

1. INTRODUCTION

Paracetamol (acetaminophen) is a widely used over-the-counter analgesic-antipyretic drug. An overdose of paracetamol is known to be the cause of acute hepatic necrosis in both experimental animals (Mitchell *et al.*, 1973a; Lim *et al.*, 1994) and humans (McJunkin *et al.*, 1976; Golden *et al.*, 1981). At therapeutic dose, paracetamol mainly undergoes glucuronidation and sulfation in the liver (Prescott, 1980). In addition, small amounts are oxidized by cytochrome P450-dependent enzyme (CYP) (Raucy *et al.*, 1989; Mitchell *et al.*, 1973a) to the reactive intermediate: *N*-acetyl-*p*-benzoquinoneimine (NAPQI), a highly reactive electrophile and powerful chemical oxidant (Dahlin *et al.*, 1984). NAPQI is formed by CYP2E1, CYP1A2 and CYP3A4. The quantitatively most significant of these is CYP2E1 (Raucy *et al.*, 1989).

NAPQI formed after the ingestion of a therapeutic dose of paracetamol is promptly detoxified by conjugation with glutathione, which is further converted to cysteine conjugate and mercapturic acid (Prescott and Critchely, 1983). After large dose, increased amounts of NAPQI are formed, resulting in cellular glutathione stores depletion faster than regeneration, leading to accumulation of toxic metabolite. In the absence of intracellular glutathione, this reactive metabolite formed covalent binding to cellular macromolecules in the hepatocytes causing cell necrosis (Davis *et al.*, 1974; Mitchell *et al.*, 1973b).

The precise mechanism by which paracetamol causes cell death remains unknown, although there are two prevailing theories that are still controversial. According to Gibson *et al.* (1996), the first theory, there are

biochemical reactions between the reactive metabolite and macromolecular cell components (proteins, lipids, DNA). The second theory, the oxidative stress theory, which believed that paracetamol metabolite caused oxidative stress in the cell and ultimately leading to its demise. There is much evidence to substantiate both theories, and the question may be to what extent each one plays a role in paracetamol toxicity.

Prevention of GSH depletion and induction of glutathione transferase and glutathione reductase are the most efficient ways of direct protection against paracetamol hepatotoxicity. Moreover, physiological antioxidant defence is offered by preventative enzymes and by chain breaking small molecules, like ascorbic acid, α -tocopherol and coenzyme Q₁₀ which have been found to protect against paracetamol-induced liver damage (Amimoto *et al.*, 1995). When reactive oxygen species are formed much more than they can be counteracted by the defence mechanism of the organism, the therapeutic use of synthetic or natural antioxidants appears to be a rational approach to the management of oxidative stress related conditions (Paya *et al.*, 1992).

P. speciosa is a tropical leguminous tree in the family of Leguminosae. In Thailand, it is known as "sator" (Smitinand, 1980). It is commonly found in many rural areas in southern Thailand. *P. speciosa* seeds have been used as food either cooked or raw in Thailand and Malaysia. It is considered to be of high nutritional value. People in the southern part of Thailand believe that the beans have antidiabetic effect and laxative effect. The protein content in *P. speciosa* seeds are approximately 8-9% of fresh weight (Suvachittanont and Pothirakit, 1988) and lectin has been purified and characterized (Suvachittanont and Peutpaiboon, 1992).

P. speciosa seeds have distinctive smell, suggesting the presence of

sulfur compounds. The sulfur containing compounds in the seeds were cysteine and their derivatives, such as glutathione, djenkolic acid and thiazolidine-4-carboxylic acid (TCA), which is often called thioproline. (Suvachittanont *et al.*, 1996).

Treatment with exogenous antioxidant may appear to be helpful in conditions related to oxidative stress (Tatsuya *et al.*, 1995; Kourounakis *et al.*, 1997; Manda and Bhatia, 2003). It is anticipated that an antioxidant would be useful as a therapeutic agent if it is available and nontoxic (Kourounakis *et al.*, 1997). Recent study showed that *P. speciosa* seeds have antioxidant activity as indicated by the superoxide scavenging activity and DPPH free radical scavenging assay by using Trolox (Trolox Equivalent Antioxidant Concentration = TEAC) as a reference (Prasatthong *et al.*, 2001). However, there was no report on possible hepatoprotective activity of this plant. Therefore, the hepatoprotective effect and antioxidant activity of both fresh and boiled *P. speciosa* seeds on paracetamol-induced hepatotoxicity in rats were examined.