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Molecular Mechanism of *Tinospora crispa* on Herb-Drug Interaction in Rat Hepatocytes

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ABSTRACT *Tinospora crispa* (Family: Menispermaceae) has been used locally as a folk medicine for diabetes mellitus. The objective of this study is to elucidate the effects of *T. crispa* on the molecular mechanism of aminopyrine metabolism in rat hepatocytes. A total of two inhibitors of the second messenger system, namely IBMX and KT-5720 were investigated on the possible pathway that could mediate the effects of *T. crispa* on hepatic Phase I metabolizing enzymes. Normal old male Sprague Dawley rats (n = 6) were used in this study. Isolated hepatocytes cells were prepared using liver perfusion technique [1]. The effect of *T. crispa* on aminopyrine *N*-demethylase activity was determined in the absence or presence of inhibitors by measuring the quantity of formaldehyde formed using the method of Nash [2]. The findings showed that *T. crispa* may act via the cAMP pathway at lower concentrations, ranging from 1.0, 10, and 100 ng/ml, but gave paradoxical results at higher concentrations (0.001 – 1.0 mg/ml).

Keywords: *Tinospora crispa*, second messenger system, hepatocytes, cAMP pathway.

ABSTRAK *Tinospora crispa* (Famili: Menispermaceae) telah lama digunakan sebagai ubat tempatan bagi penyakit diabetes mellitus. Kajian ini bertujuan untuk mengelusidasi mekanisme molekular bagi kesan *T. crispa* ke atas metabolisme aminopirin dalam hepatosit tikus. Dua perencat daripada sistem pengutus sekunder, iaitu IBMX dan KT-5720, telah digunakan untuk mengkaji kemungkinan lintasan yang diperantarakan oleh *T. crispa* pada enzim metabolisme Fasa I. Tikus Sprague Dawley jantan tua normal (n = 6) telah digunakan dalam kajian tersebut. Penyediaan hepatosit adalah merujuk kepada teknik perfusi hati tikus [1]. Kesan *T. crispa* ke atas aktiviti enzim aminopirin *N*-demetilase dengan kehadiaran perencat atau tidak akan ditentukan melalui pengukuran kepekatan formaldehid yang dihasilkan berdasarkan kaedah Nash [2]. Keputusan menunjukkan bahawa *T. crispa* kemungkinan bertindak melalui lintasan cAMP pada kepekatan yang rendah, iaitu julat 1.0, 10, dan 100 ng/ml, manakala pada kepekatan yang tinggi (0.001-1.0 mg/ml) memberi keputusan yang sebaliknya.

INTRODUCTION

Tinospora crispa (Family: Menispermaceae), commonly known as putarwali or akar seruntum (Malays), has been used traditionally as folk medicine to treat several illness, such as diabetes mellitus and hypertension [3-5]. Since some medicinal herbs and pharmaceutical drugs are therapeutic at one dose and toxic at another, concurrent use of herbs and drugs may mimic, magnify, or oppose the effect of drugs [6].

Our previous study has investigated the effect of *T. crispa* on hepatic Phase I metabolizing enzymes using aminopyrine as drug model has shown that the chloroform extract significantly increased ($P<0.01$) aminopyrine *N*-demethylase *in-vitro* activity at 0.001, 0.01, 0.1, and 1.0 mg/ml against their respective control groups in Sprague Dawley rat hepatocytes. However, their molecular mechanism of herb-drug interaction was not known. The present study elucidated the effects of *T. crispa* on the molecular mechanism of aminopyrine metabolism in rat hepatocytes. Two secondary messenger inhibitors, IBMX and KT-5720, were used to investigate the possible pathway mediated by *T. crispa* on hepatic Phase I metabolism.

MATERIALS AND METHODS

Chemicals

4-Dimethylamino-antipyrine (aminopyrine) was purchased from Sigma, USA. IBMX (3-Isobutyl-

1-Methylxanthine) and KT-5720 were obtained from Calbiochem®, Merck, Darmstadt, Germany. All other chemicals used were of analytical grade.

Animal

The hepatocytes were obtained from normal male Sprague Dawley rats, weighing 300-400 g. Food and water were provided *ad libitum* one week before the experiment began.

Plant Material

The stems of *T. crispa* were collected from rain forest of Balik Pulau, Penang.

Extraction

The powdered stems of *T. crispa* were extracted with methanol at 45°C. The concentrated methanol residue was defatted with n-hexane and subsequently partitioned with chloroform/water (2 : 1).

Sample Preparation

The animals were sacrificed and their hepatocytes were isolated following the liver perfusion technique [1]. The hepatocytes (6 x 10^3) were incubated in a 10 ml volume containing aminopyrine (25 mM), serial concentration of *T. crispa* (0.000001 – 1.0 mg/ml) and an incubation medium (physiological solution - Buffer Hank's Balanced Salt Solution [1]) for 18 minutes at room temperature (31 ± 1^{0}C). For the presence of an inhibitor, the hepatocytes (6 x 10^3) were pre-incubated with IBMX at its IC_{50} value of 1.0 x 10^{-6} M or KT-5720 at its IC_{50} value of 5.6 x 10^{-8} M for 15 minutes and then further incubated for 18 minutes at room temperature (31 ± 1 ^{0}C) in the presence of aminopyrine (25 mM) and serial concentration of *T. crispa* (0.000001 – 1.0 mg/ml). The reaction was terminated by the addition of 25 % zinc sulfate, and was followed by the addition of saturated barium hydroxide solution. After centrifugation at 1000 rpm for 5 minutes, the supernatant was taken out for the determination of liberated formaldehyde following the method of Nash [2]. The absorbance was measured at 415 nm using a microplate reader PowerWaveX 340® and its concentration was determined from the standard curve produced from 0 to 0.1 µmol/mL from the stock, 0.1 mM formaldehyde solution [10].

Calculation

The enzyme activity was expressed as µmol/min/cell [10], and the percentage of enzyme activity was calculated using the following formula:

$$\text{Enzyme Activity} = \frac{\text{Volume of supernatant (ml)x Concentration of formaldehyde (µmol/ml)}}{\text{Incubation time (min) x Amount of hepatocytes (cell)}}$$

$$\text{\% Enzyme Activity} = \frac{\text{Enzyme Activity of samples}}{\text{Enzyme activity of control groups}} \times 100$$

Statistical analysis

The results were analyzed by ANOVA and Dunnett Multiple Comparison Testing (InStat® software) and the variance between the groups in presence and absence of inhibitors was analyzed by Student's *t*-test.

RESULTS AND DISCUSSION

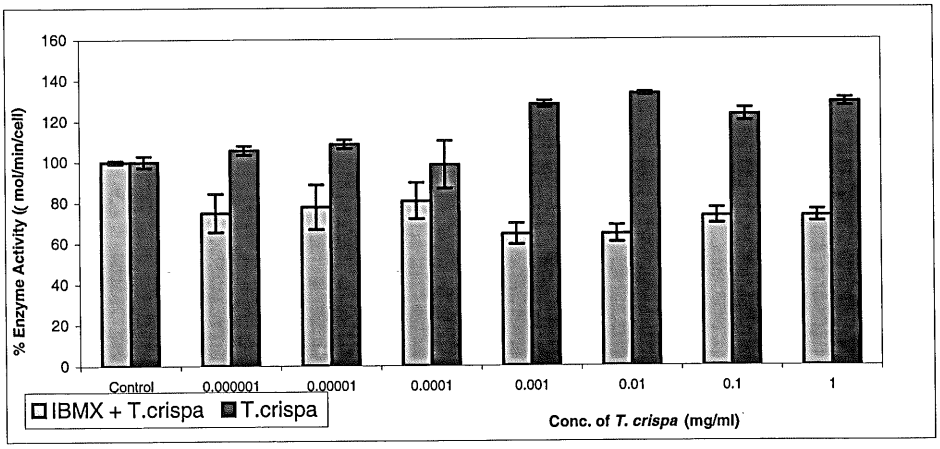
Table 1 showed the intra-group comparison of *in-vitro* effect of *T. crispa* extract on aminopyrine *N*-demethylase activity in the presence and absence of inhibitors, namely IBMX and KT-5720. The dose-response study showed that the metabolism of aminopyrine was significantly increased by *T. crispa* alone, at 0.001 (P<0.01), 0.01 (P<0.01), 0.1 (P<0.05), and 1.0 (P<0.01) mg/ml, against their respective control groups. In the presence of IBMX, significant decrease of aminopyrine *N*-demethylase activity was shown at 0.001 (P<0.01) and 0.01 (P<0.01) mg/ml against their respective control groups. In the presence of KT-5720, an inhibitor of protein kinase A (PKA), a significant change in the aminopyrine *N*-demethylase activity at 0.001 (P<0.05) and 1.0 (P<0.001) mg/ml was observed when compared to their respective control groups.

To examine the effect of *T. crispa* on the cAMP pathway, IBMX and KT-5720 were used for the present study. Theoretically, IBMX reduces the metabolism of aminopyrine, as it inhibits phosphodiesterase (PDE) in the cAMP pathway whereas, KT-5720 inhibits protein kinase A thereby increasing the metabolism of aminopyrine. From the findings, the results in the absence of IBMX (figure 1) and KT-5720 (figure 2) were not identical with the results of *T. crispa* alone, especially significant increase of activity was observed at the higher concentration of 0.001- 1.0 mg/ml. If *T. crispa* acts as PDE stimulant, it should increase the activity of aminopyrine *N*-demethylase. However, the effect

of *T. crispa* (figure 1) in the presence of IBMX showed a decrease in aminopyrine *N*-demethylase activity at higher concentration.

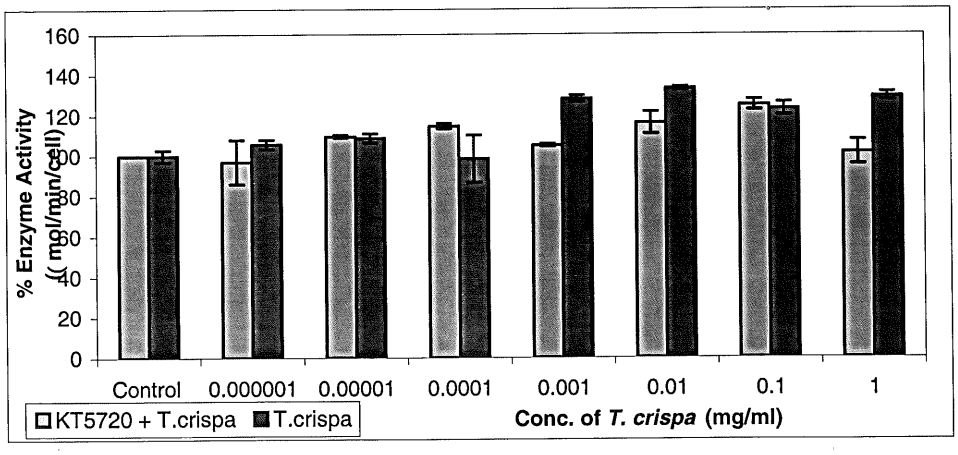
Similarly, the effect of *T. crispa* in the presence of KT-5720 did not follow the expected prediction. From Figure 2, it showed a significant decrease in aminopyrine *N*-demethylase activity instead of increasing the aminopyrine *N*-demethylase activity.

Thus, '*T. crispa* increase aminopyrine *N*-demethylase activity, possibly due to its action via the cAMP pathway at lower concentration but at higher concentrations (0.001 – 1.0 mg/ml), it may not follow the cAM Ppathway.



Value = Mean ± SEM. against their respective control groups; n = 6.

Figure 1. *In-vitro* effect of *T. crispa* on aminopyrine *N*-demethylase activity in presence and absence of inhibitor IBMX. (IBMX was prepared at IC_{50} = 1 x 10^{-6}M)



Value = Mean ± SEM. against their respective control groups; n = 6.

Figure 2. *In-vitro* effect of *T. crispa* on aminopyrine *N*-demethylase activity in presence and absence of inhibitor KT-5720. (KT-5720 was prepared at IC_{50}= 5.6 x 10^{-8}M)

Table 1. Intra-group comparison of the effect of *T. crispa* on aminopyirne *N*-demethylase activity in the presence and absence of IBMX and KT-5720.

Conc. of *T. crispa* (mg/ml)	% Enzyme Activity		
	T. crispa	*T. crispa* + IBMX	*T. crispa* + KT-5720
Control	100±2.83	100±0.77	100
0.000001	105.71±2.26	74.93±9.43	97.05±11.0
0.00001	108.78±2.22	77.87±11.02	109.59±0.72
0.0001	98.57±11.7	80.82±8.95	114.75±1.38
0.001	128.16±1.63**	64.60±5.2**	105.16±0.74*
0.01	133.26±0.79**	64.60±4.16**	116.22±5.49
0.1	123.06±3.19*	73.45±3.82	125.07±2.59
1.0	129.18±1.96**	73.45±2.87	101.47±6.08***

Value = Mean ± SEM. against their respective control groups; Dunnett Test; n = 6.
* $P<0.05$; ** $P<0.01$;*** $P<0.001$.

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