.52 ±0.06	*0.80 ±0.10	*0.76 ±0.05	0.60 ±0.06	*0.82 ±0.07
Creatinine (mg/dl)	0.90 ±0.03	0.93 ±0.05	0.96 ±0.05	0.95 ±0.09	0.89 ±0.03
Glucose (mg/dl)	132.21 ±4.97	*98.75 ±2.90	117.75 ±8.65	135.00 ±15.04	142.33 ±9.94
Cholesterol (mg/dl)	62.50 ±2.53	*73.53 ±5.33	*75.33 ±4.67	*75.0 ±3.60	58.33 ±1.71
Triglycerides (mg/dl)	138.0 ±7.53	*186.6 ±20.73	162.75 ±21.77	140.0 ±10.85	*94.25 ±3.35

All values expressed as mean ± SE

* Significant differences at $p < 0.05$

However, creatinine content did not show any overt changes in response to the treatments by Tamaron, Parathion and Confidor (Tables 1,2 and 3). These findings are in agreement with that reported by Guilhermino *et al.* (1998) for Parathion administration to rats.

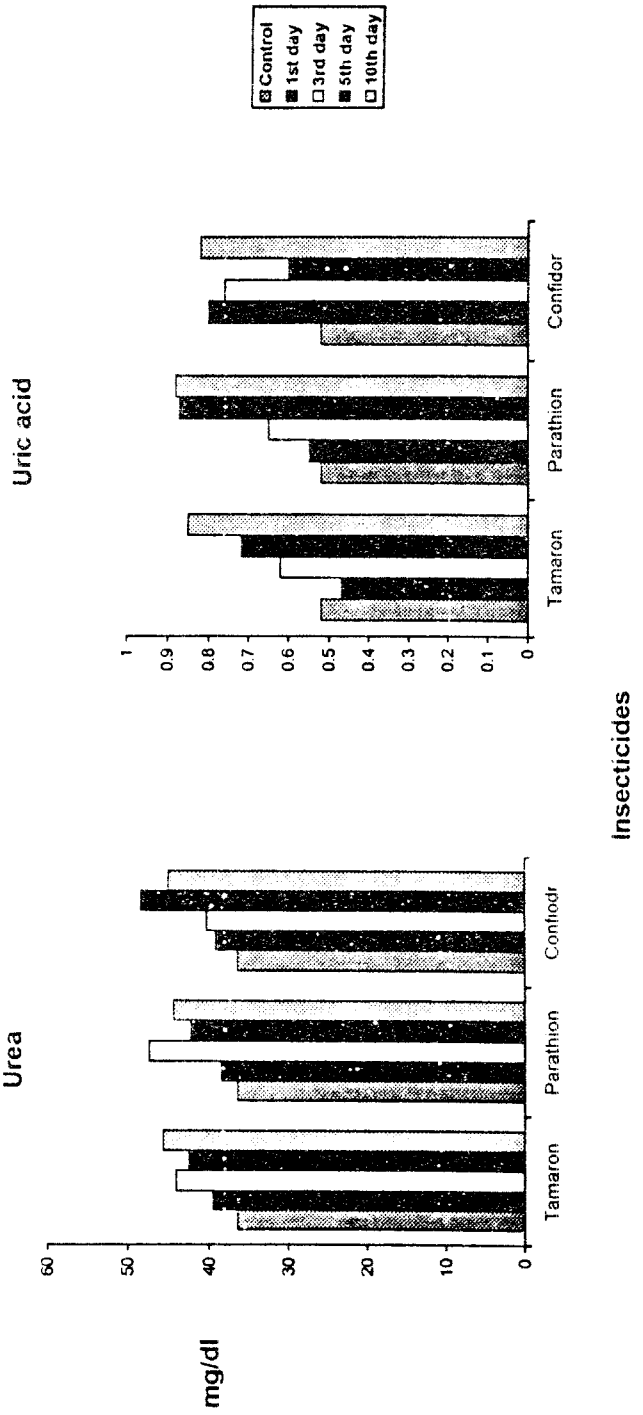

Fig (1): The effect of the daily oral administration of Tamaron, Parathion and Confidor (1/10 of LD_{50}) on some of non-protein nitrogen constituents of rabbit's serum after 1,3,5 and 10 days of administration.

Fig. (2) : The effect of the daily oral administration of Tamaron, Parathion and Confidor (1/10 of LD_{50}) on rabbit's serum glucose, cholesterol and triglycerides after 1,3,5 and 10 days of administration.

Glucose content of rabbits blood serum responded differently to the treatments by the tested insecticides (tables 1,2 and 3). Tamaron and Parathion (the organophosophorous insecticides) caused a significant increase in glucose content of rabbits blood serum at the first day of inoculation, vise versa to that noticed by confidor at the same day. However, at the third day of the experiment, glucose contents of rabbits blood serum treated with 1/10 LD_{50} of Tamaron, Parathion and Confidor decreased to 88, 74 and 89%, of the control level, respectively (Fig. 2). Then Glucose content in treated rabbits began to increase gradually to be higher than the control level at the tenth day of the treatment Insecticides may directly or indirectly play a specific role in pancreatic secreation (Matsumura, 1995), gluconeogensis process, and glycogen metabolism or glucose oxidation. It means that there was a disturbance in carbohydrate metabolism of rabbits by the daily oral administration of insecticides.

In general, daily oral administration of 1/10 LD_{50} of Tamaron, Parathion and Confidor raised up cholesterol concentration in blood serum of treated rabbits compared to the control level at the different intervals of the experiment (Tables 1, 2 and 3). However, Tamaron showed a significant increase in serum cholesterol content at the fifth day of administration whereas Parathion and Confidor produced significant increase in serum cholesterol level during all the time intervals investigated. Except for Parathion, cholesterol content of rabbits blood serum treated with Confidor or Tamaron declined, approximately, to the control level at the tenth day of the experiment (Fig. 2). The increment in cholesterol content agreed with that reported by Parker *et al.* (1984) in response to six month feeding of Fenvalerate in dogs.

There was no specific trend in the response of triglycerides content in rabbits blood serum to the treatment by 1/10 LD_{50} of Tamaron, Parathion and Confidor (Tables 1,2 and 3). However, Tamaron administration caused a significant increase in triglycerides content of rabbits blood serum at the different intervals of the experiment. This increasment was more obvious at the first and third days of Tamaron treatment (Fig. 2). On the other hand, Confidor also caused an increase in triglyceride contents in the serum of treated rabbits compared to the

control level at the different sampling dates except at the tenth day (Table 3). However, Parathion acted differently compared to Tamaron, the second organophosphate insecticides, on triglyceride content of rabbits blood serum (Table 2). Triglycerides content of rabbits blood serum were 71, 61, 139 and 104% of the control level in response to the administration of Parathion for 1, 3, 5 and 10 days respectively (Fig 2). The changes observed in serum triglycerides and cholesterol contents, in response to the treatment by any of the tested insecticides, take place in the liver due to imbalance between the normal rates of lipid synthesis, utilization and secretion (Glaser and Mager, 1972).

In summary, the results of this work indicate that Tamaron, Parathion and Confidor administration induce significant alterations in some biochemical parameters of rabbit's blood. This reflects the harmful effects of these insecticides on kidney and liver functions as well as carbohydrate and lipid metabolisms. Therefore, implementation of primary prevention programs in the Gaza Strip is urgently needed. Such programs would include training courses for farmers to improve their safety and ecological awareness, provision of suitable and cheap protective gear for farmers and monitoring of the health status of workers exposed to insecticides. In addition, more efforts must be done for frequent monitoring of ground

water contamination with pesticides and for adoption of integrated pest management (IPM) program to reduce pesticide dependence.

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الملخص العربى

دراسة مقارنة لتأثير بعض المبيدات الحشرية على سكر الجلوكوز وبعض الليبدات والمكونات النيتروجينية غير البروتينية في سيرم دم الأرانب

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تهدف هذه الدراسة إلى تقدير ومقارنة تأثير إعطاء الأرانب بعض المبيدات الحشرية (تمارون - باراثيون- كونفيدور) بجرعة قدرها 1/10 من الجرعة المميتة /كيلو جرام من وزن الجسم يوميا لمدة عشرة أيام على سكر الجلوكوز وبعض الليبدات والمكونات النيتروجينية غير البروتينية فى سيرم دم الأرانب. وقد تبين أن إعطاء كل مبيد من المبيدات الحشرية الثلاثة لمدة عشرة أيام أحدث زيادة ملموسة فى تركيز يوريا الدم مقارنة بحيوانات المجموعة المقارنة. بصفة عامة لوحظ زيادة ملموسة فى تركيز اليوريا وذلك فى اليوم الثالث من بداية التجربة، كذلك أدى إعطاء المبيدات الحشرية إلى زيادة فى تركيز حمض البوليك وبلغت هذه الزيادة أعلى ما يمكن فى اليوم العاشر

من التجربة، ومن ناحية أخرى فإن محتوى الكرياتينين لم يتغير بشكل ملحوظ إثناء إعطاء التمارون والباراثيون أو الكونفيدور أثناء جميع فترات التجربة. أما سكر الجلوكوز فقد أظهر تباينا فى الإستجابة نتيجة لإعطاء المبيدات. ومن ناحية أخرى فإن الباراثيون و الكونفيدور أحدثت زيادة ملموسة فى مستوى الكوليستيرول فى الفترات الزمنية المختلفة بينما التمارون أحدث زيادة ملموسة

فى مستوى الكولوستيرول فى اليوم الخامس من التجربـــة. أمـــا الجلســريدات الثلاثية فقد تباينت فى الاستجابة لتأثير المبيدات المستعملة. مما سبق يمكـــن أن نستنتج أن إعطاء المبيدات الحشرية لمدة عشرة أيام حتى التركـــيزات الضعيفـــة منها (1/10 من الجرعة المميتة) لها تأثير ضارا علـــى الكبـــد والكليـــة وأيـــض الكربوهيدرات والليبيدات.