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Anthelmintic, anti-inflammatory and antioxidant effects of *Garcinia mangostana* extract in hamster opisthorchiasis

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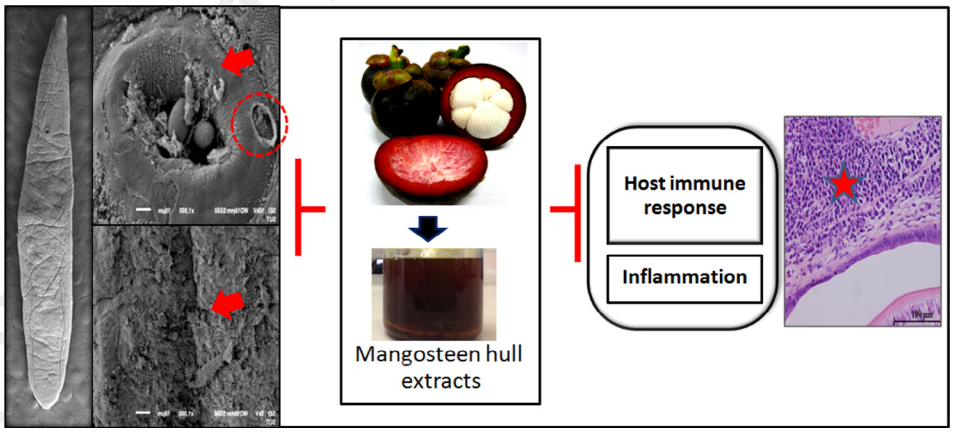
HIGHLIGHTS

- We tested the anti-*O. viverrini* activity of *G. mangostana* extracts.
- *G. mangostana* extracts interfered the *O. viverrini* tegument and reproductive organs.
- SEM shows sloughing and blebbing of *O. viverrini* tegument after treatment.
- *G. mangostana* extracts might useful for anthelmintics development in the future.

GRAPHICAL ABSTRACT



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ABSTRACT

Administration of praziquantel for treatment of liver fluke infection may affect the host, with mild and severe effects after treatment caused by host immune response. Therefore, we focused on the antioxidant property, inflammatory and anthelmintic effects of the traditional folk medicine, *G. mangostana* pericarp extract, in hamster opisthorchiasis. Syrian hamsters were divided into four groups: normal (control) (N); administered *G. mangostana* alone (GM); infected with *Opisthorchis viverrini* alone (OV); and infected with *O. viverrini* and administered *G. mangostana* extract for 1.5 months (OVGM). Hamster livers were collected 45 days after infection to determine histopathological changes, i.e. aggregation of inflammatory cells. The morphology of adult *O. viverrini* (body size and sizes of reproductive organs) was analyzed, as well as worm burden, eggs per worm and eggs per gram of feces. Toxicity was tested by kidney function (blood urea nitrogen and creatinine); the results demonstrated that *G. mangostana* had no renal toxic effect. ABTS radical-scavenging assay indicated that the extract had antioxidant property. Reduction in aggregation of inflammatory cells surrounding the hepatic bile duct, especially at the hilar region, was found in the

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OVGM group. Worm burden was similar in both infected groups (treated or untreated with *G. mangostana*), but the average size of adults in the OV group was larger than in the OVGM group; moreover, eggs per worm and eggs per gram of feces were also comparatively higher. The present study suggests that *G. mangostana* extract possesses anti-inflammatory and antioxidant properties and can interfere with parasite development by affecting adult size and egg production. This may be useful for controlling the spread of OV infection and other parasites in endemic areas.

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1. Introduction

Opisthorchis viverrini infection is found throughout Southeast Asia, especially Laos, Cambodia, Vietnam, and particularly Thailand. Humans are infected by ingesting raw cyprinid fish that are contaminated with the metacercariae of *O. viverrini*. After infection, the juveniles move to the biliary tract and can cause several hepatobiliary diseases, including cholangitis, hepatitis, lithiasis and cholangiocarcinoma (CCA). To eradicate this parasite would seem to be a relatively simple matter, but eating raw cyprinid fish is part of the culture among people living in this region, and the practice is difficult to change. Therefore, *O. viverrini* infection remains a major health problem in northeast Thailand, even though in recent years there has been mass therapy for treatment of this parasite (Vivatanasesth et al., 1982). The high prevalence of *O. viverrini* infection in this region is correlated with its having the highest incidence of CCA in the world (IARC, 2011). CCA can arise from a combination of pathological causes: infection by the parasite itself; host immune response to the parasite; immunopathology; and other factors, such as consumption of improperly fermented food, and long-term infection without treatment (Manwong et al., 2013). Several previous studies have focused on the reduction of pathology from liver fluke infection by administering anthelmintic drugs or certain medicinal plants which may possibly prevent or retard CCA development (Mahavorasirikul et al., 2010; Plengsuriyakarn et al., 2012). Curcumin (Sriraj et al., 2009) and *Thunbergia laurifolia* (Wonkchalee et al., 2012) were reported to have anti-inflammatory effects in cases of animal opisthorchiasis and CCA.

To date, there have been no reports on the anthelmintic activity of medicinal plants against *O. viverrini*. However, many other plants have been studied for anthelmintic activity: i.e. onions, garlic, chives, coconut, birch bark, pineapple, banana, mangosteen, chicory, date palm fruit, fig, pumpkin, and neem tree seeds against nematodes (*Trichuris muris* and *Angiostrongylus cantonensis*) (Klimpel et al., 2011) and trematodes (*Fasciola hepatica* and *Echinostoma caproni*) (Abdel-Ghaffar et al., 2011). Other studies have reported on the effects of ginger (*Zingiber officinale*) on different stages of *Schistosoma mansoni* (Aly and Mantawy, 2013; Mostafa et al., 2011), and the effects of water extract of black nightshade (*Solanum nigrum*) leaves on *Schistosoma mansoni* (Ahmed and Rifaat, 2005). *Piper chaba* fruits demonstrated strong activity against schistosome cercariae (Atjanasuppat et al., 2009), while *Lysimachia ramosa* Wall. (Primulaceae) was found to have an effect on the helminth parasites *Fasciolopsis buski*, *Ascaris suum* and *Raillietina echinobothrida* (Challam et al., 2010). *G. mangostana* pericarp, the hull of the mangosteen fruit, is a traditional folk medicine that has been shown to be beneficial in the treatment of infected wounds (Pedraza-Chaverri et al., 2008), has anti-colon cancer effects (Aisha et al., 2012), and possesses anti-inflammatory (Chen et al., 2008), antimicrobial (Iinuma et al., 1996) and cholesterol-lowering (Williams et al., 1995) properties. Due to these previously demonstrated properties, and because of the abundance of mangosteen hulls available in Thailand during the rainy season, the present study investigated the antioxidant and anti-inflammatory properties and anthelmintic effects of *G. mangostana* pericarp extract on hamster opisthorchiasis through observation of pathological changes, worm recovery and parasite development.

2. Materials and methods

2.1. Plant materials and preparation

Garcinia mangostana pericarp (mangosteen hulls) was collected from a local market in Khon Kaen, Thailand. The hulls were washed, dried in a hot-air oven at 60 °C for 48 h, ground, and then kept at 4 °C in a refrigerator until used. For in vivo testing, about 500 mg of *G. mangostana* powder was incubated with 1000 mL methanol for 3 weeks. After filtering through 0.45 µm filter paper, *G. mangostana* solution was evaporated on a hotplate to remove the methanol. About 10 mg of *G. mangostana* extract was dissolved in 1 mL of DMSO, and distilled water was added to 100 mL. Fresh preparations were fed to hamsters for 2 weeks at a daily dose of 100 mg/kg of body weight.

2.2. ABTS radical-scavenging activity assay

Free radical-scavenging activity of *G. mangostana* extract was determined by 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS; Fluka, Steinheim, Germany), following the method described by Chun et al. (2005) with slight modification. ABTS was dissolved in distilled water to a 7 mM aqueous solution of ABTS (30 mL) with 25 mM potassium persulfate (a blue/green chromophore with characteristic absorption at 700 nm). The mixture was kept in the dark at room temperature for 12 h before use. For the study, the ABTS working solution was diluted with ethanol to give an absorbance of (0.70 ± 0.02) at 700 nm and was equilibrated at room temperature. The reaction mixtures, incubated in 96-well plates, consisted of 50 µL of various concentrations of *G. mangostana* extract and 100 µL of ABTS/ethanol working solution (Yang et al., 2011). The mixture was stirred and incubated for 10 min in the dark; then the absorbance was measured at 700 nm. Trolox (25 µM) was used as a standard solution. The percentage inhibition of ABTS was calculated for each concentration relative to a blank absorbance (ethanol). The dose–effect results were determined using 10 different concentrations; experiments were performed 5 times. The percentage scavenging effect was calculated as:

$$\%\ \text{ABTS scavenging inhibition} = (A_1 - A_2) * 100/(A_1 - A_0)$$

where A_0 is the absorbance of the blank, A_1 is the absorbance of ABTS working solution as a positive control, and A_2 is the absorbance of the sample. The scavenging ability of the sample was expressed as scavenging concentration (SC_{50}), i.e. the effective concentration at which 50% of ABTS radicals were scavenged. Data were analyzed using CalcuSyn 2.1 software (Biosoft, Cambridge, UK).

2.3. Experimental animals

Hamsters were supplied by the Animal Care Unit, Faculty of Medicine, Khon Kaen University. Randomly selected male Syrian hamsters 7 weeks old were used. Hamsters were fed with a commercial rodent diet (C.P. Foods, Bangkok, Thailand) ad libitum and kept in galvanized metal cages containing 8 hamsters/cage. The maintenance and care of the experimental animals was in compliance with

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