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Further studies on the effect of the chemical insecticide "Tamaron" on the activity of certain digestive enzymes in the White Rat.

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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 before 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. Webb (1948); Jensen et al (1949); Hartley and Kilby (1950); Jensen and Balls (1952); Mounter et al (1957), Ooms (1961); Dombrovskii et al (1965), Gabr and Said (1972 B); Gabr et al (1972); Bunyan and Jennings (1976), Said (1979 & 1981), studied the effect of different chemical insecticides on the activity of some digestive enzymes. He found that the activity of these enzymes showed a considerable inactivation in vitro.

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 (pancreatic lipase, ileum dipeptidase and ileum maltase).

Material and Methods

A- The animals used through this study were adult male white rats (Rattus rattus) weighing about 130 g each. Certain hydrolytic enzymes (pancreatic lipase, ileum dipeptidase and ileum maltase) 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 pancreatic extract (1: 10) and intestinal mucosa extract (1:10) were used (Said,1979).

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.D.H. capillator. The error in this method is about 0.05 pH units.

C- Methods of measurement of the enzyme activity:

1- Pancreatic lipase:

The method of Willstätter et al (1923) was followed in the present work. The digestive mixture (total volume 1.5 c.c.) had the following composition:

0.140	c.c.	olive oil
0.500	c.c.	0.2 N veronal-acetate buffer
0.025	c.c.	2 % $CaCl_2$
0.025	c.c.	3 % egg albumen
0.310	c.c.	dist. H_2O
0.500	c.c.	enzyme solution.
1.5	c.c.	Total volume.

The incubation time was one hour at 37°C.

2- Ileum dipeptidase:

The method of Willstätter and Waldschmidt-Leitz 1921, and Willstatter et al 1926, was used with

some modification for measuring dipeptidase activity The digestive mixture had the following composition:

1.0	c.c.	2 % glycylglycine solution
1.0	c.c.	0.1N veronal-acetate-HCl buffer
0.5	c.c.	dist. H_2O
1.0	c.c.	enzyme solution
3.5	c.c.	Total volume.

The incubation time was one hour at 37 °C.

3- Ileum maltase:

The method of Hagedorn and Jensen (1922), was used in the present work. The digestive mixture had the following composition:

2.0	c.c.	0.4 % maltose solution
0.8	c.c.	0.1 N veronal-acetate-HCl buffer
0.8	c.c.	dist. H_2O
0.4	c.c.	enzyme solution
4.0	c.c.	Total volume

The digestive mixture was incubated at 37 °C. for exactly 10 minutes. The titration sample was 0.1 c.c.

D- 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 pancreatic lipase, ileum dipeptidase and ileum maltase were 7.2, 8.1 and 7.3, respectively.

E- 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 (pancreatic lipase, ileum dipeptidase and ileum maltase), three doses (1/10, 1/100 and 1/1000 high dose) were tested.. The high dose was 30 mg/kg body weight/day. The high dose is very near 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

1- Pancreatic lipase:

Table I displays the data which concern the effect of tamaron on the activity of pancreatic lipase (in c.c. 0.1 N KOH) prepared from normal white rats. The 1/10 high dose reduced the activity from 0.63 to 0.13 (= 79.4 % inactivation), the 1/100 high dose reduced the activity from 0.63 to 0.32 (= 50 % inactivation)

and the 1/1000 high dose reduced the activity from 0.63 to 0.43 (= 31.8 % inactivation).

2- Ileum dipeptidase:

Table 2 exhibits the data which concern the effect of tamaron on the activity of ileum dipeptidase (in c.c. 0.01 N KOH) prepared from normal animals. The 1/10 high dose reduced the activity of dipeptidase from 1.28 to 0.87 (= 32 % inactivation), the 1/100 high dose reduced the activity from 1.28 to 1.01 (=21 % inactivation) and the 1/1000 high dose reduced the activity from 1.28 to 1.13 (= 12 % inactivation).

3- Ileum maltase:

Table 3 displays the data which concern the effect of tamaron on the activity of maltase (in mg glucose) prepared from normal animals. The 1/10 high dose reduced the activity from 0.112 to 0.065 (= 42 inactivation), the 1/100 high dose reduced the activity from 0.112 to 0.084 (= 25 % inactivation) and the 1/1000 high dose reduced the activity from 0.112 to 0.092 (= 18 % inactivation).

Discussion

The effect of the chemical insecticide "tamaron" on the activity of certain digestive enzymes (pancreatic lipase,

ileum dipeptidase and ileum maltase) 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. These data (Tables 1-3) 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 enzymes (pancreatic lipase, ileum dipeptidase and ileum maltase). Pancreatic lipase seems to be the most sensitive to the inactivation effect produced by the chemical insecticide, whereas ileum dipeptidase seems to be the most resistible to such an inactivation.

b) The 1/100 high dose of tamaron produced a lower effect on pancreatic lipase, ileum dipeptidase and ileum maltase. Pancreatic lipase showed a considerable inactivation while ileum dipeptidase 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 week effect on pancreatic lipase, ileum dipeptidase and ileum maltase, yet pancreatic lipase and ileum maltase 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 "taamaron" 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; Hartley and Kilby, 1950; Jansen and Balls, 1952; Mounter et al, 1957; Ooms,1961; Dombrovskii et al, 1965; Gabr and Said, 1972 B., Gabr et al, 1972; Bunyan and Jennings, 1976 and Said, 1979 & 1981.

Summary

The effect of the chemical insecticide "tamaron" on the activity of certain digestive enzymes (pancreatic lipase, ileum dipeptidase and ileum maltase) 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 high dose produced a considerable inactivation to all the enzymes studied. Pancreatic lipase and ileum maltase seem to be the most sensitive to the inactivation effect, while ileum dipeptidase seems to be the most resistable.

The 1/100 high dose produced an effect lower than that produced by the 1/10 high dose, in all the enzymes studied. Pancreatic lipase and ileum maltase seem to be the most sensitive ones while ileum dipeptidase is the most resistible.

The 1/1000 high dose produced a very slight effect on the enzymes studied. Pancreatic lipase showed a considerable inactivation.

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Table I

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

Condition	Digestive mixture at 37°C, pH value 7.2 and time of digestion one hour.	Titration (c.c. 0.1N KOH)		Activity c.c. 0.1N KOH
		After 0 hour	After one hour	
Normal (control)	0.14 c.c. olive oil, 0.5 c.c. 0.2 N veronal-acetate buffer, 0.025 c.c. 2 % $CaCl_2$, 0.025 c.c. 3 % egg albumen, 0.31 c.c. dist. H_2O and pancreatic extract (1 ; 10).	2.71	3.34	0.63
1/10 high doae	0.14 c.c. olive oil , 0.5 c.c. 0.2 N veronal-acetate buffer, 0.025 c.c. 2 % $CaCl_2$, 0.025 c.c. 3 % egg albumin , 0.07 c.c. tamaron in H_2O , 0.24 c.c. dist. H_2O and 0.5 c.c. pancreatic extract (1 : 10).	2.75	2.88	0.13
1/100 high dose	0.14 c.c. olive oil, 0.5 c.c. 0.2 N veronal-acetate buffer, 0.025 c.c. 2 % $CaCl_2$, 0.025 c.c. 3 % egg albumen, 0.07* c.c. tamaron in H_2O, 0.24 c.c. dist. H_2O and 0.5 c.c. pancreatic extract (1:10).	2.78	3.10	0.32
1/1000 high dose	0.14 c.c. olive oil, 0.5 c.c. 0.2 N veronal-acetate buffer , 0.025 c.c. 2 % $CaCl_2$, 0.025 c.c. 3 % egg albumen , 0.07** c.c. tamaron in H_2O , 0.24 c.c. dist. H_2O and 0.5 c.c. pancreatic extract (1:10).	2.74	3.17	0.43

* 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 2

Effect of tamaron on the activity of ileum dipepetidase of normal rat.

Condition	Digestive mixture at 37℃, pH value 8.1, time of digestion one hour and titration sample 0.5 c.c.	Titration (c.c. 0.01N KOH)		Activity c.c. 0.01N KOH
		After 0 hour	After one hour	
Normal (control)	1.0 c.c. 2 % glycylglycine solution, 1.0 c.c. 0.1 N veronal-acetate buffer, 0.5 c.c. dist. H_2O and 1.0 c.c. extract of ileum mucosa (1: 10).	1.91	3.19	1.28
1/10 high dose	1.0 c.c. 2 % glycylglycine solution, 1.0 c.c. 0.1N veronal-acetate buffer, 0.07 c.c. tamaron in H_2O, 0.43 c.c. dist. H_2O and 1.0 c.c. extract of ileum mucosa (1:10).	1.86	2.73	0.87
1/100 high dose	1.0 c.c. 2% glycylglycine solution, 1.0 c.c. 0.1N veronal-acetate buffer, 0.07* c.c. tamaron in H_2O, 0.43 c.c. dist. H_2O and 1.0 c.c. extract of ileum mucosa (1:10).	1.89	2.90	1.01
1/1000 high dose	1.0 c.c. 2% glycylglycine solution, 1.0 c.c. 0.1N veronal-acetate buffer, 0.07** c.c. tamaron in H_2O, 0.43 c.c. dist. H_2O and 1.0 c.c. extract of ileum mucosa (1:10).	1.90	3.03	1.13

* 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.

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Table (3)

Effect of tamaron on the activity of ileum maltase of normal rat.

Condition	Digestive mixture at 37°C , pH value 7. 3, time of digestion 10 minutes and titration sample 0. 1 c. c.	Digestion (mg glucose) After 0 minute	Digestion (mg glucose) After 10 minutes	Activity mg glucose
Normal (control)	2. 0 c. c. 0. 4% maltose solution, 0. 8 c. c. 0. 1 N veronal-acetate buffer, 0. 8 c. c. dist. H_2O and 0. 4 c. c. extract of ileum mucosa (1:10).	0. 158	0. 270	0. 112
1/10 high dose	2. 0 c. c. 0. 4 % maltose solution, 0. 8 c. c. 0. 1N veronal-acetate buffer, 0. 07 c. c. tamaron in dist. H_2O , 0. 73 c. c. dist. H_2O and 0. 4 c. c. extract of ileum mucosa (1:10).	0. 155	0. 220	0. 065
1/100 high dose	2. 0 c. c. 0. 4% maltose solution, 0. 8 c. c. 0. 1N veronal-acetate buffer, 0. 07* c. c. tamaron in dist. H_2O , 0. 73 c. c. dist. H_2O and 0. 4 c. c. extract of ileum mucosa (1:10).	0. 160	0. 244	0. 084
1/1000 high dose	2. 0 c. c. 0. 4 % maltose solution, 0. 8 c. c. 0. 1N veronal-acetate buffer, 0. 07** c. c. tamaron in dist. H_2O, 0. 73 c. c. dist. H_2O and 0. 4 c. c. extract of ileum mucosa (1:10)	0. 162	0. 254	0. 092

* 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.