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In vitro evaluation of anthelmintic property of herbal plants against *Fasciola gigantica*

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ABSTRACT

The flukicidal property of ethno-medicinal plant extracts of *Areca catechu*, *Erythrina indica* and *Zingiber officinale* against adult stage of *Fasciola gigantica in vitro* in comparison with chemical drug, Oxyclozanide was studied based on gross visual motility and mortality with score index, gross changes and histopathology of treated flukes. The *Areca catechu* extract had 100% lethal effect at 1% , 2.5% and 5% concentration. *Areca catechu* even showed higher efficacy than oxyclozanide. However *Zingiber officinale* extract was effective only at 5% concentration and *Erythrina indica* ineffective in all the concentrations.

Key words: Anthelmintic property, *Fasciola gigantica*, Plants extract

Fasciolosis, an economically important disease of domestic livestock and occasionally man, is caused by digenean trematode of the genus *Fasciola* (liverflukes). The two species most commonly implicated as the etiological agents of fasciolosis are *F. hepatica* and *F. gigantica*. Among this, *F. gigantica* occurs commonly in India and other tropical countries. *Fasciola gigantica* causes traumatic hepatitis, hepatic fibrosis, hyperplastic cholangitis, jaundice, anaemia and edema (bottle jaw) in domestic ruminants (Dalton 1999).

It adversely affects milk production, wool quality and quantity, and in weight gain of the animal. According to *FAO report* it is estimated that 300 million bovines and 2.4 million people exposed to fasciolosis worldwide causing enormous losses amounting to more than US$ 3 billion per annum (Dalton 1999).

The effective control of *Fasciola* currently includes strategic and tactile use of anthelmintic drugs and careful management of grazing lands, including control of stocking rates and appropriate rotation strategies. Because of the limited availability of drugs, high cost, development of resistance, chemical residue in milk and meat, toxicity problem and failed snail control measures, the majority of world population depends on traditional remedies. It is estimated that some 20 000 species of higher plants are used medicinally throughout the world for controlling various diseases. In the developed world this move is in response to the production of animals free from industrial chemical inputs and the need to discover new therapeutic substance of natural origin with possibly low toxicity to man and animals (Guarrera 1999).

For these various reasons, interest in screening of medicinal plants for their anthelmintic activity remains of great scientific interest despite use of synthetic chemicals in modern clinical practice all over the world. The plant kingdom is known to provide a rich source of anthelmintic, antibacterial and insecticide (Githori *et al*. 2006).

As a contribution to the search for therapeutical alternatives for *Fasciola gigantica, the* present study envisaged to evaluate the *in vitro* activity of extracts from plants viz, *Areca catechu* (areca nut), *Erythrina indica* and *Zingiber officinale* (ginger) in comparison to currently using flukicide drug oxyclozanide.

MATERIALS AND METHODS

Source of plant extracts

Areca catechu nuts were purchased from local market. *Erythrina indica* leaves were collected from tree. *Zingiber officinale* rhizomes were purchased from local market and identified botanically before use. All the collected materials were grinded in mortar and pestle separately and aqueous extracts were filtered through gauze cloth. *Areca-catechu* was powdered and filtered through sieve; the filtrate was labeled and stored at 4°C until further use.

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Collection of liverflukes

Fasciola gigantica were collected from the bile duct of infected cattle at the slaughter house in Perambur in Chennai. They were kept in saline medium and transferred to the laboratory of department of Veterinary Parasitology. After washing the flukes several times with saline and selecting for healthy ones with normal microscopic structure and good motility were selected. They were kept in a RPMI 1640 medium until the experiment began.

Experimental design

Good motile fluke with weights ranging from 1 to 2 g were separated into 13 groups and they were kept in petri-dish containing 25 ml of RPMI 1640 medium. Each group has 5 flukes. Groups 1, 2 and 3 were the positive control with oxyclozanide of 1% (0.25 g/25 ml), 2.5% (0.625 g/25 ml) and 5% (1.25 g/25 ml). Group 4 was negative control; they were kept only in RPMI 1640 medium. Remaining groups were exposed to 3 plant extracts having 1%, 2.5% and 5% concentration, respectively. The flukes in the medium were kept in room temperature and were observed for motility at 3, 12 and 15 h of incubation.

Motility criteria

The motility was scored using the following criteria (Jiraungkoorskul *et al.* 2005) Score 3 -Moving whole body; Score 2 -Moving only parts of the body; Score1 - Immobile but alive; Score 0 -Died.

Specimen preparation for light microscopic analysis

Carmine staining: After death of flukes, 1 fluke from each group were prepared for carmine staining. The flukes were washed thoroughly with 0.1 M phosphate buffer saline, pH 7.4 and pressed in between 2 slides, tied both sides with rubber band and submerged in formalin for at least 12 h for fixing, then they were washed in running tap water overnight. The washed flukes were dehydrated with 70% alcohol 3 times and stained with acetic alum carmine dye overnight. The flukes were destained with 1% aid alcohol, washed in ammonia water, dehydrated with graded series of ethanol, cleared in xylene and mounted with DPX.They were examined for abnormalities using Olympus TL3, S2–ST dissection microscope and photographed using a dissection photomicrograph.

Histopathological analysis (Haematoxylin and eosin staining): A further 4 flukes from each group were set for paraffin embedding. They were fixed in 10% formaldehyde for 24 h, dehydrated with a ascending series of ethanol and cleared with xylene. They were then embedded in paraffin, sectioned at thickness of 5 micrometers using a rotary microtome and stained with haematoxylin and eosin.They were examined for abnormalities using light microscope and photographed.

RESULTS AND DISCUSSION

The score index for motility of *Fasciola gigantica,* gross microscopical changes and histopathological changes in various concentrations of extracts were given in Tables 1–3.

Control flukes: The normal control flukes remained active with whole body movements (score 3) from 0–12 h in RPMI medium. Ten per cent of the groups showed a decrease in motility by moving only parts of the body (score 2) at 15 h. The morphology of adult *Fasciola gigantica* was not altered grossly. The flukes showed the normal microscopic structure

Table 1. Motility score index –5% concentration

Groups 5% concentration/	After 1 h				After 2 h				After 3 h			
% of motile flukes	3	2	1	0	3	2	1	0	3	2	1	0
Areca catechu				100%								
Zingiber officinale	40%	60%					80%	20%				100%
Erythrina indica	10%				100%				100%			
Oxyclozanide control				100%								
Normal control	100%				100%				100%			

Table 2. Motility score index –5% concentration

Groups 2.5% concentration/	After 1 h				After 2 h				After 3 h			
% of motile flukes	3	2	1	0	3	2	1	0	3	2	1	0
Areca catechu				100%								
Zingiber officinale	100%				100%				100%			
Erythrina indica	100%				100%				100%			
Oxyclozanide control				100%								
Normal control	100%				100%				100%			

Table 3. Motility score index –1% concentration

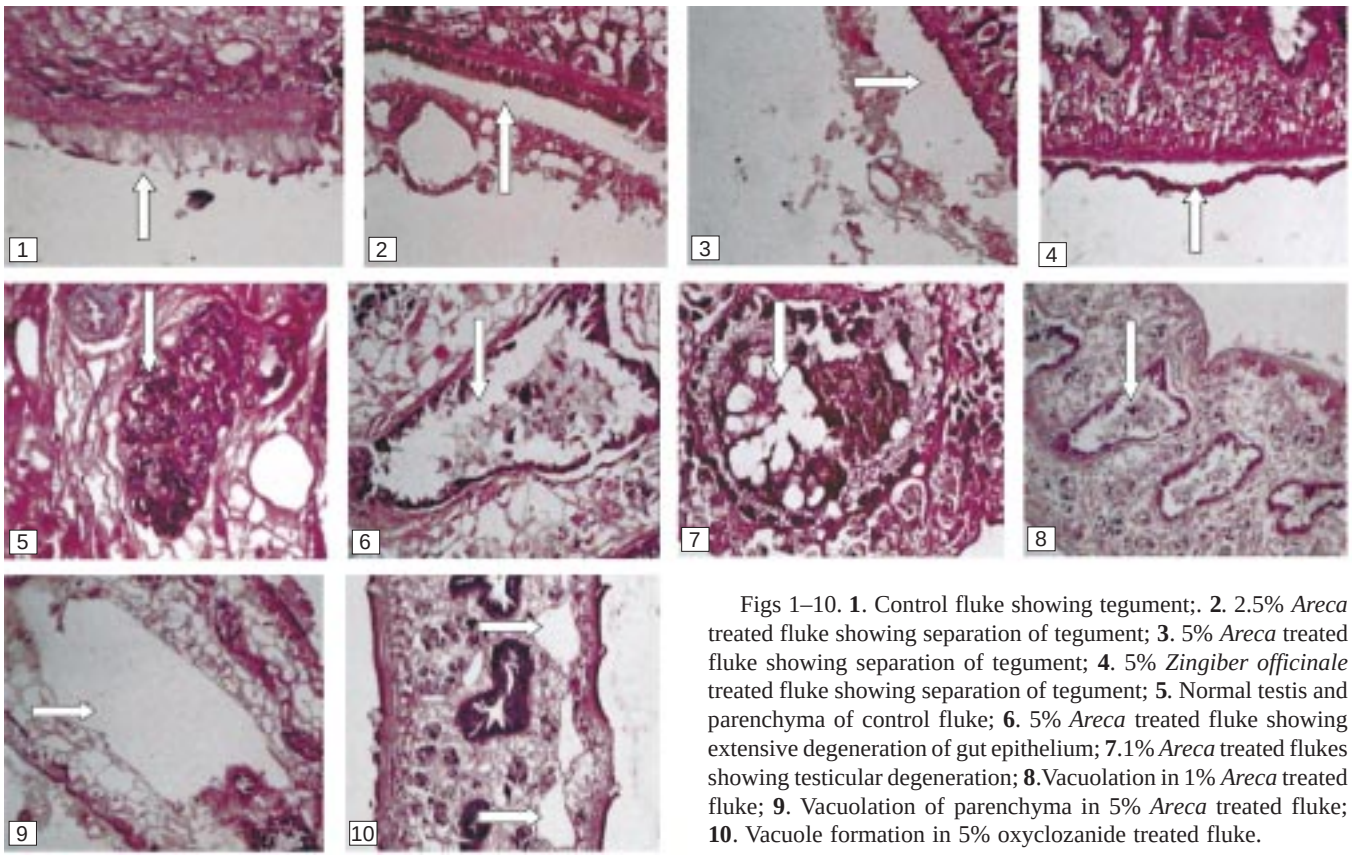
Groups 1% concentration/	After 1 h				After 2 h				After 3 h			
% of Motile flukes	3	2	1	0	3	2	1	0	3	2	1	0
Areca catechu				100%								
Zigiber officinale	100%				100%				20%	80%		
Erythrina indica	100%				100%					80%	20%	
Oxyclozanide control				100%								
Normal control	100%				100%					80%	20%	

of various organs and tegument. No histopathological changes were observed (Figs 1, 5).

The oxyclozanide treated control flukes become slender, shrunken, paralyzed and then finally died after 2 min at 5% concentration. In case of 2.5% concentration, the flukes showed paralysis and died after 3 min of exposure. The flukes were shrunken and moved only the parts of the body (score 2) and died after 4 min in 1% concentration. The flukes were undergone change in their position and paralyzed before death. Grossly the flukes showed spiny eruptions on the surface of the body, shrinkage of the fluke and breaking of testicular branches. Histopathologicaly**,** vacuole formation was noticed in the longitudinal section of fluke (Fig.10). Testes were massively degenerated and also there is disruption of villi in 5% oxyclozanide treated fluke. In 2.5% and 1% also showed the vacuolation in parenchyma, degeneration of testes, disruption of villi, but in a less intensive manner.

Effect of Areca catechu *on* Fasciola gigantica: Areca caused more severe effects and sudden paralysis on flukes. They caused mortality within 30 sec (score 0) at 5% concentration. At 2.5% concentration, flukes showed paralysis and edges of the flukes were shrunken moderately. They died within 30 sec (score 0). The flukes showed decrease in motility after 30 sec by moving only the parts of their body (score 2) in 1% concentration. Afterwards they become immobile (score 1). Finally they paralyzed and died after 2 min.

In all the concentrations of Areca treated flukes, deformation of the body shape with absence of spines on the



Figs 1–10. **1**. Control fluke showing tegument;. **2**. 2.5% *Areca* treated fluke showing separation of tegument; **3**. 5% *Areca* treated fluke showing separation of tegument; **4**. 5% *Zingiber officinale* treated fluke showing separation of tegument; **5**. Normal testis and parenchyma of control fluke; **6**. 5% *Areca* treated fluke showing extensive degeneration of gut epithelium; **7**.1% *Areca* treated flukes showing testicular degeneration; **8**.Vacuolation in 1% *Areca* treated fluke; **9**. Vacuolation of parenchyma in 5% *Areca* treated fluke; **10**. Vacuole formation in 5% oxyclozanide treated fluke.

tegument was noticed. Rupture of testicular, uterine and intestinal branches was also noticed. Histopathologically, the flukes showed the marked separation of tegument from the cuticle and also corrugated at 5% concentration (Fig. 3). The longitudinal smooth muscle showed a massive contraction leads to vacuolation of parenchyma (Fig. 9). There was severe degeneration of testis. Only few stem cells were left leaving many hollow spaces in the testis, thus spermatogenesis was reduced (Fig. 6). At 2.5% treated flukes, showed separation of the tegument, vacuolation of parenchyma all over the body and small hollow space in testis showing degeneration (Fig. 2). Flukes treated with 1% concentration showed mild separation of tegument, vacuolation in parenchyma (Fig. 8) and few hollow spaces in testis indicating testicular degeneration (Fig. 7).

Prajapathi *et al.* (2007) reported anticestodal effect of *Areca catechu* in dogs and poultry. *Areca catechu* was also proposed for treatment of liver fluke infection in ruminants (Hammond *et al.* 1997). However, the *in vitro* effect of *Areca catechu* on *Fasciola gigantica* was not studied as per the available literature.

Effect of Zingiber officinale *(Ginger) extract on* Fasciola gigantica: The ginger treated flukes become flattened after exposure to 5% concentration, but they were motile (score 3). The 60% of the flukes moved only the part of the body (score 2), while remaining 20% were immobile (score 1) at the end of 1 h. All the flukes died (score 0) after 3 h. In case of 2.% and 1% ginger, the flukes were fully active up to 12 h (score 3) as that of control groups.

Microscopically 5% concentration treated flukes showed the presence of prominent spines. Gross abnormalities were not detected in 2.5% and 1% concentration treated groups. Separation of tegument was noticed in 5% concentration flukes (Fig. 4). 2.5% and 1% treated flukes were normal. Goto *et al*. (1990) also reported destruction of digestive tract and disturbance of cuticle in Anisakis larvae. Effect of these extract on *Fasciola gigantica* was so far not studied. However, ginger showed anthelmintic activity against *Ascaris lumbricoides* (Akthar *et al.* 2000). Iqbal *et al*. (2006) reported the anthelmintic activity against gastrointestinal nematodes in sheep. The extracts also tried against *Haemonchus contortus* (Iqbal *et al.* 2001). It also showed antischistosomal and antifilarial effect (Mascolo *et al*. 1989 and Datta and Sukul 1987).

Effect of Erythrina indica *(Kalyanamurungai) extract on* Fasciola gigantica: The flukes were remained active up to 12 h (score 3) as that of normal control group in all the concentrations. No abnormal gross and histopathological changes were detected in both extract treated flukes, in all the concentrations.

The extracts of *Erythrina indica* leaves did not show any effect on *Fasciola* grossly as well as microscopically. However, another species of *Erythrina* showed anthelmintic activity (Nwude and Ibrahim 1980), thus it is evident from the results that extracts of *Areca catechu* markedly reduced the motility and caused death of flukes in all concentrations. Ginger was effective at 5% concentration only. *Erythrina indica* did not show any effect on *Fasciola gigantica*. *In vitro* effect of plant extracts on *Fasciola gigantica* was so far not studied except Kushwaha *et al*. (2004) who reported the inhibitory effect of extracts of *Carioca papaya*, *Mallotus phillippinensis* and *Azadiracta indica*.

It is concluded that, our results may be a base for developing herbal-based anthelmintics for the control of *Fasciola gigantica* in livestock. Studies need to be carried out worldwide to identify more plants with medium to high efficacy against nematode parasites. Plant products that have showed high activity against worm parasites *in vitro* need to be evaluated and tested in animal hosts. It may be not as a sole alternative to anthelmintic drugs but as a part of an integrated approach specifically designed to achieve sustainable parasite control in animal production systems.

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