CHAPTER II

LITERATURE REVIEWS

Cissus quadrangularis

Cissus quadrangularis Linn. (Phet-Sang-Khat or Phet-Cha-Sang-Khat), a plant in Vitaceae family, is an indigenous climbing herb in India, Africa and Arabic countries and has been used for medical purposes for a long time. Different parts of *Cissus quadrangularis* Linn. are used for various purposes (Figure 2-1). In India, the preparation of the dry shoots powdered of *Cissus quadrangularis* Linn. has been used for the treatment of internal ulceration and external wounds (Reddy et al., 1989) roots were used for myalgia (Nagarajo and Rao, 1990); stems were used for irregular menstruation (Quisumbing, 1951) and the plant juice was used for earache (Chopra, R.N. et al., 1965) and amenorrhea (Burhill, 1966). In Central Africa, decoction of this plant has been taken for blenorrhagia and to calm palpitation (Sillans, 1953). In South Africa, dried root of this plant has been used for anti-inflammation (Lin et al., 1999). In Arabic countries, the hot water extract of entire dried plant has been used as a "cure-all" medicine (Schmucker, 1969).

In Thailand, the plant has been used as carminative, anti-hemorrhoids and to promote fracture bone healing (Pong, 1950). Further investigation indicated that young shoots of *C. quadrangularis* have been used for dyspepsia and indigestion. Stems were used in the treatment of irregular menstruation and their paste is used traditionally as poultice on inflammatory bone fractures. Water squeezing from this plant has been used for irregular menstruation, scurvy, and epistaxis. Leave of this plant have been used in the treatment of internal ulceration and external wounds. Their roots have been used for bone fracture healing. Many reports have shown that the entire plant is considered to be used as anthelminthics, aphrodisiac, antiasthmatic, and is useful in gastrointestinal disorders such as abdominal colic pain, dyspepsia, and irregular menstruation (นันทวัน บุญยะประภัศร, 2542, Elizabeth, 2002; Sivarajan and Balachandran, 1994). In addition, some studies reported that *C. quadrangularis* was used for cancer-curing by combining

with other herbal medicine such as Sea Holly (*Acanthus ebractetus* Vahi), *Rhinacanthus nasutus* (Linn.) Kurz. and *Hydrophyum fermicarum* (สุภาภรณ์ ปิติภรณ์, 2544)

The occurrence of chemical compounds found in *C. quadrangularis* Linn.

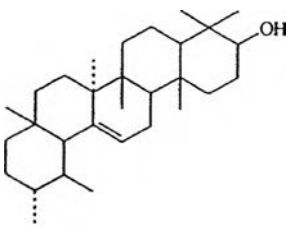
The *C. quadrangularis* stems contain high amount of calcium oxalate, Vitamin C and anabolic steroidal substances. The other chemical compounds of this plant are shown in groups as following:

1. Triterpene compounds[1,2,3,4,5]: δ-amyrin, δ-amyrone, Iso-aborenol, Friedelan-3-one, Epi-friedelinol, Lupenone, Onocer-7-ene-3-alpha-21-beta-diol, Onocer-7-ene-3-beta-21-alpha-diol, Onocer-8-ene-3-beta-21-alpha-diol-7, Taraxerol, and Taraxerol acetate.
2. Stilbene compounds[6]: Pallidol, Parthenocissin A, Quadrangularin A, Quadrangularin B, Quadrangularin C, Piceatannol, and Resveratrol
3. Steroid compounds[2,3,4]: keto-steroid, oxo-steroid and β-sitosterol
4. Flavonol compounds[6]: Kaemferol, Quercetin, Quercitrin and Iso-quercitrin.
5. Miscellaneous compounds[4]: Hepadecyl octadecanoate, Icosanyl icosanoate, tritriacontanoic acid,31-methyl, Octadec-9-ene,9-methyl, Docosan-1-ol-cyclohexane,7-hydroxy-20-oxo, tritriacontan-1-ol,31-methyl, Tricos-2-en-22-one,4-hydroxy-2methyl

1:Mehta et al., 2001 2:Bhutani, 1984 3:Pluemjai et al., 1986
4:Gupta et al., 1991 5:Gupta et al., 1990 6:Adesanya et al., 1999

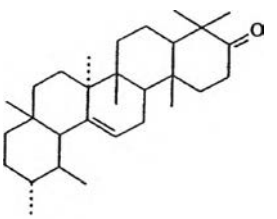
Figure 2-1 *C. quadrangularis* Linn. 1, Habit; 2, Flower bud; 3, Open flower; 4, Flower (Sivarajan and Balachandran, 1994)

Triterpenoid compounds



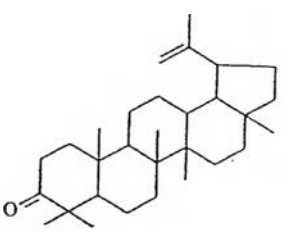
δ-amyrin

(Bhutani., 1984)



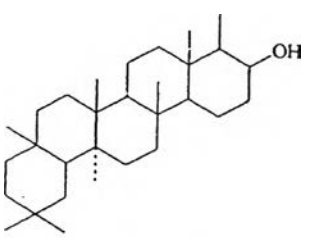
δ-amyrone

(Bhutani., 1984)



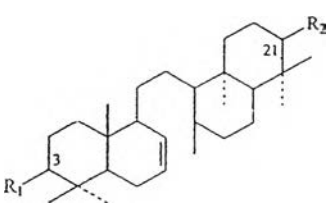
Lupenone

(Pleumjai et al., 1986)



Epi-friedelinol

(Pleumjai et al., 1986)

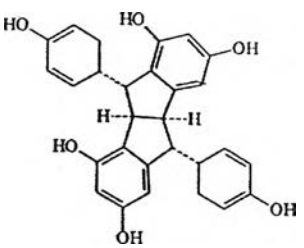


Onocer-7-ene-3-alpha-21-beta-diol

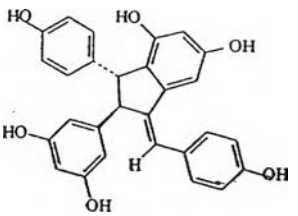
(Bhutani., 1984)

Figure 2-2 Structures of compounds identified in *C. quadrangularis*

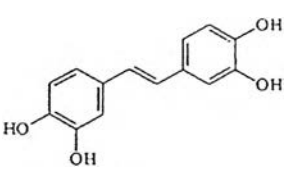
Stilbene derivatives



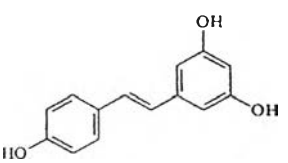
Pallidol



Parthenocissin A



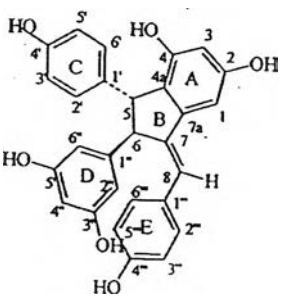
Piceatannol



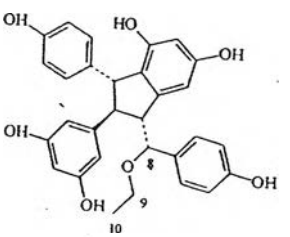
Resveratrol

Figure 2-2 (continued) Structures of compounds identified in *C. quadrangularis* (Adesanya et al., 1999)

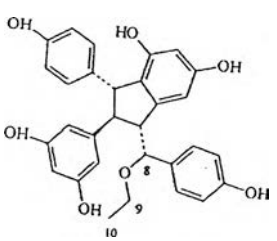
Quadrangularins



Quadrangularin A



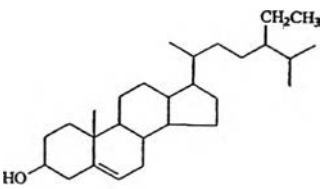
Quadrangularin B



Quadrangularin C

Figure 2-2 (continued) Structures of compounds identified in *C. quadrangularis* (Adesanya et al., 1999)

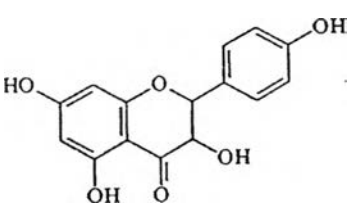
Steroid compounds



β-sitosterol

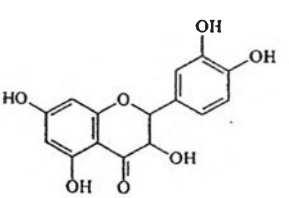
(Pleumjai et al., 1986)

Flavanol compounds



Kaemferol

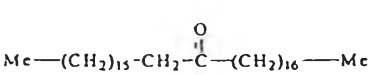
(Adesanya et al., 1999)



Quercetin

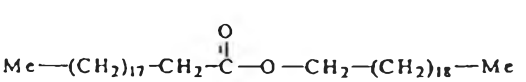
(Adesanya et al., 1999)

Alkane compounds



Hepadecyl octadecanoate

(Gupta et al., 1991)



Icosanyl icosanoate

(Gupta et al., 1991)

Figure 2-2 (continued) Structures of compounds identified in *C. quadrangularis*

Pharmacological activitiy

1. Antimicrobial and antioxidant activity

Lin et al. (1999) reported that the aqueous extract and methanolic extract of dried root of *Cissus quadrangularis* Linn. exhibited antibacterial activity with equivocal potency against *Bacillus cereus, Bacillus circulans, Bacillus pumilus, Bacillus stearothermophilus, Bacillus subtilis, Proteus mirabilis, Pseudomonas solacearum, Salmonella typhimurium, Shigella Shigella jlexneri, boydii, Staphylococcus aureus* and *Staphylococcus epidermidis in vitro.*

Furthermore, additional studies (Murthy et al., 2003) found that extract of *Cissus quadrangularis* can exhibited antioxidant activity. In these studies the extract of *C. quadrangularis* was tested for antioxidant activity using betacarotene linoleic acid model and 1, 1-diphenyl-2-picrylhydrazyl model. The ethyl acetate fraction of both fresh and dried-stems extracts at a concentration of 100 ppm showed 64.8% antioxidant activity in the beta-carotene linoleic acid system and 61.6% in the diphenyl-2-picrylhydrazyl system. This fraction was found to contain sterols, vitamin C, and tannins. The antioxidant activities of methanolic extract and aqueous extract were comparatively less significant than that of the ethyl acetate extract, and the n-hexane extract showed the least activity. Ethyl acetate extract and methanolic extract of both fresh and dried-stems of *C. quadrangularis* also exhibited antimicrobial activity against gram-positive bacteria, including, *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus,* and *Streptococcus* species. Results from this study provided an implication of using *C. quadrangularis* as an antibacterial agent and more so as an antioxidant in several applications requiring these properties. As well as in *Listeria monocytogenes* (Alzoreky and Nakahara, 2003)

2. Hypotensive activity

Bhukani et al., (1969) reported that the extraction from EtOH-H_2O (1:1) of dried aerial parts of *Cissus quadrangularis* Linn. showed hypotensive activity in dog's experiment.

3. Anti-implantation activity

Dhawan et al., (1980) reported that oral administration of a single dose of the ethanolic extract of dried aerial-parts of *Cissus quadrangularis* Linn. (340 mg/kg) to adult female rats showed anti- implantation activity.

4. Antiproliferation activity

Opoku et al., (2000) reported that the aqueous extract and methanolic extract from dried roots of *Cissus quadrangularis* Linn. showed antiproliferation activity in cell culture.

5.Antihemorrhoidal effect

This effect of *C. quadrangularis* was managed in clinical study. One hundred twenty one hemorrhoidal patients were divided into 3 groups by their stages of disease or degree of severity. Dried-stem powder of *C. quadrangularis* in capsule at the dose of 1,000 mg/day or 2,000 mg/day or 2 tablets of Daflon® twice daily, were given to the patients in each group for 2 months. After the experimental period, patients were assessed for the symptomatic improvement by scoring based on patient satisfaction following each regimens. The result demonstrated that dried-stem powder of *C. quadrangularis* at the dose of either 1,000 mg or 2,000 mg/day for 2 months, had an antihemorrhoidal activity which was comparable to the administration of Daflon® 2 tablets twice daily for 2 months (ดวงรัตน์ เชี่ยวชาญวิทย์ และคณะ, 2545)

6. Accelerating healing process effect

Deka et al., (1994), Chopra et al., (1976), and Udapa et al. (1964) also reported this effect of *C. quadrangularis* in the same manner while they carried out the experiments in different species of animals and different route of administration (intramuscular), respectively. Five hundred grams of dried-stem powder of *C. quadrangularis* were extracted with methanol using soxhlet apparatus. The dried methanolic extract was reconstituted with distilled water (100 mg/ml), filtered and used in

the study. Eight healthy mongrel dogs of either sex weighing 3-5 kg were randomly selected into two groups, control and treatment groups. Each- group consisted of 4 animals. Under general anesthesia, a closed fracture of the right forelimb of each animal was produced. Treated group received *C. quadrangularis* methanolic extract (50 mg/kg, subcutaneously) every alternate day whereas the control group received normal saline (0.5 ml/kg, S.C.). On the 11th day of fracture, two animals from each group were sacrificed for histopathological and radio logical examination whereas the rest of animals were continuing the treatment. On the 21st day of fracture, the rest of animals in control and treated group were sacrificed and performed in the same manner. The results showed that treated animals sacrificed on the 11th day of fracture revealed an appearance of bony dissolution and periosteal reaction at the fractured sites as observed by radiograph whereas, in the control group, bony dissolution was comparatively less and periosteal reaction was absent. Histopathologically, treated group exhibited initiation of osteogenesis which was absent in the control group. Radiograph of the treated group sacrificed on the 21st day of fracture revealed the presence of almost complete bridging of the fractured ends with extensive bony deposition and periosteal reaction as compared to those of the control. The treated group revealed replacement of cartilaginous cells by osteoblastic cells and union of the fractured gap at several places with the formation of new bony trabeculae, whereas bony trabeculae were absent in the control group.

7. Antiosteoporotic effect

Shirwaikar et al., (2003) showed that an extract of *C. quadrangularis* had a definite antiosteoporotic effect from the findings assessed on the basis of biomechanical, biochemical and histopathological parameters. This report was investigated in ovariectomized rat model of osteoporosis at two different dose levels of 500 and 750 mg/kg per day. Healthy female albino rats were divided into five groups of six animals each. First group was sham operated and served as control. All the other groups were ovariectomized. Group 2 was given with equivolume of saline and served as ovariectomized control. Groups 3-5 were orally treated with raloxifen (5.4 mg/kg) and

ethanolic extract of *C. quadrangularis* (500 and 750 mg/kg), respectively.

8. Anti-inflammatory activity and Analgesic effect

The preliminary anti-inflammatory activity was reported by Thisayakorn et al. (2000) The suspension of *Cissus quadrangularis* Linn. in soy bean oil was given orally at 500, 1,000, 2,000 and 3,000 mg/kg to rats. *Cissus quadrangularis* Linn. at 500 and 1,000 mg/kg gave negative results, whereas 1500, 2000 and 3000 mg/kg could slightly inhibit inflammation at 1 and 2 h. It appeared that *Cissus quadrangularis* Linn. might not inhibit acute inflammation in rats.

Singh et al, (1984) found that analgesic effect of the alcoholic fraction of *C. quadrangularis* was comparable to acetylsalicylic acid. The alcoholic extract was prepared with 70% alcohol in a soxhlet apparatus and was evaporated at low temperature and the residue was dissolved in normal saline for use in the experiments. Medium lethal dose (LD_{50}) of *C. quadrangularis* alcoholic extract in mice via oral and intraperitoneal routes was found to be 4 and 3.5 g/kg body weight, respectively. Analgesic activity was assessed in mice by the method of Haffner's tail clip and Eddy's hot plate method. *C. quadrangularis* was given orally at the dosages of 100, 200 and 400 mg/kg and intraperitoneally at the dosages of 87.5, 175 and 350 mg/kg. The results showed that analgesic effects of the extract of this plant as observed by both methods were dose related. Duration of analgesic activity was from 2-4 hours and the optimum effect was observed at $1/20^{th}$ - $1/10^{th}$ of the LD_{50}.

Toxicological effects

1. Acute toxicity

Sangdee and Lhieochaiphunt reported that the degree of acute toxicity of the aqueous extract of whole part of *C. quadrangularis* studied in mice, rats and rabbits by intraperitoneal injection and orally through gastric tube ranged from practically non toxic to slightly toxic. (พักตร์พริ้ง แสงดี และ สรศักดิ์ เหลี่ยวไชยพันธุ์, 2528).

Acute oral toxicity test of *C. quadrangularis* in rats showed no toxic signs and no mortalities occurred within the observation period of 14 days. Rats were designed to requirement defined in the Limit test of OECD guidelines (1993) and both sexes were fed with *C. quadrangularis* in the dose of 2000 mg/kg (กฤติยา อินทรเผือก, 2543).

Furthermore, acute oral toxicity test of *C. quadrangularis* was studied in mice. Ten mice (Swiss albino), 5 males and 5 females, 8 weeks of age, were used in each group. Mice were given *C. quadrangularis* in the dose of 2000 mg/kg. The result showed that no toxic signs and no mortalities occurred during 14 days of observation period (จักรพงษ์ ลิมปนุสรณ์, 2543).

2. Subacute toxicity

Subacute toxicity study of dried powder of *C. quadrangularis* was conducted according to the OECD guidelines (1993). Wistar rats, 5 weeks of age, 10 of each sex in each group, were gavaged with suspension of *C. quadrangularis* at the doses of 50, 350 and 2,450 mg/kg body weight for 30 consecutive days. Control rats were given with distilled water at the equivolume as the experimental groups. Rats in the satellite group were given dried powdered of *C. quadrangularis* at 2,450 mg/kg body weight for 30 consecutive days and observed thereafter for 14 days in order to study reversibility of adverse effects. No toxic signs and changes of growth rate, food consumption and relative internal organ weights were found in rats after treated with *C. quadrangularis* at the doses of 50, 350 and 2,450 mg/kg body weight. Clinical blood chemistry and hematology of rats were not affected after treated with the low and the medium doses of *C. quadrangularis*. The highest dose of *C. quadrangularis* used in that study caused significant changes of clinical blood chemistry and hematology such as a decrease of

serum creatinine in female rats, decrease of serum albumin in both sexes of rats and also a decrease in total white blood cell count of male rats. Level of serum, creatinine, serum albumin and total white blood cell count of rats were returned to normal levels after drug withdrawal for 14 days. Some histological findings in internal organs of rats in all groups were found but not related to the doses of the drug used.

3. Subchronic toxicity

Early studies Attawish et al. (2002) have documented subchronic toxicity of dried stem power of *C. quadrangularis* in Wistar rats of both sexes. This experiment was conducted according to OECD guidelines (1993) and 20 rats, 10 males and 10 females, 5 weeks of age, were used in each group. Rats in control group received water orally at 10 ml/kg per day whereas rats in the other 4 treated groups were orally administered with dried-stem powder of *C. quadrangularis* for 3 months at the doses of 0.03, 0.3, 3.0 and 30 g/kg body weight/day, which were equivalent to 1, 10, 100 and 1000 fold of the therapeutic dose in human, respectively. The results showed that no difference of rat body weight gain was observed between *C. quadrangularis*-treated and control groups. *C. quadrangularis* did not produce any significant dose-related changes of hematology, clinical blood chemistry, and histopathological lesion of several internal organs.

4. Mutagenic activity

Sivaswamy et al. (1991) reported that fresh fruit of *C. quadrangularis* possessed a very low mutagenic activity against *Salmonella thyphimurium* (TA 1537) and *Salmonella thyphimurium* (TA 98), but not against *Salmonella thyphimurium* (TA 1535) and *Salmonella thyphimurium* (TA 1538).

5. Chromosome aberration and clastogenic activity

C. quadrangularis extract was given to mice by intragastric route at dosage of 0.1 g/animal. The results demonstrated that *C. quadrangularis* possessed a very low chromosome aberration induction and clastogenic activity (Balachandran et al., 1991).

Analgesic Tests

There are numerous tests of nociception. Experimental studies on conscious animals are often designated "behavioral studies". Thus, describing these tests should be a simple matter of first accounting for the nature of the stimulus (electrical, thermal, mechanical, or chemical) and then describing the behavioral parameters that are measured.

Hot-plate test

The hot-plate technique which is based on the use of thermal stimulation is the test involving the stimuli on the skin. It is often used to determine analgesia that is mediated primarily in the higher centers of the brain and monitors a spinal reflex (and its central modulation) (Woolfe and MacDonald, 1944). The hot-plate test is capable of measuring a complex motor response that presumably involves both supraspinal and spinal mechanisms. There are variations of this test which employ different temperatures in order to determine nociception. The 50°C hot-plate technique also known as a "cold" plate, utilized in some studies, is used often to determine the analgesic activity of weaker analgesics such as aspirin or nonsteroidal anti-inflammatory drugs. The less consistent results associated with this test, however may deter its use. The 52°C hot-plate technique, or "warm" plate, measures the analgesic activity of mildly potent analgesics such as morphine and codeine. A hot-plate maintained at 55°C, is effective when testing potent analgesic compounds including morphine and fentanyl. Tjolse and colleagues (1991) have generated a complex variation of the hot-plate test increasing temperature. This test may be used to reliably determine the analgesic potency of weaker analgesics as well as opioids. The starting temperature is 42°C which is increased at rates that are determined by the investigator, for example 1, 2, 3, 4, 5 or 6°C/min The licking of hind paw signals the onset of pain, and this response, once observed by the investigator constitutes the end point. The time of onset and temperature that elicited the response is then recorded.

The hot-plate test is a technique that is applicable to both mice and rats, which

involves placing an animal on a preheated (50°-56°C) surface (typically an aluminum plate approximately 28 X 28 X 2 cm) that is maintained at specific temperatures by constantly circulating water through passages in the metal plate) and a clear plastic cylinder, 20 cm in diameter and 30 cm in height is placed on the surface to confine the animal during testing and recording the reaction time to the thermal stimulus or the latency of the first sign of discomfort (vocalization, jumping; or lifting, licking or shaking the paws). Animals are placed onto the platform from an elevation of 5 cm and one of two end points used: the interval between reaching the hot plate and licking of the rear paw or jumping from the surface of the metal plate. In the absence of these behaviors the trial is terminated after a defined time (45 sec is cut off time). The average of the last two baseline latencies (BL) observed within 30 min prior to any treatment is compared to a test latency (TL) recorded at various times after a given intervention. The reduction in responsiveness to noxious stimuli can then be expressed as percent maximum possible effect (%MPE) according to the formula:

$$\% \text{ MPE} = [(TL - BL) / (45 - BL)] \times 100$$

Normally, the hot-plate test is a assay of the analgesic efficacy of drugs. In contrast to the tail-flick, it simply highly integrated escape response to noxious stimuli. Analgesic will prolong the reaction time significantly and the dosage required to induce this effect in 50% of the animals (Effective Dose-50, ED_{50}) can be computed.

In addition this technique is test of nociception that involves a thermal stimulus besides the tail-flick test. Theoretically, changes in peripheral blood flow may also cause the response latency change. However, until such investigations have been performed, one should expect that similar problems may occur in the constant temperature hot-plate test in experiments where peripheral blood flow is influenced by drugs or other experimental manipulations. In the increasing temperature hot-plate test, the influence of peripheral blood flow may be reduced. In this test the temperature of the plate increases gradually from a temperature below pain threshold, and the endpoint is the plate temperature when a hind paw lick occurs, regardless of the time it takes before the response occurs. This test therefore be less, if at all, influenced by the pretest skin temperature. For instance, neurotoxic lesions or serotonergic systems do not induce an

apparent "hyperalgesia" in this test as they do in the constant temperature hot-plate test and the tail-flick test (Hunskaar et al., 1986; Bjorkum and Serge. 1998).

Tail-flick Test

The additional analgesic test, which is based on the use of thermal stimulation. The tail flick test first described by D'Amour and Smith in 1941. At the present time this test of analgesia is a typically modified version of the original D' Amour and Smith. Tail-flick test which measures the latency of a rat withdrawing its tail in response to radiant heat from a light beam focused on the dorsal tail surface. In a recent variation of the tail-flick test, an animal is placed in a restraint device such that its tail lies flat on a surface. Following the administration of the test compound, a light beam, which serves as the heat source, is applied to the tail. Response latency, the length of time from applicantion of the light, usually seconds, until the animal remove or flick its tail from the source of heat is measured and compared to the animal's pretest time (Proudfit and Anderson, 1975). In general, the mean of 2 baseline latencies (BL) recorded within 30 min prior to any manipulation is compared to test latencies (TL) observed at various times after imposition of some potentially analgesic treatment. The intensity of the radiant heat source is adjusted to yield mean BL of approximately 2.0 s. In order to prevent tissue damage the heat source is terminated after 6.0 s. The degree of reflex inhibition can then be expressed as a percentage of the maximum possible effect (%MPE) according to the formula:

$$\% \text{ MPE} = [(\text{TL}-\text{BL})/(\text{cut off time} - \text{BL})] \times 100$$

The reaction time of the tail movement varies with the intensity (power) of the source of radiant heat when it is, more intense, the temperature slope is steeper and, consequently, the reaction time is shorter and the movement is greater. The reaction time varies with the surface area stimulated: when the area increases, the reaction time decreases. Similar findings were obtained when electromyographic responses were recorded in the tail muscles. However, it is reaction time also varies with the site stimulated; paradoxically, it decreases when the stimulus is applied to increasingly distal parts of the tail even though the pathway for the afferent signals is longer. Also

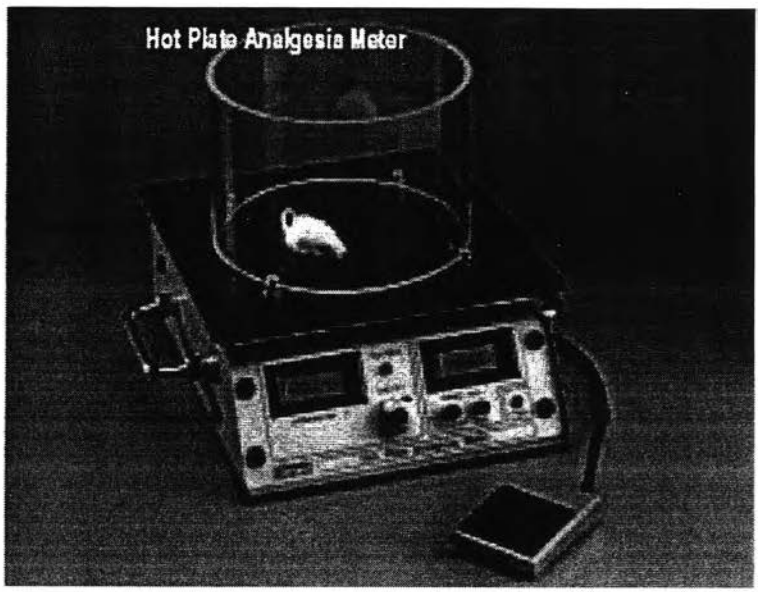
paradoxically, and perhaps as a result of this, pharmacological data can depend on the part of the tail being stimulated. Thus, it can be shown that the test is more sensitive to morphine when the distal part of the tail is stimulated than when a mire proximal part is stimulated, with the middle part giving an intermediate effect (Bars et al., 2001).

There is a distinct problem in this test, that use thermal stimulation, it is the possible influence with skin temperature like in hot-plate test. The tail skin temperature is importance in interpreting the results of the tail-flick test. At least under some circumstances, changes in skin temperature may influence the response latencies. For instance, it is imperative to take the effects of skin temperature into account when investigation factors or using drugs that influence autonomic activity, and thermo- or cardiovascular regulation, such as the monoaminergic systems. The problem may be reduced by recording the tail skin temperature and correcting the tail-flick latency data for changes in the temperature. An alternate method for correction of latencies is to establish the relationship between skin temperature and tail-flick latency in an adequate number of animals under similar experimental conditions, and subsequently to correct tail-flick latencies according to the calculated regression factor (Ren and Han, 1979; Eide and Tjolsen, 1988). This method should be used with caution, because it must be assumed that the experiment is performed under the same conditions as when the correction factor was determined.

Other agents including neuromuscular blockers, skeletal muscle relaxants may have some influence on the tail-flick test since those drugs will prevent animals from removing their tails from a heat stimulus when experiencing pain. The results may also be misinterpreted as analgesia.

Recently a group of researchers has modified the mouse tail-flick method for evaluation the duration of sensory block of local anesthetics (Grant et al., 1993). In this model, agents were administered directly at the site of neural communication between the thermal stimulation and the central processing component of this spinal reflex response. They utilized a 3D-gauge needle and administered 20 µl on each side of the tail near the sixth vertebral caudal segment. They demonstrated dose-related responses for assessing the activity of local anesthetic in mice.

a)



b)

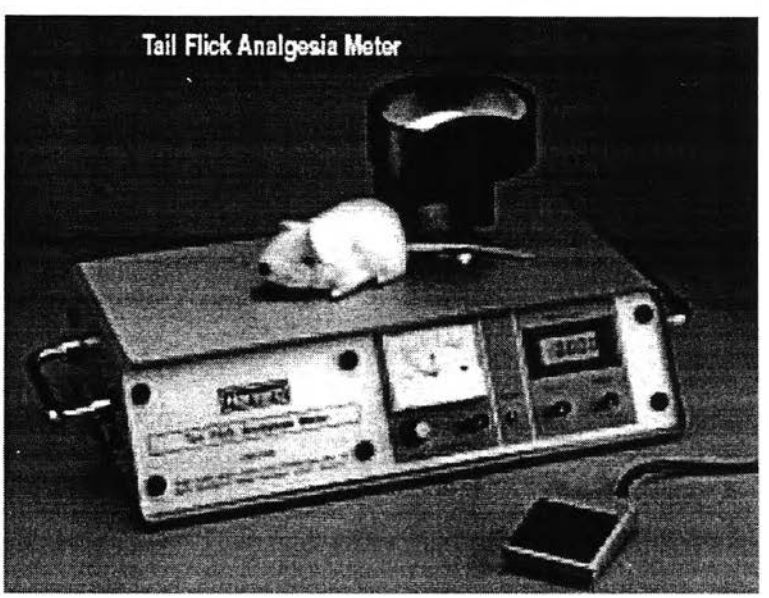


Figure 2-3 a) Hot-plate analgesia meter and b) Tail-flick analgesia meter

Paw-pressure Test

The paw-pressure test is the test based on the use of mechanical stimuli and the preferred sites for applying nociceptive mechanical stimuli is the dorsal surface of the rat's hind paw; using an "Analgesia-Meter" (Ugo-Basile, comerio-Varese, Italy) (Bars et al., 2001; Randall and Selitto, 1957). The Basile Analgesy-Meter has been designed to perform rapid precise screening of analgesic drug according to the Randall-Selitto test (Randall and Selitto, 1957) Test using constants pressure has been abandoned progressively for those applying gradually increasing pressing pressure (a certain of grams per second). This force is continuously monitored by a pointer moving along a linear scale. The force is applied to the animal's paw, which is placed on a small plinth under a cone-shaped pusher with a rounded tip. The plinth is made of teflon, which is biologically inert and has a very low friction coefficient. Thus, if the animal suddenly withdraws its paw, it slips out easily without being injured. The operator depresses a pedal-switch to start the mechanism which exerts the force. When the rat struggles, the operator releases the pedal and reads off the scale the force at which the animal experienced pain. The motor's speed is constant, thus enabling very reproducible measurements to be made. The motor stops immediately when the foot pedal is released.

Since the screw pitch is 16 mm and the motor speed is 1 rpm, the slide moves at a rate of 16 mm per second. The instrument is geometrically designed to increase the force on the paw at a rate of 16 grams per second (16x2 with one disc added, 16x3 with two, etc.). The rate at which the force increases has been found to be a satisfactory compromise for normal operation. Excessively high speed reduces the duration of the test but entails a greater degree of error caused by variations in the operator's reaction time when the pedal is released. On the other hand, if the speed is too low the animal is restrained for a longer period, which is inconvenient for the operator and may cause the animal to make spurious movements before it actually feels pain. The applied force is continuously monitored by an indicator moving along a line scale calibrated in grams x 10 with a pointer riveted to slide, e.g., 11.5 = 115 grams. The scale can be multiplied by 2 or 3 by placing on the slide one or two discs, respectively.

For instance, with one disc on the slide, the force will be 240 g, when the pointer indicates 12. With the pointer in the same position, two discs will give a reading of 360. The force is sufficient to produce indentations of the glabrous skin of the hind paw but without penetration of the skin. The application of force is stopped when the rat starts to struggle (vigorous attempt to withdraw the paw) to a noticeable degree (whether or not accompanied by shrill vocalization). The paw-pressure or paw-pinch thresholds are determined at the withdrawal response. The time at which the rat removes the paw, or struggles to do so, is recorded as the endpoint. Animals failing to react to a 250 g force are given scores corresponding to the full scale (250 g). The average of two successive determinations is taken as the latency for paw withdrawal. The animal is gently held during this test but not restrained.

With the aim of improving the sensitivity of the test, Randall and Selitto (1957) proposed comparing thresholds observed with a healthy paw and with an inflamed paw. The inflammation was induced beforehand by a subcutaneous injection into the area to be stimulated of substances such as croton oil, beer yeast, or carrageenan, the last of these being the most commonly used today. Even though it was found that the sensitivity of the method was improved, it was to the detriment of its specificity because, a priori, two different pharmacological effects analgesic and anti-inflammatory could be confused. It is therefore quite difficult to state that there has been analgesic or even "analgesic" activity. However, a comparison in the same animal of responses triggered from a healthy and an inflamed paw allows this problem to be overcome: nonsteroidal anti-inflammatory drugs (NSAIDs) are inactive on the former but do increase the (lowered) vocalization threshold when pressure is applied to the latter. One can increase the discrimination between different analgesic substances with this test by reducing the rate at which the pressure applied to the paw is increased and by increasing the time limit for subjecting the animal to the stimulus (the cut off time) (Bars et al., 2001).

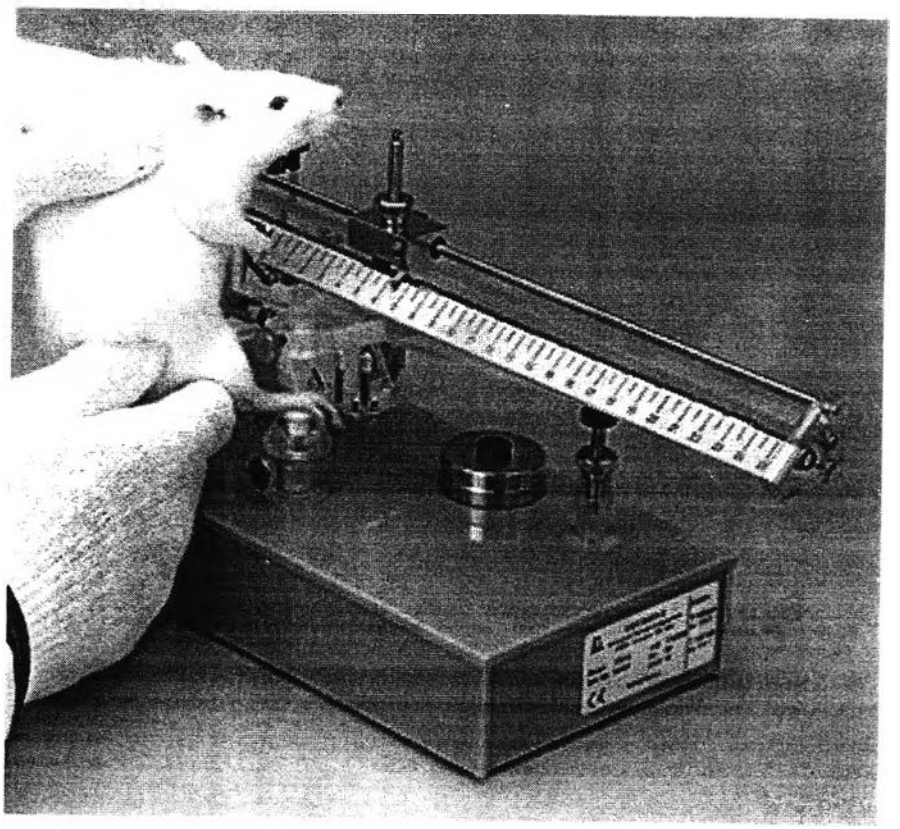


Figure 2-4 Paw pressure analgesia meter