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Effect of Sublethal Concentrations of Glyphosate and Dalapon on Protein and Aminotransferase Activity in *Pseudosuccinea columella*

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Pseudosuccinea columella (Lymnaea sp.) serves as a intermediate snail host of Fasciola hepatica, the sheep liver fluke. Fasciola hepatica's incidence is directly related to the incidence of the lymnaeid snail. The continuing rise of liver fluke infections in domestic ruminants such as cattle, sheep, goats and other vertebrates including humans has attracted much attention (Santiago and Hillyer 1988). The economic implications of this infection are very high and cause millions of dollars in losses each year.

Glyphosate is a broad spectrum herbicide, with high water solubility (1.2% at 25^0 C). It is one of the most important agricochemical discoveries of this century (Grossbard and Atkinson 1985). It is relatively nonselective and very effective on deep-root perennial species of grasses, sedges, and broadleaf weeds (Herbicide Handbook, 1989). It is very specific for plants and is relatively non toxic in animal species. However, Thompson et al. (1989) determined its LD_{50} in P. columella to be 98.9 ppm. Dalapon is a widely used herbicide for control of annual and perennial grasses. It has been used in sugarcane, sugarbeets, corn, potatoes, grapes, and many other crop and noncrop lands (Herbicide Handbook 1989). It is an aliphatic acid herbicide with high water solubility (110 g/100 mL at 22^0 C); LD_{50} in P. columella 98.7 ppm (Thompson et al. 1989).

The runoff of herbicides into the aquatic ecosystem and the tendency of aquatic organisms to accumulate these herbicides in their tissues have led to increasing investigations (Grossbard and Atkinson 1985). The exposure of non-target organisms such as P. columella to these herbicides is enhanced by their water solubility and extensive usage in the environment. These chemicals may directly affect these non-target organisms by causing morphological, physiological, immunological and biochemical changes.

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The objective of this study is to determine the effects of sublethal concentrations of glyphosate and dalapon on some biochemical functions of P. columella, namely its aminotransferase activity and protein content. Proteins exhibit functional versatility and therefore utilize a variety of biological roles. Some functions of proteins include transport, metabolic control, contraction and catalysis of chemical transformations. Transamination is involved in the synthesis of amino acids, in the degradation of most amino acids and in the exchange of amino groups. The hepatopancreas of snails is damaged extensively during larval trematode infections, and the aminotransferase index is a means of detecting this damage (Monohar 1972). Quantitation of protein and transaminase activity may serve as good diagnostic tools in determining the effects produced by xenobiotics and/or parasitic organisms on living systems.

This study will further elucidate and add new dimensions to our knowledge of the metabolic changes occurring in P. columella snails as a result of chronic exposure to glyphosate and dalapon.

MATERIALS AND METHODS

Experimental and control snails, Pseudosuccinea columella were obtained from laboratory at 3 wk of age. They were maintained in the laboratory by culturing in containers containing 1000 mL of artificial spring water (water hardness = 80-120 mg/L measured as $CaC0_3$, DO = 6-8 mg/L, pH = 6.5-8.5 and ammonia nitrate levels were maintained at 2 mg/L). Snails were exposed to sublethal concentrations of dalapon or glyphosate ranging from 1-10 mg/L (pH 6.8-7.2). All snails were fed endive lettuce leaves ad libitum and each bucket aerated (Christian and Tate, 1982; Thompson 1989).

The herbicides, glyphosate (97%) and dalapon (98.5%), were obtained from Chem Service, West Chester, Pennsylvania. A 1% stock solution in distilled water of each herbicide was prepared fresh every 3 mon and kept at 25°+ 2°C. The appropriate dilutions as needed were made from the stock solution (1-10 mg/L). Each herbicide concentration were measured and placed in fleakers (1000 mL). The herbicide solution was poured into culture containers and 10 snails were placed into each concentration and maintained. The snails laid eggs and the immature snails developed within the herbicide solutions. Hatched snails were allowed to mature in the herbicide solution.

Pseudosuccinea columella snails (4 wk old) reared in sublethal

concentrations of glyphosate and dalapon (1-10 mg/L) were randomly selected from laboratory cultures. The snails were removed from solutions, and placed on ice to desensitize them. They were immediately deshelled, weighed, and homogenized in 0.24 M Tris-HCL buffer (pH 7.4). The homogenate was diluted 1:50 with Tris-HCL buffer and served as the enzyme source. Six standards were prepared utilizing Sigma calibration standard solution (pyruvic acid). The samples were also prepared according to Sigma procedures for aspartate aminotransferase (AST)/glutamic oxaloacetic transaminase (GOT), and alanine aminotransferase (ALT)/glutamic pyruvic transaminase (GPT). Into sample test tubes, 1.0 mL of substrate (D-L aspartate and alpha-ketoglutaric acid or D-L alanine and alpha-ketoglutaric acid) were pipetted. The tubes were warmed in a water bath to 37°C. The tubes were removed from the bath and 0.2 mL of snail body fluid was added to the substrate. The tubes were placed back into the water bath and incubated for 1 hr for GOT, and 30 min for GPT. The tubes were removed at the specified time and 1.0 mL of Sigma color reagent was added to each tube and allowed to incubate for 20 min at room temperature. Spectrophotometric analysis utilizing a Shimadzu 2101 PC UV -Vis scanning spectrophotometer, was performed at 550 nm. The absorbance was then converted to Sigma-Frankel (SF) units, (one SF unit of GOT and GPT will form 4.82 x 10^{-2} μmol glutamate/minute at pH 7.5 and 25° C) based on the standard curve obtained. The DeRitis quotient was determined from a ratio of GOT:GPT.

The protein contents of P. columella snails, reared in 1-10 mg/L of glyphosate and dalapon respectively were determined. Snails reared in artificial spring water served as controls. For each concentration, approximately 20 snails were randomly selected from the laboratory cultures, and according to the methods of MacInnis and Voge, (1970) isolation of protein fractions were obtained via precipitation into various macromolecules. Snails were deshelled and the wet weight of the sample obtained. The tissues (1 g) were homogenized in 10 mL of ETOH. After centrifugation for 5 min at 500 rpm, 20 mL of chloroform-methanol was added. After vigorously shaking and centrifuging (5 min at 500 rpm), the supernatant was decanted and to the pellet 10 ml of 10% trichloroacetic acid (TCA) as added to precipitate the protein. The supernatant was decanted, and 10 mL of cold TCA was added to the pellet to further precipitate proteins. Protein concentration was estimated following the method of Lowry et al. 1951. The amount of protein in each sample was calculated as μg/mL of sample based on the standard curve.

Statistical analysis (analysis of variance and Duncan's multiple range test) were performed on all experimental data.

RESULTS AND DISCUSSION

The results presented in Table 1 show that the overall average aspartate aminotransferase activity (AST/GOT) increased significantly in all snails reared in glyphosate or dalapon for 4 wk compared with controls. However, the alanine aminotransferase (ALT/GPT) activity in snails reared in glyphosate decreased but, there were variable responses in activity in snails reared in dalapon.

Results show that there was an overall unequal increase in the total aminotransferase activity in the body fluid of snails exposed to glyphosate and dalapon. Both herbicides caused a slight decline in aminotransferase activity (GOT and GPT) in snails reared in 0.1 and 10.0 mg/L. The most significant increases were seen at the lowest concentration of glyphosate (0.1 ppm). The highest activity was observed at the lowest concentration (0.1 mg/L) for both herbicides which may indicate, that lower concentrations of these chemicals are more readily absorbed and metabolized by these snails. At 10 mg/L the aminotransferase (GOT) activity is slightly higher than at 1.0 mg/L glyphosate, but not significantly higher; however, the activity in both groups were significantly decreased from those snails reared in 0.1 mg/L. These results may indicate that over an extended period of time, there is a saturation of the toxification system in P. columella at about 1.0 mg/L for glyphosate and/or dalapon. This would explain the lack of consistent dose dependent effects of increasing concentrations of glyphosate and dalapon. There was also an increase in GPT activity in dalapon treated snails at 1.0 mg/L causing a decrease in the De Ritis quotient while there is a decrease in GPT activity in glyphosate treated snails. This suggests that there may be a subsidiary role played by this aminotransferase on exposure to these herbicides. As noted by Hamen (1968) the transamination reaction is probably the most important pathway in the metabolism of many amino acids. Transamination not only serves as a pathway for conversion of alpha-keto acids to L-amino acids, but also as an alternative means of replenishing the pyruvate pool (Monohar 1972). Of the two aminotransferases studied, the GOT activity is significantly increased ($p < 0.05$), thereby raising the De Ritis quotient to values above 1.0 in all treated animals (Tables 1). Values of this quotient greater than one (1.0) are usually indicative of hepatic injury in humans. However in snails it may be an assessment of how the

Table 1. Average protein concentration and aminotransferase activity in Pseudosuccinea columella reared in sublethal concentrations of glyphosate and dalapon.

Treatment Groups	GOT (SF Units) Glyphosate -- Dalapon	GPT (SF Units) Glyphosate--Dalapon	DeRitis (GOT:GPT) Quotient Gly. -- Dal.	Protein Conc. (µg/mL) Gly. -- Dal.
Control	110.2 ± 11.0 -- 110.4 ± 10.9	76.5 ± 5.1 -- 76.5 ± 5.1	1.4 -- 1.4	467.3 -- 467.0
0.1 mg/L	171.3 ± 22.1[ab] -- 241.0 ± 4.8[ab]	50.3 ± 6.1[a] -- 79.7 ± 4.8[a]	3.4 -- 3.0	538.7 -- 504.7
1.0 mg/L	130.0 ± 18.2[a] -- 210.7 ± 7.3[ad]	42.0 ± 2.5[a] -- 131.3 ± 11.9[acd]	3.1 -- 1.6	603.3[a] -- 495.3
10.0 mg/L	137.7 ± 17.4[a] -- 159.0 ± 10.5[a]	53.6 ± 8.1[a] -- 70.6 ± 6.4	1.6 -- 2.3	513.0 -- 494.0

Values for GOT and GPT are expressed as mean of nine trials ± SD.
Values for protein concentration are expressed as µg/ mL.
Gly = glyphosate and Dal = dalapon.
[a] Significantly different from the control ($P<0.05$).
[b] Significantly different from 1.0 and 10 ppm ($P<0.05$).
[c] Significantly different from 0.1 ppm ($P<0.05$).
[d] Significntly different from 10 ppm ($P<0.05$).

hepatopancreas of the snail is being affected by these herbicides or this may be a measure of the extent to which these herbicides may prove to be metabolic burdens to the snails at lower concentrations by causing them to drain their stores of glycogen and amino acids. This phenomenon is observed during larval trematode infections of snails (Monohar 1972). The concentration of protein in treated snails appears to decrease slightly as the concentration of both herbicides (glyphosate and dalapon) increases (Table 1). There is however, an increase observed in protein concentration of snails reared in 1.0 mg/L glyphosate. This increase may be related to the significant decrease in transaminase (GOT) activity observed in snails reared in 1.0 mg/L glyphosate compared with those reared in 0.1 mg/L glyphosate. Therefore, it may be possible that, at 1.0 mg/L, protein catabolism is maximized allowing the concentration of protein (denatured) to be increased in the snail homogenate. The exact mechanism for this response has not been determined, and further studies are necessary to determine why this occurred.

This study was undertaken to show that changes exist and therefore, there is a need for further studies to evaluate the potential usage of these herbicides as controls for F. hepatica .

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