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# HEPATOPROTECTIVE EFFECT OF *ANDROGRAPHIS PANICULATA* AND ANDROGRAPHOLIDE AGAINST CARBONTETRACHLORIDE

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## Abstract

*Hepatoprotective efficacy of* ***A. paniculata*** *and its major diterpenoid lactone, andrographolide, was studied in $CCl_4$-induced hepatotoxicity in rats comparing with glycyrrhizin. A single oral administration of dried leaf powder of* ***A. paniculata*** *(1,000 mg/kg), andrographolide (20 mg/kg) and glycyrrhizin (500 mg/kg) 1 hour before $CCl_4$ treatment significantly inhibited $CCl_4$ - induced increase in serum transaminase activities (SGOT, SGPT). No significant difference among the effects produced by different drugs in the dosage used was found. After a repeated administration for 7- consecutive days , only andrographolide exhibited inhibitory effect while* ***A. paniculata*** *and glycyrrhizin did not produce appreciable effect. The results suggest that the hepatoprotective effect of* ***A. paniculata*** *and andrographolide depends on the duration of treatment.*

**Key word index :** Hepatoprotective effect , *Andrographis paniculata* , Andrographolide , $CCl_4$ - induced hepatotoxicity in rats.

## Introduction

The liver, the largest organ in the body , is often the target organ for chemically induced injuries. In human , liver disorders may be classified as fatty liver , cell necrosis , cholestasis , cirrhosis , hepatitis and carcinogenesis (1). As the disorders associated with this organ are numerous and varied , an actual curative therapeutic agent has not yet been found. In Thailand , many herbal plants have been claimed to possess a liver protective activity , but little scientific works have been done to assess their activities.

*Andrographis paniculata* Wall.ex Nees has long been known for the relief of liver disorders (2). Its major diterpenoid lactone , andrographolide, was recently reported to exhibit inhibitory effects upon hepatotoxicity induced by an intraperitoneal administration of paracetamol-,galactosamine-(3) and $CCl_4$-intoxication (4). Glycyrrhizin which is one of the main constituents of *Glycyrrhiza glabra* L. and allied plants has also been shown to possess antihepatotoxic effect against $CCl_4$ both in vitro (5) and in vivo (6). In the present experiment , oral administration of *A. paniculata* and andrographolide were used to assess their hepatoprotective efficacy comparing with glycerrhizin in $CCl_4$-intoxicated rats.

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## Materials and methods

### Plant materials and chemicals

Dried leaf of *A. paniculata* (collected in Bangkok) was ground to fine powder. Andrographolide was isolated from the whole leaf of *A. paniculata* in the laboratory of Department of Pharmacognosy, Faculty of Pharmaceutical Science, Chulalongkorn University. All plant materials and glycyrrhizin (Sigma Chemical Company) were prepared as 1% tragacanth suspensions. $CCl_4$ (Merk) was dissolved in olive oil (1:1 , v/v).

### Experimental animals

Male Wistar rats (The National Laboratory Animal Center , 150-200 g.) were used. All animals have free access to lab chow and water until the experiment.

### $CCl_4$ - induced hepatotoxicity in rats in vivo

To determine the effect of $CCl_4$ on serum transaminase activities , separate groups of rats were treated with $CCl_4$ (0.5-2.0 ml/ kg , p.o.) or olive oil. Blood was obtained from the orbital sinus with heparinized microhaematocrit tubes at 0 , 12 , 24 and 36 hours after $CCl_4$ administration. Serum glutamate-oxaloacetate transaminase (SGOT) and serum glutamate-pyruvate transaminase (SGPT) were measured to assess the magnitude of liver damage according to the method of Reithman and Frankel (7).

### Pre-treatment of $CCl_4$ - intoxicated rats with *A. paniculata* and andrographolide

#### 1. Pre-treatment with single dosage

Dried leaf powder of *A. paniculata* (1,000 mg/kg) , andrographolide (20 mg/kg) and glycyrrhizin (500 mg/kg) were orally administered to separate groups of rats 1 hour before $CCl_4$ administration (1 ml/ kg).

#### 2. Pre-treatment with repeated dosage

Dried leaf powder of *A. paniculata* (1,000 mg/kg) , andrographolide (20 mg/kg) and glycyrrhizin (500 mg/kg) were orally administered to separate groups of rats once daily for 7- consecutive days before $CCl_4$ administration (1 ml/kg).

In both conditions ,the control rats were treated with the same volume of vehicle. Transaminase activities were determined at 24 hours after $CCl_4$ treatment.

### Statistical analysis

The results are shown in mean + SEM. Statistical significance was evaluated through unpaired Studentûs t-test and one way analysis of varience (ANOVA) using the Lest Significant Different (LSD) method ($p<0.05$).

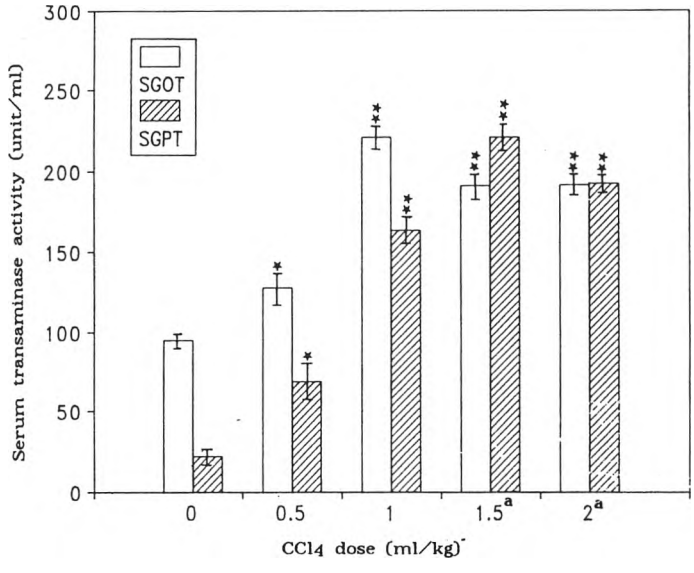
## Results and discussion

### 1. $CCl_4$ - induced hepatotoxicity in rats

As shown in Fig. 1, SGOT and SGPT activities in rats given $CC_{l4}$ (0.5-2.0 ml/kg) increased in a dose dependent manner and significantly differed from control.With high dosage (1.5 and 2.0 ml/kg) , three animals in each group died at 24 and 12 hours.

In Fig. 2, Transaminase activities after treatment with hepatotoxic dose of $CCl_4$ (1.0 ml/kg) were shown to increase gradually with time. The maximum levels were found at 12-24 hours. This response is similar to that found in mice (6). $CCl_4$ is a classical hepatotoxin used to screen a nonspecific hepatoprotective substance both *in vivo* (4,6,8) and in vitro (5, 9) test models. In rats , the dosage of $CCl_4$ used was varied from 0.25-5.0 ml/kg , and the time to assess liver damage was varied from 2-48 hours. Serum transaminases have been widely used to assess the magnitude of liver damage ,since GOT and GPT are released into the blood under hepatotoxic conditions and the amount of the released enzymes are nearly paralleled with the rate of liver damage. In the present work , the dose of 0.5-1.0 ml/kg appears to be the appropriate toxic dose of $CCl_4$ to induce liver damage in Wistar rats without lethal effect. The appropriate time to determine SGOT and SGPT was 12-24 hours after $CCl_4$ treatment.

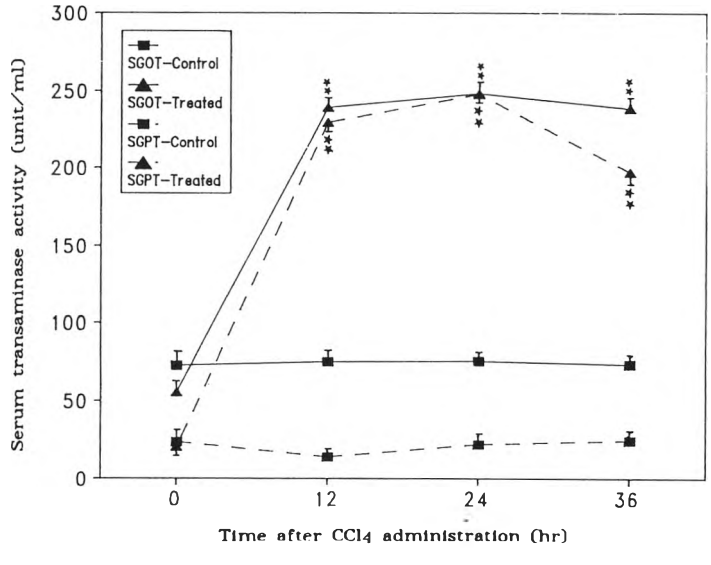
**Fig.1** Dose-dependence of $CCl_4$- induced increase of serum transaminase activitties (SGOT, SGPT) in rats.




n=5,a:n=2, three animals in each group died during the experiment. Transaminase activities were determined at 24 hr after $CCl_4$ treatment.

Significantly different from control: *p<0.05, **p<0.01(ANOVA)

**Fig.2** Time-dependence of $CCl_4$- induced increase of serum transaminase activities (SGOT, SGP) in rats.



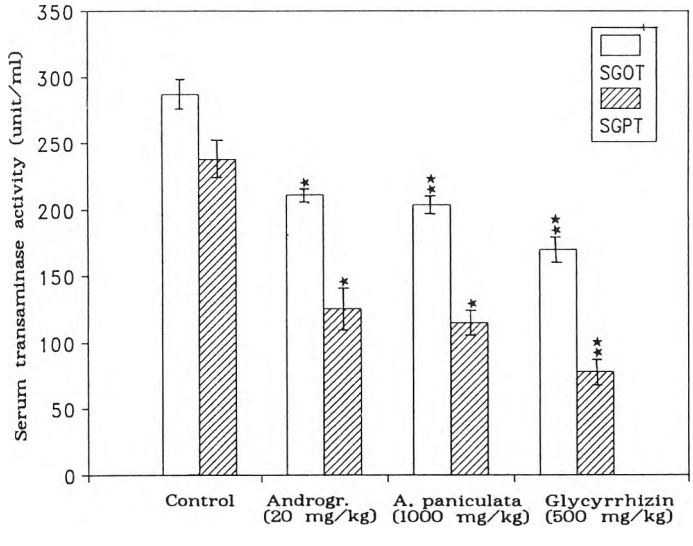
n=5,Transaminase activities were determined periodically after $CCl_4$ treatment.
Significantly different from the value before $CCl_4$ administration (t=0) and the control at the corresponding time. : ** $p<0.01$
(Unpaired Studentûs t-test)

## 2. Hepatoprotective effect of *A. paniculata* and andrographolide in $CCl_4$ - intoxicated rats

Fig.3 shows that single oral administration of either *A. paniculata* (1,000 mg/kg) , andrographolide (20 mg/kg) or glycyrrhizin (500 mg/kg) to rats 1 hour before $CCl_4$ challenge significantly reduced the $CCl_4$- induced increase in the levels of SGOT (30% , 27% and 41% of the control respectively) and SGPT (52% , 48% and 68% of the control respectively).In the dosage used,they are equally effective since no significant difference was found among their protective effects. These results also indicate that the oral administration of andrographolide is effective even in the dose lower than intraperitoneally given of the compound (100 mg/kg) in previous report (4).

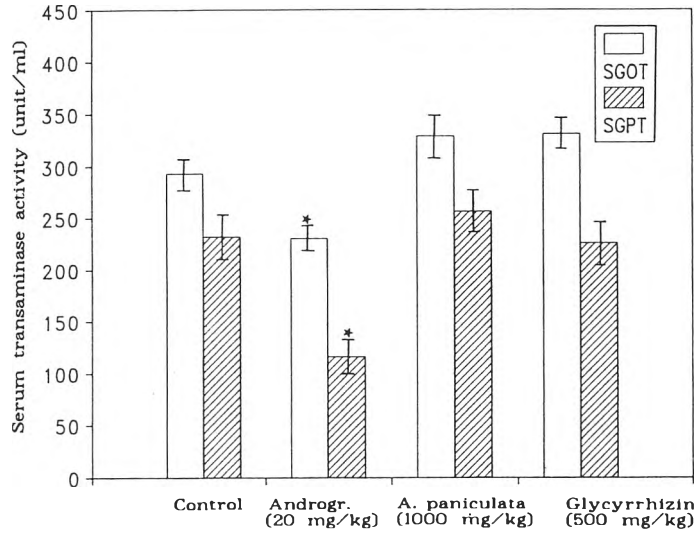
Unlike single administration ,in repeated administration of the drugs for 7- consecutive days , only andrographolide exhibited a significant reduction of the $CCl_4$- induced increase of SGOT (21.2% of the control) and SGPT (50.2% of the control). *A. paniculata* and glycyrrhizin failed to inhibit the elevation of transaminase activities (Fig.4). The results apparently demonstrate that their hepatoprotective effects depend on the duration of treatment. Various results regarding efficacy of andrographolide have been reported (4,10).Choudhury and Poddar (10) found that single administration of *A. paniculata* extract and andrographolide inhibited the $CCl_4$- induced increase of transaminase activities where as no appreciable effect was found when the drugs were administered for 15-consecutive days. Recently, Handa and Sharma (4) found that 4-consecutive days of treatment with andrographolide produce protective action against $CCl_4$.

**Fig.3** Effects of single dosage of *A. paniculata* , andrographolide and glycyrrhizin on $CCl_4$- induced increase of serum transaminase activities in rats.




n=5, Drugs were orally administered 1 hr before $CCl_4$ administration. Transaminase activities were determined at 24 hr after treatment. Significantly different from control :* $p<0.05$, ** $p<0.01$ (ANOVA)

**Fig.4** Effects of repeated dosage of *A. paniculata*, andrographolide and glycyrrhizin on $CCl_4$-induced increase of serum transaminase activities in rats.




n=5, Drugs were orally administered once daily for 7-consecutive days before $CCl_4$ administration. Transaminase activities were determined at 24 hr after $CCl_4$ treatment. Significantly different from control :* $p<0.05$ (ANOVA)

The hepatotoxicity by $CCl_4$ is known to be due to its enzymatic convertion to $CCl_3$ , the highly reactive free radicals by the cytochrome P-450 dependent monooxygenase system. The free radicals initiate chain reactions such as lipid peroxidation which in tern disrups the stucture and function of lipid in the membrane of cell organells , leading to liver damage (1).

The observations that single administration of *A. paniculata* as well as andrographolide inhibited hepatic lipid peroxidation (11) and hepatic microsomal drug metabolizing enzymes (12) which may responsible for the inhibitory action on $CCl_3$ formation and hepatoprotective effect of a single administration of *A. paniculata* and andrographolide. However, after repeated administration ( 7 days ) only andrographolide exhibited the protective effect while *A. paniculata* had no appreciable effect. The negative effect of *A. paniculata* might be due to the finding that despite the inhibitory effects after a single administration, repeated administration ( 7 days ) of *A. paniculata* exerted an inductory effect on hepatic microsomal enzyme. While it took longer time for andrographolide (15 days) to develope profound inductory effects. The faster and stronger inductory effect of *A. paniculata* may be resulted from the presence of other non-defined components in the leaf of *A. paniculata*.

The hepatoprotective effect of glycyrrhizin was also found to be due to the inhibitory effect on the hepatic microsomal enzyme and lipid peroxidation (13). Although no experimental evidence has been found , glycyrrhizin may possibly be enzyme inducer following chronic treatment thereby failing to show the protective effect.

Since the protective effect of andrographolide has been observed in various types of intoxication , like alcohol-(14) , paracetamol- and galactosamine- hepatotoxicity in which different mechanisms of intoxication are involved (3). Whether the other non-specific protective mechanisms is responsible for the hepatoprotective acitvity of the compound should be further investigated.

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# ผลของฟ้าทะลายโจรและแอนโดรกราฟโฟไลด์ ในการปกป้องตับจากพิษของคาร์บอน เตตราคลอไรด์

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## บทคัดย่อ

*ศึกษาผลในการปกป้องตับจากพิษของคาร์บอนเตตราคลอไรด์ (CCl4) ของฟ้าทะลายโจร (**A. paniculata**) andrographolide เปรียบเทียบกับ glycyrrhizin ในหนูขาว พบว่าการให้ผงใบแห้งของ **A. paniculata** (1,000 มก./กก.) andrographolide (20 มก./กก.) และ glycyrrhizin (500 มก./กก.) ทางปากครั้งเดียว 1 ชั่วโมงก่อนได้รับ CCl4 สามารถยับยั้งการเพิ่มของระดับ SGOT และ SGPT ซึ่งถูกเหนี่ยวนำโดย CCl4 ได้อย่างมีนัยสำคัญทางสถิติ โดยไม่พบความแตกต่างอย่างมีนัยสำคัญทางสถิติระหว่าง ผลที่เกิดจากการให้สารเหล่านี้ แต่เมื่อให้สารเหล่านี้ซ้ำติดต่อกันเป็นเวลา 7 วันก่อนได้รับ CCl4 พบว่ามีเพียง andrographolide ที่มีฤทธิ์ยับยั้งในขณะที่ **A. paniculata** และ glycyrrhizin ไม่มีผล ผลการศึกษานี้ชี้ให้เห็นว่าผลในการปกป้องตับของ **A. paniculata** และ andrographolide ขึ้นกับระยะเวลาในการได้รับสาร*

**กุญแจคำ** : ผลในการปกป้องตับ ฟ้าทะลายโจร แอนโดรกราฟโฟไลด์ พิษต่อตับที่เหนี่ยวนำโดยคาร์บอนเตตราคลอไรด์ในหนูขาว