Medicinal Uses of *Phyllanthus urinaria* L. And Its Component, Corilagin, in Liver Diseases

HAU Kwok Po

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Principal Supervisor: Prof. FONG Wang Fun, David

Hong Kong Baptist University

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ABSTRACT

The plant *Phyllanthus urinaria* L., commonly called leaf-flower (night-closing leaf), is an annual herb species in the family Euphorbiaceae. The plant has been used as folk medicines in many countries for several conditions such as gonorrhea, diabetes, dysentery, flu, tumours, jaundice, vaginitis, against headache, fever, conjunctivitis, menstrual disorders, dyspepsia, etc. It is excellent in treating liver and kidney ailments and used extensively for detoxification.

Acetaminophen is a commonly used drug for the treatment of patients with common cold and influenza, and pain. However, an over dose of acetaminophen may be fatal. In Chapter 2 of the study, it was investigated whether mice, administrated intraperitoneally with a lethal dose of acetaminophen, when followed by oral administration of *P. urinaria* extract, may be prevented from death. Silymarin was used as the positive reference in the animal experiments. Histopathological analysis of mouse liver sections showed that *P. urinaria* extract may protect the hepatocytes from acetaminophen-induced necrosis. Therapeutic dose of *P. urinaria* extract did not show any toxicological phenomenon on mice. Immunohistochemical staining with the cytochrome P450 CYP2E1 antibody revealed that *P. urinaria* extract reduced the cytochrome P450 CYP2E1 protein level in mice which
pre-treated with a lethal dose of acetaminophen. *P. urinaria* extract also inhibited the cytochrome P450 CYP2E1 enzymatic activity *in vitro*. Heavy metals included arsenic, cadmium, mercury and lead as well as herbicide residues were not found above their detection limits. High performance liquid chromatography identified corilagin and gallic acid as the major components of the *P. urinaria* extract. It is concluded that *P. urinaria* extract is effective in attenuating the acetaminophen induced hepatotoxicity, and inhibition of cytochrome P450 CYP2E1 enzyme may be an important factor for its therapeutic mechanism.

Where it was also demonstrated that silymarin has a comparable pharmaceutical activity as *P. urinaria* extract when they were used to rescue mice from acetaminophen induced acute liver injury. The therapeutic action of silymarin was also further compared with N-acetyl cysteine (NAC) (commonly used in clinic for emergency treatments) as a rescuer in mice after administrating lethal dose of acetaminophen for 24 hours. In Chapter 3, the results showed that silymarin could greatly improve the counteracting effects on mortality rate when compared with N-acetyl cysteine (NAC) as delayed therapy.

In Chapter 2, it was shown that corilagin was one of the major components in the water extract of *P. urinaria*. The potential *in vivo* anti-tumour activity of corilagin using the
Hep3B hepatocellular carcinoma cell line and an athymic nude mice xenograft model was studied in the Chapter 4. The purity of corilagin was confirmed by high performance liquid chromatographic analysis. Corilagin was administrated intraperitoneally for a continuous period of seven days. A significant inhibition of tumour growth was observed when treated mice were compared with control groups. Furthermore, analysis of enzymes markers of liver function, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), suggested that current therapeutic dosage of corilagin did not exert adverse effect on liver. The observations of the study support the view that corilagin is considerably effective to retard the \textit{in vivo} growth of xenografted Hep3B hepatocellular carcinoma.

Even though the \textit{in vivo} anti-tumour activity of corilagin on Hep3B hepatocellular carcinoma was demonstrated using xenograft athymic nude mice model, but its underlying mechanism of action still remains unclear. In Chapter 5, it was shown that, under the \textit{in vitro} condition using the Hep3B carcinoma cell line as the experimental model system, corilagin induced inhibition of cell growth in a dose dependent manner. Increased Annexin expression and chromosomal condensation were observed in a time and dose dependent manner. Furthermore, cell cycle alterations and caspase 3 activation were found after incubating the Hep3B cells with corilagin. Pre-incubation with the pan-caspase inhibitor could partially reverse the cytotoxic effect of corilagin. These results clearly suggest that
both caspase dependent and independent pathways are active downstream of corilagin induced cell death. Furthermore, corilagin was able to enhance the cytotoxicity of both 
cis-platin and doxorubicin on the Hep3B hepatocellular carcinoma cells.
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