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Effcts of the herbicide gallant and mercury on liver function of *Tilapia zilIi*

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Abstract

Background: Herbicides are highly toxic for both human and animal health. The increased application of herbicides in agriculture during the last decades has resulted in the contamination of both soil and water. Also do heavy metals, which represent one of the most important group of pollutants produced as a result of many industrial activities which can find their way easily to the normal aquatic environments, disturbing and damaging the existing organisms.

Material and Methods: Fishes of the species *Tilapia zillii* were exposed to sublethal concentration of herbicide Gallant (haloxyfop-ethopxy ethanol ester) (3 mg/L), mercury as mercuric chloride (6 mg/L) and a combined dose of herbicide (1.5 mg/L) and mercury (3 mg/L) for 96 hrs., in aquaria under controlled laboratory conditions. A comparative physiological study was carried out to test the toxicological effects of these pollutants on glutamic pyruvic transaminase (GPT), glutamic oxaloacetic transaminase (GOT) activities & hepatosomatic index of liver.

Results: A progressive decrease in enzyme activity as well as total protein content of liver were observed, while hepatosomatic index showed a slight insignificant increase.

Key words: Gallant, mercury, glutamic pyruvic transaminase & glutamic oxaloacetic transaminase activities and hepatosomatic index of liver.

Introduction

Modern agricultural practices in USA and many other countries have resulted in nearly unrivaled efficiency and productivity of pesticides and herbicides. Unfortunately, there is also the potential for release of these compounds to the environment and consequent adverse effects on wildlife and human populations (Dalton and Frick, 2008).

Numerous herbicides are now widely used in many parts of Egypt to control wide variety of grass. Gallant is active against a very wide range of grass weeds. Many of the treated areas contain fresh water resources like streams, lakes and ponds which harbour diverse aquatic fauna and flora.

Pollution by mercury represents a serious problem in the framework of chemical pollution of aquatic environment due to its high ability to accumulate in fish organs (Portmann, 1972; Scott and Armstrong, 1972 and Saleh, 1982) and subsequently may affect the people who feed on fish.

Numerous environmental pollutants including herbicides, fungicides, rodenticides, pesticides (organochlorine, organophosphorus, carbamate and pyrethroid) as well as heavy metals, have been reported to alter liver function (Cashman, 1990; Riviere *et al.,* 1990 and Saleh *et al.,* 1991).

Intensive studies concerning the toxic effects of herbicide gallant on fish organs are lacking. Therefore, the present study was undertaken to elucidate the deleterious effects of herbicide gallant, mercury and a combined dose of both gallant and mercury on liver function of the *Tilapia zillii*.

Material and methods

Experimental animals

Tilapia zillii fishes weighing ~ 36 g were obtained from Tawarga pond and transported immediately to adequate laboratory conditions (temperature 24 ± 1 ˚C & pH 7.5) for at least two weeks in 60 liters glass aquaria.

Preliminary toxicity tests were conducted in the laboratory under static conditions.

Chemical pollutants

1- Gallant, which is a formulated herbicide, was purchased from Dow Chemical Company, Midland, Michigan, USA. It contains the active ingredient 2-ethoxyethyl, 2-[4-(3-chloro-5-trifluoromethyl-2-pyridyloxy) phenoxy]-propionate ; known by the common name haloxyfopethoxyethyl ester or haloxyfop-EE. Code Name Dow Co. 453.
2- Mercury as mercuric chloride was purchased from Dow chemical Company, Midland, Michigan, USA.
3- All other chemicals and reagents were of analytical grade.

Expermintal protocol

Fishes were divided randomly into four groups, 36 fishes for each. The first group was kept in fresh untreated Tawarga pond's water and used as control. The second group was exposed to a sublethal dose of herbicide gallant (3 mg/L). The third one was treated with a sublethal dose of mercury 6 mg $HgCl_2$/L). A combination of half sublethal doses of the above pollutants was performed for the fourth group.

Sampling and tissue extract

Batches of 6 fishes form each group were taken, dissected at intervals of 6, 12, 24, 48, 72 and 96 hrs. postinitial exposure. Representative samples of liver were immediately removed, homogenized in distilled water to make 10% homogenate (W/V) and kept in deep freezer at -40 ◌̊C till subsequent biochemical analysis.

Methods

The method used for the determination of GPT & GOT was that described by Sabath *et al*., (1968). Total protein content was determined according to the method of Cabib and Polacheck (1984). The gutted weight of every fish was recorded. Its liver was removed and weighed. Finally, the hepatosomatic index (HSI) was calculated by the following equation formulated by Jangaard, 1967.

HSI = Wt of liver in gram × 100/Gutted Wt of fish in gram.

Statistical analysis

The data were statistically analyzed using the t-test (Parker, 1973). Results were expressed as the mean ± SE for six measures. Significance was considered at a level of $P< 0.05$.

Results

The effects of the herbicide gallant, or the heavy metal, mercury or the combination of both toxicants on GOT & GPT activities showed a significant progressive decrease when compared with the control values throughout the periods of exposure. Mercury solitary decreased the GOT activity more than the other treatments (Table 1); while the GPT activity was largely affected by mercury and gallant combination (Table 2). Similar results were obtained by Oser (1965), Street (1970), Krample (1971), El-Elaimy (1981) and Asztalos *et al.*, (1988). They attributed this reduction to hepatocellular damage.

The hepatic total protein content was significantly decreased during all the periods of experiment, except at 6 and 12 hrs intervals of exposure (mercury and gallant separately) they were both insignificant (Table 3). Similar decreases in total proteins were found with pesticides in rats (Choudhari and Chakrabarti, 1983); in cockerels (Mohiuddin and Ahmed, 1986); in pigeons and chickens (Khalifa *et al.*, 1989; Saleh *et al.*, 1991) and in fishes Bansal *et al.*, 1979).

The effect of all treated pollutants on hepatosomatic index of fish showed a slight insignificant increase with exception to the significant decrease during the last two intervals of exposure to mercury & gallant combination and the significant increase at the last period of exposure to gallant alone (Table 4).

Table 1: Liver glutmamic oxaloacetic transminase (GOT) activity (U/g wet tissue) of different fish groups.

Experimental groups	Duration in hours					
	6h.	12h.	24h.	48h.	72h.	96h.
Control	149.82±5.51	149.49±6.53	151.49±3.48	147.82±8.05	148.66±6.97	141.66±7.07
Herbicide gallant	55.42±2.21*	58.07±3.10*	59.67±0.91*	61.82±3.24*	64.00±2.58*	65.78±2.30*
Mercuric chloride	46.25±1.06*	50.25±2.60*	51.32±1.15*	40.24±1.35*	38.92±1.73*	25.57±2.02*
Gallant+ mercuric chloride	60.16±2.66*	65.24±4.14*	61.49±4.01*	67.24±2.45*	67.74±2.13*	51.07±1.50*

All results are expressed as mean ± SE of six fishes.

- statistically significant (P<0.05)

Table 2: Liver glutamic pyruvic transminase (GPT) activity (U/g wet tissue) of different fish groups.

Experimental groups	Duration in hours					
	6h.	12h.	24h.	48h.	72h.	96h.
Control	137.74±2.98	138.49±2,59	140.67±0.88	140.32±2.47	143.33±3.72	143.49±2.84
Herbicide gallant	24.01±1.66*	30.01±1.66*	49.82±0.40*	55.02±2.41*	59.01±4.07*	62.32±3.65*
Mercuric chloride	15.01±2.03*	26.50±3.05*	28.16±1.39*	60.65±3.65*	49.01±3.53*	37.32±2.80*
Gallant+ mercuric chloride	36.49±4.67*	32.32±4.18*	28.19±0.75*	17.32±2.54*	16.49±1.01*	12.32±0.75*

All results are expressed as mean ± SE of six fishes.

* statistically significant (P<0.05)

Table 3: Liver total protein content (mg/g wet tissue) of different fish groups.

Experimental groups	Duration in hours					
	6h.	12h.	24h.	48h.	72h.	96h.
Control	189±9.5	180±10.0	170±6.3	180±9.8	180±9.7	170±8.2
Herbicide gallant	190±8.6	150±18.0	130±10.0*	130±3.3*	120±20.0*	50±5,6*
Mercuric chloride	170±15.0	140±24.0	60±4.7*	50±5.6*	50±6.1*	50±5.3*
Gallant+ mercuric chloride	100±8.5*	60±5.5*	80±12*	110±12.0*	60±8.9*	50±5.6*

All results are expressed as mean ± SE of six fishes.

* statistically significant (P<0.05)

Table 4: Hepatosomatic index (HSI) of different fish groups.

Experimental groups	Duration in hours					
	6h.	12h.	24h.	48h.	72h.	96h.
Control	0.9968 ± 0.0874	1.3096 ± 0.1319	1.2579 ± 0.1439	0.9351 ± 0.0653	1.1074 ±0.0672	0.9899 ± 0.0385
Herbicide gallant	1.0574 ± 0.0949	1.0690 ± 0.1473	1.2202 ± 0.0752	1.1524 ± 0.2037	1.236 ± 0.0824	1.2351 * ± 0.0824
Mercuric chloride	1.1766 ± 0.2279	1.0336 ± 0.1670	1.2364 ± 0.1801	1.2157 ± 0.1884	1.2626 ± 0.1466	1.1089 ± 0.0441
Gallant+ mercuric chloride	1.2034 * ± 0.0116	1.4949 ± 1.408	1.1575 ± 0.1937	0.9291 ± 0.1142	0.7994 * ± 0.0599	0.8561 * ± 0.0405

All results are expressed as mean ± SE of six fishes.

statistically significant (P<0.05)

Discussion

The severe reduction in GOT & GPT activity in the liver of the treated fishes with mercury alone or the combination between mercury & gallant could be attributed to the high accumulation of mercury in the liver of fish (Saleh 1982 and Mazhar *et al.*, 1986b). The last author demonstrated that, liver damage was proved by aggregations of RBCs, dilatation and congestion of blood vessels. Hepatocellular degeneration progressed to vacuolar necrosis with inflammatory and haemo-rrhagic lesions. This was accompanied by anastomosis of hepatic circulation and increase in Kupffer cells

The herbicide and heavy metals caused a destructive effect on the lysosomal membranes of the liver cells, followed by a release of proteases and other protein splitting enzymes, which destroyed some important intracellular proteins, but did not convert them into their amino acid components. Meanwhile, there might have occurred an increased uptake of selective amino acids from the liver to rebuild these compounds for the repair processes of cells, thus accounting for the decrease in the protein content in liver of treated fishes. Previous results of earlier workers corrolate with this explanation (Awasthi *et al.*, 1984).

The importance of the transamination system lies not only in the breakdown of amino acid, but also in their biosynthesis. Thereby a direct relationship existed between the liver GOT and GPT activities and total protein content during the present study.

The slight increase in HSI values in the present data was referred to the accumulation of pollutants as suggested by many authors (Kendall, 1977 and Mazhar *et al.*, 1986a, b).

Saleh (1982) reported that, the pollution of the aquatic environment causes an increase in the hepatosomatic index. This probably means that the HSI could be used as a quick indicator for detecting pollution in the aquatic environments. As a matter of fact, the amount of pollutants in the fish liver is directly proportional to the degree of pollution in the aquatic environment by heavy metals and pesticides. Accordingly, the hepatosomatic index may be considered as a valid indicator for this type of pollution.

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تأثير مبيد الحشائش جالنت والزئبق على وظائف الكبد للسمكة البلطي من نوع *Tilapia zillii*

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لقد صممت هذه الدراسة لفحص التأثير السام لمبيد الحشائش جالنت وكذلك كلوريد الزئبق على الأسماك من نوع *Tilapia zillii* بمتوسط وزن قدره 36 جم, بعد ذلك تم تقسيم 144 سمكة عشوائيا إلى أربع مجموعات -بواقع 36 سمكة لكل مجموعة- كالتالي:-
أ - مجموعة طبيعية ضابطة.
ب - مجموعة معاملة بمبيد الحشائش جالنت بجرعة قدرها 3مجم/لتر ماء.
ت -مجموعة معاملة بكلوريد الزئبق بجرعة قدرها 6مجم/لتر ماء.
ث -مجموعة معاملة بكل من مبيد الحشائش وكلوريد الزئبق كالتالي: 1.5 مجم جالنت/لتر + 3مجم من كلوريد الزئبق/لتر ماء.
وقد تم أخذ 6 سمكات عشوائيا أيضا من كل مجموعة عند الفترات الزمنية التالية 6, 12, 24, 48, 72, 96 ساعة من بداية التجربة. حيث شرحت كل سمكة سريعا وتم فصل الكبد وذلك لعمل 10% (وزن/حجم) من مطحون الكبد لكل سمكة على حدة وذلك لتقدير نشاط إنزيمات الترانس امينيز GPT , GOT بالإضافة إلى المحتوى البروتين الكلى للكبد.

ولقد خلصت الدراسة الحالية على الأتى:-
1 - انخفاض دال في مستوى إنزيمات الترانس امينيز GPT , GOT في كبد الأسماك عند كل الفترات الزمنية للتجربة فى مجموعة الأسماك المعلجة بمبيد الحشائش وكلوريد الزئبق وكذلك فى المجموعة المعالجة بكل من مبيد الحشائش + كلوريد الزئبق.
2 - انخفاض دال في مستوى البروتين الكلي في كبد الأسماك عند الفترات 24, 48, 72 و 96 ساعة بينما كان الانخفاض غير معنوى عند الفترتين 6, 12 ساعة فى كل المجموعات المعالجة.
3 - ارتفاع غير معنوى في المعامل الكبدي للاسماك عند كل فترات التجربة ماعدا عند 96 ساعة فى المجموعة المعالجة بمبيد الحشائش جالنت, وعند 6, 72, 96 ساعة في المجموعة المعالجة بكل من مبيد الحشائش + كلوريد الزئبق فقد كان الارتفاع معنويا.