UNIVERSITI SAINS MALAYSIA
PROJEK PENYELIDIKAN JANGKA PENDEK
LAPORAN AKHIR

PENYARINGAN METABOLISME HATI DAN KAJIAN MEKANISME MOLEKUL AGEN ANTI-MALARIA ARTEMISININ DAN DIHIDROARTEMISININ

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CANSELORI
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Laporan Akhir Projek Penyelidikan Jangka Pendek

1) Nama Penyelidik: DR. ABAS HJ HUSSIN

Nama Penyelidik-Penyelidik Lain (Jika berkaitan): DR. CHAN KIT LAM

2) Pusat Pengajian/~~Pusat/Unit~~: SAINS FARMASI

3) Tajuk Projek: PENYARINGAN METABOLISME HATI DAN KAJIAN MEKANISME MOLEKUL AGEN ANTI-MALARIA ARTEMISININ DAN DIHIDROARTEMISININ

) (a) Penemuan Projek/Abstrak

(Perlu disediakan makluman di antara 100 - 200 perkataan di dalam Bahasa Malaysia dan Bahasa Inggeris. Ini kemudiannya akan dimuatkan ke dalam Laporan Tahunan Bahagian Penyelidikan & Pembangunan sebagai satu cara untuk menyampaikan dapatan projek tuan/puan kepada pihak Universiti).

SILA LIHAT LAMPIRAN A DAN B

(b) Senaraikan Kata Kunci yang digunakan di dalam abstrak:

Bahasa Malaysia	Bahasa Inggeris
.....	.....
TIKUS SPONTAN HIPERTENSIF	SPONTANEOUSLY HYPERTENSIVE RATS
INTERAKSI DRUG	DRUG INTERACTION
ARTEMISININ & DIHIDROARTEMISININ	ARTEMISININ & DIHIDROARTEMISININ
METABOLISME HATI	LIVER METABOLISM
EMBLICA OFFICINALIS	EMBLICA OFFICINALIS
.....	.....
.....	.....
.....	.....
.....	.....

5) Output Dan Faedah Projek

(a) Penerbitan *(termasuk laporan/kertas seminar)*
(Sila nyatakan jenis, tajuk, pengarang, tahun terbitan dan di mana telah diterbit/dibentangkan).

Telah dibentangkan di Seminar Kebangsaan Ke-9 Kumpulan Sebatan Semula Jadi di U.P.M pada 21-22 Oktober 1992:

i) Influence & molecular mechanism elucidation of anti-malarial agent: artemisinin on drug metabolism in spontaneously hypertensive rats.

ii) Involvement of cAMP pathway in the influence of water extract of E. officinalis on aminopyrine metabolism in normal rat hepatocytes.

iii) Molecular mechanism elucidation of the influence of the water extract of E. officinalis on drug metabolism in spontaneously hypertensive rats.

iv) Effect of anti-malarial agent, dihidroartemisinin on aminopyrine metabolism in hepatocytes of spontaneously hypertensive rats — akan dibentangkan di 10th Scientific Meeting of the MSPP di Kubang Kerian pada 10–11 Mei 1993.

(b) Faedah-Faedah Lain Seperti Perkembangan Produk, Prospek Komersialisasi Dan Pendaftaran Paten.
(Jika ada dan jika perlu, sila gunakan kertas berasingan)

Memperkembangkan maklumat ubat-ubatan tradisional terutamanya berkaitan dengan interaksi (kemungkinan) bahan tulen artemisinin atau dihidroartemisin dengan beberapa kumpulan drug moden dan implikasi kepada efikasi dan ketoksikan drug moden. Diharapkan usaha ini akan memudahkan langkah untuk pengkompilan maklumat interaksi ubatan tradisional-drug moden di negara ini supaya satu buku panduan tersebut dapat dihasilkan.

(c) Latihan Gunatenaga Manusia

i) Pelajar Siswazah —

ii) Pelajar Prasiswazah: 1 orang - projek latihan penggijazahan dan 3 orang sebagai pembantu pelajar penyelidikan

iii) Lain-Lain: —

6. Peralatan Yang Telah Dibeli:

i) Anthos filter 415 nm untuk microplate reader.
ii) Nitrous oxide cylinder

UNTUK KEGUNAAN JAWATANKUASA PENYELIDIKAN UNIVERSITI

LAMPIRAN B

Seminar Kebangsaan Ke-9
Kumpulan Sebatian Semulajadi

"Komersialisasi Hasil Penyelidikan Sebatian Semulajadi"

21-22 Oktober 1992
Pusat Pengembangan dan Pendidikan Lanjutan
Universiti Pertanian Malaysia
Serdang, Selangor

PROGRAM dan ABSTRAK

Anjuran:
Jabatan Kimia
Universiti Pertanian Malaysia
Serdang, Malaysia

INVOLVEMENT OF cAMP PATHWAY IN THE INFLUENCE OF WATER EXTRACT OF *EMBLICA OFFICINALIS* ON AMINOPYRINE METABOLISM IN NORMAL RAT HEPATOCYTES

Abas Hj Hussin, Mohd Bakri Kasim and Mohd Zaini Asmawi

School of Pharmaceutical Sciences, Universiti Sains Malaysia,
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Although modern drugs are commonly prescribed to treat illnesses, the use of herbal medicines by different ethnic groups in Malaysia still prevails. In the course of treatment, there are occasions where patients tend to take both the modern prescribed drugs and herbal preparations concurrently. The interaction that ensues may result in a decrease in the bioavailability or the accumulation, and hence toxicity, of the modern drug. With respect to this, the study was undertaken to investigate the influence of *Emblica officinalis* locally known as Buah Melaka, on the metabolism of a model drug, aminopyrine, in normal rat hepatocytes. This study also attempt to elucidate the possible molecular mechanism of action of *E. officinalis* in bringing about its influence.

Isolated hepatocytes were prepared from normal Sprague Dawley rats using the collagenase digestion method. The hepatocytes were incubated with aminopyrine in the absence and presence of the water extract of *E. officinalis*, EABM (0.0078 - 0.375 mg/ml concentration) and the metabolism of aminopyrine was assayed according to a modified method of Cochin and Axelrod[1]. Molecular mechanism of action of EABM on aminopyrine metabolism was investigated by adding protein kinase inhibitors KT-252a and KT 5720; protein kinase C activator, phorbol-12-myristate-13-acetate; theophylline and 3-isobutyl-1-methylxanthine.

Our results indicated that EABM significantly decreased ($P<0.05$) aminopyrine metabolism and the effect is dose-related. K-252a inhibited the effect of EABM suggesting that EABM influence on aminopyrine metabolism is mediated by phosphorylation reaction. KT 5720 inhibited the effect of EABM in the male but not in the female rat hepatocytes indicating that EABM effect in the male but not in the female rat, is mediated by the cAMP pathway.

In conclusion, EABM was demonstrated to lower the metabolism of aminopyrine metabolism in rat hepatocytes and the pathway involved in EABM influence is sex-differentiated.

1. J. Pharmac. exp. Ther. (1959) **125**: 416-421

INFLUENCE AND MOLECULAR MECHANISM ELUCIDATION OF ANTI-MALARIAL AGENT, ARTEMISININ, ON DRUG METABOLISM IN SPONTANEOUSLY HYPERTENSIVE RATS

Santhanathan Rajendram, Abas Hj Hussin and Chan Kit Lam

School of Pharmaceutical Sciences, Universiti Sains Malaysia,
11800 Minden. Penang.

Artemisinin or qinghaosu, as it is known in China, is noted for its anti-malarial properties and is isolated from the plant *Artemisia annua* L [Family: compositae]. Apart from being an anti-malarial agent, the plant is also used as an anti-pyretic agent and in chronic dysentery. It is also employed as a bactericide for scabies, abscesses and eye disorders. In the present study, preliminary investigations were conducted to study the influence and molecular mechanism elucidation of artemisinin on aminopyrine metabolism in hepatocytes from spontaneously hypertensive rats.

Male Sprague Dawley rats with body weight of 150-250g (12 - 16 weeks old) were used. Rats were allowed free access to food (rat chow) and tap water. The blood pressure of the rats were measured prior to the sacrifice of the animals using the tail-cuff technique. Isolated hepatocytes were prepared by using the modified collagenase digestion method. The hepatocytes were incubated (18 minutes) with aminopyrine in the absence or presence of artemisinin (10^{-11} - 10^{-6} M concentration) and the metabolism of aminopyrine was assayed according to a modified method of Cochin and Axelrod. To study the molecular mechanism of artemisinin in influencing its effect on aminopyrine metabolism, the hepatocytes were pre-incubated for 15 minutes with various compounds which include IBMX, KT 5720, TPA, staurosporine and calmidazolium before incubating with artemisinin.

Our results indicated that artemisinin is capable of increasing aminopyrine metabolism in hypertensive rats inferring that it could affect the bioavailability of aminopyrine and other drugs that undergo similar phase 1 metabolic pathway (N-demethylation) as aminopyrine. Thus, it should be made aware that interaction between artemisinin and drugs that undergo hepatic phase one N-demethylation could occur and reminded of its possible implication.

MOLECULAR MECHANISM ELUCIDATION OF THE INFLUENCE OF THE WATER EXTRACT OF BUAH MELAKA (*EMBLICA OFFICINALIS*) ON DRUG METABOLISM IN SPONTANEOUSLY HYPERTENSIVE RATS

Abas Hj Hussin, Hafsah Mustapha and Mariam Ahmad

School of Pharmaceutical Sciences, Universiti Sains Malaysia,
11800 Minden. Penang.

Emblica officinalis, locally known as Buah Melaka has been traditionally used for its cardiotonic, anti-bacterial, anti-virus, diuretic, emetic and purgative properties. A study has indicated that it is able to reduce blood pressure in hypertensive rats via inhibition of the α-adrenoceptor. With due respect to the latter, it is of our interest to investigate the effect of this plant on drug metabolism in hypertensive rats and to elucidate the possible molecular mechanisms involved.

Hepatocytes were prepared from male spontaneously hypertensive rats. The hepatocyes were then incubated with a model drug, aminopyrine, in the presence of the water extract of Buah Melaka (EABM) for 18 minutes. After the incubation, the metabolites formed were then assayed and quantitated. Cyclic AMP analogue, 3-isobutyl-1-methylxanthine (IBMX), protein kinase C activator, phorbol-12-myristate-13-acetate (TPA), Ca-ionophore (A 23187) and calmodulin-antagonist, calmidazolium were added into the incubation cocktail to investigate the possible pathway involved through which EABM exert its influence on aminopyrine metabolism.

Our results indicated that EABM is able to reduce aminopyrine metabolism in spontaneously hypertensive rats. This effect of EABM is altered by IBMX, TPA, A 23187 and calmidazolium.

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Poster Session of: _____
Poster Board Number: _____
Oral Session: ✓ (A4)
on 10.5.93 (date) at 11.45 - 1.00 (time)

ABSTRACT FORM

NOTES:

1. Underline name of registered participant who will present paper
2. Please type neatly within this space (See Sample)

Title: EFFECT OF ANTI-MALARIAL AGENT, DIHIDROARTEMISININ, ON AMINOPYRINE METABOLISM IN HEPATOCYTES OF SPONTANEOUSLY HYPERTENSIVE RATS

Author(s): Abas Hj Hussin and Chan Kit Lam

Institution(s): School of Pharmaceutical Sciences, Universiti Sains Malaysia, 11800 Penang.

Text:

Artemisinin is the clinically active antimalarial constituent isolated from the plant *Artemisia annua* L. [Family : Compositae]. Dihidroartemisinin, obtained by sodium borohydride reduction of artemisinin, was reported to be more therapeutically active than the parent compound. A study was conducted to investigate the influence of dihidroartemisinin on aminopyrine metabolism in hepatocytes obtained from normal and spontaneously hypertensive rats (SHR).

Hepatocytes were obtained from normal and SHR male, 40 weeks old rats.The hepatocytes were incubated with dihidroartemisinin and the metabolism of aminopyrine metabolism was assayed by the method of Cochin and Axelrod (1959). Mechanism of action of dihidroartemisinin was investigated by the addition of staurosporine, KT-5720, trifluoroperazine and phorbol-12-myristate-13-acetate.

The results indicated that dihidroartemisinin significantly ($P<0.01$) reduced aminopyrine metabolism in hepatocytes from normal and SHR rats at concentration as low as 10^{-13}M. Preincubation with staurosporine, KT-5720, trifluoroperazine and phorbol-12-myristate-13-acetate did not significantly influence the effect of dihidroartemisinin. The results indicated that dihidroartemisinin is able to inhibit hepatic N-demethylation of aminopyrine and possibly other drugs that undergo the similar pathway.

References:

References

1. Cochin,J and Axelrod, J. (1959) J Pharmac. Exp. Ther. 125:416

3. Return original abstract plus TWO photocopies
4. Submission deadline is 27th March, 1993.
5. Please indicate mode of presentation by ticking in the appropriate box

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Poster

LAMPIRAN A

Pendahuluan

Projek ini ialah satu kerjasama diantara pengarang dengan seorang pensyarah Disiplin Kimia Farmaseutik, PPS Farmasi, Prof. Madya Dr Chan Kit Lam, untuk mengembangkan maklumat tentang agen anti-malaria, artemisinin dan derivatif sintetiknya, dihidroartemisinin. Pokok *Artemisia annua* telah berjaya dikulturkan didalam tabung uji oleh kumpulan Chris Teoh, USM dan maklumat kimia dan farmakologinya sedang giat diselidiki oleh para penyelidik tempatan dan antarabangsa.

Objektif Penyelidikan

i) menyelidiki pengaruh artemisinin dan dihidroartemisinin terhadap metabolisme hati, satu model drug moden, aminopirin yang diketahui menjalani metabolisme N-demetilasi, didalam tikus spontan hipertensif (SHR). Kajian mekanisme tindakan kedua-dua bahan ini diperingkat molekul juga telah dikaji.

ii) mengkaji pengaruh serta kajian mekanisme molekul ekstrak air Buah Melaka, EABM *(Emblica officinalis)* terhadap metabolisme aminopirin didalam tikus normal dan SHR. Kajian ini ialah sambungan daripada kajian geran jangka pendek pertama saya yang telah pun ditamatkan.

Kaedah

Kajian in-vitro yang dijalankan melibatkan penggunaan hepatosit tikus jantan normal Sprague Dawley dan tikus spontaneously hypertensive rats (SHR). Umur tikus-tikus yang digunakan ialah lebih kurang 40 minggu dan berukuran berat badan 250-350 g. Hepatosit yang perolehi telah dieramkan dengan bahan tulin artemisinin atau dihidroartemisinin (yang dihasilkan oleh Prof. Madya Dr Chan Kit Lam) atau dengan EABM didalam kehadiran aminopirin. Metabolisme aminopirin kemudiannya di asai dengan mengikut Kaedah Cochin dan Axelrod (1959). Untuk mengkaji mekanisme molekul artemisinin, dihidroartemisinin atau EABM serta kajian mekanisme molekul mereka, hepatosit-hepatosit telah di preeramkan dengan beberapa bahan seperti IBMX, KT 5720, TPA, calmidazolium, staurosporine, trifluoperazine, K 252a, theophylline atau A-23187.

Tekanan darah tikus-tikus normal dan SHR telah ditentukur dengan menggunakan Kaedah Tail-Cuff.

Keputusan & Perbincangan

Kesan artemisinin terhadap metabolisme aminopirin didalam tikus SHR

Keputusan kami menunjukkan bahawa artemisinin meningkatkan secara signifikan metabolisme aminopirin. Ini ketara dengan peningkatan aktiviti aminopirin N-demetilase pada kepekatan bermula 10^{-9}M artemisinin. Artemisinin (10^{-8}M) menghasilkan puncak aktiviti enzim dan kepekatan melebihi 10^{-8}M menunjukkan penurunan aktiviti enzim tetapi masih lagi tinggi daripada kawalan ($P < 0.05$) (Rajah 1). Didalam kehadiran IBMX, pengaruh artemisinin (10^{-8}M) terhadap aktiviti aminopirin N-demetilase telah dibalikkan, dan direncat sepenuhnya pada kepekatan IBMX serendah 10^{-11}M (Rajah 2).

Ini turut dilihat dengan kesan artemisinin 10^{-8}M didalam kehadiran aktivator protein kinase C, TPA (Rajah 3). Bagaimana pun, julat rencatan aktiviti aminopirin N-demetilase oleh TPA adalah lebih rendah berbanding dengan IBMX (banding Rajah 2 dan 3).

Kedua-dua perencat protein kinase A, KT 5720 dan perencat kalmodulin, kalmidazolium, menurunkan secara signifikan aktiviti enzim aminopirin N-demetilase (Rajah 4 dan 5) manakala perencat protein kinase C, staurosporine menurunkan kesan artemisinin (10^{-8}M) ke paras kawalan (Rajah 6).

Kesimpulannya, artemisinin berupaya untuk meningkatkan metabolisme aminopirin didalam tikus hipertensif dan ini boleh menurunkan bioperolehan aminopirin dan drug-drug lain yang menjalani lintasan N-demetilasi yang sama. Kesan-kesan artemisinin dicadangkan diperantarakan sekurang-kurangnya, oleh siklik AMP dan diasilgliserol dan peranan protein kinase masih perlu dikaji. Tidak diketahui samada interaksi ini boleh berlaku didalam manusia dan samada keputusan yang diperolehi adalah relevan secara klinikal dan dicadangkan agar kajian ini dapat disambung keperingkat manusia.

Kesan dihidroartemisinin terhadap metabolisme aminopirin didalam tikus hipertensif spontan

Keputusan kami menunjukkan bahawa dihidroartemisinin mengurangkan secara signifikan ($P<0.01$) metabolisme aminopirin didalam hepatosit tikus normal, hipertensif spontan dan diabetik pada kepekatan serendah 10^{-13}M. Pre eraman dengan staurosporine, KT 5720, trifuoroperazine dan TPA tidak mempengaruhi kesan dihidroartemisinin ini. Keputusan-keputusan ini menunjukkan bahawa dihidroartemisinin berupaya merencat N-demetilasi hepar aminopirin dan mungkin

juga drug-drug lain yang menjalani lintasan yang serupa.

Kesan EABM terhadap metabolisme aminopirin didalam tikus normal.

Didalam tikus normal 10 bulan, EABM menurunkan secara signifikan ($P<0.05$) metabolisme aminopirin. Kesan EABM ini tidak berbeza didalam tikus jantan atau betina (banding Rajah 1 dan 2). Didalam tikus normal 5 bulan, EABM cuma mengurangkan secara signifikan ($P<0.01$) metabolisme aminopirin didalam tikus jantan tetapi tidak didalam tikus betina (banding Rajah 3 dan 4).
K-252a merencat kesan EABM menandakan bahawa pengaruh EABM terhadap metabolisme aminopirin diperantarakan oleh tindak balas penfosforilan (Rajah 5). Perencat sepesifik protein kinase A, KT 5720, membalikkan pengaruh EABM terhadap metabolisme aminopirin didalam tikus jantan tetapi tidak didalam tikus betika 10 bulan menandakan pengaruh EABM terhadap metabolisme aminopirin didalam tikus jantan diperantarakan oleh lintasan protein kinase A (Rajah 6).
Kesimpulannya, EABM telah didapati menurunkan metabolsime aminopirin didalam hepatosit tikus normal dan lintasan yang terlibat didalam kesan EABM adalah bersifat seks. Faktor-faktor umur dan seks mungkin mementukan kesan EABM terhadap metabolisme aminopirin.

Kesan EABM terhadap metabolisme aminopirin didalam tikus hipertensi spontan

Pada kepekatan dibawah 0.0313 mg/ml, EABM telah didapai tidak mempunyai kesan terhadap metabolisme aminopirin. Bagaimana pun, peningkatan kepekatan EABM akan merencat dengan signifikan metabolisme aminopirin (Rajah 1).
IBMX (Rajah 2) didapati merencat kesan EABM pada kepekatan 0.0078 mg/ml tetapi tidak mempengaruhi kesan EABM pada kepekatan 0.25 mg/ml (banding Rajah 1 dan 3). Peningkatan kepekatan kalmidazolium menurunkan secara signifikan ($P<0.01$) kesan 0.0078 mg/ml EABM berbanding kawalan. Kesan penurunan EABM (0.25 mg/ml) telah direncat sama sekali oleh kalmidazolium (Rajah 4 dan 5).
Kesan EABM (0.0078 mg/mldan 0.25 mg/ml) terhadap metabolisme aminopirin tidak dipengaruhi oleh TPA (Rajah 6 dan 7).
Didalam kehadiran A 23187, EABM (0.0078 dan 0.25 mg/ml) tidak mengubah metabolism aminopirin (Rajah 8 dan 9).
Kesimpulannya, keputusan yang diperolehi menunjukkna bahawa EABM berupaya untuk mengurangkan metabolisme aminopirin didalam hati tikus. Ini akan berakhir dengan akumulasi drug didalam peredaran darah dan akan membawa kepada ketoksikan drug. Ia juga menunjukkna bahawa pengaruh EABM terhadap metabolisme aminopirin diperantara oleh lintasan cAMP dan kalmodulin pada kepekatan EABM

rendah (0.0313 mg/ml) dan oleh lintasan kalmodulin pada kepekatan EABM tinggi (0.065 mg/ml).

Kesemua ekstrak dan bahan-bahan tulin kecuali artemisinin, menurunkan metabolisme aminopirin. Drug-drug yang diketahui menjalani tindak balas N-demetilasi didalam hati dijangka akan mengalami pengurangan metabolisme sekiranya kedua-dua ekstrak tumbuhan dan drug diambil bersama atau satu selepas yang lain. Penyelidikan ini cuma dijalankan keatas haiwan dan kajian ke atas manusia perlu dikembangkan.

LAMPIRAN A

Introduction

This project is a collaboration between the author and another lecturer from the Pharmaceutical Chemistry discipline, PPS Farmasi, Assoc Prof Dr Chan Kit Lam, to develop more information on the anti-malarial agent, artemisinin and its synthetic derivatives, dihydroartemisinin. The plant *Artemisia annua* has been successfully cultured in the test-tube by Dr Chris Teoh group from USM and information on the chemistry and pharmacology of these substances are being actively investigated by many workers, locally and internationally.

Research Objectives

i) to investigate the influence of artemisinin and dihydroartemisinin on liver metabolism of a modern drug model, aminopyrine, which is known to undergo N-demethylation reaction, in spontaneously hypertensive rats (SHR). The molecular mechanism of action of both substances were also investigated.
ii) to investigate the influence and elucidate the molecular mechanism of action of the water extract of Buah Melaka, EABM (*Emblica officinalis*) on aminopyrine metabolism in normal and SHR rats. This work is an extension work of the research done in the author's previous short term grant.

Method

In-vivo research was carried out and involved the use of hepatocytes obtained from normal male Sprague Dawley and spontaneously hypertensive rats. The rats used were about 40 weeks old and of body weight ranging from 250-350g. Hepatocytes obtained were incubated with the pure compound artemisinin and dihydroartemisinin (obtained from Prof Madya Dr Chan Kit Lam) or EABM in the presence of aminopyrine. Aminopyrine metabolism was then assayed by the Cochin & Axelrod Method (1959). In order to investigate the molecular mechanism of artemisinin, dihydroartemisinin or EABM, the hepatocytes were preincubated with IBMX, KT 5720, TPA, calmidazolium, staurosporine, trifluoroperazine, K-252a, theophylline and A 23187.

Blood pressure of normal and SHR rats were measured by using the tail cuff method.

Results and discussion

Effect of artemisinin on aminopyrine metabolism in SHR rats

Our result indicated that artemisinin affected significant increase in aminopyrine metabolism. This is indicated by the increased in aminopyrine N-demethylase activity at concentration beginning 10^{-9} M artemisinin ($P<0.05$). Artemisinin (10^{-8}M) caused a pesk in the enzyme activity and concentration above 10^{-8}M showed a lowering in the enzyme activity but are still significantly above the control (Figure 1).

In the presence of IBMX, artemisinin (10^{-8}M) influence on aminopyrine N-demethylase activity was significantly reversed and in fact, totally inhibited at concentrations as low as 10^{-11} M IBMX (Figure 2).

This was similarly seen with the effect of artemisinin (10^{-8}M) in the presence of protein kinase C activator, TPA (Figure 3). However, the extent of inhibition of the activity of aminopyrine N-demethylase by TPA was much lower when compared to IBMX (compare Figure 2 and 3).

Both protein kinase A inhibitor, KT 5720 and calmodulin inhibitor, calmidazolium, significantly lowered the activity of the enzyme aminopyrine N-demethylase (Figure 4 and 5) whereas protein kinase C inhibitor, staurosporine, reduces the effect of artemisinin (10^{-8}M) to the control level(Figure 6).

In conclusion, artemisinin is capable of increasing aminopyrine metabolism in hypertensive rats inferring that it could reduce the bioavailability of aminopyrine and other drugs that undergo similar phase 1 N-demethylation pathway. These effect of artemisinin is suggested to be mediated , at least, by cyclic AMP and diacylglycerol and the role of protein kinases still needs to be investigated and explored. It is not known whether this type of interaction would occur in human and whether it is clinically relevant. It is suggested that in the future, this study should be extended to human.

Effect of dihydroartemisinin on aminopyrine metabolism in spontaneously hypertensive rats

Our results indicated that dihydroartemisinin significantly ($P<0.01$) reduced aminopyrine metabolism in hepatocytes from normal, spontaneously hypertensive and diabetic rats at concentration as low as 10^{-13}M. Preincubation with staurosporine, KT-5720, trifluoroperazine and TPA did not significantly influence the effect of dihidroartemisinin. The results indicated that dihydroartemisinin is able to inhibit hepatic N-demethylation of aminopyrine and possibly other drugs that undergo the similar pathway.

Effect of EABM on aminopyrine metabolism in normal rats

In 10 months old normal rats, EABM significantly ($P < 0.05$) reduced aminopyrine metabolism. These effect of EABM is not differentiated in the male or female rats (compare Figure 1 and 2). In the 5 months old normal rats, EABM only significantly reduced ($P < 0.01$) aminopyrine metabolism in the male but not in the female rats (compare Figure 3 and 4).

K-252a inhibited the effect of EABM suggesting that EABM influence on aminopyrine metabolism is mediated by phosphorylation reaction(Figure 5). Specific protein kinase A inhibitor, KT 5720, reverses the influence of EABM on aminopyrine metabolism in the male but not in the female rats (10 months old) suggesting that the influence of EABM on aminopyrine metabolism in the male rats is mediated via the protein kinase A pathway (Figure 6).

In conclusion, EABM was demonstrated to lower the metabolism of aminopyrine in rat hepatocytes and the pathway involved in EABM influence is sex-differentiated. Age and sex factors may determine the effect of EABM on aminopyrine metabolism.

Effect of EABM on aminopyrine metabolism in spontaneously hypertensive rats

At concentrations below 0.0313 mg/ml, EABM exhibited no significant effect on aminopyrine metabolism when compared to control. However, further increase of EABM concentration showed significant reduction ($P<0.05$ and $P<0.01$) on aminopyrine metabolism (Figure 1).

Addition of IBMX, as shown in Figure 2, significantly reduced the effect of EABM (0.0078 mg/ml) but did not affect EABM effect on aminopyrine metabolism at 0.25 mg/ml EABM concentration (compare Figure 1 and 3).

Increasing concentration of calmidazolium significantly reduced ($P<0.01$) EABM's (0.0078 mg/ml) effect when compared to control. However, the reducing effect of EABM (0.25 mg/ml) was inhibited by all concentrations of calmidazolium (Figure 4 and 5).

The effect of EABM (0.0078 mg/ml) on aminopyrine metabolism was unaltered by TPA when compared to control (Figure 6). The effect of EABM (0.25 mg/ml) was also unaffected in the presence of TPA (compare Figures 1 and 7).

In the presence of A 23187, EABM (0.0078 mg/ml) exhibited no significant alteration in aminopyrine metabolism when compared to control (Figure 8) and non of the concentrations of A 23187 alter the reducing effect of EABM (0.25 mg/ml) on aminopyrine metabolism (Figure 9).

In conclusion, our result suggested that EABM is capable of reducing aminopyrine metabolism in rat liver. This will ultimately result in accumulation of the drugs in the circulation and may lead to drug toxicity. Our result also indicated that EABM's influence on aminopyrine metabolism is probably mediated by the cAMP- and

calmodulin-associated pathway at low concentratons (0.0313 mg/ml) and by calmodulin-associated pathway at high concentrations above 0.065 mg/ml.

All the extracts and pure compounds except artemisinin tested, decreased the metabolism of aminopyrine. Drugs that are known to undergo N-demethylation reaction in the liver are expected to experience a similar reduction in their metabolism if both plant extracts and drugs are taken together or taken subsequently. However the investigaton was only conducted in animals and further work need to be carried out in human being.

INVOLVEMENT OF cAMP PATHWAY IN THE INFLUENCE OF WATER EXTRACT OF EMBLICA OFFICINALIS ON AMINOPYRINE METABOLISM IN NORMAL RAT HEPATOCYTES

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AIM

1) To study the influence of Emblica officinalis (Buah Melaka) on aminopyrine metabolism in an animal model

2) To elucidate the possible mechanism of action of E. officinalis in exerting its influence on liver aminopyrine metabolism

METHODOLOGY

- normal male and female Sprague Dawley rats
- Age : 5 months old (150 - 190 g)
  10 months old (240 - 300g)

in-situ liver perfusion with collagenase

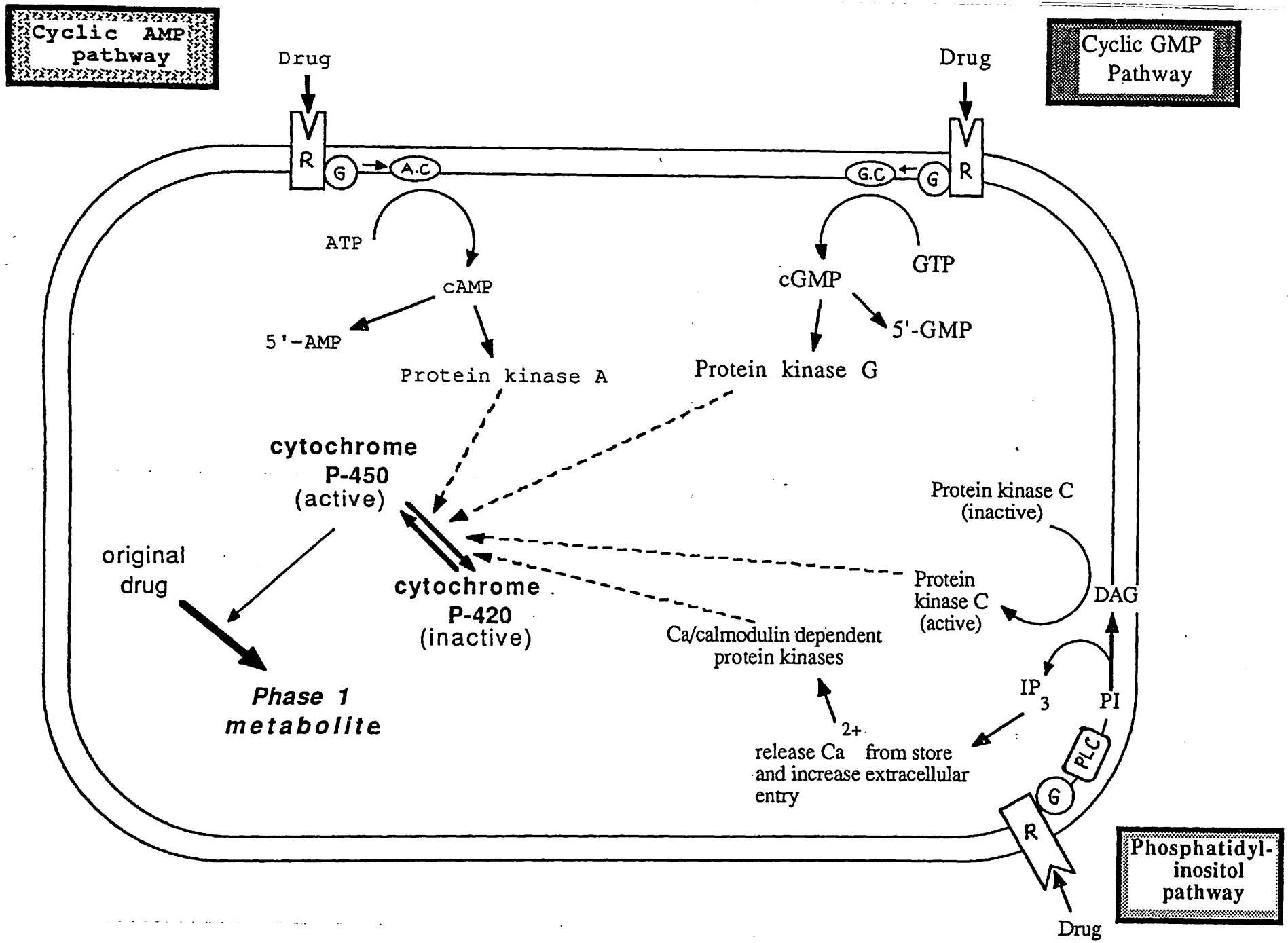
HEPATOCYTES

Hepatocytes + incubation medium + aminopyrine + ekstrak air Buah Melaka (EABM) in the absence / presence of K-252a / KT 5720 / IBMX

EABM : 0.0078 - 0.375 mg/ml
No.hepatocytes/incubation 17,500.
Aminopyrine concentration 5 mM
Incubation time : 15 min.

Metabolites formed were measured colorimetrically

Results were expressed as aminopyrine N-demethylase activity (nmol / million cells / minute)


Cyclic AMP pathway
Drug
R
G
A.C
ATP
cAMP
5'-AMP
Protein kinase A
cytochrome P-450 (active)
original drug
Phase 1 metabolite
cytochrome P-420 (inactive)
Cyclic GMP Pathway
Drug
R
G.C
G
GTP
cGMP
5'-GMP
Protein kinase G
Protein kinase C (inactive)
Protein kinase C (active)
DAG
Ca/calmodulin dependent protein kinases
IP3
PI
2+
release Ca from store and increase extracellular entry
PLC
G
R
Drug
Phosphatidyl-inositol pathway

Emblica officinalis (Pokok Buah Melaka)

Taxanomy :

Division : Spermatophyta

Sub-division : Angiospermae

Class : Dicotyledoneae

Order : Geraniales

Family : Euphorbiaceae

Genus : Emblica

Species : Officinalis

Uses : antibacteria, antivirus, expectorant, diuretic, dyspepsia, purgative, headache, antipyretic, antihypertensive.

PREPARATION OF EKSTRAK AIR BUAH MELAKA (EABM)

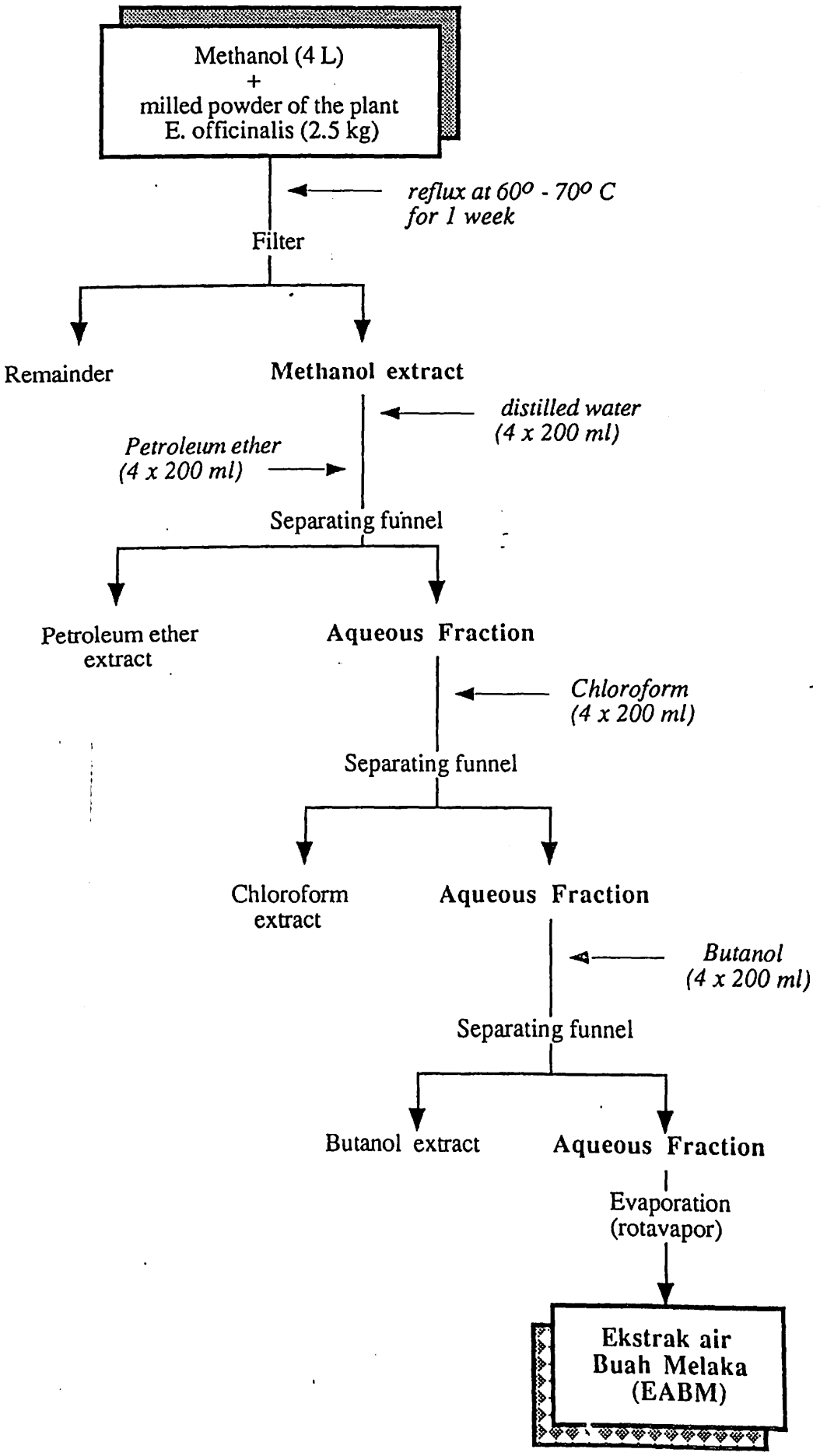


Figure 1. The effect of EABM on the metabolism of aminopyrine in hepatocytes obtained from 10 months old normal male rats.

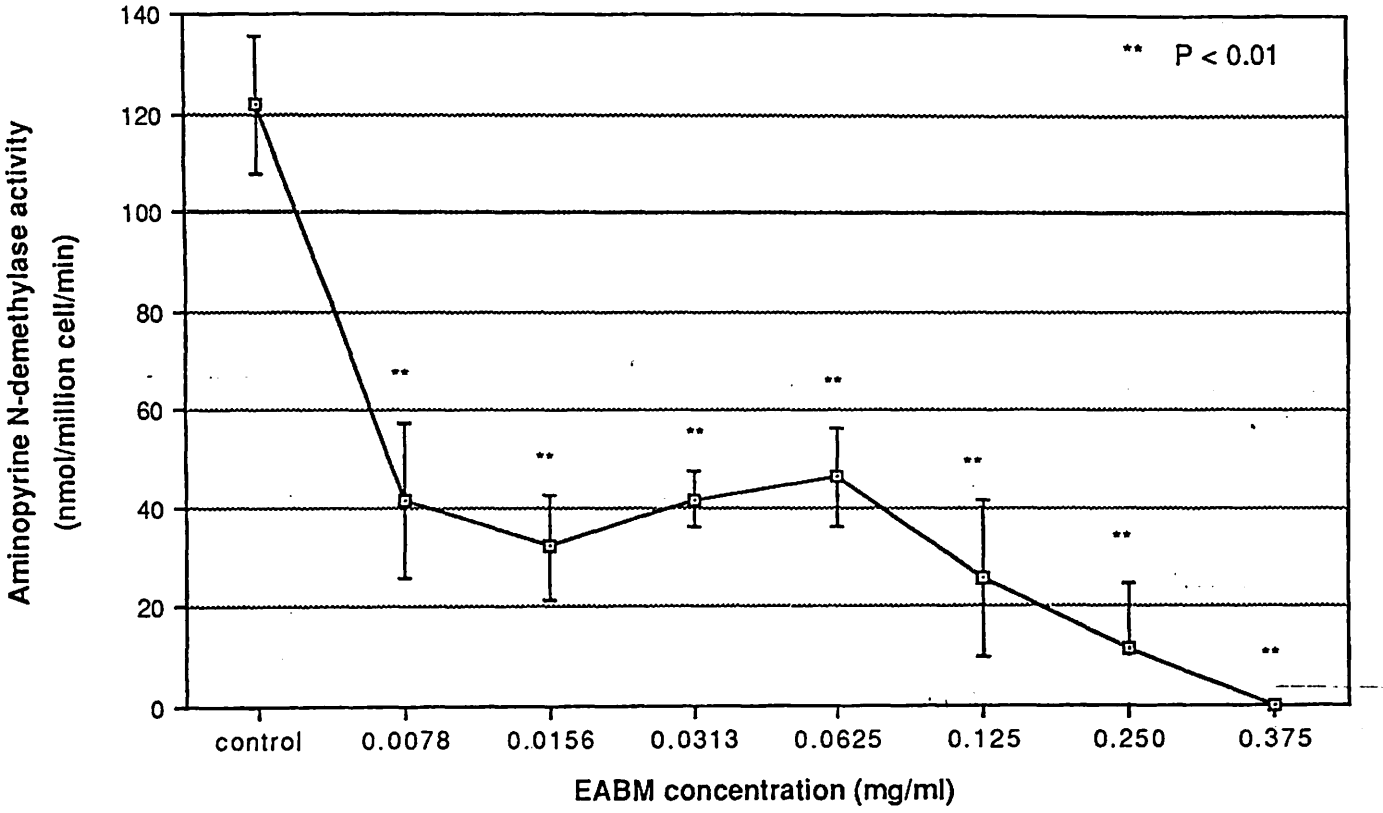


Figure 2. The effect of EABM on the metabolism of aminopyrine in hepatocytes obtained from 10 months old normal female rats.

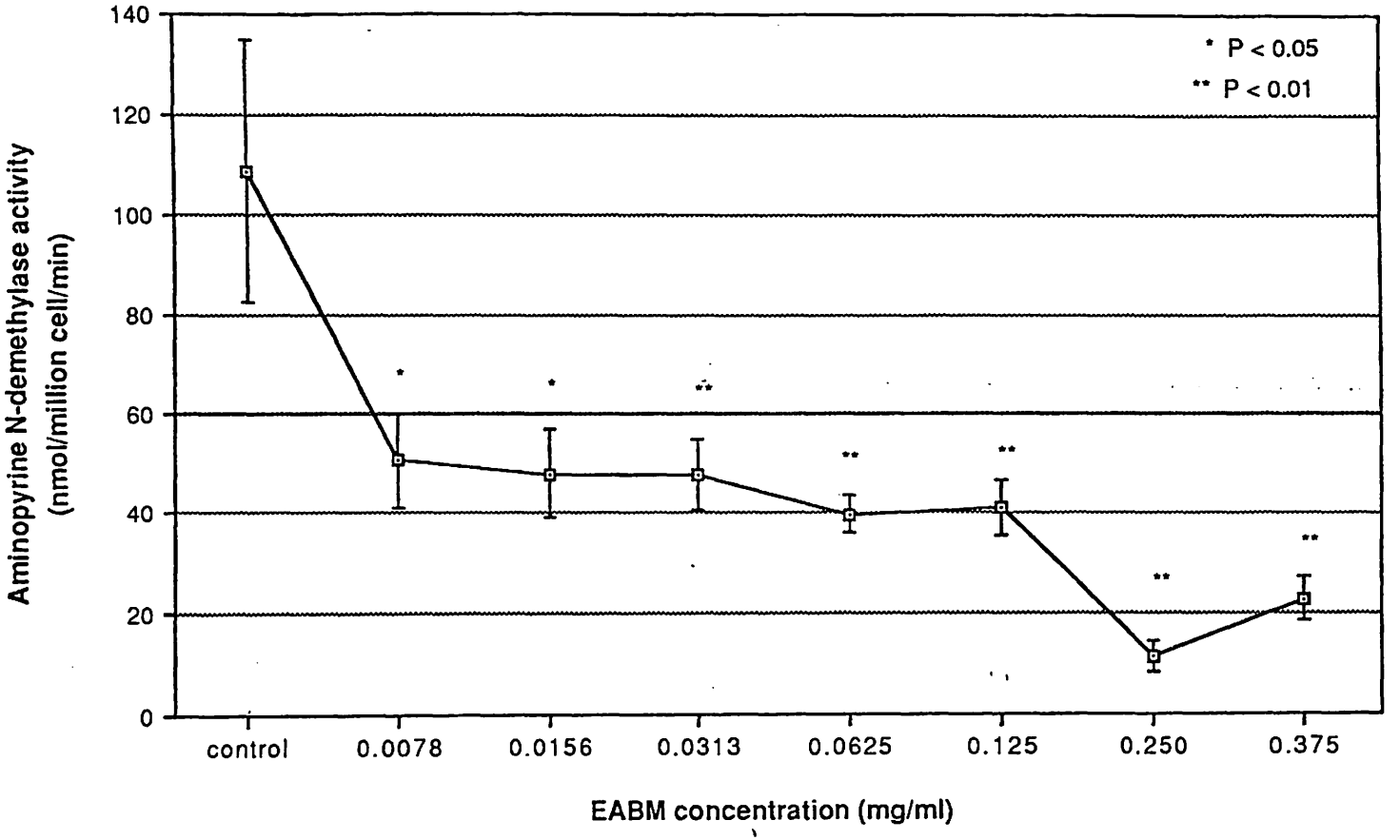


Figure 3. The effect of EABM on the metabolism of aminopyrine in hepatocytes obtained from 5 months old normal male rats

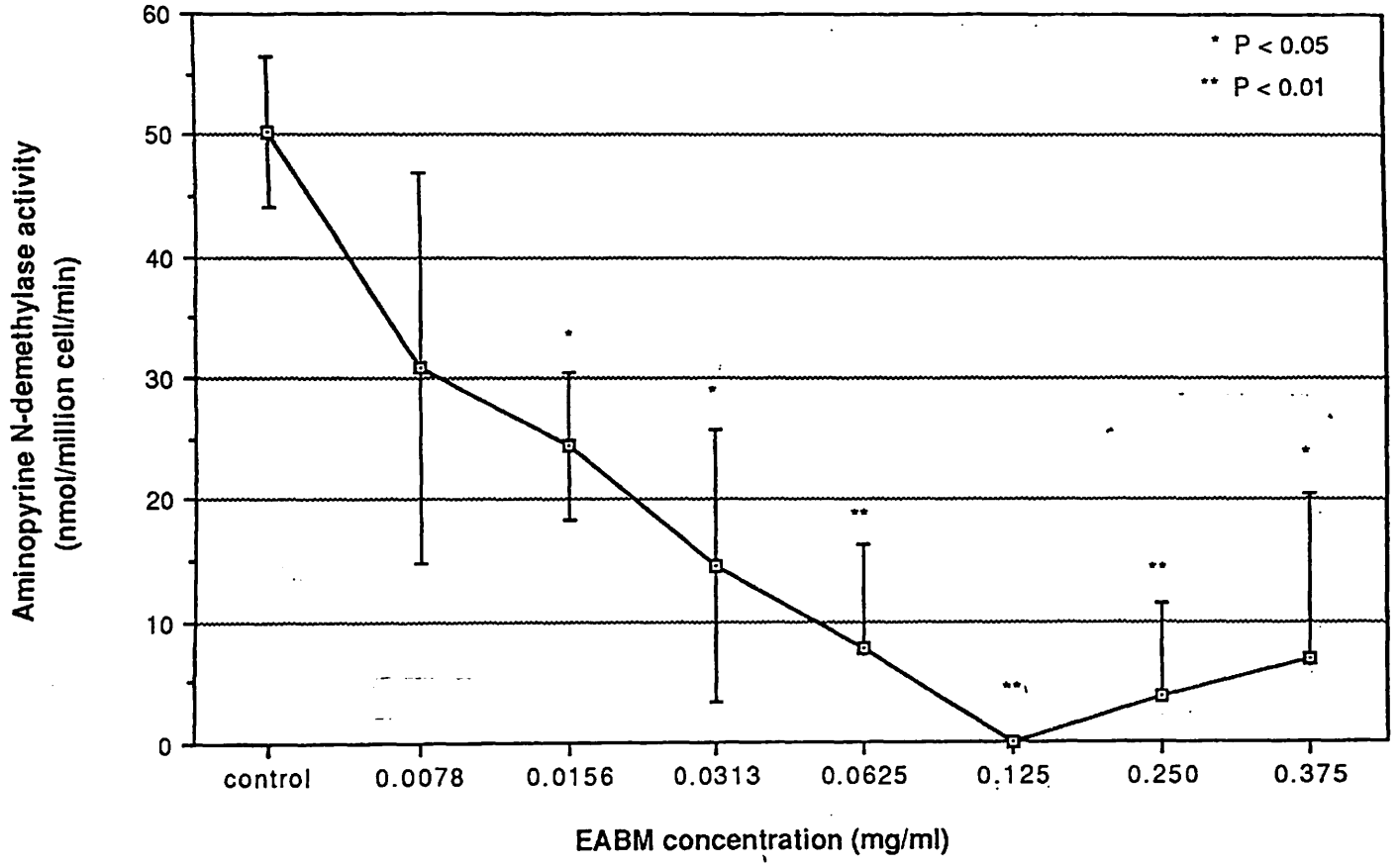


Figure 4. The effect of EABM on the metabolism of aminopyrine in hepatocytes obtained from 5 months old normal female rats

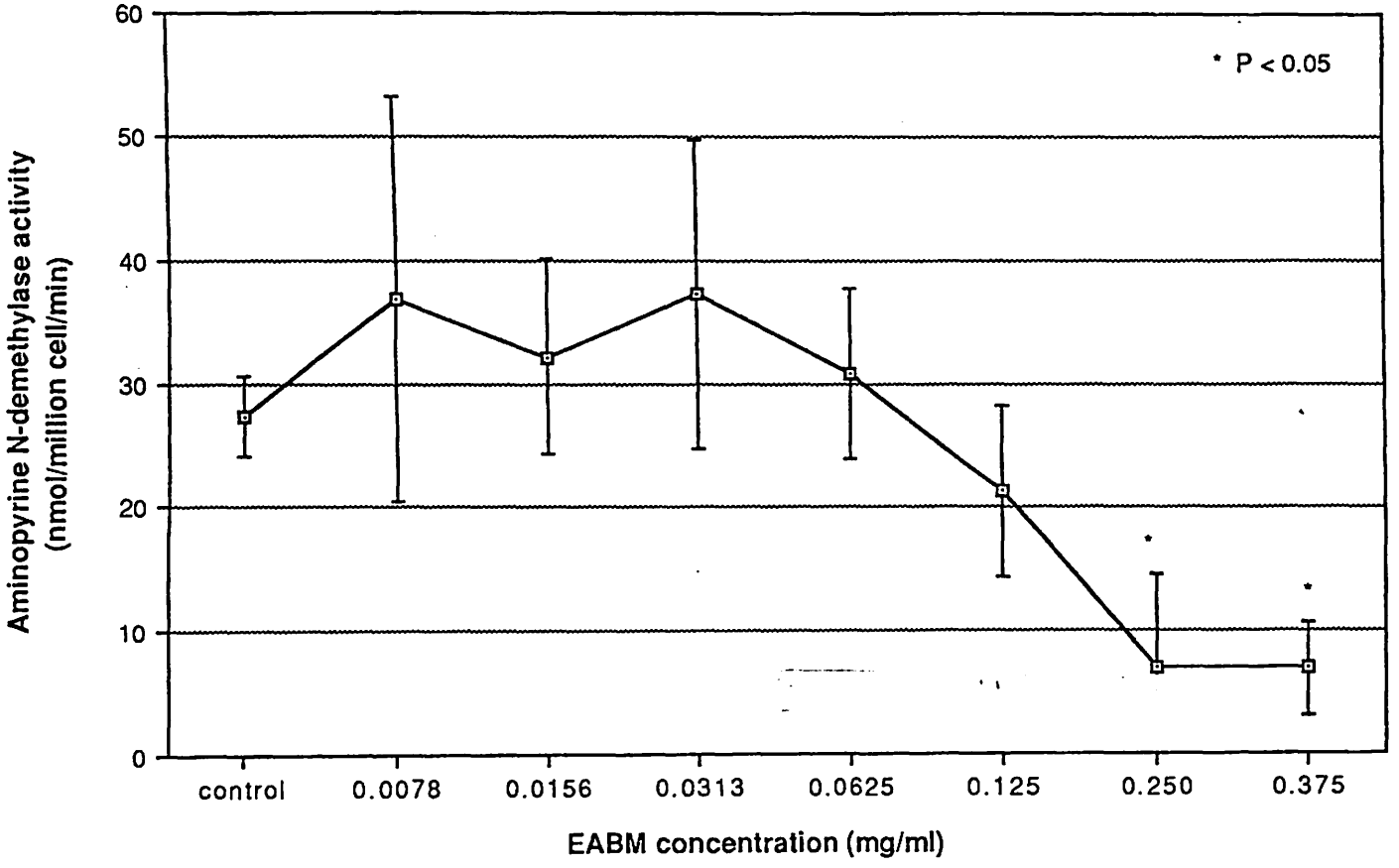


Figure 5. The effect of EABM on the metabolism of aminopyrine in the presence of K-252a (20 nM) in hepatocytes obtained from 10 months old normal male and female rats

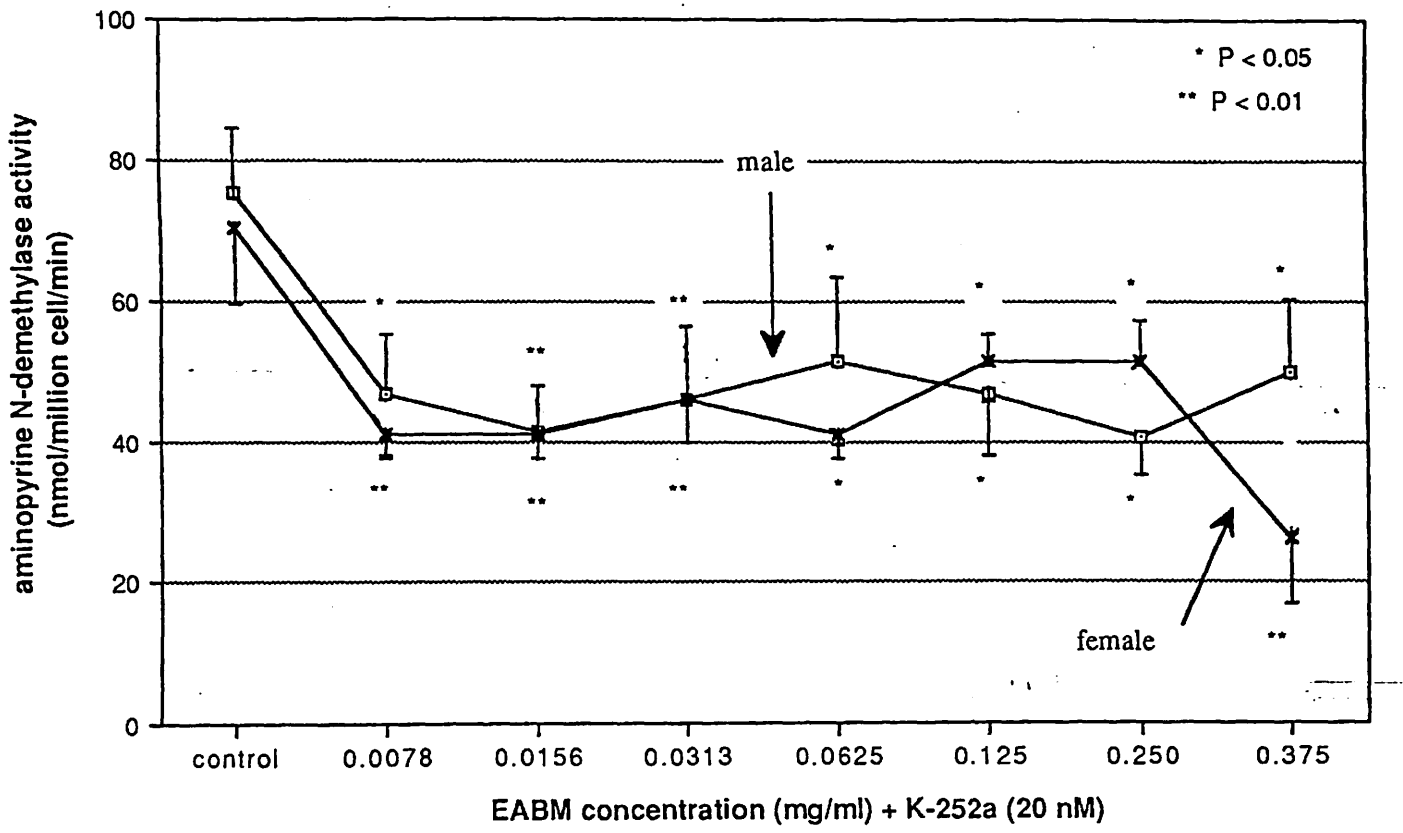


Figure 6. The effect of EABM on the metabolism of aminopyrine in the presence KT 5720 (60 nM) in hepatocytes obtained from 10 months old normal male and female rats

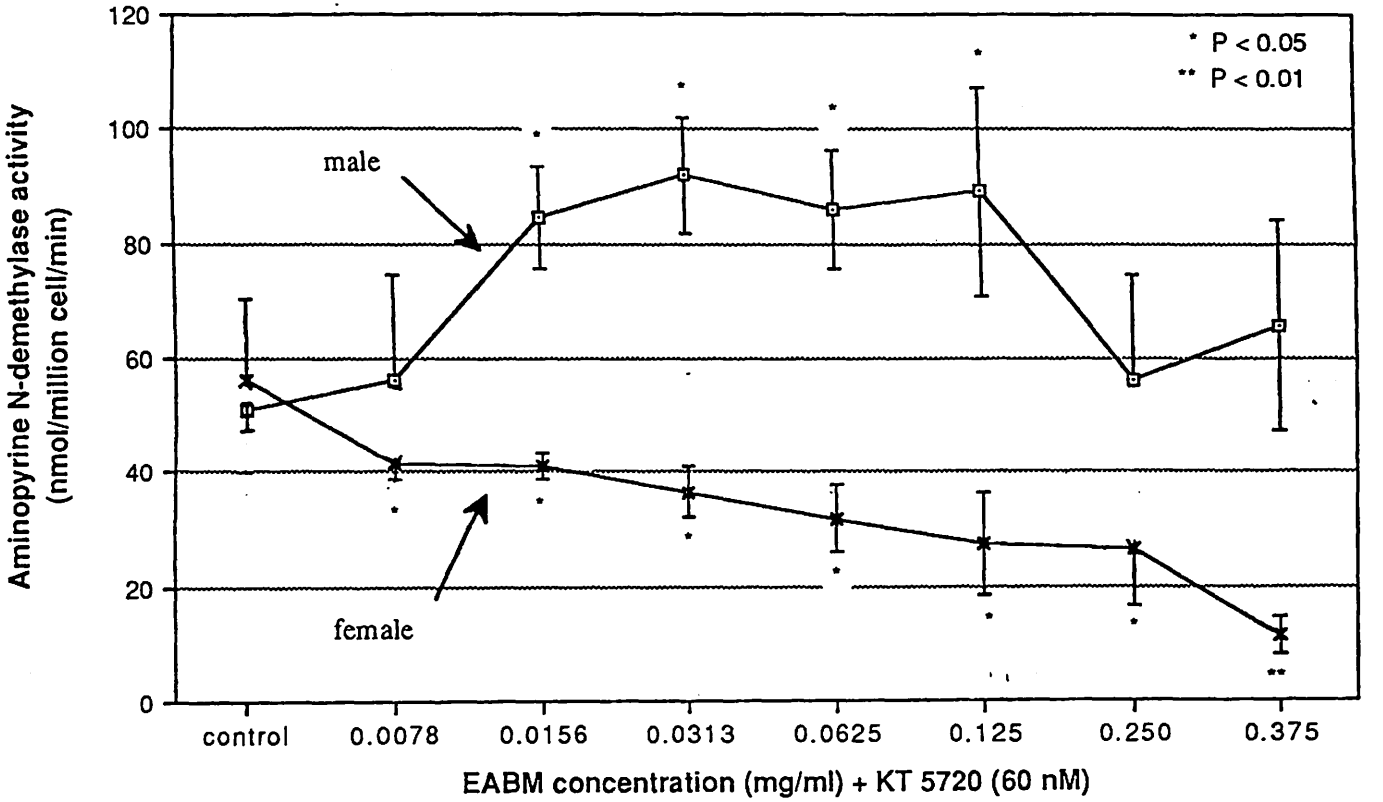
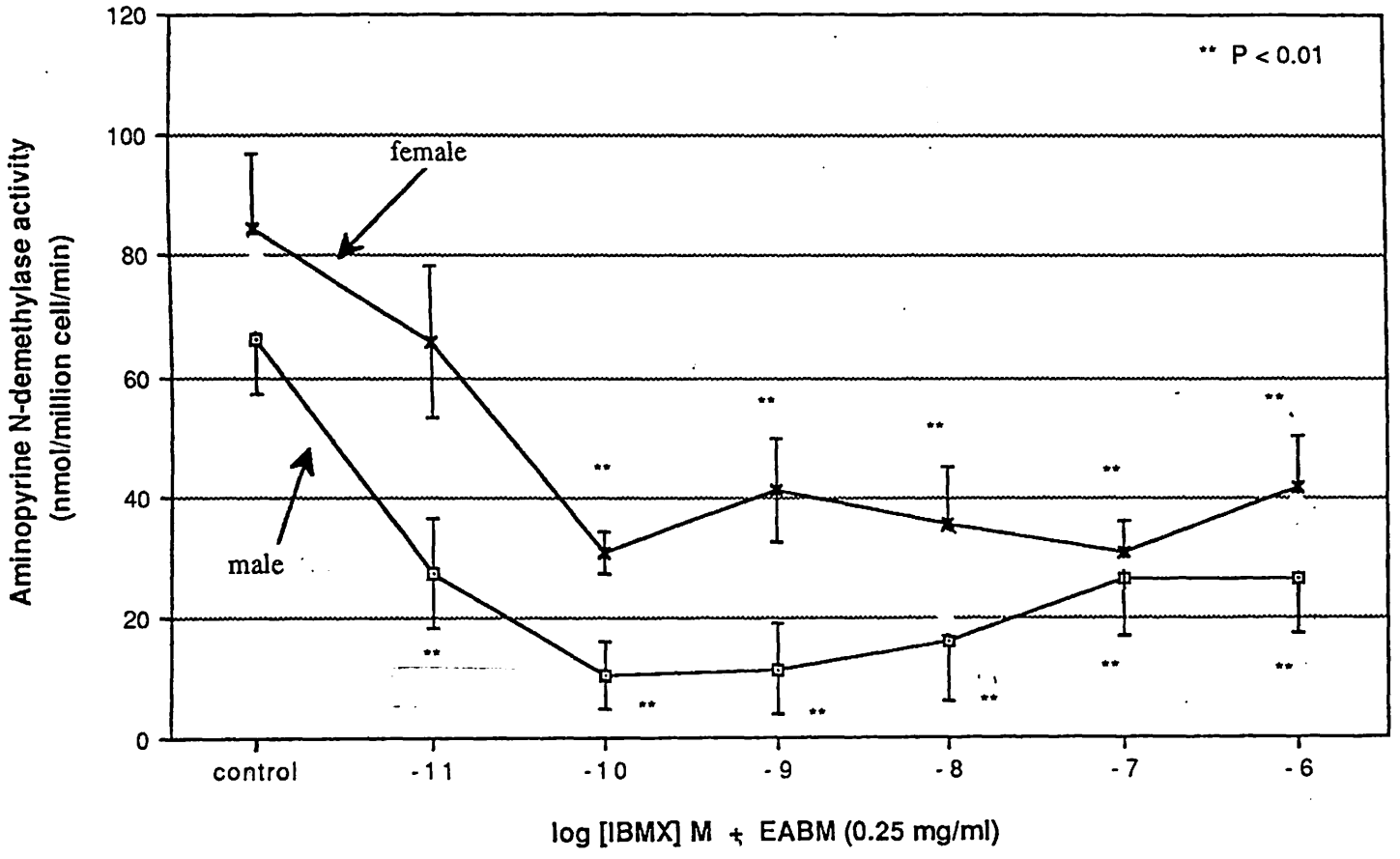


Figure 7. The effect of EABM (0.25 mg/ml) on aminopyrine metabolism in the presence of 3-isobutyl-1-methylxanthine (IBMX) in hepatocytes obtained from 10 months old normal male and female rats



SUMMARY

1) Age and sex factors may determine the effect of EABM on aminopyrine metabolism.

   a) In the 10 months old group, EABM affected significant decrease in aminopyrine metabolism in both the male and female rats.

   b) However, in the 5 months old group, EABM affected significant reduction on aminopyrine metabolism in the male rats only whereas in the female rats, no significant alteration when compared to control was observed.

2) Specific protein kinase A inhibitor, KT-5720, reverses the influence of EABM on aminopyrine metabolism in the male but not in the female rats (10 months old) suggesting that the influence of EABM on aminopyrime metabolism in the male rats is mediated via the protein kinase A.

MOLECULAR MECHANISM ELUCIDATION OF THE INFLUENCE OF THE WATER EXTRACT OF BUAH MELAKA (EMBLICA OFFICINALIS) ON DRUG METABOLISM IN SPONTANEOUSLY HYPERTENSIVE RATS

Abas Hj Hussin, Hafsah Mustapha and Mariam Ahmad

INTRODUCTION

Although modern drugs are commonly prescribed to treat illnessess, the use of herbal medicines by different ethnic groups in Malaysia still prevails. In the course of treatment, there are occasions where patients tend to take both modern prescribed drugs and herbal preparations concurrently. The interaction that ensues may result in a ***decrease in the bioavailability or the accumulation, and hence toxicity***, of the modern drug.

Disease states such as diabetes, thyroid disorders and ***hypertension*** have been shown to affect the metabolism of drugs. *Emblica officinalis*, locally known as Buah Melaka, has been shown to reduce the blood pressure of spontaneously hypertensive rats (SHR) via inhibition of the α-adrenoceptor.

With respect to this, the ***objectives*** of this study are to investigate the influence of *E. officinalis* on the metabolism of a model drug, aminopyrine, in hepatocytes obtained from SHR rats and to elucidate the possible molecular mechanism of action of E. officinalis in bringing about its influence.

METHODS

The blood pressure was measured by means of the tail-cuff technique[1] using Model 179 Blood Pressure Analyser, IITC Life Science, U.S.A. Isolated hepatocytes were prepared from male spontaneously hypertensive rats (15-20 weeks old; 150 - 250 g; bred in the School of Pharmaceutical Sciences, Universiti Sains Malaysia) by using the collagenase digestion method[2].

The hepatocytes were incubated with aminopyrine (5 mM) in an incubation medium for 18 minutes in the absence or presence of the water extract of *E. officinalis* (EABM) (at concentrations between 0.0078 - 0.375 mg/ml) and the metabolism of aminopyrine assayed according to the modified method of Cochin and Axelrod[3] and the results were expressed in nmol HCHO formed/million cells /min.

The effect of EABM on aminopyrine metabolism was then tested in the presence of cAMP analogue, 3-isobutyl-1-methylxanthine (IBMX); a protein kinase C activator, phorbol-12-myristate-13-acetate (TPA); a calcium ionophore, A 23187 and a calmodulin-inhibitor, calmidazolium. Two concentrations of EABM (0.0078 mg/ml and 0.25 mg/ml) were tested in the presence of the above substances (10^{-11} - 10^{-6} M). The hepatocytes were pre-incubated with the above substances for 15 minutes prior the incubation with EABM.

Means and standard deviations were calculated and statistical analysis were performed by means of Students *t*-test.

RESULTS

At concentrations below 0.0313 mg/ml, EABM exhibited no significant effect on aminopyrine metabolism when compared to control. However, further increase of EABM concentration showed significant reduction ($P<0.05$ and $P<0.01$) in aminopyrine metabolism (Fig. 1).

Addition of IBMX, as shown in figure 2, significantly reduced the effect of EABM (0.0078 mg/ml) but did not affect EABM effect on aminopyrine metabolism at 0.25 mg/ml EABM concentration (compare Fig. 1 and 3).

Increasing concentration of calmidazolium significantly reduced ($P<0.01$) EABM's (0.0078 mg/ml) effect when compared to control. However, the reducing effect of EABM (0.25 mg/ml) was inhibited by all concentrations of calmidazolium (Fig. 4 and 5).

The effect of EABM (0.0078 mg/ml) on aminopyrine metabolism was unaltered by TPA when compared to control (Fig. 6). The effect of EABM (0.25 mg/ml) was also unaffected in the presence of TPA (compare Fig. 1 and 7).

In the presence of A 23187, EABM (0.0078 mg/ml) exhibited no significant alteration in aminopyrine metabolism when compared to control (Fig. 8) and non of the concentrations of A 23187 alter the reducing effect of EABM (0.25 mg/ml) on aminopyrine metabolism (Fig. 9).

Figure 1. The effect of EABM on the metabolism of aminopyrine in hepatocytes obtained from male spontaneously hypertensive rats

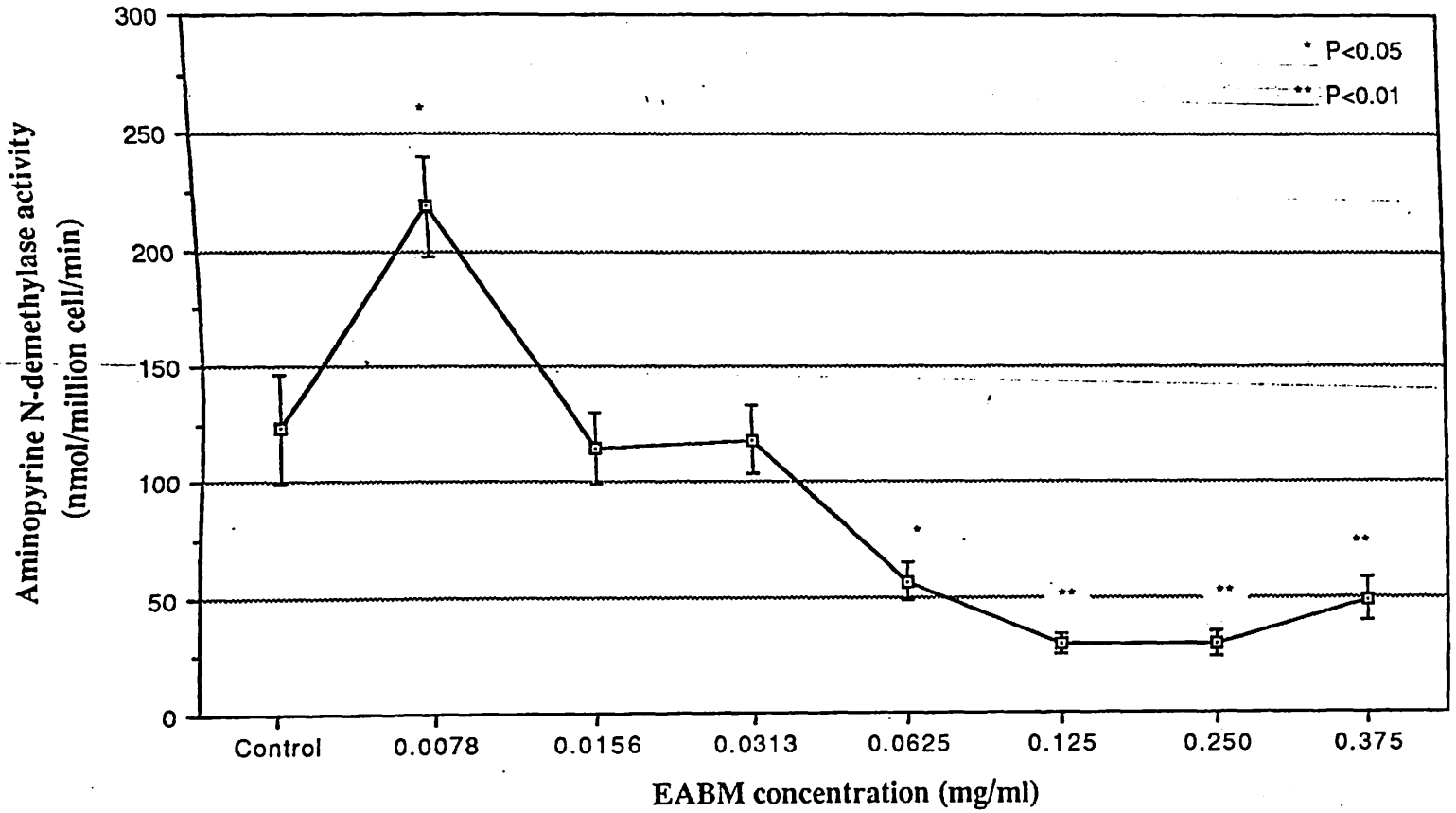


Figure 3. The effect of EABM (0.25 mg/ml) on aminopyrine metabolism in the presence of 3-isobutyl-1-methylxanthine (IBMX) in hepatocytes obtained from male spontaneously hypertensive rats

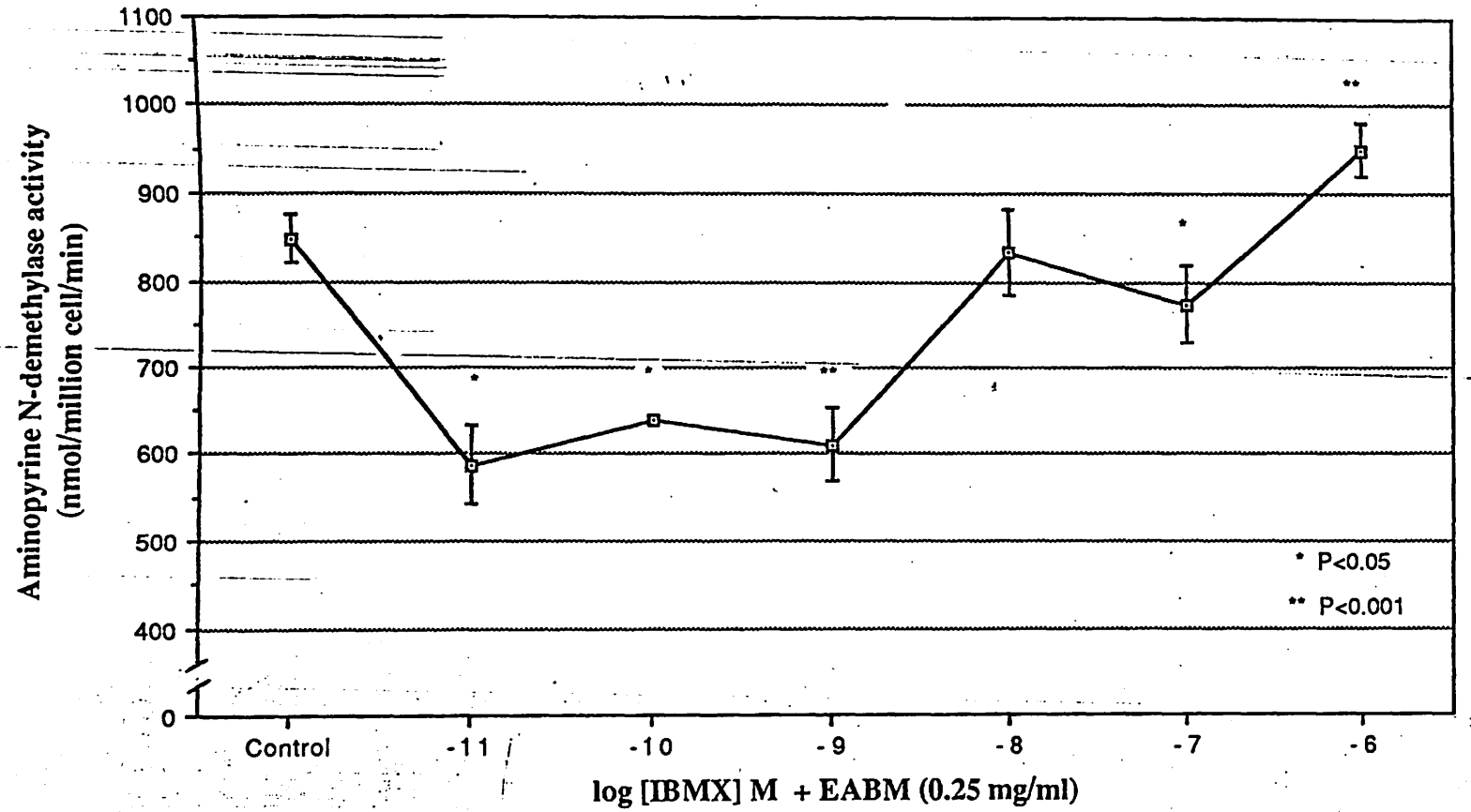


Figure 4. The effect of EABM (0.0078 mg/ml) on aminopyrine metabolism in the presence of calmidazolium in hepatocytes obtained from male spontaneously hypertensive rats

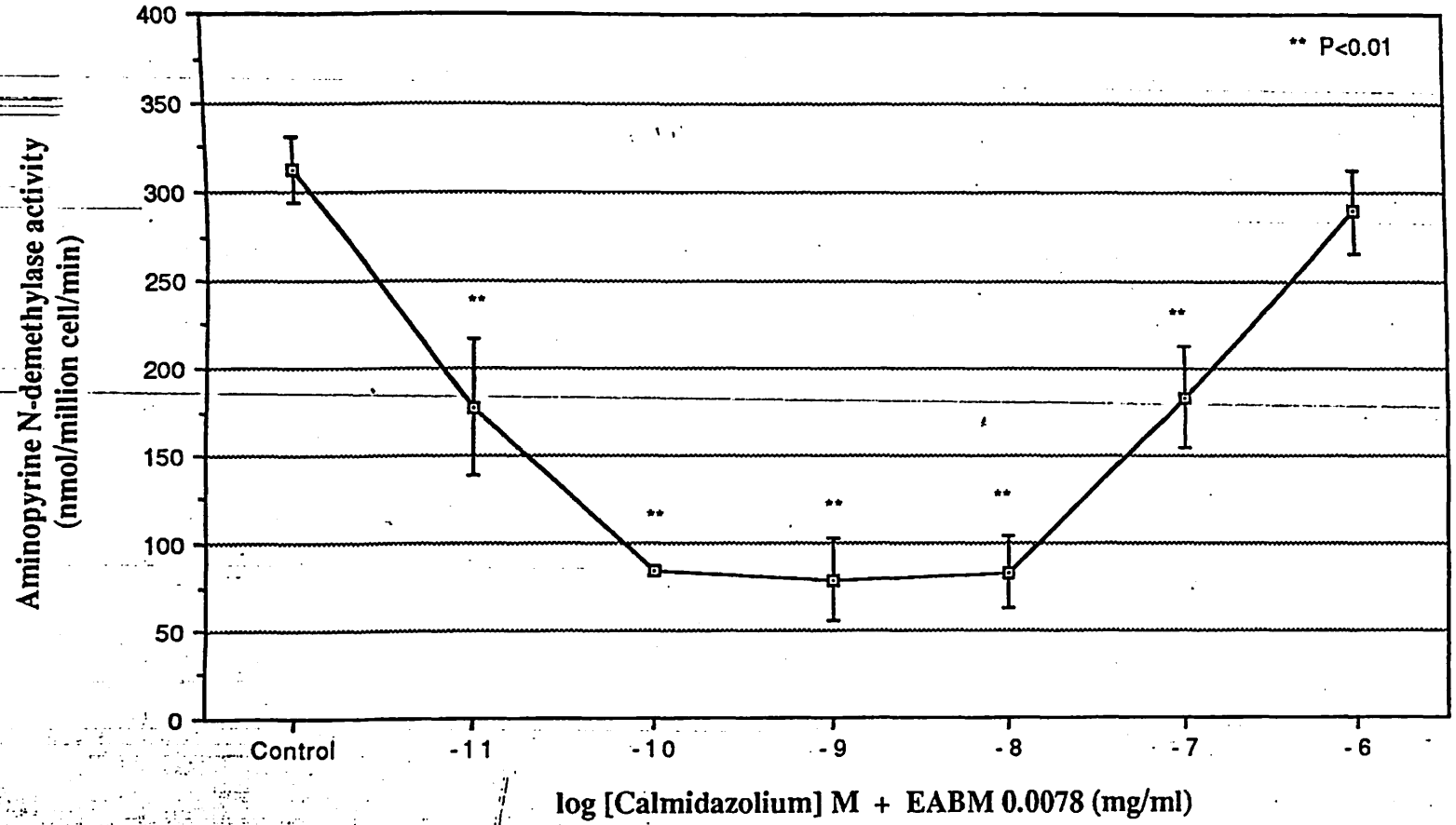


Figure 5. The effect of EABM (0.25 mg/ml) on aminopyrine metabolism in the presence of calmidazolium in hepatocytes obtained from male spontaneously hypertensive rats

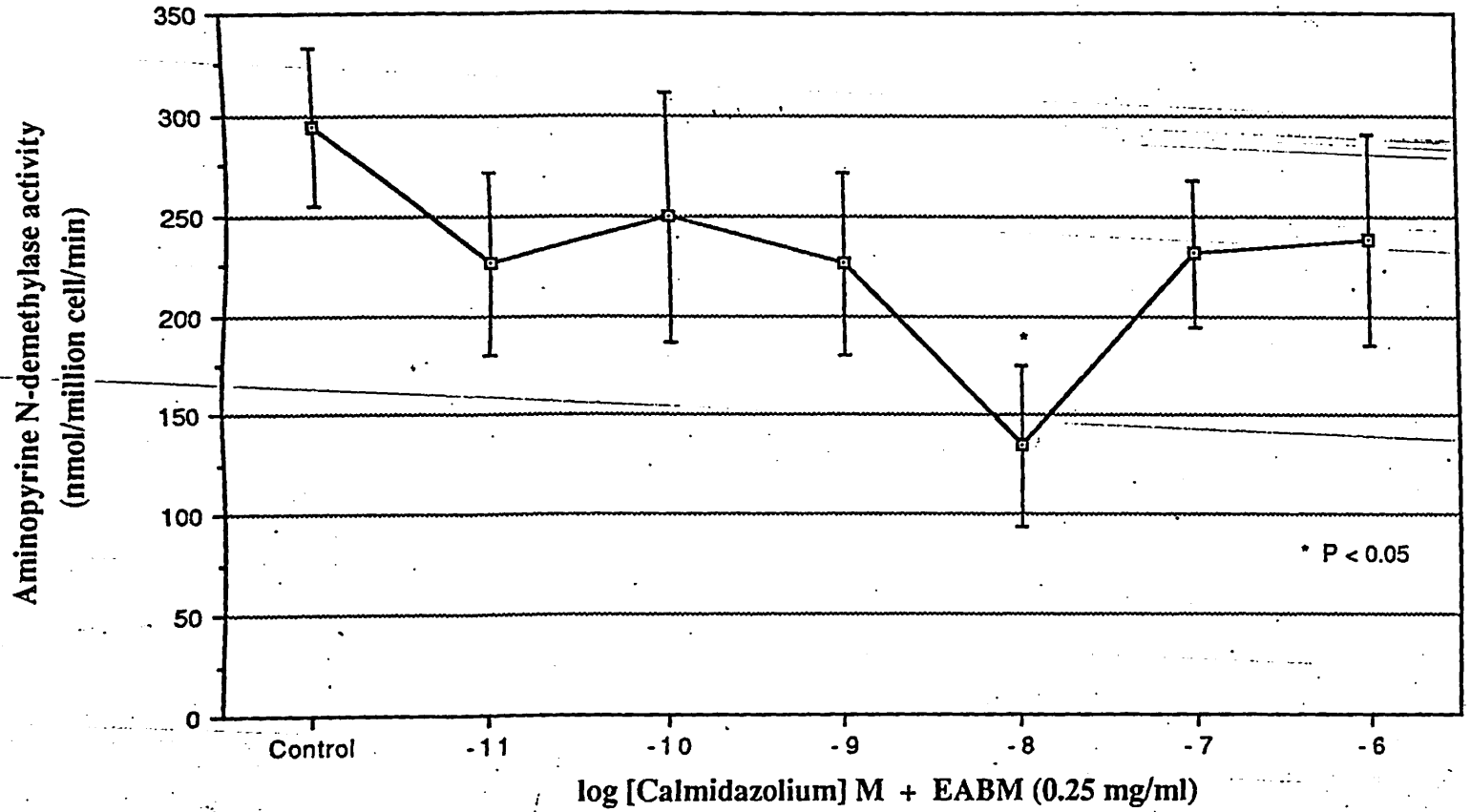


Figure 6. The effect of EABM (0.0078 mg/ml) on aminopyrine metabolism in the presence of phorbol-12-myristate-13-acetate (TPA) in hepatocytes obtained from male spontaneously hypertensive rats

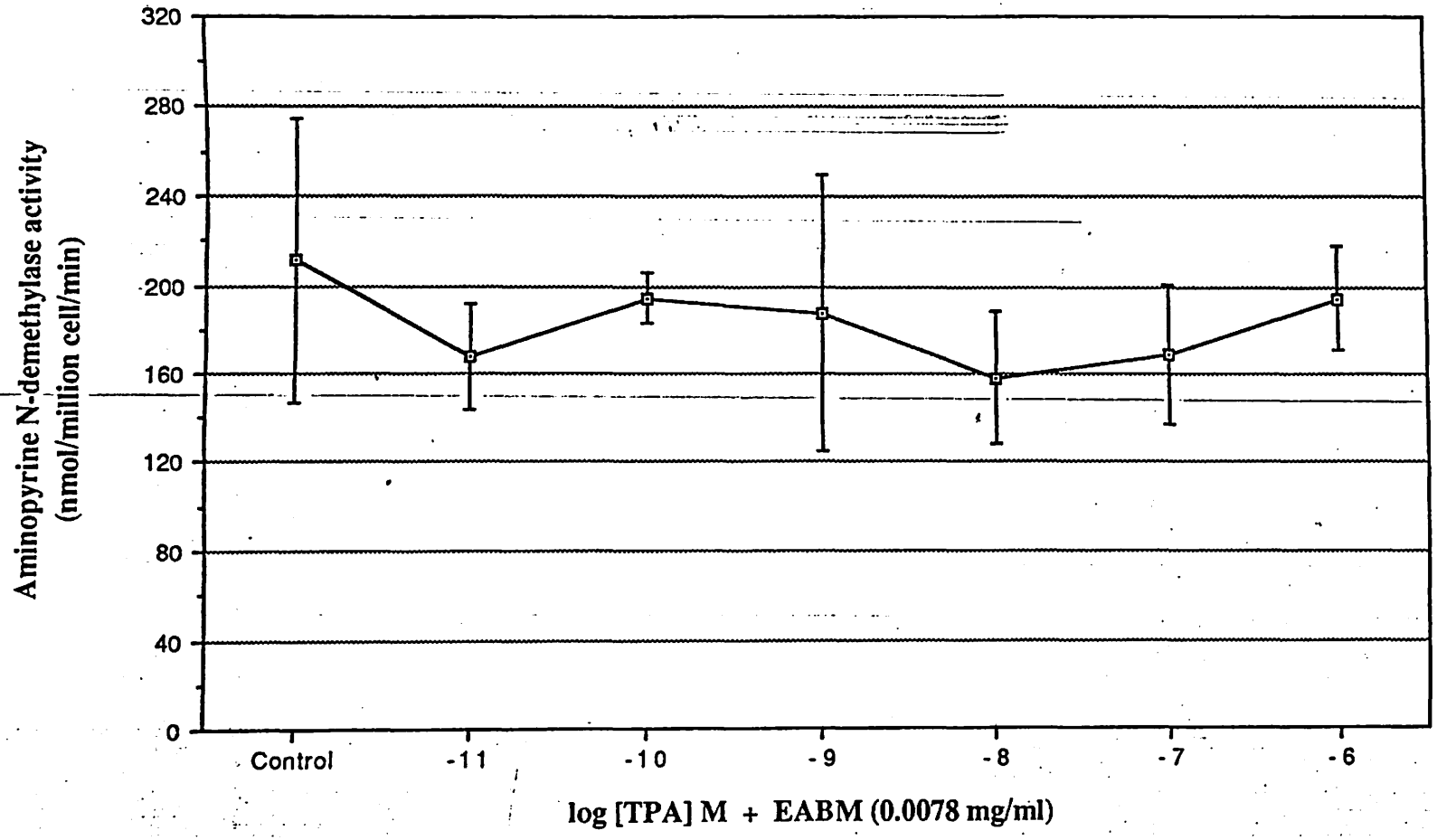


Figure 7. The effect of EABM (0.25 mg/ml) on aminopyrine metabolism in the presence of phorbol-12-myristate-13-acetate (TPA) in hepatocytes obtained from male spontaneously hypertensive rats

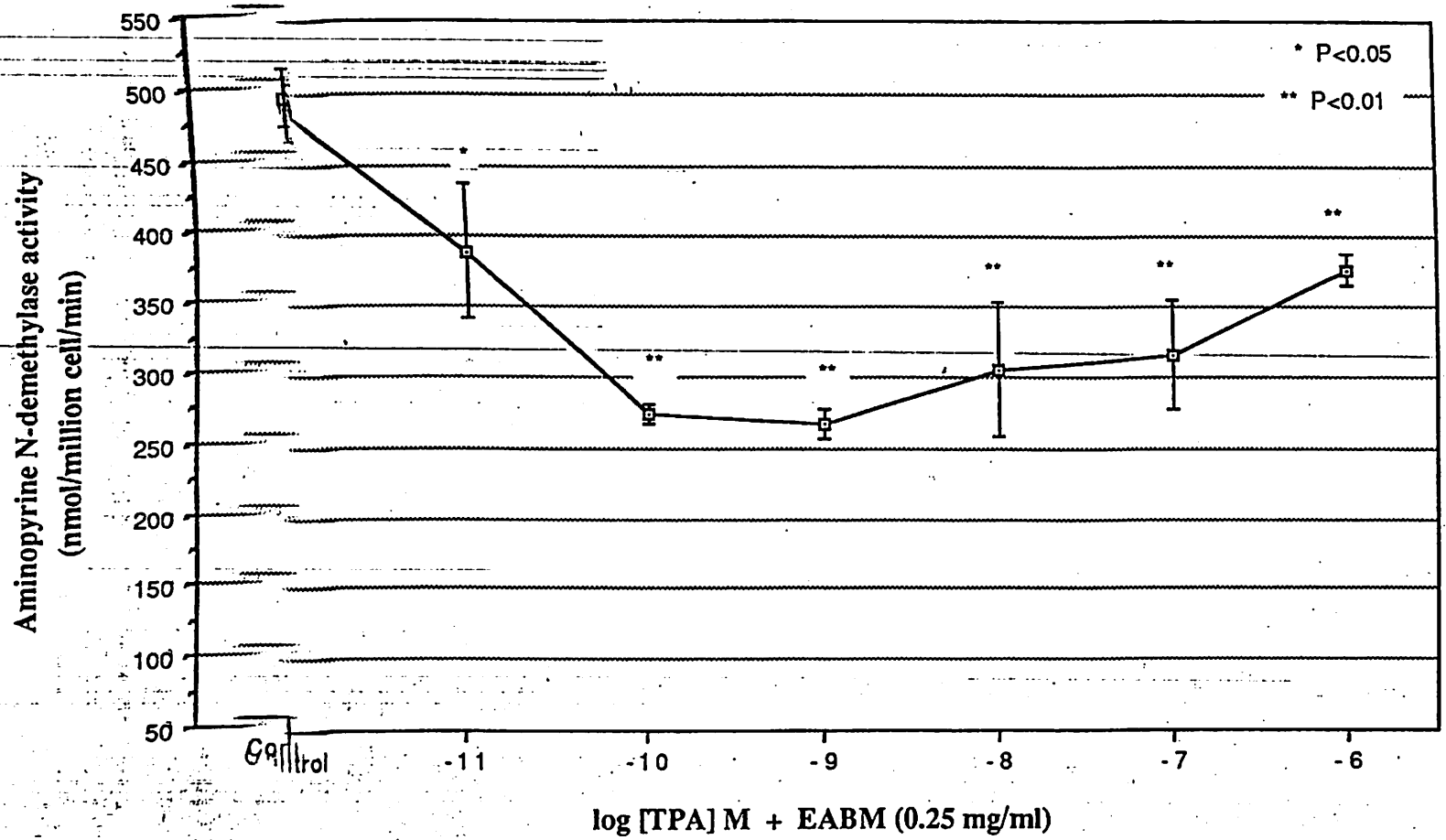


Figure 8. The effect of EABM (0.0078 mg/ml) on aminopyrine metabolism in the presence of A 23187 in hepatocytes obtained from male spontaneously hypertensive rats

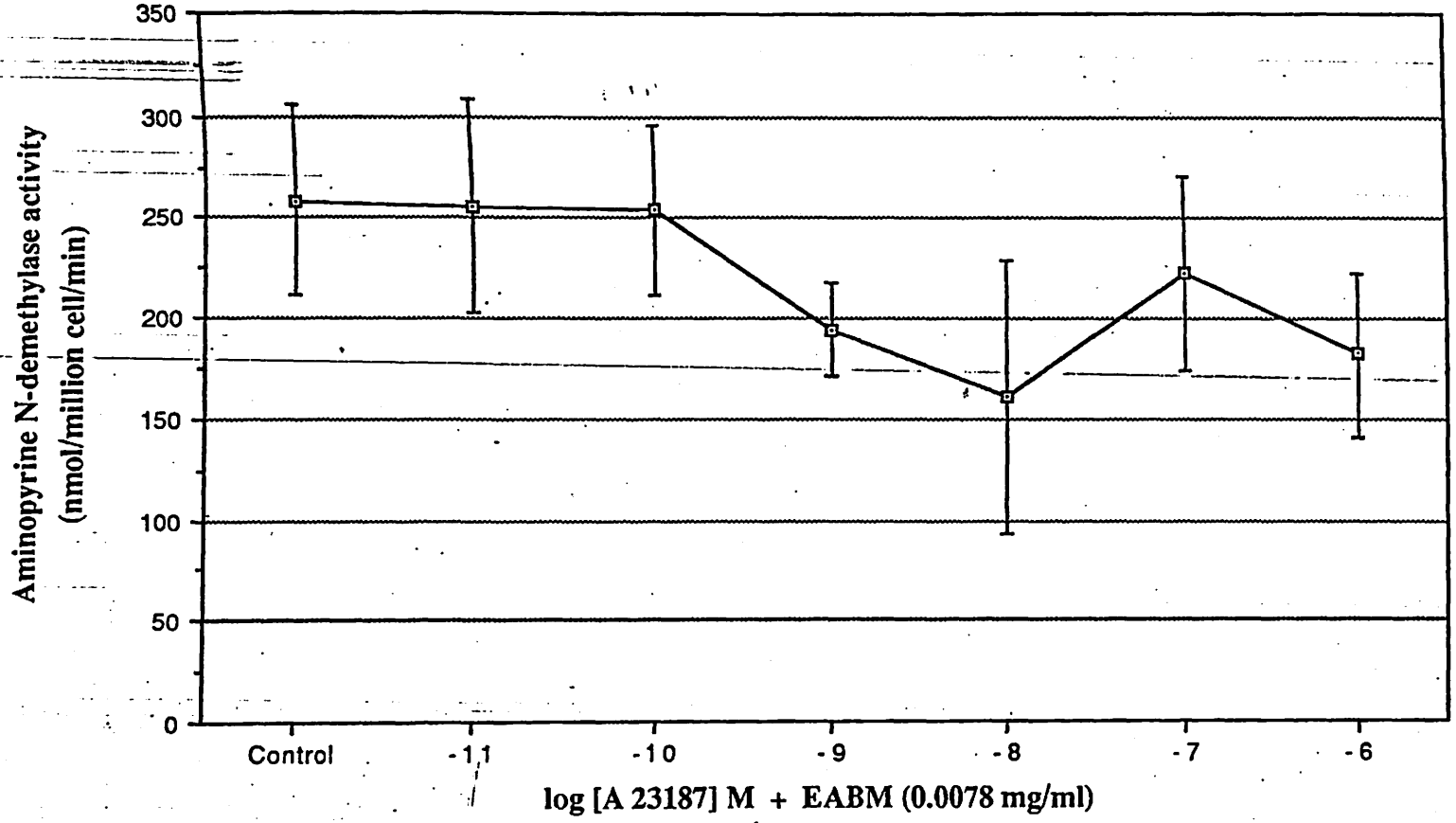
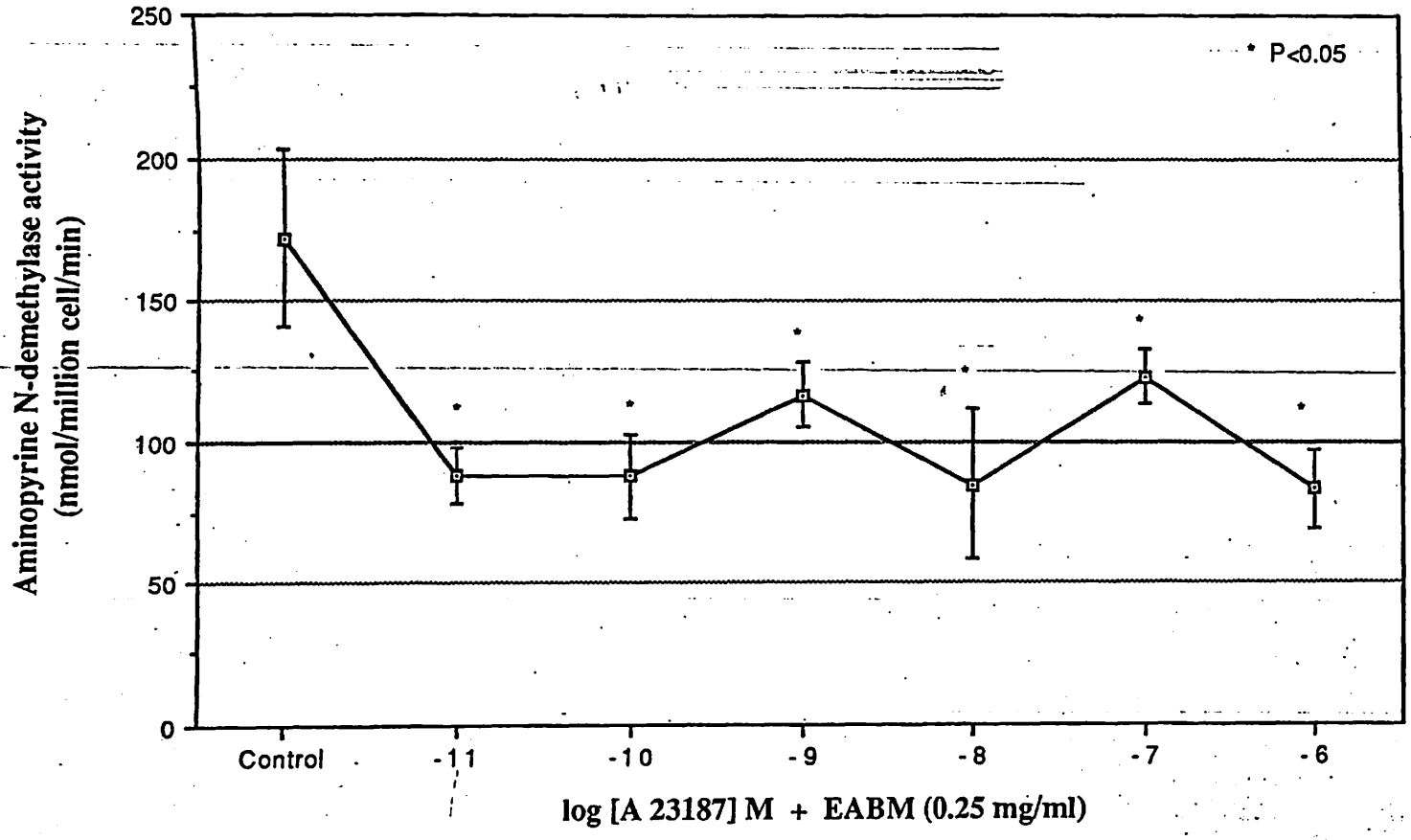


Figure 9. The effect of EABM (0.25 mg/ml) on aminopyrine metabolism in the presence of A 23187 in hepatocytes obtained from male spontaneously hypertensive rats



DISCUSSION

The rats used were evidently hypertensive. The systolic and diastolic blood pressure were 196.5 ± 12.36 mmHg and 147.5 ± 33.91 mmHg (n = 8) respectively. Our results indicated that EABM is able to reduce aminopyrine metabolism in hepatocytes of spontaneously hypertensive rats. At concentrations of 0.0625 mg/ml and above, EABM was able to reduce the activity of aminopyrine N-demethylase. As such, if Buah Melaka were to be taken (for its anti-hypertensive property) together with **drugs** that undergo similar liver metabolic phase 1 pathway as aminopyrine (e.g. diazepam, imipramine, procainamide and chlorpromazine), there is a possibility that there will be reduction and hence, accumulation and eventually, toxicity of the latter drugs.

Phosphorylation of the protein components of the liver biotransformation system has been found to be important in drug metabolism. Phosphorylation was found to cause conversion of cytochrome P-450 to its denatured form, cytochrome P-420. At 0.0078 mg/ml concentration, EABM's effect on aminopyrine metabolism was not altered by TPA and A 23187 indicating that protein kinase C and extracellular calcium intrusion are not involved, at least, at the lower concentrations, in the influence of EABM on aminopyrine metabolism. However, the addition of IBMX and calmidazolium resulted in about 40 % and 75 %

reduction in aminopyrine metabolism indicating the involvement of the cAMP- and calmodulin-associated pathway and possibly cross-talk between the two systems by EABM in eliciting its effect.

At 0.25 mg/ml, EABM's effect on aminopyrine metabolism was not significantly altered in the absence and presence of IBMX, TPA and A23187 (compare Fig. 1 with Fig. 3, 7 and 9). However, all concentrations of calmidazolium were able to inhibit the effect of EABM on aminopyrine metabolism (compare Fig. 1 and 5). This results indicate that the reducing effect of EABM on aminopyrine metabolism is probably mediated by the calmodulin-associated system.

REFERENCES

1. Pfeffer, J.M, Pfeffer, M.A. and Frohlich, E.D. (1971) Journal of Laboratory and Clinical Medicine, **78**: 957-962

2. Hussin, A.H and Skett, P. (1987) Biochemical Pharmacology, **36**: 3155-3159

3. Cochin, J. and Axelrod, J. (1959) J. Pharmac. exp. Ther. **125**: 416-42

CONCLUSION

Our result suggested that EABM is capable of reduc aminopyrine metabolism in rat liver. There is a possibil that *other drugs* that undergo similar N-demethylati process in the phase 1 metabolism in the liver wou undergo similar reduction in their liver metabolism. Th will ultimately result in accumulation of the drugs in th circulation and may lead to drug toxicity. Our result als indicated that EABM's influence on aminopyrin metabolism is probably mediated by the cAMP- an calmodulin-associated pathway at low concentration (< 0.0313 mg/ml) and by calmodulin-associated pathway a high concentrations above 0.0625 mg/ml.

INFLUENCE AND MOLECULAR MECHANISM ELUCIDATION OF ANTI-MALARIAL AGENT, ARTEMISININ, ON DRUG METABOLISM IN SPONTANEOUSLY HYPERTENSIVE RATS

Santhanathan Rajendram, Abas Hj Hussin and Chan Kit Lam

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INTRODUCTION

Artemisinin also called artemisinine or qinghaosu, a sesquiterpene lactone with an unusual endoperoxide linkage, is the clinically active antimalarial constituent isolated from the Chinese medicinal herb, Qinghao or *Artemisia annua* L [family : compositae]. It is particularly useful in the treatment of cerebral malaria where rapid reversal of the parasitemia and restoration to conciousness of the patient is critical. Beside being used as an antimalarial agent, it is also used as an anti-pyretic agent, bactericide for scabies, abscesses and eye disorders, and in the treatment of chronic dysentery.

The utilization of traditional herbal preparations have been in practise for centuries by different ethnic groups in Malaysia and is still popular despite the rapid development and improvement of the health sector in this country. The recent introduction of government regulation to register all traditional medicinal preparations have added a new dimension to the use of these type of preparations in therapy.

The **objectives** of this study are to investigate the possible interaction between naturally-derived medicinal constituent *and* the so-called 'modern drug' and the influence of the former on the liver metabolism of the latter. This study also intend to investigate the possible molecular mechanism of action of the former in bringing about its effet on the liver metabolism of the latter in hepatocytes of spontaneously hypertensive rats.

METHODS

The blood pressure was measured by means of the tail-cuff technique using Model 179 Blood Pressure Analyser, IITC Life Science, U.S.A. Isolated hepatocytes were prepared from male spontaneously hypertensive rats (15-20 weeks old; 150 - 250 g; bred in the School of Pharmaceutical Sciences, Universiti Sains Malaysia) by using the collagenase digestion method.

The hepatocytes were incubated with aminopyrine (5 mM) in an incubation medium for 18 minutes in the absence or presence of artemisinin (at 10^{-11} - 10^{-6} M concentrations) and the metabolism of aminopyrine assayed according to the modified method of Cochin and Axelrod and the results were expressed in nmol HCHO formed/million cells /min.

The effect of EABM on aminopyrine metabolism was then tested in the presence of cAMP analogue, 3-isobutyl-1-methylxanthine (IBMX); a protein kinase C activator, phorbol-12-myristate-13-acetate (TPA); a protein kinase C inhibitor, staurosporine; a protein kinase A inhibitor, KT 5720 and a calmodulin-inhibitor, calmidazolium. Artemisinin (10^{-8} M) was then tested in the presence of the above substances with concentration range of 10^{-11} - 10^{-6} M. The hepatocytes were pre-incubated with the above substances for 15 minutes prior the incubation with artemisinin.

Means and standard deviations were calculated and statistical analysis were performed by means of Students *t*-test.

RESULT

Our result indicated that artemisinin affected significant increase in aminopyrine metabolism. This is indicated by the increased in aminopyrine N-demethylase activity at concentration beginning 10^{-9} M artemisinin ($P < 0.05$). Artemisinin (10^{-8} M) caused a peak in the enzyme activity and concentration above 10^{-8} M showed a lowering in the enzyme activity but are still significantly above the control level (Figure 1).

In the presence of cyclic AMP analogue, 3-isobutyl-1-methylxanthine (IBMX), artemisinin (10^{-8} M) influence on aminopyrine N-demethylase activity was significantly reversed and in fact, totally inhibited at concentrations as low as 10^{-11} M IBMX (Figure 2).

This was similarly seen with the effect of artemisinin (10^{-8} M) in the presence of protein kinase C activator, phorbol-12-myristate-13-acetate (TPA) - see Figure 3. However, the extent of inhibition of the activity of aminopyrine N-demethylase by TPA was much lower when compared to IBMX (compare Figure 2 and 3).

Both protein kinase A inhibitor, KT 5720 and calmodulin inhibitor, calmidazolium, significantly lower the activity of the enzyme aminopyrine N-demethylase (Figure 4 and 5) whereas protein kinase C inhibitor, staurosporine, reduces the effect of artemisinin (10^{-8} M) to the control level (Figure 6).

Figure 1. Dose-response effect of artemisinin on the metabolism of aminopyrine in hepatocytes obtained from male spontaneously hypertensive rats

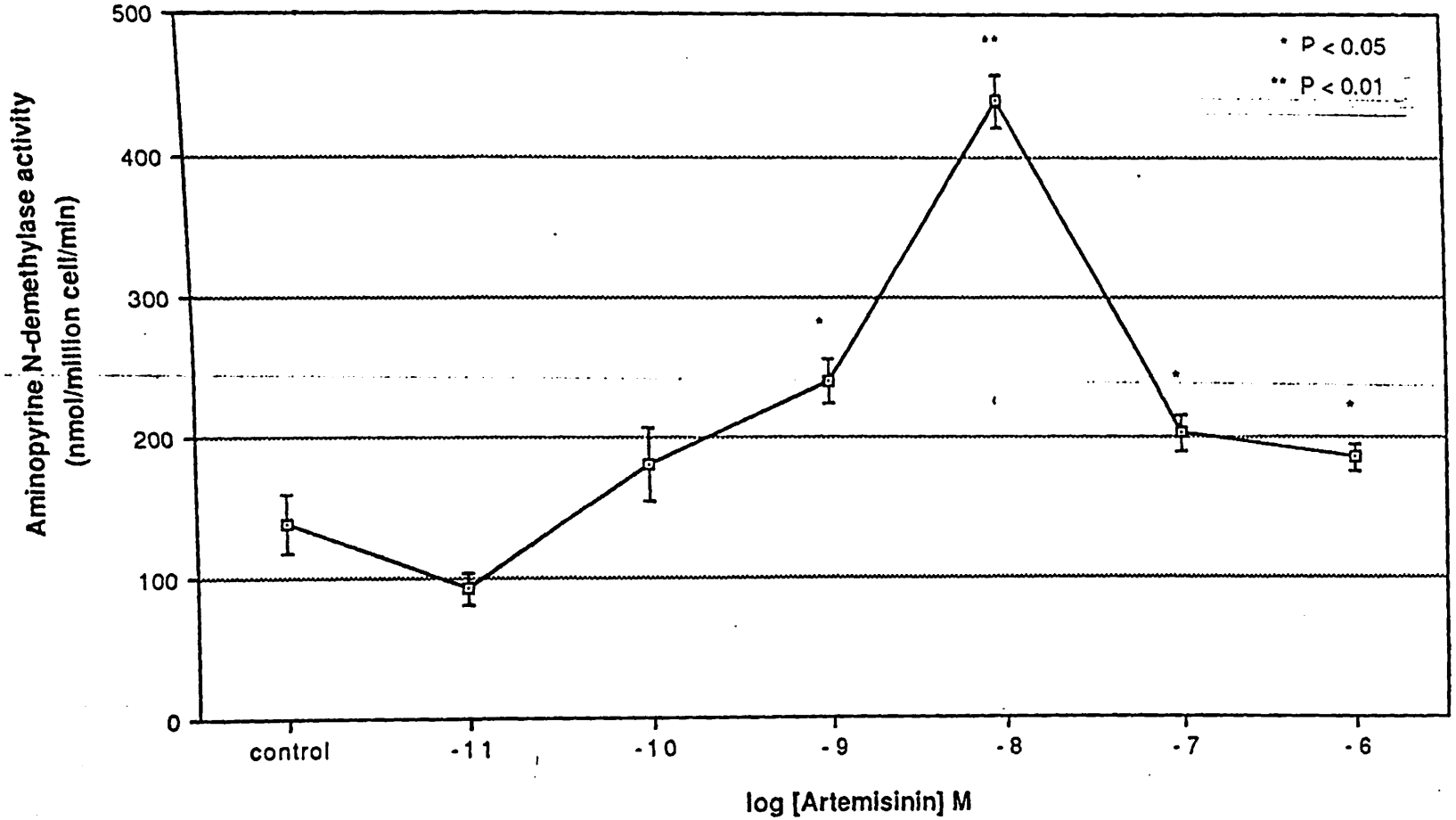


Figure 2. The effect of artemisinin (10^{-8} M) on aminopyrine metabolism in the presence of 3-isobutyl-1-methylxanthine (IBMX) in hepatocytes obtained from male spontaneously hypertensive rats

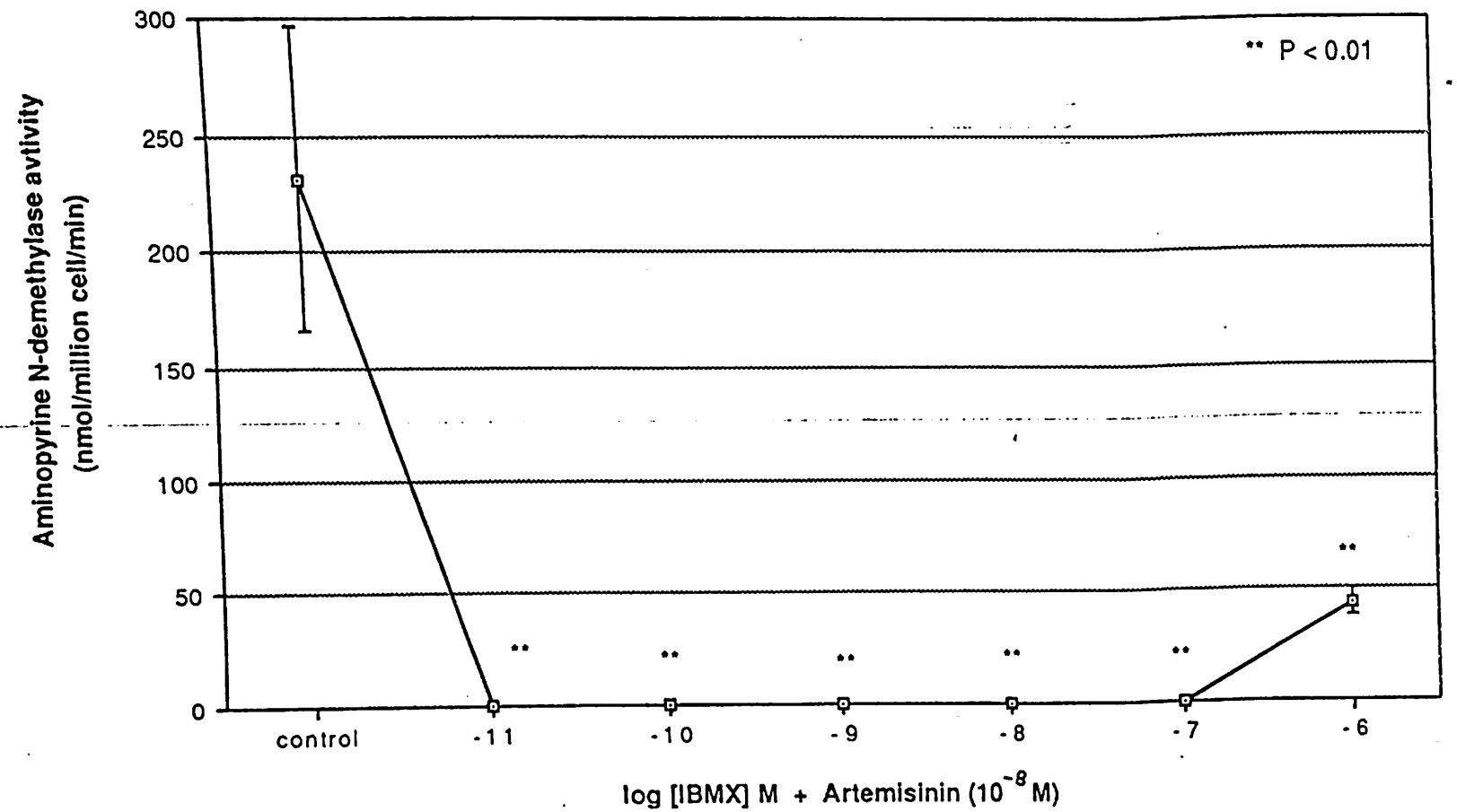


Figure 3. The effect of artemisinin (10^{-8}M) on aminopyrine metabolism in the presence of protein kinase C activator, phorbol-12-myristate-13-acetate, TPA, in hepatocytes obtained fom male spontaneously hypertensive rats

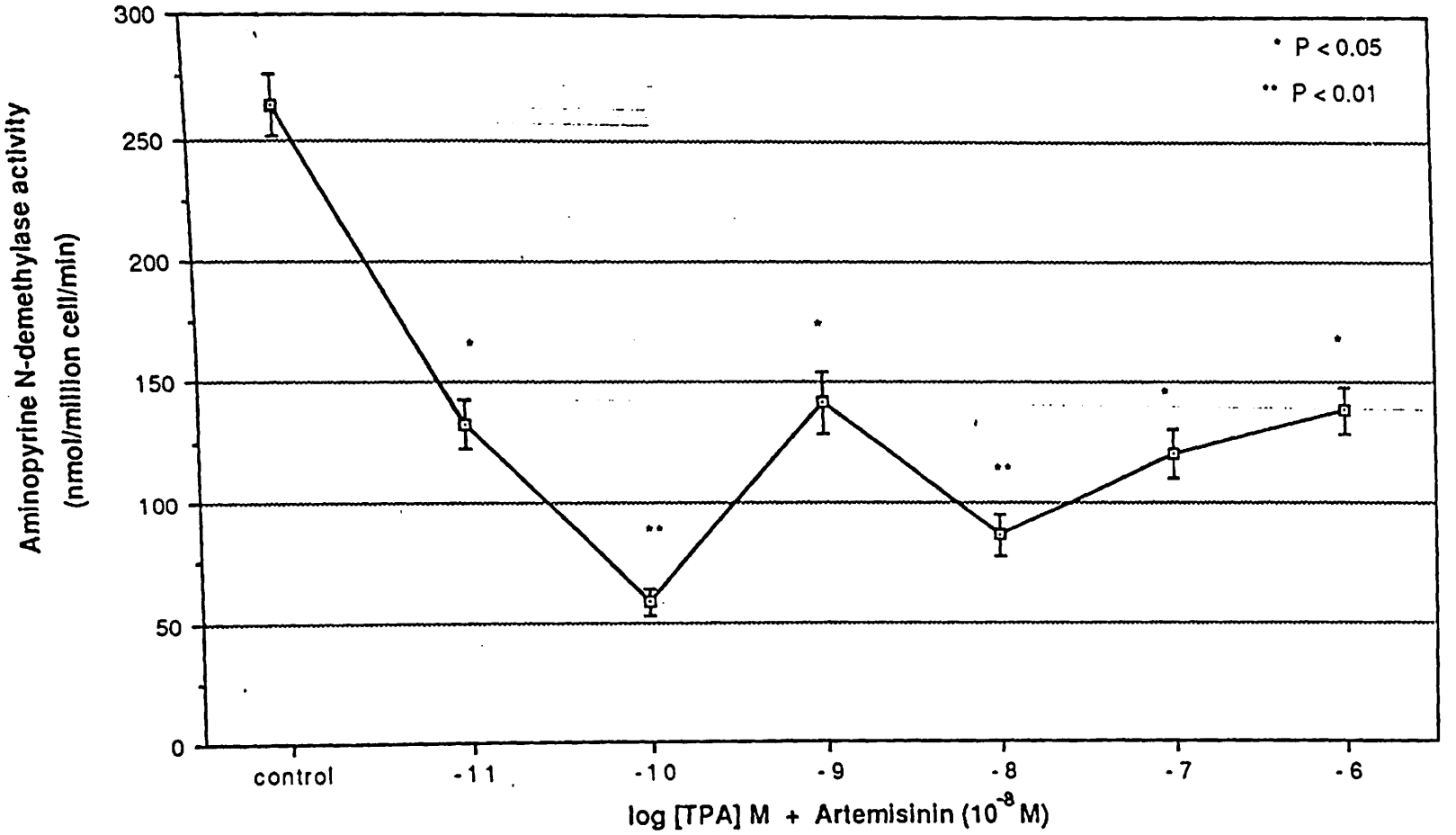


Figure 5. The effect of artemisinin (10^{-8} M) on aminopyrine metabolism in the presence of calmodulin inhibitor, calmidazolium, in hepatocytes obtained from male spontaneously hypertensive rats

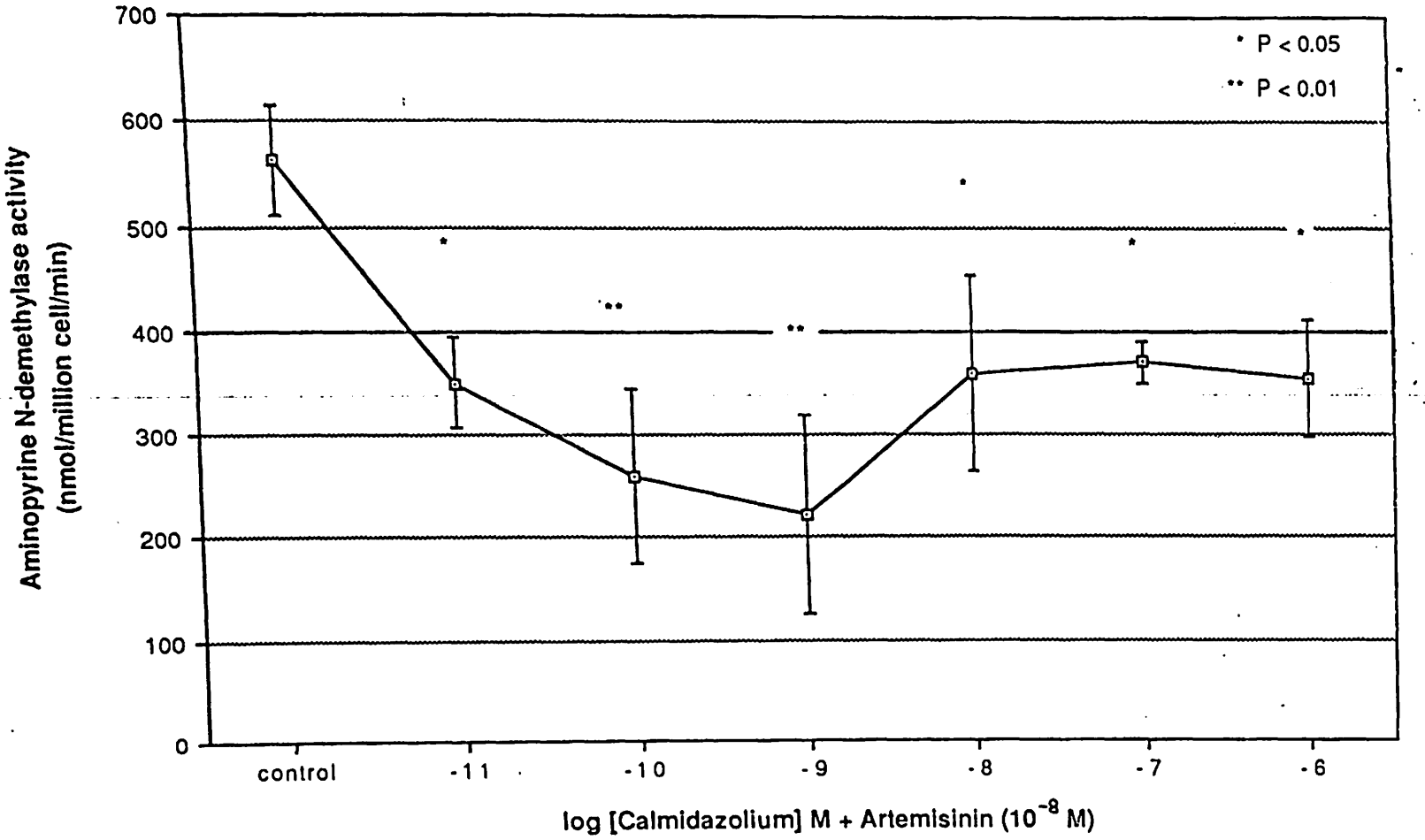
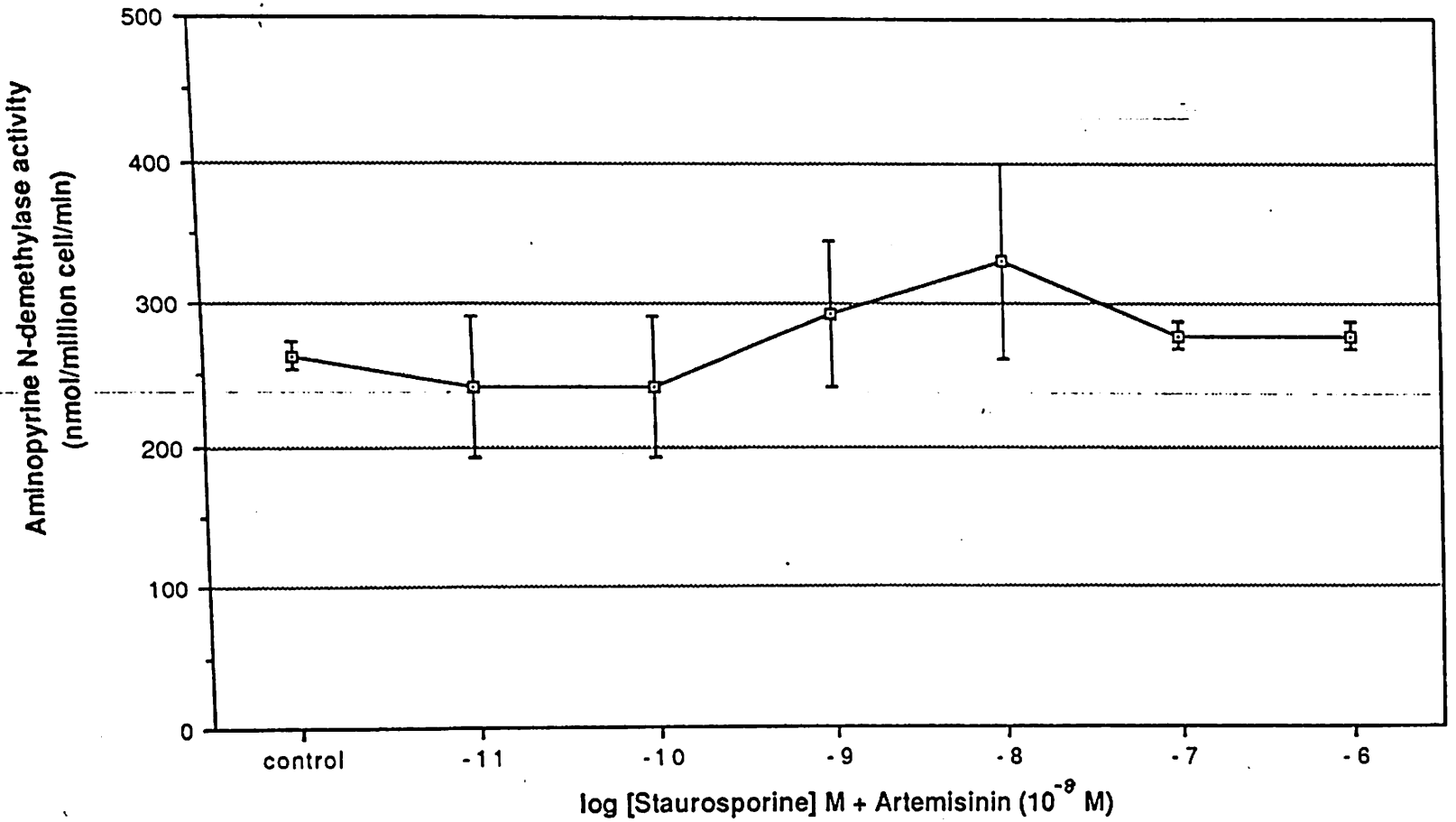


Figure 6. The effect of artemisinin (10^{-9}M) on aminopyrine metabolism in the presence of protein kinase C inhibitor, staurosporine, in hepatocytes obtained from male spontaneously hypertensive rats



DISCUSSION

The rats used were evidently hypertensive. The systolic and diastolic blood pressure were 206.5 ± 15.23 mmHg and 134.5 ± 40.46 mmHg (n = 8) respectively. Our results indicated that artemisinin is able to **increase** aminopyrine metabolism in hepatocytes of spontaneously hypertensive rats. Physiological concentrations of artemisinin (10^{-8} - 10^{-9} M) were able to increase aminopyrine demethylation of the phase 1 liver metabolism. Other drugs that undergo phase 1 N-demethylation process also have the potential to be affected by artemisinin. Consequent to these effect of artemisinin, the concentrations of these drugs in the circulation will be reduced.

Both IBMX and TPA expectedly reduced the activity of aminopyrine N-demethylase. Both IBMX and TPA reduced artemisinin effect on aminopyrine metabolism but the total inhibition of aminopyrine N-demethylase activity by IBMX indicate the relative involvement of cyclic AMP in the mechanism of action of artemisinin when compared to diacylglycerol. IBMX and TPA are expected to activate protein kinase A and C respectively and this in turn will increase the phosphorylation of cytochrome P-450 (active form of the metabolising enzyme) to P-420 (inactive form of the metabolising enzyme).

However, the effect of artemisinin (10^{-8}) on aminopyrine metabolism in the presence of protein kinase A inhibitor, KT 5720; protein kinase C inhibitor, staurosporine; and calmodulin inhibitor, calmidazolium, were not as anticipated. Theoretically, these inhibitors are expected to potentiate the effect of artemisinin. Further study is needed to explore the role of the protein kinases in the mechanism of artemisinin.

CONCLUSION

Artemisinin is capable of increasing aminopyrine metabolism in hypertensive rats inferring that it could *reduce the bioavailability* of aminopyrine and other drugs that undergo similar phase 1 N-demethylation pathway. These effect of artemisinin is suggested to be mediated, at least, by cyclic AMP and diacylglycerol and the role of protein kinases still needs to be investigated and explored. It is not known whether this type of interaction would occur in human and whether it is *clinically relevant*. It is suggested that in the future, this type of study should be extended to human.

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