SHORT REPORTS

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PHARMACOLOGICAL PROPERTIES OF THE AQUEOUS EXTRACT OF *ERYTHROPHLEUM SUCCIRUBRUM* GAGNEP LEAVES

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Abstract

The aqueous extract of Erythrophleum succirubrum *Gagnep leaves given to mice by intraperitoneal route was shown to cause poisoning. The* LD_{50} *of the extract in mice was 20 g/kg body weight. The extract given to anesthetized rats, 0.5 g/kg body weight intravenously produced a significant increase in blood pressure. In isolated preparations, the extract stimulated rat ileum, rat atria and rat phrenic nerve-hemidiaphragm. The stimulation in rat atria and phrenic nerve-hemidiaphragm was followed by inhibition.*

The genus Erythrophleum, which belongs to the family Caesalpiniaceae, comprises many species which are widely distributed throughout West Africa, Philippines, Australia and Indo-china[1]. Leaves of some species have been the cause of mass poisoning of livestock, such as *E. couminga* Baill. in Madagascar and the Seychelles Islands, while some are devoid of toxic properties, such as *E. densiflora* Merrill of the Philippines and *E. fordii* Oliver of Indo-china[1].

E. succirubrum Gagnep, Known as "Saat" or "Phan Saat" by the people in Northeastern part of Thailand, is a handsome tree which is believed to be the cause of death in some children and livestocks

Since the plant is so easily accessible to children and cattle that they may accidentally consume it is important to have data on its toxicity and pharmacological properties.

In the present study the toxicity and some pharmacological properties of *E. succirubrum* Gagnep leaves were investigated in the experimental animals.

Adult albino rats of Fisher strain (300 - 400 g) and Swiss albino mice (35 - 40 g) were used. All of them were bred at the Faculty of Medicine, Khon Kaen University.

E. succirubrum Gagnep leaves were weighed, chopped into small pieces, and blended for 15 min with distilled water, approximately three times of the leaves weight. The paste was filtered through cotton gauze. The cloudy liquid was evaporated on water bath to reduce to one-sixth of the original volume, and then centrifuged at 3000 g for 10 minutes. The supernatant was used as the 2 : 1 aqueous extract. All of the doses of the extract which are mentioned in the text are expressed in terms of the fresh weight of *E. succirubrum* Gagnep leaves.

The study of acute effects of *E. succirubrum* Gagnep extract in mice was carried out by injecting the extract intraperitoneally. At the dose of 10 g/kg body weight, mice showed signs of quivering, generalized weakness, and difficulty in breathing with obvious forceful movement of the respiratory muscles. The group treated with higher dose showed the signs of hyperreflexia, and tonic and clonic seizure with spastic paralysis before death, the LD_{50} of the aqueous extract, determined by the method described by Reed and Muench[2] was 20 g/kg body weight.

The extract (filtered through the 0.45 u membrane) given intravenously 0.5 g/kg body weight to the anesthetized rats (prepared as described by the Staff of Department of Pharmacology University of Edinburgh and McLeod[3]) caused an increase in blood pressure and a decrease in heart rate which returned to normal in 4 min as recorded by Statham Pressure Transducer, Grass Model Tachograph 7 P 4 F and 7 DAF, and Grass Model 7 P 122 B Polygraph (Grass Instrument, Quincy Mass., U.S.A.). At the dose 2 g/kg body weight similar changes in blood pressure and heart rate were observed. Additionally, the electrocardiogram (recorded by Grass Model 7 P 6 C and 7 DAF) was changed into a typical sinus arrest and AV block arrhythmia. The electrocardiogram was completely recovered in 30 min as shown in Fig. 1.

The *in vitro* studies were carried out using the methods described by the Staff of Department of Pharmacology University of Edinburgh and Perry,[4]. The contractile activities of the preparations were recorded by means of a force transducer (Grass Instrument Co. Model FT-10 and polygraph).

The extract with a concentration of 13.3 mg/ml produced a positive inotropic on isolated rat atria followed by the cardiac standstill and initially produced a stimulation followed by inhibition in isolated rat phrenic nerve-hemidiaphragm preparation when the muscle was neurally stimulated (using the supramaximal rectangular wave pulse of 0.5 msec duration and frequency of 30 ppm by Grass Model SD 9 stimulator). A time course of neuromuscular inhibition was shown in Fig. 2. The inhibition produced by the extract could not be antagonized by neostigmine.

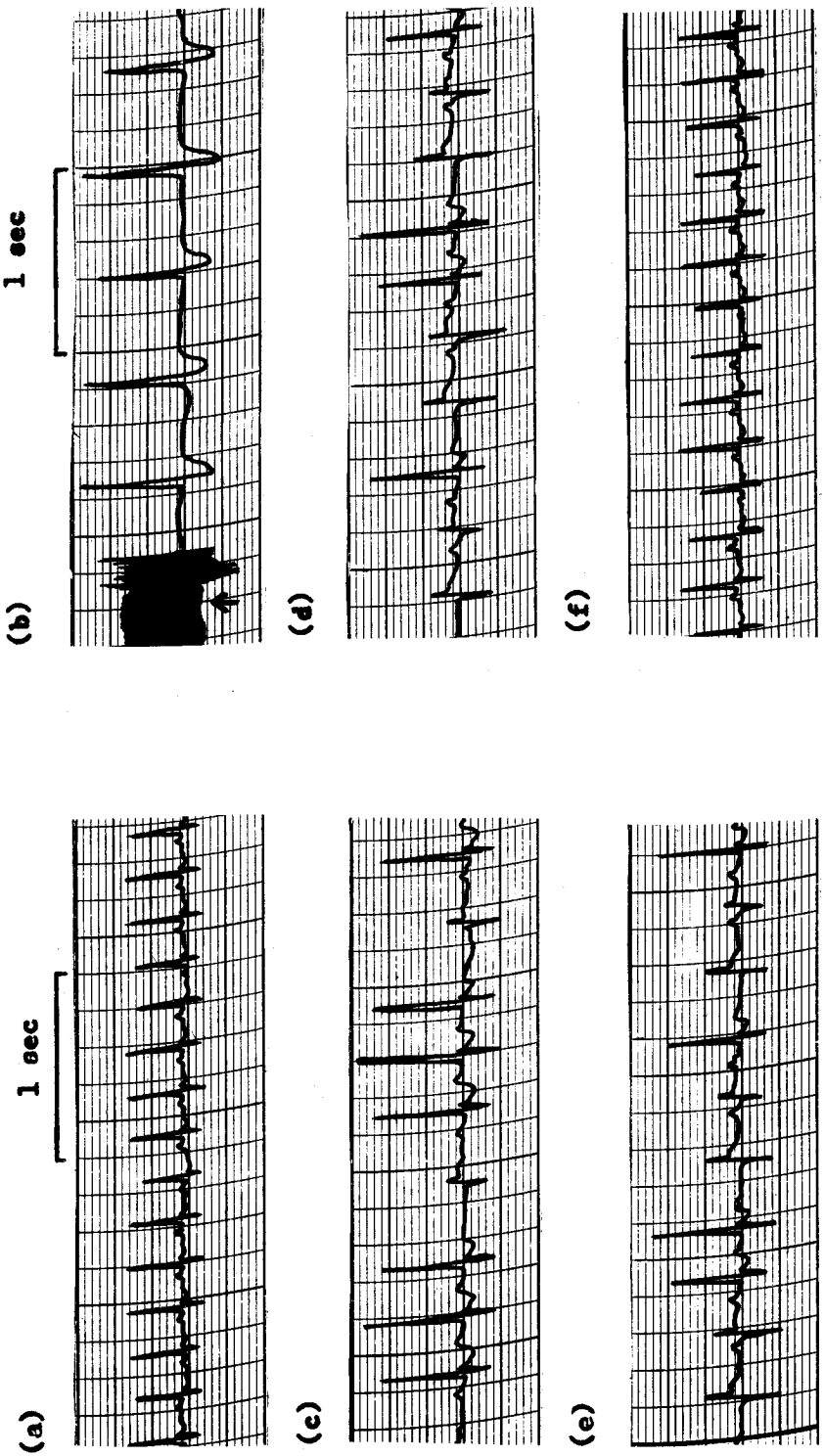

Fig. 1. The change in electrocardiogram in an anesthetized rat produced by the aqueous extract of *E. succirubrum* Gagnep leaves (2 g/kg):
(a) the normal electrocardiogram pattern, (b) intravenous injection (c) 1 min later, (d) 2 min later, (e) 3 min later, and (f) 30 min later.

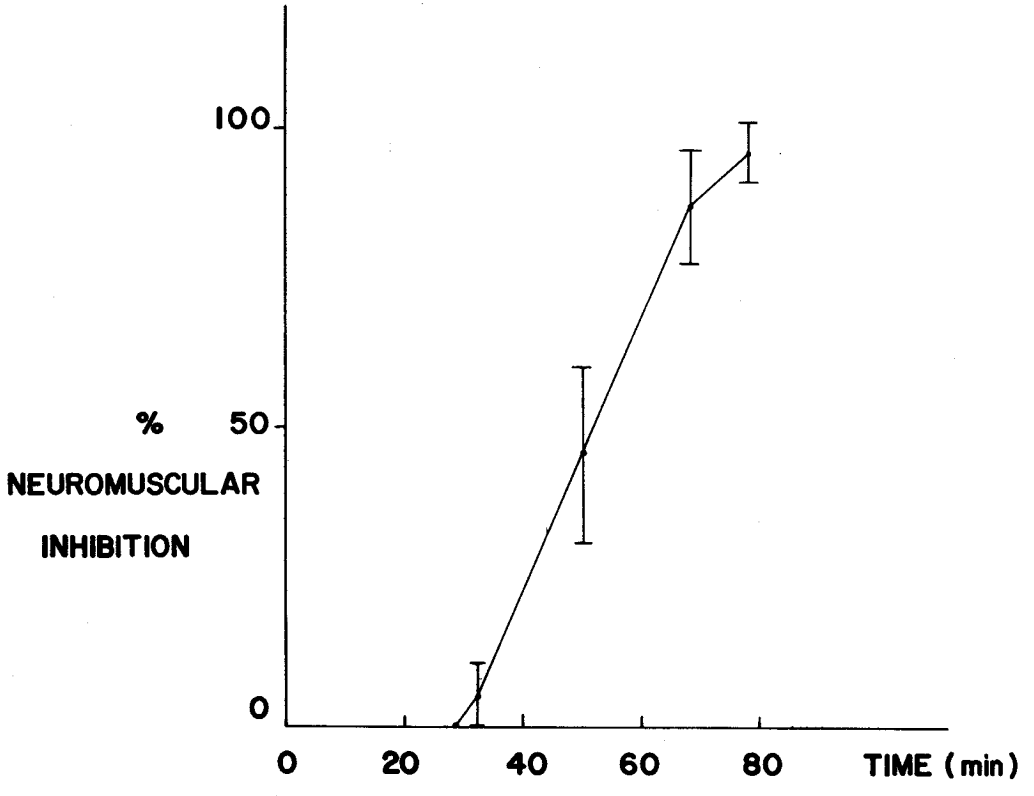

Fig. 2. Time course of neuromuscular inhibition (mean ± S.D.) produced by the aqueous extract of *E. succirubrum* Gagnep leaves (13.3 mg/ml). The muscle was stimulated indirectly through the phrenic nerve with supramaximal rectangular pulses.

The extract with a concentration of 24 mg/ml produced a marked contraction of rat ileum which could by antagonized by antazoline mesylate (40 μg/ml) but not by atropine (40 μg/ml).

This study shows that consumed *E. succirubrum* Gagnep could be a cause of death in animals. Acute toxicity in mice had both respiratory and central effects. At present, the primary cause of death is not clarified. From the effects of the extract in isolated rat atria and phrenic nerve-hemidiaphragm preparations, death may be due to cardiac or respiratory failure or both.

A survey of literature[5-11] revealed that many types of chemical and pharmacological work have been carried out on some Erythrophleum species. Alkaloids from the bark, seeds, and leaves were isolated and their structures were identified. Some of them, coumidine and cassaine, have potent cardiotonic activity[11]. So far there has been no chemical investigation on *E. succirubrum* Gagnep. In this report, we would like to mention our preliminary finding that the aqueous extract shows positive result to many alkaloidal tests (10 % phosphotungstic acid, Mayer's reagent, Wagner's reagent and Dragendorff solution) and triterpenoid test (Liebermann Burchard's reagent).

Further studies of the active ingredients in the *E. succirubrum* Gagnep, the mechanism of action, and the antagonists remain to be accomplished. Information on these will be very useful in the case of treatment of accidental poisoning. The possible use of the plant for medicinal purpose in the future is intriguing as well.

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บทคัดย่อ

จากการศึกษาถึงพิษและฤทธิ์ทางเภสัชวิทยาของน้ำสะกัดใบพันซาด *(Erythrophleum succirubrum* Gagnep, Casealpiniaceae) พบว่า เมื่อให้น้ำสะกัดฉีดเข้าช่องท้องของหนีถีบจักร (mice) จะทำให้เกิดอาการพิษขึ้นอย่างเห็นเด่นชัด ปริมาณใบพันซาดที่ทำให้หนูถีบจักรตายเป็นจำนวนครึ่งหนึ่งมีค่าเท่ากับ 20 กรัม ต่อน้ำหนักตัว 1 กิโลกรัม การให้น้ำสะกัดเข้าเส้นเลือดดำในหนูขาวปริมาณ 0.5 กรัม ต่อน้ำหนักตัว 1 กิโลกรัม จะทำให้ความดันโลหิตเพิ่มขึ้น ในอวัยวะแยกน้ำสะกัดใบพันซาดสามารถกระตุ้นลำไส้เล็กส่วน ileum ของหนู และกระตุ้นตามด้วยการยับยั้งการทำงานของหัวใจห้องบนของหนู และกล้ามเนื้อกระบังลมที่กระตุ้นด้วยไฟฟ้าผ่านทางเส้นประสาท phrenic