Effects of Piper longum fruit, Piper sarmentosum root and Quercus infectoria nut gall on caecal amoebiasis in mice

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Abstract

The anti-amoebic effects of crude methanol extracts of Piper longum fruit, Piper sarmentosum root and Quercus infectoria nut gall against Entamoeba histolytica infecting the caecum of mice were studied. Caecal amoebiasis in mice was induced by injection of Entamoeba histolytica trophozoites directly into the caecum. The mice were then treated orally with the extract, a standard drug (metronidazole), or vehicle p.o. for five consecutive days, beginning 24 h after the infection and were examined on the sixth day. At a dose of 1000 mg/kg per day, the extracts of Piper longum fruit, Piper sarmentosum root and Quercus infectoria nut gall had a curative rate of 100, 40 and 26%, respectively. At a concentration of 500 and of 250 mg/kg/day, extract from Piper longum fruit was still effective in 93 and 46% of the cases, respectively, while extract from Piper sarmentosum root at a dose of less than 1000 mg/kg per day did not cure any mice from amoebiasis. Extract of Quercus infectoria nut gall at a concentration of 500 and of 250 mg/kg per day cured 26 and 13% of mice, respectively. Metronidazole at a concentration of 125 and of 62.5 mg/kg per day had a curative rate of 100 and 60%, respectively. The severity of caecal wall ulceration was reduced in mice which received the extract and metronidazole as compared to the control animals.

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1. Introduction

Among parasitic infections, amoebiasis ranks third worldwide in lethal infection, after malaria and schistosomiasis (Walsh, 1988; Petri and Mann, 1993). Although it is asymptomatic in 90% of cases, about 50 million people are estimated to suffer from the symptoms of amoebiasis such as haemorrhagic colitis and amoebic liver abscess (Ravdin, 1995). These infections result in 50 000–100 000 deaths annually. In South Africa and India, the disease is rather common (Walsh, 1986).

During 1987–1997, in Thailand, there were more than 50 000 cases of dysentery reported each year and approximately 400 people died. For about 90% of the cases, the cause of dysentery is unknown; however, Entamoeba histolytica was detected in 2–3% of cases (Anon, 1997). Among children under five years who were admitted with acute diarrhoea in a hospital, Entamoeba histolytica was confirmed in 7.8% of the cases (Suwatanat, 1997). The estimated number of infected cases may be much higher due to the lack of a sensitive and specific diagnostic test (Petri et al., 2000).

The most effective and commonly used drug for treatment of intestinal protozoa infection is metronidazole (Tracy and Webster, 1996). However, this drug has been reported to cause mutagenicity in bacteria (Legator et al., 1975) and is carcinogenic in rodents (Rustia and Shubik, 1972; Shubik, 1972). It has been reported that the human pathogenic bacterium, Helicobacter pylori, becomes resistant to metronidazole in vitro (Zwet et al., 1994). Moreover, it seems to act as an immunosuppressive agent in experimental rats, both in cell-mediated and humoral immune responses (Saxena et al., 1985). These are the main reasons why there is a need to develop a safe and effective alternative antiamoebic agent.

For people in developing countries, medicinal plants are popular because their products are safe and widely available at low cost. Some compounds extracted from medicinal plants already play an important role against infectious diseases e.g. quinine from Cinchona sp., and artesunicin from Artemisia annua; both are effective against malaria. In the present study, Piper longum (Linn.) fruit (PLF), Piper sarmentosum (Roxb.) root (PSR) and Quercus infectoria (Oliv.)
nut gall (Qin) were selected because these species are routinely used to cure bloody diarrhea in Thai traditional medical practice. It is, therefore, of interest to scientifically evaluate their effect on amoebiasis for potential antiamoebic activity in vivo. The selected plants were extracted with methanol and tested against caecal amoebiasis in mice. The anti-amoebic effect of the extract was compared with the standard drug metronidazole.

2. Materials and methods

2.1. Isolation and cultivation of Entamoeba histolytica

The culture of Entamoeba histolytica used in this experiment was isolated from the bloody stool diarrhea of a patient from Maharaj hospital, Nakorn Srithammarat, Thailand. Briefly, the untreated fecal samples were collected after diagnosis as Entamoeba histolytica infection and cultured in Boeck & Drbohlav medium with some modification as described elsewhere (Sawangjaroen et al., 1993). Calf bovine donor serum (10%) was used instead of horse serum. The culture was incubated at 37°C and Entamoeba histolytica trophozoites along with their associated bacteria were sub-cultured every 24/48 h.

2.2. Preparation of extracts from medicinal plants

Plf and Qin were purchased from the medicinal plant store while Psr was collected from the area around Hatyai, Songkhla, Thailand. Voucher specimens for Plf, Qin and Psr have been deposited at The Prince of Songkla University Herbarium, Department of Biology, Faculty of Science, Prince of Songkla University, Hatyai, Songkhla, Thailand under voucher specimen numbers K. SAWANGJAROEN 1 (PSU), K. SAWANGJAROEN 2 (PSU) and K. SAWANGJAROEN 3 (PSU), respectively. The plants (or parts) were washed, cut into small pieces and dried in sunlight or in an oven at 50°C maximal. Each plant material was subsequently pulverized and macerated in absolute methanol, at the ratio of 1 kg of plants per 3 l of methanol. The supernatants were collected after 7 days and the remaining plant was macerated again. This procedure was repeated twice. Whole methanol extract from each plant was filtered and evaporated to dryness under a low pressure with a rotary evaporator, at 55°C. The extracts were then stored at 4°C until use. Methanol extraction of Plf, Psr and Qin gave 22.8, 44.5 and 46.7% yield, respectively.

2.3. Inoculation procedure

Female Swiss albino mice, weighing between 25 and 35 g, aged 1–1.5 months were used throughout the experiment. The mice were prepared for Entamoeba histolytica infection according to the method of Ray and Chatterjee (1981) with a slight modification. Briefly, 24 h before the commencement of the surgery, the mice (25–35 g) were starved, and, in the morning and evening, the mice were pretreated orally with 0.5 ml of 25% MgSO4 in distilled water. On the next day, the mice were anesthetized by an intraperitoneal injection of pentobarbital sodium 40 mg/kg. Laparotomy was performed to expose the caecum. The suspension of actively motile Entamoeba histolytica at the volume of 0.2–0.3 ml containing 2.0 × 104–2.5 × 105 trophozoites was injected directly into the caecum. The caecum was then returned into the peritoneal cavity, the abdominal muscle was closed and the skin sutured. Rat pellets and drinking water were provided ad libitum. The mice were randomly selected for the treatment and control groups.

2.4. Effects of crude extracts and metronidazole on amoebiasis in mice

Extract of Plf, of Psr and of the standard drug, metronidazole in tablet form, were suspended in a 20% gum acacia solution in distilled water. The extract of Qin was suspended in distilled water. All treatment were administered daily p.o. using a feeding tube, for five consecutive days, beginning 24 h after infection with Entamoeba histolytica. The doses of plant extract used were 1000, 500, 250 and 125 mg/kg body weight per day and for metronidazole 125 and 62.5 mg/kg per day. The control animals were treated with 20% gum acacia solution in distilled water (for Plf, Psr and metronidazole) and with distilled water (for Qin). Fifteen animals were used for each treatment. On the sixth day, the animals were sacrificed by cervical dislocation and the caecum was carefully examined macroscopically for lesions and the content structure. The severity of infection was scored according to the method of Neal ranging from 0 for normal to 4 for severe structure destruction (Neal, 1951). The presence of Entamoeba histolytica trophozoites in the caecum was observed under light microscope. In the absence of Entamoeba histolytica trophozoites, a small amount of caecum content was transferred into a fresh medium and cultured for 24–48 h and this was then examined for trophozoites under light microscope.

3. Results and discussion

The effects of extracts from Plf, Psr and Qin against experimental caecal amoebiasis in mice are shown in Table 1. The results from the present study demonstrate that methanol extracts from selected medicinal plants are effective against Entamoeba histolytica in mice as evaluated by the number of mice cured and the reduction of severity of the mice caecal content and caecal wall lesions in comparison to the untreated mice. The anti-amoebic effects of all extracts are clearly dose-dependent. Most of the published data on medicinal plants against Entamoeba histolytica in vivo is based on the rat model (Sohni et al., 1995; Ghoshal et al., 1996). Our study shows for the first time that the selected...
Table 1

<table>
<thead>
<tr>
<th>Test materials</th>
<th>Dose (mg/kg per day)</th>
<th>Number of mice cleared/treated (% cured)</th>
<th>Average caecal score* (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Contents</td>
</tr>
<tr>
<td><em>Piper longum</em> fruit</td>
<td>125</td>
<td>0/15 (0)</td>
<td>0.4 (0–1)</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>7/15 (46)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>14/15 (93)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>15/15 (100)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td><em>Piper sarmentosum</em> root</td>
<td>125</td>
<td>0/15 (0)</td>
<td>2 (2–2)</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0/15 (0)</td>
<td>2 (2–2)</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0/15 (0)</td>
<td>1.4 (0–2)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>6/15 (40)</td>
<td>0.2 (0–1)</td>
</tr>
<tr>
<td><em>Quercus infectoria</em> nut gall</td>
<td>125</td>
<td>0/15 (0)</td>
<td>1.06 (0–2)</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>0/15 (0)</td>
<td>0.62 (0–2)</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>0/15 (0)</td>
<td>0.25 (0–2)</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>4/15 (26)</td>
<td>0.01 (0–2)</td>
</tr>
<tr>
<td>Metronidazole</td>
<td>62.5</td>
<td>9/15 (60)</td>
<td>0.06 (0–1)</td>
</tr>
<tr>
<td></td>
<td>125</td>
<td>15/15 (100)</td>
<td>0 (0–0)</td>
</tr>
<tr>
<td>Untreated control</td>
<td>-</td>
<td>0/20 (0)</td>
<td>2.55 (2–3)</td>
</tr>
</tbody>
</table>

*Caecal scores were graded upon the following criteria (Neal, 1951).

Wall: normal, 0; slight thickening, 1; marked local thickening and contraction, 2; extensive thickening and contraction, 3; caecum shapeless (extensive ulceration with abscess formation), 4.

Contents: normal, 0; slightly less solid than normal, 1; slightly mucoid, 2; mucoid (some solid matter present), 3; no solid matter (white or yellow mucus only), 4.

medicinal plant also reduces the severity of caecum due to *Entamoeba histolytica* infection in mice.

The pooled controls of 20 mice were all positive for amoebae at the time of sacrifice. This amoebic infection generally produced score of caecal content and caecal wall ranging between 2 and 3 with the average of 2.55 and 2.40, respectively. This indicates the virulence of the strain of *Entamoeba histolytica* used in this study. Although, this strain was originally isolated from human bloody stool diarrhea, it was still infective in mice. It is generally known that axenic strain of *Entamoeba histolytica* becomes non-invasive after prolonged cultivation in vitro (Phillips et al., 1972; Phillips, 1973). We found that the amoebae isolated from the control mice infected with this strain of *Entamoeba histolytica* was still virulence and could be used subsequently.

In the present study, mice treated with metronidazole at a concentration of 125 mg/kg per day for 5 days were successfully cured from amoebiasis, confirming that this strain of *Entamoeba histolytica* was still sensitive to this drug. Our results on efficacy of metronidazole were similar to the studies of several investigators whose studies on caecal amoebiasis were performed, both in rats and mice models (Bhopale et al., 1995; Ghoshal et al., 1996).

The extract from Plf appeared to be the most effective at a concentration of 1000 mg/kg per day, as this dose cleared all *Entamoeba histolytica* from the intestine of mice on the day of examination. This is comparable to metronidazole at the dose 125 mg/kg per day. Although treatment with extract from Plf at a concentration of less than 1000 mg/kg per day did not cure all animals, the caecal content and caecal wall of these mice appeared normal indicating the effectiveness of the extract against the parasites. The use of this extract to treat amoebiasis may at least help in reducing severity occurred in the intestine. Our finding from this study on the effect of Plf on *Entamoeba histolytica* is consistent with those previously reported that an ethanol extract of Plf at a concentration of 1000 mg/ml per day can cure 90% of rats infected with *Entamoeba histolytica* (Ghoshal et al., 1996). Although Plf is effective for the treatment of amoebiasis in rodents, the mode of actions of Plf extract against *Entamoeba histolytica* is unknown. An in vitro study showed that allcin from freshly crushed garlic inhibited the activity of cysteine proteinases, an important contributor to amoebic virulence (Ankri et al., 1997). In addition, piperine which is widely known to be a major constituent of Plf is not effective as an amoebicide either in vitro or in vivo (Ghoshal et al., 1996). Further investigations are therefore needed to identify an active compound of this extract and to determine whether the alteration of the enzyme activity is the target mode of action of this extract.

The methanol extracts from Psr and Qin appeared to be effective against caecal amoebiasis in mice in this study. However, the effect of these extracts on the amoebiasis seem to be much less potent than that of Plf. It is unlikely therefore that their antidysenteric activity occur solely from the antiamoebic activity. Their mode of actions on the treatment of dysentery have yet to be determined.

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References


