EFFECTS OF *MORINGA OLEIFERA* SUPPLEMENTATION COMBINED WITH RESISTANCE EXERCISE ON IMMUNE RESPONSES AND BONE TURNOVER MARKERS AMONG YOUNG MEN

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By

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LIST OF ABBREVIATIONS

1CTP	– Cross-linked C-terminal telopeptide of Collagen alpha-1
ALP	– Alkaline phosphatase
ANOVA	– Analysis of variance
BMI	– Body mass index
$CD19^+$	– B lymphocyte cells
$CD3^+$	– T lymphocyte cells
$CD4^+$	– T helper cells
$CD8^+$	– T cytotoxic cells
Ig	– Immunoglobulin
MO	– *Moringa oleifera*
NK	– Natural killer cells
SD	– Standard deviation
SPSS	– Statistical package for social science
TNF-α	– Tumour necrosis factor alpha
WBC	– White blood cells

KESAN GABUNGAN PENGAMBILAN SUPLEMEN *MORINGA OLEIFERA* DAN SENAMAN RINTANGAN KEATAS TINDAK BALAS IMUN DAN PENANDA PEROLEHAN TULANG DALAM KALANGAN LELAKI MUDA

ABSTRAK

PENGENALAN: Fungsi imun yang baik dan tulang yang sihat diperlukan untuk mengekalkan rutin harian seseorang. Setakat ini, tiada kajian yang telah dijalankan untuk menyiasat manfaat yang mungkin daripada gabungan pengambilan suplemen *Moringa oleifera* dan senaman rintangan terhadap tindak balas imun dan penanda perolehan tulang dalam kalangan lelaki muda. **TUJUAN:** Oleh itu, kajian ini dijalankan untuk menentukan kesan gabungan suplemen *Moringa oleifera* dan senaman rintangan, berbanding suplemen *Moringa oleifera* sahaja (MO), senaman rintangan sahaja (Ex) dan kumpulan kawalan (C) terhadap tindak balas imun dan penanda perolehan tulang dalam kalangan lelaki muda. **KAEDAH:** Empat puluh orang lelaki muda sedentari (umur: 22.1 ± 1.3 tahun) telah direkrut dan dibahagikan secara rambang kepada empat kumpulan berasingan: plasebo tanpa senaman kawalan (C), senaman rintangan sahaja (Ex), suplemen *Moringa oleifera* sahaja (MO) dan gabungan senaman rintangan dan suplemen *Moringa oleifera* (MOEx). Peserta-peserta dalam kumpulan Ex dan MOEx menjalani latihan senaman rintangan 3 kali seminggu selama 6 minggu yang terdiri daripada 10 senaman berlainan. Para peserta dikehendaki melakukan 3 set senaman rintangan dengan 10-15 ulangan untuk setiap set. Peserta-peserta dalam kumpulan MO dan MOEx mengambil suplemen *Moringa oleifera* (300 mg 100% serbuk daun *Moringa oleifera* dalam setiap kapsul) dan peserta-peserta

dalam kumpulan C dan Ex mengambil plasebo 2 kali sehari selama 6 minggu. Ketinggian, berat badan, tekanan darah dan kadar degupan jantung para peserta semasa berehat diukur pada pra dan pasca ujian. Sampel darah selepas semalaman berpuasa dikumpulkan pada pra dan pasca ujian. Sampel darah telah digunakan untuk analisis jumlah bilangan sel darah putih, limfosit, monosit, T limfosit ($CD3^+$, $CD4^+$ dan $CD8^+$), dan B limfosit ($CD19^+$). Sampel darah juga telah digunakan untuk analisis kepekatan fosfatase alkali (ALP), 'osteocalcin' (OC) dan 'cross-linked C-terminal telopeptide of collagen alpha-1' (1CTP). Analisis 'mixed ANOVA' digunakan untuk menentukan perbezaan diantara dan didalam kumpulan. **KEPUTUSAN:** Hasil kajian menunjukkan bahawa gabungan senaman rintangan dan pengambilan suplemen *Moringa oleifera* tidak memberi kesan signifikan ($p > 0.05$) yang lebih baik terhadap tindak balas imun dan penanda perolehan tulang berbanding dengan kumpulan kawalan, senaman rintangan sahaja dan pengambilan suplemen *Moringa oleifera* sahaja. **KESIMPULAN:** Enam minggu gabungan pengambilan suplemen *Moringa oleifera* dan senaman rintangan tidak menghasilkan kesan yang baik terhadap tindak balas imun dan penanda perolehan tulang dalam kalangan lelaki muda.

EFFECTS OF *MORINGA OLEIFERA* SUPPLEMENTATION COMBINED WITH RESISTANCE EXERCISE ON IMMUNE RESPONSES AND BONE TURNOVER MARKERS AMONG YOUNG MEN

ABSTRACT

INTRODUCTION: Proper immune function and healthy bone is required for maintaining daily routine of an individual. To date, no study has been carried out to investigate possible benefits of *Moringa oleifera* supplementation combined with resistance exercise on immune responses and bone turnover markers among young men. **PURPOSE:** Therefore, the present study was carried out to determine the effects of *Moringa oleifera* supplementation combined with resistance exercise (MOEx) compared to *Moringa oleifera* supplementation alone (MO), resistance exercise alone (Ex) and control (C) groups on immune responses and bone turnover markers among young men. **METHODS:** Forty sedentary young men (age: 22.1 ± 1.3 years old) were recruited and were randomised into four separate groups: placebo without exercise control (C), resistance exercise alone (Ex), *Moringa oleifera* supplementation alone (MO) and resistance exercise with *Moringa oleifera* supplementation (MOEx). Participants in the Ex and MOEx groups performed resistance exercise 3 times per week for 6 weeks which consisted of 10 different exercises. Participants were required to perform 3 sets of 10-15 repetitions of resistance exercise per set. Participants in the MO and MOEx groups consumed *Moringa oleifera* (300 mg of 100% *Moringa oleifera* leaf powder each capsule) and participants in the C and Ex groups consumed placebo twice daily for 6 weeks. Participants' body height, weight, blood pressure and

resting heart rate were measured at pre- and post-tests. Blood samples were collected at pre- and post-tests after an overnight fast. Blood samples were used to analyse total WBC, lymphocytes, monocyte, T lymphocyte ($CD3^+$, $CD4^+$ and $CD8^+$) and B lymphocyte ($CD19^+$) counts. Blood samples were also used to analyse concentrations of alkaline phosphatase (ALP), osteocalcin (OC) and cross-linked C-terminal telopeptide of collagen alpha-1 (1CTP). A mixed analysis of variance (ANOVA) was performed to determine the significance of the differences between and within groups. **RESULTS:** It was found that combination of resistance exercise and *Moringa oleifera* did not significantly ($p > 0.05$) induce better effects on immune responses and bone turnover markers concentration compared to control, resistance exercise alone, and MO supplementation alone. **CONCLUSION:** Six weeks of combined MO supplementation and resistance exercise did not significantly enhance immune responses and bone turnover markers among sedentary young men.

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND OF THE STUDY

Recently, researchers have identified that *Moringa oleifera* is a type of plant with countless health benefits. It is a source of nutrition and it contains medicinal properties that are useful for human health (Abdull Razis *et al*., 2014). It has been reported that almost all parts of *Moringa oleifera* can be used as a source of nutrition and provide a useful product for humans (Abdull Razis *et al*., 2014; Varmani & Garg, 2014). Every part of *Moringa oleifera* can be used for certain nutritional and/or medicinal purposes. This herb has been reported as anti-inflammatory, antimicrobial, antioxidant, anticancer, hepatoprotective, anti-ulcer, diuretic, antiurolithiatic, and antihelmintic by Farooq *et al*. (2012). Besides, calcium, copper, iron, potassium, magnesium, manganese, and zinc are crucial minerals that are present in *Moringa oleifera* (Mahmood *et al*., 2010). Additionally, previous studies have shown that *Moringa oleifera* and its components have an attribute for wound healing, anti-inflammatory, antioxidant, antimicrobial, antitumor, and antiulcer properties (Varmani & Garg, 2014).

Immune functions are part of homeostasis network, to fight against pathogens. A previous study shows that *Moringa oleifera* has beneficial effects on immune functions. There was one previous study which reported that *Moringa oleifera* can significantly reduce immunosuppression by stimulating both cellular and humoral immunity (Gupta *et al*., 2010). According to Sudha *et al*. (2010), both low and high doses of *Moringa oleifera* stimulates the immune system by acting through cellular and humoral immunity in animals. However, low dose of the extract was found to be

more effective compared to high dose. This could be due to the presence of toxicant such as isothiocyanate and glycoside cyanides that may pose stress at high concentration and hence reduce the antioxidant potential of *Moringa oleifera*. Another study by Ojeka *et al*. (2016) reported that *Moringa oleifera* extract has a possible role to enhance the capacity of the host to fight the invading parasite or bacteria. Based on the result of a previous study by Yang *et al*. (2006), an increase in consumption of *Moringa oleifera* can improve nutritional status and strengthen immune functions to fight infectious diseases.

Proper nutrition is also important to improve and maintain bone health in young and older population respectively. Beta-carotene, amino acids, vitamin C, potassium, and calcium are several nutrients that are contained in *Moringa oleifera* leaves which are important for bone health (Brown *et al*., 2016). It was reported that in one serving of *Moringa oleifera* leaves, there is 22% daily value of Vitamin C, 41% daily value of potassium, 61% daily value of magnesium, 71% daily value of iron, and 125% daily value of calcium (Idealbite, 2014). Patel *et al*. (2013) reported that *Moringa oleifera* has a beneficial effect on bone integrity and it is effective in prevention of osteoporosis. In addition, *Moringa oleifera* fruit extract has been shown to have an ability in preventing bone loss in rats (Patel *et al*., 2015).

Besides nutrition, exercise is also one of the factors that can enhance immune function and improves bone health. One of the examples of exercise that is commonly practiced nowadays is resistance exercise. It is one of the most important contributors to promote health and prevent diseases. According to Calle and Fernandez (2010), resistance training is associated with reduced risk of low-grade inflammation related diseases such as cardiovascular disease and type 2 diabetes. Immune function can also be enhanced through a combination of nutrition and exercise. However, data is scarce

on the effect of nutritional supplement and exercise on immune function parameters. Nevertheless, it has been reported by Liew *et al*. (2013) and Mohamed and Ooi (2013) that combination of honey or chocolate malt drink and circuit training programme had resulted beneficial effects on immune functions in young males. Thus, the authors suggested that chocolate malt drink and honey supplementation can be recommended to young population to enhance their immune functions. Resistance exercise has also been shown to have beneficial effects on bone health. A previous study by Whipple *et al*. (2004) suggested that moderate intensity resistance training may reduce bone resorption, leading to a favourable change in overall bone turnover for at least 8 hours post exercise in untrained young men.

Understanding the effectiveness of the combination between botanical product supplementation and resistance exercise on immune response and bone health has potential benefits for public health. To date, information on *Moringa oleifera* and resistance exercise on immune functions and bone metabolism are lacking. So, the aim of this study was to examine the effects of 6 weeks *Moringa oleifera* supplementation on immune responses and bone turnover markers in response to resistance exercise among young men.

1.2 PROBLEM STATEMENT

Nowadays, numerous previous studies reported on the benefit of moderate exercise and good dietary intake in enhancing immune responses and bone health. It was reported that *Moringa oleifera* is rich in minerals and possess anti-inflammatory, antimicrobial and antioxidant properties. It was also reported that *Moringa oleifera* can enhance immune function. However, the information regarding the effects of *Moringa oleifera* supplementation combined with resistance exercise on immune responses and bone turnover markers among sedentary young men are lacking. Thus, it is important to investigate the possible health benefits of *Moringa oleifera* supplementation. Therefore, the present study was proposed.

1.3 OBJECTIVES OF THE STUDY

1.3.1 General objective

To determine the effects of 6 weeks *Moringa oleifera* supplementation combined with resistance exercise (MOEx) compared to *Moringa oleifera* supplementation alone (MO), resistance exercise alone (Ex) and placebo without resistance exercise control (C) groups on immune responses and bone turnover markers among sedentary young men.

1.3.2 Specific objectives

1. To determine the effects of 6 weeks *Moringa oleifera* supplementation combined with resistance exercise (MOEx) compared to *Moringa oleifera* supplementation alone (MO), resistance exercise alone (Ex) and placebo without resistance exercise control (C) groups on immune responses i.e. total white blood cell (WBC), monocyte, total lymphocyte, total T lymphocyte cells ($CD3^+$), T helper ($CD4^+$), T cytotoxic ($CD8^+$) and B lymphocyte ($CD19^+$) cells count among sedentary young men.

2. To determine the effects of 6 weeks *Moringa oleifera* supplementation combined with resistance exercise (MOEx) compared to *Moringa oleifera* supplementation alone (MO), resistance exercise alone (Ex) and placebo without resistance exercise control (C) groups on serum bone formation markers (alkaline phosphatase and osteocalcin) and bone resorption marker (Cross-linked C-terminal telopeptide of Collagen alpha-1) among sedentary young men.

1.4 RESEARCH QUESTIONS AND HYPOTHESES OF THE STUDY

RQ_1: Is there any significant difference between C, Ex, MO, and MOEx groups on immune responses among sedentary young men after 6 weeks.

H_{O1}: There is no significant difference between C, Ex, MO, and MOEx groups on immune responses among sedentary young men after 6 weeks.

H_{A1}: There is significant difference between C, Ex, MO, and MOEx groups on immune responses among sedentary young men after 6 weeks.

RQ_2: Is there any significant difference between C, Ex, MO, and MOEx groups on concentration of serum bone formation markers and bone resorption marker among sedentary young men after 6 weeks.

H_{O2}: There is no significant difference between C, Ex, MO, and MOEx groups on concentration of serum bone formation markers and bone resorption marker among sedentary young men after 6 weeks.

H_{A2}: There is significant difference between C, Ex, MO, and MOEx groups on concentration of serum bone formation markers and bone resorption marker among sedentary young men after 6 weeks.

1.5 SIGNIFICANCE OF THE STUDY

Proper immune function and healthy bone is required for maintaining daily routine (everyday activities) of an individual. Since to date, no study has been carried out to investigate possible benefits of *Moringa oleifera* supplementation combined with resistance exercise on immune responses and bone turnover markers among sedentary young men, therefore, the present study is warranted. It is hoped that results obtained from this study can be used in planning exercises and nutritional promotion programmes to increase immune functions and bone health among sedentary young men.

1.6 OPERATIONAL DEFINITIONS

Resistance exercise programme: A circuit training with 10 different exercises in 10 stations using elastic bands (5 stations) and dumbbells (5 stations) as the resistance. The ten stations were arranged alternately with emphasis on strengthening the upper and lower extremities. The participants were required to perform 3 sets of 10-20 repetitions per stations for 3 sessions per week for a total of 6 weeks.

Supplementation: *Moringa oleifera* (MO) or placebo capsules were randomly given to the participants. The supplementation regimen was 7 days per week for 6 weeks.

***Moringa oleifera*:** Participants in MO and MOEx group consumed four capsules of MO (1200mg; 300mg per capsule) for 7 days per week for 6 weeks.

Placebo: Participants in the C and Ex group consumed placebo for 7 days per week for 6 weeks.

Immune responses: Full blood count analysis (total WBC, monocyte and total lymphocyte) by using Automated Hematology Analyzer and immunophenotyping analysis (total T lymphocytes ($CD3^+$), T helper ($CD4^+$), T cytotoxic ($CD8^+$) and B lymphocyte ($CD19^+$)) by using a Flow Cytometer.

Bone turnover markers: Analysis of blood parameters of serum bone formation markers (alkaline phosphatase and osteocalcin) and serum bone resorption marker (serum Cross-linked C-terminal telopeptide of Collagen alpha-1).

Sedentary young men: A group of young men aged between 20 to 26 years old who exercised less than 2 times per week prior to the study period (Bennett et al 2006).

CHAPTER 2

LITERATURE REVIEW

2.1 IMMUNE SYSTEM

The human body is consistently exposed to microorganisms from the environment that is inhaled, swallowed, or inhabit our skin and mucous membranes (Parkin & Cohen, 2001). Maintaining homeostasis in the body requires ceaseless battle against harmful agents in our internal and external environment. Subsequently, the immune system is one of the imperative systems to shield the human body from the foreign substances and microbes such as bacteria, viruses, fungi, parasites that exist in nature (Calder & Kew, 2002).

Generally, functions of immune system are to recognise, assault, and demolish components that are unfamiliar to the host (Lollo *et al*., 2012). As mentioned by Tortora and Derrickson (2011a), immunity is the ability to avoid harm or disease through our defence system. There are two general types of immunity, which are innate immunity and adaptive immunity. These two types of immunity have their own diverse parts to secure the human body. Both innate and adaptive immunity work synergistically and are fundamental for an optimal function of the immune response (Wolach, 2012).

The two parts of immunity involve various blood-borne factors (complement, antibodies and cytokines) and cells. These cells are generally termed leucocytes or white blood cells (WBC). Leucocytes fall into two general classes which are phagocytes which include granulocytes (neutrophils, basophils and eosinophils), monocytes, macrophages and lymphocytes. Lymphocytes are classified as T lymphocytes, B lymphocytes, and natural killer cells. T lymphocytes are further

divided into helper T cells (these are recognised by the presence of the molecule CD4 on their surface) and cytotoxic T cells (these are recognised by the presence of CD8 on their surface). All cells of the immune system originate in bone marrow (Calder & Kew, 2002).

2.1.1 Innate immunity

Innate (non-specific) immunity refers to defences that are present at birth (Tortora & Derrickson, 2011a). It is the first line of defence against infectious agents. It is present prior to exposure to pathogens and its movement is not upgraded by such exposures (Calder & Kew, 2002). It also does not involve specific recognition of a microbe and acts against all microbes similarly (Tortora & Derrickson, 2011a). In some cases, it is used to incorporate physical, chemical and microbiological barriers, but more usually encompasses the components of the immune system which are neutrophils, monocytes, macrophages, complement, cytokines and acute phase proteins which give quick host defence (Parkin & Cohen, 2001). Furthermore, it represents immunity's early warning system and is intended to keep infectious agents from obtaining entrance into the body and in the event that they do enter, with their quick elimination to those that do get access (Calder & Kew, 2002; Tortora & Derrickson, 2011a).

The elimination can happen by direct destruction of pathogens by complement, by toxic chemicals such as superoxide radicals and hydrogen peroxide released by phagocytes or by toxic proteins released by natural killer cells and by engulfing pathogens by the process of phagocytosis, which is made more proficient by coating the invading pathogen with host proteins like complement or antibodies, and their subsequent destruction (Calder & Kew, 2002).

2.1.2 Adaptive immunity

Adaptive immunity includes lymphocytes (a type of white blood cell) called T lymphocytes (T cells) and B lymphocytes (B cells) (Tortora & Derrickson, 2011a). The recognition of antigens is by T lymphocytes and by antibodies (immunoglobulin (Ig)) produced by B lymphocytes (Calder & Kew, 2002). Generally, adaptive immunity refers to the defence that includes specific recognition of a microbe once it has ruptured the innate immunity defence, which recognises it as being unfamiliar to the host. It depends on a specific response to a specific microbe whereby it will adapt or change to handle it (Tortora & Derrickson, 2011a). It also includes a segment of memory, with the end goal that if the antigen is encountered again (i.e. there is reinfection), the response is quicker and more grounded than the initial response (Calder & Kew, 2002).

Lymphocytes are divided into two types which are B cells and T cells. Both are developed in primary lymphatic organs (red bone marrow and the thymus) from pluripotent stem cells that begin in red bone marrow (Tortora & Derrickson, 2011a). The B cells multiply and mature into antibody-producing cells (plasma cells) (Calder & Kew, 2002) and it finishes their advancement in red bone marrow, a process that proceeds throughout life (Tortora & Derrickson, 2011a). Plasma cells produce antibody which serves to kill toxins, avoid organisms adhering to mucosal surfaces, enacts complement, opsonises bacteria for phagocytosis, and sensitises tumour and contaminated cells for antibody-dependent cytotoxic assault by killer cells (Parkin & Cohen, 2001). The B cells response to an antigen is named humoral immunity (Calder & Kew, 2002).

Meanwhile, T cells are developed from pre-T cells that move from the red bone marrow into the thymus, where they mature. There are two noteworthy sorts of mature T cells that leave the thymus which are helper T cells (Th) and cytotoxic T cells (Tc) (Tortora & Derrickson, 2011a). At that point, T cells multiply and are capable straightforwardly to destroy virally infected cells (Tc) or control the action of different cells involved in the response (Th). The T cell response is named cell-mediated immunity (Calder & Kew, 2002).

The Th is also known as $CD4^+$ T cells and the Tc cells is also known as $CD8^+$ T cells. The $CD4^+$ T cells are the organising cells of the immune response, perceiving foreign antigen, and activating other parts of the cell-mediated immune response to annihilate the pathogen. They likewise have a noteworthy impact in activation of B cells. Meanwhile, $CD8^+$ T cells are engaged in an antiviral and conceivably antitumor activity. Both types have a major role in the control of intracellular pathogens (Parkin & Cohen, 2001).

2.2 BONE

The grown-up human skeleton has a sum of 206 bones, excluding the sesamoid bones. The appendicular skeleton has 126 bones, the axial skeleton has 74 bones, and the auditory ossicles has six bones (Clarke, 2008). The skeleton is made up of a few unique tissues working together which are bone or osseous tissue, cartilage, dense connective tissues, epithelium, adipose tissue and nervous tissues. The skeleton serves a variety of functions that are critical for human body. Bones involved four distinctive kinds of cells which are osteogenic cells, osteoblasts, osteocytes and osteoclasts (Tortora & Derrickson, 2011b).

2.2.1 Functions of bone

Bone serves an assortment of functions. It serves as the basic structure for the body by supporting soft tissues and giving attachment points for the tendons of most skeletal muscles (Clarke, 2008; Datta *et al*., 2008; Tortora & Derrickson, 2011b). It is important to protect an essential internal organs from injury (Clarke, 2008). For instance, cranial bones secure the brain, vertebrae ensure the spinal cord and the rib cage secures the heart and lungs (Tortora & Derrickson, 2011b). Besides, bone also serves to allow movement and locomotion by providing levers for the muscles (Clarke, 2008; Tortora & Derrickson, 2011b).

Other than that, bone tissue stores around 99% of the body's calcium. On demand, bone discharge minerals into the blood to keep up critical mineral balances (homeostasis) and to distribute the minerals to other parts of the body (Tortora & Derrickson, 2011b). Along these lines, bone is essential in the maintenance of mineral homeostasis and acid-base balance (Clarke, 2008). In addition, bone also works as blood cell production. Within certain bones, connective tissues called red bone marrow produces red blood cell, white blood cell and platelets, and the process known as haemopoiesis (Tortora & Derrickson, 2011b).

2.2.2 Bone cells

There are four types of cells present in bone tissue which are osteogenic cells, osteoblasts, osteocytes and osteoclasts. Osteogenic cells are unspecialised bone stem cells derived from mesenchyme, the tissue from which every connective tissue is shaped. They are the only bone cells to experience cell division, the subsequent cells form into osteoblasts. Osteogenic cells are found along the inward segment of the

periosteum in the endosteum and in the canals inside bone that contain blood vessels (Tortora & Derrickson, 2011b). Osteoprogenitor cells offer ascent to and keep up the osteoblasts that synthesise new bone matrix on bone-forming surfaces (Clarke, 2008).

Osteoblasts are known as a bone-building cell (Tortora & Derrickson, 2011b). They synthesise and emit type I collagen and other matrix proteins which are needed to build the extracellular matrix of bone tissues (Clarke, 2008). They also start calcification process. As osteoblasts encircle themselves with the extracellular matrix, they wind up noticeably caught in their secretions and move toward becoming osteocytes (Tortora & Derrickson, 2011b). Meanwhile, osteocytes represent terminally differentiated osteoblasts (Clarke, 2008). It is a mature bone cell and the main cells in bone tissues and function to keep up its day by day metabolism, for example, the exchange of nutrients and wastes with the blood (Tortora & Derrickson, 2011b). As indicated by Clarke (2008), osteocytes ordinarily express osteocalcin, galectin 3, CD44 (a cell adhesion receptor for hyaluronate), and a few other bone matrix proteins.

Osteoclasts are the only cells that are known to be equipped for resorbing bone (Clarke, 2008). It is a huge cell derived from the fusion of as many as 50 monocytes (a type of white blood cell) and is concentrated in the endosteum. In favour of the cell that faces the bone surfaces, the osteoclast's plasma membrane is deeply folded into a ruffled border. Here the cell discharges powerful lysosomal enzymes and acids that digest the protein and mineral components of the underlying extracellular bone matrix. This breakdown of bone extracellular matrix named as resorption. Resorption is part of the normal development, maintenance and repair of bone (Tortora & Derrickson, 2011b).

2.2.3 Bone remodelling

Bone is an exceptionally powerful structure experiencing constant remodelling. Remodelling starts before birth and proceeds until death (Clarke, 2008). In youth, there is a very high rate of bone turnover in which formation surpasses resorption. In young adulthood, formation and resorption are in approximate balance, however, with maturing, there is a net loss of bone. The rate of bone turnover, collagen matrix, size, structure, geometry and density all consolidate to determine the bone's general mechanical capacity. Deformities in these process will bring about illnesses, for example, osteoporosis, Paget's disease of bone, osteopetrosis and osteogenesis imperfect (Datta *et al*., 2008).

Bone experience longitudinal and radial growth, modelling and remodelling during life. Longitudinal and radial growth happen during youth and adolescence. Longitudinal growth occurs at the growth plates, where cartilage multiplies in the epiphyseal and metaphyseal regions of long bones, before consequently experiencing mineralisation to form primary new bone. And then, modelling is the process by which bones change their general shape in response to physiologic impacts or mechanical forces, prompting continuous modification of the skeleton to the forces that it encounters. Bones may widen or change axis by removal or addition of bone to the appropriate surfaces by independent action of osteoblasts and osteoclasts in response to biomechanical forces. During bone modelling, bone formation and resorption are not tightly coupled. Bone modelling is less frequent than remodelling in adults (Clarke, 2008).

Meanwhile, remodelling is a process of a development of new bone tissue and breaking down of old bone tissue to maintain bone quality and mineral homeostasis. It includes bone resorption, which is the removal of minerals and collagen fibres from

bone by osteoclasts and then, bone deposition or formation, which is the addition of minerals and collagen fibres to a bone by osteoblasts. In this way, bone resorption results in the destruction of the bone extracellular matrix while bone deposition results in the formation of a bone extracellular matrix (Tortora & Derrickson, 2011b).

Bone resorption and bone deposition or formation are critical to prevent accumulation of bone micro-damage (Clarke, 2008). At any given time, about 5% of the total bone mass in the body is being remodelled. Remodelling takes place at various rates in various regions of the body (Tortora & Derrickson, 2011b). This bone turnover also enables the bone to repair itself, for example after fracture, and to adapt to the forces placed on it (Datta *et al*., 2008). It expels injured bone, replacing it with new bone tissue. Also, remodelling might be activated by factors such as exercise, sedentary lifestyle and changes in diet (Tortora & Derrickson, 2011b).

2.3 NUTRITIONAL SUPPLEMENTATION AND IMMUNE RESPONSE

Great and solid nourishment is essential for a human body that may influence the immune system through the activation of cells, modification in the production of signalling molecules and gene expression (Valdes-Ramos *et al*., 2010). As indicated by Ranjit (1997), nutrition is a critical determinant of immune responses in which absence of proper nutrition turned into the normal reason for immunodeficiency. Furthermore, lack of appropriate nutrition or otherwise called as malnutrition diminishes immune defences against attacking pathogens and makes the individual more susceptible to disease (Gleeson *et al*., 2004). Immune functions additionally can be lessened through over nutrition and obesity (Ranjit, 1997).

Additional intake of nutrients through nutritional supplementation for adequate intake of iron, zinc and vitamins A, E, B6 and B12 are particularly vital for the maintenance of immune function, yet excess intakes of some micronutrients can also impair immune function and have other adverse effects on well-being (Gleeson *et al*., 2004). A previous study by Muhamad *et al*. (2015) reported that *Eurycoma longifolia* may improve immune functions after a bout of endurance running in the heat by increasing natural killer (NK) cell count. This previous study suggested that nutritional supplementation like *Eurycoma longifolia* can be used to improve immune functions particularly after strenuous exercises which may impair immune functions.

Moreover, Liew *et al*. (2013) found that chocolate malt drink supplementation elicited significant effects on immune functions among young males by increasing the number of lymphocytes and its subsets populations. Chocolate malt drink supplementation has a rich source of flavonoids which is vital to enhance immune function in individuals. Therefore, the authors concluded that there is a positive relationship between healthy nutritional intake and immune responses.

2.4 NUTRITIONAL SUPPLEMENTATION AND BONE

Nutritional supplementation is important for individuals to help them to get the adequate nutrition in their daily life. Deficiency of vitamins and minerals in dietary intake can lead them to many diseases, especially during aging. As mentioned before, osteoporosis is one of the common bone diseases that happens among the elderly. Sufficient vitamins and minerals are needed for the bone to become healthy and stronger. Calcium and vitamin D supplements have long been recognised as the cornerstone for prevention and management of osteoporosis and fractures (Sunyecz, 2008).

Dietary sources of vitamin D, calcium and protein provide some protection against bone atrophy or weakening (Hurwitz, 2016). The additional dietary sources of calcium and vitamin D can be supplied through the nutritional supplementation. Regarding the effects of nutritional supplementation on bone health, a few previous studies demonstrated that supplementation elicited positive effects on bone. A previous study by Bunout *et al*. (2006) reported that a special nutritional supplement, providing additional measures of calcium, vitamin D, vitamin K and isoflavones, enhanced markers of bone turnover in osteoporotic elderly subjects.

The previous study by Fazlini *et al*. (2013) reported that nutritional supplementation can supply sufficient intake of vitamins in individuals. The authors showed that the result of calcium supplementation leads to an increased daily intake of calcium among the subjects and helped to maintain the serum calcium level within normal range. Calcium is a major constituent of bone. In this way, it would make sense that calcium supplementation was helpful in maximising peak bone mass, retaining acquired bone mass, and thus reducing the risk of osteoporosis. Moreover, as indicated by Williamson *et al*. (2017), Vitamin D plays a critical role in skeletal homeostasis and the Vitamin D supplementation is used worldwide to maintain optimal bone health.

There are a couple of past investigations officially reporting the positive consequences of nutritional supplementation on bone health either in human or animal studies. In an animal study, Williamson *et al*. (2017) revealed that dietary vitamin D_3 supplementation may increase bone health by enhancing bone material quality and supports the use of vitamin D_3 supplementation, during adolescence, for achieving a higher peak bone mass in adulthood and thereby preventing osteoporosis.

In a human study, calcium supplementation significantly slowed axial and appendicular bone loss in normal post-menopausal women (Reid *et al*., 1993). In addition, another study reported that the utilisation of calcium, or calcium in combination with vitamin D supplementation, can be used for the preventive treatment of osteoporosis in individuals aged 50 years or older (Tang *et al*., 2007). Besides, calcium supplementation on children additionally could improve the rate of increase in bone mineral density (Johnston *et al*., 1992).

2.5 *MORINGA OLEIFERA*

Moringa oleifera is a type of herb that serves as multi-purpose herbal plant utilised as human food and an alternative for medicinal purposes worldwide (Abdull Razis *et al*., 2014). It is a natural and the most broadly developed variety of genus *Moringa* belonging to family Moringaceae, which is local to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan (Fahey, 2005; Mahmood *et al*., 2010). *Moringa oleifera* can be found in numerous tropical and subtropical nations (Torondel *et al*., 2014). It is referred to by various names such as drumstick tree and horseradish tree in English (Varmani & Garg, 2014). *Moringa oleifera* is a quickly growing evergreen tree with averaged height about 5-10 meters in a year (Ganatra *et al*., 2012). It can develop well in the humid tropics or hot dry land (Farooq *et al*., 2012) and it has a soft trunk, white corky bark, bipinnate leaf and a fragrant white in colour flowers (Varmani & Garg, 2014).

Moringa oleifera is a gigantic source of nutritional components. It is believed that almost all parts from *Moringa oleifera* can be used as a source of nutrition and provide a useful product for humans (Abdull Razis *et al*., 2014; Varmani & Garg,

2014). According to El-Sohaimy *et al*. (2015), the leaves of *Moringa oleifera* have huge nutritional value such as phytochemicals, vitamins, minerals, proteins and amino acids and it is considered as natural energy booster for human around the world who are lacking in many nutritional supplements, for example, protein, carbohydrate, lipids and fibres. Fibre is crucial as a part of the diet in order to clean the digestive tract by removing potential carcinogens from the body and subsequently prevents the absorption of excess cholesterol. In addition, fat and carbohydrate are extremely significant as main sources of energy for the human body.

Besides, *Moringa oleifera* leaves also contained a high level of minerals including sodium, potassium, magnesium, phosphorus, iron, zinc, copper, calcium and manganese. It also contains essential amino acids such as lysine, methionine, phenylalanine, histidine, leucine, isoleucine and valine, and an effective vitamin production in reasonable concentrations for human requirements. Therefore, the leaves can be used to combat malnutrition, especially among infants and nursing mothers (El-Sohaimy *et al*., 2015). The leaves also offering incredible potential for the individual who is nutritionally at risk and might be viewed as a protein and calcium supplement (Ganatra *et al*., 2012).

Moringa oleifera is sometimes called 'the miracle tree' since it functions as medicinal in addition as a functional food. It possesses an amazing healing ability for various ailments and even some chronic diseases (Abdull Razis *et al*., 2014). It has been used for a considerable length of time for nutritional as well as medicinal purposes. In addition, Varmani and Garg (2014) stated that *Moringa oleifera* and its components possess wound healing, anti-inflammatory, antioxidant, antimicrobial, and antihelminthic, antipyretic, anti-diabetic, antihypertensive, lipid-lowering, antifertility, antitumor, hepatoprotective and antiulcer properties. Thus, it can be

concluded that *Moringa oleifera* has numerous applications in the medicinal field and it is genuinely has advantages for mankind and hence should be taken as a high quality gift of nature at low value (Mahmood *et al*., 2010).

2.5.1 *Moringa oleifera* and immune function

There are some previous studies reporting that *Moringa oleifera* has beneficial effects on immune functions. Sudha *et al*. (2010) investigated the immunomodulatory activity of methanolic leaf extract of *Moringa oleifera* in experimental models of cellular and humoral immunity in animals. In this study, the animal was receiving either ethanolic extract of Ocimum sanctum or low dose (250 mg/kg) or high dose (750 mg/kg) of methanolic extract of *Moringa oleifera* or none. The outcome demonstrated that both low and high dose of *Moringa oleifera* stimulates the immune system by acting through cellular and humoral immunity in animals. However, the extract was found to be more effective at low dose compared to high dose. This could be due to the presence of toxicant such as isothiocyanate and glycoside cyanides that may pose stress at high concentration and hence reducing the antioxidant potential of *Moringa oleifera*.

Similarly, a previous study by Ojeka *et al*. (2016) on animals also reported that *Moringa oleifera* acts as an immune booster on rats. The result in this previous study shows that the *Moringa oleifera* extract has potential to boost the capacity of the host to fight the invading parasite or bacteria. In addition, as indicated by Mahajan and Mehta (2010), ethanolic extract of seeds of *Moringa oleifera* additionally suppress the cellular and humoral response in mice and it shows the possibility to inhibit macrophage phagocytic activity.

Other than that, in an intervention study using a rat model that was done by Yang *et al*. (2006), a diet containing 5% Moringa powder was compared to a 5% common cabbage diet and a nutrient-sufficient diet without vegetables. Following 3 weeks of intervention, the result demonstrated that the *Moringa* diet reduced blood triglycerides and enhance the immune response. From the outcome, it is believed that consumption of *Moringa oleifera* leads to a better immune response compared to consumption of vegetables that are rich in fibre but low in nutrient content, like common cabbage. Thus, the researchers suggested that *Moringa oleifera* should be promoted for greater consumption to improve nutrition and strengthen immune functions in order to fight infectious diseases.

According to Sreelatha *et al*. (2011), who had conducted a study on the impacts of *Moringa oleifera* on human cancer cells, the result of the study found that *Moringa oleifera* has a potential for cancer chemoprevention. This was due to *Moringa oleifera* have strong antiproliferation and potent induction of apoptosis. Another example of the previous study on humans reporting that *Moringa oleifera* powder supplementation might act as an immune stimulant for patients that are suffering from HIV infection (Burger *et al*., 2002). Despite numerous animal studies conducted to investigate beneficial effects of *Moringa oleifera* on immune function, to date, human studies in this field is lacking. Thus, the present study was proposed to be carried out.

2.5.2 *Moringa oleifera* and bone

Moringa oleifera is a tropical plant where, its leaves contain several of nutrients such as beta-carotene, amino acids, vitamin C, potassium, calcium and multiple important micronutrients that are essential for bone health (Brown *et al*., 2016). To

date, limited previous studies have been carried out to determine the effects of *Moringa oleifera* on human bone. Patel *et al*. (2013) reported that *Moringa oleifera* can affect bone integrity as it increased ALP activity as well as induced bone formation and it potently prevents osteoporosis. In addition, another study reported that *Moringa oleifera* intervention showed a reduction in pain, swelling and tenderness of the fractured jaw that were measured through radiographic interpretation (Singh *et al*., 2011).

Previous studies have been carried out in examining the efficacy of *Moringa oleifera* on bone strength in animals. Nkukwana and colleagues (2014) conducted a study on the effects of dietary *Moringa oleifera* leaf meal supplementation on tibia strength, morphology and inorganic content of broiler chickens. A total of 2400 one-day-old broiler chickens were distributed to five dietary treatments; T1 (positive control), T2 (starter (1 – 5 g of *Moringa oleifera* /kg feed)), T3 (grower (3 – 15 g of *Moringa oleifera* /kg feed)), T4 (finisher (5 – 25 g of *Moringa oleifera* /kg feed)) and T5 (negative control). The researchers found that there was a positive correlation observed between tibia weight, tibia length and the level of phosphorus and calcium in the tibia from birds that consumed *Moringa oleifera* supplementation. The result obtained in this study provided evidence that *Moringa oleifera* can enhance nutrient utilization efficiency, increase mineral bioavailability and support bone strength.

In another animal study, Patel *et al*. (2015) investigated the osteoprotective and antidiabetic activities of *Moringa oleifera* plant extract. Diabetes mellitus has been known to be related with a high risk of osteoporosis. The authors have explored the effects of different parts of *Moringa oleifera* (leaf, fruit, and flower extract) on bone health markers in diabetic osteoporosis rats. Off all the three parts of *Moringa oleifera,* fruit extract was found to be highly significant in reducing the glucose levels and

osteoclastic bone marker and increasing the osteoblastic bone marker in rats. The outcome demonstrates that *Moringa oleifera* fruit extract has an important role in preventing bone loss in rats.

2.5.3 Dosage of *Moringa oleifera* supplementation

To date, studies on human with regards to *Moringa oleifera* supplementation are very limited. However, animal studies showed that water extract of the leaves at a dosage between 150-200 mg/kg body weight of oral intake is deemed as optimal. Based on this, a preliminary human dose was estimated at 1,600-2,200 mg for a 68 kg person, 2,100-2,900 mg for a 91 kg person, and 2,700-3,600 mg for a 114 kg person (Kurtis, 2016). In addition, there was a study (animal and *in vitro*) where researchers investigated the toxicity potentials of the *Moringa oleifera* (Asare *et al*., 2012). This study reported that for human consumption, *Moringa oleifera* is genotoxic at supra-supplementation levels of 3,000 mg/kg body weight. In the present study, the dosage of *Moringa oleifera* given to the participants were as prescribed on the bottle by the manufactured company, i.e. a total of 1200 mg of leaf extract of *Moringa oleifera* per day. This value is below the genotoxic level and safe for human consumption.

2.6 CIRCUIT TRAINING AND RESISTANCE EXERCISE

Regular physical activity is one of the most important contributors to promote health and prevent diseases. The risk of developing chronic diseases such as cardiovascular disease, diabetes and some types of cancers can be reduced by being physically active (Gjevestad *et al*., 2015). Nowadays, resistance training is becoming a popular form of exercise as its role in improving athletic performance, as well as serves a lot of health-related benefits for most populations including adolescents,