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SHORT COMMUNICATION

Effect of fingerroot on reducing inflammatory cells in hamster infected with *Opisthorchis viverrini* and *N*-nitrosodimethylamine administration

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Abstract The active compound in fingerroot is effective in the treatment of many inflammatory diseases. The aim of our present study was to evaluate the efficacy of fingerroot on reducing histopathological changes in hamsters that were infected with the liver fluke *Opisthorchis viverrini* or were administered *N*-nitrosodimethylamine (NDMA), and then treated with fingerroot. Light microscopic observation and liver function tests for alanine transaminase (ALT), alkaline phosphatase (ALP), and direct bilirubin were investigated. The results of histopathological changes show that fingerroot has anti-inflammatory properties—in the case of *N*-nitrosodimethylamine administration on day 30—by reducing the aggregation of inflammatory cells surrounding the hepatic bile ducts, which correlates with a decreased serum ALT level. The decrease of direct bilirubin levels in hamsters treated with fingerroot suggests that fingerroot may enhance biliary contraction. The present study found that fingerroot clearly reduces the inflammatory cells in hamsters that were administered NDMA, but not in the case of *O. viverrini* infection. This finding suggests that fingerroot has anti-inflammatory property, but not in the case of hamster opisthorchiasis.

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Introduction

Cholangiocarcinoma (CCA) is a rare but highly fatal disease, with the greatest prevalence observed in Southeast Asia, including Thailand (IARC 1994). Most CCA cases are due to infection with *Opisthorchis viverrini*, which is one of the primary risk factors. Humans are infected by ingestion of raw cyprinoid fish which contain the infective stage known as metacercaria. After *O. viverrini* metacercariae ingestion, the excysted juveniles migrate to the bile canal at the duodenum and grow to adulthood at the common bile duct or gallbladder, an area which is suitable for their survival. At the early stages of infection, liver changes are due to the inflammatory response (eosinophils, monocytes, and neutrophils) around the juvenile flukes in the intrahepatic bile ducts. The severity of inflammation gradually increases and reaches a maximum at about 3–4 weeks post-infection, as evidenced by the accumulation of mononuclear cells and eosinophils which infiltrate the intrahepatic bile ducts. The virulence of the disease also depends on the number of parasites and the duration of infection, which involves the host's immune response such as cytokine expression and free radicals (Pinlaor et al. 2004). Chronic infection with *O.*

viverrini for many years is associated with hepatobiliary diseases (Sripa 2003), including the development of hepatobiliary cancer and CCA. Because of the prevalence of the behavior of eating raw fish, the infection rate of this parasite remains high. Therefore, reducing the pathogenesis from an *O. viverrini* infection may be one of the preferred options to reduce the risk of CCA development.

Our previous report (Boonjaraspinyo et al. 2009) showed that a traditional medicine, turmeric, reduced the inflammatory cells surrounding the hepatic bile duct. Thus, the present study was performed to evaluate the efficacy of another traditional medicine, fingerroot, whose antioxidant active compounds are: panduratin A; cardamonin; 2',6'-dihydroxy-4'-methoxychalcone; 2',4'-dihydroxy-6'-methoxychalcone; 4-hydroxypanduratin A (Shindo et al. 2006); and flavanones such as pinocembrin, pinostrobin, alpinetin, 5-hydroxy-7-methoxyflavanone, and 5,7-dihydroxyflavanone (Mahidol et al. 1984). Fingerroot is widely used for treatment of many inflammatory diseases (Murakami et al. 1995; Yanti et al. 2009; Tuchinda et al. 2002). In the present study, histopathological changes and liver function tests—alanine transaminase (ALT), alkaline phosphatase (ALP) and direct bilirubin—were observed.

Materials and methods

Parasite preparation

The parasites were prepared following the method in the previous report (Boonmars et al. 2009). In brief, *O. viverrini* metacercariae were obtained from naturally infected cyprinoid fish in an endemic area of Khon Kaen, northeast Thailand. Fresh fish were digested in 1% pepsin/HCl and incubated at 37°C for 1 h, then filtered and precipitated with normal saline in a sedimentation jar. Afterwards the metacercariae—oval-shaped, with large, black excretory bladders—were identified under a dissecting microscope.

Fingerroot diet

Fingerroot was acquired from a farm in Loei province, Thailand. The rhizomes were sliced into small pieces, dried by sunlight for a day, and then ground into powder. Ten kilograms of fresh fingerroot yielded 700 g of fingerroot powder. Seven kilograms of mouse pellets (from Charoen Pokphand Co., Ltd., Thailand) were ground and mixed with fingerroot powder (583.31 g). The fingerroot compound was dissolved with ethyl alcohol and the sweet taste increased by a glucose solution (water containing 300 g of dissolved sucrose). The fingerroot diet was compressed into a block to make pellets.

Infection with *O. viverrini*

Twenty hamsters were given 50 *O. viverrini* metacercariae by oral intragastric intubation, as in the previous protocol (Boonmars et al. 2007, 2008), and fed with the assigned diet.

Induced inflammation by administration of *N*-nitrosodimethylamine

Twenty of the hamsters were administered 12.5 ppm of *N*-nitrosodimethylamine (NDMA; Wako, Japan) daily in their water until the animals were sacrificed.

Animal groups

Sixty hamsters were divided into six groups: (1) uninfected control (N); (2) administered a fingerroot diet alone (FING; treated control); (3) administered *N*-nitrosodimethylamine alone (NDMA); (4) administered *N*-nitrosodimethylamine and a fingerroot diet (NDMA+FING); (5) infected with *O. viverrini* alone (OV); and (6) infected with *O. viverrini* and administered a fingerroot diet (OV+FING). Hamsters were treated as the designed groups and sacrificed on days 30 and 60, for collection of their whole liver tissues for observation of histopathological changes and their sera for liver function tests. The protocol was approved by the Animal Ethics Committee of the Faculty of Medicine, Khon Kaen University, Thailand (Ethical Clearance No. AEKKU48/2552).

Light microscopic observation

Hamster liver tissues from each group were analyzed by light microscopic observation, as in previous reports (Boonmars et al. 2007, 2008).

Biochemical estimation

Measurement of serum liver enzymes

Hamster sera were obtained to determine liver damage by evaluation of ALT, ALP, and direct bilirubin analysis at the Chemistry Room, Community Laboratory, Faculty of Associated Medical Sciences, Khon Kaen University.

Statistical analysis

The data of histopathological changes, serum ALT, ALP, and direct bilirubin levels were analyzed and presented as means± SD. Statistics were analyzed from five hamsters using one-way ANOVA (SPSS version 13.0, USA). Values were considered statistically significant when $p<0.05$.

Results

Effect of fingerroot on liver histopathology of *O. viverrini* infection and NDMA administration in hamster models

Analysis of histopathological changes focused on the aggregation of inflammatory cells surrounding the hepatic bile ducts. Histopathological changes in the uninfected group or normal control (Fig. 1 (A, D)) were similar to those observed in the FING group (Fig. 1 (G, J)) at both time points (1 and 2 months). The aggregation of inflammatory cells surrounding the hepatic bile ducts was observed at both time points in groups receiving NDMA administration (Fig. 1 (B, E)) and *O. viverrini*-infected groups (Fig. 1 (C, F)). The NDMA+FING group at 1 month showed only few or no inflammatory cells surrounding the intrahepatic bile ducts (Fig. 1 (H)). However, a decrease in inflammatory cells was observed in the group of OV+FING at 1 month (Fig. 1 (I)). This reduction of inflammatory cells was not observed in the groups of NDMA+FING at 2 months (Fig. 1 (K)) and at 2 months post-*O. viverrini* infection (Fig. 1 (L)).

Fingerroot diet effect on liver enzymes

Table 1 shows the activities of serum ALT, ALP, and concentration of direct bilirubin which correlate with histopathological changes (Fig. 1). The serum markers (ALT, ALP, and direct bilirubin) in the FING group remained within normal levels. Serum ALT levels increased about three- to sixfold after hamsters were administered NDMA, and two- to threefold after *O. viverrini* infection when compared with values in the uninfected control and in those administered FING alone. There was a significant decrease in serum ALT in the group of NDMA+FING at 1 month.

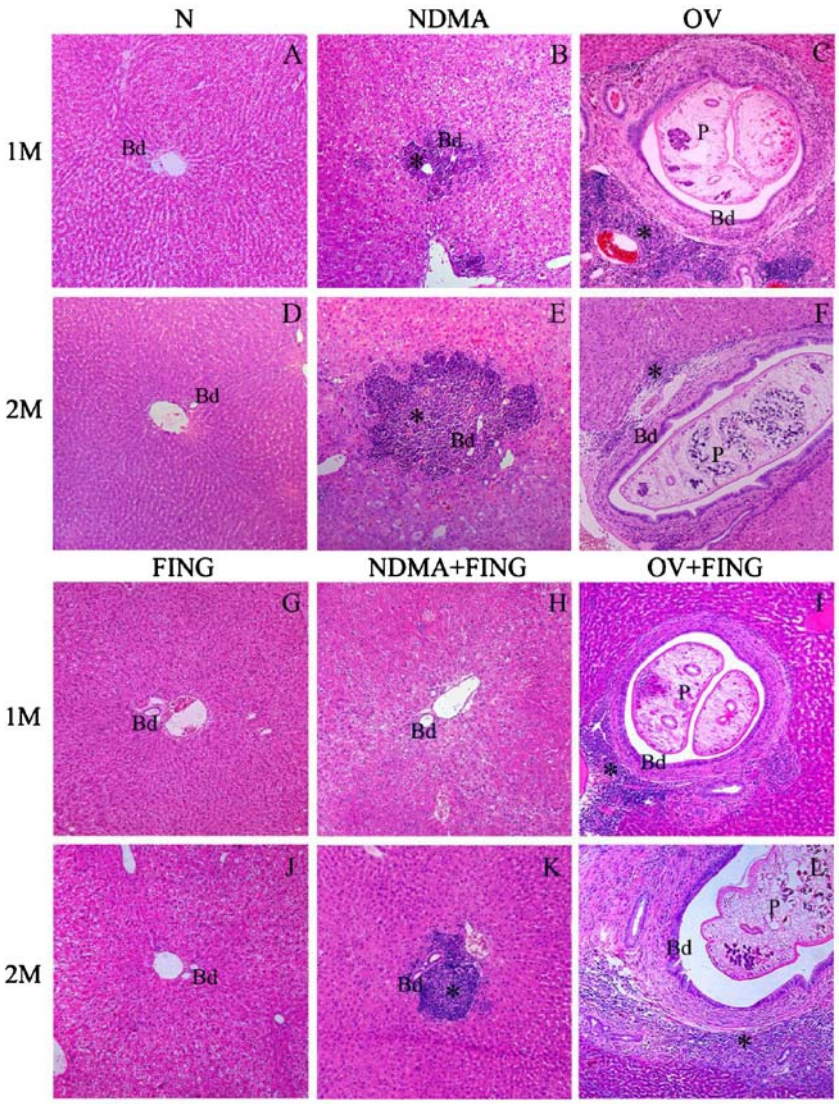

Fig. 1 Histopathological changes in test groups: administered fingerroot alone (*FING*; *G*, *J*); administered NDMA alone (*NDMA*; *B*, *E*); administered NDMA plus fingerroot (*NDMA+FING*; *H*, *K*); infected with OV (*OV*; *C*, *F*); infected with OV plus FING (*OV+FING*; *I*, *L*); and normal control (*N*; *A*, *D*). *Bd* bile duct, *P* parasite, * inflammatory cell (magnification ×10)

Table 1 Liver function tests

Experimental group	Alanine transaminase (U/L)		Alkaline phosphatase (U/L)	Direct bilirubin (mg/dL)
	30 days Mean±SD	60 days Mean±SD	60 days Mean±SD	60 days Mean±SD
N	157.33±52.00[a]	69.67±22.19[a]	55.33±11.06[a]	0.50±0.17[a]
FING	57.80±5.54[a]	52.00±2.65[a]	50.67±3.51[a]	0.35±0.70[a]
NDMA	899.00±456.99[d]	348.33±133.58[b]	72.67±30.44[a]	0.20±0.35[a]
NDMA+FING	384.00±82.15[b]	280.00±133.37[b]	57.33±39.72[a]	0.40±0.42[a]
OV	550.00±231.06[c]	418.67±326.82[c]	51.67±15.82[a]	0.87±0.35[b]
OV+FING	413.00±238.00[c]	448.00±181.43[c]	48.00±4.00[a]	0.30±0.17[a]

Serum alanine transaminase, alkaline phosphatase, and direct bilirubin levels in the group of administration of fingerroot alone (FING), administration of NDMA alone (NDMA), administration of NDMA plus fingerroot (NDMA+FING), infected with OV (OV), infected with OV plus fingerroot (OV+FING) compare with normal control (N). Mean with different superscripts are significantly different ($P<0.05$).

Serum ALP levels in the FING, NDMA, NDMA+FING, OV, and OV+FING groups remained within normal levels. However, all of the groups which were administered fingerroot seemed to show a decreased ALP level. The direct serum bilirubin level (which was lower in groups given a fingerroot diet than in the untreated groups) shows that fingerroot enhanced bile flow and biliary contraction. Serum direct bilirubin levels in the FING, NDMA, NDMA+FING, and OV+FING groups remained within normal levels. Serum direct bilirubin levels increased about two- to fourfold after infection when compared with values in the OV+FING, NDMA+FING, NDMA, FING, and the uninfected control groups.

Discussion

The present study clearly shows that fingerroot has anti-inflammatory and biliary contraction-enhancing properties. This was evident in the hamster model in which NDMA was administered at an early stage of treatment. The pathological changes evidenced by a reduction of the inflammatory cells surrounding the hepatic bile ducts was correlated with a decrease in the results of the liver function tests ALT and direct bilirubin, as well as a decrease in serum ALP due to decreased liver cell damage.

Examination of pathological changes in the livers revealed the anti-inflammatory property of fingerroot in hamsters with inflammatory cells induced by NDMA; but this property could not be observed in hamsters infected with *O. viverrini*. The histopathological results showed that fingerroot has no toxic side effects in normal hamsters; this was supported by the liver function test results, and agreed with a previous report that fingerroot has no toxic effect in rat models (Chandra et al. 2007). The administration of NDMA was directly toxic on liver tissue, and induced the infiltration of inflammatory cells surrounding the biliary tree and hepatic tissue at days 30 and 60. These results agreed with Boonmars et al. (2009). Moreover, NDMA administration in rats induced chronic inflammation and led to liver tumors (Peto et al. 1991), bile duct proliferation, and liver fibrosis (George et al. 2001). The anti-inflammatory property of fingerroot was obviously demonstrated by the reduction of inflammatory cells in hepatic tissue compared with the untreated control group at day 30 (Fig. 1 (H)). Decreased inflammatory cells (Fig. 1) led to decreased serum ALT, ALP, and decreased direct bilirubin levels (Table 1) in all groups treated with a fingerroot diet. This result agrees with previous reports that the extract compounds from fingerroot showed inhibitory effects in the Epstein-Barr-virus-activated test (Murakami et al. 1995), and inhibition of *Porphyromonas gingivalis* in preventing periodontal inflammation (Yanti et al. 2009). Panduratin A, an antioxidant compound, had a significant anti-inflammatory activity in 12-*O*-tetradecanoylphorbol 13-acetate-induced ear edema in rats (Tuchinda et al. 2002). In addition, pinostrobin, the active compound found in fingerroot oil and in the powdered rhizomes, is a potent inducer of quinone reductase in murine hepatoma cells (Fahey and Stephenson 2002). Quinone reductase is an important phase II enzyme detoxifier, while an increase of this enzyme can deactivate radicals and electrophilic quinones in normal cellular processes (Cuendet et al. 2006).

The histopathology of hamsters infected with *O. viverrini* was similar to previous reports (Boonmars et al. 2007, 2008, 2009), that at 30 days post-infection a peak of inflammatory cells (mononuclear cells and eosinophils) was observed surrounding the hepatic bile ducts, as well as epithelial hyperplasia, goblet cell metaplasia, adenomatous metaplasia, and thickened periductal fibrosis (Fig. 1 (C)), findings which correspond with increased serum ALT level.

A slight decrease in inflammatory cells during *O. viverrini* infection was observed when a fingerroot diet was administered. However, this result was different from that observed during the administration of NDMA and fingerroot together. One possible explanation is that different inflammatory inducers may have different host immune response mechanisms. The metabolized product from NDMA is directly toxic to the liver, and subsequently enhances inflammatory cell response; whereas *O. viverrini* induces a host immune response, which enhances inflammatory cells surrounding the hepatic bile duct. Moreover, the present study shows the property of fingerroot to enhance bile flow and gallbladder contraction through decreased direct bilirubin level.

The present study demonstrates the advantages of fingerroot as an anti-inflammatory in hamsters that were administered NDMA, as well as in those infected with *O. viverrini* at an early stage, leading to a reduction in liver pathology.

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