Malaysian Journal of Pharmaceutical Sciences,
Vol. 3, No. 1, 25—33 (2005)

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EFFECTS OF *ANDROGRAPHIS PANICULATA* EXTRACT ON URIDINE DIPHOSPHATE GLUCURONOSYLTRANSFERASE ACTIVITY

NORZAINAH AHMAD[1*], SABARIAH ISMAIL[2] AND ABAS HJ HUSSIN[3]
[1] Advanced Medical and Dental Institute, [2]Centre for Drug Research,
[3]School of Pharmaceutical Sciences,
Universiti Sains Malaysia, 11800 USM Pulau Pinang, Malaysia

The effects of the ethanolic extract of the aerial parts of Andrographis paniculata (AP) on glucuronidation reaction in rat liver microsomes were examined under both in vivo and in vitro conditions. In vitro, AP ethanolic extract at 5 µg/mg protein significantly increased p-nitrophenol (p-NP) uridine diphosphate glucuronosyltransferase (UGT) activity whilst AP at 50 ug/mg protein had no effect on p-NP UGT activity. However, AP ethanolic extract at 500 µg/mg protein significantly decreased p-NP UGT activity. In vivo, administration of single doses of AP ethanolic extract (75 mg/kg/day, p.o.) to adult male Sprague-Dawley rats (n = 7, 140 ± 22 g, body weight) for 15 consecutive days significantly decreased p-NP UGT activity (treated: 1.97 ± 0.15 nmol/min/mg versus control: 2.50 ± 0.15 nmol/min/mg protein). There were no significant changes in body or liver weights of the rats.

Keywords: *Andrographis paniculata, UGT, p-NP glucuronidation*

INTRODUCTION

Glucuronidation is an important phase II reaction for the deactivation and elimination of a wide variety of xenobiotics and endobiotics in humans and other mammals. The enzyme responsible for glucuronidation is uridine diphosphate glucuronosyltransferase (UGT). UGTs are encoded by a complex multigene family and these microsomal enzymes are located in the endoplasmic reticulum of cells from a number of tissues (Tephly and Burchell 1990).

Multiple forms of UGTs have been observed in most species studied and these forms are responsive to the inductive and inhibitory effects of many endogenous and exogenous factors such as hormone, growth factor and nutrition (Nebert 1994). Modulation of UGTs may change the pharmacological and toxicological effects of xenobiotics in humans and result in serious drug-drug interactions.

* Corresponding author: Norzainah Ahmad, e-mail: norzainah@notes.usm.my

Andrographis paniculata (AP) (*hempedu bumi* or *akar cerita*), from the *Acanthaceae* family, has been used as a traditional medicine in India, China and South East Asia for diseases such as fever, malaria, asthma, bronchitis, dysentery, dyspepsia, hepatitis and filariasis (Tang and Eisenbrandt 1992). An *in vitro* study has reported that AP extract has significant activity against human immunodeficiency virus (HIV) (Chang and Yeung 1988).

Several studies have reported pharmacological activities such as reduction of hexabarbital or phenobarbital sleeping time (Chaudhuri 1978), inhibition of drug metabolising enzymes (Choudhury *et al.* 1987) and liver antiperoxidative potential (Roy and Poddar 1984) of the diterpenes found in the plant. Since this extract is often taken with conventional therapeutic drugs, it is very important to explore potential herb-drug interaction. The present studies were thus undertaken to investigate both *in vivo* and *in vitro* effects of the ethanolic extract of AP on UGT activity towards *p*-nitrophenol (*p*-NP) in rat liver microsomes. *p*-NP is a commonly used substrate for determination of UGT activity because its disappearance upon glucuronidation can be followed spectrophotometrically (Bock *et al.* 1983).

METHODS

Chemicals

All the chemicals used were of standard analytical purity grade. Uridine diphosphate glucuronic acid (UDPGA) and *p*-NP were supplied by Sigma Chemical Co., St Louis, MO, USA.

Preparation of AP extract

The ethanolic extract of AP was a kind gift from Professor Chan Kit Lam, Pharmaceutical Chemistry Discipline, School of Pharmaceutical Sciences, Universiti Sains Malaysia (USM). Dried and powdered aerial parts of AP (1 kg) supplied by Landward Engineering Sdn Bhd (Melaka, Malaysia) were extracted with 95% ethanol at 60°C in a soxhlet extractor for 5 days (8 hrs daily). The ethanolic extract (160 g of the ethanol extract was obtained from 1 kg starting material) was then used in both the *in vivo* and *in vitro* studies.

Experimental Design

In vivo treatment

Eight weeks old male Sprague-Dawley rats (142–164 g, body weight) were bred in animal house, School of Pharmaceutical Sciences, USM. All rats were kept under normal condition, maintained on a standard laboratory diet and had free access to tap water *ad libitum*. Rats (n = 7) were treated (administered orally) with a single daily dose of AP ethanolic extract (75 mg/kg/day) for 15 consecutive days. The corresponding control rats (n = 7) were treated with distilled water through the same route. The animals of both experimental and control groups were sacrificed 24 hrs after last treatment.

In vitro treatment

Rat liver microsomes were preincubated with ethanolic extract of AP (5 μg/mg, 50 μg/mg or 500 μg/mg protein) at 37°C for 20 min. The same volume of vehicle was added to the microsome in the control tubes and preincubated under similar conditions as described for the experimental tubes.

Preparation of rat liver microsomes

Microsomes were prepared using calcium precipitation technique (Gibson and Skett 1986). Microsomal protein content was determined according to the method of Lowry *et al.* (1951).

Enzyme assay

UGT activity towards *p*-NP was assayed using modified methods (Bock *et al.* 1983; Guo *et al.* 1992). Full enzyme activation was achieved by preincubating microsomes (2.5 mg/mL) with *p*-NP (0.5 mM) in the presence of 0.125% Triton X-100 for 2 min. The mixture was then incubated at 37°C in the presence of 5 mM $MgCl_2$ in 0.1 M Tris-HCl (pH 7.4) in a final volume of 0.2 mL for 15 min. Reactions were started by the addition of 3 mM UDPGA. The absorbance of *p*-NP was read at 405 nm on a Powerwave X340 microplate reader. The absorbance difference between the control samples (without microsomal protein) and the samples incubated in the presence of microsomal protein represents the amount of *p*-NP consumed through glucuronide formation. The assay was performed under conditions leading to linear reaction rates with time and protein concentration. Final calculations were expressed in nmol *p*-NP conjugated/min/mg protein.

Statistical analysis

Results were expressed as nmol *p*-NP conjugated/min/mg protein as compared to the control. Means and standard deviations were calculated, and the comparison between the means of treated group and the control was done using the Student's t-test at a significant level of 0.05.

RESULTS AND DISCUSSION

Rat liver microsomes are valuable models to evaluate the effects of herbal extracts on hepatic drug metabolising enzymes. This system has been used to evaluate the effects of extracts of rosemary (Debersac *et al.* 2001), green tea (Embola *et al.* 2002) and major flavonoids of *Scutellariae radix* (Ueng *et al.* 2000) on UGT activity with *p*-NP as a substrate.

AP extract has been reported to inhibit rat hepatic phase I drug metabolising enzymes such as aniline hydroxylase, N-demethylase and O-demethylase under *in vitro* and short term *in vivo* conditions (Chaudhuri 1978). An 80% hydroalcohol extract of AP has also been shown to increase the activity of a phase II drug metabolising enzyme, glutathione-S-transferase (GST) in mice livers after treatment of AP extract at 50 mg/kg body wt/day and 100 mg/kg body wt/day for 14 days (Singh *et al.* 2001). However, there are no reports on either *in vitro* or *in vivo* effect of AP extract on UGT activity. This study was done to evaluate the potential of AP ethanolic extract to alter *p*-NP UGT using rat liver microsomes.

Figure 1 demonstrates the *in vitro* effect of the ethanolic extract of the aerial parts of AP on UGT activity. It is evident from Figure 1 that AP at 5 μg/mg protein significantly increased ($p < 0.05$) UGT activity towards *p*-NP. Concentration of AP at 50 μg/mg did not produce any appreciable effect on UGT activity. However, the percentage of UGT activity was significantly decreased ($p < 0.05$) with the increase in the concentration of AP at 500 μg/mg protein. Although the current study could not offer an explanation for the trend observed, i.e. a crossover from stimulation to inhibition of *p*-NP-UGT activity as the AP concentration increases, a similar observation with UGT1A1 activity was reported by Willams *et al.* (2002). Sixteen structurally diverse compounds (modulators) including 8 flavonoids were examined for their potential as activators and/or inhibitors of UGT1A1-catalysed estradiol-3-glucuronidation over a wide range of modulators concentrations. The results showed that estradiol-3-glucuronidation activity was activated or inhibited depending on the

modulator concentrations used. *In vitro* study showed that estradiol-3-glucuronidation was stimulated to 180% of the untreated value at 10 μM of 17α-ethynylestradiol (17 α-EE; modulator), attenuated to 125% at 50 μM of 17α-EE and inhibited to 80% of its control value at 75 μM of 17α-EE.

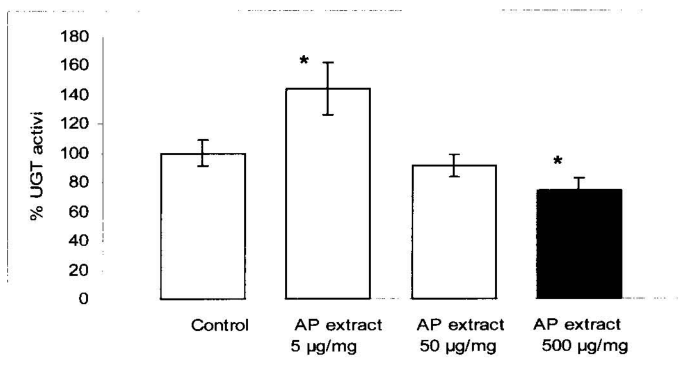

Fig. 1: *In vitro* effect of ethanolic extract AP on *p*-NP glucuronidation in rat liver microsomes. Results are presented as percent of control activity (in the absence of AP extract). Data expressed as mean ± SD (n = 5).
* Significantly different from control, $p < 0.05$.

Figure 2 shows that, after feeding 75 mg/kg body wt/day for 15 days of the ethanolic extract of AP to rats, UGT activity towards *p*-NP was significantly decreased in the *in vivo* study. UGT activity of treated and control rats were 1.97 ± 0.15 nmol/min/mg and 2.50 ± 0.15 nmol/min/mg protein, respectively. The value decreased 21% compared to control (Fig. 3). However, the inhibition of the activity was not accompanied by any significant change ($p > 0.05$) in body or liver weight of the rats (Table 1).

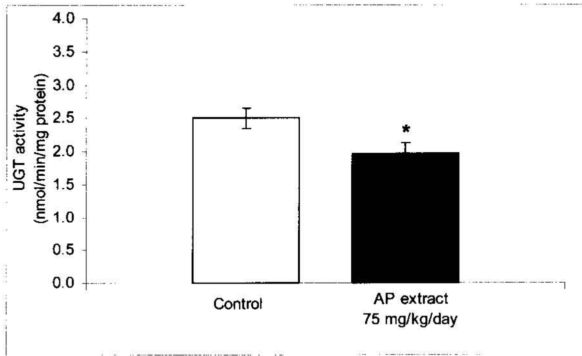

Fig. 2: *In vivo* effect of ethanolic extract AP on *p*-NP glucuronidation in rat liver microsomes. Results are expressed as mean ± SD (n = 5).
* Significantly different from control, p < 0.05.

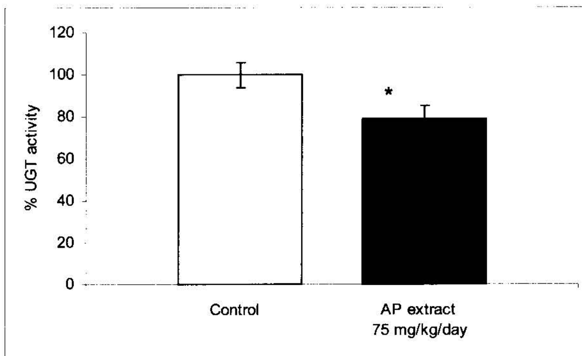

Fig. 3: *In vivo* effect of ethanolic extract AP on *p*-NP glucuronidation in rat liver microsomes. Results are presented as percent of mean ± SD (n = 5).
*Significantly different from control, p < 0.05.

Table 1: *In vivo* Effect of Ethanolic Extract of AP (75 mg/kg/day, 15 Days Treatment) on Body Weight, Liver Weight and Food Intake of Rats

	Control	**Treated**
Weight of rats (g)	210.00 ± 27.94	193.30 ± 22.37
Ratio of liver weight/body weight (%)	7.16 ± 1.11	7.13 ± 1.48
Weight of livers (g)	3.42 ± 0.4 6	3.69 ± 0.62
Amount of food taken/rat/day	17.20 ± 2.10	15.53 ± 1.49
Increment of body weight (g)	64.70 ± 12.96	53.70 ± 19.51

Results are expressed as mean ± SD (n = 7). All values are not significantly different (p > 0.05) from controls.

AP extract contained as its primary chemical constituents, lactones such as andrographolide and deoxyandrographolide along with flavonoids (Reddy *et al.* 2003). One of the flavonoids that had been isolated from AP plant was wogonin (Gupta *et al.* 1983). Wogonin isolated from the root of *Scutellariae baicalensis* had been found to inhibit UGT activity (Ueng *et al.* 2000). It is possible that wogonin is present in this AP ethanolic extract and thus it could be responsible for the decrease in UGT activity observed. However, other chemical components in the extract could also contribute to the decrease. Further studies to isolate and quantify the amount of wogonin in the ethanolic extract of AP could be carried out to confirm this finding.

The exact reasons for the reduced activity of UGT under both *in vitro* and *in vivo* conditions are not clear at this time. Decrease of UGT activity in the presence of AP may be related to the competition for UGT. Whether or not a decrease in the amount of the co-factor UDPGA occurred due to the presence of AP could not be assessed in this experiment since UDPGA was added in excess in the incubation tubes.

Not much information is available on the safety and efficacy of herbal products used alone or in combination with commonly prescribed drugs, despite the recent widespread use of herbal medications. Recently, a slight reduction (20–30%) in glucuronidation was observed in human colon carcinoma cell line treated with 5% solutions of herbal teas (Okamura and Tamura 2004). The reasons for the decrease were thought to be caused by some components in the herbal teas interacting directly with UGT enzymes within the cells, and by regulation of gene expression at a transcriptional and/or translational level (Okamura and Tamura 2004).

In this study, approximately 20% decrease in *p*-NP UGT activity was observed in the *in vivo* and at highest dose in the *in vitro* studies. Since alteration of hepatic glucuronidation has implications with regard to the bioavailability of carcinogens as well as therapeutic drugs in humans, further studies to elucidate the mechanism by which AP alters glucuronidation are therefore necessary.

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