# CHAPTER 1 INTRODUCTION

## 1.1 Background

Osteoporosis is characterized by the loss of bone mass and strength that leads to fragility fractures, probably existed throughout human history but only recently became a major clinical problem as the increasing of human age (Raisz, 2005). It is a major growing health problem for elderly women associated with ovarian hormone deficiency following menopause and the most common cause of age related bone loss in women (Shirwaikar *et al*., 2010). The major cause of osteoporosis is a lack of certain hormones, particularly estrogen in women and androgen in men (Shirwaikar *et al*., 2003). One of the other factors that could be expected to be the cause of osteoporosis is due to the actions of female animals sterilization (ovariectomy) which is usually done on pets, both dogs and cats that will decrease estrogen level of the body. The effect of a decrease in estrogen level will increase bone resorption resulting in the occurrence of osteoporosis.

Especially the role in osteoclast for this case. Estrogen is an inhibitor of bone resorption that decreases both osteoclast numbers and activity (Krassas and Papadopoulou, 2001). Estrogen deficiency will lead to increase osteoclastogenesis and continue to lose bone (Oursler, 2003). Also effects in the form of decreased absorption of calcium in the intestines, which leads to increase parathyroid hormone levels and increase bone degradation in postmenopausal women (Meiyanti, 2010). After osteoporosis occurred it will easily cause bone fracture. In

these conditions, calcium requirement is quite high because in addition to be used for the improvement of osteoporosis condition as well as to the process of fracture healing. It was feared would interfere with the process of blood calcium homeostasis and then caused metabolism disorders.

Calcium is an essential element in the human body and is necessary to many cell functions. It is a vital component of bone architecture and is required for deposition of bone mineral throughout life. Although the body stores more than 99% of its calcium in the bones and teeth, it is also found in the extracellular fluid or plasma. Bone resorption increased to restore plasma levels in time of the plasma level decreases. Sufficient intake of calcium is necessary to maintain this balance. Calcium is absorbed in the small intestines with the aid of vitamin D (Kasper *et al*., 2005).

According to Hardy *et al* (1993) the blood calcium level was significantly reduced immediately after fracture. The level of ionised calcium in the blood is important for calcification, it will be reduced immediately after fracture and increased thereafter, during development of callus to facilitate the process of fracture calcification. Both calcium and phosphorous are transported to blood from gastrointestinal cells. Mineral homeostasis requires for the transport of calcium, magnesium, and phosphate across their target cells in bone, intestine, and kidney.

The regulation of bone and bone mineral metabolism results from the interactions among three important hormones, there are parathyroid hormone (PTH), calcitonin, and vitamin D at three target organs, bone, kidney, and

Gastrointestinal (GI) tract, to regulate the bone calcium and phosphorus. The calcium and phosphorus components are derived from the blood and which is from nutritional sources (Kini and Nandeesh, 2012).

Several studies have been conducted on the influence of osteoporosis on fracture healing. Experimental studies of bone fracture healing in osteoporotic animal models have shown reduced callus mass and reduced strength when compared with healing of similar fractures in animals with normal bone mass (Walsh *et al*., 1997;Namkung *et al*., 2001). The number of osteoclasts in the fracture calluses of the osteoporotic bone was significantly higher. These findings indicate that osteoporosis influences the healing of fractures, which may contribute to delayed healing of the fracture (Islam *et al*., 2005).

Many synthetic agents such as estrogens in hormone replacement therapy, selective estrogen receptor modulators like raloxifen have been developed to treat osteoporosis. Enhancement of estrogen will raise the absorption of calcium in intestine. But each one of them is associated with side effects such as hypercalcemia, hypercalciurea, increase risk of endometrial and breast cancer, breast tenderness, menstruation, thromboembolic events, vaginal bleeding and hot flushes (Shirwaikar *et al*., 2003). Then, it would be most helpful to explore naturally occurring substances especially of plant origin that could prevent bone loss and free from any adverse effects.

*Cissus quadrangularis* (CQ) has a role on estrogenic receptors of the bone in fracture healing process. Efficacy of CQ on early ossification and remodeling of bones have been observed that CQ acts by stimulation of metabolism and increase

uptake of the minerals calcium, sulpher and strontium (Mishra *et al*., 2010). *Cissus quadrangularis* has the effect of increasing the stimulation of all the cells of mesenchyma origin, namely the fibroblasts, the chondroblasts and osteoblasts which gives the effect of an increasing of the fibroblastic phase (first week), collagen phase (second week) and osteochondroital phase (third and fourth weeks) (Mishra *et al*., 2010).

Based on the background above, it is necessary to do more research on the capability of *Cissus quadrangularis* extract to maintain homeostasis blood calcium level as fracture femur therapy on ovariectomized rat (*Rattus norvegicus*).

## 1.2 Statement of Problem

1. Is there a difference level of homeostasis of blood calcium levels in a fracture femur on ovariectomized rat (*Rattus norvegicus*) between osteoporosis bone and normal bone?
2. Does *Cissus quadrangularis* extract have the capability as fracture femur therapy on ovariectomized rat (*Rattus norvegicus*) to maintain homeostasis blood calcium level?

## 1.3 Theoretical Base

The most common cause of osteoporosis arises from estrogen deficiency that begins some years in time of menopause. Estrogen deficiency accelerates the normal turnover of bone tissue, but the activity of bone resorbing cells (osteoclasts) is greater than that of bone forming cells (osteoblasts). Then the

clinical features of osteoporosis is a consequence of the increase occurrence of bone fractures (Kanis, 2010).

Giannoudis *et al*., (2007) showed experimental studies on the effect of osteoporosis on fracture healing have been carried out on ovariectomized rats. These studies have shown that ovariectomy significantly reduces bone mass and the mechanical strength of the bone. Fracture healing appears to be delayed with callus mineralization and biomechanical properties. Both estrogen and calcium deficiencies are important risk factors in the pathogenesis of osteoporosis. Ovariectomy plus calcium deficiency results in great decrease in bone volume (Mazzeo *et al*., 1988).

According to O'Loughlin and Morris (1998) research, ovariectomized rats were unable to achieve the same calcium balance or absorption as the normal rats. Ovariectomy reduced calcium balance due to increase faecal calcium excretion, a consequence of reduced intestinal calcium absorption. Trabecular bone thus would be the preferred source of the additional calcium required to maintain homeostasis when this cannot be accomplished by increase intestinal absorption or decreased urinary excretion (Khosla *et al*., 2010).

Calcium is one of the main bone forming minerals and supply to bone (Prentice, 2004). Calcium plays a key role in a wide range of biologic functions, one of the most important functions is in skeletal mineralization. Majority of total body calcium is present in the skeleton as calcium-phosphate complexes, primarily as hydroxyapatite, which is responsible for much of the material properties of bone. Bone continuously remodels by coordinated cellular

mechanisms to adapt its strength to the changing needs of growth and physical exercis. Old, damaged, and unneeded bone is removed by resorption, and new bone is deposited by formation. Diseases affecting either or both of these processes lead to disturbed calcium homeostasis (Peacock, 2010).

The extracts of CQ stem showed anti-inflammatory properties and were used in enhancing osteoblast proliferation, bone fracture healing, ossification of fetal bone and increasing the thickness of trabecular bone (Varoni *et al*., 2012). Extract of CQ contains a high percentage of calcium ions and phosphorus, both essential for bone growth. Calcium ions, phosphorous and phytoestrogens present in this plant extract used in the process of ossification and very useful in bone fracture healing process (Rao *et al*., 2007). The plant extract also facilitated extracellular matrix mineralization, which was more pronounced in the presence of osteogenic media and the study proved that CQ accelerates fracture healing and also causes early remodeling of fracture callus (Singh *et al*., 2013).

## 1.4 Aim of Research

1. To determine the difference level of homeostasis of blood calcium levels in fracture femur on ovariectomized rat (*Rattus norvegicus*) between osteoporosis bone and normal bone.
2. To determine the capability of *Cissus quadrangularis* extract as fracture femur therapy on ovariectomized rat (*Rattus norvegicus*) to maintain homeostasis blood calcium level.

## 1.5 Outcome of Research

To provide knowledge about the homeostasis level of blood calcium level between normal bone compared with osteoporosis bone and the benefits of *Cissus quadrangularis* extract as alternative therapy medicine to maintain homeostasis blood calcium level in cases of osteoporotic fractures.

## 1.6 Hypothesis

1. There is no difference in the level of homeostasis of blood calcium levels in a fracture femur on ovariectomized rat (*Rattus norvegicus*) between osteoporosis bone and normal bone.
2. *Cissus quadrangularis* extract have the capability as fracture femur therapy on ovariectomized rat (*Rattus norvegicus*) to maintain homeostasis blood calcium level.