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EFFECTS OF NICLOSAMIDE AND *EUCALYPTUS CAMALDULENSIS* ON *BIOMPHALARIA GLABRATA*, THE SNAIL INTERMEDIATE HOST OF *SCHISTOSOMA MANSONI*

XIAONONG ZHOU[a], E.SUCHART UPATHAM[b], MALEEYA KRUATRACHUE[b] AND PRAPEE SRETARUGSA[c]

a) Jiangsu Institute of Parasitic Diseases, Wuxi, Jiangsu 214064, People's Republic of China.

b,c) Departments of Biology [b] and Anatomy,[c] Faculty of Science, Mahidol University, Bangkok 10400, Thailand.

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ABSTRACT

Toxicity studies were carried out on the snail Biomphalaria glabrata *using niclosamide (bayluscide) and the extract from dried leaves of* Eucalyptus camaldulensis. *The snails were exposed to Niclosamide and to aqueous and methanolic extracts of* E. camaldulensis *for 24 h and recovered for 48 h. In niclosamide, the LC_{50} and LC_{90} values were 0.063 and 0.126 mg/l, respectively. In aqueous extract, the LC_{50} and LC_{90} values were 3049.65 and 5568.33 mg/l; while in methanolic extract, they were 71.59 and 96.73 mg/l. Histological changes were observed in the intestines, digestive glands and ovotestes of the molluscicides treated snails. In the intestines, gaps were formed between the epithelial cells and the connective tissue resulting in the derangement of the ciliated cells. In the digestive glands, the digestive cells became irregular in shape; and both digestive and calcium cells showed considerable shrinkage. The ovotestes of the molluscicides treated snails showed reduction in numbers of spermatozoa and oocytes.*

INTRODUCTION

Most of the 74 countries of the developing world where schistosomiasis is endemic and where over 600 million people are exposed to the risk of infection have agriculturally based economies. It is generally agreed that chemotherapy and snail control should be coordinated to achieve maximum sustained reduction in prevalence and intensity of infection[1]. At present, the chemical arsenal consisted of only one molluscicide (Niclosamide) is recommended by World Health Organization WHO for use in the control programme.

In recent years, research on plant molluscicides has become multi - disciplinary since plant molluscicides may be cheaper and more readily available than synthetic ones. Many developing countries are reluctant to embark on chemical snail control programmes using costly synthetic compounds[2]. Moreover, indigenous, rather than imported materials are desirable, especially as the strategy for effective schistosomiasis control programmes which must be based on sustained and long-term inputs. Several plants have been studied for molluscicidal activity, including *Phytolacca dodecandra, Ambrosia maritima, Anacardium occidentalis* and *Swartzia madagascarensis*. However, not enough information is known about

the chemical composition of the active substance, variation within species, methods of preparation and toxicological properties[1].

Eucalyptus trees (*Eucalyptus* spp., Myrtaceae) are among the most prolific trees around settlements in many parts of the tropics and subtropics, where their wood is widely used for fuel, lumber and poles[3]. Its essential oil obtained from the leaves is used in pharmaceutical preparations (such as antiseptics, cough drops), in perfumes and in the futation process for concentration ores. The aqueous extract of *Eucalyptus* spp. had some molluscicidal activity against *Biomphalaria* snails[4]. It is, hence, likely that more potential molluscicidal activeness will be shown if their active ingredients can be more efficiently extracted[3].

Niclosamide (Bayluscide) has been proven to be an extraordinary molluscicide. Its degree of activity exceeds that of other "available" molluscicides by 10 or more times. It was commercialized in the form of ethanolamine salt and formulated as a 70 % wettable powder (Bayluscide, Bayer 73). In snails, the chemical is taken up primarily by the soft tissues. Lethal concentrations for snails irreversibly block the uptake of oxygen, while lower concentrations stimulate respiration[5]. Nevertheless, its mode of action with respect to the histological changes has received relatively little attention.

The present study was undertaken in order to investigate the effects of extracts of Eucalyptus camaldulensis and Niclosamide, if any, on the intestine, digestive gland, and ovotestis of *Biomphalaria glabrata*, the intermediate host of *Schistosoma mansoni*.

MATERIALS AND METHODS

Animals

Snails used in this study were laboratory-bred *B. glabrata*, which were cultured in aerated glass aquaria and fed with lettuce in the Center for Applied Malacology and Entomology, Department of Biology, Faculty of Science, Mahidol University, Bangkok, Thailand.

Molluscicides

E. camaldulensis were collected from the jungle in Northern Thailand. The dried leaves of *E. camaldulensis*, the specimens of which were deposited in the Plant Museum of Malacology and Entomology, Faculty of Science, Mahidol University, were weighed and homogenized in Quickier Mini Blender prior to dissolving in distilled water for 24 h and filtering. The supernatant, as aqueous extract, was obtained for immersion test. The remains of the plant were dissolved in methanol again for 24 h. After the supernatant was removed and evaporated with rotary evaporator, the methanol extract was obtained for immersion test.

Niclosamide used was 70 % wettable powder (Bayluscide) purchased from Bayer Company, Germany.

Immersion test

The tests were performed according to the methodology recommended by WHO[6]. Six snails, *B. glabrata* (about 1.5 ± 0.2 cm in size) were exposed in series of concentration with two snails per bottle which contained 200 ml dechloorinated tap water, pH 7.2±0.3. After

being exposed for 24 h at 22°± 2°C, the snails were thoroughly washed and kept under further observation for 48 h, and then final mortality was determined. The LC_{50} and LC_{90} values were calculated by probit analysis computing programme[7].

Observation on histological changes in snails

Ten live snails exposed to molluscicides (*E. camaldulensis* and Niclosamide) at LC_{50} concentration for 24 h and ten normal snails were used for this study. They were relaxed in dry ice for 30 min and then their shells were removed. The intestines, digestive glands and ovotestes were dissected out and processed for light microscopic examination.

The intestine, digestive gland and ovotestis were fixed in Bouin's fluid for 5 h. The tissues were then washed several times with 70% alcohol, dehydrated in a graded series of ethanol, and infiltrated with dioxane. Finally, they were embedded in paraffin. Sections were cut on a rotary microtome at 5 - 6 μm thickness and stained with hematoxylin and eosin. Examination and photography were performed with Olympus Vanox and Leizt Orthoplan microscopes.

RESULTS

Molluscicidal activity

The mortality of *B. glabrata* increases with the concentration of molluscicides after exposure to aqueous and methanolic extracts of *E. camaldulensis* for 24 h and recovered for 48 h. In aqueous extract, the LC_{50} and LC_{90} values are 3049.65 and 5568.33 mg/l, respectively, whereas in methanolic extract the LC_{50} and LC_{90} values are 71.59 and 96.73 mg/l, respectively, which were 42.6 and 57.5 times lower than those in aqueous extract (Table 1).

The dosage-response curves of *B. glabrata* exposed to aqueous and methanolic extracts of E. camaldulensis are shown in Figs. 1 and 2. The remains of *E. camaldulensis*, after being extracted by water and methanol, could not kill the snails until up to 250,000 mg/l.

In Niclosamide, the LC_{50} and LC_{90} values were 0.063 and 0.126 mg/l,respectively(Table 1).Thedosage-response curve is shown in Fig.3. No dead snails were detected in the control group which were treated with dechlorinated tap water only.

Histological changes

Changes in the intestine

Observation was emphasized on the mid-intestine. There are two types of mucous gland cells intermingled with ciliated

cells in the epithelium of mid-intestine. The first type was goblet-shaped with two types of granules. The second type was similar in size and nuclear features but with only one type of granules. All these epithelial cells are connected with muscle layer by connective tissue (Fig. 4).

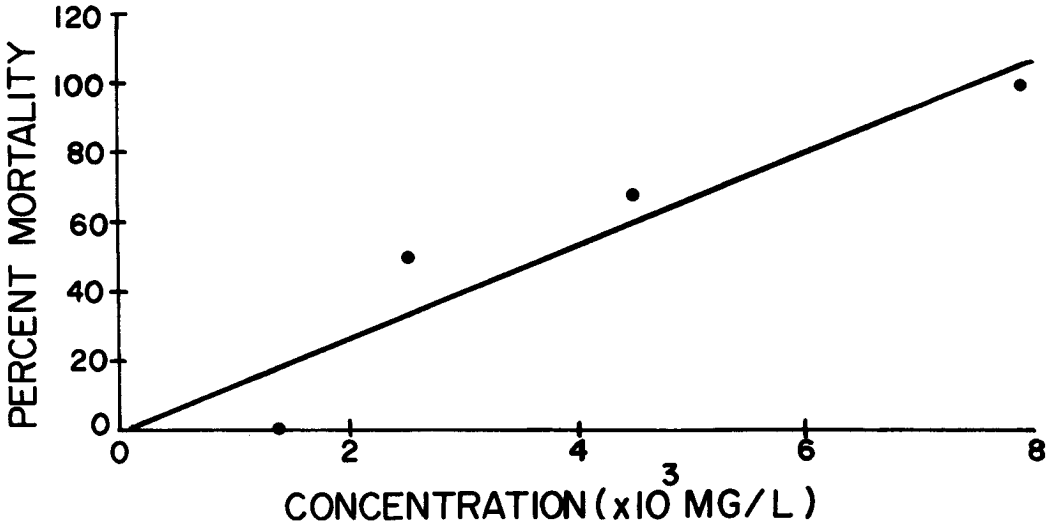

Fig.1 The dosage-response curve of *Biomphalaria glabrata* exposed to aqueous extract of *Eucalyptus camaldulensis*.

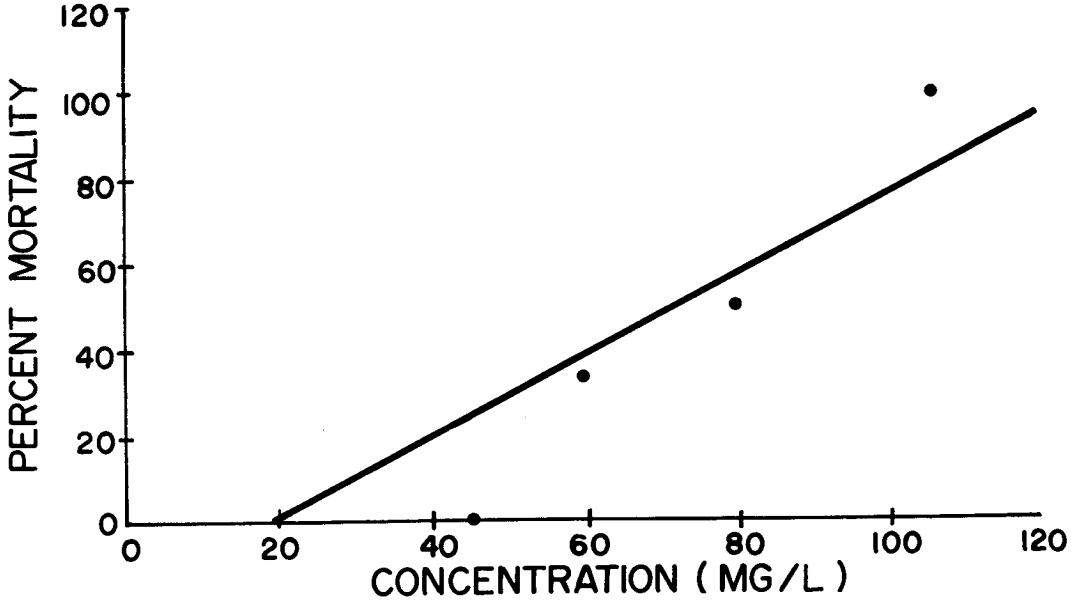

Fig.2. The dosage-response curve of *Biomphalaria glabrata* exposed to methanolic extract of *Eucalyptus camaldulensis*.

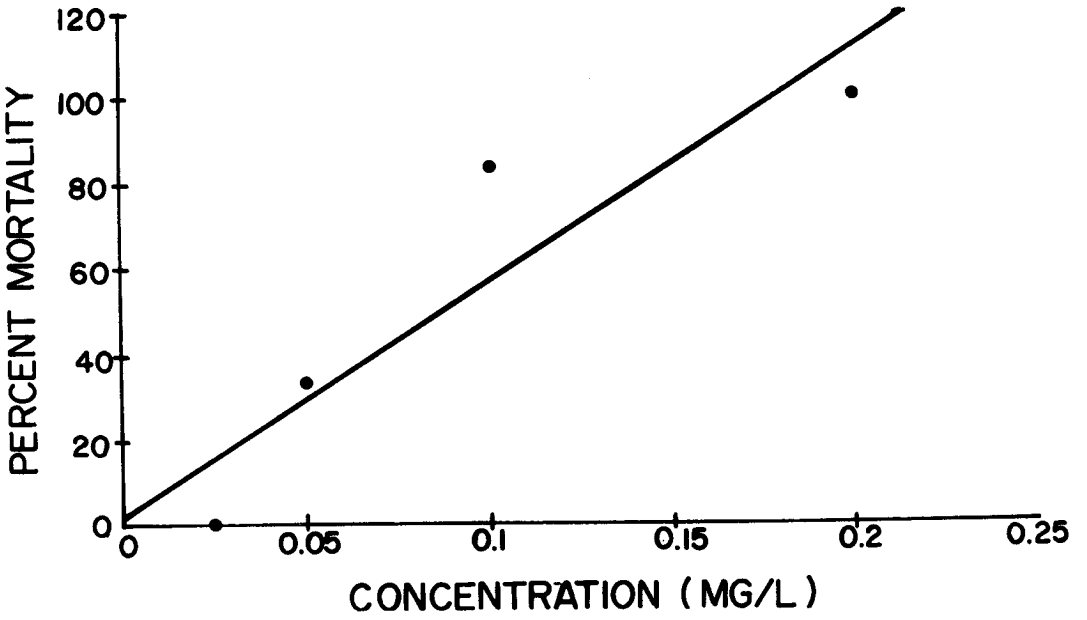

Fig.3. The dosage-response curve of *Biomphalaria glabrata* exposed to Niclosamide.

Table 1. Mortality of *Biomphalaria glabrata* exposed to molluscicides for 24 hours

Aqueous extract of *E. camaldulensis*		Methanolic extract of *E. camaldulensis*		Niclosamide	
Concentration (mg/l)	Mortality (%)	Concentration (mg/l)	Mortality (%)	Concentration (mg/l)	Mortality (%)
0	0	0	0	0	0
$1x10^3$	0	40.00	0	0.025	0
$2x10^3$	50.00	60.00	33.33	0.050	33.33
$4x10^3$	66.67	80.00	50.00	0.100	83.33
$8x10^3$	100.00	100.00	100.00	0.200	00.00
LC_{50}	3,049.65	71.59		0.063	
LC_{90}	5,568.33	96.73		0.126	

In the *E. camaldulensis* and Niclosamide treated snails, the muscle fibers of the intestine become relaxed and the gaps between the epithelial cells and the connective tissue appear, resulting in the derangement of the ciliated cells (Fig. 5). In addition, both mucous cells and ciliated cells appear irregular in shape and some show considerable shrinkage (Fig. 5).

Changes in the digestive gland

The digestive gland is of compound branched tubular type (Fig. 6). A very thin layer of circular muscle fibers surrounds each digestive tubule. The cells constituting the wall of the tubule are arranged around irregular lumina and easily recognizable as two main types: acidophilic "digestive cells" and basophilic "calcium cells" (Fig. 7).

The digestive cells are long, columnar and club-shaped, with domed distal apices and flat bases by which they rest on a very thin basement membrane. Their nuclei are basal, usually oval, but may be elliptical or spheroidal. The calcium cells are darkly stained and located at the periphery of the tubule of digestive gland in small groups. These cells contained large bodies that are calcareous (Fig. 7).

In the *E. camaldulensis* and Niclosamide treated snails, the digestive cells in the digestive gland become irregular in shape. Some of the digestive cells are broken and the secretory material leaks into the lumen (Fig.8). Both digestive and calcium cells show shrinkage and, subsequently, the lumen of the digestive tubule appears relatively large and filled with secretory material (Fig. 9).

Changes in the ovotestis

Ovotestis consists of many follicles known as acini, part of which are embedded in the digestive gland (Fig.10). In each acinus, there are both male and female germ cells. The germinal epithelial cells are consisted of two types, namely, the Sertoli cells and the spermatogenic cells. The latter consist of the spermatogonia, spermatocytes, spermatids and spermatozoa (Fig. 11). Female germ cells consist of two types: the oocyte and the follicle cells. Oocyte is a very large cell with prominent nucleolus, pale cytoplasm and is surrounded by the follicle cells (Fig. 11). There are numerous spermatozoa and a large number of oocytes in the ovotestis.

In the *E. camaldulensis* and Niclosamide treated snails, the ovotestis shows reduction in numbers of acini (Fig.12). In addition, the numbers of Sertoli cells, spermatozoa and oocytes also decrease and appear to be degenerated in the acinus (Fig.13).

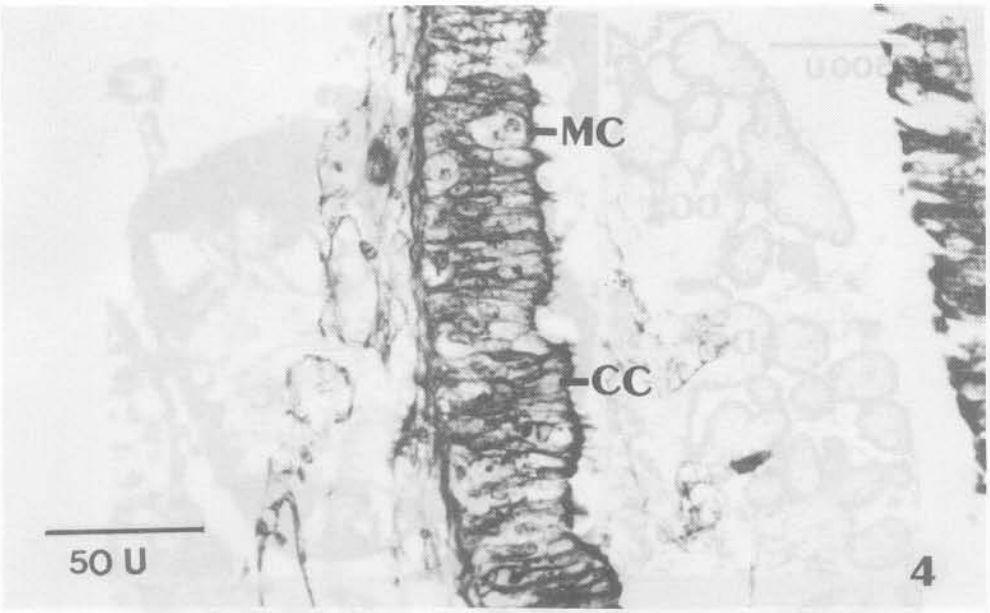

Fig.4. The mid-intestine of normal snails showing epithelium on basement membrane consisting of ciliated cells (CC) and mucous cells (MC).

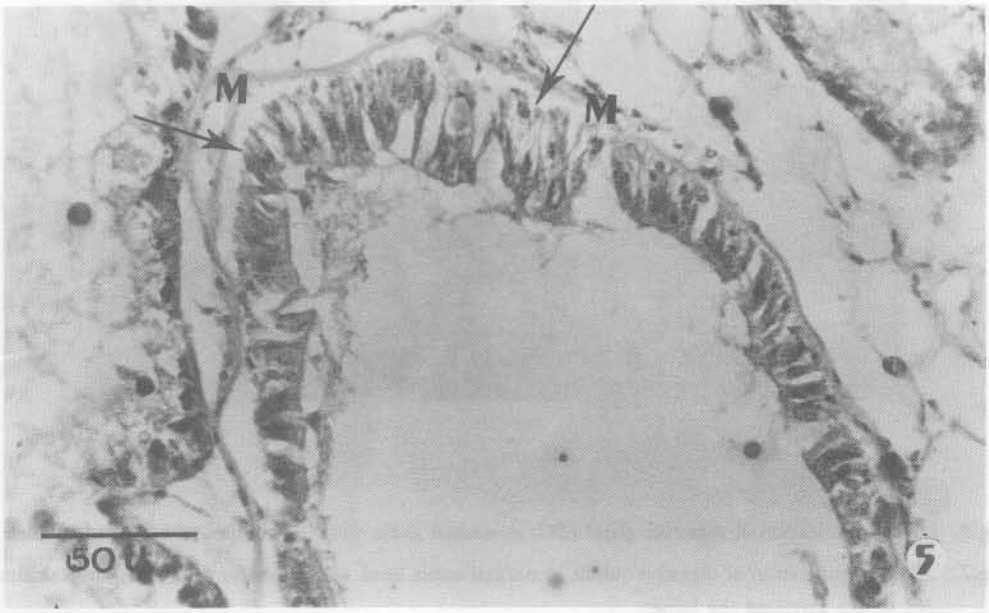

Fig.5. The mid-intestine of snails treated with molluscicides showing gaps (arrows) between epithelial cells and muscle layer (M). Note the irregular-shaped epithelial cells which are separated from one another with intercellular spaces.

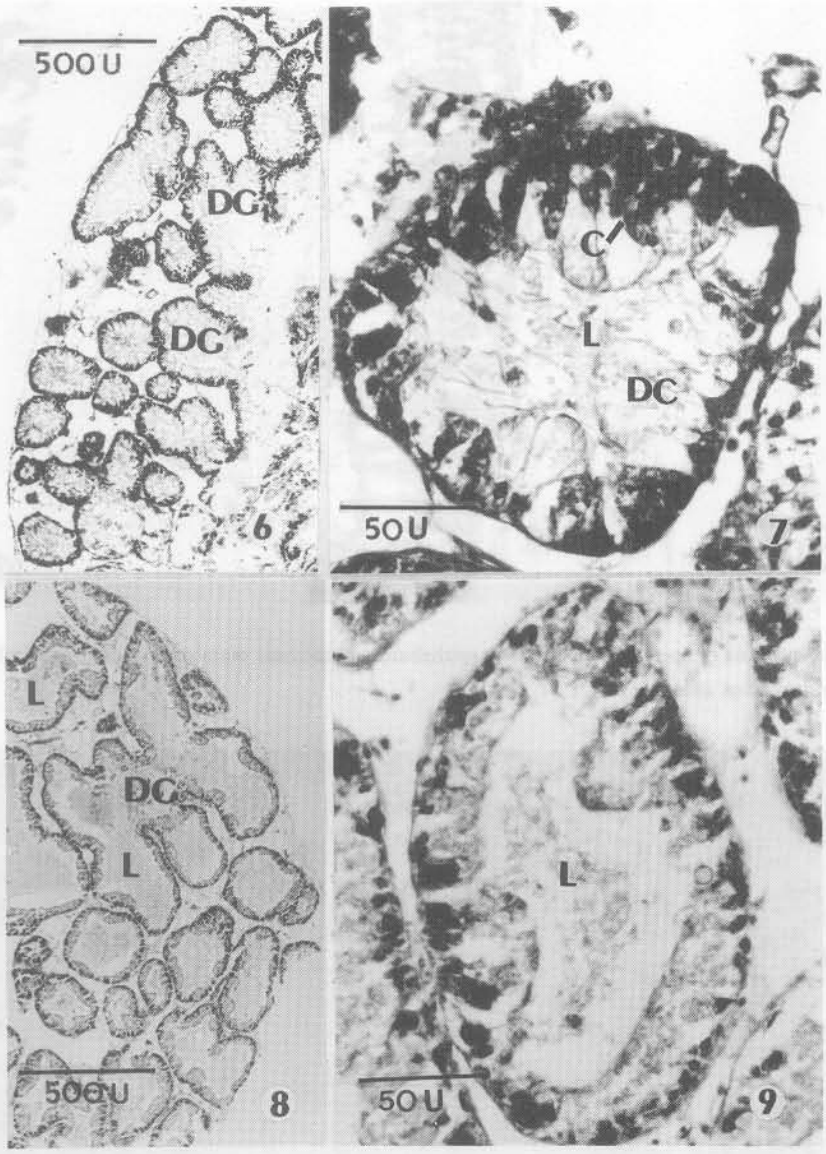

Fig.6. Low magnification of digestive gland (DG) in normal snails showing compound branched tubules.

Fig.7. High magnification of digestive tubule in normal snails lined with digestive cells (DC) and calcium cells (C) which surround the lumen.

Fig.8. Low magnification of digestive gland (DG) in snails treated with molluscicides. Note the presence of secretory material in the wider lumen (L).

Fig.9. High magnification of digestive tubule in snails treated with molluscicides. Both digestive and calcium cells show shrinkage. Note the wide lumen (L) with secretory material.

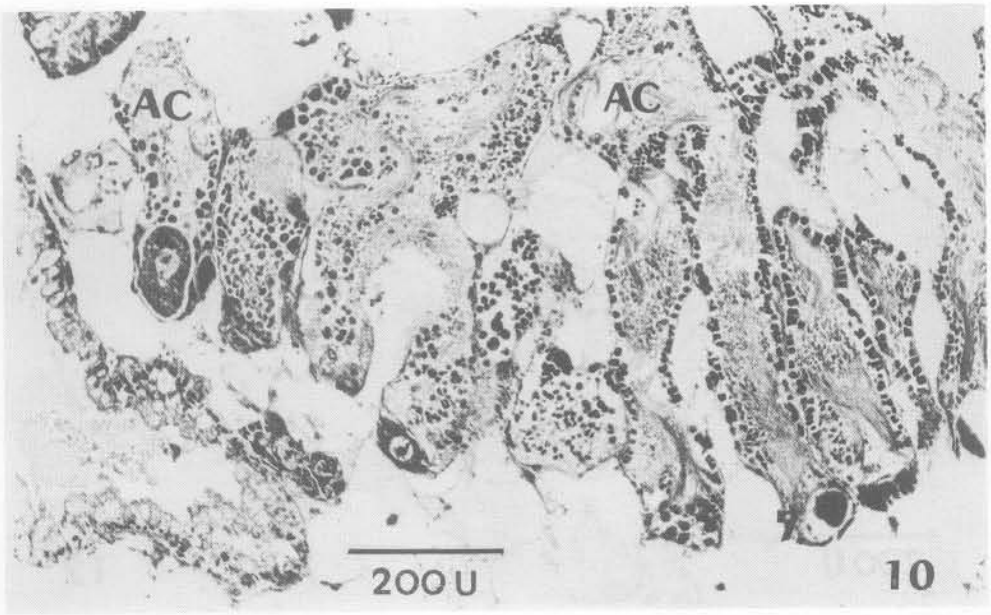

Fig.10. Ovotestis of normal snails is composed of several acini (AC).

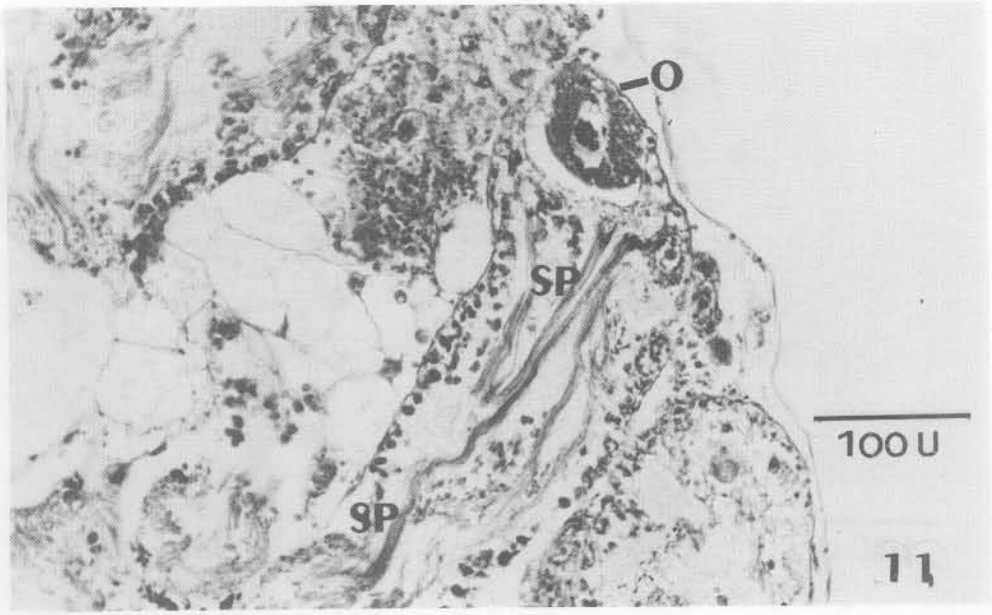

Fig.11. High magnification of acinus of ovotestis in normal snails. Note the presence of spermatozoa (SP) and oocytes (O).

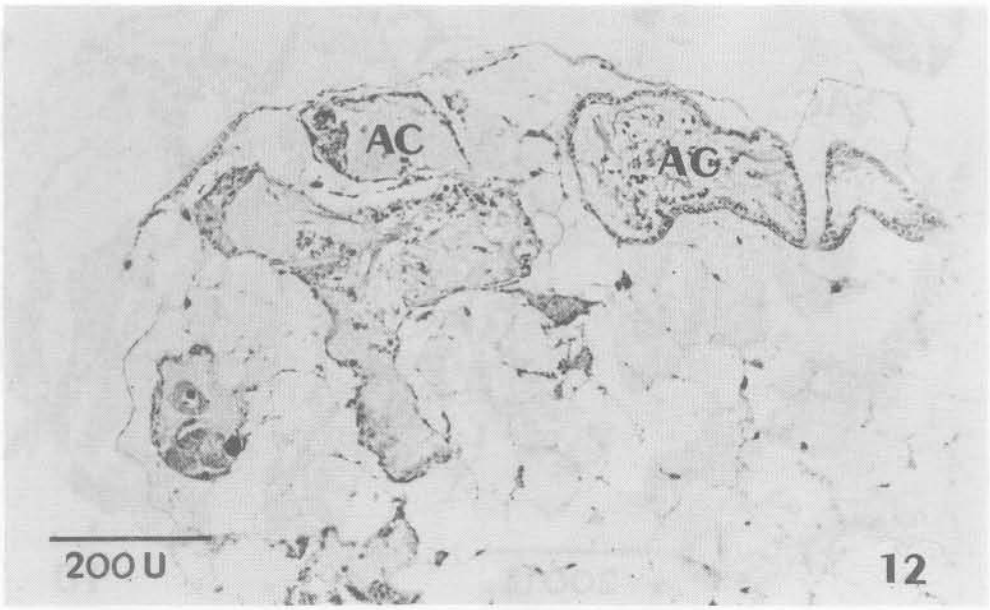

Fig.12. Ovotestis of snails treated with molluscicides. Note the reduction in numbers of acini (AC).

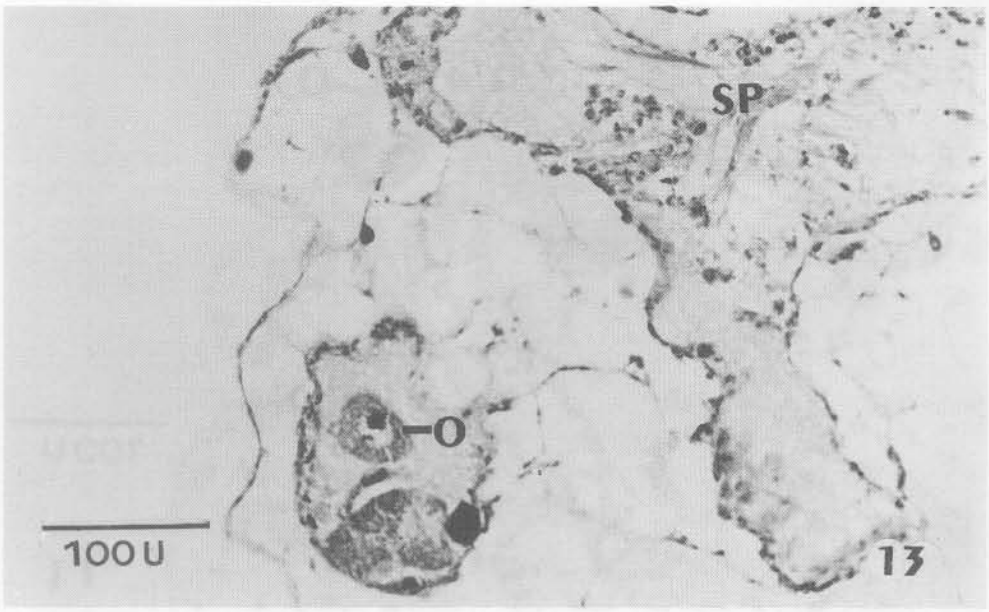

Fig.13. High magnification of acini of ovotestis in snails treated with molluscicides. Note degenerating spermatozoa (SP) and oocyte (O).

DISCUSSION

The present study demonstrated that methanolic extract of *E. camaldulensis* exhibited higher molluscicidal activity (LC_{50}=71.59) than the aqueous extract. According to the criteria suggested by Farnsworth et al.[8], the results are positive if a plant extract exerted any percentage kill at a concentration of 100 mg/l or less. They were weak if more than 100 mg/l of the extract were required to kill snails, and were negative if there was no kill at any test level. Therefore, in the present study, the aqueous extract of *E. camaldulensis* showed weak activity (LC_{50}=3049.65), which was similar to the results of Broberg[4] who studied the aqueous extracts of *Eucalyptus* spp. which showed molluscicidal effect at 1000 mg/l on *B. glabrata*.

Many workers had studied the components of essential oil from the leaves of *Eucalyptus* spp. which mainly consisted of cineole, citronellal, cyclohexanol, thujene and alloocimene[9]. In the present study, the methanolic extract showed 42.6-57.5 times higher molluscicidal activity than the aqueous extract, indicating that some active components which could be dissolved in methanol occurred in the essential oil of *E. camaldulensis* leaves. It is considered that the methanolic extract of *E. camaldulensis* leaves is one of the promising molluscicides, at least against *B. glabrata*. Further studies to test the compounds extracted from its leaves by chromatography or spectroscopic methods should be taken into consideration in the future.

Eucalyptus is mostly native to Western Australia. But because of its multiple usage, now it has migrated into many countries including tropical and subtropical countries. The leaves of Eucalyptus species could be used as animal feed because they contained water, protein, lipid, sugar, fiber and ash[10]. This suggests that Eucalyptus leaves are less toxic to animals as well as to people, and so they should be safe when used as molluscicides in the field.

The results on the histological study demonstrated that similar tissue damage on the *B. glabrata* snails occurred when exposed to either methanolic extract of *E. camaldulensis* or Niclosamide at the LC_{50} concentration for 24 hours.

Pesticides considerably affected the intestine and hepatopancreas of the marine bivalves[11]. In the intestine, both mucous and ciliated cells lost their connection with the muscle fibers; the mucous cells were reduced in size and the connective tissue became irregular[11]. Similar results have been observed in the intestine of *B. glabrata* snails treated with either *E. camaldulensis* or Niclosamide in the present study.

It is suggested that the damage of the digestive gland, especially on digestive cells observed in the present study influences its functions of secretion, excretion, absorption, digestion and storage of fat and glycogen. The effect of pesticides on the hepatopancreas of the marine bivalves showed that both digestive cells and darkly staining generative cells of the bivalve hepatopancreas lost their shape and exhibited a considerable shrinkage[11]. This tissue damage in the digestive gland as a result of molluscicides differs from the damage as a result of being parasitized by larval trematodes of which the destruction was mechanical damage, extracorporeal digestion or ingestion, lysis and autolysis[12].

In the reproductive system, histopathological changes of both Sertoli and spermatogenic cells in the acinus of the ovotestis of *B. glabrata* suggested that spermatogenesis as well as spermiogenesis are affected by both molluscicides, *E. camaldulensis* and Niclosamide. In the parasitized bivalves, the ova appeared degenerate and were smaller than those of the control[13]. In the present study, oocytes of the snails treated with both molluscicides are reduced in numbers and become degenerate. This also suggested that oogenesis as well as ovulation are affected by molluscicides.

ACKNOWLEDGEMENTS

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