Indian Journal of Animal Sciences **82** (4): 363–366, April 2012

Light and scanning electron microscopic studies on the effect of *Acacia arabica* against *Cotylophoron cotylophorum*

L VEERAKUMARI[1], R ASHWINI[2] and K LALHMINGCHHUANMAWII[3]

Pachaiyappa's College, Chennai, Tamil Nadu 600 030 India

Received: 5 May 2011; Accepted: 29 October 2011

ABSTRACT

Cotylophoron cotylophorum (Fischoeder 1901) is a gastrointestinal parasite, primarily occurring in the rumen of livestock. Paramphistomosis, the disease caused by this digenetic trematode is responsible for considerable economic losses. The objective of this study is to investigate the effect of *Acacia arabica* on the surface topography and internal architecture of *Cotylophoron cotylophorum.* Structural analysis of the control and drug-treated flukes was carried out using light microscopy and scanning through electron microscope. In light microscopic observation, severe vacuolization in the parenchyma and oral sucker are obvious. Lesions were also observed in testis. Morphological analysis by scanning via electron microscope revealed distortions in the tegument, crater formations and appearance of ridges.

Key words: *Acacia arabica, Cotylophoron cotylophorum,* Paramphistomosis

Cotylophoron cotylophorum is a gastrointestinal parasite, present in the rumen of livestock. It causes paramphistomosis in cattle, sheep and goats. Paramphistomosis has been a neglected trematode infectious disease; recently, it emerged as an important cause of productivity loss (Anuracpreeda *et al*. 2008). Acute infection causes decreased appetite, listlessness, weight-loss, fluid foul-smelling diarrhoea, dehydration resulting in death of the animal. Early diagnosis and treatment with appropriate anthelmintic is essential.

Acacia arabica (Lam.) (*babul* or gum arabic) is a leguminous tree found naturally in the Deccan part of India. It is used as an astringent, spasmolytic, demulcent, anthelmintic and abortifacient in Indian traditional medicine system. In folk medicine, gum arabic has been reported to be used internally for the treatment of inflammation of the intestinal mucosa, and externally to cover inflamed surfaces (Gamal el-din *et al.* 2003).

The present work was aimed at studying the anthelmintic effect of *Acacia arabica* against *C.cotylophorum.* The morphology and internal anatomy of *Acacia arabica* ethanol extract (*Aa*EE) treated flukes were studied using light and scanning electron microscope.

MATERIALS AND METHODS

Collection and in vitro *maintenance*

C.cotylophorum was collected from the rumen of the infected sheep slaughtered at Perambur abattoir, Chennai. Adult live worms collected were washed thoroughly in physiological saline and maintained in Hedon-Fleig solution of pH 7 (Veerakumari 1996).

Collection of plant material and preparation of extract

Barks of *Acacia arabica* were procured from local authorized dealer, identified and authenticated by botanist in Captain Srinivasa Moorthy Drug Research Institute for Ayurveda and Siddha, Arumbakkam, Chennai. Vouchered specimens are deposited in herbarium at Pachaiyappa's college, Chennai, India.

About 2 kg of barks of *A.arabica* were cleaned, shade dried, coarsely powdered and extracted exhaustively (48 h) in solvents of increasing polarity (hexane, chloroform, ethyl acetate and ethanol) successively. The supernatant was filtered through Whatmann No 1 filter paper and the filtrate was dried to evaporate. The organic solvent was evaporated in rotary evaporator Evator. The residue obtained was dried to powder using Lyodel lyophiliser. Preliminary investigations based on the motility of the drug-treated flukes revealed that *A.arabica* ethanol extract (*Aa*EE) is highly effective against *C.cotylophorum*.

Sample preparation: Ten mature flukes were incubated in *Aa*EE (10 µg ml^{-1}) for 8 h. Appropriate controls were also maintained simultaneously. Control and drug-treated flukes were examined under light and scanning electron microscope.

Light microscopic studies: Control and drug-treated flukes were fixed in 10% formalin and processed for histological

Present address: [1]Associate Professor and Head (veerakumari_2002@yahoo.co.in), Unit of Parasitology, [2,3] Junior Research Fellow. P G and Research Department of Zoology.

examination by employing the method of Lillie (1965). Histological sections were examined and photographed under high power compound microscope, LABO.

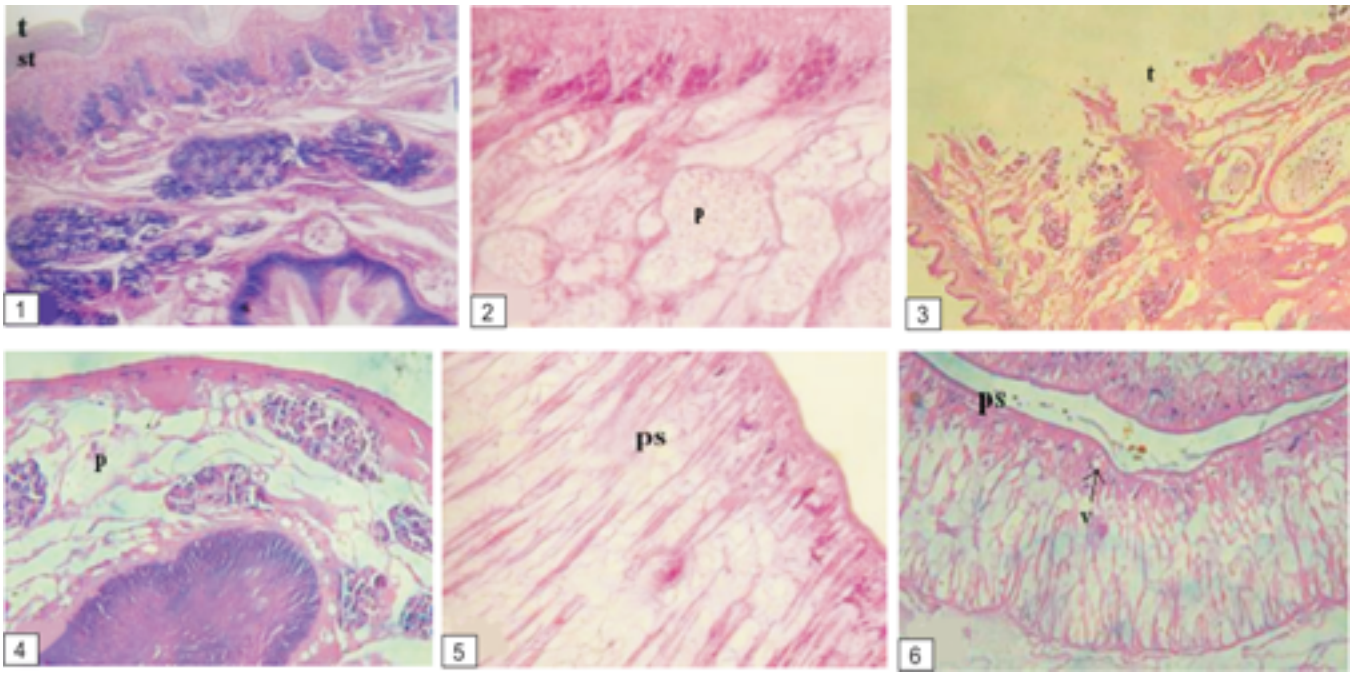
Scanning electron microscopic studies: Control and drug-treated flukes were fixed in 2.5% glutaraldehyde (pH 7.2) for 18 h and washed thrice in 0.1M phosphate buffer for 15 min. They were subsequently dehydrated in graded alcohol series. The flukes were then transferred to100% alcohol overnight and air-dried in vacuum desiccator for 48 h. The dried samples were mounted on a brass stub and coated with gold under vacuum for 4 min at 30 mA deposition. Stubs were then transferred to scanning electron microscope, observed and photographed at various magnifications.

RESULTS AND DISCUSSION

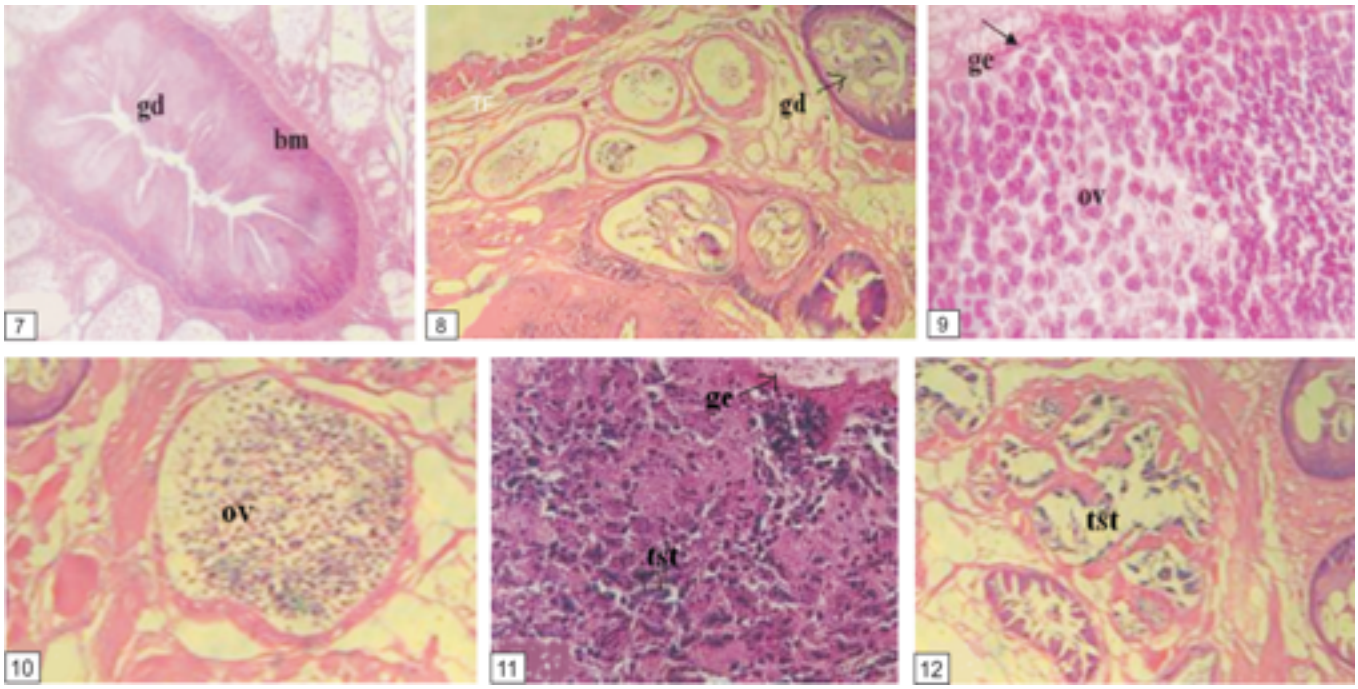
The internal architecture and morphological alterations in *Aa*EE-treated flukes were studied (Figs 1–20).

Light microscopic observations

Control flukes showed smooth spineless tegumental synctium followed by basal membrane, sub-tegumental layer, longtitudinal muscle and parenchyma (Figs 1, 2). *Aa*EE-treated flukes revealed prominent conformational changes. Severe disruptions were seen in the tegumental synctium. The basal infolds are swollen and damage to the epithelium were evident (Fig. 3). Incubation of flukes in *Aa*EE caused disruption of the microvilli in the tegument, which led to tegumental detachment. Tegumental detachment may lead to complete breakdown of cells in the parenchyma leaving vacuolated areas (Veerakumari and Paranthaman 2004). Vacuolisation around the basal infolds and parenchyma is clearly visible in the drug-treated flukes (Fig. 4). Presence of vacuoles in the parenchyma could distort the structural integrity of the flukes. The musculature of the oral sucker of the control flukes were well developed (Fig. 5) whereas widespread swelling and blebbing was observed in the oral sucker of drug-treated flukes. Appearance of vacuoles in the region surrounding the oral sucker was also clearly visible (Fig. 6). *Aa*EE paralyses the musculature of oral sucker resulting in paralysis of the worms. Hence the flukes are forced to survive in a stressed environment. Blebbing is a common stress reaction mounted in response to anthelmintic treatment, where secretory bodies are rapidly transported to, and released from, the apical plasma membrane in an effort to replace damaged membrane and maintain the integrity of the tegumental surface (McConville *et al*. 2006). Blebbing and damage to the tegument impair its physiological function. Gastrodermis of the untreated flukes was uniformly arranged with a thin cytoplasmic layer (Fig. 7) but the gastrodermis of *Aa*EE-treated fluke was severely affected. Disruptions in the gastrodermis were more prominent. Erosions and degenerations of tissues was also characterized (Fig. 8). Gastrodermis is the major site for localization of acid phophatases which plays an important role in the transport of glucose. *Aa*EE could interfere with the absorptive process in the flukes by their inhibitory effect on the activity of



Figs 1–6. **1.** Untreated fluke showing smooth tegument (t), followed by sub-tegumental layer (st) (H&E ×140); **2.** Untreated fluke showing intact parenchyma (p) (H&E ×170); **3.** *Aa*EE-treated fluke showing distorted tegument (t) (H&E ×100); **4.** *Aa*EE-treated fluke showing vacuolisation in parenchyma (p) (H&E ×400); **5.** Untreated fluke showing posterior sucker (ps) with strongly developed musculature (H&E ×180); **6.** *Aa*EE-treated fluke showing vacuoles (v) in the posterior sucker (ps) (H&E ×400).

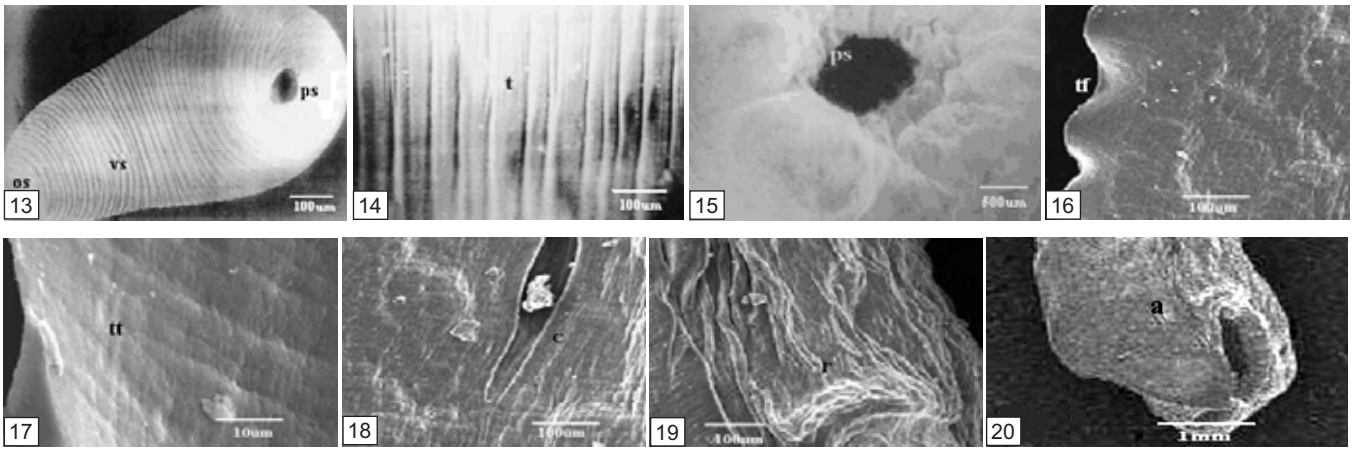


Figs 7–12. **7.** Untreated fluke showing lumen lined by columnar epithelium gastrodermis (gd) and rests upon basement membrane (bm) (H&E ×120); **8.** *Aa*EE-treated fluke showing distortions and necrosis in gastrodermis (gd) (H&E ×400); **9.** Untreated fluke showing intact ovary (ov) lined with germinal epithelium (ge) (arrows) (H&E ×100); **10.** Vacuolated testis (tst) and ovary (ov) in *Aa*EE-treated flukes (H&E ×400); **11.** Untreated fluke showing testes (tst) with germinal epithelium(ge) (arrow) (H&E ×120); **12.** Vacuolated testis (tst) and ovary (ov) in *Aa*EE-treated flukes (H&E ×400).

phophatases. Ovary of the control flukes were round and lined by germinal epithelium (Fig. 9). Blebs were observed in the ovaries of the drug-treated flukes (Fig. 10). Damage to the eggs and destruction of yolk cells due to *Aa*EE were also visualized. The male genitalia of the control flukes comprised 2 pre-ovarian tandem testes, which were lobed. Spermatocytes were liberated into the lumen from the germinal epithelium of the testes (Fig. 11). Lesions and vacuoles were also observed in the testes (Fig.12). Furthermore, vacuolization in the *ovary* and *testes* may affect the reproductive potential of *Cotylophoron cotylophorum*.

Scanning electron microscopic observations

Scanning electron micrographs of the control flukes showed pear-shaped structure with a broad round posterior end and a narrow anterior end (Fig. 13). The tegument



Figs **13–20** Scanning electron micrographs of *Aa*EE-treated flukes. **13.** Untreated fluke showing posterior sucker (ps), oral sucker(os) and ventral sucker (vs) (×100); **14.** Untreated fluke showing smooth tegument (t) (×100); **15.** Untreated fluke showing posterior sucker (ps) (×500); **16.** Dorsal surface showing tegumental folding (tf) ×250; **17.** Dorsal surface showing thickened tegument (tt) (×2000); **18.** Lateral surface showing the presence of crater (c) ×250; **19.** Ventral view showing ridges (r) on the tegument (×200); **20**. Ventral surface showing distortions in acetabulum (a) (×18).

covering the body is transversely folded, smooth and spineless (Fig. 14). The oral sucker is terminally positioned with dense sensory papillae (Fig.15).

The scanning electron micrographs revealed degenerative alterations in the surface topography of *Aa*EE- treated flukes. At higher magnifications, extensive folding on the tegumental surface was evident. Morphological changes in the tegumental surface were clearly visible (Fig. 16). Tegument of the drug-treated flukes appeared to be thick (Fig. 17). Damage to the tegumental surface may disrupt the physiological process associated with the tegument including osmoregulation, protection, secretion and synthesis (Halton 2004). Crater formation was observed in the lateral side of the *Aa*EE-treated fluke. The crater is highly invaginated with large pits (Fig. 18). Appearance of pits is due to the rupture of the sensory papillae. Damage to the papillae would undoubtedly disrupt many of the sensory function such as food detection and sexual reproduction (Shalby *et al*. 2010). Ridges are prominent on the ventral surface on the drug-treated flukes. It appears to be convoluted with folds and grooves on the anterio-mid ventral surface. Small ridges are separated from one another by variable size pits (Fig. 19). The ventral sucker of the drug-treated flukes has thick muscularis rims covered with wide transverse folds (Fig. 20). Similarly, Veerakumari and Paranthaman (2004) reported prominent changes in the oral sucker, posterior sucker and on the ventral surface of *C.cotylophorum* treated with oxyclozanide and niclosamide. The thickening of the folds is due to the swelling of the basal in fold of the tegumental synctium (Shalby *et al*. 2009). Ventral sucker helps the parasite to adhere to rumen and prevent the parasite displacement from the host (Balasubramanian and Ramasamy 2010). Damage to the acetabulum may lead to dislodgement of the flukes from the ruminal wall.

The present study elucidates the anthelmintic efficacy of *Acacia arabica* on *Cotylophoron cotylophorum*. Structural analysis of the control and drug-treated flukes confirmed various degrees of degeneration which includes necrosis and vacuolation of tissues. It may be concluded that *Aa*EE during their absorption, affect the structural conformation of the flukes. Thus plant-based medicines such as *Acacia arabica* could be used as an effective anthelmintic to treat paramphistomosis.

ACKNOWLEDGMENTS

We gratefully acknowledge Department of Science and Technology (DST), for funding this project. Our thanks are due to Mrs Amudha Manohar, Lab technician, Sri Kanchi Kamakoti, Child trust Hospital, Chennai and Dr Suresh Gandhi, Lecturer, Department of Geology, University of Madras, Chennai for their assistance in light microscopy and scanning electron microscopic studies.

REFERENCES

Anuracpreeda P, Wanichanon C and Sobhon P. 2008. *Paramphistomum cervi*: Antigenic profile of adults as recognized by infected cattle sera. *Experimental Parasitology* **118**: 203–07.

Balasubramanian P and Ramasamy P. 2010. Surface topography and tegumental morphology of adult digenetic trematode of Indian strain of *Fasciola gigantica* Cobbold. *Indian Journal of Science and Technology* **3**: 21–25.

Gamal el-din A M, Mostafa A M, Al-Shabanah O A, Al-Bekairi A M and Nagi M N. 2003. Protective effect of arabic gum against acetaminophen-induced hepatotoxicity in mice. *Pharmacological Research* **48**: 631–35.

Halton D W. 2004. Microscopy and helminth parasite. *Micron* **35**: 361–90.

Lillie R D. 1965. *Histopathologic Technic and Practical Histochemistry*. 3rd edn. McGraw-Hill Book Co., New York.

McConville M, Brennan G P, McCoy M, Castillo R, Hernandez-Campos A, Ibarra F and Fairweather I. 2006. Adult triclabendazoleresistant *Fasciola hepatica*: surface and subsurface tegumental responses to *in vitro* treatment with the sulphoxide metabolite of the experimental fasciolicide compound alpha. *Parasitology* **133**:195–208.

Shalby H A, El Namaky A H, Kamel R O A and Derbala A A. 2010. Tegumental surface change in adult *Paramphistomum microbothrium* following *in vitro* administration of artemether. *Journal of Helminthology* **84**: 115–22.

Shalby H A, El Namaky A H and Kamel R O A. 2009. *In vitro* effect of artmether and triclabendazole on adult *Fasciola gigantica*. *Veterinary Parasitology*. **160**: 76–82.

Veerakumari L and Paranthaman D. 2004. Light and scanning electron microscopic studies on the effects of niclosamide and oxyclozanide on *Cotylophoron cotylophorum* (Fischoeder, 1901). *Journal of Veterinary Parasitology* **18**: 1–12.

Veerakumari L. 1996. '*In vitro* studies on the effect of some anthelmintics on *Cotylophoron cotylophorum* (Digenea: Paramphistomidae).' A structural and biochemical analysis. Ph.D. thesis submitted to the University of Madras, Chennai.