ANTHELMINTIC ACTIVITY OF ESSENTIAL OILS OF CYMBOPOGAN NARDUS AND AZADIRACHTA INDICA ON FASCIOLA GIGANTICA

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ABSTRACT

The efficacy of essential oils of Cymbopogan nardus (citronella) and Azadirachta indica (neem) on Fasciola gigantica was assessed in vitro. The essential oil of citronella showed a potential anthelmintic activity whereas neem oil did not show any significant effect. Study revealed that 1% citronella oil showed anthelmintic effect on live Fasciola gigantica. The in vitro anthelmintic effect was evaluated in comparison with control and score index was made based on gross motility. The morphological variation and histopathological changes in treated and control fluke was studied.

Key words: Citronella oil, Neem oil, Anthelmintic activity, Fasciola gigantica

INTRODUCTION

Fasciolosis has been recognized as an important helminthic disease of livestock causing significant losses to livestock owners, on account of poor growth and lower productivity of domestic ruminants. It is estimated that 300 million bovines and 2.4 million people are exposed to Fasciolosis world wide causing enormous loss, amounting to more than US $ 3 billion per annum. The disease is mainly caused by Fasciola gigantica in animals in India (Dalton, 1999). Effective control of this parasite includes strategic use of anthelmintic drugs and control of snail intermediate host. However problems have emerged with the use of chemical drugs, notably the development of resistance in parasite, chemical residues and toxicity problem as well as cost of drugs for treatment in animals.

For these various reasons, screening of medicinal plants for their anthelmintic activities remains of great scientific interest. A number of medicinal plants have been used in man and animals (Akthar et al., 2000). Citronella oil is used in traditional medicine as an antiseptic, bactericidal, deodorant, tonic, stimulant, weedicide and acaricide (Thorsell et al., 2006). Neem oil is also used in traditional medicine as fly repellant (Sharma et al., 1993). Kushwaha et al. (2004) studied the effect of extracts of carioca, papaya, Mallotus philipiensis and Azadirachta indica on Fasciola gigantica in vitro. Alcoholic extracts of Allium sativum and Piper longum showed inhibitor effect on F. gigantica (Singh et al., 2007). However effect of plant oils on F. gigantica was not studied elsewhere. Under these circumstances, the present study is taken up to evaluate the anthelmintic property of the herbal plant oils in vitro in comparison with flukicide drug oxyclozanide against F.gigantica.
MATERIALS AND METHODS

Collection of Liver flukes

_Fasciola gigantica_ were collected from the bile duct of infected cattle at the slaughterhouse in Perambur in Chennai. They were kept in normal saline and transferred to the laboratory of department of Veterinary Parasitology. After washing the flukes several times with normal saline, the healthy ones with normal microscopic structure and good motility were selected. They were kept in a RPMI 1640 medium (Gibco, USA) until the experiment began.

Source of plant oils

Citronella oil was obtained from dried grass of _Cymbopogon nardus_ (Graminae). The oil was extracted by steam distillation process of finely chopped fresh dried plant. Neem oil was extracted from the dried seeds of _Azadirachta indica_.

Experimental design

Anthelmintic activity was studied by _in vitro_ petri dish method as described by Jiraungkoorskul _et al._ (2005) and Githiori _et al._ (2006). Ten flukes were taken for each group in petri dishes with RPMI-1640 medium and exposed to each of the following treatment group at room temperature at a concentration of 1% of Citronella oil, neem oil, oxyclozanide drug powder treated control and normal RPMI-1640 control. The inhibition of motility and/or mortality of flukes were observed after 3, 12, 15 hrs and score index was made. The morphological and histopathological variation of flukes was studied after the experiment. The experiment was repeated thrice with fresh flukes.

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
- **Score 1** - Immobile but alive
- **Score 0** - Died

Specimen preparation for light microscopic analysis

**Carmine staining**

After death of flukes, five flukes from each group were prepared for carmine staining. The flukes were washed thoroughly with 0.1M phosphate buffered saline, pH 7.4 and pressed in between two slides, tied both sides with rubber band and immersed in 10% formalin for at least 12 hrs for fixing. Then, they were washed overnight in running tap water to remove the formalin. The washed flukes were dehydrated with ascending grades of alcohol three times and stained with acetic alum carmine stain overnight. The flukes were destained with 1% acid 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 (Tokyo, Japan) and photographed using a Leica dissection photomicroscope.

**Haematoxylin and eosin staining**

A further four flukes from each group were set for paraffin embedding. They were fixed in 10% formalin for 24 hrs, dehydrated with ascending series of ethanol and cleared with xylene. They were then embedded in paraffin, sectioned longitudinally at thickness of 5 micrometers using a rotary microtome and stained with haematoxylin and eosin. They were examined for abnormalities using an Olympus CH 40 light microscope (Tokyo, Japan) and photographed.

RESULTS AND DISCUSSION

Gross visual motility

The movement of the treated and control flukes were observed and motility score index was.
Fig 1. Control fluke uterus showing fully yolk packed eggs.

Fig 2. Citronella treated fluke uterus showing shrinkage of yolk material with various shapes in egg.

Fig 3. Control fluke showing tegument with spin.

Fig 4. Neem oil treated fluke tegument shows breakage of spines.

Fig 5. Vacuolation of parenchyma and sloughing of spines in citronella treated fluke.

Fig 6. Intestinal caeca with villi of control fluke.
The flukes (100%) in control and neem oil treated group remained active with whole body movements from 0 to 3 hrs (score 3). Citronella oil and oxyclozanide treated flukes were dead within 3hrs (score 0). Forty per cent of neem oil treated flukes showed motility by moving only parts of the body (score 2) and remaining 60% were died (score 0) at 12 hrs. The RPMI-1640 normal control flukes (80%) showed motility by moving only parts of the body (score 2) and remaining 20% flukes died (score 0). All the neem oil treated and RPMI-1640 control flukes were died at 15hrs (score 0).
Light microscopic studies

The gross changes in carmine stained flukes were observed under light microscope. Grossly deformation of the body shape was observed in treated groups after death. Disintegration of Intestinal, uterine and testicular branches was noticed in citronella oil treated group. Eggs were blackened inside uterus and sloughing of spines from the cuticle was also observed. In case of neem oil group, spines of the flukes sloughed off from the tegument. Testicular branches were also separated. Spiny eruptions on the surface of the body and shrinkage of the fluke were noticed in oxyclozanide control group.

The flukes were sectioned longitudinally and were explored for any histopathological changes in their organs under microscope. Morphological changes in the tegument layer and spines were observed in most of oil treated groups. Total disappearance of spines from cuticle (Fig, 5), complete desquamation of gut epithelial cells and absence of villi in intestinal caeca (Fig.7) were noticed in citronella oil treated flukes. They also showed disintegration of eggs if uterus with half moon shaped shrinkage of yolk (Fig.2). The neem oil treated flukes showed the blebbing of teguments, breakage of spines (Fig.4) and erosion of intestinal villi (Fig.8). Vacuole formation was noticed in oxyclozanide control group (Fig.9).

From the in vitro trials, it was concluded that citronella oil showed flukicidal effect similar to that of oxyclozanide. It affects the tegument, intestinal caeca and uterus. However comparatively neem oil showed less effect on flukes than citronella oil. Literature showed that, these oils were tried for controlling various ectoparasites (Abdul- Shafy and Zayed, 2002; Kim et al., 2004). However the effect of these oils on endoparasites was not studied earlier. The mechanism of action of these oils was not clearly understood. This in vitro study may act as a base for evaluation of effect of these oils in vivo against Fasciola gigantica. Toxicological studies of these oils also are carried out though

<table>
<thead>
<tr>
<th>Groups</th>
<th>% of Motile flukes</th>
<th>After 3 hrs</th>
<th>After 12 hrs</th>
<th>After 15 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Citronella oil</td>
<td>100%</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Neem oil</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxyclozanide control</td>
<td>100%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>100%</td>
<td>80%</td>
<td>20%</td>
<td>60%</td>
</tr>
</tbody>
</table>

Table.1
Motility Score index

After 3 hrs | After 12 hrs | After 15 hrs
3 | 2 | 1 | 0 | 3 | 2 | 1 | 0 | 3 | 2 | 1 | 0

Anthelmintic activity of...
Chenopodium oil was used for many years in UK to treat nematode parasite infections in monogastric animals including humans and mustard oil was tried for treating helminthic parasites (Gibson, 1965; Ketzis et al., 2002). These oils may be used to enrich feedstuffs like silage and hay and also incorporated in feeds as a binder for the preparation of feed pellets to treat fasciolosis in animals.

REFERENCES


