EFFECT OF EUPHORBIA PEPLUS PLANT EXTRACT AND THE ANTIHELMENTHIC PRAZEQUANTEL ON THE DEFENCE SYSTEM OF BIOMPHALARIA ALEXANDRINA SNAIL

Ibrahim B. Helal, Mostafa H. ELMehlawy, ElSayed T. Rizk, and Gehan M.EL-Khodary
Zoology Department, Faculty of Science, Tanta University, Tanta, Egypt*

Keywords: Plant molluscicide Euphorbia peplus, Praziquantel, Haemocytes, defence system, B. alexandrina

ABSTRACT

The effect of sublethal concentrations of E. peplus water suspension and praziquantel water solution on the defense system of Biomphalaria alexandrina was examined by laboratory experiments.

The mean number of haemocytes decreased insignificantly after the first week of snail exposure to the plant extract. Thereafter, a significant increase occurred after the second week of exposure. Treatment with praziquantel showed a significant increase in haemocytes after the 3rd and 4th weeks of exposure. Haemocytosis represents a response to external stress or certain stimuli. The haemocytes showed behavioral as well as structural changes after long exposure to the tested molluscicides. The cells had many cytoplasmic inclusions and produce many long spike-like pseudopodia. Haemocytes exhibited an enhanced tendency to aggregate in clusters. Such alterations may be indicative of a state of haemocytes activation. The total plasma protein concentration after treatment with E. peplus and Praziquantel increased plasma than in control snails while no changes in protein concentration of the digestive gland extract. This may be a result of an imbalance in the rate of protein synthesis and the rate of its degradation.

The treatment with E. peplus and Praziquantel caused a marked increase in acid phosphatase after the 2nd and 4th weeks. On the other hand, alkaline phosphatase activity increased after the 4th week. This could be explained as defensive and lysosomal activity of the haemocytes. After treatment with the tested substances the plasma and digestive gland extract were capable to agglutinate sheep red
blood cells coated with miracidial and cercarial antigens at high titers during the whole period of the experiment. Such agglutinins may have a protective function, perhaps through precipitating the toxic molecules produced by such substances and / or helping to mitigate the impaired haemocytes function.

**INTRODUCTION**

*Euphorbia peplus.* is an annual wild herb common in Egypt and belongs to family Euphorbiaceae. The molluscicidal activity of this plant has been studied, using different parts of the plant and extraction processes (Shoeb *et al.,* 1982; El-Nahhas 1988 and Rizk, 1998). Meanwhile, Praziquantel (4H pyrazino (2.1-a) isoquinoline -4-one, 2-cyclohexyl carbonyl 1. 2, 3, 6, 7, 11 b hexahydro) is an antischistosomal drug, most commonly used in Egypt. Its action on schistosomes is mainly on the tegument which is known to be the protective layer of the worm. Few attempts on using of praziquantel as a molluscicide are very scarce. Taema (1996) recommended its use against *B. alexandrina* and showed that its solution caused 100 % mortality at a concentration of 100 ppm after 72 hr on both *B. alexandrina* and *Lymnaea* sp. Mostafa and Gad (1997) found that 25 mg/g of Praziquantel induced high rates of snail mortality (3.8 % survival after 6 weeks). Its found activity was time-dependent and when exposure was increased from 24 to 72 hours the LC90 values decreased from 205 to 130 ppm.

Circulating haemocytes react against foreign bodies, digest and transport nutrients, accumulates various toxic substances such as heavy metals, pesticides, molluscsides (Malker and Chong, 1974). These cells are also involved in adsorption and digestion of food in some tissues and in removal of residual materials from cells and organs of the body (Yonow and Renwrantz, 1986).

Circulating haemocytes represent the primary mediators of cellular defense reactions in molluscs. The biochemical mechanism (s) by which molluscan haemocytes recognize foreign matter appears to depend, at least in part, on; (1) Homologous plasma opsonic proteins, especially those with lectin-like properties (Yang and Yoshino, 1990 b). (2) The presence of haemocyte membrane-associated molecules which are capable of binding directly to foreign particles (Coustau and Yoshino, 1994).

Some studies have been made on the composition of plasma of molluscs (Michelson, 1966; Lee and Cheng 1972 and Gress and
EFFECT OF E. PEPLUS PLANT EXTRACT AND THE PRAZEQUANTEL ON B. ALEXANDRINA

Cheng, 1973). Such studies revealed that the plasma of B. glabrata includes a number of protein fractions. The changes in the number and concentrations of protein and amino acids were regarded to be immune factors of snails (Friedle, 1961).

The snail's total protein concentration was changed by heavy metals (Tolba et al., 1997). The total protein content was recorded to be decreased by prolonged exposure to some plant molluscicides as Calendula micrantha (Rawi et al., 1996), Abrus precatorius, Agremone mexicana and Nerium indica (Singh and Singh 1999). On the other hand, El-Emam and Ebeid (1989) showed that the natural molluscicide (Calendula) and the synthetic molluscicide (Mollotox) increased the total protein concentrations of B. alexandrina. Rawi et al. (1993) reported that the sub-lethal concentrations of the pesticides lannate 90, Cuprosan 311 and Dithane M45 decreased the protein content in haemolymph of B. alexandrina and increased with the use of Diuron, 2,4-D and DDT. El-Mehlawy and Rizk (2000) observed a marked increase in total protein concentration of B. alexandrina under the effect of diazinon for 25 days.

Among the constituents of humoral immunity are the lysosomal hydrolases. Hydrolytic enzymes detected in haemocytes from gastropods are alkaline and acid phosphatase, peroxidases, lysozyme, glucuronidase, lipase, aminopeptidase and amylase (Cheng et al., 1978, Cheng and Butler 1979; Granath and Yoshino, 1983 a and b; Cheng and Dougherty, 1989). Changes of hydrolase enzyme activity affect the snails haemocytes reaction against the molluscicidal treatment. El-Emam and Ebeid (1989) showed (Molotox) and Calendula caused a significant increase of acid phosphatase in the haemolymph of B. alexandrina.

Singh et al. (1992) found that, the latex of some Euphorbiales reduces the acid and alkaline phosphatases in the nervous tissue of Lymnaea acuminata. Al-Sharkawy et al. (1996) reported that the alkaline phosphatase of B. alexandrina digestive gland decreased after exposure to Ammi majus. Atlam (2000) stated that B. alexandrina haemocytes after acute exposure to E. peplus showed lower activity of both acid and alkaline phosphatase. Finally, El-Mehalawy and Rizk (2000) showed a decrease in alkaline phosphatase activity and increase in acid phosphatase after long-term exposure of B. alexandrina to diazinon.

A standard technique for measuring the defence response of invertebrate haemolymph is the agglutination assay (Loker et al.,
Many invertebrates possess circulating multivalent factors capable of agglutinating vertebrate erythrocytes and other target cells. This activity is often associated with molecules with lectin properties. In some cases the agglutinins showed opsonic activity when tested against relatively small particles such as bacteria and yeast (Yang and Yoshino. 1990 a, b). Thus, the present study was conducted to evaluate the toxic effect of these two substances on the snail defense system and its possible response to *Schistosoma mansoni* larval stages.

**MATERIAL AND METHODS**

Ninety healthy adult *B. alexandrina* snails (size 7.5 – 8.5 mm) were obtained from “Teodor Bilharse institute – Department of snail control” (Embaba- Cairo) for this test. Snails were exposed to 20 ppm of *E. peplus* water extract and 10 ppm of Praziquantel water solution. For each of above concentrations, snails were used at a density of 10 snails per liter in 3 replicates for a period of 4 weeks.

Snail haemolymph samples were taken by cardiac puncture as described by Loker and Hertel (1987) from ten exposed and unexposed snails (controls) at 7,14, 21 and 30 days post-exposure. The haemolymph collected from both treated and control snails was centrifuged at 10000 g for 10 min (Zelck et al., 1995 ). The plasma was stored in a freezer at 10 °C until used. The number of haemocytes/mm³ of haemolymph was counted in a Bürker-Turk haemocytometer (Van der Knaap et al., 1981). Monolayers of haemocytes were prepared and stained with Giemsa’s stain for 20 min according to the method of Abdul-Salam & Michelson (1983). A minimum of 100 haemocytes in a 2-mm field of view was counted for each snail and the percentage of each category was calculated.

Miracidial and cercarial antigens were prepared according to the methods described by Heyneman *et al.* (1971). The total water-soluble protein of miracidia antigen was 5.4 μg/ml and the total water-soluble protein of cercariae antigen was 1.5 μg/ml. Extracts of the digestive gland was prepared, in phosphate buffer solution (PBS) at pH 7.4, according to the method described by Michelson (1964).

The indirect haemagglutination test (IHA) shows good reproducibility and high sensitivity for detection of helminths antibodies, where sheep erythrocytes (SRBCs) coated with the particulate antigen were used as target cells as described by Bing *et al.* (1967) and modified by Preston and Duffus (1975).
Acid phosphatase (AcP) was estimated according to the method of Kind and King (1954) using test kits purchased from Biomerieux Sa, France. Alkaline phosphatase (AkP) was estimated according to the method of Weichtselaum, (1946) using test kits from the above source.

Colourmetric determination of total protein content was estimated according to the principle of Lowery et al. (1951).

The analysis of variance (ANOVA) test was performed to find out the significance of two factors effects.

RESULTS

The number of haemocytes changed significantly with time (P< 0.02) in control snails and those exposed to sublethal concentration of *E. peplus* water suspension and praziquantel water solution. Moreover, the number of haemocytes changed significantly (P = 0.000) after treatment with the two substances. Furthermore, interaction between the two factors had also significant (P = 0.000) effect on the number of these cells.

The mean values of control snails varied from 187 to 262 cells/mm$^3$ from the 1st to the 4th week of experiment (Table 1, Fig. 1). The number of haemocytes of snails treated with *E. peplus* water suspension decreased insignificantly to 102 cells/mm$^3$ after the 1st week of exposure. Thereafter, a significant increase by about 3 folds occurred after the 2nd week of exposure. Then, the number of haemocytes fluctuated around the control value after the 3rd and 4th week. In contrast, haemocytes of snails treated with praziquantel water solution began to increase significantly, over that of the control, after the 3rd and 4th week of exposure reaching 332 and 360 cells/mm$^3$ respectively.

As seen in several monolayers of haemocytes, some cells remained round while others spread over the glass surface and formed more and large pseudopods. The present investigation revealed the presence of three morphological distinct types of haemocytes namely; granulocytes, hyalinocytes and round cells. Granulocytes were the most common type. They were characterized by their large size and the presence of prominent pseudopods. The cytoplasm was slightly basophilic and contained few granules and various vacuoles (Figs 4 A&C). The second type, the hyalinocytes had thin, relatively short filopodia and a well spread hyaline cytoplasm surrounding a central
nucleus (Fig. 1 B). The third type includes some undifferentiated or round cells with very small size and a spherical nucleus surrounded by basophilic cytoplasm, in which no granules or vacuoles were seen (Fig. 4 A).

The exposure of snails to *E. peplus* water suspension and Praziquantel water solution for two weeks showed behavioral as well as structural changes in the haemocytes. The cells had many cytoplasmic inclusions. They produced many long, spike–like pseudopodia and spread greatly over the glass surface (Fig. 5).

Moreover, after exposure of snails to *E. peplus* water suspension and Praziquantel water solution for longer time (4 weeks), haemocytes exhibited an enhanced tendency to aggregate in clusters and interconnected by the tips of their spikes of pseudopods (Fig. 6).

Exposure of snails to either *E. peplus* or praziquantel elevated the plasma total protein (Table 2). The marked elevation was noticed after 2 weeks of exposure to the tested molluscicides, where the concentrations of plasma protein after treatment with *E. peplus* water suspension and praziquantel were increased by about 30%. In comparison to the control values, no valuable changes in protein concentrations of digestive gland extracts were noticed. Meanwhile, marked changes in acid and alkaline phosphatase activities of the haemolymph were reported as shown in Table (2) the activity levels of acid phosphatase in control snails were 0.776 and 0.918 U/L at the second and fourth weeks respectively. After treatment with *E. peplus* water suspension, an increase in the activity of the enzyme reached 1.746 and 1.4307 U/L respectively, while, after treatment with Praziquantel water solution, an increase in the acid phosphatase activity reached 1.3025 U/L after the second week and 1.6401 U/L after the fourth week of exposure.

On the other hand, the alkaline phosphatase activity of haemolymph decreased by 57.3 % and 96 % at the second week after treatment with *E. peplus* and praziquantel water suspensions respectively. At the fourth week, the activity of the alkaline phosphatase increased to 8.74 and 5.66 U/ 100 ml respectively. The enzyme level of control snails was 4.27/U/100 ml.

Analysis of variance of haemagglutination assays performed on the examined snails during the whole period of experiments showed that the two treatments induced a significant increase in haemagglutinins of snail haemolymph and digestive gland extract against both miracidial and cercarial antigens over the control values.
EFFECT OF *E. PEPLUS* PLANT EXTRACT AND THE PRAZIQUEXANTEL ON *B. ALEXANDRINA*

However, the haemagglutination titers were not affected with time or with combination of time and treatment.

After treatment with *E. peplus* water suspension, the plasma was capable to agglutinate sheep red blood cells (SRBCs) coated with miracidial and cercarial antigens at high titers (*P* < 0.01) during the whole period of the experiment, while the plasma of control group agglutinated SRBCs at low titers (Figs 2 A&B). The mean value of the log10 plasma haemagglutinins of treated snails varied from 2.41 to 2.91 against both miracidial and cercarial antigens (Tables, 4&5). Moreover, *E. peplus* water suspension induced the digestive gland extract to agglutinate SRBCs coated by miracidial and cercarial antigens at high titers (*P* < 0.03). In treated snails, the Log10 digestive gland haemagglutinins increased from 0.7 to 1.71 against miracidial antigen and from 0.4 to 2.01 against cercarial antigen at the first week of exposure respectively (Figs. 3 A&B).

The effect of praziquantel water solution on the agglutinating activity of haemolymph and digestive gland extract of *B. alexandrina* against miracidial and cercarial antigens is presented in Tables (4 and 5). The obtained results showed that Log10 haemolymph haemagglutinins of treated snails ranged from 2.11 to 2.81 for miracidial antigens and from 2.51 to 2.91 for cercarial antigens. While, the Log10 haemagglutinins of the digestive gland extract varied from 1.17 to 1.81 against miracidial antigens and from 1.71 to 2.01 against cercarial antigens.

**DISCUSSION**

Molluscan haemocytes are known to play a major role in immunological defense reactions (Cheng *et al.*, 1975; Sminia *et al.*, 1983; Ottoviani, 1989; Giamberini *et al.*, 1996 and Borges and Andrade, 2003). The chronic effect of *E. peplus* showed a slight decrease in circulating haemocytes after the first week of exposure. This decrease may be due to haemolysis, as described by Atlam (2000), after the acute exposure to *E. peplus*. After the second week of exposure to *E. peplus* and after the third and fourth week of exposure to Praziquantel, significant increases in haemocytes were observed. A similar increase in circulating haemocytes was detected by haemolymph withdrawal (Sminia, 1972), by urethane treatment (Granath and Yoshino, 1985), by temperature (Stumpy and Gilbertson, 1978), and by infection (Van der Knaap *et al.*, 1987 and...
Haemocytosis in snails represents a response to external stress or certain stimuli and may originate from a variety of biotic (e.g., infection) or abiotic sources (Wolmarans and Yssel, 1988). This haemocytic response may be due to stimulation of the haematopoietic organ to produce a number of haemocytes (Lie et al., 1975) and/or a strong mobilization of existing tissue haemocytes into the circulation (Coustau and Yoshino, 1994).

The treatment with *E. peplus* and praziquantel increased the cytoplasmic inclusions, spread size and formation of aggregates of *B. alexandrina* haemocytes. Such alterations may be indicative of a state of haemocytes activation. Similar observations were demonstrated by Abdul-Salam and Michelson (1980), Van der Knaap et al. (1987), Boehmler et al., (1996) after trematode infections. The increase of cytoplasmic inclusions of the granulocytes in the present results may be a sign of lysosomal activity of the cell. Feng et al. (1971) reported that the electron - dense granules of the granulocytes could be considered as lysosomes with hydrolase enzymes concerning with intracellular digestion of phagocytic materials in the cells of *Crassostrea virginica*. The formation of aggregates of haemocytes may be due to the attraction and arrangement of these cells around the extracellular substances which were derived from toxic materials, as described by Abdul-Salam and Michelson (1980) during larval trematode infection.

The present results showed an increase in the total plasma protein of *B. alexandrina* after treatment with *E. peplus* and praziquantel but no changes in protein concentrations of the digestive gland extract. The increased total protein concentrations reflect the elevated levels of other serum enzymes (Cheng and Dougherty, 1989). Similar results were reported in snails treated with organophosphorus compounds (El-Mehlawy and Rizk, 2000). Rawi et al. (1993) interpreted the increase in protein content after exposure to DDT, 2, 4-D and Diuron might be a result of an imbalance in the rate of synthesis and the rate of degradation of protein.

After the exposure of the two tested molluscicides, the snail haemolymph showed higher elevation of acid phosphatase after 2nd and 4th weeks of exposure and alkaline phosphatase after the 4th week only. These increases are correlated with the granular increase and lysosomal activity of the haemocytes while the cytoplasmic inclusions of these cells may be lysosomes containing hydrolytic enzymes. This increase of the enzymatic activity agrees with the hypothesis that the increase of hydrolase synthesis during
phagocytosis enhances digestion and lysing of engulfed materials. Moreover, the increases in acid and alkaline phosphatase activity indicate that these enzymes may participate the defense mechanism system of snails (Cheng and Bulter, 1979). The increase in acid phosphatase was supported by Singh and Agarwal (1989). They concluded that its increase after snail treatment by anticholinesterase compounds may be due to the direct action on cell and lysosomal membranes thus releasing acid phosphatase. Also, Al-Sharkawy et al. (1996) showed that A. meyus caused a marked increase of alkaline phosphatase activity in the haemolymph. This increase may be a consequent of cell damage exposed to the plant extract as a result of increased cytotoxic oxygen radicals. So increased haemolymph activities of the enzymes could be a result of their release from damaged cell. Therefore, it can be speculated that the toxic substances in E. peplus and Praziquantel have a similar action.

Moreover, the present results indicated that E. peplus and praziquantel induced high titers of snail agglutinins specific to miracidial and cercarial antigens, in plasma and digestive gland extracts. These elevated plasma agglutination titres correlated with the elevation of circulating haemocytes were similarly noticed by Loker et al. (1987) and Arreguin-Espinosa et al. (2001).

Haemocytes are likely candidates to produce molecules with agglutinating activity (Van der Knaap et al., 1981). From the available literatures, the mechanism of elevated hemagglutinins under the influence of molluscicides have not been studied before. Yet, such agglutinins may have protective function, perhaps through precipitating the toxic molecules produced by the tested molluscicides and/or helping to mitigate the impaired haemocytes function. Similar findings were demonstrated by Loker et al. (1992) after infection of B. glabrata with Echinostoma paraenset

REFERENCES


EFFECT OF *E. PEPLUS* PLANT EXTRACT AND THE PRAZIQUENLTEL ON *B. ALEXANDRINA*


Sminia, T. (1972). Structure and function of blood and connective tissue cells of the freshwater pulmonate Lymnaea stagnalis studied by electron microscopy and enzyme histochemistry. Z. Zeilforsch., 130:452-497.


### Table (1): Effect of exposure of E. peplus water suspension and Praziquantel water solution on the total number of haemocytes/mm$^3$ of B. alexandrina.

<table>
<thead>
<tr>
<th>Exposure Period (week)</th>
<th>Control Mean no. of haemocytes/mm$^3$ ± SD</th>
<th>Treated with E. peplus Mean no. of haemocytes/mm$^3$ ± SD</th>
<th>Treated with Praziquantel Mean no. of haemocytes/mm$^3$ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week</td>
<td>1.87x10$^2$ ± 5.97</td>
<td>1.4x10$^2$ ± 5.64</td>
<td>2.36x10$^2$ ± 7.64</td>
</tr>
<tr>
<td>2nd week</td>
<td>6.69</td>
<td>6.28</td>
<td>6.97</td>
</tr>
<tr>
<td>3rd week</td>
<td>7.36</td>
<td>7.43</td>
<td>6.43</td>
</tr>
<tr>
<td>4th week</td>
<td>8.64</td>
<td>7.45</td>
<td>6.23</td>
</tr>
</tbody>
</table>

* Significant at P < 0.01

### Table (2): Effect of exposure to E. peplus water suspension and Praziquantel water solution on the total protein in the plasma and digestive gland extract of B. alexandrina.

<table>
<thead>
<tr>
<th>Exposure Period (week)</th>
<th>Control Plasma Total Protein</th>
<th>Treated with E. peplus Plasma Total Protein</th>
<th>Treated with Praziquantel Plasma Total Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week</td>
<td>5.9</td>
<td>6.28</td>
<td>5.64</td>
</tr>
<tr>
<td>2nd week</td>
<td>6.23</td>
<td>4.95</td>
<td>7.45</td>
</tr>
<tr>
<td>3rd week</td>
<td>7.42</td>
<td>7.64</td>
<td>6.43</td>
</tr>
<tr>
<td>4th week</td>
<td>8.64</td>
<td>7.45</td>
<td>6.23</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exposure Period (week)</th>
<th>Control Digestive Gland Total Protein</th>
<th>Treated with E. peplus Digestive Gland Total Protein</th>
<th>Treated with Praziquantel Digestive Gland Total Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week</td>
<td>6.61</td>
<td>6.84</td>
<td>7.45</td>
</tr>
<tr>
<td>2nd week</td>
<td>4.545</td>
<td>4.78</td>
<td>5.88</td>
</tr>
<tr>
<td>3rd week</td>
<td>7.45</td>
<td>7.63</td>
<td>6.43</td>
</tr>
<tr>
<td>4th week</td>
<td>6.64</td>
<td>6.84</td>
<td>7.45</td>
</tr>
</tbody>
</table>

Significant at P > 0.01

Mean no. of haemocytes/ mm$^3$ ± SD

EFFECT OF E. PEPLUS EXTRACT AND THE PRAZIQUENTAL ON B. ALEXANDRINA

Table (1): Effect of exposure of E. peplus water suspension and Praziquantel water solution on the total number of haemocytes/mm$^3$ of B. alexandrina.

Table (2): Effect of exposure to E. peplus water suspension and Praziquantel water solution on the total protein in the plasma and digestive gland extract of B. alexandrina.
Table (3): Effect of exposure of *B. alexandrina* to sublethal concentrations of *E. peplus* water suspension and Praziquantel water solution on the levels of acid and alkaline phosphatase of haemolymph.

<table>
<thead>
<tr>
<th>Factors</th>
<th>Acid Phosphatase (U/L)</th>
<th>Alkaline phosphatase (U/100 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2nd week</td>
<td>4th week</td>
</tr>
<tr>
<td>Control</td>
<td>0.77</td>
<td>0.9182</td>
</tr>
<tr>
<td>Treated with <em>E. peplus</em></td>
<td>1.746</td>
<td>1.4307</td>
</tr>
<tr>
<td>Treated with Praziquantel</td>
<td>1.3025</td>
<td>1.6401</td>
</tr>
</tbody>
</table>

Table (4): Log10 haemagglutination titers of haemolymph and digestive gland extract treated with *E. peplus* water suspension and Praziquantel water solution against miracidial antigens.

<table>
<thead>
<tr>
<th>Exposure Period (week)</th>
<th>Control haemolymph</th>
<th>Control digestive gland extract</th>
<th>Treated with <em>E. peplus</em> haemolymph</th>
<th>Treated with <em>E. peplus</em> digestive gland extract</th>
<th>Treated with Praziquantel haemolymph</th>
<th>Treated with Praziquantel digestive gland extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>1&lt;sup&gt;st&lt;/sup&gt; week</td>
<td>0.3±0.3</td>
<td>0.7±0.17</td>
<td>2.71±0.52*</td>
<td>1.71±0.17*</td>
<td>2.11±0.3*</td>
<td>1.17±0.17*</td>
</tr>
<tr>
<td>2&lt;sup&gt;nd&lt;/sup&gt; week</td>
<td>0.5±0.35</td>
<td>0.3±0.3</td>
<td>2.51±0.46*</td>
<td>1.8±0.3*</td>
<td>2.61±0.46*</td>
<td>1.61±0.17*</td>
</tr>
<tr>
<td>3&lt;sup&gt;rd&lt;/sup&gt; week</td>
<td>0.6±0.3</td>
<td>0.3±0.3</td>
<td>2.41±0.6*</td>
<td>1.61±0.17*</td>
<td>2.71±0.3*</td>
<td>1.81±0.3*</td>
</tr>
<tr>
<td>4&lt;sup&gt;th&lt;/sup&gt; week</td>
<td>0.3±0.3</td>
<td>0.1±0.17</td>
<td>2.91±0.17*</td>
<td>1.3±0.17*</td>
<td>2.81±0.35*</td>
<td>1.17±0.35*</td>
</tr>
</tbody>
</table>

*Significant at P < 0.001
Table (5): Log10 haemagglutination titers of haemolymph and digestive gland extract treated with *E. peplus* water suspension and Praziquantel water solution against cercarial antigens.

<table>
<thead>
<tr>
<th>Exposure Period (week)</th>
<th>Control</th>
<th>Treated with <em>E. peplus</em></th>
<th>Treated with Praziquantel</th>
<th>Treated with <em>E. peplus</em></th>
<th>Treated with Praziquantel</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week</td>
<td>0.5±0.17</td>
<td>0.4±0.17</td>
<td>2.7±0.3*</td>
<td>1.8±0.3</td>
<td>2.5±0.46*</td>
</tr>
<tr>
<td>2nd week</td>
<td>0.6±0.3</td>
<td>0.8±0.46</td>
<td>2.9±0.17*</td>
<td>1.8±0.3</td>
<td>2.8±0.35*</td>
</tr>
<tr>
<td>3rd week</td>
<td>0.7±0.46</td>
<td>0.6±0.3</td>
<td>2.9±0.17*</td>
<td>1.7±0.3</td>
<td>2.8±0.17</td>
</tr>
<tr>
<td>4th week</td>
<td>0.6±0.3</td>
<td>0.9±0.3</td>
<td>2.9±0.17*</td>
<td>1.7±0.3</td>
<td>1.7±0.46</td>
</tr>
</tbody>
</table>

Significant at P < 0.04
EXPLANATION OF FIGURES (2-5).

Fig. (2). Control snail (haemocytes stained with Giemsa)
   A) Granulocytes (g) and undifferentiated cells (u). X400
   B) Hyalinocytes (h) X250
   C) Granulocytes (g) X100

Fig. (3). Haemocytes of treated snail for 2 weeks showed many cytoplasmic inclusions and long pseudopodia.
   A) Haemocytes of treated snail with *E. peplus* X1000
   B) Haemocytes of treated snail with *E. peplus* X1000
   C) Haemocytes of treated snail with Praziquantel. X1000.

Fig. (4). Haemocytes of treated snail after 4 weeks of exposure
   A) Haemocytes of treated snail with *E. peplus*. X400
   B) Haemocytes of treated snail with Praziquantel. X1000.
(Fig. 1): Number of *B. alexandrina* haemocytes after treatment with *E. peplus* water suspension and praziquantel water solution.
Fig. 2: Haemaglutination titres of *Biomphalaria alexandrina* digestive gland after treatment with *E. peplus* water suspension and praziquantel water solution against miracidial and cercarial antigen.
(Fig. 3): Haemaglutination titres of *Biomplaria alexandrina* plasma after treatment with *E. peplus* water suspension and praziquantel water solution against meracidial and cercarial antigen.
Fig. (4): Control snail (haemocytes stained with Giemsa)
A) Granulocytes (g) and undifferentiated cells (u). X400.
B) Hyalinocytes (h). X250  
C) Granulocytes (g). X100
Fig. (5): Haemocytes of treated snail for 2 weeks showed many cytoplasmic inclusions and long pseudopodia.  
A) Haemocytes of treated snail with *E. peplus* X1000.  
B) Haemocytes of treated snail with *E. peplus* X1000.  
C) Haemocytes of treated snail with Praziquantel. X1000.
Fig. (6): Haemocytes of treated snail after 4 weeks of exposure
A) Haemocytes of treated snail with *E. papulosis*. X400.
B) Haemocytes of treated snail with Praziquantel. X1000.