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Study on acute toxicity, anti-oxidant and anticancer activity of ginger in *Opisthorchis viverrini*-carcinogen induced cholangiocarcinoma in hamsters

Tullayakorn Plengsuriyakarn[1]*, Vithoon Viyanant [1], Smarn Tesana [2], Veerachai Eursithichai[1], Wanna Chaicharoenkul[1], Arunporn Itharat[3], Kesara Na-Bangchang[1]

[1] Pharmacology and Toxicology Unit, Graduate Program in Biomedical Sciences, Faculty of Allied Health Sciences, Thammasat University, Pathumthani 12121, Thailand
[2] Department of Parasitology, Faculty of Medicine, Khon Kaen University, khon Kaen, Thailand
[3] Applied Thai Traditional Medicine center, Faculty of Medicine, Thammasat University, Pathumthani 12121, Thailand
* Presenting Author

Abstract

The study aimed to investigate acute toxicity and anti-cholangiocarcninoma activity of the crude ethanolic extract of ginger (*Zingiber officinale* Roscoe). The extract was resuspended in distilled water before given to hamsters (5 males and 5 females in each group) *via* intragastric gavage. Ethanolic extract of ginger was shown absence of toxicity at the maximum dose of 5,000 mg/kg body weight. The investigation of anti-cholangiocarcinoma activity was carried out in *Opisthorchis viverrini*-dimethylnitrosamine (DMN) induced-cholangiocarcinoma (CCA) hamster model. The crude extract (at the dose of 1,000, 3,000 and 5,000 mg/kg body weight daily or every alternate day for 30 days) was fed to animals at 12 weeks after induction, with confirmation of cholangiocarcinoma by histopathological examination at autopsy. Body weight, food and water consumption were recorded daily. The pathogenesis change was examined by hematoxylin-eosin stained at autopsy. Study is underway to conclude on the anticancer activity of the extract. The anti-oxidant activity was also evaluated using the free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. The ethanolic extract of ginger exhibited moderate anti-oxidant activity with mean (SD) IC_{50} (concentration which produced 50% inhibition of oxidative activity) of 26.68 ± 0.16 μg/ml.

Keywords: cholangiocarcinoma, *Opisthorchis viverrini*, dimethylnitrosamine , anti-cholangiocarcinoma activity, anti-oxidant, acute toxicity, hamsters

Introduction

Cholangiocarcinoma (CCA) is an uncommon adenocarcinoma which arises from the epithelial cells of bile ducts anywhere along intrahepatic and extrahepatic biliary tree excluding the papilla of Vater and the gall bladder (1). *Opisthorchis viverrini* (OV) infection is a high risk factor of cholangiocarcinoma (CCA) (2). Although CCA is a relatively rare cancer worldwide, the highest incidence rate is observed in north-east region of Thailand where the prevalence of infection with OV is also highest (2-3). Thai medicinal plants have been increasingly applied as an alternative treatment for various diseases particularly cancer. The aim of the present study was to study on acute toxicity of the crude ethanolic extract of ginger as well as their anticancer activity in OV-carcinogen induced cholangiocarcinoma in hamsters. In addition, the anti-oxidant activity of the extract was also evaluated.

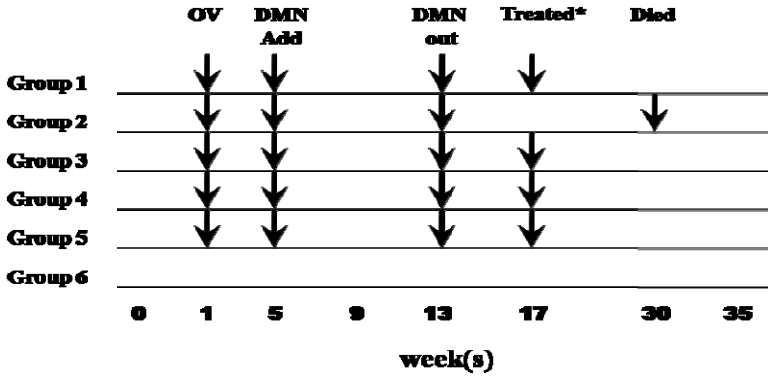

Figure 1. Shematic diagram showing sequences of treatment in each group of hamsters. The arrows (↓) represent the intervention introduced: OV= infection with 50 *Opisthorchis* viv*errini* metacercariae; DMN add = administration of dimethylnitrosamine; DMN out = withdrawal of dimethylnitrosamine; Treated = administration of the ethanolic extract; Died = time of start of death in each hamster

Methods

Acute Toxicity and Anticancer Activity: Syrian golden hamsters (National Laboratory Animal Centre, Thailand), aged 6–8 weeks, weighting 105–120 g were used throughout the experiment. The extract was resuspended in distilled water and given to hamsters *via* intragastric gavage. The acute toxicity (5) of the crude ethanolic extract of ginger was evaluated in a total of 10 (5 males and 5 females) hamsters at the highest dose of 5,000 mg/kg body weight. Distilled water-Tween 80 was given to animals in the control group (5 males and 5 females). Animals were observed individually after dosing at least once during the first 30 minutes, then periodically during the first 24 hours, and thereafter, daily for a total of 14 days (5). At the end of the observational period, the animals were sacrificed under inhalation of ether solution and autopsy was carried out on all the animals. The anticancer activity of the crude ethanolic extract of ginger was evaluated in a total of 80 *Opisthorchis viverrini* (OV)-dimethylnitrosamine (DMN) induced cholangiocarcinoma in hamsters (5 males, 5 females in each group) (Figure 1). The first five groups were OV-infected hamsters, in which 50 metacercariae of OV were fed by intragastric gavage. Four weeks after infection, all were fed with drinking water containing 12.5 *ppm* of DMN daily for 8 weeks (6). Four weeks after DMN withdrawal, hamsters were fed with the ethanolic extract of ginger (prepared in distilled water-Tween 80) at the dose of 1,000, 3,000 and 5,000 mg/kg body weight daily and every alternate day for 30 days (6 groups, 5 males and 5 females each). In the control groups (5 males and 5 females each), animals were treated with distilled water (gastric gavage) and 5-fluorouracil (subcutaneous injection). Body weight, food and water consumption were recorded daily. The progression of CCA was confirmed by pathogenesis changes examined by hematoxylin-eosin stained at autopsy. The study is underway to evaluate the survival time and survival rate (primary endpoint parameters) of the treated compared with control groups.

Anti-oxidant Activity: The anti-oxidant activity was evaluated using the colorimetric free radical, 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay (7). The decrease in DPPH (scavenging activity) was measured as a reduction in absorbance at the UV wavelength 517 nm.

Results

The ethanolic extract of ginger at the highest dose of 5,000 mg/kg body weight showed no apparent acute toxicity during the observational period. Stomachache and slower movement were however observed during the first administration of the ethanolic extract of

ginger, which lasted until one hour after administration. In the hamsters with OV-carcinogen induced cholangiocarcinoma in all groups, average daily intake of water and food, as well as the average of body weight were similar, but were significantly decreased when compared with the non-induced group (5 males and 5 females). With regards to the anti-oxidant action, the ethanolic extract of ginger exhibited moderate activity with mean (SD) IC_{50} (concentration which produced 50% inhibition of oxidative activity) of 26.68 ± 0.16 μg/ml.

Discussion and Conclusion

The ethanolic extract of ginger is considered non-toxic in the acute toxicity model. The observed disturbance of locomotion may be a result of local irritation of stomach epithelial cells. The progression of chlolangiocarcinoma induced by OV infection in combination with the carcinogen DMN had significant influence on the growth of animals. Study is underway to conclude on the anticancer activity of the extract. The anti-oxidant activity was also evaluated using the free radical DPPH assay and results showed the ethanolic extract of ginger to exhibit moderate anti-oxidant activity. It is noted however that, in a previous study, phenolic extract of ginger was shown to produce more potent activity with IC_{50} of 0.64 μg/ml (8).

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