Egypt. J. Aquat. Biol. & Fish., Vol.7, No.4 : 501- 527 (2003) *ISSN 1110 – 6131*

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 LC_{90} 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, molluscicides (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

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 (Mollotox) 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.*,

1994). 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/mm^3 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 Weitchselaum, (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 numbers 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 3[illegible] 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 spead 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 repoted 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.

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

Helal and Abd El-Maksoud.1999). 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 verginica.* The formation of aggregates of haemocytes may be due to the attraction and arrangment 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 2^{nd} and 4^{th} weeks of exposure and alkaline phosphatase after the 4^{th} 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. mcyus* 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

Abdul-Salam. J. M. and Michelson, E. H. (1980). *Biomphalaria glabrata* amoebocytes: Effect of *Schistosoma mansoni* infection on *in vitro* phagocytosis. J. Invertebr. PathoL. *35*:241-248.

Abdul-Salam, J. M. and Michelson, E, H. (1983). *Schistosoma mansoni* : immunoflourescent detection of its antigen reacting with *Biomphalaria glabrata* amoebocytes. Exp. Parasitol., 55 : 132 - 137.

Al-Sharkawy. I. M.; Mansour, M. A. and Tabl. G. A. (1996). Preliminary laboratory investigation of the molluscicidal activity of some widdy distributed in the Nile Delta. J. Egypt. Ger. Soc. Zool., 20 (A) : 245 -266.

Al-Sharkawy, I. M. and Rizk. E. T. (1996). Comparative study on the effecacy of *Ammi mqjus* water extract in the control of *Biomphalaria alexandrina*, *Bulinus truncatus* and *Lymnaea caillaudi* and its lethality to some non target species. J. Union Arab Biol., Cairo, *6*(A)Zool. : 577- 597.

Arreguin-Espinosa, R. ; Fenton, B. ; Vazquez-Contreras, E. ; Arreguin, B. and Garcia-Hernandez, E. (2001). PFA, a novel mollusk agglutinin, is structurally related to the ribosome-inactivating protein superfamily. Arch. Biochem. Biophys., *15*:394(2).151-155.

Atlam. A. E. (2000). Comparative studies on the toxic effect of some plant molluscicides on the target and non-target fresh water snails. Ph. D. thesis, Faculty of Science, Tanta University. Tanta, Egypt.

Bing. 0. H. ; Weyand. J. G. M. and Stavitsky. A. B. (1967). Haemagglutination with aldehyde fixed erthrocytes for assay of antigens and antibodies. Proc. Soc. Exp. Bio.Med., *124*:1166- 1170.

Boehmler. A. M. ; Fryer, S. E. and Bayne. C. J. (1996). Killing of *Schistosoma mansoni* sporocysts by *Biomphalaria giabrata* hemolymph *in vitro* : alteration of hemocyte behavior after poly -L- lysine treatment of plastic, and kinetics of killing by different host strains. J. ParasitoL. *82* : 332 - 335.

Borges C. M. and Andrade Z. A. (2003). Extra-cellular matrix changes in *Schistosoma mansoni*-infected *Biomphalaria glabrata*. Mem. Inst. Oswaldo Cruz., *98*(1).135-139.

Cheng. T. C. and Butler. M. S. (1979). Experimentally induced elevations in acid phosphatase activity in hemolymph of *Biomphalaria glabrata* (Mollusca). J. Invertebr. Pathol. *34*:119 - 124.

Cheng, T. C. and Doughertg. W. J. (1989). Ultrastructural evidence for the destruction of *Schistosoma mansoni* sporocysts associated with elevated lysosomal enzyme levels in *Biomphalaria glabrata*. J. Parasitol., *75*(6) : 928-941.

Cheng. T. C. ; Guida. V. G. and Gerhart. P. L. (1978). Aminopeptidase and lysosome activity levels and serum protein concentrations in *Biomphalaria glabrata* (Mollusca) challenged with bacteria. J. Invert. Pathol., *32*: 297 - 302.

Cheng. T. C. ; Rodrich. G. E. ; Foley. D. E. and Koehler, S. A. (1975). Release of lysosome from hemolymph cells of *Mercenaria mercenaria* during phagocytosis, J. Invertebr. Pathol., *25*: 261 - 265.

Coustau, C. and Yoshino, T. P. (1994). Surface membrane polypeptides associated with hemocytes from *Schistosoma mansoni* ~ susceptible and - resistant strains of *Biomphalaria glabrata* (Gastropoda). J. Inverteber. Pathol., *63*: 82-89.

El-Emam. M. A. and Ebeid, F. A. (1989). Effect of *Schistosoma mansoni* infection, starvation and molluscicides on acid phosphate. transaminases and total protein in tissues and hemolymph of *Biomphalaria alexandrina*. J. Egyptian Soc. ParasitoL., *19*(1): 139-147.

El-Mehlawy. M. H. and Rizk. E. T. (2000). Toxicity of the organophosphorous pesticide diazinon on schistosomiasis snail vector *Biomphalria alexanderina*. Proc. Int. Conf. Biol. Scien., *1*(2):2000 : 153 - 162.

El-Nahhas, H. A. (1988). Effects of some plants and their extracts on intermediate hosts of Schistosomiaisis and Fascislasis. M. Sc. Thesis. Faculty of Science. Menoufia University, Menoufia. Egypt.

Feng. S. Y. ; Feng. J. S. ; Burke. C. N. and Khairallah, L. H. (1971). Light and electron microscopy of the leukocytes of *Crassostrea virginica*. Z. Zeilforsch., *120*: 222 - 245.

Friedl. F. E. (1961). Studies on larval Fascioloides magna. IV . Chromatographic analysis of free amino acids in the haemolymph of a host snail. J. parasitol., *45*:773 - 776.

Giamberini, L. ; Auffret. M. and Pihan. J. C. (1996). Haemocytes of the freshwater mussel, *Dreissena polymorpha* : cytology. cytochemistry and X- ray microanalysis. J. Moll. Stud., *62*:367 - 379.

Granath. W. 0. and Yoshino, T. P. (1983a). Characterization of molluscan phagocyte subpopulations based on Lysosmal markers. J. Exp. ZooL, *226*: 205 - 210.

Granath, W. 0. and Yoshino. T. P. (l983b). Lysosomal enzyme activities in susceptible and refractory strains of *Biompholaria glabrata* during the course of Infection with Schistosoma mansoni. J. Parasitol., *69*:1018- 1026.

Granath. W. 0. and Yoshino. T. P. (1985). *Biomphalaria glabrata* (Gastropoda) : Effect of urethane on the morphology and function of haemocytes, and on susceptibility to *Schistosoma mansoni* (Trematoda]. J. Inv. Pathol., *45*: 324-330.

Gress. F. M. and Cheng, T. C. (1973). Alteration of total serum proteins and protein fractions in *Biomphalaria glabrata* parasitized by *Schistosoma mansoni*. J. Invertebr. Pathol., *22*: 382 - 390.

Heyneman, D. : Faulk, W. P. and Fudenberg, H. H. (1971). *Echinostoma lindoense*: larval antigens from the snail intermediate host, *Biomphalaria glabrata*. Exp. Parasitol., *29*:480-492.

HelaL B. B. and Abd El-Maksoud. Y. D. (1999). Studies on the internal defense system in *Lymnaea natalensis* the snail intermediate host of *Fasciola gigantica*. Egypt. J. Zool., 32: 303-318.

Kind, P. R. and King, E. G. (1945). Colorimetric determination of alkaline and acid phosphatase activaties. J. Clin. Path. 7:322.

Lee. F. 0. and Cheng. T. C. (1972). Alteration of total protein and hemoglobin in the hemolymph of infected *Biomphalaria glabrata*. Exp. ParasitoL, *31*: 203-216.

Lie. K. J. ; Heyneman. D. and Yau, P. (1975). The origin of amebocytes in *Biomphalaria glabrata*. J. Parasitol., *63*: 574 - 576.

Loker. E. S. ; Cimino. D. F. and Hertel, L. A. (1992). Excretory-secretory products of *Echinostoma paraensei* sporocytsts mediate interference with *Biomphalaria glabrata* hemocytes functions. J. Parasitol., *78*:104-115.

Loker. E. S. ; Couch, L. and Hertel. L. A. (1994). Elevated agglutination liters in plasma of *Biomphalaria glabrata* exposed to *Echinostoma paraensei*: Characterization and functional relevance of a trematode- induced response, parasitol., *108*:17-26.

Loker, E. S. and Hertel. L. A. (1987). Alterations in *Biomphalaria glabrata* plasma induced by infection with the digenetic termatode *Echinostoma paraensei*. J. Parasitol., *73*:503 - 513.

Lowery, O. H. ; Roseprough, N. J. ; Farr. A. L. and Randall, R. J. (1951). Protein measurment with folin phenol reagent. J. Biol. Chem., *193*:265-275.

Malker, E. A. and Chong. T. C. (1974). Medical and economical malacology ". Academic Press. New York.

Michelson, E. H. (1964). Miracidia-immobllizing substances in extracts prepared from snails infected with *Schistosoma mansoni*. Am. J. Trop. Med. Hyg., *13*:156- 170.

Michelson, E. H. (1966). Characterization of the hemolymph antigens of *Australorbis glabratus* by disk electrophoresis and immunoelectrophoresis. Ann. Trop. Med. Parasitol., *60*: 280-287.

Mostafa. B. B. and Gad. H. S. M. (1997). Effect of UV-irradiation ، gamma irradiation and praziquantel on infected *Biomphalaria alexandrina* snails. J. Egypt. Soc. Parasitol., *27*(1). 35-46.

Ottaviani, E. (1989). Haemocytes of the freshwater snail *Viviparus ater* (Gastropoda, Prosobranehia). J. Moll. Stud., *55*:379-382.

Preston. J. M. and Duffus, W. P. H. (1975). Diagnosis of *Schistosoma bovis* infection in cattle by an indirect haemagglutination test. J. HelminthoL, *49*: 9 - 17.

Rawi. S. M. ; El-Gindy. H. I. and Abd-El-Kader. A. (1993). The effect of some molluscicides on total protein .albumin, and total lipid of the snail *Biomphalaria alexandrina*. J. Egypt. Ger. Soc. ZooL, *(13)*D : 273 - 288.

Rawi. S. M. ; El-Gindy. H. I. and Abd-El-Kader. A. (1996). New possible molluscicides from Calendula micrantha officinalis and Ammi majus. II Molluscicidal .physiological, and egg-laying effects against *Biomphalaria alexandrina* and *Bulinus truncatus*. Ecotoxicol. Environ. Saf.. *35*(3): 261 - 267.

Rizk E. T. (1998). Schistosomiasis control : evaluation of the molluscicidal activity of a plant extract (*Sesbania sesban*) against *Biomphalaria alexandrina*. J. Egypt. Ger. Soc. Zool., *27*(D) :91 - 107.

Shoeb. H. A. ; El-Emam. M. A. and Osman, N. S. (1982). The molluscicidal activity of Euphorbiaceae. 3- *Euphorbia peplus*. Egypt J. Bilh., *9*(1) : 41- 54.

Singh. A. ; Agarwal, R. A. ; and Singh, A. (1992). Toxicity of the latex of Euphorbiales. Effect on acid and alkaline phosphatases of the snail *Lymmaea acuminata*. Biol. Agricult, Horicult., *88*(3) : 211 - 219.

Singh, D. K. and Agarwal, R.A. (1989). Toxicity of piperonyl butoxide - carbaryl synergism on the snail *Lymnaea acuminata*. Int. Revue. Ges. Hydrobiol., *74*(6): 689 - 699.

Singh, S. and Singh, D. K. (1999). Effect of molluscicidal components of *Arbus precatorius*, *Argemone mexicana* and *Nerium indicum* on certain biochemical parameters of *Lymnaea acuminata*. Phytother. Res., *13*(3): 201-213.

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.

Sminia, T. ; Van Der Knaap, W. P. and Van Asselt. L. A. (1983). Blood cell types and blood cell formation in gastropod mollusks. Dev. Comp. Immnol., *7*:665-668.

Stumpy. J. L. and Gilbertson, D. E. (1978). Hemocytes of *Biomphalaria glabrata* : Factors affecting variability .J. Invert. Pathol., *32*: 177-181.

Taema, A. (1996). Effect of antihelmenthiic drugs on different stages of some digenetic trematodes and their intermediate hosts. M. Sc. thesis. Faculty of Science, Tanta University, Tanta n Egypt.

Tolba. M. R. ; Beder. M .and Mossa. M. (1997). Effect of some heavy metals on respiration, mean enzyme activity and total protien of the pulmonate snails *Biomphalaria alexandrina* and *Bulinus truncatus*. J. Egypt. Ger. Soc. Zool., *24*(D): 17 - 35.

Van der Knaap. W. P. W. ; Boerrigter-Barendsen. L. H. ; Van der Hoeven. D. S. P. and Sminia, T. (1981). Immunocytochemical demonstration of a humoral defence factor in blood cells (amoebocytes) of the Pond-snail, *Lymnaea stagnalis*. Cell and Tissue Res.296 -219,291 .

Van der Knaap, W. P. W. ; Meuleman. E. A. and Sminia, T. (1987). Alternations in the internal defence system of the pond snail *Lymnaea stagnalis* induced by infection with schistosome *Trichobilharzia ocellaia*. ParasitoL Res., *73*: 57 - 65.

Weichselbaum, T. E. (1946). Determination of total protein in serum. Am. J. Clin. Path., *16*:40.

Wolmarans. C. T. and Yssel. E. (1988). *Biomphalaria glabrata* : Influence of selected abiotic factors on leukocytosis .J. mvertebr. Pathol., *51*:10-14.

Yang. R. and Yoshino, T. P. (1990 a). Immunorecognition in the freshwater bivalve, *Corbicula flummea*. I. Electrophoretic and immunologic analysis of opsonic plasma components. Dev. Comp. ImmunoL, *14*:385 -395.

Yang. R. and Yoshino. T. P. (1990 b). Immunorecognition in the freshwater bivalve, *Corbicula Jiuminea* II. Isolation and characterization of plasma opsonin with hemagglutinating activity. Dev. Comp. ImmunoL, *14*:397 - 404.

Yonow. N. and Renwrantz. L. (1986). Studies on the haemocytes of *Acteon tomat*. His. J. Moll. Stud., *52*:150- 155.

Zelck, U. E. ; Becker, W. and Bayne, C. J. (1995). The plasma proteins of *Biomphalaria glabrata* in the presence and absence of *Schistosoma mansoni*. Dev. Comp. Immunol., *19*(3):181-194.

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*.

Exposure Period (week)	Mean no. of haemocytes / mm^3 ± SD		
	Control	Treated with *E. peplus*	Treated with Praziquantel
1st week	$1.87x10^2$±57.95	$1.02x10^2$±10.41	$3.09x\ 10^2$±76.53
2nd week	$1.4x10^2$±57.46	$4.52x10^2$±151.68*	$2.39x10^2$±97.12
3rd week	$2.36xl0^2$±42.64	$1.95xl0^2$±31.22	$3.32x10^2$±19.36*
4th week	$2.62x10^2$±39.89	$2.25xl0^2$±31.22	$3.87x10^2$±31.22*

* 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*.

Exposure period (week)	Control		Treated with *E. peplus*		Treated with Praziquantel	
	plasma	digestive gland	plasma	digestive gland	plasma	digestive gland
1st week	5.9	6.61	6.5	6.58	5.6	6.97
2nd week	6.28	4.545	8.19	6.84	8.2	5.78
3rd week	6.23	7.45	7.42	7.63	7.23	7.48
4th week	5.64	6.43	8.64	7.66	7.36	6.69

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.

Factors	Acid Phosphatase(U/L)		Alkaline phosphatase (U/100 ml)	
	2nd week	4th week	2nd week	4th week
Control	0.77	0.9182	3.28	4.27
Treated with *E. peplus*	1.746	1.4307	1.4	8.74
Treated with Praziquantel	1.3025	1.6401	0.12	5.66

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

Exposue Period (week)	Control		Treated with *E. peplus*		Treated with Praziquant	
	haemolymph	digestive gland extract	haemolymph	digestive gland extract	haemolymph	Digestive gland extract
1st week	0.3±0.3	0.7±0.17	2.71±0.52*	1.71±0.17*	2.11±0.3*	1.17±0.17
2nd week	0.5±0.35	0.3± 0.3	2.51± 0.46*	1.8±0.3*	2.61± 0.46*	1.61±0.17
3rd week	0.6±0.3	0.3± 0.3	2.41± 0.6*	1.61±0.17*	2.71±0.3*	1.81±0.3*
4th week	0.3± 0.3	0.1±0.17	2.91±0.17*	1.3±0.17*	2.81±0.35*	1.17±0.35*

*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.

Exposure Period (week)	Control		Treated with *E. peplus*		Treated with Praziquant	
	haemolymph	digestive gland extract	haemolymph	digestive gland extract	haemolymph	digestive gland extract
1st week	0.5±0.17	0.4±0.17	2.71±0.3*	2.01±0.17*	2.51±0.46*	1.81±0.3
2nd week	0.6±0.3	0.8± 0.46	2.91±0.17*	1.81±0.3*	2.81±0.35*	2.01±0.17*
3rd week	0.7± 0.46	0.6± 0.3	2.81±0.17*	1.5±0.3*	2.81±0.35*	1.91±0.17*
4th week	0.6±0.3	0.9± 0.3	2.91±0.17*	1.71±0.35*	2.91±0.17	1.71±0.46*

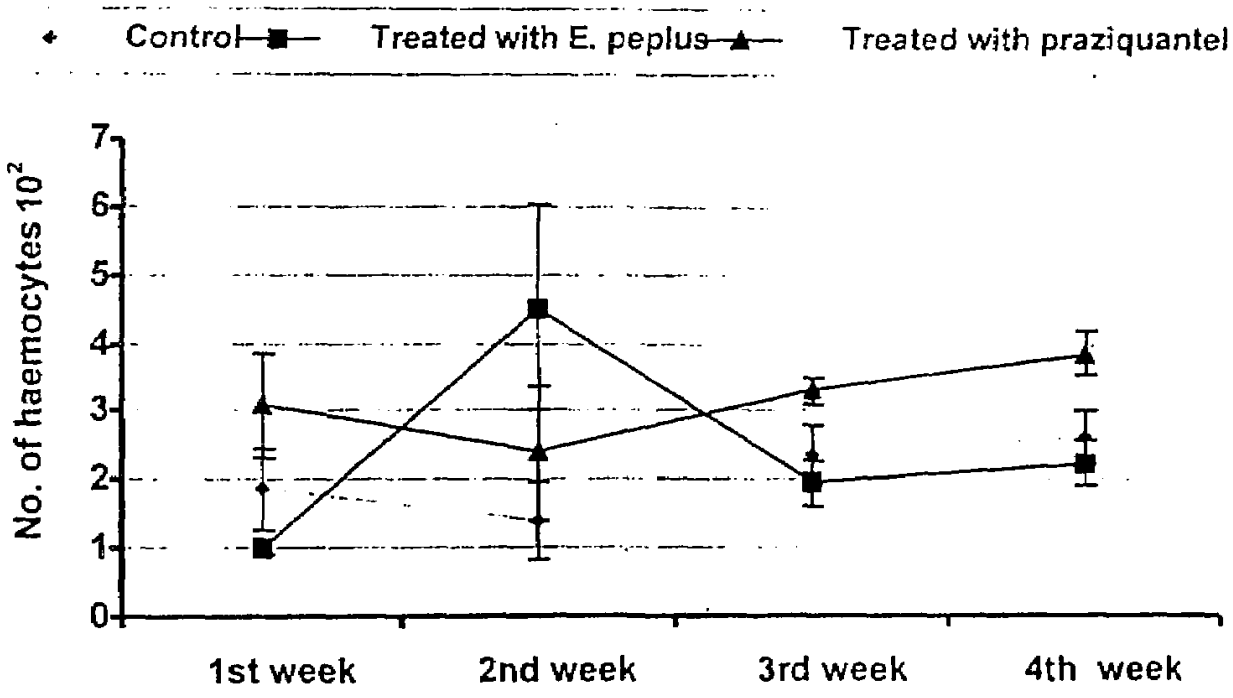
*Significant at $P < 0.04$

EXPLANATION OF FIGURES (2-5).

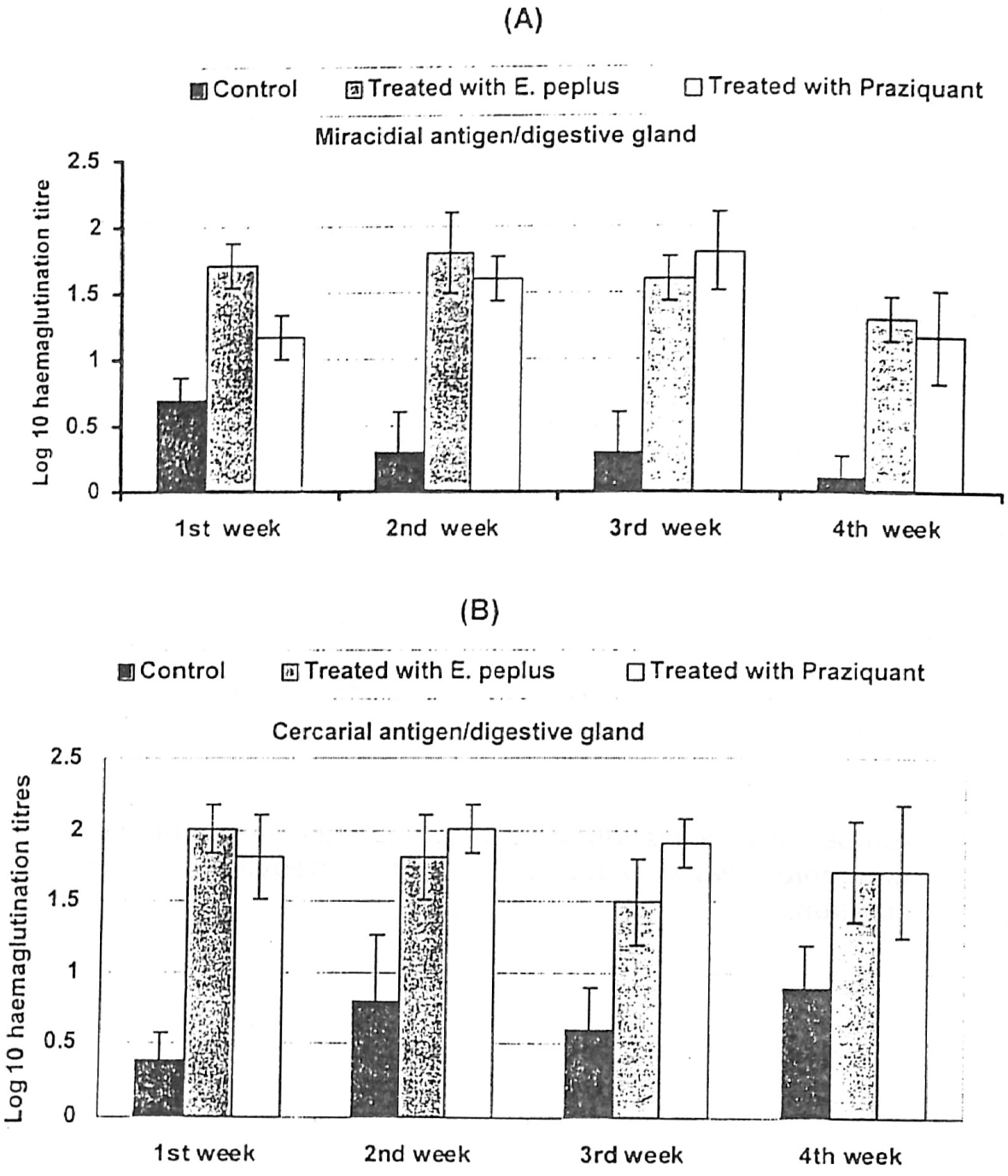
Fig. (2). Control snail (haemocytes stained with Giemsa)
A) Granulocytes (g) and undifferentiated cells (u). X400
B) Hyalinocytes (h) X250
C) Granulocytes (g) X100

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.

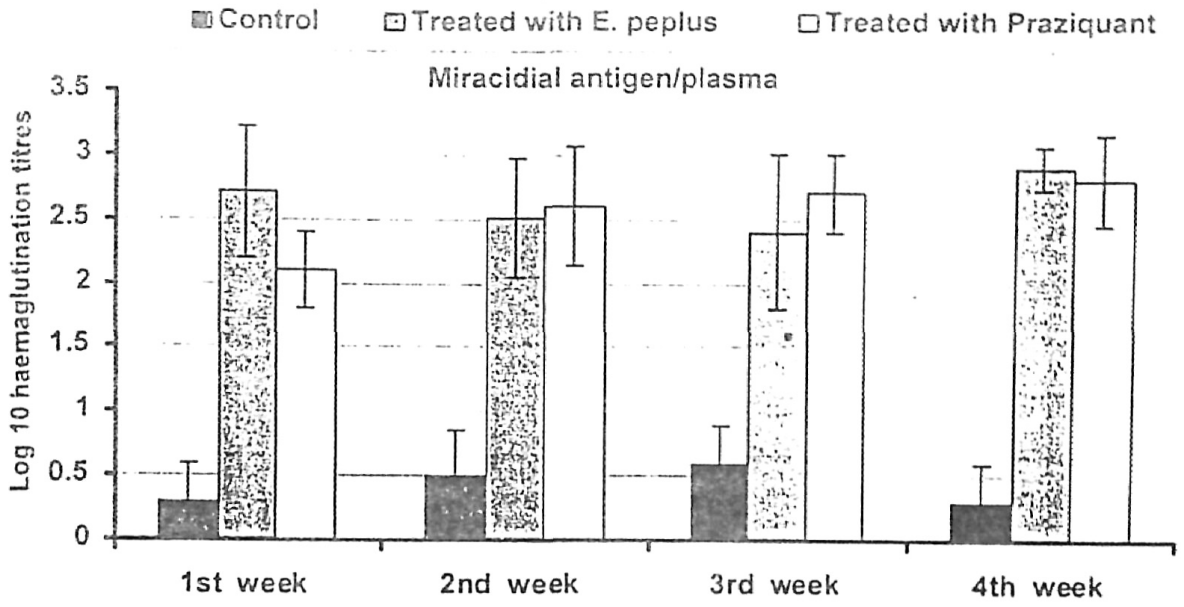


(Fig. 1): Number of *B. alexandrina* haemocytes after treatment with *E. peplus* water suspension and prazequantel water solution.

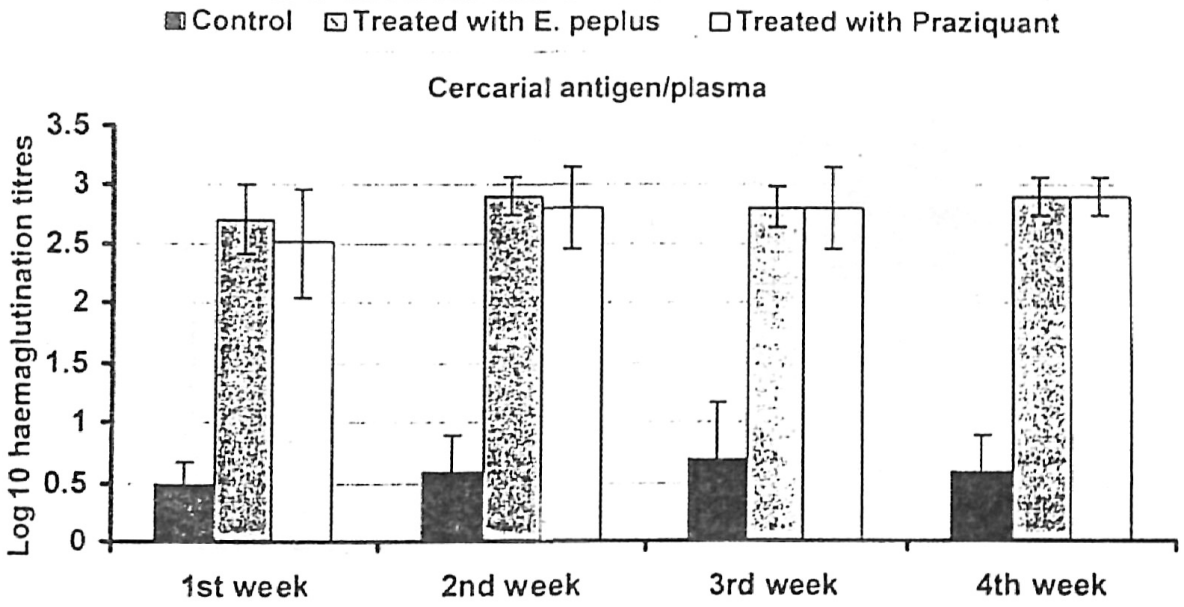


(Fig. 2): Haemaglutination titres of *Biomplaria alexandrina* digestive gland after treatment with *E. peplus* water suspension and praziquantel water solution against meracedial and cercarial antigen.

(A)



(B)



(Fig. 3): Haemaglutination titres of *Biomplaria alexandrina* plasma after treatment with *E. peplus* water suspension and praziquantel water solution against meracedial and cercarial antigen.

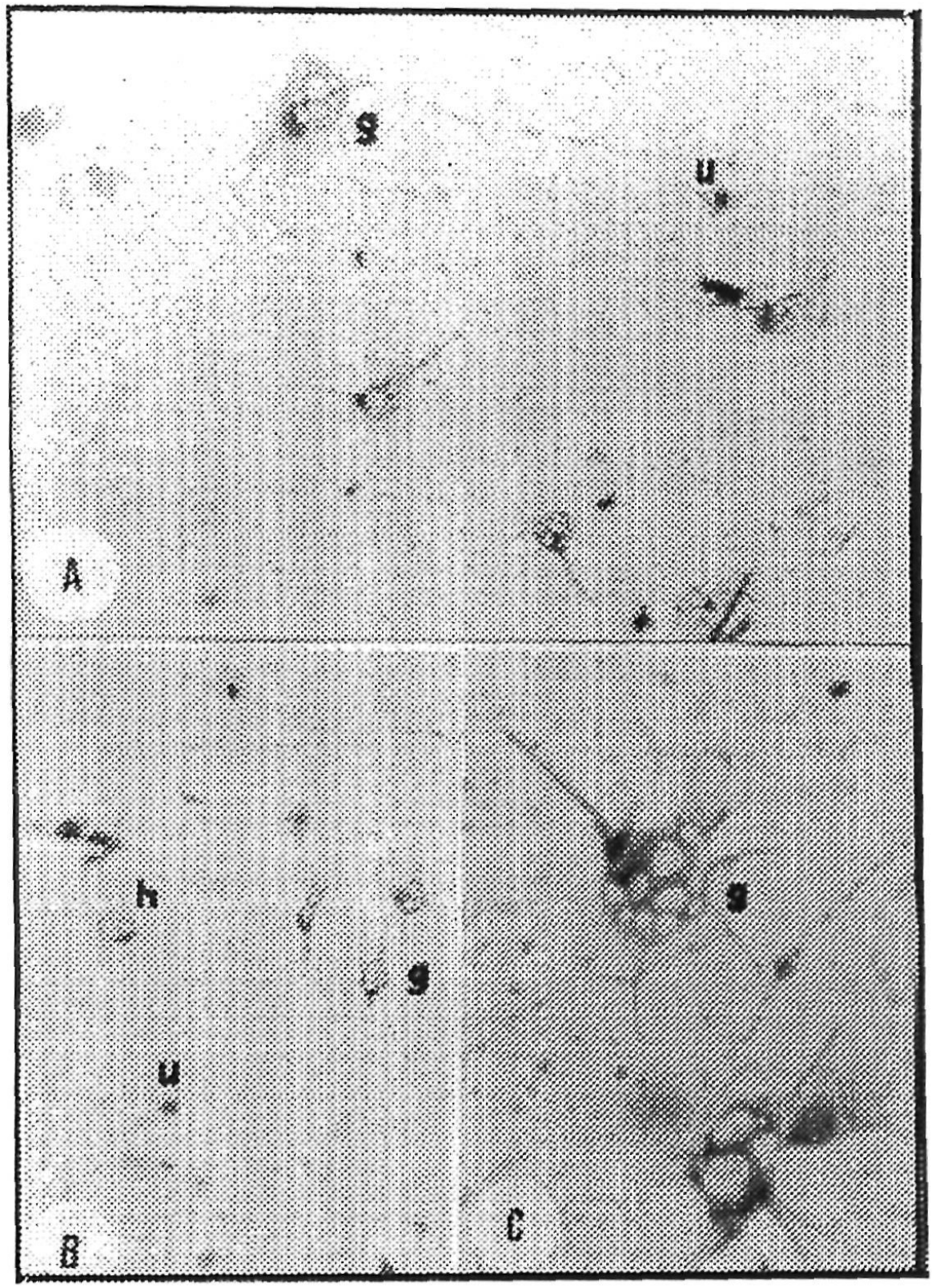


Fig. (4): Control snail (haemocytes stained with Giemsa)
A) Granulocytes (g) and undifferentiated cells (u). X400.
B) Hyalinocytes (h). X250 C) Granulocytes (g). X100

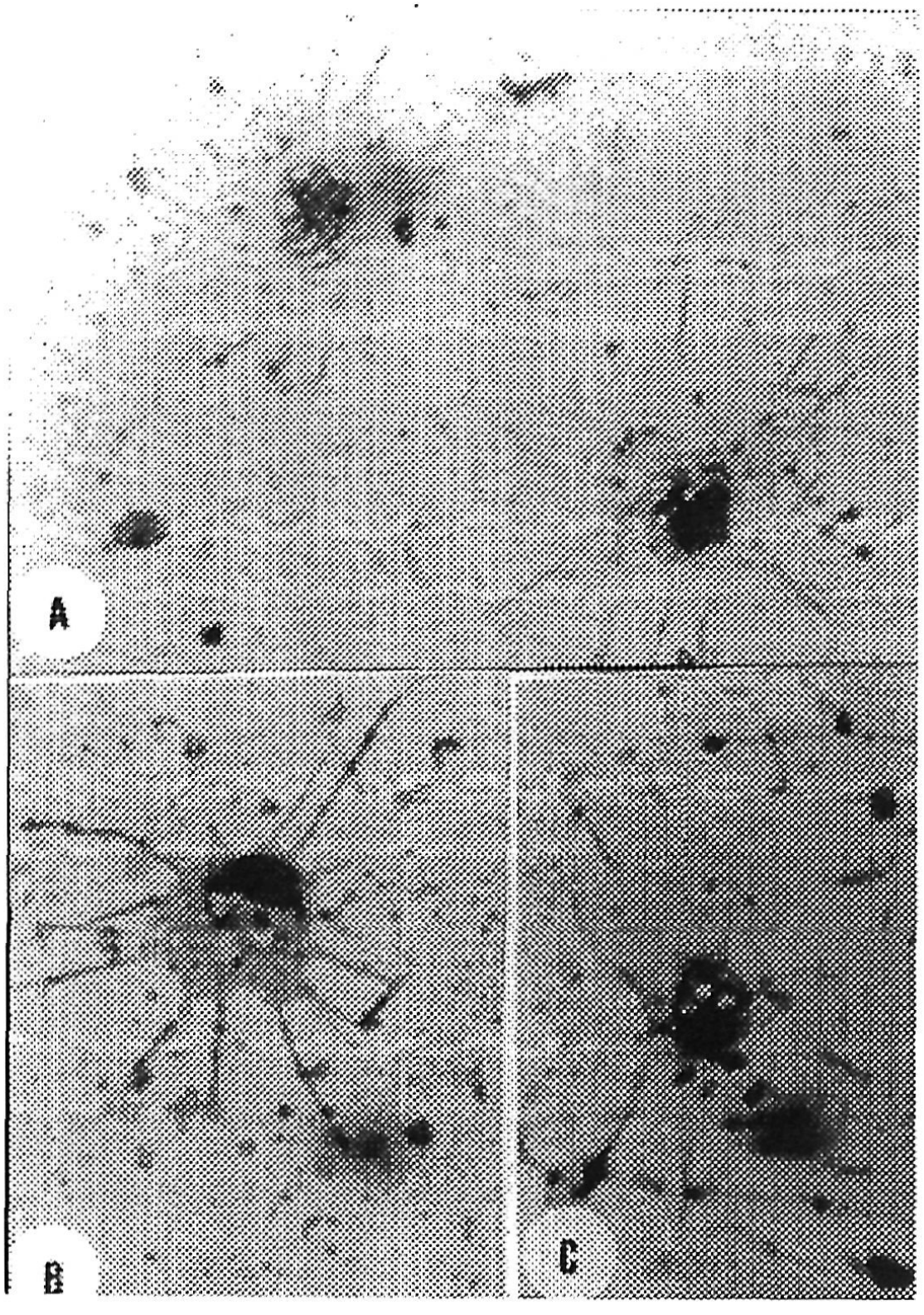


Fig. (5): Haemocytes of treated snail for 2 weeks showed many cytoplasmic inclusions and long pseudopodia.
A) Haemocytes of treated snail with *E. peplus* X1000.
B) Haemocytes of treated snail with *E. peplus* X1000.
C) Haemocytes of treated snail with Praziquantel. X1000.

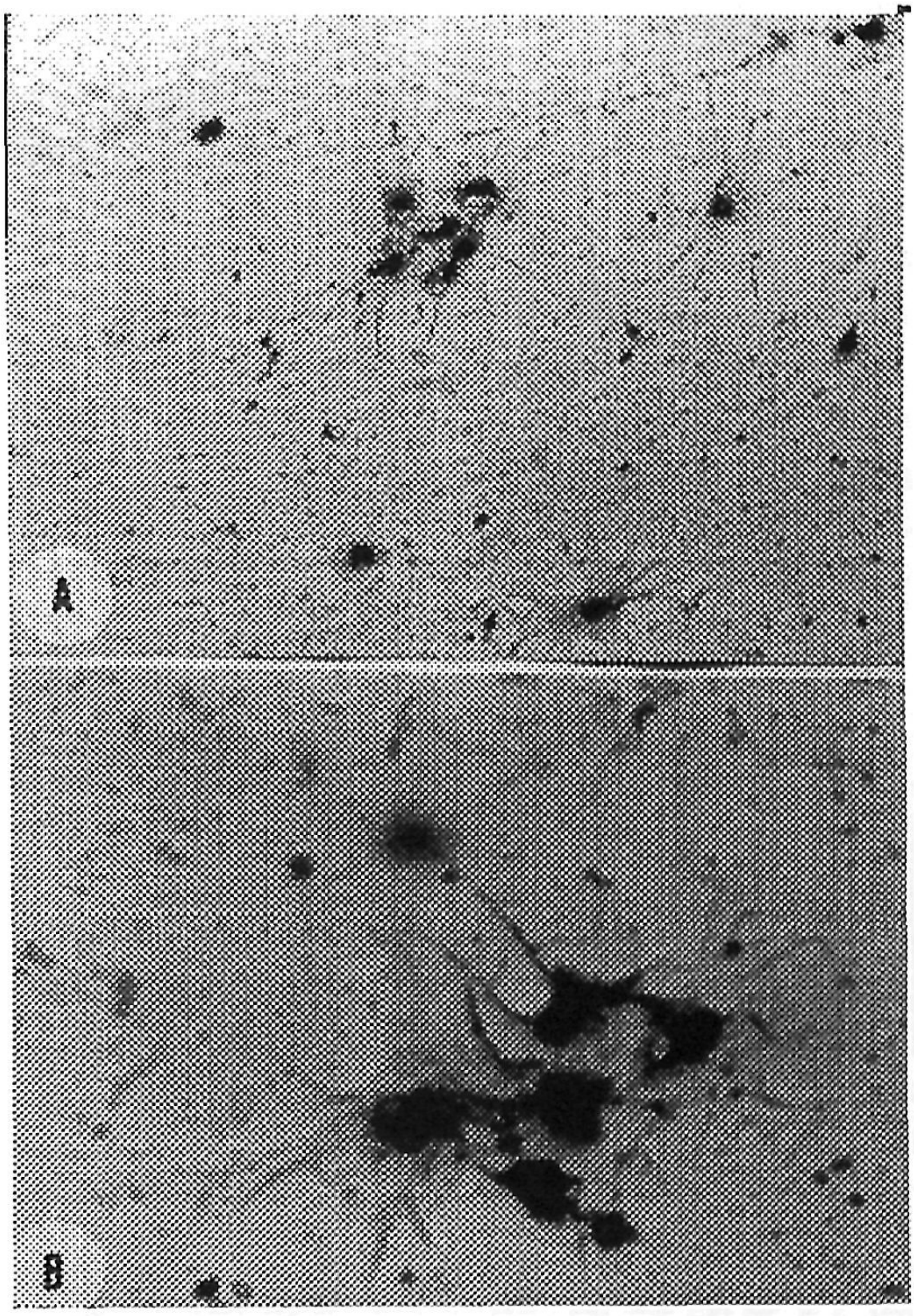


Fig. (6): Haemocytes of treated snail after 4 weeks of exposure

A) Haemocytes of treated snail with *E. poplus*. X400.

B) Haemocytes of treated snail with Praziquantel. X1000.