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Further studies on the potency of certain digestive enzymes prepared from the white rat treated with the chemical insecticide "tamaron"

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Introduction

The present work is a part of a series of investigations which deal with the effect of the chemical insecticides on the physiology of digestion. Gabr and Said (1972, A,B,C.) on a study which concerns the effect of some chemical insecticides (DDT, lindane and malathione) on the peptic and oxyntic cells in mammals, found that these insecticides have a hazardous effect on the structure and function of these cells. In 1972, Gabr et al. studied the effect of the previously mentioned insecticides on the potency of certain digestive enzymes (pepsin, trypsin, salivary and pancreatic amylase and pancreatic lipase) in the white rat Rattus rattus. They found the potencies of those enzymes showed a considerable reduction. Said (1979), carried out experiments on the white rat Rattus rattus to study the effect of DDT, lindane and malathione on the potency of certain digestive enzymes (maltase and dipeptidase). He

found a reduction in the potencies of these enzymes. In 1981, Said experimented the previously mentioned insecticides on the potency of certain digestive enzymes prepared from the domestic pigeon Columba livia domestica. The enzymes are liver esterase, ileum esterase and pancreatic lipase. The doses applied were 1/10, 1/100 & 1/1000 of the approximate lethal dose (high dose) for each of these chemical insecticides. The enzymes of the 1/10 high dosed treated animals showed a considerable reduction in their potencies. The enzymes of the 1/1000 high dosed treated animals showed a lower reduction in their potencies.

In this work it is aimed to see the effect of the chemical insecticide "tamaron" on the potency of certain digestive enzymes (pancreatic lipase, ileum dipeptidase and ileim maltase) in the white rat Rattus rattus.

Material and Methods

A- Preparation of enzyme solution:

The animals used through this study were adult male white rats (Rattus rattus) weighing about 130 g each. All the animals were kept on the same normal diet (bread, milk(NIDO) and water), during the experiment, to avoid

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* The trem potency designates the change in the activity of the enzyme prepared from insecticide treated animals.

the probable interference of the effect of food kind. The animals were kept in the laboratory in cages about one week before use. In each experiment not less than 30 animals were used after killing them by a blow on their heads. Certain hydrolytic enzymes (pancreatic lipase, ileum dipeptidase and ileum maltase) were prepared from these rabbit animals. Water extracts of fresh pancreatic extract (1:10) and intestinal mucosa extract (1:10) were used.

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 Willstatter et al.(1923) was used 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.	0.2N 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 Willstätter et al. 1926, wss used for measuring dipeptidase activity. The digestive mixture had the following composition.

1.0	c.c.	2 % glycyl glycine 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. 0.1N 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 activities of pancreatic lipase, ileum dipeptidase and ileum maltase were at 7.2, 8.1 and 7.3, respectively (Said 1979).

E. Effect of the chemical insecticide on the digestive enzymes:

For studying the effect of tamaron on the potency of the hydrolytic enzymes, 3 doses (1/10, 1/100 and 1/1000 high dose) were tested. The high dose in mg/Kg. body weight/day was 30. This high dose is very near to the LD_{50} for tamaron (Hassan, 1983). The animals were given daily the 1/10 high dose, the 1/100 high dose or the 1/1000 high dose orally for 60 days.

Results

1. Pancreatic lipase:

Table 1 displays the date which concern the potency of pancreatic lipase prepared from tamaron treated animals as compared with those prepared from normal animals. The 1/10 high dose reduced the potency from 0.67 to 0.12 (= 82 % reduction), the 1/100 high dose reduced the potency from 0.65 to 0.39 (= 40 % reduction) and the 1/1000 high dose reduced the potency from 0.63 to 0.43 (= 32 % reduction).

2. Ileum dipeptidase:

Table 2 indicates the data which concern the potency of ileum dipeptidase prepared from tamaron treated animals as compared with those prepared from normal animals. The 1/10 high dose reduced the potency from 1.27 to 0.70 (=45% reduction) and the 1/100 high dose reduced the potency from 1.28 to 0.87 (= 32 % reduction), while the 1/1000 high dose reduced the potency from 1.25 to 0.99 (= 21 % reduction).

3. Ileum maltase:

Table 3 shows the data which concern the potency of ileum maltase prepared from tamaron treated animals as compared with those prepared from normal animals. The

1/10 high dose reduced the potency from 0.108 to 0.051 (= 53 % reduction), the 1/100 high dose reduced the potency from 0.106 to 0.066 (= 38 % reduction) and the 1/1000 high dose reduced the potency from 0.105 to 0.076 (= 28 % reduction).

Discussion

The results of the present investigation clearly show the reduction in the potencies of the digestive enzymes (pancreatic lipase, ileum dipeptidase and ileum maltase) extracted from tamaron treated animals. These results could be explained as follows:

a) The enzymes of the 1/10 high dose treated animals showed a considerable reduction in the potencies. It is clear that the pancreatic lipase was the most reduced by this chemical insecticide, while ileum dipeptidase showed the least reduction.

b) The enzymes of the 1/100 high dose treated animals, showed the lowest reduction in their potencies as compared with their correspondings for 1/10 high dose treated animals.

c) The enzymes of the 1/1000 high dose treated animals, showed a slight reduction in their potencies as compared with their correspondings for 1/10 and 1/100 high dose treated animals.

The previously mentioned results were in agreement with those obtained by other workers dealing with the effect of certain chemical insecticides on the potency of the digestive enzymes (Dombrovskii et al., 1965; Platonova, 1970; Gabr and Said, 1972A; Gabr et al., 1972; Amitabha and Konar, 1973; Zakirov et al., 1974, 1975; Said, 1979 and 1981).

Summary

1. The enzymes of 1/10 high dose treated animals with tamaron showed a considerable reduction in their potencies. The potencies of pancreatic lipase and ileum maltase were the most reduced by tamaron, while that of ileum dipeptidase is of a lower reduction.

2. The enzymes of 1/100 high dose treated animals, showed a lower reduction in their potencies than that of 1/10 high dose treated animals. The potencies of pancreatic lipase and ileum maltase were the most reduced by tamaron, while that of ileum dipeptidase is still of a lower reduction.

3. The enzymes of 1/1000 high dose treated animals, showed a lowest reduction in their potencies as compared with their correspondings for 1/10 and 1/100 high dose treated animals.

4. The present study is of importance for clarifying one of the probable causes of digestive troubles widely observed nowadays.

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Table I

The potency of pancreatic lipase of tamaron treated white rat

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Digestive mixture 1.5 c.c. containing: 0.5 c.c. pancreatic extract (1:10), 0.125 gm (= 0.14 c.c.) olive oil, 0.5 c.c. 0.2N veronal-acetate buffer, 0.025 c.c. 2 % $CaCl_2$, 0.025 c.c. 3 % eggalbumen and 0.31 c.c. distilled water. Time of digestion one h. Temperature 37°C. pH value 7.2.

Condition	Titration (c.c. 0.1N KOH)		Potency c.c.0.01N KOH
	After 0 hour	After One hour	
Normal (control of 1/10 high dose)	1.65	2.32	0.67
Treated 1/10 high dose	1.68	1.80	0.12
Normal (control of 1/100 high dose)	1.70	2.35	0.65
Treated 1/100 high dose	1.73	2.12	0.39
Normal (control of 1/1000 high dose)	1.62	2.25	0.63
Treated 1/1000 high dose	1.68	2.11	0.43

Table 2

The potency of ileum dipeptidase of tamaron treated white rat

Digestive mixture 3.5 c.c. containing: 1.0 c.c. extract of ileum mucosa (1:10), 1.0 c.c. 2 % glycyl-glycine, 1.0 c.c. 0.1N veronal acetate - HCl buffer and 0.5 c.c. dist. H_2O.

Titration sample 0.5 c.c. Mean pH value 8.1. Temperature 37°C. Time of digestion one h.

Condition	Titration (c.c. 0.01N KOH		Potency c.c. 0.01N KOH
	After 0 hour	After One hour	
Normal (control of 1/10 high dose)	1.85	3.12	1.27
Treated 1/10 high dose	1.88	2.58	0.70
Normal (control of 1/100 high dose)	1.86	3.14	1.28
Treated 1/100 high dose	1.90	2.77	0.87
Normal (control of 1/1000 high dose)	1.96	3.21	1.25
Treated 1/1000 high dose	1.89	2.88	0.99

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Table 3

The potency of ileum maltase of tamaron treated white rat

Digestive mixture 4.0 c.c. containing: 0.4 c.c. extract of ileum mucosa (1:10), 2.0 c.c. 0.4 % maltose, 0.8 c.c. 0.1M ' veronal- acetate- HCl buffer and 0.8 c.c. dist. H_2O.

Titration sample 0.1 c.c. pH value 7.3. Temperature 37 37°C. Time of digestion 10 minutes.

Condition	Digestion(mg glucose) after 0 minute	Digestion(mg glucose) After 10 minutes	Potency mg glucose
Normal (control of 1/10 high dose)	0.165	0.273	0.108
Treated 1/10 high dose	0.170	0.221	0.051
Normal (control of 1/100 high dose)	0.158	0.264	0.106
Treated 1/100 high dose	0.160	0.226	0.066
Normal (control of 1/1000 high dose)	0.160	0.265	0.105
Treated 1/1000 high dose	0.168	0.244	0.076