# RESULTS

## A. Experiments *in vitro*

*1. Effect of the ethanol extract from C. petasites and reference drugs on histamine-induced contraction of isolated guinea-pig tracheal strip preparation*

The antagonistic effect on the histamine-induced tracheal contraction, which represents the bronchodilator activity of the ethanol extract was evaluated in comparison with reference drugs (terbutaline, aminophyline and papaverine). A dose of histamine which caused submaximal contraction of the tracheal muscle (0.3 μg/ml) was used to induce bronchoconstriction. The results obtained revealed that the ethanol extract and reference drugs exhibited a significant antagonistic effect on the histamine-induced tracheal contraction as shown in Figure 5. The percent relaxation of the histamine-induced tracheal contraction of the ethanol extract and reference drugs gradually increased as higher concentrations were used. The relaxant effects of all test drugs on the tracheal muscle are summarized in Table 1. The doses of the ethanol extract, terbutaline, aminophylline and papaverine which caused almost the same relaxation (>80%) on histamine-induced tracheal contraction were found to be 2.0 mg/ml, 20.0 ng/ml, 80.0 μg/ml and 2.0 μg/ml, respectively.

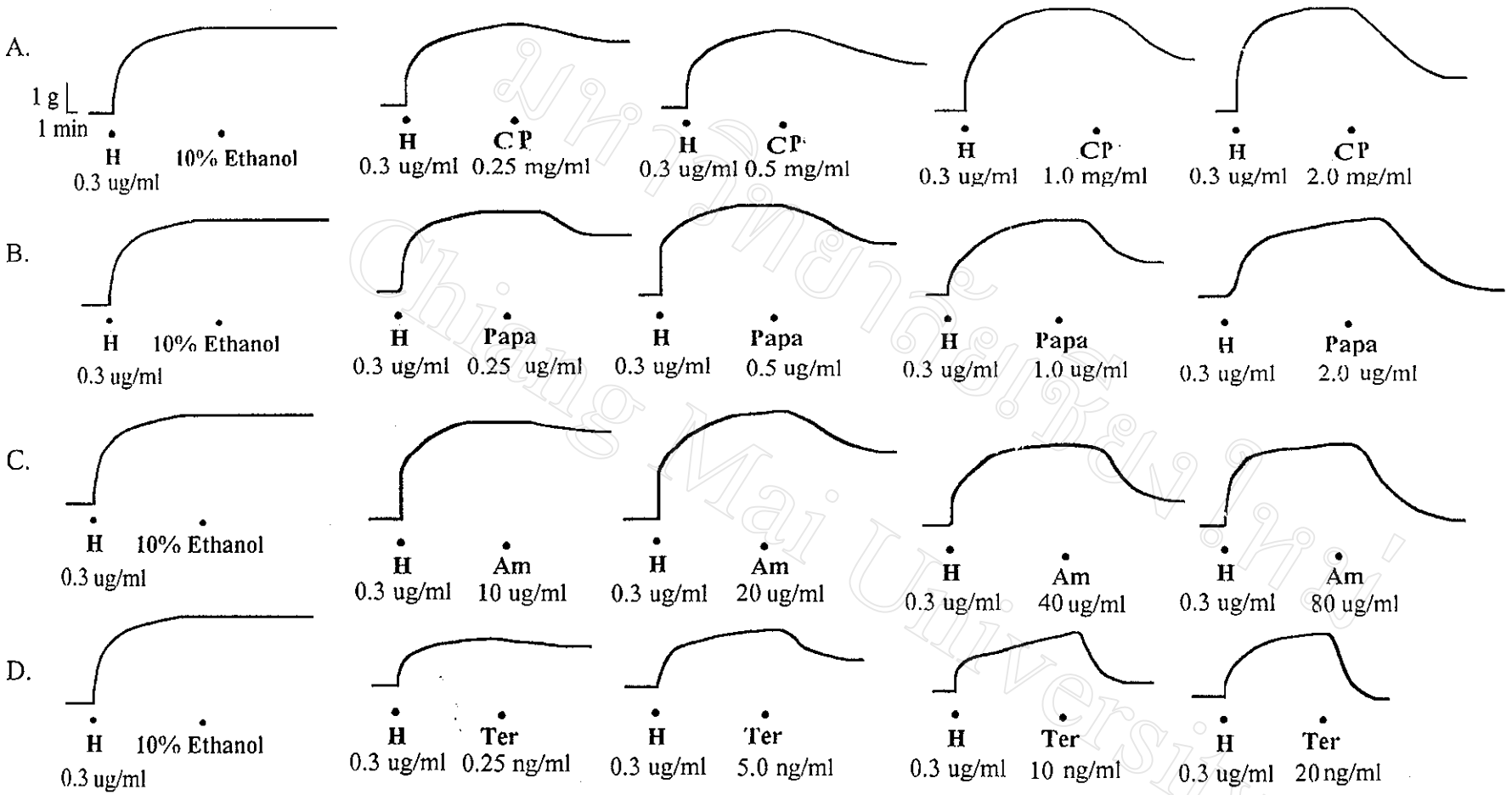


**Figure 5.** Effect of the ethanol extract from *C. petasites* (CP) (panel A), papaverine (Papa) (panel B), aminophylline (Am) (panel C), and terbutaline Ter) (panel D) on histamine (H)-induced contraction of isolated guinea-pig trachealstrip

**Table 1.** Effect of the ethanol extract from *C. petasites* and reference drugs (terbutaline, aminophylline and papaverine) on histamine-induced contraction of isolated guinea-pig tracheal strip

| Drugs | Relaxation (%) |
|---|---|
| Ethanol extract (mg/ml) | |
| 0.25 | 19.77 ± 0.49 |
| 0.50 | 33.69 ± 1.54 |
| 1.00 | 55.32 ± 3.76 |
| 2.00 | 84.56 ± 0.38 |
| Terbutaline (ng/ml) | |
| 2.50 | 25.27 ± 0.94 |
| 5.00 | 51.91 ± 1.21 |
| 10.00 | 80.00 ± 2.04 |
| 20.00 | 100.00 ± 0.00 |
| Aminophylline (μg/ml) | |
| 10.00 | 20.67 ± 0.33 |
| 20.00 | 44.32 ± 1.10 |
| 40.00 | 73.22 ± 1.03 |
| 80.00 | 92.74 ± 1.03 |
| Papaverine (μg/ml) | |
| 0.25 | 35.83 ± 1.02 |
| 0.50 | 50.96 ± 0.97 |
| 1.00 | 78.96 ± 0.71 |
| 2.00 | 98.88 ± 0.66 |

Value expressed as mean ± S.E.M., (n = 4)

Dose of histamine = 0.3 μg/ml

*2. Comparison of the dose-response relationship of the ethanol extract from C. petasites and reference drugs on the histamine-induced tracheal contraction*

The dose-response relationship of the ethanol extract and reference drugs was expressed as a linear regression equation, $Y = a + bX$ and the correlation coefficient (r) value as shown in Figure 6. According to their correlation coefficient values (r), it is suggested that the bronchodilator effect of all test drugs is dose-related ($p<0.05$).

The doses of the ethanol extract, terbutaline, aminophylline and papaverine which caused a 50% relaxation on histamine-induced tracheal contraction ($EC_{50}$) were found to be 0.7, $4.8\times10^{-6}$, $2.3\times10^{-2}$ and $4.2\times10^{-4}$ mg/ml, respectively. Terbutaline was found to be the most potent bronchodilator followed by papaverine, aminophylline and the ethanol extract. A comparison of the slope of the linear regression lines of test drugs was done by employing the method of Tallarida and Murray (1986). It was found that the linear regression line of the dose-response relationship of the ethanol extract was parallel with those of terbutaline, aminophylline and papaverine ($p>0.05$).

*3. Comparison of the bronchodilator effect of the ethanol extract from C. petasites and reference drugs on the histamine-induced tracheal contraction in the presence of propranolol (β-adrenergic antagonist)*

A dose of the ethanol extract (2.0 mg/ml), terbutaline (20.0 ng/ml), aminophylline (80 μg/ml) and papaverine (2.0 μg/ml) which exerted a

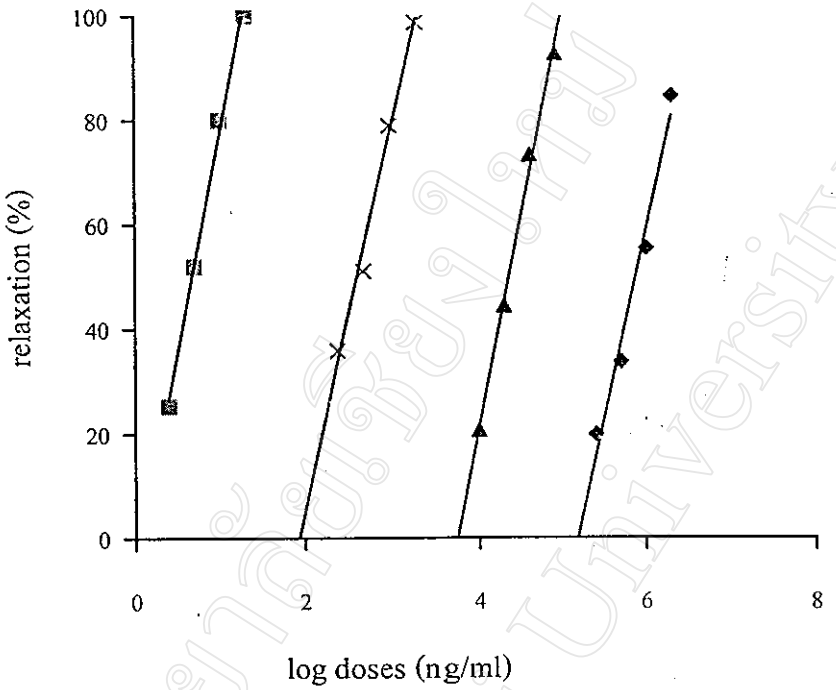


**Figure 6.** Comparison of the dose-response regression lines of the ethanol extract from *C. petasites* ( ◆——◆ ), terbutaline (■——■), aminophylline (▲——▲) and papaverine ( x——x ) on the histamine-induced contraction of isolated guinea-pig tracheal strip. The dose-response regression line was expressed by a linear regression equation of Y = a + bX, and the correlation coefficient (r):

ethanol extract Y = 71.7X – 370.94 r = 0.988 $EC_{50}$ = 0.7 mg/ml

terbutaline Y = 83.8X – 6.91 r = 0.998 $EC_{50} = 4.8 \times 10^{-6}$ mg/ml

aminophylline Y = 81.4X – 304.76 r = 0.998 $EC_{50} = 2.3 \times 10^{-2}$ mg/ml

papaverine Y = 72.1x – 139.41 r = 0.994 $EC_{50} = 4.2 \times 10^{-4}$ mg/ml

maximum relaxation of the histamine-induced contraction was used to evaluate their effect on a $\beta_2$–adrenergic receptor. Determination of the dose of propranolol, a β-adrenergic antagonist, which could effectively block the effect of terbutaline (20.0 ng/ml) was first determined and found to be 0.5 μg/ml.

In the presence of propranolol, the ethanol extract, aminophylline and papaverine still possessed the antagonistic effect on the histamine-induced tracheal contraction (Figure 7). The results obtained, therefore, suggest that the bronchodilator effect of ethanol extract is not exerted via the $\beta_2$-adrenergic receptor stimulation.

*4. Effect of the ethanol extract from C. petasites and reference drugs on acetylcholine-induced contraction of isolated guinea-pig tracheal strip preparation.*

The bronchodilator effect of the ethanol extract was also tested on acetylcholine-induced tracheal contraction. The dose of acetylcholine (0.06 μg/ml) which produced the submaximal contraction was first determined and used to induce tracheal contraction. The ethanol extract and reference drugs (atropine, aminophylline and papaverine) exhibited a significant antagonistic effect on the acetylcholine-induced tracheal contraction (Figure 8). The percent relaxation of acetylcholine-induced tracheal contraction of the ethanol extract and reference drugs as shown in Table 2 gradually increased as higher concentrations were used. The doses which caused maximum relaxation (>80%) of the acetylcholine-induced tracheal contraction of the ethanol extract, atropine, aminophylline

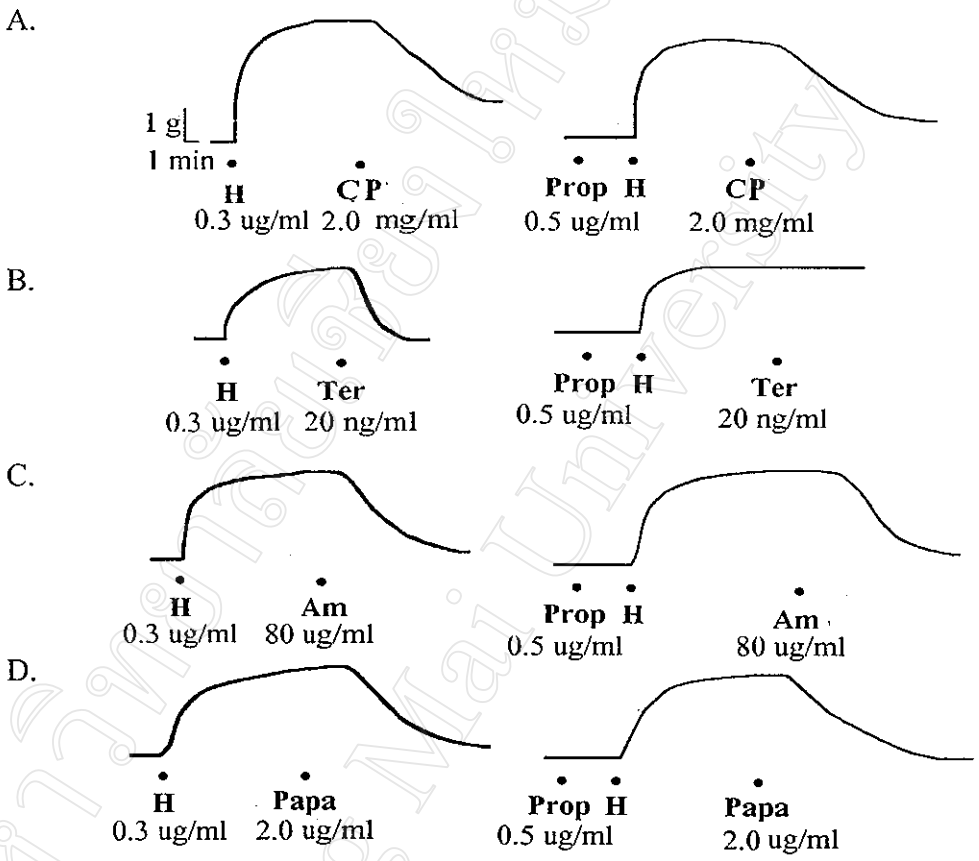


**Figure 7.** Antagonistic effect of the ethanol extract from *C. petasites* (CP) (panel A), terbutaline (Ter) (panel B), aminophylline (Am) (panel C), and papaverine (Papa) (panel D) on histamine (H)-induced contraction of isolated guinea-pig tracheal strip in the presence of propranolol (β-adrenergic antagonist) (Prop)

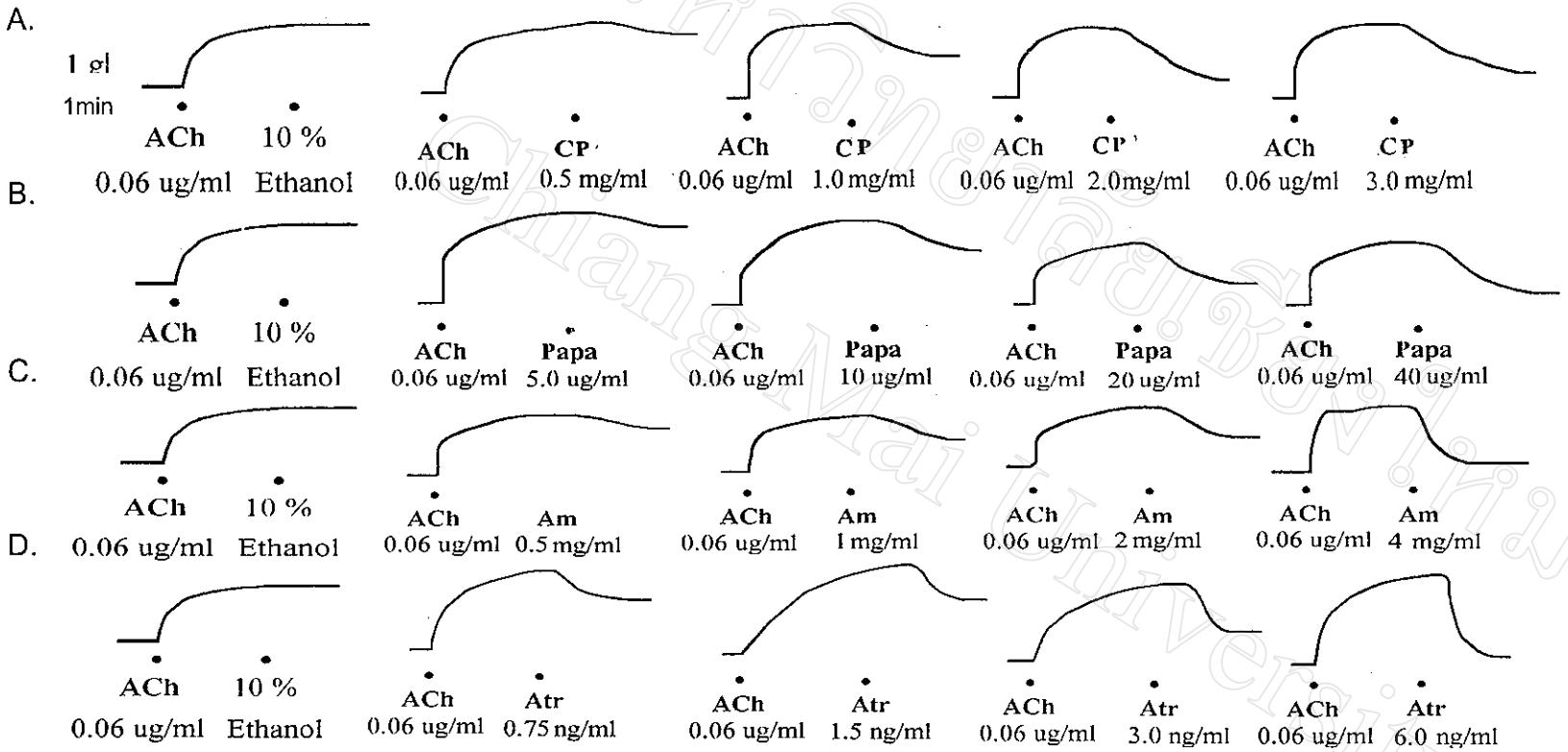


**Figure 8.** Effect of the the ethanal extract from *C. petasites* (CP) (panel A), papaverine (Papa) (panel B), aminophylline (Am) (panel C), and atropine (Atr) (panel D) on acetylcholine (ACh)-induced contraction of isolated guinea-pig tracheal strip

**Table 2.** Effect of the ethanol extract from *C. petasites* and reference drugs (atropine, aminophylline and papaverine) on acetylcholine-induced contraction of isolated guinea-pig tracheal strip

| Drugs | Relaxation (%) |
|---|---|
| Ethanol extract (mg/ml) | |
| 0.50 | 35.66 ± 1.87 |
| 1.00 | 49.51 ± 3.59 |
| 2.00 | 78.01 ± 0.46 |
| 3.00 | 87.16 ± 0.42 |
| Atropine (ng/ml) | |
| 0.75 | 13.60 ± 0.43 |
| 1.50 | 24.73 ± 0.47 |
| 3.00 | 50.69 ± 1.02 |
| 6.00 | 97.95 ± 0.73 |
| Aminophylline (mg/ml) | |
| 0.50 | 24.83 ± 0.48 |
| 1.00 | 42.05 ± 1.00 |
| 2.00 | 66.46 ± 1.54 |
| 4.00 | 98.00 ± 2.00 |
| Papaverine (μg/ml) | |
| 5.00 | 24.91 ± 1.07 |
| 10.00 | 33.53 ± 1.51 |
| 20.00 | 74.16 ± 3.79 |
| 40.00 | 93.82 ± 3.81 |

Value expressed as mean ± S.E.M., (n = 4)

Dose of acetylcholine = 0.06 μg/ml

and papaverine were found to be 3.0 mg/ml, 6.0 ng/ml, 4.0 mg/ml and 40 μg/ml, respectively.

*5. Comparison of the dose-response relationship of the ethanol extract from C. petasites and reference drugs on the acetylcholine-induced tracheal contraction*

The dose-response relationship of the ethanol extract and reference drugs on acetylcholine-induced tracheal contraction was evaluated similarly to that on the histamine-induced tracheal contraction. The linear regression lines of the dose-response relationship of ethanol extract and reference drugs were determined and graphically illustrated according to their regression equations in Figure 9.

According to their correlation coefficient values (r), it is suggested that the antagonistic effect of all test drugs is dose-related. By comparison of the $EC_{50}$ values of the test drugs on acetylcholine-induced tracheal contraction, atropine was found to be the most potent bronchodilator followed by papaverine, ethanol extract and aminophylline, of which $EC_{50}$ values were $2.3\times10^{-6}$, $1.2\times10^{-2}$, 0.9 and 1.1 mg/ml, respectively.

A comparison of the slope of linear regression lines of test drugs was done by employing the method of Tallarida and Murray (1986). It was found that the linear regression line of the dose-response relationship of the ethanol extract was parallel with those of atropine, aminophylline and papaverine ($p>0.05$).

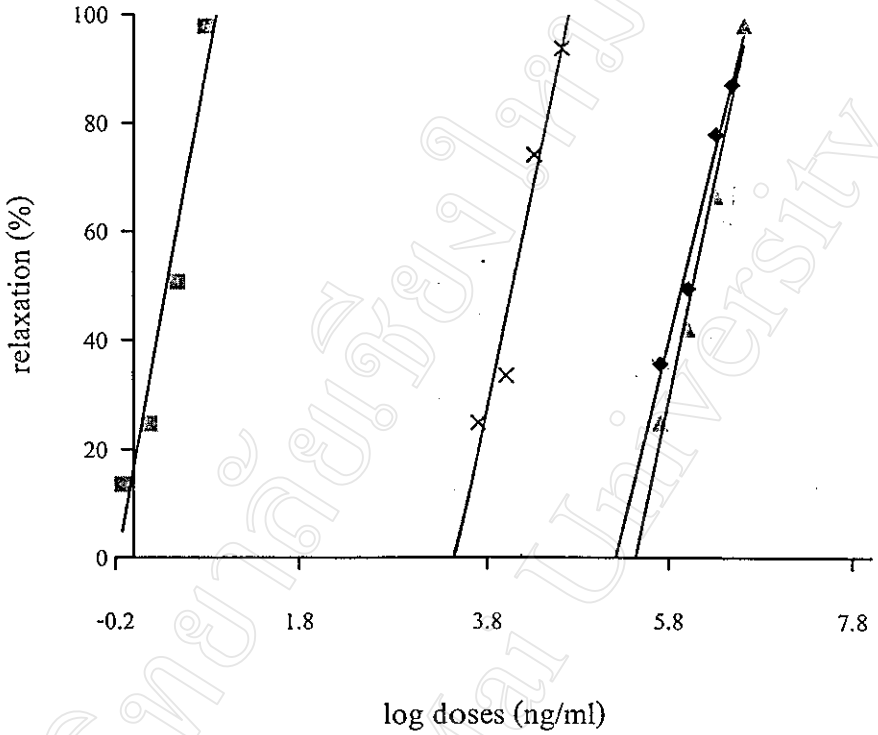


**Figure 9.** Comparison of the dose-response regression lines of the ethanol extract from *C. petasites* ( ◆——◆ ) atropine, ( ■——■ ), aminophylline ( ▲——▲ ) and papaverine ( ×——× ) on the acetylcholine-induced contraction of isolated guinea-pig tracheal strip. The dose-response regression line was expressed by a linear regression equation of Y = a + bX, and the correlation coefficient (r):

ethanol extract $Y = 65.6X - 362.98$ $r = 0.990$ $EC_{50} = 0.9$ mg/ml

atropine $Y = 92.7X + 16.48$ $r = 0.960$ $EC_{50} = 2.3 \times 10^{-6}$ mg/ml

aminophylline $Y = 81.0X - 440.58$ $r = 0.992$ $EC_{50} = 1.1$ mg/ml

papaverine $Y = 82.2X - 284.48$ $r = 0.973$ $EC_{50} = 1.2 \times 10^{-2}$ mg/ml

6. *Comparison of the protective effect of the ethanol extract from C. petasites and atropine (antimuscarinic agents) on the acetylcholine-induced tracheal contraction*

The doses of the ethanol extract (3.0 mg/ml) and atropine (6.0 ng/ml) which produced a maximum relaxation of acetylcholine-induced tracheal contraction were used. The results obtained, as demonstrated in Figure 10, show that the ethanol extract could not inhibit the effect of acetylcholine-induced tracheal contraction. Atropine, an antimuscarinic agent, completely blocked the effect of acetylcholine-induced contraction. It is suggested that the bronchodilator effect of the ethanol extract is not exerted via muscarinic receptors.

**B. Experiments *in vivo***

*1. Effect of the ethanol extract from C. petasites and reference drugs on histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pig*

The dose of histamine which produced a maximum increase in intratracheal pressure which represents bronchoconstriction was first determined and found to be 8 μg/kg. The first dose of histamine, given intravenously prior to the administration of the test drugs, was used as a control value for determination of the antagonistic effect of test drugs as shown in Figure 11. Figure 11B shows the antagonistic effect of chlorpheniramine, an $H_1$ receptor antagonist, on histamine-induced. It was found that chlorpheniramine in a dose of 25 μg/kg could completely block

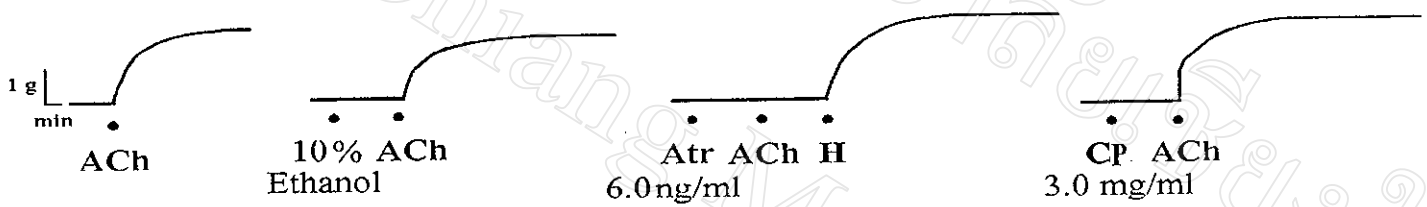


**Figure 10.** Antagonistic effect of the ethanol extract from *C. petasites* (CP) and atropine (Atr) on acetylcholine (ACh)-induced contraction of isolated guinea-pig tracheal strip

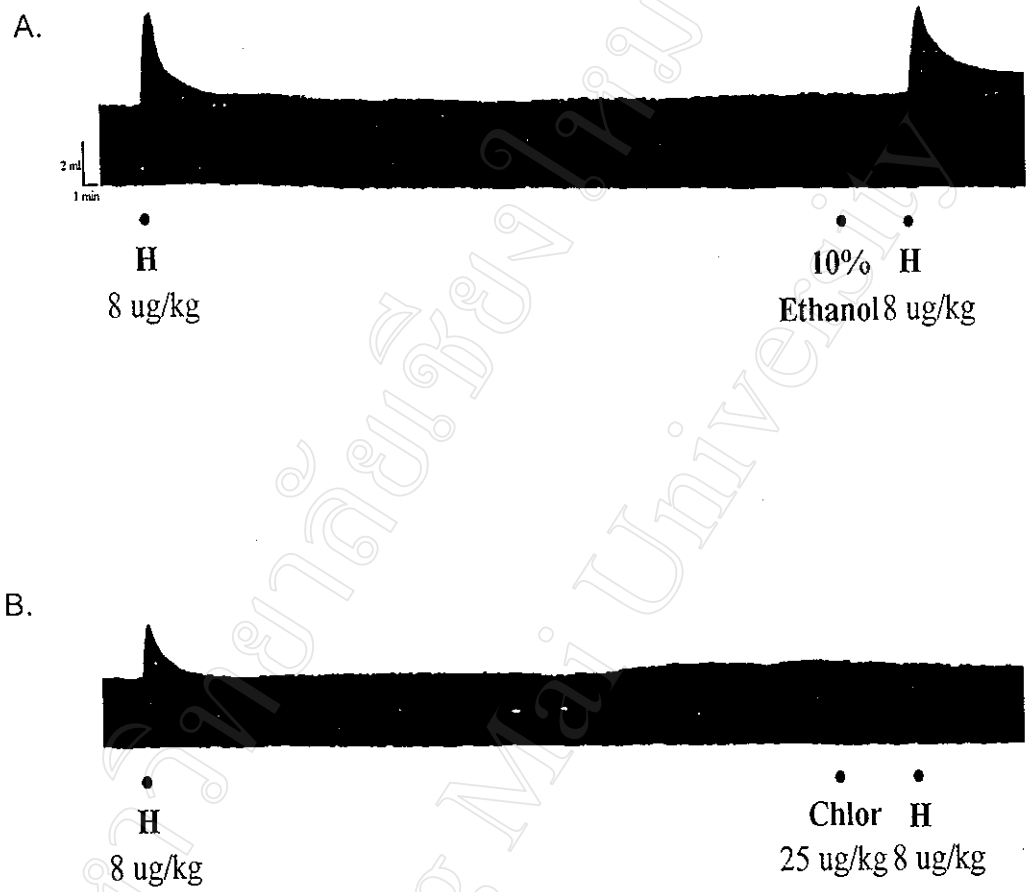


**Figure 11.** Increase in intratracheal pressure in response to the first and the second doses of histamine (A) and the blocking effect of chlorpheniramine (Chlor) on histamine (H)-induced bronchonstriction (B) in pentobarbital anesthetized guinea-pigs

the effect of histamine. Test drugs were given 2 min before the administration of the second dose of histamine, which was given 20 minutes after the first one. The antagonistic effect of the ethanol extract and reference drugs were linearly related to the dose as shown in Figure 12, 13, 14 and 15 as well as in Table 3. The doses of the ethanol extract, terbutaline, aminophylline and papaverine which caused maximum inhibition of a histamine-induced bronchoconstriction were found to be 320 mg/kg, 2.5 μg/kg, 80 mg/kg and 20 mg/kg, respectively.

*2. Comparison of the dose-response regression lines and the effective doses of the ethanol extract from C. petasites and reference drugs on the histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs*

The dose-response relationship of the antagonistic effect in terms of peak height intratracheal pressure response (PIPR) was expressed by a linear regression equations, $Y = a + bX$ and the correlation coefficient value (r), as shown in Figure 16. The peak height intratracheal pressure response was determined from the difference between the peak response value and the basal resting value of each dose of histamine. The marked positive correlation coefficient values ($r = 0.999$, 0.993, 0.971 and 0.996 for the ethanol extract, terbutaline, aminophylline and papaverine, respectively) suggest that the antagonistic effect of the ethanol extract and reference drugs on the histamine-induced bronchoconstriction in term of PIPR is dose-related ($p < 0.05$).

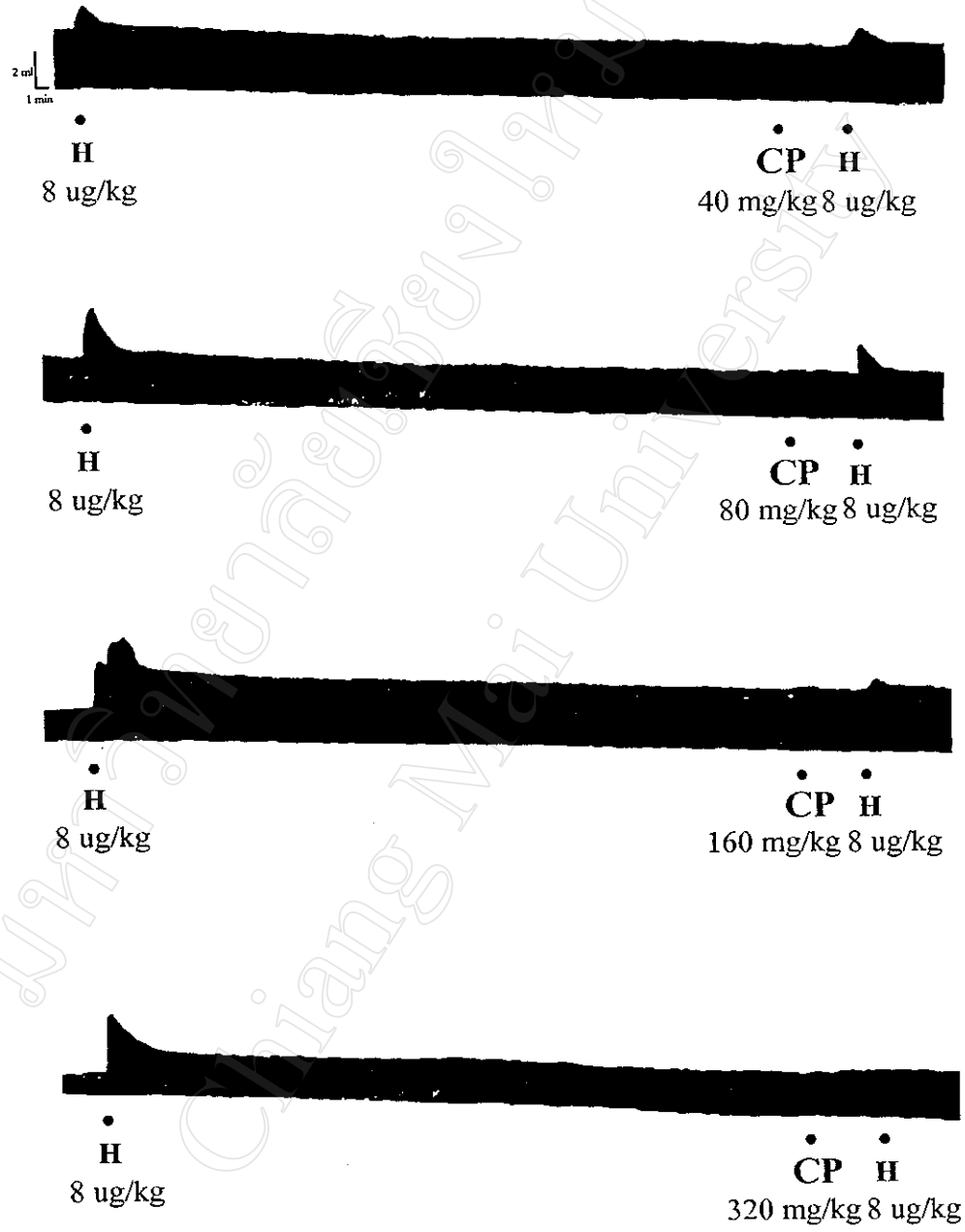


**Figure 12**. Effect of the ethanol extract from *C. petasites* (CP) on the histamine (H)-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs

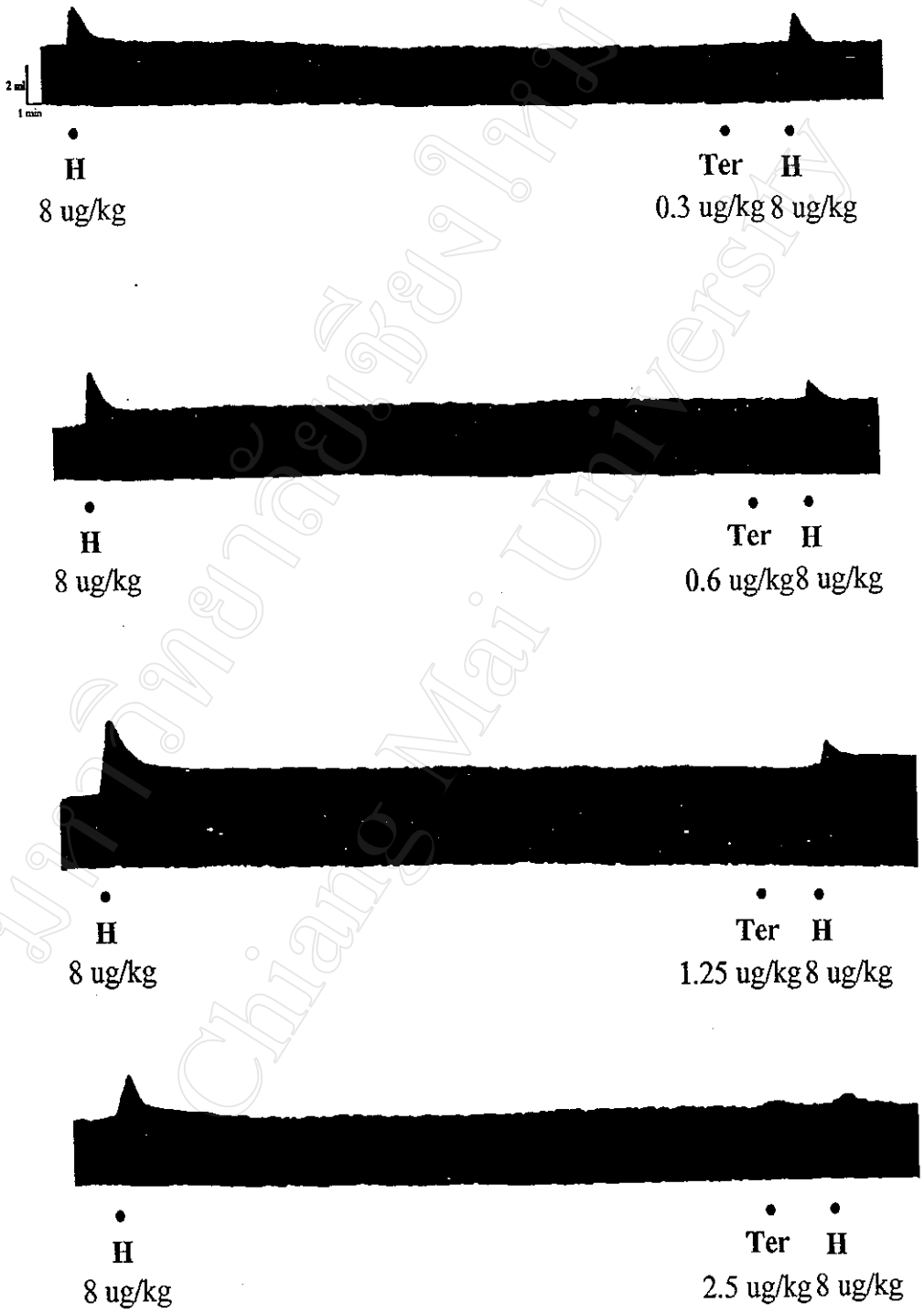


**Figure 13.** Effect of terbutaline (Ter) on the histamine (H)-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs

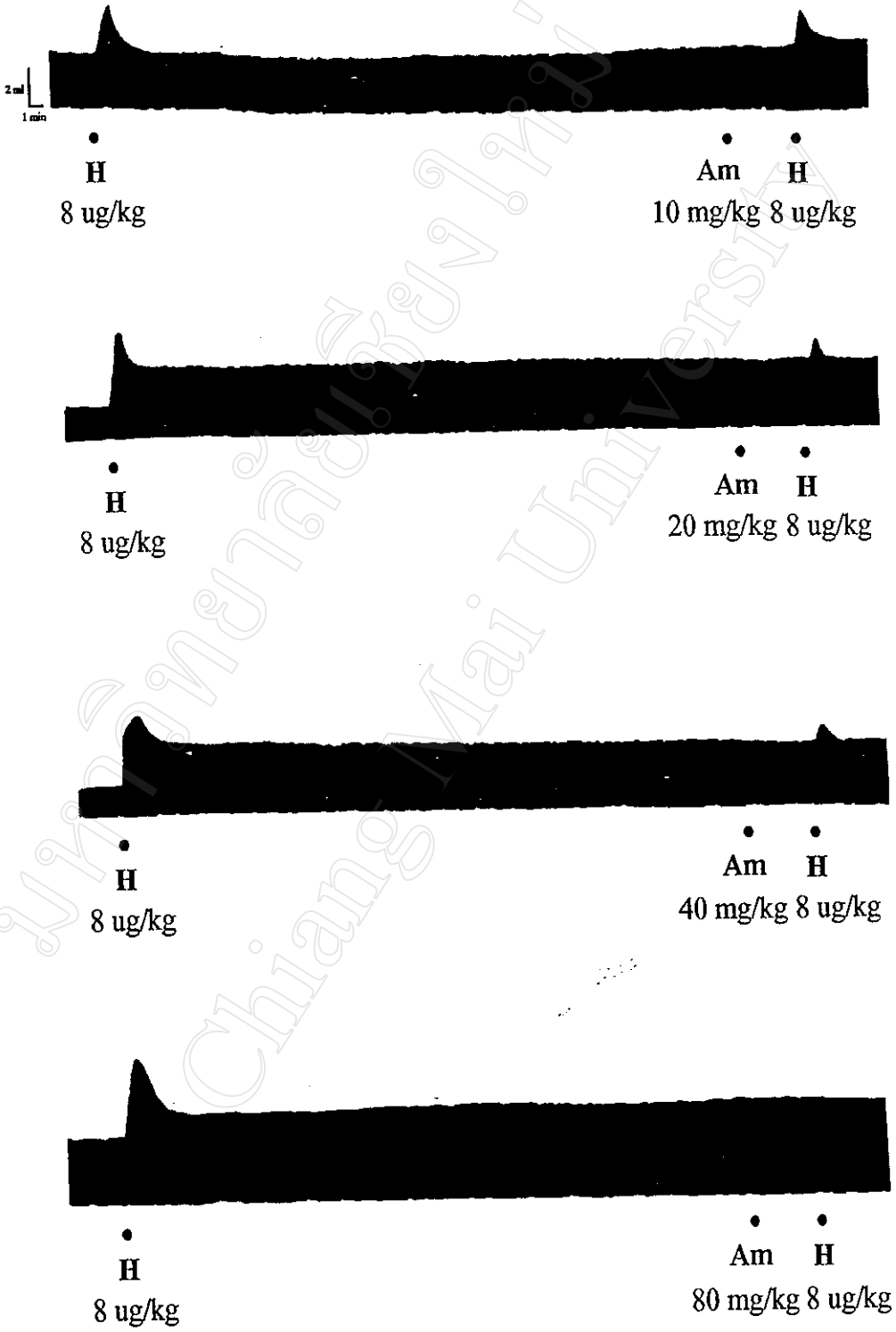


**Figure 14.** Effect of aminophylline (Am) on the histamine (H)-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs

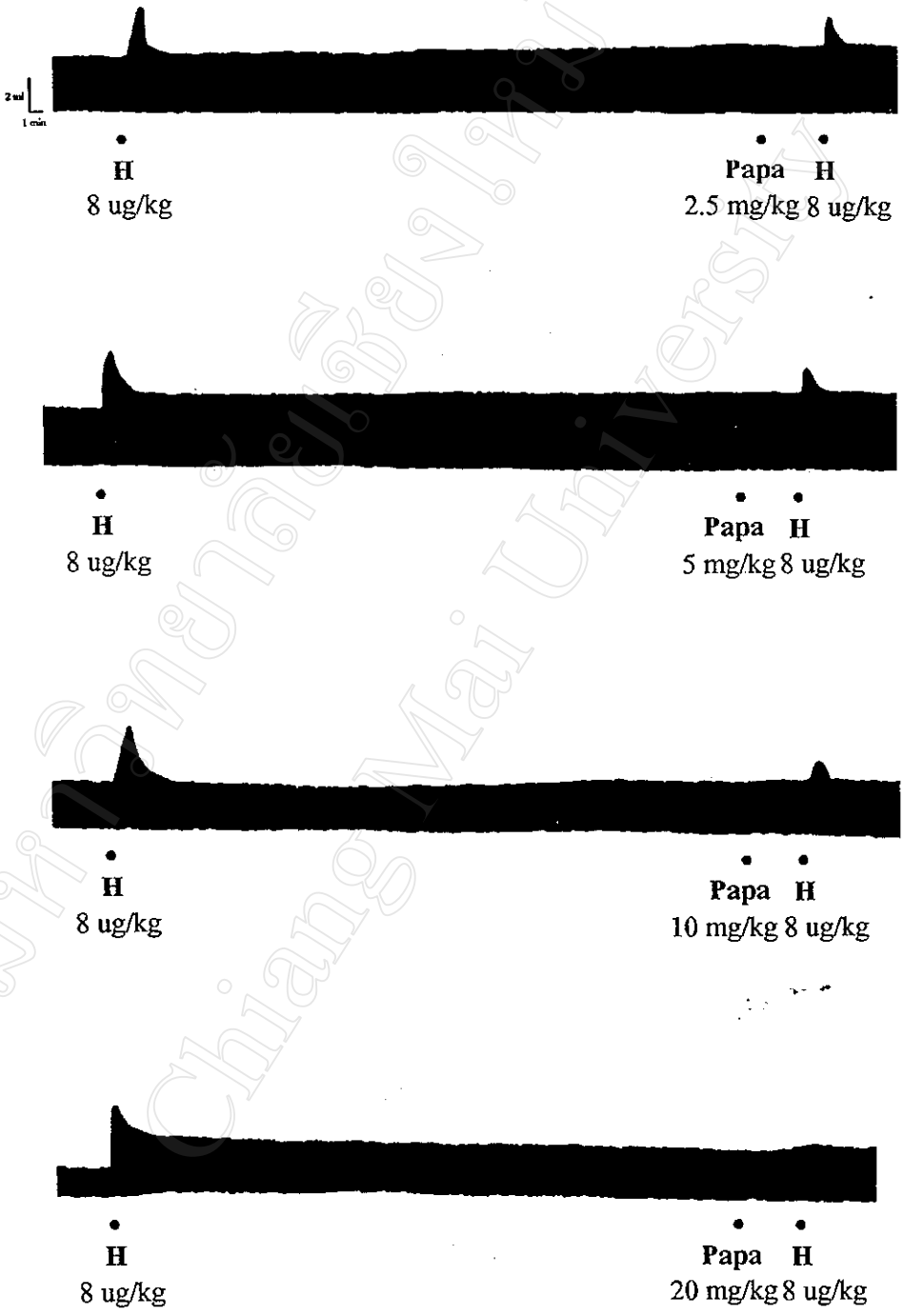


**Figure 15.** Effect of papaverine (Papa) on the histamine (H)-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs

**Table 3.** Effect of ethanol extract from *C. petasites* and reference drugs (terbutaline, aminophylline and papaverine) on histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs

| Drugs | Inhibition (%) |
|---|---|
| Ethanol extract (mg/kg) | |
| 40.00 | 15.38 ± 2.32 |
| 80.00 | 46.43 ± 2.06 |
| 160.00 | 74.53 ± 3.31 |
| 320.00 | 100.00 ± 0.00 |
| Terbutaline (ng/kg) | |
| 0.30 | 23.37 ± 1.25 |
| 0.60 | 40.81 ± 1.29 |
| 1.25 | 60.80 ± 2.71 |
| 2.50 | 88.75 ± 6.58 |
| Aminophylline (mg/kg) | |
| 10.00 | 30.95 ± 1.38 |
| 20.00 | 41.78 ± 1.29 |
| 40.00 | 63.75 ± 2.76 |
| 80.00 | 100.00 ± 0.00 |
| Papaverine (mg/kg) | |
| 2.50 | 16.31 ± 1.35 |
| 5.00 | 49.36 ± 2.42 |
| 10.00 | 68.90 ± 2.73 |
| 20.00 | 97.22 ± 2.78 |

Value expressed as mean ± S.E.M., (n = 4)

Dose of histamine = 8.0 μg/kg

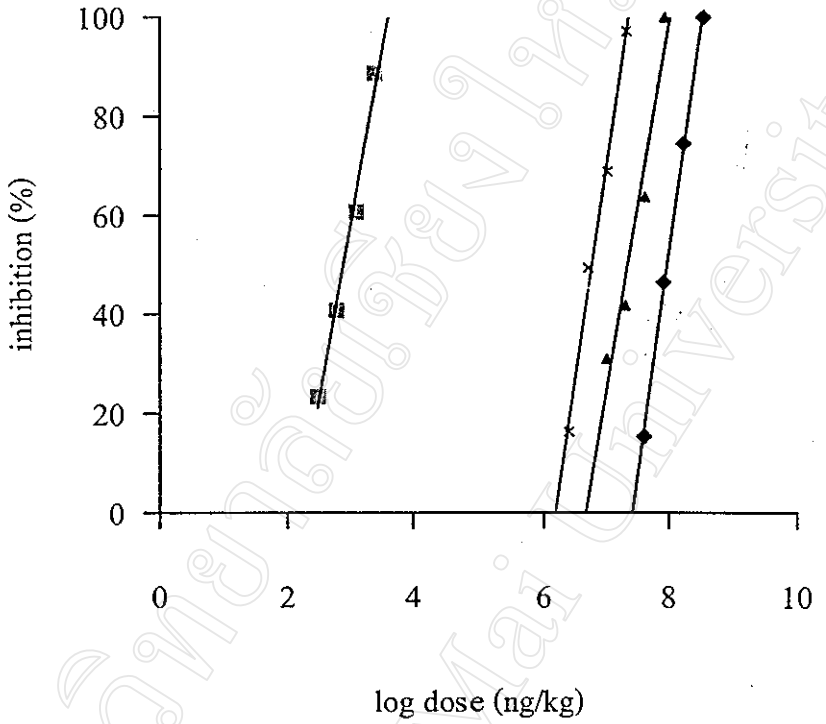


**Figure 16.** Comparison of the dose-response regression lines of the ethanol extract from *C. petasites* ( ◆——◆ ), terbutaline ( ■——■ ), aminophylline ( ▲——▲ ) and papaverine ( ×——× ) on the histamine-induced bronchoconstriction in pentobarbital anesthetized guinea-pigs. The dose-response regression line was expressed by a linear regression equation of Y = a + bX, and the correlation coefficient (r):

ethanol extract Y = 94.0X – 518.93 r = 0.999 $ED_{50}$ = 90.5 mg/kg

terbutaline Y = 70.1X – 152.77 r = 0.993 $ED_{50} = 7.7 \times 10^{-4}$ mg/kg

aminophylline Y = 76.4X – 509.86 r = 0.971 $ED_{50}$ = 21.5 mg/kg

papaverine Y = 87.4X – 540.90 r = 0.996 $ED_{50}$ = 5.7 mg/kg

The order of the antagonistic potency of these test drugs, in term of PIPR, from high to low is as follows; terbutaline, papaverine, aminophylline and the ethanol extract, of which the $ED_{50}$ values were found to be $7.7\times10^{-4}$, 5.7, 21.5 and 90.5 mg/kg, respectively.

The parallelism between dose-response curve of the ethanol extract and reference drugs was analyzed by the method of Tallarida and Murray (1986), and it was found that the dose-response curve of the ethanol extract, in term PIPR, was parallel with those of terbutaline, aminophylline and papaverine ($p>0.05$).

*3. Effect of the ethanol extract from C. petasites and reference drugs on the cumulative dose of methacholine-induced broncoconstriction in pentobarbital anesthetized rats*

The first cumulative doses of MeCh challenged set (1.5, 3 and 4.5 μg) were given intravenously to test the sensitivity of the animals. The second set (3, 6 and 9 μg) was administration similary to the first set in order to determined the antagonistic effect of the test drugs which were pretreated 2 min before. Figure 17A illustrates the increase in intratracheal pressure which representing bronchoconstriction in the response to the first and second set of cumulative MeCh-dose. Peak height intratracheal pressure response (PIRR) was determined from the difference between the peak response value and the basal resting value of each single dose.

Figure 17B shows the blocking effect of atropine on the second set of cumulative MeCh-induced bronchoconstriction. It was found that the complete blockade (100%) on the bronchoconstriction induced by MeCh

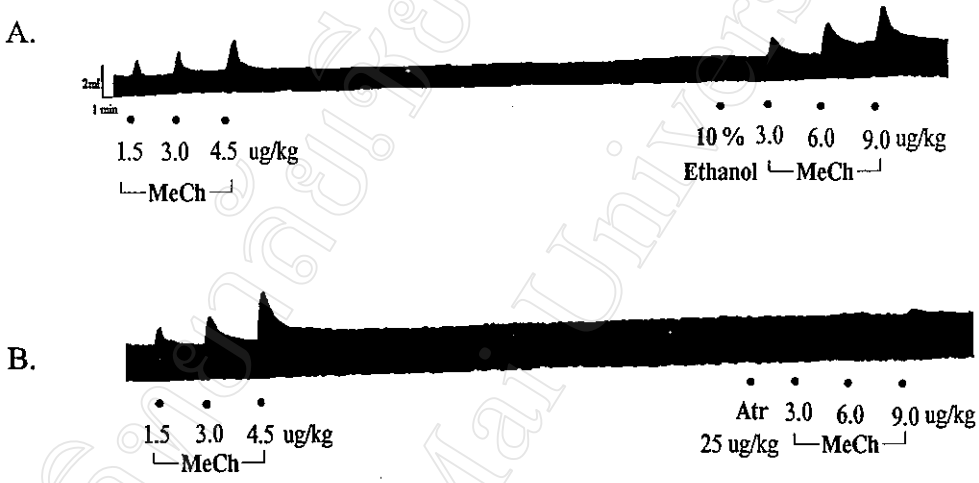


**Figure 17.** Increase in intratracheal pressure in response to the first set (1.5, 3, 4.5 μg) and the second set cumulative doses (3, 6, 9 μg) of methacholine (MeCh) (A) and the blocking effect of atropine (Atr) on the second cumulative doses of methacholine-induced bronchonstriction (B) in pentobarbital anesthetized rats

was seen when 25 μg/kg of atropine was used. The antagonistic effect of test drugs was determined by comparing the PIPR in the response to the second set of MeCh-induced between drug-treated group and control group (received 10% ethanol).

Five groups of animals were used for testing of antagonistic effect of various doses of the ethanol extract on cumulative MeCh-induced bronchoconstriction. It was found that all groups of animals exhibited similar response to the first set of MeCh-doses in term of PIPR. In other experiments using reference drugs (terbutaline, aminophylline and papaverine), each group of animals exhibited the response to the first set MeCh-dose similarly to the groups of animals in experiments with the ethanol extract.

The ethanol extract at the dose of 40, 80, 160 and 320 mg/kg caused a statistically significant inhibition of dose-related MeCh-induced increase in intratracheal pressure when given intravenously 2 min before the second set of MeCh challenges (Figure 18, Table 4). The inhibitory effect, in term of PIPR, on each dose of cumulative MeCh-dose was found to depend on the doses of the ethanol extract used, as shown in Table 4.

Pretreatment of the animals with various doses of reference drugs 2 min before the administration of the second set of MeCh-doses could also effectively inhibit MeCh-induced bronchoconstriction. Figure 19 and Table 5 show antagonistic effect of terbutaline on cumulative MeCh-induced bronchoconstriction. The inhibitory effect of terbutaline on MeCh-induced bronchoconstriction was found to depend on its doses used. Similary, other reference drugs, aminophylline and papaverine could

effectively block the bronchoconstriction induced by cumulative MeCh-doses as shown in Figure 20 and Table 6, as well as Figure 21 and Table 7, respectively. Their inhibitory effects were also found to depend on their doses used. At the dose of 80 mg/kg, aminophylline completely blocked the bronchoconstriction induced by the first dose (3 μg) of the second set of MeCh-doses.

*4. Hippocratic screening test*

The changes in general behaviour of the rats were observed following intraperitoneal injection of the ethanol extract from *C. petasites* at the doses of 2,000 mg/kg, 2,500 mg/kg, 3,000 mg/kg, 4,000 mg/kg and 5,000 mg/kg. Five doses of ethanol extract were given to the rats by an intraperitoneal route. Table 8 shows the signs and symptoms observed at 5, 15, 30 and 60 min; 2, 4, and6 h after the ethanol extract administration. The dose of 2,000 mg/kg did not cause any detectable changes, whereas the dose of 5,000 mg/kg was found to be a lethal dose causing death of one rat within 2 h and two rats within 24 h and 2 days, (n = 4). Before death the rat showed signs of respiratory arrest and cyanosis. The autopsy revealed that the internal organs i.e. liver, lungs, spleen and gastro-intestinal tract were normal in both size and colour and did not show any unusual signs.

Seven days after dosing, all alive animals were sacrificed and necropsied. The internal organs were gross examined and found to be normal in both size and colour and did not show any unusual signs.

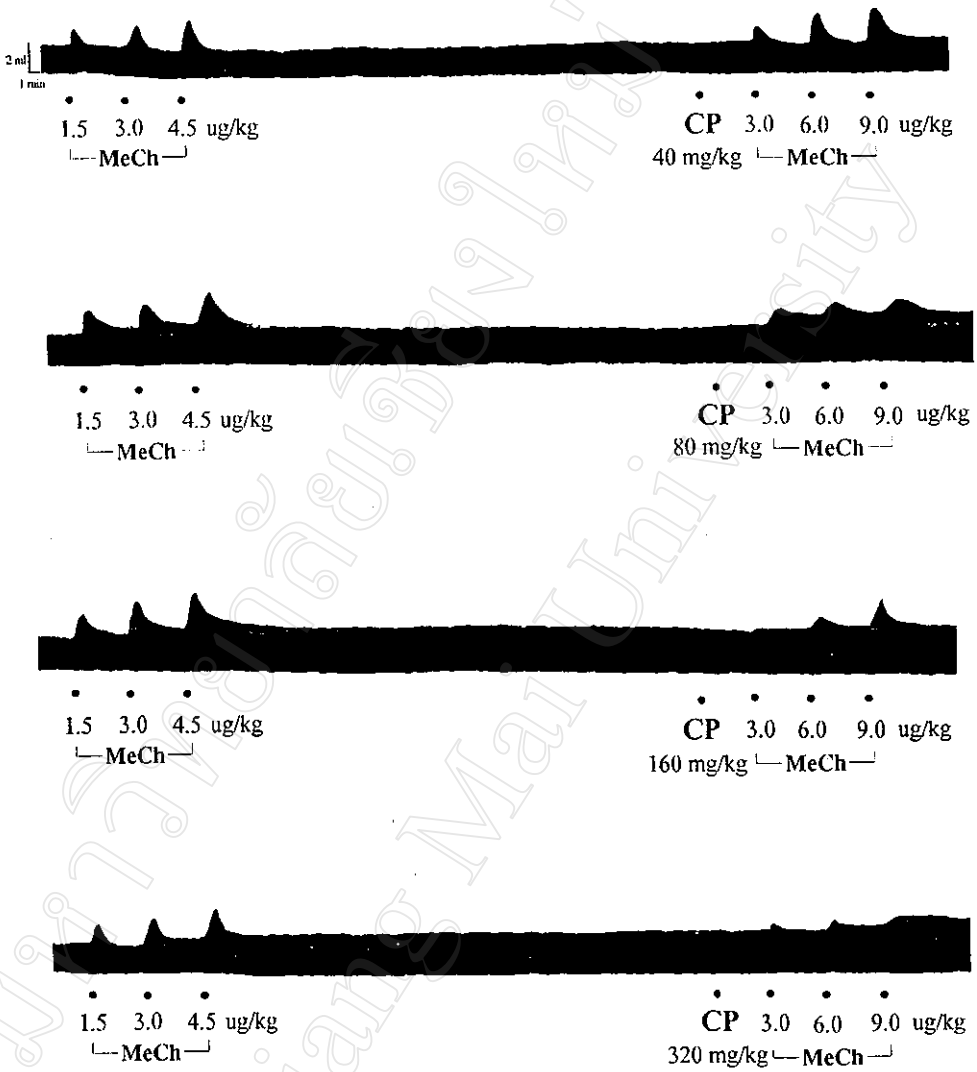


**Figure 18.** Effect of the ethanol extract from *C. petasites* (CP) on the dose-related methacholine (MeCh)-induced brochoconstriction in pentobarbital anesthetized rats

**Table 4.** Effect of the ethanol extract from *C. petasites* on the cumulative doses of methacholine-induced bronchoconstriction in pentobarbital anesthetized rats

| MeCh Doses (μg/kg) | PIPR (mm) | | | | |
|---|---|---|---|---|---|
| | Control | Ethanol extract (mg/kg) | | | |
| | 10% ethanol | 40 | 80 | 160 | 320 |
| First set | | | | | |
| 1.5 | 3.13 ± 0.43 | 3.00 ± 0.14 | 3.50 ± 0.20 | 3.88 ± 0.32 | 3.33 ± 0.51 |
| 3.0 | 5.00 ± 0.41 | 4.25 ± 0.48 | 4.63 ± 0.24 | 5.88 ± 0.31 | 4.25 ± 0.48 |
| 4.5 | 6.75 ± 0.33 | 6.18 ± 0.33 | 6.25 ± 0.25 | 7.00 ± 0.46 | 6.00 ± 0.62 |
| Second set | | | | | |
| 3.0 | 3.38 ± 0.25 | 2.50 ± 0.13* | 2.38 ± 0.14* | 1.00 ± 0.20* | 0.63 ± 0.23* |
| 6.0 | 5.75 ± 0.32 | 4.38 ± 0.24* | 3.75 ± 0.14* | 2.38 ± 0.23* | 1.75 ± 0.14* |
| 9.0 | 7.38 ± 0.26 | 6.25 ± 0.25* | 4.63 ± 0.22* | 4.75 ± 0.14* | 2.13 ± 0.32* |

The ethanol extract was given 2 min before the second set of cumulative doses of MeCh.

Mean ± S.E.M from 4 rats in each group are given. Statistical significance refers to difference from the control group: * $p<0.05$.

PIPR: peak height of the intratracheal pressure response.

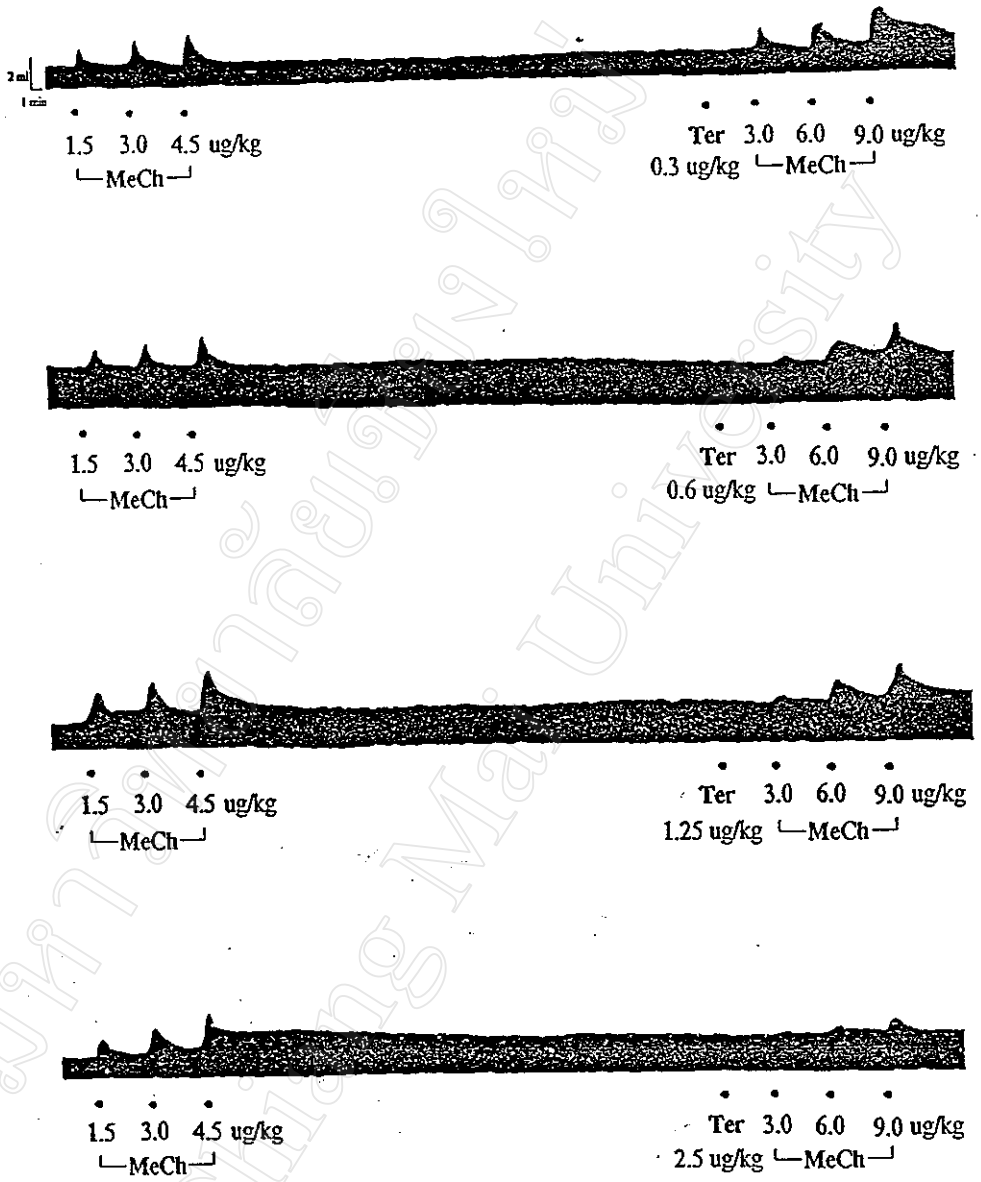


**Figure 19.** Effect of terbutaline (Ter) on the dose-related methacholine (MeCh)-induced brochoconstriction in pentobarbital anesthetized rats

**Table 5.** Effect of the terbutaline on the cumulative doses of methacholine (MeCh)-induced bronchoconstriction in pentobarbital anesthetized rats

| MeCh Doses (μg/kg) | PIPR (mm) | | | | |
|---|---|---|---|---|---|
| | Control | Terbutaline (μg/kg) | | | |
| | 10% ethanol | 0.30 | 0.60 | 1.25 | 2.50 |
| First set | | | | | |
| 1.5 | 3.13 ± 0.43 | 2.45 ± 0.21 | 2.65 ± 0.22 | 3.00 ± 0.20 | 2.88 ± 0.13 |
| 3.0 | 5.00 ± 0.41 | 4.00 ± 0.20 | 4.25 ± 0.25 | 5.05 ± 0.27 | 4.63 ± 0.31 |
| 4.5 | 6.75 ± 0.33 | 6.51 ± 0.12 | 6.20 ± 0.24 | 6.89 ± 0.26 | 6.12 ± 0.28 |
| Second set | | | | | |
| 3.0 | 3.38 ± 0.25 | 2.32 ± 0.18* | 1.50 ± 0.29* | 0.50 ± 0.29* | 0.13 ± 0.13* |
| 6.0 | 5.75 ± 0.32 | 4.25 ± 0.14* | 3.13 ± 0.31* | 3.25 ± 0.25* | 1.50 ± 0.20* |
| 9.0 | 7.38 ± 0.26 | 6.38 ± 0.20* | 6.13 ± 0.33* | 5.13 ± 0.31* | 2.88 ± 0.43* |

Terbutaline was given 2 min before the second set of cumulative doses of MeCh.

Mean ± S.E.M from 4 rats in each group are given. Statistical significance refers to difference from the control group: * $p<0.05$.

PIPR: peak height of the intratracheal pressure response.

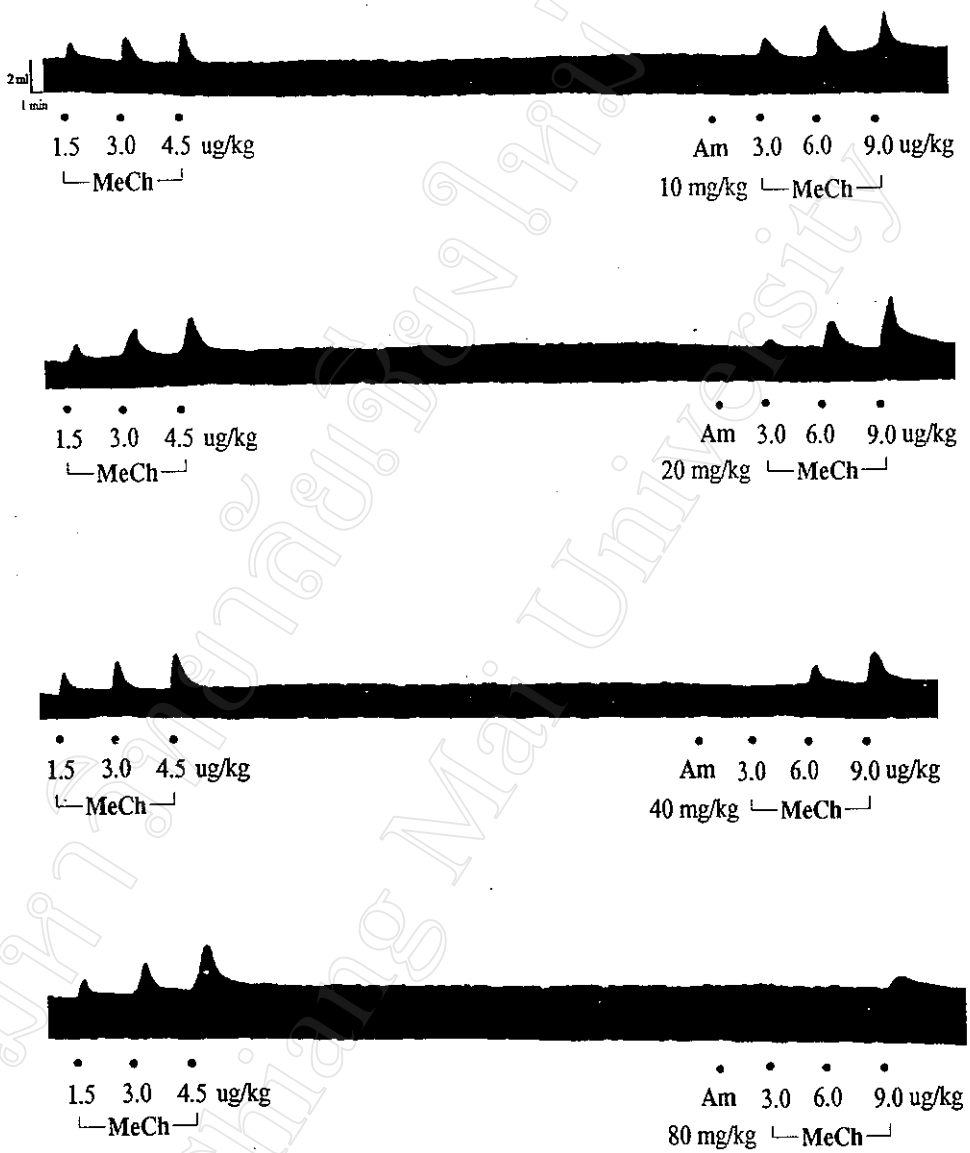


**Figure 20.** Effect of aminophylline (Am) on the dose-related methacholine (MeCh)-induced brochoconstriction in pentobarbital anesthetized rats

**Table 6.** Effect of the aminophylline on the cumulative doses of methacholine (MeCh)-induced bronchoconstriction in pentobarbital anesthetized rats

| MeCh Doses (μg/kg) | PIPR (mn) | | | | |
|---|---|---|---|---|---|
| | Control | Aminophylline (mg/kg) | | | |
| | 10% ethanol | 10 | 20 | 40 | 80 |
| First set | | | | | |
| 1.5 | 3.13 ± 0.43 | 2.68 ± 0.13 | 2.75 ± 0.14 | 3.00 ± 0.20 | 2.80 ± 0.12 |
| 3.0 | 5.00 ± 0.41 | 4.38 ± 0.31 | 4.70 ± 0.24 | 4.82 ± 0.11 | 4.61 ± 0.22 |
| 4.5 | 6.75 ± 0.33 | 6.70 ± 0.24 | 6.25 ± 0.32 | 6.66 ± 0.25 | 7.42 ± 0.26 |
| Second set | | | | | |
| 3.0 | 3.38 ± 0.25 | 2.50 ± 0.29 | 1.63 ± 0.23* | 0.50 ± 0.20* | 0.00 ± 0.00* |
| 6.0 | 5.75 ± 0.32 | 4.50 ± 0.24* | 4.25 ± 0.32* | 2.63 ± 0.24* | 0.50 ± 0.20* |
| 9.0 | 7.38 ± 0.26 | 6.29 ± 0.18* | 6.25 ± 0.25* | 4.63 ± 0.27* | 2.25 ± 0.25* |

Aminophylline was given 2 min before the second set of cumulative doses of MeCh.

Mean ± S.E.M from 4 rats in each group are given. Statistical significance refers to difference from the control group: * $p<0.05$.

PIPR: peak height of the intratracheal pressure response.

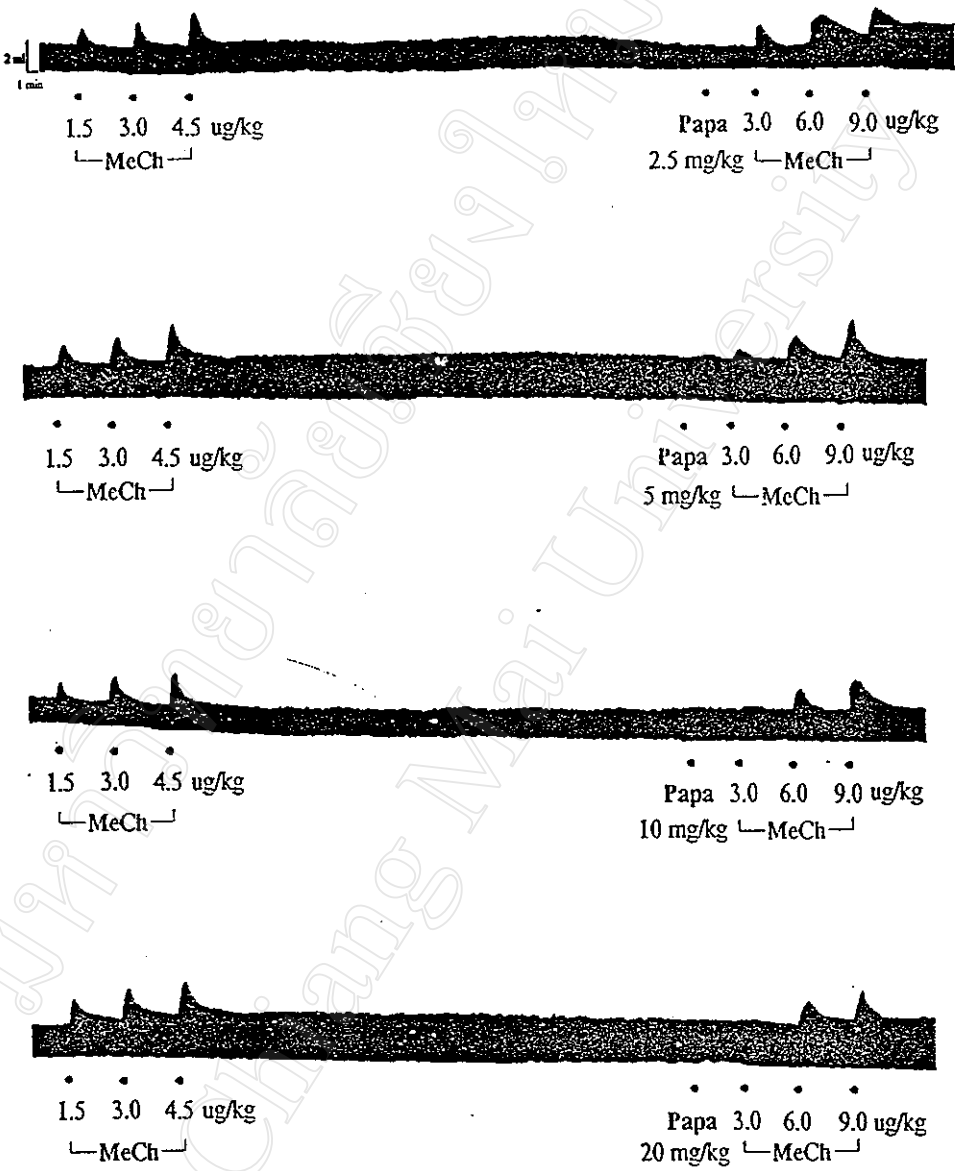


**Figure 21.** Effect of papaverine (Papa) on the dose-related methacholine-induced brochoconstriction in pentobarbital anesthetized rats

**Table 7.** Effect of the papaverine on the cumulative doses of methacholine (MeCh)-induced bronchoconstriction in pentobarbital anesthetized rats

| MeCh Doses (μg/kg) | PIPR (mm) | | | | |
|---|---|---|---|---|---|
| | Control | Papaverine (mg/kg) | | | |
| | 10% ethanol | 2.5 | 5 | 10 | 20 |
| First set | | | | | |
| 1.5 | 3.13 ± 0.43 | 3.00 ± 0.22 | 3.86 ± 0.19 | 2.88 ± 0.31 | 3.64 ± 0.34 |
| 3.0 | 5.00 ± 0.41 | 4.32 ± 0.14 | 4.50 ± 0.20 | 4.28 ± 0.24 | 4.88 ± 0.13 |
| 4.5 | 6.75 ± 0.33 | 6.35 ± 0.32 | 6.66 ± 0.27 | 5.98 ± 0.33 | 6.63 ± 0.24 |
| Second set | | | | | |
| 3.0 | 3.38 ± 0.25 | 2.50 ± 0.14* | 2.13 ± 0.31* | 1.25 ± 0.24* | 0.75 ± 0.32* |
| 6.0 | 5.75 ± 0.32 | 5.38 ± 0.23 | 4.13 ± 0.32* | 3.75 ± 0.32* | 3.25 ± 0.25* |
| 9.0 | 7.38 ± 0.26 | 7.00 ± 0.41 | 6.38 ± 0.27* | 6.25 ± 0.48* | 5.63 ± 0.23* |

Papaverine was given 2 min before the second set of cumulative doses of MeCh.

Mean ± S.E.M from 4 rats in each group are given. Statistical significance refers to difference from the control group: * $p<0.05$.

PIPR: peak height of the intratracheal pressure response.

**Table 8.** Hippocratic screening test of the ethanol extract from *C. petasites* in rats

| Signs and symptoms | Control | Response after Dosage | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 5 min | 15 min | 30 min | 60 min | 2 h | 4 h | 6 h |
| ***Ethanol extract (2,500 mg/kg)*** | | | | | | | | |
| Decrease of motor activity | 0 | 0 | 0 | +1 | +1 | +1 | +1 | +1 |
| Decrease of respiratory rate | 120/min | 0 | 0 | +1 | +1 | +1 | +1 | +1 |
| Micturition | 0 | 0 | 0 | +1 | +1 | +1 | 0 | 0 |
| ***Ethanol extract (3,000 mg/kg)*** | | | | | | | | |
| Decrease of motor activity | 0 | +1 | +1 | +1 | +1 | +1 | +1 | +1 |
| Loss of righting reflex | 0 | +1 | +1 | +1 | +1 | +1 | +1 | +1 |
| Decrease of respiratory rate | 120/min | 0 | 0 | +1 | +1 | +1 | +1 | +1 |
| Loss of screen grip | 0 | 0 | +1 | +1 | +1 | +1 | +1 | +1 |
| Lacrimation | 0 | 0 | 0 | +1 | +1 | +1 | 0 | 0 |
| Salivation | 0 | 0 | + | 0 | + | 0 | 0 | 0 |
| Micturition | 0 | +1 | 0 | 0 | +1 | 0 | 0 | 0 |
| ***Ethanol extract (4,000 mg/kg)*** | | | | | | | | |
| Decrease of motor activity | 0 | +1 | +1 | +1 | +1 | +1 | +2 | +2 |
| Loss of righting reflex | 0 | +1 | +1 | +1 | +1 | +1 | +2 | +2 |

**Table 8.** (continue)

| Signs and symptoms | Control | Response after Dosage | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 5 min | 15 min | 30 min | 60 min | 2 h | 4 h | 6 h |
| Decrease of respiratory rate | 120/min | +1 | +1 | +1 | +1 | +1 | +2 | +2 |
| Loss of screen grip | 0 | +1 | +1 | +1 | | +1 | +1 | +2 |
| Fasciculation | 0 | 0 | 0 | +1 | +1 | +1 | 0 | 0 |
| Lacrimation | 0 | 0 | +1 | 0 | +1 | +1 | +1 | 0 |
| Salivation | 0 | 0 | + | 0 | + | + | + | 0 |
| Micturition | 0 | 0 | +1 | 0 | +1 | +1 | +1 | +1 |
| ***Ethanol extract (5,000 mg/kg)*** | | | | | | | | |
| Decrease of motor activity | 0 | +1 | +1 | +1 | +2 | +2 | +3 | +2 |
| Loss of righting reflex | 0 | +1 | +1 | +1 | +2 | +2 | +3 | +3 |
| Decrease of respiratory rate | 120/min | +1 | +1 | +1 | +2 | +2 | +3 | +3 |
| Loss of screen grip | 0 | +1 | +1 | +1 | +2 | +2 | +3 | +3 |
| Fasciculation | 0 | 0 | 0 | 0 | +1 | +1 | +1 | 0 |
| Lacrimation | 0 | 0 | 0 | 0 | +1 | +1 | +1 | +1 |
| Salivation | 0 | 0 | +1 | +1 | +2 | +2 | +1 | 0 |
| Micturition | 0 | +1 | 0 | +1 | +1 | +2 | +1 | +1 |

**Table 8.** (continue)

| Signs and symptoms | Control | Response after Dosage | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | 5 min | 15 min | 30 min | 60 min | 2 h | 4 h | 6 h |
| Diarrhea | 0 | 0 | 0 | 0 | +1 | +1 | 0 | 0 |
| Cyanosis | 0 | 0 | 0 | 0 | 0 | + | 0 | 0 |

The ethanol extract was injected (intraperitoneally) in non-fasted Sprague-Dawley rats (180-200 g). Each dose consisted of 4 rats.

Dose 5,000 mg/kg causing death of one rat within 2 h and two rats within 24 h and 2 days

**Footnote for Table 8**

Signs and symptom indicate degree, ranging from:

*Decrease of motor activity*

+1 = dose not move spontaneously, but when handled will move rapidly

+2 = when handled will move slowly

+3 = when handled will move sluggishly

+4 = when handled will not move at all

*Loss of righting reflex,*

+1 = can be placed only on one side

+2 = can be placed on either side equally well

+3 = can be placed on back as well as either side

+4 =cannot be aroused from back position by a hind leg toe pinch

*Loss of screen grip*

+1 = falls at first shake of screen

+2 = falls off when screen has been inverted

+3 = falls off when screen is at a 90° angle

+4 = falls off as the screen is tilled to a 45° angle

*Decrease of respiratory rate*

+1 = 10% decrease in respiratory rate

+2 = 20% decrease in respiratory rate

+3 = 40% decrease in respiratory rate

+4 = 80% decrease in respiratory rate

*Fasciculation*

+1 = definite response in one area

+2 = response seen in more than one area, but movement is not continuous

+3 = fasciculation seen in several areas (hind legs, back, neck) stimultaneously with response persisting in at least one area continuously

+4 = continuous total involvement

*Lacrimation*

0 = absence

+1 = eye lipswet, no dropping note

+2 = maximal response with lacrimal actively dripping down from the eyes

*Salivation*

+ = jaws and fur not noticeable wet, but moisturing of the filter paper

+1 = jaws and chin fur wet, no dropping note

+2 = maximal response with salivation actively dripping down from the jaws

*Micturition and diarrhea*

+1 = pellet when flicked off of paper flooring of the rink leaves a small but detectable stain

+2 = semi-firm pellet leaves a stain roughly the size of the pellet

+3 = soft pellet and the stain exceeds the boundary of the pellet

+4 = a shapeless feces mass with pronounced staining of the paper

*Cyanosis*

0 = absence

+ = color of ears, feet and oral mucosa is a dark redbown