

## 5. DISCUSSION

Paracetamol 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). The mechanism of cell damage appears to be mediated by the metabolic activation of paracetamol, via cytochrome P450 (CYP) activity especially, CYP2E1, CYP1A2, CYP3A4, to a highly reactive toxic metabolite (*N*-acetyl-*p*-benzoquinoneimine, NAPQI), which is normally conjugated with GSH and excreted in the urine. As the dose of paracetamol is increased, cellular GSH stores become progressively depleted. When GSH stores are below a critical level (about 20-30% of normal level), they are no longer adequate to sustain detoxification of NAPQI. Then, the covalent binding of NAPQI with cellular macromolecules, especially proteins, and the production of oxidative stress within the hepatocytes will occur as a result of the electrophilic and oxidant properties of NAPQI. Cellular necrosis appears to be the major cause of paracetamol-induced hepatotoxicity and lipid peroxidation also is involved.

The aims of this study was to investigate the protective effect of *P. speciosa* on paracetamol-induced hepatotoxicity in rats as a result of the sulphur containing compounds in *P. speciosa*, cysteine and their derivatives such as glutathione, djenkolic acid and thiazolidine-4-carboxylic acid (TCA) (Suvachittanont *et al.*, 1996). Since glutathione and cysteine derivative such as *N*-acetylcysteine (Lauterburg *et al.*, 1983; Hazelton *et al.*, 1986a; Roberts *et al.*, 1987) and TCA (Nagasawa *et al.*, 1984) have been reported to have hepatoprotective effect. However, the results showed that *P. speciosa* did not

protect paracetamol-induced hepatotoxicity in rats, moreover, it may increase the toxicity of paracetamol.

In the present study, paracetamol at a single dose of 500 mg/kg given intraperitoneally caused about 3 folds increase in AST and ALT in rats at 12 hours indicating that it could cause liver injury. Since the reduced glutathione in liver was depleted to about 57.2% of control (fresh preparation) 45.8% (boiled preparation) at 3 hours and completely recovered at 12 hours after paracetamol injection, it suggested that the injury was mild to moderate and reversible. This result was in agreement with Noriega *et al.*, (2000), who have reported that liver glutathione was maximally depleted to 38% of normal in rats treated with paracetamol (400 mg/kg, i.p.) at 3 hours and returned to nearly normal levels at 15 hours. Lipid peroxidation and histology of the liver which were not altered in this study also supported that liver function and structure were not destroyed. According to Dahm and Jones, (1996), the critical level of glutathione is about 20-30% of normal, below this point the disruption of cellular structure and function occurs. Furthermore, paracetamol at dose 500 mg/kg given intraperitoneally is not enough for produced sever liver injury. *P. speciosa* at the dose of 6 g/kg either as single dose orally or single daily dose for 7 days had no effect on the enzymes suggesting that it do no harm to the liver, which is in accordance with the previous report (Suvachittanont and Pothiruckit, 1988) that rats pretreated with *P. speciosa* at the doses of 2.5 to 25 g/kg for 73 days were normal.

We have found that *P. speciosa* did not posses protective effect against paracetamol-induced hepatotoxicity. On contrary, it could increase paracetamol toxicity. The activities of enzymes AST and ALT in rats pretreated with *P. speciosa* for 1 hour before paracetamol did not significantly different from paracetamol treated group. Moreover, rats received paracetamol after

pretreatment with *P. speciosa* for 7 days, either fresh or boiled, showed more toxicity, as illustrated by the higher levels of AST and ALT than those rat that received only paracetamol. The rise in serum levels of AST, ALT and ALP has even attributed to the damaged structural integrity of the liver (Chenoweth and Hake, 1962) because these are cytoplasmic in location and are released into circulation after cellular damage (Sallie *et al.*, 1991). In laboratory animal species, ALT is specific for liver damage. Serum ALT increase rapidly when the liver is damaged, whereas serum levels of AST are elevated to some degree in almost all types of liver disease. Some non-hepatic causes of elevated AST are seen in myocardial infarction (MI), severe angina, skeletal muscle necrosis and renal necrosis (Kram and Keller, 2001). Rats received paracetamol after pretreated with *P. speciosa* for 7 days, either fresh or boiled, serum levels of AST increased more than serum levels of ALT, the enzyme AST may leaked from another organ and released into blood circulation. This result suggests that although *P. speciosa* consist of thiol compounds such as cysteine, glutathione and TCA (Suvachittanont *et al.*, 1996), it can not help protecting the liver from the toxic metabolite of paracetamol (NAPQI). This might be due to the insufficient amount of thiol compounds. As shown by the similar amount of glutathione depletion at 3 hours among paracetamol in the control group (57.2% in fresh experiment and 45.8% of control in boiled experiment), fresh, 1 hour and 7 days, (56.78% and 56.36% of control) and boiled 1 hour and 7 days, (47.06% and 43.28% of control) *P. speciosa* pretreated groups. Moreover, the glutathione recovery in boiled *P. speciosa* pretreated group is much slower than the other two groups (24 vs 12 hours). According to Suvachittanont *et al.* (1996), in boiled *P. speciosa*, formaldehyde and cysteine were condensed to form TCA, which may lead to less available of cysteine, glutathione precursor. However, it is noted that at 12 hours after

paracetamol injection, the glutathione level seems to be increased, although not significantly, in rats pretreated with fresh *P. speciosa* as compared with paracetamol group (2.03-2.22 vs 1.76  $\mu\text{mole/g}$  liver), which suggested that the glutathione synthesis was increased after *P. speciosa* pretreatment. Since glutathione half-life is about 1-5 hours (Reed and Fariss, 1984), thus it is suspected that the daily dose of *P. speciosa* might be insufficient to produce appropriate amount of glutathione at the critical time of depletion. Further investigation should be performed with multiple daily doses. Thiazolidine-4-carboxylic acid has been reported to protect mice against paracetamol induced hepatotoxicity (Nagassawa *et al.*, 1984). Although the mechanism of this protection by TCA is not known, it is believed to be due to the uncovering of its mask sulphhydryl group *in vivo*. However, the report of this study seems to have opposite result, because boiled *P. speciosa* when pretreated with paracetamol seems to increase the toxicity of paracetamol. Moreover, some other toxic metabolite of TCA, *N*-formylcysteine, which was shown to have central nervous system toxicity are suspected to play a role (Nagassawa *et al.*, 1984).

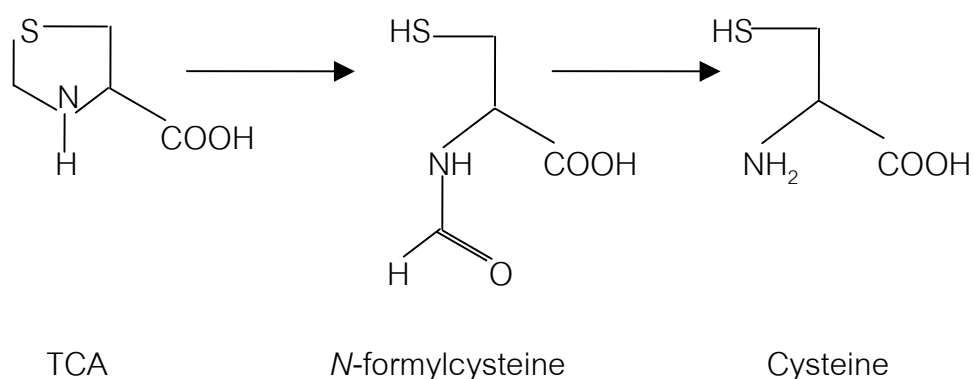


Figure 13 Metabolism of thiazolidine-4-carboxylic acid (TCA)

Although many reports showed the increase in lipid peroxidation in many tissues after paracetamol intoxication (Wendel *et al.*, 1982; Thelen and

Wendel, 1983; Noriega *et al.*, 2000), but in our study the lipid peroxidation was not increased in all *P. speciosa* pretreated groups as compared to the normal control group, which is in accordance with Dahm and Jones, (1996), who noted that lipid peroxidation did not occur until glutathione levels were depleted to 20-30% of normal. Since the glutathione in the present study was depleted maximally to about 40%, it is possible that the lipid peroxidation was not affected.

Since the histological pattern of livers were not different among all groups. It is suggested that paracetamol at the dose of 500 mg/kg, i.p. used in this study, did not cause liver cells destruction at 12 hours post dose although it alter the permeability leading to the leakage of intracellular enzymes, as shown by the mild increase in enzymes activities, nearly normal glutathione level and no change in lipid peroxidation. *P. speciosa* as well, although caused more increase in enzymes activities, it did not destroy the liver cells.

Several plants have been proved to protect against chemical-induced hepatotoxicity such as *Acanthus ilicifolius* and *Ligustrum robustum*. One of the possible hepatoprotective mechanisms involved their antioxidant activities (Babu *et al.*, 2001; Lau *et al.*, 2002). Concerning with the antioxidant activity of *P. speciosa*, Prasatthong *et al.* (2001) showed that methanol extract of fresh *P. speciosa* seeds posses far more antioxidant activity than aqueous extract (95.0 vs 6.2 TEAC, mmole/g edible part). In the present study using DPPH assay, we found that both fresh and boiled *P. speciosa* showed negligible radicals scavenging activity ( $EC_{50} = 862.5$  and  $337.5$   $\mu\text{g/ml}$ , respectively). This data supports the lack of protective activity of *P. speciosa* on paracetamol-induced hepatotoxicity.

As it was demonstrated that *P. speciosa* tend to potentiate paracetamol-induced hepatotoxicity, it is noteworthy to postulate its mechanism for further investigation. According to paracetamol pathway, the possible mechanism(s) involved in increasing toxicity are as follows:

(a) Inhibition of glucuronidation and/or sulfation as proposed by Bray and Rosengren, (2001) who reported that retinol potentiate paracetamol-induced hepatotoxicity in mouse was caused by decrease in hepatic UDPGA available for glucuronide conjugation. Maziasz *et al*, (1991) also showed that some hepatotoxicant such as CCl<sub>4</sub>, aflatoxin and cadmium decrease sulfation pathway in rats.

(b) Induction of cytochrome P450, especially CYP2E1, as reported by Perrot *et al.*, (1989) that chronic alcohol intake increased hepatic CYP2E1 activity. The severity of paracetamol-induced hepatotoxicity was enhanced by chronic alcohol intake (Schmidt *et al.*, 2002), and even a therapeutic dose of paracetamol may lead to hepatotoxicity.

It is concluded that, *P. speciosa* seeds, although containing thiol compounds which are the precursors of glutathione and have been reported to have hepatoprotective activity (Suvachittanont *et al.*, 1996; Nagasawa *et al.*, 1984), was shown by this study not to possess hepatoprotective effect against paracetamol, moreover, it might potentiate the toxicity which is varied according to the preparation, boiled preparation is more potentiabile than fresh *P. speciosa*. This may be due to some changes of the ingredients in *P. speciosa* after boiling. Therefore, administration of *P. speciosa* at high dose and for prolong usage should be awared and further investigation on the mechanisms of the toxicity should be carried out.