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EFFECT OF THE CHEMICAL INSECTICIDE "TAMARON" ON THE ACTIVITY OF CERTAIN DIGESTIVE ENZYMES IN THE WHITE RAT.

By

Monir E. A. Gabr, (Ph.D.), Anwar A. Said (Ph.D.)
and Ahmed R. E. Hassan (M.Sc.)
Faculty of Science, Ain Shams University, Cairo, Egypt.

Introduction

It is a well known fact that chemical insecticides have a hazardous effect on man and useful animals, when these chemical insecticides find their way into the body in amounts very far below the lethal doses and accumulated in the body. These hazardous effects are variable and do concern the different body systems and organs.

The present study is a contribution to what have been carried out by other workers in the field of the so called digestive toxicology. The early experiments of Webb (1948) showed a strong action of alkyl fluorophosphonate on milk lipase (77% inactivation), whereas it had no effect on both pancreatic amylase and lipase. In 1949, Jansen et al. found that a concentration of diisopropyl fluorophosphate of 4×10^{-4}M, caused a 80% inhibition of trypsin, while a concentration of 8×10^{-6}M caused 50% inhibition to chymotrypsin. Mounter et al. (1963) mentioned that diisopropyl phosphorof-

luoridate and other organophosphorous compounds react rapidly with the proteolytic enzymes causing inhibition which reached to 50 % for both trypsin and chymotrypsin. Dombrovskii et al., (1965) gave a dose of parathion which is 0.45 mg/rat/day for 60 days to albino rats; histochemical analysis revealed a sharply reduced phosphatase and esterase activities. Using frozen extracts of pig liver, Mendoza and Shields (1970) mentioned that esterase was sensitive to inhibition by the carbamates studied at the manogram to picogram levels. Gabr and Said (1972B) found that some chemical insecticides (DDT, Lindane and malathione) have a hazardous effect on the function of the peptic and oxyntic cells in mammals. In 1972, Gabr, et al. studied the effect of the previously mentioned insecticides on the activity of certain digestive enzymes (pepsin, trypsin, salivary and pancreatic amylase and pancreatic lipase) in the white rat Rattus rattus. They found that the activities of these enzymes showed a considerable reduction. Bunyan and Jennings (1976) found that liver and brain esterases were inhibited in the pheasant (Phasianius colchicus and pigeon (Columba livia) which fed with lethal and sublethal doses of 6 widely used carbamates (aldicarb, aminocarb, methiocarb, primicarb, propoxur and zectran).

The present work was carried out on the white rat (Rattus rattus) and dealt with the direct effect of the

chemical insecticide "tamaron" on the activity of certain digestive enzymes (pepsin, trypsin, salivary and pancreatic amylase and pancreatic esterase) prepared from normal animals.

Materials and Methods

A- The animals used through this study were adult male white rats (Rattus rattus) weighing about 130 g each. Certain hydrolytic enzymes (salivary amylase, pepsin, trypsin, pancreatic amylase and pancreatic esterase) were prepared from these animals.

All the animals were kept on the same normal diet , during the experiment, to avoid the probable interference of the effect of food kind. This diet consisted of bread, milk (NIDO) and water. The animals were kept in the laboratory in cages about one week before use. For obtaining the digestive juices, free from food remains, animals were starved for at least two days. In each experiment not less than 30 animals were used after killing them by a blow on their heads.

Water extracts of fresh salivary glands(1:10), stomach mucosa extract (1:10) and pancreatic extract (1:10)were used. Enterokinase was prepared from duodenal mucosa extract according to the prescription of Waldschmidt-Lietz (1924) , treated with acetone and ether.

B- Buffers :

A number of different buffer solutions were used in the present work. The nature of each buffer used in the different experiments is indicated in the tables giving the experimental results.

The measurement of the pH values was done by the B.L.H. capillator. The error in this method is about 0.05 pH units.

C- Methods of measurement of the enzymes activity:

1. Peptidases :

The method of Willstätter and Waldschmidt-Leitz (1921) and Willstätter et al., (1926) was used (with some modification) for measuring peptic and tryptic activities.

For pepsin, the digestive mixture (total volume 5.0 c.c.)had the following composition:

2.5	c.c.	egg albumen 3 %
1.0	c.c.	citrate-HCl buffer (1N sod. citrate and 2.5 N HCl)
1.0	c.c.	distilled water
0.5	c.c.	enzyme solution
5.0	c.c.	Total volume.

The incubation time of 20 h was chosen since it was found to be the most suitable one.

For trypsin, the digestive mixture was made in the following way :

0.3 c.c. enzyme solution.

0.1 c.c. enterokinase solution.

0.35 c.c. buffer solution (0.2 N ammonia-ammonium chloride.

0.15 c.c. distilled water

The above mentioned solutions were mixed together (total volume 0.90 c.c.) and the pH value of the mixture was 8.0. The mixture was put in the thermostate at 37 °C for activation. After 30 minutes, the pH was adjusted to the desired value by the addition of 0.6 c.c. 0.1N veronal acetate buffer prewarmed to 37°C, after which 1.5 c.c. of 6% casein solution (Waldschmidt-Leitz, 1924) was added. The total volume of the digestive mixture was therefore 3.0 c.c. The buffer solutions were prepared according to Michaelis (1922 and 1931). The time of incubation was half an hour.

2- Carbohydrases :

Amylase:

The method of Hagedorn and Jensen (1922) was used in the present work.

For salivary amylase the digestive mixture had the following composition:

2.0	c.c.	2% starch solution.
0.2	c.c.	buffer solution (0.1 N veronal-acetate-HCl)
1.3	c.c.	distilled water (1.4 c.c. for pancreatic amylase).
0.5	c.c.	enzyme solution (0.4 c.c. for pancreatic amylase).
4.0	c.c.	Total volume

The digestive mixture was incubated at 37°C for exactly 10 minutes. The titration sample is of 0.05 c.c. It had been shown by Pucker and Finch (1938) and confirmed by Keddis (1952 and 1956) that each mg maltose had the same reducing action on potassium ferricyanide as 0.75 mg glucose. Using this factor, the extent of digestion was expressed as "increase in mg maltose" calculated by multiplying each figure of "increase in mg glucose" by the factor 100/75.

3- Esterases :

Pancreatic esterase:

The method of Willstatter, et al , (1923) was followed in the present work. The digestive mixture (total volume 5.0 c.c.) had the following composition :

1.00	c.c.	enzyme solution.
0.05	c.c.	ethyl acetate
1.00	c.c.	0.1N veronal-acetate buffer
1.00	c.c.	2% $CaCl_2$ and
1.95	c.c.	distilled water
5.00	c.c.	Total volume.

The digestive mixture was incubated at 37°C for 60 minutes. The titration sample was 0.5 c.c.

4- Estimation of the optimal pH values of the enzymes:

All the experiments of the present study were carried out at the optimal pH of the enzymes. The optimal activity of pepsin, trypsin, salivary amylase, pancreatic amylase and esterase were 2.1, 8.0, 7.1, 6.8 and 7.5, respectively.

5- Effect of the chemical insecticide on the digestive enzymes :

For studying the effect of the chemical insecticide "tamaron" on the activity of the hydrolytic enzymes (salivary and pancreatic amylases, pepsin, trypsin and pancreatic esterase), three doses (1/10, 1/100 and 1/1000 high dose) were testad. - The high dose was 30 mg/kg body weight/ day. This high dose is very near to the LD_{50} for the chemical insecticide (Hassan, 1983). The doses of the insecticide were added to the digestive mixture instead of a part of distilled water.

Results

a- Peptidases:

1. Pepsin :

Table I displays the data which concern the effect of tamaron on the activity of pepsin (in c.c. 0.1N KOH) prepared from normal white rats. The 1/10 high dose reduced the activity from 0.83 to 0.52 (=37.4 %) inactivation), the 1/100 high dose reduced the activity from 0.83 to 0.69 (=16.9% inactivation) and the 1/1000 high dose reduced the activity from 0.83 to 0.72 (= 13.3%) inactivation).

2- Trypsin:

Table 2 exhibits the data which concern the effect of tamaron on the activity of trypsin (in c.c. 0.02 N KOH) prepared from normal animals. The 1/10 high dose reduced the activity of trypsin from 0.97 to 0.90 (=7.2% inactivation), the 1/100 high dose reduced the activity from 0.97 to 0.91 (= 6.2% inactivation), and 1/1000 high dose reduced the activity from 0.97 to 0.95 (2.1% inactivation).

b. Carbohydrases:

1. Salivary amylase.

Table 3 displays the data which concern the effect of tamaron on the activity of salivary amylase (in mg maltose) prepared from normal animals. The 1/10 high dose reduced the activity of salivary amylase from 0.117 to 0.064(=45.3%

inactivation), the 1/100 high dose reduced the activity from 0.117 to 0.087 (=25.6% inactivation), and the 1/1000 high dose reduced the activity from 0.117 to 0.112(=4.3% inactivation).

2. Pancreatic amylase :

Table 4 shows the data which concern the effect of tamaron on the activity of pancreatic amylase (in mg maltose) prepared from normal white rats. The 1/10 high dose reduced the activity of pancreatic amylase from 0.159 to 0.081 (= 49.1% inactivation), the 1/100 high dose reduced the activity from 0.159 to 0.109 (=31.5% inactivation) and the 1/1000 high doss reduced the activity from 0.159 to 0.137 (=13.8% inactivation).

c- Esterases:

Pancreatic esterase:

Table 5 displays the data which concern the effect of tamaron on the activity of pancreatic esterase(in c.c. 0.01 N KOH) prepared from normal animals. The 1/10 high dose reduced the activity of pancreatic esterase from 0.38 to 0.20 (=47.4% inactivation), the 1/100 high dose reduced the activity from 0.38 to 0.27 (=29.0% inactivation) and the 1/1000 high dose reduced the activity from 0.38 to 0.34(=10.5% inactivation).

Discussion

The effect of the chemical insecticide "tamaron" on the activity of certain digestive enzymes (pepsin, trypsin, salivary amylase, pancreatic amylase and pancreatic esterase) of the white rat Rattus rattus; was carried out. The doses applied were 1/10, 1/100 and 1/1000 of the approximate lethal dose of tamaron. The approximate lethal dose of tamaron was 30 mg/kg body wt./day. The data in Table 6 indicate the following :-

Concerning the direct effect of tamaron on the activity of the digestive enzymes it could be noticed that:

a) The 1/10 high dose produced a considerable inactivation to all enzyme (pepsin, trypsin, salivary and pancreatic amylase and pancreatic esterase). Pancreatic amylase seems to be the most sensitive to the inactivation effect produced by the chemical insecticide, whereas trypsin seems to be the most resistible to such an inactivation.

b) The 1/100 high dose of tamaron produced a lower effect on pepsin, trypsin, salivary and pancreatic amylase and pancreatic esterase. Pancreatic amylase showed a considerable inactivation while trypsin seems to be the most resistible to the inactivation effect produced by this chemical insecticide. It is clear that the inactivation effects for the 1/100 high dose are lower than their correspondings for the 1/10 high dose.

c) The 1/1000 high dose of the chemical insecticide generally have a weak effect on pepsin, trypsin, salivary and pancreatic amylase and pancreatic esterase, yet pepsin and pancreatic amylase showed a considerable inactivation. It is clear that the inactivation effects for the 1/1000 high doses were lower than their correspondings for the 1/10 and 1/100 high doses.

The above mentioned results which concern the inactivation of the experimented on digestive enzymes, through the effect of the applied chemical insecticide "tamaron" agree with the results obtained by other workers dealing with the effect of certain chemical insecticides on the digestive enzymes (Webb, 1948; Jansen et al, 1949; Kounter et al.,1965; Dombrovskii et al.,1965; Mendoza and Shields, 1970; Gabr and Said, 1972B; Gabr et al;1972; Bunyan and Jennings,1976).

Summary

The effect of the chemical insecticide "tamaron" on the activity of certain digestive enzymes (pepsin, trypsin, salivary and pancreatic amylase and pancreatic esterase) of the white rat Rattus rattus was carried out. The doses applied were 1/10, 1/100 and 1/1000 of the approximate lethal dose of the chemical insecticide. The approximate lethal dose of tamaron for rats was 30 mg/kg body wt./day.

The 1/10 high dose produced a considerable inactivation to all the enzymes studied, Pancreatic amylase and pancreatic esterase seem to be the most sensitive to the inactivation effect, while trypsin seems to be the most resistible.

The 1/100 high dose produced an effect lower than that produced by the 1/10 high dose, in all the enzymes studied.

Pancreatic amylase and pancreatic esterase seem to be the most sensitive ones, while trypsin is the most resistible.

The 1/1000 high dose produced very slight effect on enzymes studied. Pancreatic amylase and pepsin showed a considerable inactivation.

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Women's Coll. Ann. Rev.
Vol. 15 (1990).

Table 1

Effect of tamaron on the activity of pepsin of normal rat.

Condition	Digestive mixture at 37°C, pH value 2.1, time of digestion 20 hours and titration sample 0.5 c.c.	Titration (c.c.0.1N KOH)		Activity
		After 0 hour	After 20 hours	c.c. 0.1N KOH
Normal (control)	2.5c.c 3% eggalbumen, 1.0 c.c. citrate-HCl buffer, 1.0 c.c. dist. H_2O and 0.5 c.c. water extract of stomach mucosa (1:10)	5.89	6.72	0.83
1/10 high dose	2.5 c.c. 3% eggalbumen, 1.0 c.c. citrate-HCl buffer, 0.07 c.c. tamaron in H_2O, 0.93 c.c. dist. H_2O and 0.5 c.c. water extract of stomach mucosa (1:10).	5.97	6.49	0.52
1/100 high dose	2.5 c.c. 3% eggalbumen, 1.0c.c. citrate-HCl buffer, 0.07c.c. tamaron in H_2O, 0.93 c.c. dist. H_2O and 0.5 c.c. water extract of stomach mucosa. (1:10)*	6.12	6.81	0.69
1/1000 high dose	2.5 c.c. 3% egg albumen, 1.0c.c. citrate-HCl buffer, 0.07** c.c. tamaron in H_2O, 0.93 c.c. dist. H_2O and 0.5 c.c. water extract of stomach mucosa (1:10).	6.65	7.37	0.72

* The concentration of this preparation is 1/10 of that used for the 1/10 high dose.

** The concentration of this preparation is 1/10 of that used for the 1/100 high dose.

Vol. 15 (1990).

Table 1

Effect of tamaron on the activity of pancreatic trypsin of normal rat.

Condition	Digestive mixture at 37°C., pH value 8.0, time of digestion one hour and titration sample 0.5 c.c.	Titration(c.c.0.02N KOH)		Activity c.c. 0.02N KOH
		After 0 hour	After one hour	
Normal (control)	0.3 c.c. pancreatic extract (1:10),0.1 c.c.enterakinase solution, 0.35 c.c. 0.2N ammonium buffer,0.15c.c. dist. H_2O, 0.8 c.c. 0.1 N veronal-acetate buffer and 1.5c.c. 6% casein.	1.07	2.04	0.97
1/10 high dose	0.3c.c. pancreatic extract (1:10),0.1 c.c.enterokinase solution,0.35c.c. 0.2N ammonium buffer, 0.07 c.c.tamaron in dist. H_2O, 0.08 c.c. dist. H_2O, 0.6 c.c. 0.1 N veronal-acetate buffer and 1.5 c.c. 6% casein.	1.09	1.99	0.90
1/100 high dose	0.3c.c. pancreatic extract (1:10),0.1 c.c. enterokinase solution,0.35 c.c. 0.2N ammonium buffer,0.07 c.c. tamaron in dist. H_2O, 0.08 c.c. dist. H_2O, 0.6 c.c. 0.1 N veronal-acetate buffer and 1.5 c.c. 6% casein.	1.12	2.03	0.91
1/1000 high dose	0.3 c.c. pancreatic extract (1:10),0.1c.c. enterokinase solution,0.35 c.c. 0.2N ammonium buffer, 0.07c.c. tamaron in dist. H_2O, 0.08 c.c. dist. H_2O, 0.6 c.c. 0.1N veronal-acetate buffer and 1.5 c.c. 6% casein.	1.22	2.17	0.95

• The concentration of this preparation is 1/10 of that used for the 1/10 high dose.

•• The concentration of this preparation is 1/10 of that used for the 1/100 high dose.

Table 3

Effect of tamaron on the activity of salivary amylase of normal rat.

Condition	Digestive mixture at 37°C., pH value 6.8, time of digestion 10 minutes and titration sample 0.05 c.c.	Digestion (mg glucose)		Activity	
		After 0 minute	After 10 minutes	mg glucose	mg maltose
Normal (control)	2.0c.c. 2% starch solution, 0.2c.c. 0.1 N veronal-acetate buffer, 1.3 c.c. dist. H_2O, and 0.5 c.c. salivary gland (parotid) extract[2] (1:10).	0.025	0.113	0.088	0.117
1/10 high dose	2.0c.c. 2% starch solution, 0.2c.c. 0.1 N veronal-acetate buffer, 0.07 c.c. tamaron in dist. H_2O, 1.23 c.c. dist. H_2O, and 0.5 c.c. salivary gland (parotid) extract[2] (1:10).	0.024	0.072	0.048	0.064
1/100 high dose	2.0 c.c. 2% starch solution, 0.2c.c. 0.1 N veronal-acetate buffer, 0.07 c.c. tamaron in dist. H_2O, 1.23 c.c. dist. H_2O, and 0.5 c.c. salivary gland (parotid) extract[2] (1:10).	0.027	0.092	0.065	0.087
1/1000 high dose	2.0 c.c. 2% starch solution, 0.2 c.c. 0.1N veronal-acetate buffer, 0.07 c.c. tamaron in dist. H_2O, 1.23 c.c. dist. H_2O, and 0.5 c.c. salivary gland (parotid) extract.[2]	0.027	0.111	0.084	0.112

* The concentration of this preparation is 1/10 of that used for the 1/10 high dose.

** The concentration of this preparation is 1/10 of that used for the 1/100 high dose.

Table 4

Effect of tamaron on the activity of pancreatic amylase of normal white rat.

Condition	Digestive mixture at 37°C, pH value 7.1, time of digestion 10 minutes and titration sample 0.05 c.c.	Digestion (mg glucose)		Activity	
		After 0 minute	After 10 minutes	mg. glucose	mg maltose
Normal (control)	2.0 c.c. 2% starch solution, 0.2 c.c. 0.1N veronal-acetate buffer, 1.4 c.c. dist. H_2O, and 0.4 c.c. pancreatic extract (1:10).	0.041	0.150	0.119	0.159
1/10 high dose	2.0 c.c. 2% starch solution, 0.2c.c.0.1 N veronal-acetate buffer, 0.07 c.c. tamaron in dist. H_2O, 1.33 c.c. dist. H_2O, and 0.4 c.c. pancreatic extract (1:10)	0.031	0.092	0.061	0.081
1/100 high dose	2.0 c.c. 2% starch solution, 0.2c.c.0.1 N veronal-acetate buffer, 0.07 c.c. tamaron in dist. H_2O, 1.33 c.c. dist. H_2O, and 0.4 c.c. pancreatic extract (1:10).	0.038	0.120	0.082	0.109
1/1000 high dose	2.0c.c. 2% starch solution, 0.2 c.c.0.1 N veronal-acetate buffer, 0.07 c.c. tamaron in dist. H_2O, 1.33 c.c. dist. H_2O, and 0.4 c.c. pancreatic extract (1:10).	0.036	0.139	0.103	0.137

* The concentration of this preparation is 1/10 of that used for the 1/10 high dose.

** The concentration of this preparation is 1/10 of that used for the 1/100 high dose.

Table 5

Effect of tamaron on the activity of pancreatic esterase of normal white rat.

Condition	Digestive mixture at 37°C., pH value 7.5, time of digestion 60 minutes and titration sample 0.5 c.c.	Titration (0.01N KOH)		Activity c.c. 0.1 N KOH
		After 0 hour	After one hour	
Normal (control)	0.05 c.c. ethyl acetate, 1.0 c.c. 0.1N veronal-acetate buffer, 1.0 c.c. 2% $CaCl_2$ solution, 1.95c.c. dist. H_2O and 1.0c.c. pancreatic extract (1:10).	0.98	1.36	0.38
1/10 high dose	0.05 c.c. ethyl acetate, 1.0 c.c. 0.1N veronal-acetate buffer, 1.0 c.c. 2% $CaCl_2$ solution, 0.07 c.c. tamaron in dist. H_2O, 1.88c.c. dist. H_2O and 1.0 c.c. pancreatic extract (1:10).	0.98	1.18	0.20
1/100 high dose	0.05 c.c. ethyl acetate, 1.0 c.c. 0.1 N veronal-acetate buffer, 1.0 c.c. 2% $CaCl_2$ solution, 0.07c.c. tamaron in dist. H_2O, 1.88 c.c. dist. H_2O and 1.0 c.c. pancreatic extract (1:10).	0.98	1.25	0.27
1/1000 high dose	0.05 c.c. ethyl acetate, 1.0 c.c. 0.1N veronal-acetate buffer, 1.0 c.c. 2% $CaCl_2$ solution, 0.07 c.c. tamaron in dist. H_2O, 1.88c.c. dist. H_2O and 0.1 c.c. pancreatic extract (1:10).	0.98	1.32	0.34

* The concentration of this preparation is 1/10 of that used for the 1/10 high dose.

** The concentration of this preparation is 1/10 of that used for the 1/100 high dose.

Table 6

Effect of the Chemical Insecticide "Tamaron" on the activity of the digestive enzymes.

Enzymes	Percentage of inactivation of the enzyme by the chemical insecticide.		
	1/10 high dose	1/100 high dose	1/1000 high dose
Pepsin	37.4	16.9	13.3
Trypsin	7.2	6.2	2.1
Salivary amylase	45.3	25.6	4.3
Pancreatic amylase	49.1	31.5	13.8
Pancreatic esterase	47.4	29.0	10.5