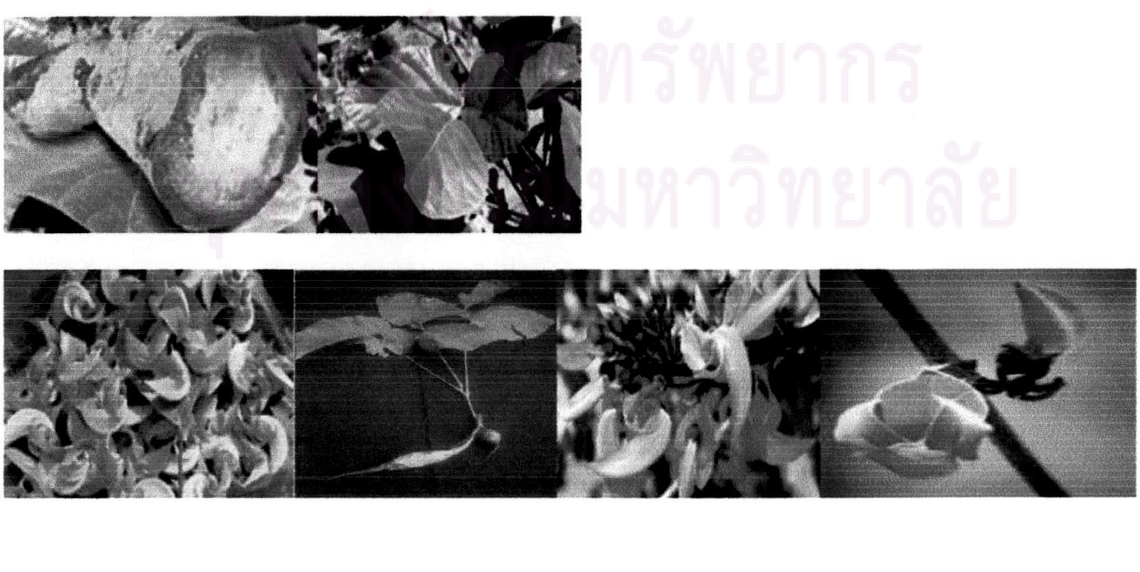
CHAPTER II

THEORY AND LITERATURE REVIEW

Butea superba Roxb. is a herb in the family Leguminosae, subfamily Papilionaceae. It has a common name as Red Kwao Krua. It has the characteristics of being a crawler that wraps itself around large trees. One branch has 3 leaves; the flowers are of a yellowish orange color and the plant grog's out in the open. The long root of the plant is buried under the round-like roots of a yam. This type of plant reproduces through seeds and the separation of its roots. This plant can be found growing in forests in the northern and eastern regions of Thailand. The root and stem of the plant are believed to be medicines for strength and power. This plant has come to be known as one type of miracle herb. Since *Butea superba* helps to enhance the human health, it was considered to be an influential factor in analyzing the chemical constituents of this herb.

Figure 1 Characteristics of *Butea superba* Roxb.



The tuberous root of *Butea superba* was found to contain 5 groups of chemical constituents : carboxylic acid , steroid , steroid glycoside , flavonoid and flavonoid glycoside (Thanatip, 1994)(Table 2.1). β- sitosterol , campesterol and stigmasterol, a steroid group, were reported to be the anti – inflamatory agent, enhancing activity of T – Helper – 1 (TH – 1) cell, and reducing blood sugar by increase insulin secretion (Attern Med.,2001). That three chemicals also have a protective effect on the common cancer (Awad and Fink, 2000), such as colon (Awad , Chen ,Fink and Hennessey, 1996 ;) and prostate (Awad, Fink, Wiliam and Kim , 2001).

Table 1. Summary of the chemical constituents of *B.superba* (Thanatip, 1994)

Category	**Chemicals**
Carboxylic acid	Straight chain carboxylic acid (C22-C26)
Steroid	Campesterol, stigmasterol, B-sitosterol
Steroid glycoside	B-sitosterolyl 1-3-o-B-D-glucopyranoside, Stigmasteryl 1-3-o-B-D-glucopyranoside
Flavonoid	3, 7, 5'-trihydro – 4'-methoxyflavone
Flavonoid glycoside	3-5'-dihydroxy-4'-methoxyflavone-7-o-B-D-glucopyranoside

In 1998, Yadava *et al.* studied a new bio- active flavonoid glycoside from the stems of *Butea superba*. It showed an antimicrobial activity against plant pathogenic fungi; *Trich viride, Asprgillus fumigatus, A. niger, A. terreus, Penicillium expansum, Helmitnhosporium oryzae, Botxitis cinerea, Rhizopus oligosporus, R, chinensis, Kelbsiella pneumoniae, and Fusearium moniliforme*, grampositive bacteria;

Streplococcus pyogenes, Staphylococcus aureus, and Bacillus subtilis, and gram-negative bacteria; *Escherichia coli, Proteus vulgaris, Klebsiella pneumoniae, Pseudomonas aeruginosa.* The maximum inhibitory effect was occurred in *H. oryzne, A. niger, B. cinera* and gram – positive bacteria.

In 2000, Roengsamaran *et al.* studied the activity of flavonoid and flavonoid glycoside from tuber root of *Butea superba.* These compounds showed the higher inhibitory effects on cAMP phosphodiesterase than caffeine and theophylline *in vitro*.

In 2003, Ingkaninan *et al.* studied the inhibitory activity of methanolic extracts of *Butea superba* on acetylcholinesterase in Thai people. The results showed that the extract of *Butea superba* has the 50 – 65 % inhibitory activity on acetylcholinesterase. Generally, acetylcholinesterase inhibitor has been used as a drug for the treatment of Alzheimer's disease.

In 1999, Manosroi *et al.* investigated the LD50 of *Butea superba* in rats. The LD50 was 20 gm/kg.

In 2003, Pongpanparadon *et al.* studied the genotoxic effects of *Butea superba* at doses of 2, 20, 200 and 1,000 mg/kg/day for 9 weeks in rats using the micronucleus test and lethal test. The results showed that 1,000 mg/kg/day of *Butea superba* was significantly induced the formation of micronuclei in polychromatic erythrocytes more effective than the distilled water treatment group (control group). None of these doses had a toxic effect on male reproductive function. There were no differences in the number of implantation sites and dead fetuses produced by female rats that mated with *Butea superba* treated male rats compared to the control group.

In 2003, Cherdshewasart and Nimsakul studied the effect of *Butea superba* on erectile dysfunction in Thai men. They found that there was a significant upgrading in 4 to 5 in the descriptive evaluation of the international index of erectile function – 5 questionnaires. They also found that 82.4 % of the patients exhibited a noticeable improvement on erectile dysfunction from the sexual record.

From the previous reports, it is tempting to speculate that *Butea superba* has an effect to the reproductive functions in male animals as well as in men. It is therefore important to understand the male reproductive system and hormone regulation.

Male reproductive system

The reproductive system in male includes the testis, excurrent duct system and accessory glands. The testis is composed of seminiferous tubules and the interstitial cells of Leydig which are present in the angular spaces between the tubules. Before puberty, the testes are small and have a rubbery consistency. During puberty, the testes increase in size, mainly under the influence of FSH, and their consistency becomes comparable to muscle.

The testis performs two functions that are to a large extent complementary: the proliferation of spermatozoa and the secretion of sex steroid hormones. The sex steroid hormones determine the physiologic state of the accessory ducts and glands and usually condition the appearance of the secondary sex characters (Turner, 1971).

The continuation of each seminiferous tubule near the mediastinum is devoid of germ cells. This short portion is known as the straight tubule (tubulus rectus). The tubule recti coalesce within the mediastinal connective tissue to form a reticular network termed rete testis, whose content is drained by efferent ductules of the testis to the head of the epididymis. The efferent ductules and the epididymis are not simple drainage tubes but have important functions in the maturation of spermatozoa. The accessory glands are the seminal vesicle, the prostate and the bulbourethral glands of Cowper, all of which open into the excurrent duct system. The seminal vesicle is highly convoluted, and secretory epithelium is embedded in thick smooth muscle layers. The lumen of the gland is drained by the short excretory duct, which joins the vas deferens. After these two tubes merge, their continuation is termed ejaculatory duct that penetrate the prostate and join the prostatic portion of the urethra. Similar to the seminal vesicles, the prostate stores its secretory product in the lumen and suddenly propels it into the urethra at the time of ejaculation. The development and function of these organs are androgen – dependent (Kacsoh, 2000).

The major hormone that produced by testis

The major hormone that produced by testis is androgens. The androgens comprise a class of steroids with distinct structural and functional features. The principal testicular androgen is testosterone (T). It is synthesized from acetate and cholesterol by the Leydig cells of the interstitial tissue.

In man, 4-10 mg of T is secreted daily. The hormone rapidly enters both the blood and lymph, but although the concentrations in each are similar, the major quantitative output is to the blood, with its greater flow. However, the lymphatic flow is important as the lymph drainage pathways carry T adjacent to the testicular excurrent ducts and the male accessory sex glands, which are stimulated by it. Not all of T goes into the blood and lymph.

Female reproductive system

Because this study was also performed in female rats, the basic knowledge on female reproductive system is also indispensable. The reproductive system of females consists of the ovaries and the duct system. The later not only receives the eggs ovulated by the ovary and conveys them to the site of ultimate implantation, the uterus, but also receives the sperm and conveys it to the site of fertilization, the oviduct. The ovary functions chiefly in the production of mature eggs and in the elaboration of hormones that regulate the reproductive tract and secondary sex characters, condition the mating reactions, and exert other metabolic effects. Neither the gametogenic nor endocrine functions are continuous processes; they fluctuate rhythmically during the life of the individual. The periodic changes in the female tract are determined by cyclic variations in the pituitary gland and ovaries. The intervals are called estrous cycles in subprimate species and menstrual cycles in human and other primates. Although both kinds of cycles are regulated by the same or similar pituitary and ovarian hormones, they differ in important details and will be discussed later.

The female genital tract consists of a pair of fallopian tubes (oviducts), the uterus, and the vagina. Anatomically the uterus in human is an approximately pear-shaped organ, which has two main portions, the corpus uteri and the cervix uteri. Whereas the uterus shape in rats is different from that of human, a bilateral horn, the anatomy is the same. The uterine cavity is surrounded by three layers of the corpus and fundus of the uterus, the endrometrium, the myometrium and the perimetrium.

The vagina can be divided into two parts: the vestibule and the posterior vagina. The muscular coat is less well developed in the vagina than the other portions of the duct system. It consists of a thin layer of longitudinal fibers and a thicker layer of circular fibers. In normally cycling females, the epithelial lining of the vagina undergoes periodic changes, which are controlled by hormones secreted by the ovaries (Nalbandov, 1964).

The synthesis and secretion of sex steroid hormones in females

Sex steroids in female are synthesized by the cooperative function of granulosa and theca cells. In ovary, the maturing ovarian follicles and (after ovulation) the corpus luteum are the major steroidogenic tissue. The maturing ovarian follicle consists of two adjacent steroidogenic cell populations: the epithelial granulosa cells, and the mesenchyme – derived theca interna cells. The steroidogenic cells of corpus luteum are derived from their preovulatory counterparts and are termed granulosa lutein and theca lutein cells, respectively. The main secreted sexual steroid hormone before ovulation is estradiol(E_2). The main steroid hormones produced by the corpus luteum are progesterone(P_4) and E_2. Normal sex hormone production is achieved by the coordinated function of granulosa and theca cells both before and after ovulation (Kacsoh, 2000).

The Hypothalamic – pituitary - gonadal axis (HPG axis)

The function of hypothalamus

The hypothalamus is a relatively small area at the base of the brain. It is a part of the diencephalon and lies between the midbrain (caudally) and the forebrain. Despite its small size, the hypothalamus is extremely complicated structure with many diverse functions, which include the regulation of sexual and ingestive behaviors, the control of body temperature and the integration of autonomic activity. Each function is associated with one or more small areas of the hypothalamus consisting of aggregations of neurons, called hypothalamic nuclei. The functions of the hypothalamus associated with reproduction involve the supraoptic, paraventricular, arcuate, ventromedial and suprachiasmatic nuclei, and also two less easily defined areas, the medial anterior hypothalamic and medial preoptic areas. These nuclei have either direct neural or indirect vascular connections with the pituitary gland. The gonadotropin – releasing hormone (GnRH) is a decapeptide hormone that produced from the neurons in the hypothalamus. GnRH is assumed to be the most important final common mediator of all influences on reproduction conveyed through the central nervous system (Johnson and Everitt, 1988).

The function of pituitary

The pituitary gland lies in the hypophyseal fossa of the sphenoid bone, overlapped by a circular fold of duramater, the diaphragma sellae, which has a small central opening through which the pituitary stalk, or infundibulum, passes. Infundibulum is the part that connected the pituitary gland to the hypothalamus.

The anterior lobe of the pituitary contains a variety of cell types. The gonadotrophs are one among them. There are remaining the intricate jobs of finding out how the pituitary gland causes the cyclic behavior of the ovary. According to this interpretation, the pituitary gland secretes the gonadotropic complex, which consists of two distinct substances. The two hormones were therefore named, with reference to their effects on the ovary, the follicle- stimulating hormone (FSH) and luteinizing hormone (LH). Later it was shown that both hormones are also secreted by the pituitary gland of males (Nalbandov, 1964).

Regulation of sex steroid hormones and gonadotrophin secretion in females and males

In puberty, the main determinants of gonadal steroid hormone secretion are the pituitary gonadotropins. The rates- limiting step of steroidigenesis in the gonad is depended on LH. LH receptors mediate this action in all gonadal cells: Leydig cell, theca interna, theca lutein, and granulosa lutein cells (Nalbandov, 1964).

In males, the only target of LH is the Leydig cell, the source of all testicular steroid hormones. The only target of FSH is the nonsteroidogenic, sertoli cell, which provides the epithelial lining of the seminiferous tubules. The sertoli cells secrete the protein hormone inhibin B in response to FSH. LH stimulates T secretion from the Leydig cells (Nalbandov, 1964).

In contrast, in females FSH is the physiologic stimulus estrogen secretion before ovulation. The only target of FSH in females is the granulose cell. FSH stimulates the proliferation of granulose cells, the secretion of inhibin B, and the expression of aromatase. The circulating estrogens secreted directly from the ovaries serve at of locally converted androgen (Nalbandov, 1964).

The both T and estrogen exert biologic actions, including a negative feedback on the secretion of gonadotropins both at the hypothalamus and pituitary levels. The rise is largely attributable to removal of T and estrogen, as infusion of this hormone results in the rapid decline of FSH and LH levels. Most of the negative feedback action of T is mediated by estrogen receptors after the local aromatization of T into 17β - estradiol. But full negative feedback also requires androgen receptor – mediated action (Nalbandov, 1964).

In females, if plasma concentrations of E_2 increase greatly, for example 200-400% above those seen in the early follicular phase of the cycle in human or diestrus phase in rodents, FSH and LH secretions are enhanced, not suppressed. Under these conditions, a surge of LH and FSH is produced. The term positive feedback is often used to describe the relationship whereby high levels of E_2 increase the secretion of gonadotrophins, and thereby of E_2 (Johnson and Everitt, 1988).

The estrous cycle of rats

The estrous cycle of the rat is completed in four to five days, although the timing of the cycle may be influenced by exteroceptive factors such as light, temperature, nutritional status, and social relationships. In species having such short cycles, the ovaries contain follicles in various stages of formation, as well as corpora lutea of several past estrous cycles. The cycle is roughly divisible in four stages (Turner, 1971):

1. Estrus. This is the period of heat, and copulation is permitted only at this time. The condition lasts from 9 to 15 hours and is characterized by a high rate of running activity. Under the influence of FSH, a dozen or more ovarian follicles grow rapidly. Behavioral changes include quivering of the ears and lordosis, or arching the back in response to handling or the approaches by the male. The uteri undergo progressive enlargement and become distended owing to the accumulation of luminal fluid. Many mitoses occur in the vaginal mucosa and, as new cells accumulate, the superficial layers become squamous and cornified. The latter cells are exfoliated in the vaginal lumen, and their presence in *vaginal smears* in indicative of estrus. During late estrus, there are cheesy masses of cornified cells (Co) with degenerate nuclei present in the vaginal lumen, but few if any leukocytes are found during estrus. Ovulation occurs during estrus and is preceded by histologic changes in the follicle suggestive of early luteinization. Much of the luminal fluid in the uteri is lost before ovulation.

2. Metestrus. This occurs shortly after ovulation and is intermediate between estrus and diestrus. The period lasts for 10 to 14 hours and mating is usually not permitted. The ovaries contain corpora lutea and small follicles, and the uteri have diminished in vascularity and contractility. Many leukocytes (L) apperar in the vaginal lumen along with a few cornified cells.

3. Diestrus. The lasts 60 to 70 hours, during which functional regression of the corpora lutea occurs. The uteri are small, anemic, and only slightly contractile. The vaginal mucosa is thin, and leukocytes migrate through it, giving a vaginal smear consisting almost entirely of these cells.

4. Proestrus. This heralds the next heat and is characterized by functional involution of the corpora lutea and preovalutory swelling of the follicles. Fluid collects in the uteri and they become highly contractile. The vaginal smear is dominated by nucleated epithlial cells (O), which occur singly or in sheets (Figs 2).

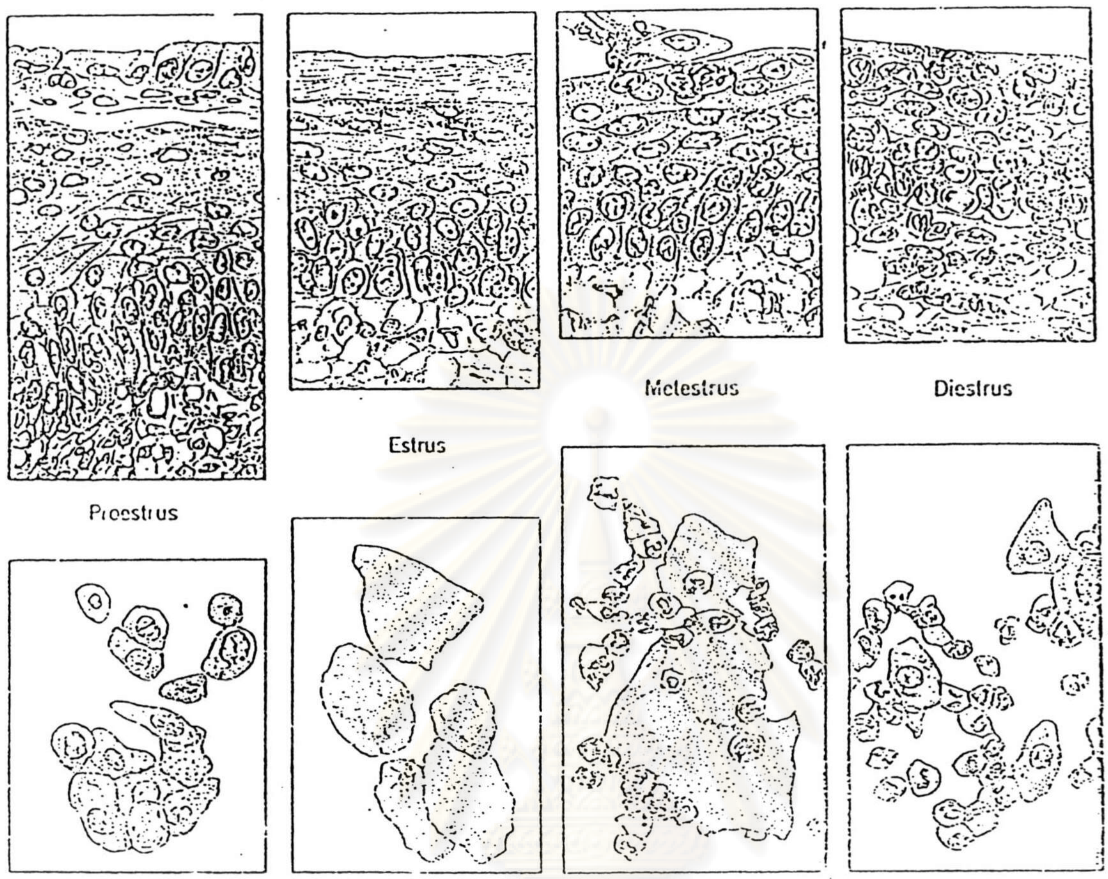


Figure 2. Types of cells in the different stages of estrous cycle by vaginal smear

O represented the nucleated cells in proestrus period

Co represented the cornified squamous cells in estrus period

L represented the Leukocyte cells in metestrus and diestrus period

In this study, changes of vaginal cytology in female rats were used as an indicator for the response of sex steroid hormones, especially for estrogens.