CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Botanical Description of Garlic

Garlic, Allium sativum of the Liliaceae family, is widely cultivated perennial herb. It is native to Asia and naturalized in South Europe and North America. Its leafless flower stem grows to a height of 60 cm. The garlic bulb has a membraneous skin enclosing up to 20 bulblets, called cloves. The bulb has a pungent aroma and taste and is a classic flavouring agent in cooking.

Nutritional Values

Garlic bulbs contain various kinds of nutrients, for example, carbohydrate (in the form of cellulose), fat, protein, vitamins, and minerals. The nutritional composition and the composition of vitamins and minerals of garlic bulbs are shown in Tables 1 and 2, respectively.

Chemical Constituents

Chemical investigations have shown that garlic consists of the essential oil of which sulfur-containing components of different distilled fractions are diallyl sulfide ($C_6H_{10}S$), diallyl tetrasulfide ($C_6H_{10}S_4$), etc.

Table 1. Nutritional Composition of Garlic Bulbs.

Nutrient	Percent
Energy	84 cal/100 gm
Humidity	77
Protein	3.4
Fat	1.1
Cellulose	10.7
Vitamins	0.4
Minerals	0.8
Volatile oils and others	6.6

Table 2. Composition of Vitamins and Minerals in 100 gm of Garlic Bulbs.

Vitamin and Mineral	Unit (I.U. or mg)
Vitamin A	16 I.U.
Carotene	50 I.U.
Thiamine	0.22 mg
Riboflavin	0.08 mg
Vitamin C	20 mg
Calcium	18 mg
Sulfur	67 mg
Iron	1.7 mg

(Semmler, 1892). The primary odoriferous component of crushed garlic cloves is diallyl disulfide oxide or diallyl thiosulfoxide ($C_6H_{10}S_2O$, M.W. 162) (Fig. 1a), which is called "allicin" (Cavallito et al., 1944). Allicin is formed enzymatically by alliinase from an amino acid called "alliin", (+)-s-allyl-L-cysteine sulfoxide (Fig. 1b) (Stoll and Seebeck, 1951). Pyruvate and ammonia are also simultaneously formed in the chemical reaction (Fig. 1c).

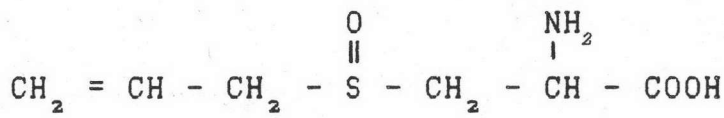
Pure allicin is an oily liquid, not stable at room temperature and decomposes into different products, eg. ajoen and vinyldithiines. It is unstable toward alkalies, yielding the disulfide and sulfur dioxide. It is soluble in most organic solvents such as alcohols, ketones, ethers, halogenated hydrocarbons, and aromatic hydrocarbons (Jansen et al., 1987).

In order to obtain reliable quantitative determination of allicin it has to be considered that solvents used for extraction may affect the activity of the alliinase present. Alliinase is not inactivated by pentane, diethyl ether or chloroform whereas a methanol/water mixture with a content of more than 80 % methanol inhibits the enzymes (Jansen et al., 1987). It has been claimed that garlic extraction with chloroform gives pure allicin and preservation of allicin extract with povidone makes it stable for about one year. The allicin extract obtained can

be examined by thin-layer chromatography (TLC) at relative flow of 0.70-0.75 (Poolsanong, 1984).

a.

b.



c.

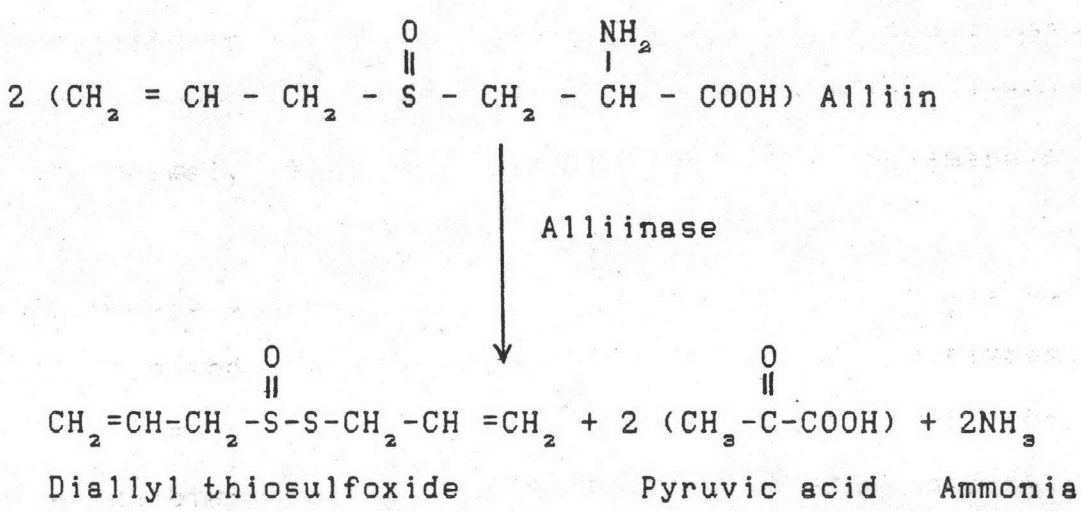


Fig. 1. a. Chemical structure of allicin.
b. Chemical structure of alliin.
c. Chemical reaction of alliin-alliinase system.

Pharmacological Effects

Garlic is widely used as a condiment and food. It has been used in folk medicine since ancient times and is still used in many parts of the world. Several investigations have demonstrated that the active principle in garlic is allicin. Garlic extract exerts some antibacterial activities against Mycobacterium tuberculosis (Rao, Rao, and Venkataramon, 1946), Salmonella typhimurium and Escherichia coli (Johnson and Vaughn, 1969). It has been also shown to possess antifungal properties against yeast-like fungi, dermatophytes, and dimorphic fungi (Moore and Atkin, 1977). Allicin has been reported to have antitumor activity since it blocked cyclophosphamide-induced genotoxicity (Goldberg and Josephy, 1986) and it selectively inhibited glutathione-dependent PGH_2 to PGE_2 isomerase in mammary adenocarcinoma cells (Shalinsky, McNamara, and Agrawal, 1989).

The hypocholesterolemic (Augusti and Mathew, 1974; Bordia, 1981) and hypoglycemic (Jain and Vyas, 1975) properties of garlic oil extract have been reported. These effects may be explained by the inhibition of cholesterol and fatty acid biosynthesis in liver enzymes and chicken hepatocytes (Qureshi et al., 1983). It has been also demonstrated that garlic oil extract had the lowering effects on the levels of two enzymes, alkaline phosphatase and alcohol dehydrogenase in the serum, liver and kidney of

rats fed on high sucrose and alcohol diets (Agoda and Osuji, 1986). The oil extract is of the advantage to use in the partial treatment of diabetes (Mathew and Augusti, 1973) and to use in the prevention of atherosclerosis and coronary heart disease (Jain, 1978; Bordia and Verma, 1980).

Garlic also has some cardiovascular-modulating activities. It has been demonstrated increasing fibrinolytic activity (Watananukul et al., 1988) and decreasing platelet aggregation (Bordia, 1978). Both effects may be useful in the prevention and treatment of atherosclerosis and coronary heart disease.

Effects on Reproductive System

In male, it has been demonstrated that chronic administration of garlic powder resulted in spermatogenic arrest in albino rats (Dixit and Joshi, 1982). Furthermore, it has been shown to possess spermicidal effect (Qian et al., 1986).

The study conducted on growth of reproductive system in rats, 40 days of age, fed on garlic solution at doses of 1, 5, 10, 50, 100 and 150 mg/day for 7 and 10 days showed that weight of ovaries and uterus did not change significantly. It was concluded that garlic did not stimulate the growth of ovaries and uterus and/or pituitary gonadotropins (Borvonsin and Thongkukiat, 1986).

In ancient times, garlic was used, concerning reproductive effects, as emmenagogue (Saha, Savini, and Kasinathan, 1961), abortifacient (Prakash and Mathur, 1976), and aphrodisiac agents (Czajka et al., 1978). Studies in animals on the effects of garlic on uterine motility have demonstrated estrogenic activity in ovariectomized rat (Lorenzo and Orellana, 1955), immature rat (Tewari, Mapa, and Chaturvedi, 1976) and guinea pig (Bickoff, 1963). The experiment in isolated guinea pig uterus demonstrated that 50 mg/ml of garlic extract was equivalent to 0.003 I.U. of oxytocin in its contractile action (Saha, Savini, and Kasinathan, 1961). In vitro study has demonstrated increasing in rate of contraction of rat uterus during proestrus and diestrus (Borvonsin and Chumpolbunchorn, 1988). In human, alcoholic extract of garlic increased contraction of nonpregnant uterus (Tinao and Terren, 1955; Lorenzo et al., 1958; Ayensu, 1978).

Nevertheless, some studies have reported controversially. Water extract of garlic has been demonstrated decreasing uterine motility in pregnant and nonpregnant albino rat and guinea pig (Sharaf, 1969). 95 % alcoholic extract and petroleum ether extract of garlic at doses of 150-200 mg/kg had no antifertility activity in female albino rats (Prakash and Mathur, 1976).

Patterns of the Contraction of Human Uterine Muscle

Uterine contraction has been studied extensively, mostly from animal models. In vitro study of human myometrial contraction revealed different patterns of contraction waveforms which seemed to relate to days of the menstrual cycle. The myometria from pregnant and menopausal women were inactive. Those from women during the first half of their menstrual cycle (proliferative phase) showed high frequency and low amplitude contractions while those from women during the second half of their menstrual cycle (secretory phase) showed low frequency and high amplitude contractions (Lohsiriwat and Anumanrajadhon, 1986).

Regulation of Human Myometrial Contraction

The contractility of myometrium is thought to be regulated by an interaction of myogenic, neurogenic and hormonal control systems (Garfield, 1984). According to myogenic control, it is generally accepted that Ca^{2+} - dependent regulation of interaction between myosin and actin filaments is fundamental to smooth muscle contraction. Myometrial contraction, like that of vascular smooth muscle, is initiated by an increase in the concentration of intracellular free calcium (Bolton 1979) which forms a complex with calmodulin. This activates a kinase which in turn catalyses the phosphorylation of myosin, allowing its interaction with actin and hence, contraction of the smooth

muscle cell (Kamm and Stull, 1985).

Studies on neurogenic control of myometrial contraction have shown that the human uterus is innervated by predominant adrenergic and cholinergic motor nerves from the autonomic nervous system. It is evident that the myometrium is innervated by tetrodotoxin-sensitive excitatory nerves consisting of alpha-adrenergic and cholinergic components. The presence of the nerves is decreased in pregnancy (Morizaki et al., 1989). During different phases of the menstrual cycle, in postmenopausal women and term pregnancy, it has been reported that the affinity and a number of alpha 1-adrenergic receptors were unchanged, whereas the number of alpha 2-adrenergic receptors increased concomitantly with circulating plasma estradiol levels (Bottari et al., 1983). Cholinergic and alpha-adrenergic receptors cause uterine contraction, whereas beta-adrenergic receptors induce relaxation.

The existing data on the hormonal factors involved in uterine contraction indicate that estrogens, progesterone, oxytocin, prostaglandins and relaxin play a major role. Prostacyclin relaxes the nonpregnant myometrium *in vitro* and may also do so *in vivo*, although intravenous infusion of prostacyclin has no effect upon the uterine contractility in nonpregnant or pregnant subjects (Ylikorkala and Makila, 1985). Concerning with the onset and maintenance of parturition, it is evident that estrogens, relaxin, and the

prostaglandins are particularly involved in cervical ripening, while prostaglandins, progesterone and oxytocin are more involved in regulating myometrial contractility. Progesterone dominance during pregnancy is associated with a firm closed cervix, few myometrial gap junctions, low calcium levels in the cells, and a quiescent myometrium (Steer, 1990). Progesterone and the estrogens play only a facilitatory role in the initiation of labor. Previous evidence suggested that oxytocin is the most importance for the initial phase of labor whereas increased synthesis of PGF-2 alpha is essential for the progression of labor. The finding of maximal oxytocin receptor density in the myometrium in labor adds strong support to the notion that oxytocin is the trigger for uterine contraction (Fuchs and Fuchs, 1984).

Rationale

Effects of allicin, the most active ingredient of garlic (Stoll and Seebeck, 1951), on the contraction of isolated human myometrial preparations have been rarely studied despite documentary evidence in traditional medicine for its ecbolic, including emmenagogue and abortifacient properties. Most of the studies have been devoted to animal models, especially rat and guinea pig. *In vivo* and *in vitro* studies in animals have demonstrated estrogenic effect of garlic (Lorenzo and Orellana, 1955; Saha, Savini, and Kasinathan, 1961; Bickoff, 1963; Tewari, Mapa, and

Chaturvedi, 1976; Borvonsin and Chumpolbunchorn, 1988). In human, alcoholic extract of garlic increased contraction of nonpregnant uterus (Tinao and Terren, 1955; Lorenzo et al., 1958; Ayensu, 1978). Nevertheless, it has been controversially reported that water extract of garlic decreased uterine motility in pregnant and nonpregnant albino rat and guinea pig (Sharaf, 1969). 95 % alcoholic extract and petroleum ether extract of garlic at doses of 150-200 mg/kg were reported to have no antifertility activity in female albino rats (Prakash and Mathur, 1976). The contrary effects may be explained by difference in the solvents used for the extraction of garlic and hence, difference in the derived products.

However, studies of the effects of allicin on uterine contraction were mostly undertaken in animals, especially rat and guinea pig. Little information is also available on the possible mechanism of its action. Thus, the present study was conducted in the isolated human uterine muscle for the study of the effects of allicin on its contraction. In the second part of this study, mechanism of action of allicin was also investigated.

Objectives of the Study

1. To study the effects of allicin extracted from garlic on the contraction of isolated human uterine muscle during proliferative phase of the menstrual cycle.

2. To investigate the mechanism of action of allicin on the contraction of isolated human uterine muscle, via muscarinic receptor, alpha - adrenergic receptor, beta-adrenergic receptor or calcium channel.