DISCUSSION

Asthma is a common disease that affects a large part of the world 's population. Although an exact definition is difficult to establish, it is a lung disease most clearly characterized by episodes of increased airflow obstruction in which the sufferer is highly sensitive to a variety of airway irritants. This leads to bronchoconstriction, which if sufficiently severe can lead to wheeze and breathlessness (Wardlaw, 1993).

Although the exact cause of asthma is fairly known, the evidence points to a genetically susceptible individual exposed to an environmental trigger which can cause an inflammatory reaction in the airways and lead to bronchospasm. In the management and treatment of asthma, drugs are of great importance. Although much emphasis is put on avoiding triggering factors, drugs are needs to minimize the suffering symptoms. Two main classes of drugs are currently used to control the disease; anti-inflammatory drugs (preventers) to suppress the inflammatory response and drugs used intermittently to reverse bronchospasms (relievers). The current understanding of asthma has led to a consensus that the appropriate treatment is a combination of both types of drugs (Wardlaw, 1993).

Since asthma has been affecting people worldwide, it can be expected that many cultures, especially developing countries, have looked for cures in their local environment. Many medicinal plants have been used to treat asthma (Panthong *et al.*, 1986). Some of them have been evaluated for this traditional use. The results obtained showed their efficacy as

bronchodilators and has led to the isolation of new chemical compounds (Akah *et al.*, 1997; Dai *et al.*, 1997; Eman *et al.*, 1997; Erazo *et al.*, 1997; Hamasaki *et al.*, 1997; Govidan *et al.*, 1999). *C. petasites* is one of medicinal plants used in Thailand as a tea or an alcoholic extract for the treatment of bronchial asthma and inflammation of muscle and joints. Although *C. petasites* has been found to yield several compounds, including terpenoids, a steroid and flavonoids (Manzoor, 1966), no report has been made on a biological evaluation of the plant. The present study was undertaken to evaluate the anti-asthmatic property of *C. petasites* on animal models both *in vitro* and *in vivo*.

It is known that few if any animals get asthma as a natural disease, thus any animal model of asthma is considered artificial. Therefore, an animal model of bronchial asthma should parallel to the human reaction in as many respects as possible. The two main animal models used are guinea-pig and rat (Church, 1975). Commonly used bronchoconstrictive inducers are histamine and acetylcholine or methacholine for the guinea-pig (Curry, 1946; Herxheimer, 1951) and acetylcholine or methacholine for rat (Jameison, 1962; Salonen and Mattila, 1981).

In the present study, to demonstrate and to investigate the bronchodilator activity of the ethanol extract from *C. petasites*, the isolated guinea-pig tracheal strip served as the *in vitro* model, whereas the guinea-pigs as well as the rats under pentobarbital anesthesia, in which the pulmonary resistance or dymamic lung compliance was recorded, served as the *in vivo* models.

Drugs which are known to possess smooth muscle relaxant activity and can effectively antagonize experimental bronchoconstriction were used as reference drugs for the investigation of the bronchodilator effect of the ethanol extract. These drugs include terbutaline (β_2-adrenergic agonist), atropine (antimuscarinic agent), aminophylline (methylxanthine) and papaverine (spasmolytic agent). All reference drugs effectively counteracted histamine- and acetylcholine- or methacholine-induced bronchoconstriction *in vitro* as well as *in vivo*.

Drugs with smooth muscle relaxant activity can cause bronchodilation, but by different mechanisms. It has been proposed that the relaxation of smooth muscle and the increase in cyclic AMP are causually related (Hogg, 1982). Terbutaline, aminophylline and papaverine are known to cause an increase in intracellular cyclic AMP. Their effects are, however, mediated by different mechanisms. Activation of β_2-adrenergic receptors by β_2-adrenergic agonists cause activation of adenylyl cyclase with a subsequent increase in intracellular cyclic AMP (Barnes, 1989). An increase in intracellular cyclic AMP by aminophylline and papaverine was originally thought to occur through an inhibition of phosphodiesterase which catalyzes cyclic AMP to 5'-AMP (Butcher and Sutherland, 1962). Recently, it was suggested that aminophylline may cause bronchodilation by antagonizing adenosine at the P_1-receptor which was thought to be A_{2B}-subtype (Igor *et al.*, 1998). Cyclic AMP, via activation of protein kinase A, inhibits phosphorylation of myosin and reduces the intracellular calcium concentration, thereby producing bronchodilation (Barnes, 1992).

Stimulation of muscarinic receptor causes an increase of the intracellular levels of cyclic GMP and as a consequence calcium in the cytosol is increased by the mobilization of Ca^{2+} from intracellular pools and the flux of Ca^{2+} from extracellular pools (Schultz, 1977). The bronchodilator activity of atropine results from the blockade of muscarinic receptors on bronchial smooth muscle via specific competitive antagonism of acetylcholine (Gross, 1988), resulting in interference of cyclic GMP mediated bronchospasm and thus potentiates bronchial muscle relaxation. It is therefore apparent that any effective bronchodilators share a mechanism of action in a final common pathway which is a reduction of the intracellular free Ca^{2+} concentration (Pakes *et al.*, 1980).

The parallelism of the linear regression lines of the dose-response relationship of test substances and reference drugs are usually used to provide suggestive evidence about the mechanism of action. In the histamine-induced tracheal contraction of isolated guinea-pig tracheal strip preparation, all test drugs were found to exhibit a dose-related antagonistic effect. Comparison of the dose-response regression lines of these test drugs (Figure 6), suggests that the dose-response regression line of the ethanol extract was parallel with those of terbutaline, aminophylline and papaverine. In the presence of propranolol, a β-adrenergic receptor antagonist, the ethanol extract still possessed the antagonistic effect on the histamine-induced tracheal contraction. This result suggests the different mechanisms of bronchodilator effect of the ethanol extract and terbutaline. In other words, the bronchodilator effect of ethanol extract is not mediated via β_2-adrenergic receptor stimulation.

In the guinea-pig tracheal strip preparation, acetylcholine was also used as the bronchoconstrictive inducer. Atropine, an antimuscarinic agent was used as reference drug in this study, together with other i.e. aminophylline and papaverine. Comparison of the parallelism of the linear regression lines of the dose-response relationship of test drugs (Figure 9), showed that the linear regression line of the ethanol extract was parallel to those of atropine, aminophylline and papaverine. Nevertheless, the ethanol extract could not prevent the effect of acetylcholine-induced tracheal contraction whereas atropine could completely block this effect. This finding suggests that the bronchodilator effect of the ethanol extract was not exerted via muscarinic receptor. According to the parallelism of the dose-response regression lines of all test drugs which suggests the similar mechanism of action, it postulated that the ethanol extract might share some mechanism of the bronchodilator activity with other reference drugs in a final common pathway of bronchodilatation (Barnes, 1992).

Since the processes involved in asthma are complex and still uncompletely defined and there are many chemical mediators released locally during these complex responses, the bronchoconstriction induced in the whole animal models should bear the closest mechanical similarity to human asthma (Salonen, 1985). In the present study, the bronchodilator effect of the ethanol extract and reference drugs was investigated in two different animal species, guinea-pigs and rats, in order to increase the relevance of the results. A reproducible obstruction of airway was induced by an intravenous administration of two different pharmacological agents, histamine in guinea-pig and methacholine in rat. In the guinea-pig model, the bronchodilator activity of the ethanol extract and reference

drugs was compared. Chlorpheniramine, an H_1-receptor antagonist, was also used to show the blocking effect against histamine-induced bronchoconstriction in guinea-pigs (Figure 11B). Although histamine was the first allergic mediator to be implicated in asthma, H_1-receptor antagonists are little use for the treatment of symptomatic asthma and have no place in the treatment of acute or chronic severe asthma. The reason is that antihistamines have no effect on other mediators and it might be difficult to achieve high enough lung concentrations by the oral or parenteral route (Paterson *et al.*, 1979). Besides this, high doses of antihistamines produce various undesirable side effects.

In the present study all test drugs exhibited the protective effect against bronchospasm induced by histamine in a dose-related manner in terms of PIPR. The dose-response regression line of the ethanol extract in the term of PIPR was found to be parallel to those of terbutaline, aminophylline and papaverine. However, the *in vitro* experiment, showed that the bronchodilator effect of the ethanol extract was not mediated via β_2-adrenergic receptor. This result may give the suggestion of the bronchodilator activity of the ethanol extract that, its mechanism of action might be related to the other reference drugs in a final common pathway of bronchodilatation (Barnes, 1992).

In the experiment in rats, Salonen and Mattila (1981) found that the methacholine-induced bronchoconstriction and bradycardia were totally antagonized with atropine thus suggesting that the bronchoconstrictive action of methacholine was mainly due to the activation of muscarinic receptors in the bronchial tree and heart. The present study supports this finding that atropine could completely prevent the methacholine-induced

bronchoconstriction as shown in Figure 17B. Methacholine was given in a cumulative manner in order to prevent the marked depressor effect on the cardiovascular system, by injecting two sets of the drug intravenously. These two sets of cumulative doses caused a mild, moderate and submaximal rise in PIPR values and this parameter of the bronchoconstrictive response was found to be increased in a does-related manner in each set of drug. Test drugs were administered 2 min prior to the second set of methacholine doses. The protective effect of the ethanol extract and all reference drugs against the second set of accumulative methacholine-induced bronchoconstriction was found to be moderately effective. No complete protection was seen even with the highest doses of test drugs used in this study. Nevertheless, the inhibitory effect of all test drugs on the methacholine-induced bronchoconstriction in terms of PIPR was found to be dose-related. The reason for a noncomplete protection of the bronchoconstriction by test drugs might be due to an insufficiency of the doses used. Anyhow, larger doses of these drugs could not be administered because of the cardiovascular effects. Tachycardia or hypotension usually occurred in concomitance with the use of bronchodilators such as terbutaline and aminophylline (Norn and Stahl, 1979; Svensson *et al.*, 1989; Homer, 1998). Tachycardia comes from either a direct action of drugs on cardiac muscle or a reflex occurring from hypotension, which is usually caused by drugs with smooth muscle relaxant activity. Both tachycardia and hypotension caused by bronchodilators are futher increased by adding the second set of methacholine-doses. The animals, therefore, could die easily if the larger doses of these test drugs were intravenously administered.

Several compounds including terpenoids, steroids and flavonoids have been found in *C. petasites* (Manzoo, 1966). Flavonoids are natural products found to be biochemical constituents of plants. Many studies on the effects of flavonoids showed that they possess a wide range of pharmacological activities including that on smooth muscle and on the release, synthesis or activity of many inflammatory mediators (Yoshimoto *et al.*, 1983; Macander, 1986). It is therefore possible that flavonoids which are frequently found as constituents of plants, are responsible for the bronchodilator effects of the ethanol extract of *C. petasites*.

In addition to the study of the bronchodilator effect, the ethanol extract was also studied in conscious animals to investigate its effect on the general behaviour by employing the Hippocratic screening test. Intraperitoneal injection of the ethanol extract at doses of 2,500, 3,000 and 4,000 mg/kg to the rats produced mild changes, such as decrease of respiratory and motor activity, slight loss of righting reflex and screen grip. Marked CNS depression leading to respiratory arrest was seen with the dose of 5,000 mg/kg. Other mild effects observed following high doses of the ethanol extract from *C. petasites* were lacrimation, micturation and diarrhea. The symptoms observed indicates that high doses of *C. petasites* affects the autonomic nervous system.

In conclusion, the present investigation revealed the bronchodilator effect of the ethanol extract from *C. petasites* on some experimental animal models which were suggested to be good models for evaluation of bronchodilators (Burns and Doe, 1978; Lulich and Paterson, 1980; Salonen, 1985). It has been proved that the relaxant effect of the ethanol extract on tracheo-bronchial muscle was not mediated via the

β_2-adrenergic receptor stimulation, since in the presence of propranolol, a β-adrenergic antagonist, the antagonistic effect on histamine-induced tracheal contraction was still observed. In addition, the ethanol extract does not act by blocking the muscarinic receptor. In the study to measure the protective effect of the ethanol extract against experimental bronchospasm *in vivo*, it was found that the ethanol extract exhibited effective protection against both histamine- and methacholine-induced bronchoconstriction. The positive results obtained in both animal models used indicate the therapeutic potential of the ethanol extract from *C. petasites* for the treatment of bronchial asthma. Anyhow, it is necessary to note that this study was carried out only under a few experimental models and in two species of animals. Further study needs the use of the other inducer which are known to play important roles in asthma such as prostaglandins, leukotrienes and platelet-activating factor (Kreutner *et al.*, 1989; Tavares-Murta *et al.*, 1993) and applying other experimental models which have been suggested to resemble human asthma.