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SOME OF BIOCHEMICAL CHANGES INDUCED BY THEOPHYLLINE AND FUROSEMIDE IN THE LAND SNAIL, *Monacha obstructa*

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ABSTRACT

The biochemical effects of two compounds i.e. theophylline and furosemide were studied on the glassy clover snail *Monacha obstructa.* Animals were treated with sub-lethal concentration (¼ LC_{50}) of each tested compound using contact (thin film) technique. Some biochemical parameters were measures at different periods after treatment.

Results showed that the two tested compounds gradually reduced the activity of aspartate amino transferase (AST) and peroxidase enzymes with the prolongation of periods after treatment, while the contrary occurred with alanine aminotransferase (ALT) as both compounds enhanced its activity proportionally than control. Concerning lactic acid dehydrogenase (LDH), results showed severe reduction in its level after 1, 2, 3 and 4 days post-treatment. Also catalase enzyme took adverse way as its activity increased in the 1^{st}, 2^{nd}, 3^{rd} and 4^{th} days for both compounds. On the other hand, levels of the total protein and total lipid were significantly or insignificantly decreased post-treatment with the two tested compounds.

INTRODUCTION

Land snails are injurious pests to a wide variety of field crops, vegetables, fruit, orchards and ornamental flowers and shrubs (Miller *et al.* 1988). These animals attack plants at their different growth stages and reduce their yields. The most effective control method is application by chemical compounds which cause bad health, environmental pollution and toxic effects on non- target organisms.

Some compounds e.g. theophylline and furosemide showed promising efficiency for control land snails (Khidr, 2010).Coffee and tea plants contain theophylline, another member of the Xanthine family, which bears structural and pharmacological similarity to "caffeine".

Theophylline is found in a very small amounts in tea but has a stronger effect on the heart and respiratory system than caffeine and is used as a bronchial dilator drugs in therapy for respiratory diseases under variety of brand names (Eldridge *et al.* 1983).

Therefore, the present study aimed to investigate the biochemical namely theophylline and furosemide on some enzymes, total protein and total lipid to throw a light on the mode of action of these chemicals in the terrestrial snail *Monacha obstructa.*

MATERIALS AND METHODS

Tested compounds:

- Theophylline: 1 H-purine-2, 6- dione, 3, 7- dihydro- 1, 3- dinuthyl..
- Furosemide: 4-chloro-N-C-2- Furylmethyl)-5- sulfamoyl onthronilic acid)
-The two compounds were obtained from Amriya company for Pharmacenutical Industries as pure powder.

Tested Animals:

Adult individuals of the glassy clover snail*, Monacha obstructa* were collected from untreated clover field at El-Remaly village, Qeuesna district, Menoufia Governorate. The snails were transferred to laboratory, kept in glass boxes and fed on fresh lettuce leaves (El-Deeb *et al*. 2003). For each treatment, 40 healthy snails were allocated and divided into four replicates, each of 10 individuals.

Contact application:

The snails were treated with sub-lethal concentration (¼ LC_{50}) of each compound using thin layer film technique according to Ascher and Mirian (1981), as the tested concentration i.e. 0.1% for theophylline and furosemide was applied in Petri-dishes using water (khidr, 2010). Two ml of each compound concentration were spread on inner surface of a petri-dish by moving the dish gently in circles. Water was evaporated under room conditions in a few minutes leaving a thin layer film of the tested compounds. The snails were exposed to the candidate concentration of both tested compound for 72h. A parallel control test was conducted using plain water.

Biochemcial studies:

Samples preparation:

Samples were prepared according to Bergrneyer (1963). Ten snails were homogenized for 3 minutes with 10ml of phosphate buffer, PH 7 at 1-4ºC and centrifuged at 3500 rpm for l0 minutes. The whole extract sample was cleaned through centrifugation several times. The extraction process takes not more than 24h. under cooling.

According to Ponting and Joslyn (1948) absorbencies at 530 nm were read within 30 second intervals at 20 C. The solution tested containing 2 ml tissue extract, 5ml acetate buffer pH 5.4 (0.02 M), 1 ml guaiacol (0.1 M), 1 ml hydrogen peroxide (0.5 M) and 1 ml distilled water. A blank was conducted containing distilled water instead of hydrogen peroxide.

Determination of AST and ALT:

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) was determined according to the method of Reitman and Frankel (1957) using commercial reagents.

Determination of LDH:

Lactic acid dehydrogenase (LDH) was assayed using the colorimetric method described by Cabaud and Wroblewski (1958).

Determination of total proteins and lipids:

Total proteins were colorimetrically determined according to Gornall *et al.* (1968), while total lipids were assayed by the method of Zollner and Kirsch (1962).

Determination of Peroxidase:

A direct colorimetric of guaiacol oxidation was used for each of the tested compound on some enzymes activities to clarify physiological response in the land snail *M. obstructa*. AST, ALT, LDH, total protein and total lipid were determined. Also, the oxidoreductase enzymes activities peroxidase and catalase were measured at different periods 1, 2, 3 and 4 days post-treatment. A paralled control test was conducted.

Determination of catalase:

A direct colorimetric method was used according to Saunders (1964). The solutions tested containing 1ml tissue extract, 1ml hydrogen peroxide 0.01 M (substract) and 5 ml phosphate buffer pH 6.8 (0.0067M) were cooled to 0ºC, 1ml of 2N sulphuric acid (this arrests peroxide decomposition) was added with vigorous stirring. The mixture was nitrated with 0.0005 N permanganate to the first persistent pink color.

Statistical analysis was done using the Student "t" test according to Snedecor and Cochran (1967) and Hill (1971)**.**

RESULTS AND DISCUSSION

Effect on AST, ALT and LDH enzymes:

Alterations in the activity of a separate aminotransferase (AST) and alanine aminotransferase (ALT) are known to be helpful in the diagnosis of hepatic diseases and infarcts of the heart. Data in Table (1) show the effect of sub-leathal concentration (¼ LC_{50}) of the two compounds theophylline and furosemide on the activity of AST, ALT and LDH enzymes in the land snail *M. obstructa*. Results indicate that theophylline treatment reduced the AST activity gradually with prolongation the period after treatment as the enzyme activity decreased than control with 21.8, 34.6, 47.4 and 53.8 % after 1, 2, 3 and 4 days post-treatment, respectively. Concerning the effect on the ALT enzyme, the contrary occurred as the same treatment raised the enzyme activity proportionally to 13.6, 20.3, 40.7 and 50.8 % after 1, 2, 3 and 4 days, respectively than control. Regarding furosemide compound, data took the same trend as AST activity reduced by27.6, 33.9, 35.9 and 39.7% than control after 1, 2, 3 and 4 days of treatment, respectively. On the other hand, the activity of ALT enzyme increased than control with 22.0, 54.2, 61.0 and 64.4 % after 1, 2, 3 and 4 days, respectively. Similar results were observed by Khidr *et al*. (2005) and Gabr *et al*. (2007). El Deeb *et al.* (2003) recorded that methomyl compound decreased the AST activity to 26.7, 26.7 and 13.3 % for *M. obstructa* after 24, 48 and 72h post-treatment, respectively. Tilkion *et al*. (1983) stated that the amount of AST was directly proportional to the number of cells damaged and the intervals after administration. Also, Amer *et al.* (1994) reported that the increase of AST and ALT activity may be referred to the diffusion of this enzyme from its intracellular sites due to damage caused by the insecticide on the sub-cellular level. In contrast, the decrease of the enzyme level may be due to either, the diffusion of these enzymes from the liver to the blood and then trough the kidney to outside with the urea or /and due to the decrease in its synthesis due to liver tissue disorders.

Table (1): Effect of ¼ LC_{50} of theophylline and furosemide on the activity of AST, ALT and LDH enzymes in *Monacha obstructa* at different periods post- treatment.

Days after treatment	Compound	AST (µ/L)		ALT(µ/L)		LDH(µ/L)	
		Mean	% Decrease	Mean	% Increase	Mean	% Decrease
1	Theophylline	12.2*	21.8	6.7*	13.6	90.3*	14.9
	Furosemide	11.3*	27.6	7.2*	22.0	87.2*	17.8
2	Theophylline	10.2*	34.6	7.1*	20.3	90.3*	14.9
	Furosemide	10.3*	33.9	9.1**	54.2	64.2**	39.5
3	Theophylline	8.2**	47.4	8.3**	40.7	67.7**	36.2
	Furosemide	10.0**	35.9	9.5**	61.0	57.7**	45.6
4	Theophylline	7.2**	53.8	8.9**	50.8	54.2**	48.9
	Furosemide	9.4**	39.7	9.7**	64.6	49.7**	53.2
Control		15.6	-	5.9	-	106.1	-

$$\% \text{ Decrease or Increase} = \frac{\text{Control-treated}}{\text{Control}} \times 100$$

*** Significant (*p< 0.05)* ** Highly Significant(p< 0.01)**

Regarding the lactic acid dehydrogenase (LDH), results showed that its level reduced by 14.9(17.8), 30.0(39.5), 36.2(45.6), and 48.9(53.2) % after 1, 2, 3 and 4 days of treatment, by theophylline and furosemide, respectively than control. LDH is an enzyme concerned with the reduction in the presence of reduced diphosphonuclotide (DPNH) of alpha –kito and alpha gamma dikito acids. LDH activity of serum, serous effusions and cerebrospinal fluid may be measured by the reduction in the presence of pyrovic acid to lactic acid. Alteration in the lactic dehydrogenase (LD) activity and serous effusions have been reported in various diseases. The measurement of LD activity may be helpful in the diagnosis and prognosis of myocardial infarction, acute hepatitis, leukemia, meningitis and other diseases (Cabaud and Wroblewski, 1958). Amer *et al.* (1994) mentioned that the increase in the activity of LDH might be due to the effect of the insecticide on the membranes of the intracellular organelles and on the membrane of the cell itself, increasing its permeability to the LDH enzyme which appeared in the liver at first and in the serum after wards. Also, they reported that LDH activity decreased in the liver tissue, while it increased in the serum. This quite expected since the insecticide may cause damage to the liver cells leading to the appearance of the enzyme in the serum.

Effect on total protein and total lipid:

Plasma protein serves as source for rapid deplacement of tissue proteins during tissue depletions, as buffers in acid base balance and as transporters for the constituents of the blood such as lipid, vitamins, hormones and certain enzymes. Also, lipids play extremely important roles in normal function of cell. Not only lipid serve as highly reduced storage forms

of energy, but they also play on intimate role in the structure of cell membranes and the organelles found in the cell (Wilson, 1986 and Warnick & Carter, 1972).

The effect of sub lethal concentration (¼ LC_{50}) of the two tested compounds on the total protein and total lipid in *M. obstructa* is shown in Table (2). Data showed that theophylline treatment insignificantly decreased total protein with 2.9, 5.7, 10.0 and 17.1 % after 1, 2, 3 and 4 days post-treatment, respectively. On the other side furosemide compound significantly decreased total protein by 8.6, 12.9, 21.4 and 27.1% compared to the control after 1, 2, 3 and 4 days of treatment, respectively. Concerning total lipid, the two tested compounds took one way as they significantly decreased total lipid with values of 46.4, 47.9, 49.3 and 55.0%, for furosemide comparatively with control after 1, 2, 3 and 4 days post-treatment, respectively. While non-significant changes were observed in total lipid of *M. obstructa* as affected by theophylline compound after the same periods.

The previous data proved that the fluctuation in the level of both total protein and total lipid might be resulted from in balance between the rate of synthesis and rate of degradation. Sexena *et al*. (1989) and Gabr *et al*. (2007) reported that the depression in total lipid may be due to decline in lipid synthesizing capacity and / or due to an increase in the hydrolysis of hepatic lipid to combat the stress conditions.

Table (2): Effect of ¼ LC_{50} of theophylline and furosemide on the total protein and total lipid in *M. obstructa* at different periods post-treatment.

Days after treatment	Compounds	Total protein (g/100ml)		Total lipid (g./100ml.)	
		Mean	% Decrease	Mean	% Decrease
1	Theophylline	0.68	2.9	0.88	37.1
	Furosemide	0.64*	8.6	0.79*	46.4
2	Theophylline	0.66	5.7	0.85	39.3
	Furosemide	0.61*	12.9	0.73*	47.9
3	Theophylline	0.63	10.0	0.78	44.3
	Furosemide	0.55*	21.4	0.71**	49.3
4	Theophylline	0.58	17.1	0.76	45.7
	Furosemide	0.51**	27.1	0.63**	55.0
Control		0.7	-	1.4	-

- ***Significant (*p< 0.05)* ** Highly Significant(p< 0.01)**

Effect on peroxidase and catalase activities:

The oxidoreductase enzymes peroxidase and catalase play an important role in the conversion of hydrogen peroxidase H_2O_2 to H_2O. The importance of this reaction is attributed to the toxic effect of H_2O_2 on the life cells. H_2O_2 attack the unsaturated fatty acids of the cell membrane causing its oxidation and injury. Data in Table (3) revealed the effect of sub-lethal concentration (¼ LC_{50}) of both tested compounds on the activity of respiratory enzymes (peroxidase and catalase). Results indicated that peroxidase activity gradually decreased with increasing the period after treatment with the two tested compounds.

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Peroxidase activity recorded 77.5(43.7), 63.4(81.2), 45.0(75.4) and 35.6(57.6) increase than normal levels for theophylline and furosemide after 1, 2, 3 and 4 days post-treatment, respectively. Theophylline proved more effective on peroxidase than furosemide as it reduced the enzyme activity with high ratio at the all tested periods. Concerning catalase enzyme, it showed adverse pattern as its activity increased gradually with 105.6 and 133.0, 161.9 and 152.8 and 155.6, 183.0 % for theophylline and vertemic compounds after 1, 2 and 3 days post treatment, respectively. Thereafter, it decreased to 107.7 and 135.9 at fourth day for both compounds, respectively to be approximately near the control range (100%).

AbdEl-Aal (2004), Gabr *et al*. (2007), found that the Niclosamide molluscicide increased the activity of peroxidase and catalase after 24h with 183.3% and 137.5%, respectively; then, peroxidase activity gradually decreased to reach 64.1% after 96h while catalase activity increased to reach its maximum 150% after 72h.,. thereafter decreased to 103.1% post 96h. From the previous results it could be concluded that theophylline was more effective than furosemide against *M. obstructa* in most tested biochemical parameters. This variation in the toxic effect may be due to type of compound and its chemical structure.

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دراسه بعض التغيرات البيوكيميائية الناتجة عن تاثيرالثيوفليين والفورزاميد فى القوقع الارضى موناكا ابستراكتا

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أجرى هذا البحث لدراسة التغيرات البيوكيميائيةفى قوقع البرسيم الزجاجى بعد تعرضه للتركيز التحت مميت (¼ LC50) لكل من مركب الثيوفليين والفورزاميد وذلك بطريقة الملامسة واخذت القياسات على فترات مختلفة من المعاملة.

وقد تبين من النتائج أن كلاً من الثيوفليين والفورزاميد قد أحدث انخفاض تدريجى فى انزيم البيروكسيديز وانزيم (AST) مع زيادة الفترة بعد المعاملة بينما حدث العكس مع انزيم (ALT) حيث ارتفع نشاطه طردياً مقارنة بامعاملة الضابطة وكذلك سجل انخفاض شديد فى النشاط الأنزيمى (LDH) بعد فترة المعاملة باربعة أيام بينما اتخذ انزيم الكتاليز مساراً عكسيا تجاه الإزدياد لكل من الثيوفليين والفورزاميد خلال فترات المعاملة0

من ناحيةاخرى أشارت النتائج إلى حدوث انخفاض معنوى فى محتوى البروتين والدهون للفيورزاميد بينما أظهر الثيوفليين انخفاض غير معنوى لكلا من المادتين بعد فترة المعاملة .

قام بتحكيم البحث

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Table (3): Effect of ¼ LC_{50} of theophylline and furosemide on peroxidase and catalase activities in *M. obstructa* at different periods post-treatment.

Compound	Enzyme	Control		Days post-treatment							
		O.D	% activity	1		2		3		4	
		O.D	% activity	O.D	% activity	O.D	% activity	O.D	% activity	O.D	activity
Theophylline	Peroxidase	0.191	100	0.148	77.5*	0.121	63.4**	0.086	45.0**	0.068	35.6**
	Catalase	0.142	100	0.15	105.6*	0.189	133*	0.23	161.9*	0.153*	107.7
Furosemide	Peroxidase	0.191	100	0.179	93.7	0.155	81.2	0.144	75.4*	0.11	57.6**
	Catalase	0.142	100	0.217	152.8*	0.221	155.6**	0.26	183.0*	0.193	135.9

O.D = optical density
% Activity = OD (treated)/ OD (control)x 100
*** Significant (P < 0.05)** highly significant (P < 0.01).**