Chapter I

Introduction

Kloi (*Dioscorea daemona, D. hirsuta, D. hispida*), the plant of Dioscoreaceae family generally known as 'wild yam' , is localized in the tropical or warm temperature countries, including Thailand. According to the information obtained from people in the rural area of northern part of Thailand, tubers of kloi is consumed as main food in place of rice (Panthong and Chansirisri, 1975). Generally, the tuber is composed of 60–80 % water, 23 % carbohydrate, 2 % protein, and a small amount of fat (Nelson, 1951).

Two alkaloids have been reported as major constituents isolated from the plants of dioscoreaceae family, which are dioscorine ($C_{13}H_{19}O_2N$; DCR) and dioscine ($C_{13}H_{21}O_2N$). DCR was first isolated from *D. hirsuta* (Henry, 1949) and then later from *D. hispida* (Pavovat, 1973). Dioscine is an isomer of dihydrodioscorine. Both have been reported to produce toxic effects but DCR is more potent than dioscine (Pinder, 1953; Boardbent and Schneiden, 1958).

DCR has molecular weight of 221.29 with chemical structure shown in Figure 1, and the melting point of 43.5°C. With this relatively low

melting point, DCR usually appears in liquefied form as a pale yellow liquid with aromatic smell (Bevan, Boardbent, and Hirst, 1956; Bevan and Hirst, 1958; Stecher, 1968).

Toxic Effects of Kloi and Dioscorine

Toxic effects as results of kloi consumption have been repeatedly reported. The toxic effects include ; nausea, dizziness, vomiting, sweating, palpitation, diarrhea, weakness and syncope (Ketusinh, 1942). Prolonged eating of kloi is claimed to produce malnutrition and anemia (Panthong, 1973; Chansirisri, Panthong, and Tejasen, 1975; Panthong and Chansirisri, 1975).

Among various species of kloi ; *D. bulfifera, D. elephantipes and D. hemicrypta,* were found to possess some hemolytic action which is the effect of a toxic substance, saponin (Watt and Breyer–Brandwijk, 1962).

Preliminary study of the toxic effects using crude water extract of kloi shows the following results ;

1. Effects of aqueous kloi extract on the central nervous system

Aqueous kloi extract injected intravenously produces effects on the CNS which are characterized by over stimulation followed rapidly by depression. Suppression of motor activity is observed after a low toxic

dose, while lethal dose of the extract results in convulsions and death from respiratory arrest. Diazepam is the most potent prophylactic antidote (Chansirisri and Tejasen, 1977).

2. Effects of aqueous kloi extract on the peripheral nervous system

The 50 % (w/v) aqueous kloi extract produced complete neuromuscular blockade following the addition of kloi in tissue bath of an isolated phrenic nerve diaphragm preparation. This effect could be antagonized by neostigmine. It was suggested that kloi acted on both motor nerve terminal and endplate by inhibition of ACh release from motor nerve terminal and competitive inhibition of ACh at cholinergic receptor on the endplate (Ridtitid, 1977; Ridtitid and Apisariyakul, 1978).

3. Effects of aqueous kloi extract on the cardiovascular system

Intravenous injection of aqueous kloi extract in anaesthetised rat produced a biphasic response of blood pressure which was found to be dose related. A transient hypertensive phase occurred initially, followed by a more prolonged hypotensive phase. It appeared that kloi exerted the hypertensive action by a direct stimulation on the alpha-adrenergic receptor of vascular smooth muscle (Taesotikul and Kanjanapothi, 1977).

The red blood counts, the hematocrit values and the blood smears of the rat after four months of oral administration of aqueous kloi extract showed no significant differences from control (Chansirisri et al., 1975).

4. Effects of aqueous kloi extract on body growth

Adult rats of both sexes continuously fed with aqueous kloi extract showed no significant difference of growth during the first two months. However, after two months, the weight gain was significantly less than that comparing to the control group, which might indicate chronic intoxication in the experimental pair of rats (Tejasen and Thongtharb, 1979).

Effects of alcoholic extract of kloi

The effects of alcoholic extract of *D. daemona* was studied in various experimental animals. In frog when injected subcutaneously, low doses produced excitation while high doses caused tonic convulsion and death. In rat when injected intravenously, lethal dose showed signs of hyperpnea, pale ears, restlessness, tremor, violent twitching, clonic and tonic convulsion, respiratory arrest and death. In dog when injected intravenously, low doses showed signs of hyperpnea, salivation, bradycardia, hypersensitivity of reflexes, followed by depression, while high doses produced vomiting, salivation, depression and sleeping in conscious dogs. The lethal dose produced depression followed by clonic and tonic convulsion, finally, death from respiratory arrest. These results indicated that kloi caused stimulating effects at all levels of the CNS, cerebral cortex, medulla oblongata and spinal cord (Ketusinh, 1942).

Effects of DCR : Analeptic actions

Pharmacological studies of alkaloid DCR were performed in urethane anaesthetized rat. Intravenous injection of DCR increased arterial blood pressure and respiratory rate, while the heart rates of both *in vivo* and *in vitro* preparations were not affected. These data indicated that DCR exerts its effect on central vasomotor center and might be used as an analeptic drug (Anothayanontha, 1979).

Toxicological studies of dioscorine hydrobromide (DCR.HBr) were also performed in mice receiving intraperitoneal injection. DCR.HBr had strong CNS stimulant effect, resulting in convulsions and death with LD_{50} of 31.6 mg/kg. The alkaloid caused hypokinesia in the nonconvulsing period of its course of action. Probability of death decreased following pretreating the mice with diazepam, phenobarbital sodium, pentobarbital sodium and phenyltoin. Probable use of this alkaloid as an analeptic was discussed (Bhovadhi, 1979; Tantisira et al., 1979). Effects of DCR.HBr under action of pentobarbitone were studied in rats and mice. It was found that coma levels of respiratory rate, heart rate and blood pressure of pentobarbitone treated rats were restored following injection of DCR.HBr. In mice, DCR.HBr increased LD_{50} of pentobarbitone significantly. DCR.HBr may be use as an analeptic antidote in pentobarbitone intoxication (Hokierti, 1980).

All these data indicated that DCR.HBr could be used as an analeptic antidote. Analeptic properties of DCR was compared with two classical analeptics, bemigride and picrotoxin. In mice, symptoms produced by intravenous injection of these three compounds were hypokinesia, respiratory stimulation, hypersensitivity to external stimuli. Following high doses, tonic and clonic convulsions and death, occurred. DCR possessed the highest respiratory stimulant index among these three compounds. All compounds decreased the sleeping time produced by pentobarbitone, with picrotoxin being the most potent antagonist. LD_{50} of pentobarbitone reduced significantly after the administration of these compounds. In pentobarbitone anaesthetised rat changes in the depressed pattern of respiratory rate, heart rate, blood pressure and EEG were observed following DCR injection. The depressed respiration was restored. Heart rate and blood pressure increased significantly. Flattened EEG was converted to a wave form EEG (Tantisira et al., 1984).

In this study, cerebellar Purkinje cells were used as target neurones for elucidating the mechanism of action of DCR owing to the findings that it contains receptors for a variety of neurotransmitters, viz : glutamate, aspartate, GABA, glycine, taurine, 5–HT, NA and some other peptides.

The basic neuronal connection of the cerebellar cortex are summerized in Figure 2 and 3. In brief, there are two main sources of input to the cerebellar cortex : climbing fibers and mossy fibers. Climbing fiber inputs exert a strong excitatory effect on single Purkinje cell, whereas

another source of excitation on this cell comes from mossy fibers input mediated through granule cells which originate the parallel fibers whose endings form synapses on Purkinje cell dendrites. The basket and stellate cells are also excited by granule cells via the parallel fibers and their outputs inhibit Purkinje cell discharge. Golgi cells are excited by the mossy fiber collaterals and parallel fibers, and inhibited by purkinje cell collaterals. These cells inhibit granule cells (Eccles, 1973; Szentagothai and Arbib, 1974; Mountcastle, 1980).

GABA is know to be a major inhibitory neurotransmitter in the cerebellum. There is convincing evidence that GABA is present in Purkinje cells and released by this cell upon stimulation (Obata et al., 1967; Otsuka et al., 1971; Ribak, Vaughn, and Saito, 1978). In addition, neuropharmacological evidence indicates that GABA may also be the inhibitory transmitter released from golgi cells, basket cells, and possibly from some stellate cells (Bisti, Iosif, and Strata, 1971). However, GABA may not be the only transmitter released by stellate cells. Selective destruction of these cells by x-irradiation is followed by a substantial reduction of taurine in the molecular layer (Nadi, McBride, and Aprison, 1977). This would be consistent with the possibility that taurine is a (the) transmitter released by at least some stellate cells, especially as Purkinje cells are quite sensitive to this amino acid (Okamoto, Quastel, and Quastel, 1976), particularly when applied to their dendrites (Frederickson et al., 1978).

Several kinds of evidence point to glutamate as the excitatory transmitter released by the granule cell whose parallel fibers terminated on Purkinje cell dendrites. Purkinje cell are highly sensitive to L-glutamate particularly in the regions of dendrites (Chujo, Yamada, and Yamamoto, 1975). In addition, there is high concentration of glutamate in the cerebellum, especially in the molecular and the granular layers (Nadi et al., 1977). Selective reduction in tissue (or synaptosomal) glutamate contents is observed in animals that lack of granule cells and their axon owing to genetic defect (Roffer-Tarlov and Sidman, 1978; Roffer-Tarlov et al., 1979), or as a consequence of a viral infection (Young et al., 1974), or after selective x-irradiation (Rohde et al., 1979).

There is now extremely good evidence that NA is an inhibitory transmitter on cerebellar Purkinje cell, and 5-HT may also be a transmitter in the cerebellar cortex. Histochemical and autoradiographic studied of the rat cerebellum have revealed the presence of NA and 5-HT containing nerve fibers in molecular and Purkinje cell layers both *in vitro* and *in vivo* (Tebecis, 1974). Noradrenergic pathway originates predominantly from the locus coeruleus (Olson and Fuxes, 1971), and 5-hydroxytrptaminergic pathway presumably from the raphe nuclei (Dahlstrom and Fuxes, 1965). Direct iontophoretic applications of NA and 5-HT to Purkinje cells decrease their discharge rate (Kawamura and Provinin, 1970), and single or repetitive stimulation of locus coeruleus inhibit the firing of most Purkinje cells (Bloom, Hoffer, and Siggins, 1972).

Since evidence from neurochemical study shows that GLY can be taken up by golgi cells (Hokfelt and Ljungdahl, 1972). Possibility exists that this amino acid may be another transmitter in this brain area. Iontophoretic application of GLY depressed the firing of Purkinje cells but the response was less effective than that of GABA (Kawamura and Provinin, 1970). In consideration of ASP, Purkinje cells can be strongly excited by this amino acid, although neurochemical evidence make it unlikely that it is released by parallel fiber (Krnjevic, 1982). However, when climbing fibers are destroyed selectively either by decrease in human (Perry et al., 1977), or by the action of 3-acetyl-pyridine in rats (Nadi et al., 1977; Rea, McBride, and Rohde, 1980), the most significant observation is a consistent reduction in ASP. Hence ASP can be considerated the most possible contender for being climbing fiber's transmitter.

The present study is undertaken to investigate the actions of DCR on Purkinje cells by means of extracellular recording in conjuction with microiontophoretic technique. In view of current idea about possible antagonistic function of DCR, the present experiments are also designed to test the affects of DCR on neuronal responses induced by "classical" neurotransmitter substances reportedly reactive in the cerebellum (see above).

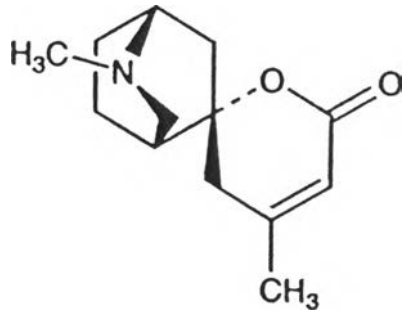

Figure 1. Chemical Structure of Dioscorine. (From The Merck Index, 1996).

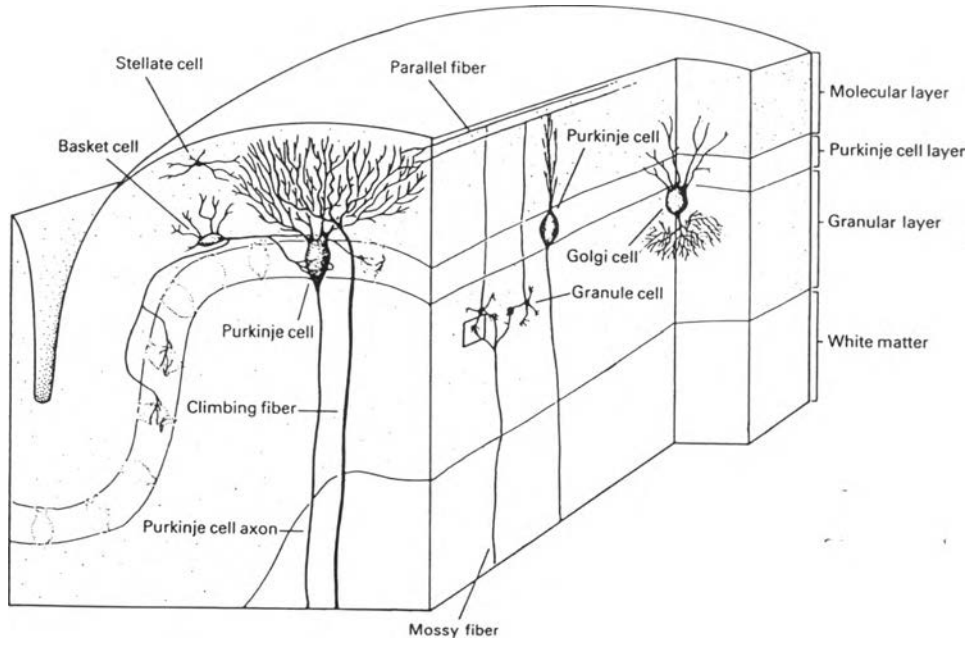

Figure 2. Schematic view of a single cerebellar folium, in both longitudinal and transverse planes, illustrates the general organization of the cerebellar cortex. The cerebellar cortex is organized into three layers and contains five types of neurons (modified from Kandel, Schwartz, and Jessell, 1991).

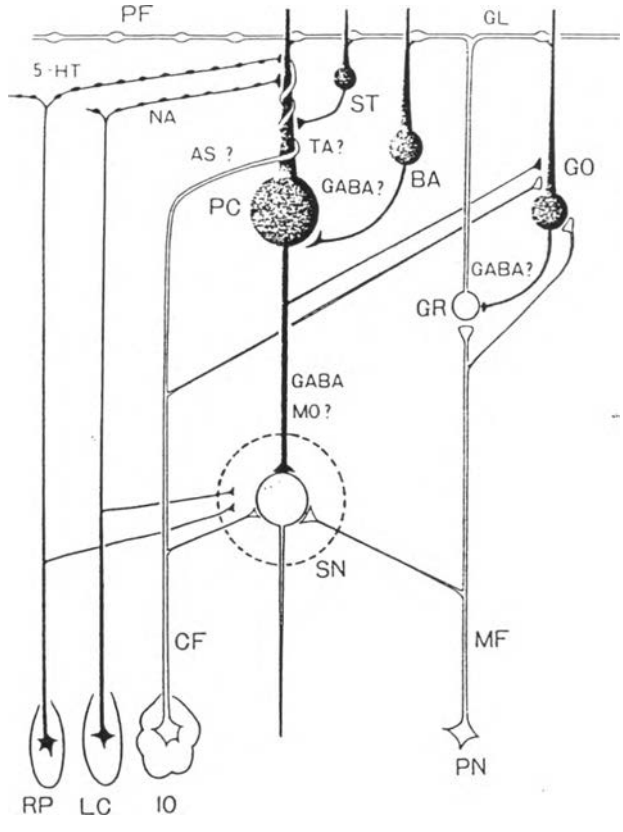

Figure 3. Basic neuronal circuitry and putative neurotransmitters in the cerebellum. PC, Purkinje cell; GO, Golgi cell; BA, basket cell; ST, stellate cell; GR, granule cell; PF, parallel fiber; MF, mossy fiber; CF, climbing fiber; SN, vestibular or cerebellar nuclear cell; PN, precerebellar neuron which issues mossy fibers; IO, inferior olive; LC, locus coeruleus; RP, raphe nuclei. Inhibitory neurons and synapses are in *black*, and excitatory ones have been left *unfilled*. Candidates for neurotransmitter substances are indicated for some synapses. *Question mark* indicates that criteria for identification have not yet been fulfilled. GL, glutamate; AS, aspartate; TA, taurine; MO, motilin; NA, noradrenaline; 5–HT, serotonin (from Ito, 1984).