CHAPTER II

LITERATURE REVIEW

I. Hydrophobic base

In the past fifty years ago hydrophobic base were made from petrolatum which is a by-product of petroleum industry. In structure, petrolatum is a two-phase colloidal system of oil and wax, the latter component acts as a gel former preventing a separation of liquid from solid hydrocarbon. Hydrophobic base prepared by petrolatum have many advantages such as extreme inertness, low cost, lack of skin sensitization and ready availability. Even if petrolatum have many advantages it also have disadvantage too. Because petrolatum is of natural origin, the variation about its source and the method by which it is refined are not controllable.

The United States Pharmacopeia recognizes petrolatum melting point between 38 - 60 ° C. Petrolatum of low melting point is a liquid at some periods during a summer while petrolatum of high melting point may be very sticky and difficult to spread. From the disadvantage of petrolatum, wax-thickened mineral oil were developed for replacing petrolatum. There are many materials which will thicken mineral oil for example ozokerite, vegetable wax and paraffin wax but the result also not satisfactory.

Polyethylene, aliphatic hydrocarbon polymer, is suitable polymer to form hydrophobic base with mineral oil. Polyethylene molecular weight 21000 were heated with mineral oil at 130 ° C for about 3 hours with good agitation. The hot solution is allowed to cool until 110 ° C and then shock-cooled to about 50 ° C. Shock-cooling, a term used to describe a very rapid cooling of the hot melt, for cooling rate 10 ° C per second is accomplished by spreading the hot solution in a thin film on a metal surface which is water cooled to a constant temperature, and removing the film from the surface when it reaches 50 ° C. The critical factors in producing a good hydrophobic base are (a) the molecular weight of polyethylene, (b) the

percentage of polyethylene, (c) viscosity of mineral oil and (d) the condition of cooling (Multimer et al.,1956).

Hydrophobic base maintain a desirable consistency over a wide temperature range (-15 to 60 ° C). It will not liquefy at temperatures occurring in tropical climates and will not harden excessively when exposed to relatively low temperatures. Because hydrophobic base dose not liquefy at body temperature, it is easily applied to the skin. From in vitro studies, hydrophobic base containing active constituents such as salicylic acid, sulfanilamide and sulfathiazole showed greater release of the incorporated drug than petrolatum (Foster et al.,1951).

Data obtained from previous studied showed that the method manufacturing hydrophobic base requires highly specialized equipment and high processing temperature ;therefore, the new simplified process for preparing polyethylene - mineral oil gels were purposed. Polyethylene molecular weight 1500-21000 were mixed with mineral oil by using high shear mixer. The use of lower molecular weight polyethylene make it possible to reduce the dissolution temperature from 130 ° C to 90-95 ° C and holding time from three hours to one hour. The gels prepared by the high shear process are much more stable at all storage temperature than those prepared by conventional mixing (Thau et al.,1956).

Rheological properties of semisolid produced by polyethylene and mineral oil were studied by several workers. Continuous shear viscometry was used to describe rheological characteristic of hydrophobic base. Hydrocarbon base containing 5 % polyethylene showed appropriate rheological characteristic. Flow behavior of hydrophobic base demonstrate a pseudoplastic characteristic. Viscosity of hydrophobic base were increased with increasing of percentage of polyethylene while when the temperature were increased viscosity of hydrophobic base were decreased (Davis et al.,1980). Besides factors also influencing viscoelastic property of hydrophobic base were temperature, method of manufacture and excipients in formulation (Davis et al.,1981).

II. Gelling agents

There are many gelling agents used in pharmaceutical formulation. Gelling agents are classified according its chemical nature of the molecule as following: (a) most natural gum such as acacia, carrageenan and xanthan gum, (b) cellulose derivatives such as sodium carboxy methyl cellulose (SCMC), hydroxyl ethyl cellulose (HEC), hydroxyl propyl cellulose (HPC) and hydroxyl propyl methyl cellulose (HPMC), (c) polyethylene and its polymer which use in gelling oil, (d) polypeptides such as gelatin and (e) synthetic block copolymers, like poloxamer.

Five types of gelling agents which were chosen in this study are SCMC, gelatin, xanthan gum, pectin and chitosan. There are varies in the properties as following:

Sodium Carboxy Methyl Cellulose (SCMC)

SCMC is an anionic polymer available in variety of grades that differ in molecular weight and degree of substitution. Gel characteristic, such as firmness and elasticity, depend on molecular weight and polymer concentration. Sequestrants are useful in controlling the availability of free cations and preventing polymer precipitation.

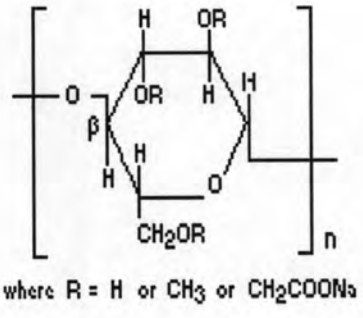


Figure 1 Structure of SCMC

Gelatin

Gelatin is used widely as a bodying agent and gel former in food industry, and in pharmaceutical products. Solution of gelatin will form gel at low temperature. Gel prepared from gelatin using in pharmaceutical products as suppositories base. Gelation temperature and melting point of gel prepared from gelatin is about 20-40 ° C. These temperatures will increase with increasing of the concentration and molecular weight of gelatin. Viscosity of gel prepared from gelatin also depends on concentration and molecular weight of gelatin.

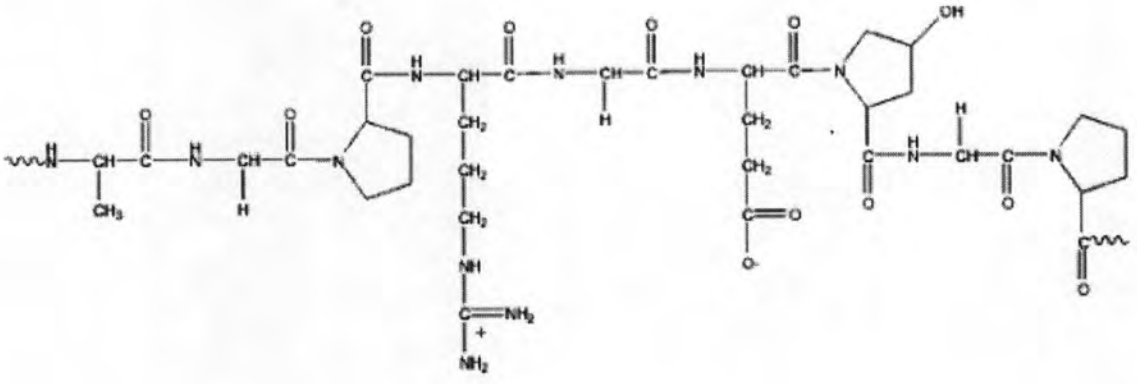


Figure 2 Structure of gelatin

Xanthan gum

Although xanthan gum is used most frequently as a stabilizer in suspensions and emulsions at concentration below 0.5 %, higher concentrations in aqueous media (1% and above) yield viscous solution that are jellylike in nature (Zatz and Knapp,1984). Xanthan gum is produced by bacterial fermentation, and its availability and quality are not subject to many of the uncertainties that affect other natural products. Thermally reversible gels result from combination of xanthan with guar or locust bean gum.

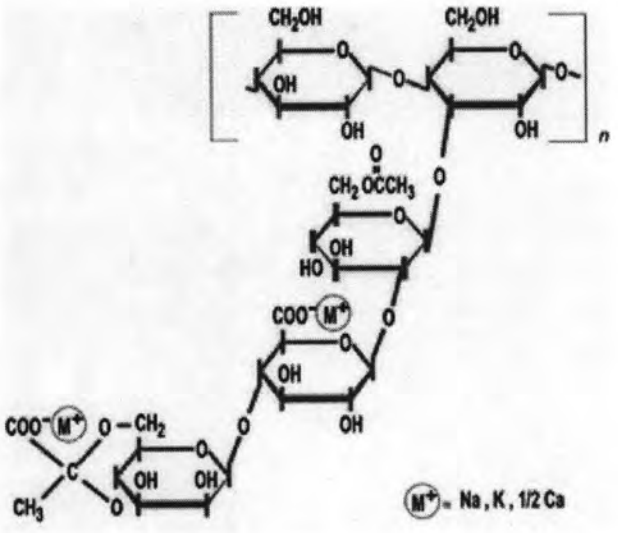


Figure 3 Structure of xanthan gum

Pectin

Pectin, the polysaccharide extracted from the inner rind of citrus fruit or apple pomance (USP 23), may be used in pharmaceutical jellies as well as in foods. The gel is formed at an acid pH in aqueous solutions containing calcium and possibly another agent that acts to dehydrate the gum (Towle and Christensen,1973). Pectin used in pharmaceutical or cosmetic products should be free of such additives.

Gel formation is more extensive in pectins with a low methoxy content. Such properties as gel strength depended on a host of factors, which include concentration of additives and pH, in addition to the characteristics of raw materials (Towle and Christensen,1973).

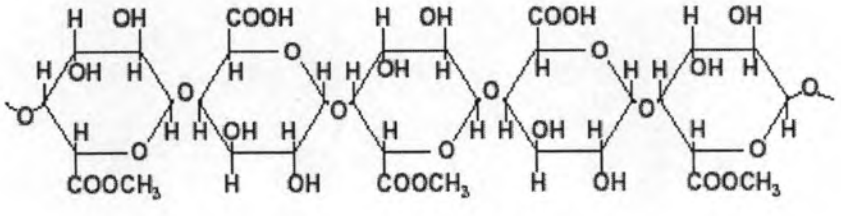
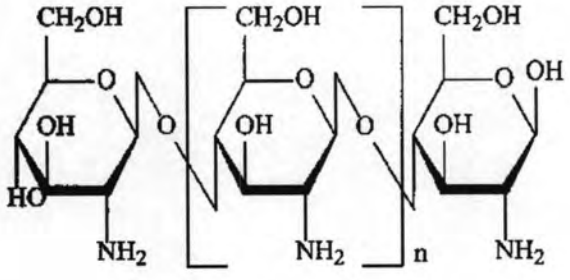


Figure 4 Structure of pectin

Chitosan

Chitosan as shown in Figure 5 is a natural biopolymer derived from the outer shell of crustaceans (Standford,1992). Chitin is extracted and partially deacetylated to produce chitosan. Unlike most gum, chitosan carries a positive charge at pH below it pKa of 6.5 and is attracted to a variety of biological tissues and surfaces containing saliasic acid that are negative charged. Chitosan can not dissolve in water, dilute acid, dilute alkaline and other organic solvents but soluble in concentrated acid. Concentrated aqueous solution have a gel-like consistency (Lllum.1998). Chitosan hydrogel could acts as a dressing for would occlusion and accelerator in healing process (Ishihara et al., 2002). Beside, chitosan could also accelerate open wound healing in dogs (Okamoto, 1995 and Ueno, 1999).



Chitosan

Figure 5 Structure of chitosan

III. ACTIVE CONSTITUENTS

ASIATICOSIDE

Centella asiatica (Linn) Urban, the synonym *Hydrocotyle asiatica* Linn., was classified in Umbelliferae family. It widely distributed in eastern asia, central america and eastern south america. The shriveled leaf fragments are brown-green to gray-green. The pieces of stolen are thin, and the fragments of the inflorescences bear 1-2 mm long involucral bracts. This low-growing (only 10-50 cm high) perennial herb has reniform, long-petioled leaves and pedunculate umbels bearing small white to purplish pink flowers (Brinckmann and Lindenmaier, 2004).

The active principles of *Centella Asiatica* are asiatic acid, asiaticoside, madecassic acid and madecassoside. There are pentacyclic triterpines belonging to the β-amyrin ursolic acid group as shown in Figure 6. Different uses are claimed for the plant, these compounds are known their major clinical indications for treatment of wounds, gastric ulcers, keroids, chronic venous insufficiency, varicose vein and it use as a constituent of brain tonic for the mentally retarded (Inamdar et al.,1996).

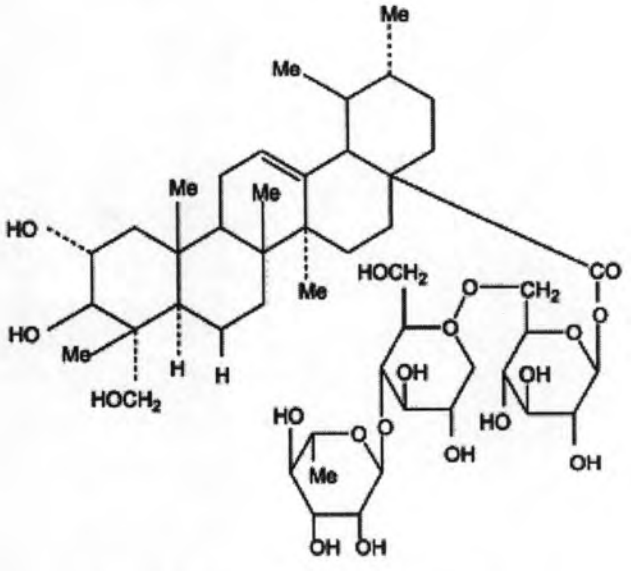


Figure 6 Structure of asiaticoside

Tritrated extract of *Centella Asiatica* (TECA) and total triterpenic fraction of *Centella Asiatica* (TTFCA) are the extracts of *Centella Asiatica* that were studied generally. The TECA is combination of 40 % of asiaticoside, 30 % of madecassic acid and 30 % of asiatic acid while TTFCA is compose of 40 % of asiaticoside and 60 % of madecassic acid and asiatic acid in a ratio that is not clearly defined.

The preparations of *Centella Asiatica* used in conventional medicine are available in oral dosage form, topical agents and in the form of injections.

Titrated extract of *Centella Asiatica* (TECA) is a poorly water – soluble drug extracted from *Centella Asiatica*. TECA solution formulated by dissolving the TECA in propylene glycol are known to be clinically effective on systemic scleroderma , abnormal scar formation and keroids by strongly inhibiting the biosynthesis of acid mucopolysaccharides and collagens in carrageenin granulomas (Sasaki et al., 1972; Tallat and abbas, 1971 ; Kiesswetter, 1964). The intramuscular injection of propylene glycol-based preparation produced swelling, pain and stiffness on the injection site due to hypertonicity and tissue damage. The micellar solution of TECA formulated by solubilizing TECA into a mixture of propylene glycol and ethoxylated hydrogenated castor oil could reduce the pain arising from the intramuscular injection (Kim et al., 1997). Because it was physically unstable during storage the micellar system with mixture of surfactants were developed to solve this disadvantage. Preparation of micellar system with a mixture of surfactants can have the advantage of controlling the hydrophile-lipophile balance (HLB) in proportion to the mixing ratio of surfactants. Tween 20 and tween 85 were used as surfactants to develop a physically stable mixed micellar system (Kim et al., 2001).

Wound Healing Agent

Healing is a physiological process that does not normally require much help but the wound may have opportunity to infection and other complications. So, the agents that expediting healing are required. Some diseases such as diabetes, immunocompromised conditions, ischaemia and some conditions like malnourishment, aging, local tissue damage lead to delay in healing process. Some diseases specially require healing agent which can facilitate healing. Asiaticoside extracted from the plant *Centella Asiatica* has been studied for the wound healing activity in normal as well as in diabetes animals. The plants selected as wound healing agents were based on its traditional medicinal use and reported pharmacological activities like fibroblast proliferation (Veechai et al.,1984) and stimulation of collagen synthesis (Maquart et al.,1990). Topical formulation of asiaticoside significantly enhanced the rate of wound healing as assessed by increase in collagen synthesis and tensile strength of the wound tissue in normal animal as well as in diabetes animal. In addition, the same results were occurred in normal animal that received asiaticoside formulation by oral route (Shukla et al.,1999).

Basic fibroblast growth factor (bFGF) is one of local growth factors that helps in the wound healing process. It is possible that asiaticoside may have a growth factor like activity or has the ability to stimulate the expression of growth factor like basic fibroblast growth factor. Asiaticoside promoted angiogenesis which plays an important role in wound healing and newly formed blood vessel in both in vitro and in vivo models as indicated by histological studies and new vessel formation in chick chorioallantoic membrane (CAM) model (Shukla et al.,1999).

When applied madecassol, an ointment formulation based on *Centella Asiatica* plant extract, locally on the wounds in rat it could reduce the proliferation of granulation and increased tensile strength. Moreover, it decreased the wound area of the skin necrosis induced by burn (Inamdar et al.,1996).

Gastric ulcer healing

Gastric ulcer healing is an orchestrated complex process. It involves resolution of inflammation and repair of gastric tissues through granulation tissue formation, re-epithelialization and extracellular matrix remodeling. Angiogenesis, the regrowth of blood vessels, occurred into ulcerated area plays an important role in the acceleration of ulcer healing because the neovasculature promotes nutrient supply to the healing tissue. Basic fibroblast growth factor (bFGF) stimulate epithelial cell proliferation and angiogenesis thus are important to the healing of gastric ulcer (Buntrock et al.,1982). Cheng and Koo, 2000 reported that asiaticoside inhibited ethanol induced gastric mucosal lesions. Moreover, in the acetic acid induced gastric ulcer rats, which are pathologically similar to chronic gastric ulceration in human, asiaticoside reduced the size of the ulcers in a dose-dependent manner. Epithelial cell and angiogenesis were promoted and bFGF was also upregurated in the ulcer tissues in rats treated with asiaticoside (Cheng et al., 2004).

Varicose Veins

Varicose veins are dilated, tortuous superficial veins that result from defective structure and function of the valves of the saphenous veins, from intrinsic weakness of the vein wall, from high intraluminal pressure. Varicose veins can be categorized as primary or secondary. Primary varicose vein originate in the superficial system and occur two to three times as frequently in woman as in men. Approximately half of patients have a family history of varicose veins. Secondary varicose veins result from deep venous insufficiency and incompetent perforating veins or from deep venous occlusion causing enlargement of superficial veins that are serving as collaterals (Kasper et al., 2005).

Patients with venous varicosities are often concerned about the cosmetics appearance of their legs. Symptoms consist of a dull ache or pressure sensation in the leg after prolonged standing; it is relived with leg elevation. The legs feel heavy, and mind ankle edema develops occasionally. Extensive venous varicosities may cause skin ulcerations near the ankle. Visual inspection of the legs in the dependent position usually confirms the presence of varicose veins

Varicose veins can usually be treated with conservative measures. Symptoms often decrease when the legs are elevated periodically, when prolonged standing is avoided, and when elastic support hose are worn. Small symptomatic varicose veins can be treated with sclerotherapy, in which a sclerosing solution is injected into the involved varicose vein and a compression bandage is applied. Surgical therapy should be reserved for patients who are very symptomatic, suffer recurrent superficial vein thrombosis, and/or develop skin ulceration (Kasper et al., 2005).

Centella Asiatica has been reported to improve the blood circulation in the lower limbs. Stimulation of collagen synthesis in the vein wall resulted in an increase in vein tonicity and a reduction in the capacity of the vein to distend. Total triterpenic fraction of *Centella Asiatica* (TTFCA) has been noted to reduce ankle edema, foot swelling, and capillary filtration rate, as well as to improve microcirculatory parameters in subjects with reported venous insufficiency of the lower extremities (Cesarone et al., 1992).

Chronic Venous Insufficiency

Chronic venous insufficiency, usually occurred in conjunction with varicosis, is a particularly common disease picture, especially in civilized countries. The condition is preceded by manifest or silent deep vein thromboses, which cause changes in the vessel wall and lead to destruction of the venous valves. This gives rise to a typical complex of symptom which manifest themselves as expressions of vascular stasis. The typical symptom include local edemas, especially in patients with static stresses, calf cramps, pain, sensations of fatigue and heaviness, and leg itch. In the late stages of disease, chronic leg ulcers occur as an expression of impaired perfusion. According to current scientific opinion, weakness of the connective tissue is not treatable. Support and compression are therefore the foremost therapeutic measures, followed by sclerosis and surgery (varicotomy). However, the symptom complex of venous disease can be effectively treated using selected herb drugs before invasive measures become necessary (Weiss and Fintelmann, 2000).

Total triterpenic fraction of *Centella Asiatica* (TTFCA) may have benefits on objective and subjective parameters in associated with chronic venous insufficiency. The treatment group experienced a significant decrease in resting flux, improvement in venoarteriolar response and decrease in leg volume. No significant changes were observed in ambulatory venous pressure or refilling time (Cesarone et al., 2001). Subjects with venous insufficiency received TTFCA three times daily by oral route for 4 mouths were found to have significantly decreased capillary filtration rates and ankle edema (De Sanctis et al., 2001).

Table 1 Clinical studies of *Centella Asiatica* in patients (Ulbricht and Basch, 2005)

Condition treated	**Author, Year**	**Dose and administration**	**N**	**SS**
Varicose veins, CVI	Marastoni, 1982	Centellase 2 tabs tid	34	Yes
Varicose veins, CVI	Pointel, 1987	TECA 120 mg bid	94	Yes
Varicose veins, CVI	Belcaro, 1989	TTFCA 120 mg/day	99	Yes
Varicose veins, CVI	Belcaro, 1990	Centellase 60mg tid	62	Yes
Varicose veins, CVI	Belcaro, Grimaldi, 1990	TTFCA 60mg bid	44	Yes
Varicose veins, CVI	Cesarone, 1994	Centellase 60 mg bid	90	Yes
Varicose veins, CVI	De Sanctis, 1994	Centellase 60 or 120 mg/day	25	Yes
Varicose veins, CVI	Cesarone, 2001	TTFCA 60mg bid	40	Yes
Varicose veins, CVI	De Sanctis, 2001	TTFCA 60mg tid	52	Yes

N = Number of patients, SS = Statistically Significant

bid = twice daily, tid = three times daily, CVI = Chronic Venous Insufficiency

Mangostin

Mangosteen, *Garcinia mangostana L.*, is a tree classified in family Guttiferae. Mangosteen found in Sri lanka and other South East Asian countries is very popular due to its delicious fruits. Not only the good taste of this fruit but it also has many medicinal properties. The fruit hull (pericarp) of this plant is used as an anti - inflammatory agent, astringent, healing skin infection, healing wound and in the treatment of diarrhea (Mahabusarakum et al,1986 and Mahabusarakum et al,1987). The fruit hull of this plant has been reported to contain the major product, mangostin. Mangostin, xanthone derivatives, were yellowish powder and odorless. From the molecular structure shown in Figure 7, it can be seen that mangosteen is a hydrophobic compound which is insoluble in water, but is highly soluble in many organic solvents such as alcohol, ether and acetone. There are many studies that revealed a number of pharmaceutical activities of this chemical for example cardiotonic, antimicrobial, anti-hepatotoxic and anti-inflammation (Yoshida et al.,1995).

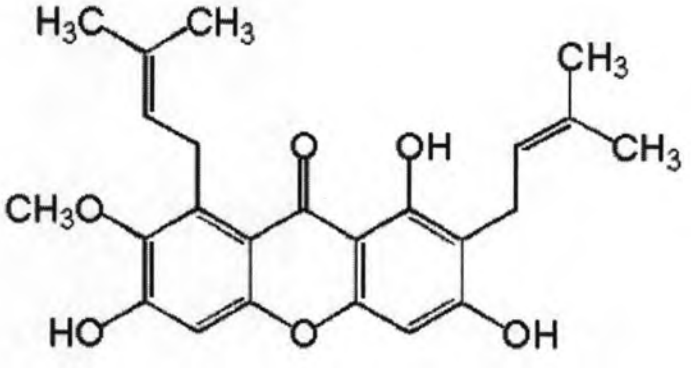


Figure 7 Structure of mangostin

Antimicrobial activities of mangostin were studied by several workers. Iinuma et al, 1996 reported that extracts of *Garcinia mangostana* showed inhibitory effects against the growth of *S. aureus* and methicillin- resistant *Staphyllococcus aureus* (MRSA). Minimum inhibitory concentration (MIC) of mangostin inhibiting the growth of MRSA were 1.57-12.5 μg/ml. The activity of mangostin against MRSA is nearly equal to that of the antibiotic vancomycin (MIC 3.13-6.25 μg/ml). When mangostin and antibiotic (vancomycin) were used in combination the anti-MRSA activity of mangostin was increased by the presence of vancomycin at a concentration of 0.32 μg/ml. The same result was reported that the minimum inhibitory concentration, MIC_{90} of mangostin on MRSA was 3.125 μg/ml and mixture of mangostin gave the MIC_{90} value of 1.48 μg/ml which was equivalent to MIC_{90} of vancomycin (Phongpaichit et al., 1994).

Garcinia mangostana extracts showed promising antibacterial activities against both *Propionibacterium acne* and *Staphyllococcus epidermidis* which triggering an inflammation of skin and causing acne vulgaris. The MIC value were the same (0.039mg/ml) for both bacterial species. So, this plant might be an alternative treatment for acne (Chomnawang et al., 2005).

It was found that mangostin at a dose of 50 mg/kg exhibits pronounced anti –inflammatory activity in carrageenin-induced paw edema, cotton pellet implantation and granuloma pounch models (Shankaranarayan et al., 1979). Moreover, mangostin showed the anti –inflammatory activity three times of aspirin and this activity equal to that of phenylbutazone (Attchara, 1988).

In preliminary toxicological study of mangostin revealed that extracts of mangostin were not harmful to experimental animals (Sornprasit et al.,1987). In clinical studies, in a 2- week study of 66 subjects with ecthyma randomized to receive topical cream containing 2 % of mangostin, a significant decrease of sign and symptom of ecthyma were seen in patient (83.33%). The activity of mangostin cream is equal to that of gentamycin cream (Mahabusarakam et al., 1986).

IV. Oral Ulcers

There are many oral ulcer diseases in the present time. Each disease will show different violation and require different treatment too. Apthous ulcer is a disease that occurs frequently but not harmful. Even if recent recurrent ulceration and oral lichen planus are rarely found disease but it require the special treatment by specialist.

Apthous ulcers are very common and easy to recognize. They may be single or multiple round ulcerations with yellow-gray fibrinoid centers surrounded by red halos. Found on non keratinized mucosa, (eg. buccal and labile mucosa) the lesion may be 1-2 mm. in diameter and cause painful. The cause of this disease remains uncertain but may be associated with stress, constipation, chronic fever and autoimmune. The painful stage lasts 7-10 days but may recurrent monthly or several time a year. Treatment is nonspecific. Topical steroids such as 0.1 % triamcinolone acetonide or 0.05% fluocinonide in an adhesive paste appear to provide symptomatic relief (Lawrence et al., 2000)

Oral lichen planus is a common chronic inflammatory disease of oral mucosa (Scully and Elkom.,1985). The erosive type of oral lichen planus can be particularly painful and may interfere with eating, speaking and swallowing (Loitz and O' Leary.,1986). The pathology of oral lichen planus which found in patients are reticular, plaque – like, atrophic, and ulcerative lesions (Lodi et al., 2002). Long – standing erosive and atrophic oral lichen planus can in a few instance be transformed into squamous cell carcinoma (Krutchkoff et al., 1978). Although the etiology of lichen planus still unknown, there are indications that it may be associated with stress, some systemic disease, drug and immunologic disorders (Walsh et al.,1990). Hypothesis for the immunophathogenesis of oral lichen planus are illustrated in Figure 8.

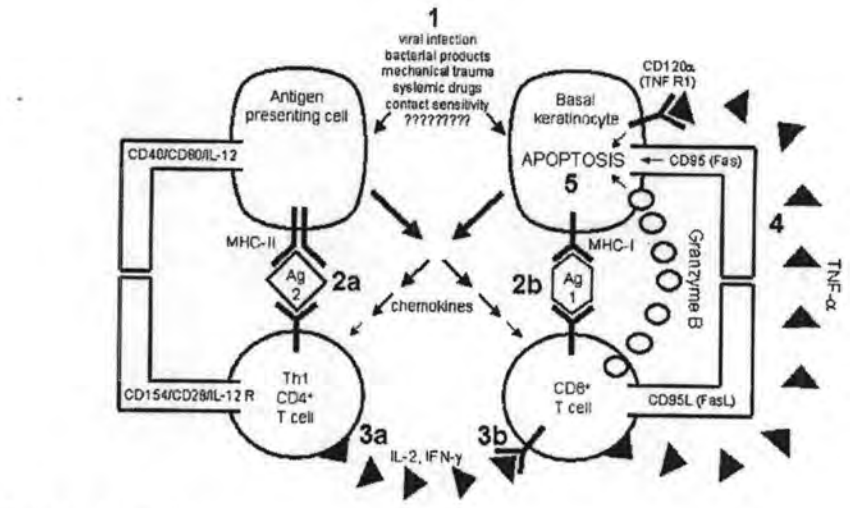


Figure 8 Hypothesis for the immunophathogenesis of oral lichen planus

Antigen presenting cell (APC) and basal keratinocytes are activated by viral infection, bacterial product, mechanical trauma, systemic drugs, contact sensitivity or an unidentified agents. After activation activated APCs and keratinocytes secrete chemokines that attract lymphocytes into the developing oral lichen planus lesion. Activated APCs present antigen associated with MHC class II to $CD4^+$ T cells (2a). Activated basal keratinocytes present antigen associated with MHC class I to $CD8^+$ T cells (2b). CD40 and CD80 coexpression and interleukin (IL) 12 secretion by MHC class II $^+$APCs promotes a T helper-1 (Th 1) $CD4^+$ T-cell response. Th1 $CD4^+$ helper T cells secrete IL-2 and interferon-gamma (INF-gamma) (3a)., which bind their respective receptor on $CD8^+$ T cells (3b). Activated antigen-specific $CD8^+$ cytotoxic T cells express FasL or secrete granzyme B or TNF – alpha (4) that trigger basal keratinocyte apoptosis (5) (Lodi et al., 2005). Treatment regimens have been attempted to improve the refractory lesions but complete cure has not been accomplished because of its recalcitrant nature (Lozada-Nor and Miranda.,1997). Therefore, potent topical steroid applications such as 0.1 % fluocinolone acetonide in oral base have been widely used for reducing pain and inflammation (Thongprasom et al., 2002).