COMBINED EFFECTS OF A CIRCUIT TRAINING PROGRAMME AND HONEY SUPPLEMENTATION ON IMMUNE FUNCTIONS IN YOUNG MALES

HUSNA AFIFAH BT MOHAMED

SCHOOL OF HEALTH SCIENCES

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By

HUSNA AFIFAH BT MOHAMED

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KEBERKESANAN GABUNGAN PROGRAM LATIHAN LITAR DAN PENGAMBILAN MADU KE ATAS FUNGSI IMUNITI DI KALANGAN LELAKI MUDA

ABSTRAK

PENGENALAN: Selain daripada fizikal aktiviti, tahap imuniti boleh dipertingkatkan dan dikekalkan melalui pengambilan nutrisi yang mencukupi. Setakat ini, pengetahuan tentang kesan gabungan program latihan litar dan pengambilan madu ke atas imuniti lelaki muda masih terhad. **MATLAMAT**: Kajian ini dijalankan untuk mengkaji kesan gabungan suatu program latihan litar dan pengambilan madu ke atas fungsi imuniti di kalangan lelaki muda. **KAEDAH :** Empat puluh subjek lelaki muda yang terdiri daripada 10 orang setiap kumpulan (n=10) : kumpulan kawalan yang tidak bersenam dan tidak mengambil madu (C), kumpulan yang mengambil madu tanpa senaman (H), kumpulan yang bersenam tanpa mengambil madu (Ex), kumpulan yang bersenam dan mengambil madu (HEx). Program latihan litar dijalankan oleh subjek sebanyak 3 kali semingu selama 6 minggu. Madu Tualang diambil oleh subjek pada dosej 20g yang dilarutkan dalam 300 ml air kosong/hari, 7 hari/minggu selama 6 minggu dalam kumpulan H dan HEx. Subjek dalam HEx mengambil 300 ml minuman madu yang mengandungi 20g madu 30 minit sebelum bersenam. Sebaik sahaja sebelum 6 minggu tempoh kajian, parameter-parameter antropometri subjek diukur. Sampel darah diambil sebelum dan selepas kajian untuk penentuan sel darah putih, limfosit, monosit, basofil, dan sel NK untuk menentukan tahap fungsi imuniti. **KEPUTUSAN** : Tiada perubahan yang signifikan diperhatikan dalam sel darah putih dan limfosit dalam kumpulan C, H, Ex dan HEx. Dalam H, Ex dan HEx, terdapatnya nilai basofil yang tinggi dan signifikan pada pasca ujian dibandingkan dengan pra ujian.

Walaubagaimanapun, peratusan peningkatan basofil adalah paling tinggi di HEx di antara kumpulan. Tambahan pula, peratusan perbezaan yang paling tinggi di antara pra dan pasca ujian dalam monosit dan sel NK dapat diperhatikan di HEx di antara kumpulan. **KESIMPULAN:** Gabungan program latihan litar dan pengambilan madu (HEx), kumpulan senaman sahaja (Ex) dan pengambilan madu sahaja (H) boleh memberi manfaat yang baik pada tahap fungsi imuniti berbanding kumpulan kawalan yang tidak bersenam dan tidak mengambil madu (C). Walaubagaimanapun, gabungan program latihan litar dan pengambilan madu (HEx) mungkin lebih bermanfaat ke atas tahap fungsi imuniti berbanding senaman sahaja (Ex) dan pengambilan madu sahaja (H). Maka, gabungan di antara senaman latihan litar dan pengambilan madu (HEx) mungkin berpotensi untuk dicadangkan demi meningkatkan fungsi imuniti di kalangan subjek lelaki muda.

COMBINED EFFECTS OF A CIRCUIT TRAINING PROGRAMME AND HONEY SUPPLEMENTATION ON IMMUNE FUNCTIONS IN YOUNG MALES

ABSTRACT

INTRODUCTION: Besides physical activity, immune level can be enhanced and maintained through adequate nutritional intake. To date, little is known about combination effects of exercise and honey supplementation on immune functions in young males. **PURPOSE:** This study was carried out to investigate the effects of a combined circuit training programmne and honey supplementation on immune functions in young males. **METHODS** : Forty young male subjects were assigned into four groups, with ten subjects per group (n=10): sedentary without honey supplementation control (C), sedentary with honey supplementation (H), circuit training programme without honey supplementation (Ex), circuit training programme with honey supplementation (HEx) groups. Circuit training programme was performed by the subjects three times per week for six weeks. Tualang Honey was consumed by the subjects at a dosage of 20 g diluted in 300 ml of plain water per day, seven days per week for six weeks in (H) and (HEx) groups. Subjects in (HEx) consumed honey drink 30 minutes before performing exercise. Immediately before six weeks of experimental period, subjects' anthropometry parameters were measured. Blood samples were taken before and after experimental period in order to measure white blood cells, lymphocytes, monocytes, basophil, and natural killer cells to determine the level of immune functions. **RESULTS:** There were no significant changes in white blood cells and lymphocytes in C, H, Ex and HEx groups. In H, Ex and HEx, there were significant higher

values of basophil in post test compared to pre test. Nevertheless, the percentage increment in basophil was highest in HEx among the groups. Additionally, greatest percentages difference between pre and post test in monocyte and NK cell were observed in HEx among the groups. **CONCLUSION:** Combined circuit training exercise with honey supplementation (HEx), circuit training exercise alone (Ex) and honey supplementation alone (H) may elicit beneficial effects on immune functions compared to control (C). However, combined circuit training exercise with honey supplementation (HEx) may elicit more beneficial effect on immune functions than exercise alone (Ex) and honey alone (H). Thus, combination of a circuit training programme and honey supplementation may has potential to be recommended for increasing immune functions in young males subjects.

CHAPTER 1

INTRODUCTION

Immune system has evolved to protect us from pathogens. Intracellular pathogens infect individual cells for example, viruses, whereas extracellular pathogens divide extracellularly within tissues or the body cavities, eg. bacteria. Leucocyte are central to all immune responses, and other cells in the tissue also participate by signaling to the lymphocytes and responding to the cytokines released by T cells and macrophages (Male *et al*, 2006).

Moderate exercise has been linked to a positive immune system response and a temporary boost in the production of macrophages, the cells that attack bacteria. It is believed that regular, consistent exercise can lead to substantial benefits in immune system health over the long-term (Quinn, 2008). During moderate exercise immune cells circulate through the body more quickly and are better able to kill bacteria and viruses. After exercise ends, the immune system generally returns to normal within a few hours, but consistent, regular exercise seems to make these changes a bit more long-lasting (Quinn, 2008).

Acute exercise, both endurance and resistance, could induce a marked increase in circulating leucocyte count (Petridou *et al.*, 2006). The cell surface expression of adhesion molecules makes an important contribution to such changes by altering patterns of leucocyte trafficking and redistribution (Petridou *et al.*, 2006).

Honey is a natural sweetener comprising vitamins, mineral enzymes, carbohydrates. It also contains natural antioxidant properties which can destroy biologically destructive chemical agents such as cancer, and protect against cellular damage and possibly prevent the development of chronic diseases. Honey has antioxidant and antibacterial properties which can help to improve digestive system, so that one can stay healthy and fight disease (Tan, 2007). It was reported that, honey may increase immune function, treat or prevent anemia, and can help to boost gastrointestinal ulcer healing (Neff, 2007).

To date, no studies have been undertaken to determine the effects of combined circuit training programme and Tualang honey supplementation on immune function in young males. Thus the present study was proposed.

1.1 OBJECTIVE OF THE STUDY

To determine the combined effects of a circuit training programme and honey supplementation on immune functions through blood white blood cell, lymphocytes, monocytes, basophil and natural killer (NK) cells in young males.

1.2 SIGNIFICANCE OF THE STUDY

It is hoped that results obtained from this study can be use for formulating guidelines in planning exercise and nutritional promotion programmes for increasing immune function of young males.

1.3 HYPOTHESIS

Ho: There are no significant differences in immune function parameters in combined circuit training and honey supplementation group compared to sedentary without supplementation control, honey alone and circuit training alone group.

Ha: There are significant differences in immune function parameters in combined circuit training and honey supplementation group compared to sedentary without supplementation control, honey alone and circuit training alone group.

1.4 OPERATIONAL DEFINITIONS

Circuit training programme: It consists of 2 circuits of exercise, with 10 different exercises in 10 station per circuit, one hour per session, 3 times per week for a total of six weeks.

Immune function: Measurements of blood parameters, i.e. white blood cells, lymphocytes, monocytes, basophil, natural killer cells.

Honey supplementation: Malaysian local honey (Tualang Honey, FAMA) (Appendix A) was consumed at the dosage of 20g per day, seven days per week for six weeks by the subjects.

Young male subjects: A group of Malaysian males between 19-25 years old were recruited.

CHAPTER 2

LITERATURE REVIEW

2.1 IMMUNE SYSTEM

Immune system probably is developed as a means of self-identification and of maintaining homeostasis. The immune system covers the body's responses to foreign or novel molecules, usually proteins, called immunogens, microorganisms, including viruses, bacteria, fungi, and parasites, tumor growth, tissue transplantation, and allergens. The immune response to any challenge requires complex communication and coordination among tissues, cells, and messenger molecules throughout the body. For instance, specialised immune cells, called helper T lymphocytes (T_H), recognise and are activated by the foreign protein on the phagocytes surface (Mackinnon, 1992).

In ones body, carried within the blood and lymph and populating the lymphoid organs are various white blood cells or leukocytes that participate in the immune response. Of these cells, the antigen-specific lymphocytes possess the attributes of diversity, specificity, memory, and self-nonself recognition the hallmarks of an adaptive immune response. Other leukocytes also play important roles, some as antigen-presenting cells and others participating as effector cells in the elimination of antigen by phagocytosis or the secretion of immune effector molecules. Some leukocytes especially T lymphocytes secrete various protein molecules called cytokines. These

molecules act as immunoregulatory hormones and play important roles in the coordination and regulation of immune responses (Kindt *et al.*, 2007).

Exercise causes many profound changes in parameters of immune function, the nature and magnitude of such changes depend on several factors including the immune parameter of interest; type, intensity, and duration of exercise, fitness level or exercise history of the subject, environmental factors such as ambient temperature and the time course of measurement (Nieman and Pedersen, 2000).

The immune response can be divided into two broad functions, i.e. innate immunity and adaptive immunity.

2.1.1 Innate immunity

Innate immunity is the first aspect of the immune system encountered by an invading microorganism, cells involved in innate immunity can recognise and deal with 'nonself' without prior exposure. Innate immunity does not improve with repeated exposure. Innate immunity involves three general mechanisms to prevent infection, structural barriers preventing entry of pathogenic organisms, chemical means (pH and soluble factors) that create an inhospitable environment for microorganisms, phagocytic cells that recognise and kill microorganisms (Mackinnon, 1992).

Numerous internal components are also features of innate immunity. Such elements are interferons, fever and other substance released by leukocytes as well as a variety of serum proteins such as beta-lysine, the enzyme lysozyme, polyamines and the kinins, among others. All of these elements either affect pathogenic invaders directly or enhance the effectiveness of host reactions to them. Other internal elements of innate immunity include phagocytic cells such as granulocytes, macrophages and microglial cells of the central nervous system, which participate in the destruction and elimination of foreign material that has penetrated the physical and chemical barriers (Benjamini *et al.*, 2000).

2.1.2 Adaptive Immunity

Adaptive immunity is characterised by specificity to the infectious agent and it generates memory of prior exposure. The adaptive response improves with repeated exposure and is the basis for immunisation to prevent disease. The adaptive immunity involves action of immune cells, such as lymphocytes and macrophages that inactivate and destroy microorganisms by several mechanisms. Memory B cells are generated by the first exposure to the foreign agent and subsequent exposure produces a faster and more effective response. The acquired immune response can be broadly divided into responses mediated either by humoral agents such as antibodies or by immune cells that activate other immune cells to combat foreign agents and can directly kill foreign or infected cells (Mackinnon, 1992).

There are three major cell types involved in adaptive immune and that complex interactions among these cell types are required for the expression of the full range of immune responses. These are mainly the B lymphocytes, T lymphocytes and macrophages. B and T lymphocytes are responsible for the specificity exhibited by the immune response. The macrophages cells do not have antigen-specific receptors as do the lymphocytes, their important function is to process and present the antigen to the specific receptors on T lymphocytes (Benjamini *et al.*, 2000).

There are two arms of adaptive immunity, humoral and cellular immunity which is to eliminate the antigen.

2.1.2.1 Humoral Immunity

Humoral immunity is mediated by serum antibodies, which are the proteins secreted by the B cell compartment of the immune response. B cells are initially activated to secrete antibodies after the binding of antigens to specific membrane immunoglobulin (Ig) molecules (B cell receptors), which are expressed by these cells (Benjamini *et al.*, 2000).

The important element involved in humoral immunity is the complement system. The reaction between antigen and antibody serves to activate this system, which consists of a series of serum enzymes, the end result of which is lysis of the target or enhanced phagocytosis by phagocytic cells. The activation of complement also results in the recruitment of highly phagocytic polymorphonuclear cells, which constitute part of the innate immune system. These activities maximise the effective response made by the humoral arm of immunity against invading agents (Benjamini *et al.*, 2000).

2.1.2.2 Cellular Immunity

The antigen-specific arm of cellular immunity consists of the T lymphocytes. Unlike B cells, which produce soluble antibody that circulates to bind its specific antigens, each T cell, bearing many identical antigen receptors called T cell receptors circulates directly to the site of antigen and performs its function when interacting with antigen. There are several subpopulations of T cells, each of which may have the same specificity for an antigenic determinant although each subpopulation may perform different functions. This is analogous to the different classes of immunoglobulin molecules that may have identical specificity but different biologic functions. The functions of T cells include; cooperation with B cells to enhance the production of antibodies, inflammatory effects, cytotoxic effects, regulatory effects and signal via cytokines (Benjamini *et al.*, 2000).

2.2 EFFECTS OF EXERCISE ON IMMUNE FUNCTIONS

Circuit weight training usually consists of two to three circuits of 10 to 15 different weight training exercises in 25 to 30 minutes. At each station, 10 to 15 repetitions at moderate exercise intensity with 40% to 55% of the one repetition maximum are performed rapidly, i.e. 30 seconds, and there is a minimum amount of rest between the exercise stations for example 15 to 30 seconds (Gettman *et al.*, 1998).

Circuit weight training programs are designed to increase strength, muscular endurance, power and cardiorespiratory endurance (Gettman *et al.*, 1998). The author also mentioned that a circuit weight training program usually has 6-15 stations per circuit. The circuit is repeated two to three times so that the total time of continuos exercise is 20-30 minutes. At each station, a load is selected that fatigues the muscle group in approximately 40%- 55% of 1 RM. There is a 15-20 seconds rest period between exercise stations (Gettman *et al.*, 1998).

Moderate aerobics exercise training has been shown to elicit beneficial outcomes in both the prevention and rehabilitation of many diseases in elderly (Mazzeo *et al*, 1998), and limited preliminary evidence suggests that exercise training or a physically active lifestyle may enhance certain NK cell and T lymphocyte function in the elderly (Shinkai *et al*, 1997).

Benoni *et al* (1995), noted a significant increase in circulating neutrophils after exercise in trained subjects but not in untrained subjects who performed 10 minutes of cycling at the same relative exercise intensity with heart rate of 150 beats per min.

It has been reported that exercise could induce an increase in the number of circulating neutrophils related to the intensity of the physical activity. Tauler *et al* (2002) show that an exhaustive exercise such as duathlon competition or a cycling mountain stage increased the neutrophil counts about fourfold.

Resistance exercise produces transient perturbations in immunity, including alterations in circulating leukocyte numbers, cytokine concentration and some measures of cell function (Koch, 2009). Nieman (1998) in their study have documented an increased risk of upper respiratory tract infection (URTI) with high volumes and intensity of endurance exercise.

Experimental studies have shown that a regular exercise program of brisk walking can increase many defenses of the immune system, including the antibody response and the natural killer (T cell) response (Leonard, 1996). Fortunately, the intensity and duration of exercise needed to support the immune system is less than that needed to provide the best cardiovascular benefits. Thus, even relatively low levels of aerobic exercise can protect your immune system. Twenty to 30 minutes of brisk walking five days per week is an ideal training program for maintaining a healthy immune response (Leonard, 1996).

The effects of exercise on immune function depend on many factors, including frequency and intensity of exercise (Nieman, 1997). Regular moderate physical activity has positive effects, at least on some measures of immunity, and has been shown to reduce risk of upper respiratory infection. However, very intense and prolonged exercise, such as running a marathon or overtraining, in the short term, actually increase the risk of developing infections (Nieman, 1998). The positive effects of moderate exercise on immunity may also partly explain the apparent reduced susceptibility to cancer of physically active people (Shepard and Shek, 1998).

Immune system changes that apparently are related to the intensity of exercise have been identified (Huddleston, 2001). Moderate endurance exercise, such as brisk walking, stimulates positive changes in the function and numbers of various immune system cells, such as natural killer cells (NK), one of the body's first lines of defenses against viruses. It is also associated with prolonged improvement in the killing capacity of neutrophils, one of the most efficient phagocytes, cells that kill foreign microorganisms and initiate the immune response. These changes may be related to the release of hormones such as endorphins and enkephalins that help to regulate immune function (Huddleston, 2001).

On the other hand, high intensity exercise may have a negative impact on immune function. Immune marker changes that suggest the increased risk for high intensity exercise include, lower measures of immunoglobulins, antibodies like those found and measured in saliva, depressed NK cell activity and decreased neutrophil phagocytic activity. These negative effects may also be related to the release of stress related hormones such as cortisol and adrenocorticotropic hormone (ACTH) which have immunosuppressive characteristics (Huddleston, 2001).

Immune system responses to strenuous exercise include increases in natural killer (NK) cell number and cytotoxicity, and suppressor/cytotoxic lymphocytes as well as decreased proliferative response to antigenic triggers. Task and recovery periods for both acute psychological stress or severe exercise show that immune status is negatively impacted during recovery (Perna *et al.*, 1997).

2.3 HONEY AND IMMUNE FUNCTION

Honey is a natural sweetener comprising vitamins, mineral enzymes, carbohydrates. It also contains natural antioxidant properties which can destroy biologically destructive chemical agents such as cancer, and protect against cellular damage and possibly prevent the development of chronic diseases. Honey has antioxidant and antibacterial properties which can help to improve digestive system, so that one can stay healthy and fight disease (Tan, 2007). It is reported that, honey may increase in immune function by increasing hemoglobin count and treat or prevent anemia, and can help to boost gastrointestinal ulcer healing (Neff, 2007).

CHAPTER 3

MATERIALS AND METHODS

3.1 SUBJECTS

Forty young Malaysian male subjects with age ranging from 19 to 25 year old were recruited in this study. The inclusion criteria of the subjects including the subjects have to be free from any health problems, and they did not have the habit of taking honey as daily supplementation prior to the experiment. The subjects were assigned into four groups, with each group consisting of ten subjects. They were assigned into the control and experimental groups. Each subject was given a detail explanation about the objectives, procedures, benefits, risks and possible discomforts experienced in this study. Ethical Approval (Appendix B) and subjects' information and consent forms (Appendix C) approved by the Universiti Sains Malaysia Research and Ethical Committee were distributed to all the subjects. Subjects were reminded regarding their participation in this study as being voluntary and they were permitted to stop being a part of this study at any time during the course of the study period.

FLOW CHART OF RESEARCH PROCEDURE

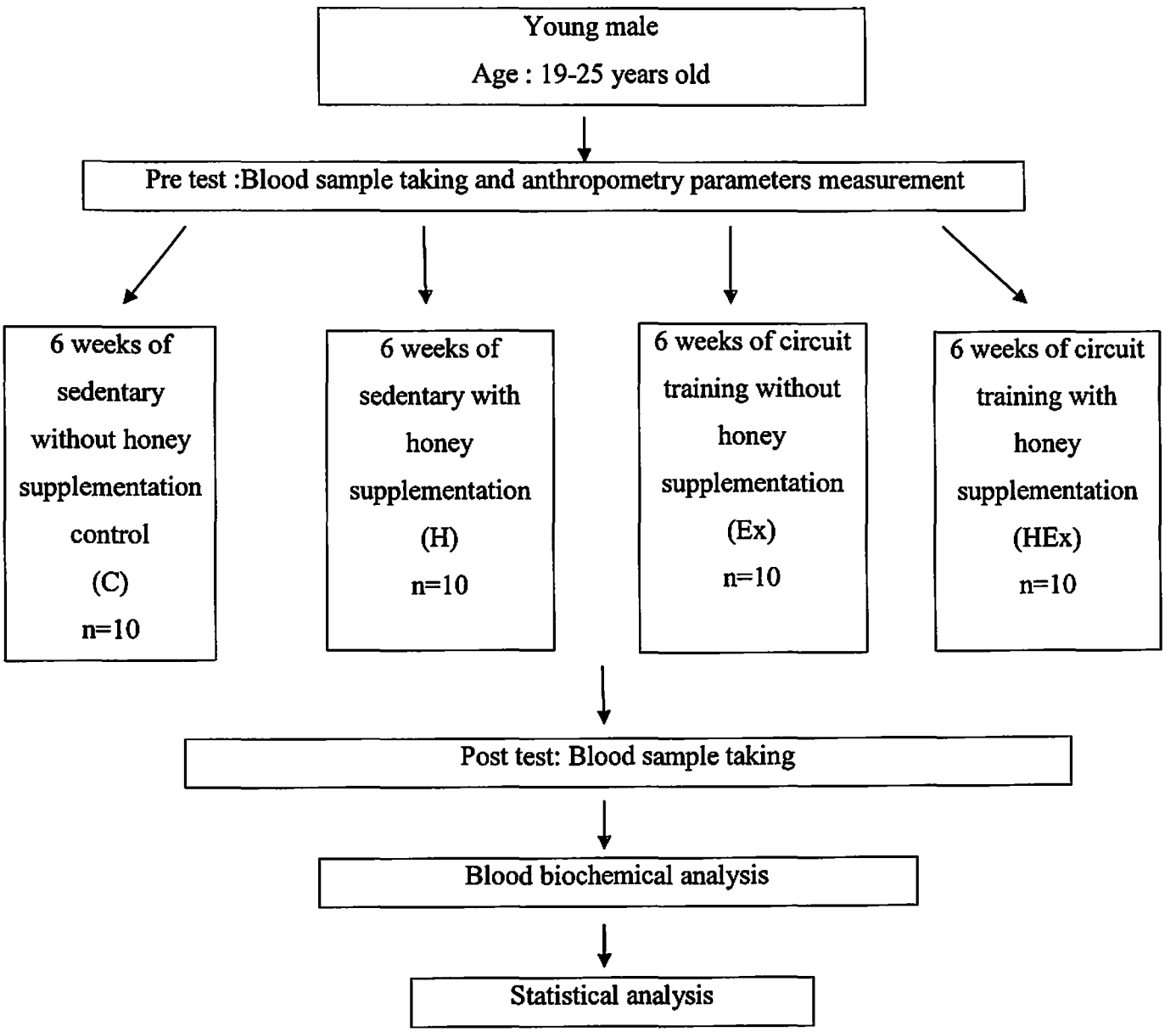

Figure 3.1: Flow chart of the experimental design

3.2 EXPERIMENTAL DESIGN

3.2.1 Subjects grouping

In the present study, the subjects were assigned into four groups, with ten subject per group (n=10): six weeks of sedentary without honey supplementation control (C), six weeks of sedentary with honey supplementation (H), six weeks of circuit training exercise without honey supplementation (Ex), six weeks of circuit training exercise with honey supplementation (HEx) groups.

3.2.2 Blood sample taking, and anthropometric measurements

Immediately before six weeks of experimental period, all the subjects were required to have the blood sample taking, anthropometric measurement sessions in the Exercise and Sport Science Laboratory, School of Health Sciences, Health Campus, Universiti Sains Malaysia. Blood taking was carried out again after 6 weeks of experimental period.

In each blood taking, 2 ml of blood was taken from each subject. The whole blood samples were sent to Immunology Laboratory in Immunology Department, School of Medical Sciences, Universiti Sains Malaysia for immune functions analysis.

After the blood taking, subjects physical and physiological measurements such as height, weight, body mass index (BMI) and percentage body fat were carried out on the same day. The subjecte's body heights were measured by stadiometer (Seca 220. Germany), the body weight, BMI and percentage body fat were measured by a digital bioelectric impedance analysis device (Kanada Scan).

3.2.3 Circuit training programme

The subjects in both the exercise without supplementation group (Ex) and honey supplementation with exercise group (HEx) were required to carry out circuit training sessions, one hour per session (5.30 p.m to 6.30 p.m), three times per week for six weeks.

The exercise sessions started with 10 minutes of warm-up and ended with 5 minutes of cooling down activities. The circuit training programme consisted of two circuits. In each circuit, subjects performed 10 different exercises in 10 different stations (one type of exercise per station, each subject spent 30 seconds in one particular station). The work rest ratio was 1:2, where subjects exercised for 30 seconds for one activity, and rested for one minute before continued with the next activities. Resting time between circuits was five minutes. The activities that involved in circuit training were elastic bend exercise, leg elastic bend, free-weight dumbbell triceps extension, rope skipping, free-weight dumbbell concentration curl, sit-up, back extension, burpee, push-up and split squat. The intensity of circuit training programme was estimated by using heart rate monitor (polar watch, S710, US) wore by one subject throughout the circuit training programme.

3.2.4 Honey Supplementation

Tualang honey, a Malaysian local product was used in this study. Three hundred ml of honey drink which containing 20 g of Tualang honey was cosumed by the subjects of honey supplementation alone group (H) and honey supplementation with exercise group (HEx) per day, seven days per week for six weeks. Subjects in (HEx) consumed 300 ml of honey drink 30 minutes before performing exercise. The mixture of honey was prepared by mixing 20g of honey with 300ml of plain water.

3.2.5 Blood Biochemical Analysis

Both blood testing sessions before and after the 6 weeks were conducted in the morning at 8.30 a.m after a 12 hour fast. The blood was withdrawn by the laboratory technologist in the Exercise and Sport Science Laboratory. About 2 ml of blood were drawn from each subject from the antecubital vein in seated position. Blood taking for subjects in Ex and HEx were carried out 18-20 hours (8-10 am) after performing exercise.

Blood samples collected into EDTA tubes and were brought to the immune laboratory and processed on the same day of collection. The EDTA contained whole blood was used for full blood count analysis, and determination of natural killer (NK) cells. An automated hematology analyser (Sysmex XS-800i) was used for full blood count analysis of white blood cell, lymphocyte, monocytes and basophil. Whereas, analysis of natural killere (NK) cells was carried out by using a flow cytometer (BD FACS Cantor II, Becton Dickinson, USA).

3.2.6 Statistical Analysis

Statistical software in the Statistical Package for Social Sciences (SPSS) Version 18.0 was used for the statistical analysis. Repeated measure ANOVA were performed to determine the significance of the difference between and within groups. Statistical significance was accepted at $p < 0.05$. All data are expressed as means ± standard deviation (SD).

3.2.7 Calculation of sample size

Sample size of the present study was calculated by G Power software. The power of the study was set at 80% with confident interval. The calculated sample size was 9 subjects per group. However, it was estimated that the drop out rate due to stop of participation in the study by the subjects during experimental period would be 10%, therefore, the actual number of the subjects recruited in this study was 10 subjects per group.

CHAPTER 4

RESULTS

4.1 SUBJECTS' ANTHROPOMETRIC DATA

A total of 37 young male subjects with mean age 21.65±1.51 years old completed the study. One subject from control group (C), one from honey group (H) and one subject from exercise with honey supplementation group (HEx) respectively discontinued the programme due to other commitment. Anthropometric data obtained from all the subjects (n=37) are summarized in Table 4.1. The mean anthropometric values ± SD obtained were as follows : body height: 169.76±6.06 cm, body weight: 68.12±13.41 kg, BMI : 23.55±4.06 kg/m^2 and percentage of body fat : 18.88±5.99 %.

Table 4.1 Anthropometric data obtained from all the subjects (N=37)

Parameters	Mean ± SD
Height (cm)	169.76±6.06
Weight (kg)	68.12±13.41
BMI (kg/m^2)	23.55±4.06
Percentage body fat (%)	18.88±5.99

4.2 IMMUNE PARAMETERS

4.2.1 White Blood Cells

Results of white blood cells in all the groups at pre and post tests are presented in Table 4.2 and Figure 4.1. In pre test, there were significant differences ($p<0.05$) between Ex and HEx groups compared to respective C group respectively. However, no significant difference was observed between H and C. In post test, there were significant differences ($p<0.05$) between Ex and HEx groups compared to respective C group respectively. However, no significant difference was observed between H and C. After 6 weeks of experimental period, there were no significant differences between pre and post test in white blood cells in all C, H, Ex and HEx groups.

Table 4.2: White blood cells at pre and post tests (Mean ± SD)

Groups	White Blood Cell (10^3/uL)			
	Pre test	Post test	Mean difference between pre and post test (±SD)	Percent difference compared to pre-test (%)
Control (C)	5.84±1.68	6.16±0.99	0.32±1.33	+5.48
Honey (H)	6.31±1.04	6.34±0.87	0.03±1.07	+0.50
Exercise (Ex)	7.07±1.28*	7.28±1.42*#	0.21±0.87	+2.98
Combined Honey and Exercise (HEx)	7.10±2.13*	7.63±1.33*#	0.52±2.16	+7.38

+, significantly different from respective control group ($p<0.05$)

#, significantly different from respective honey group ($p<0.05$)

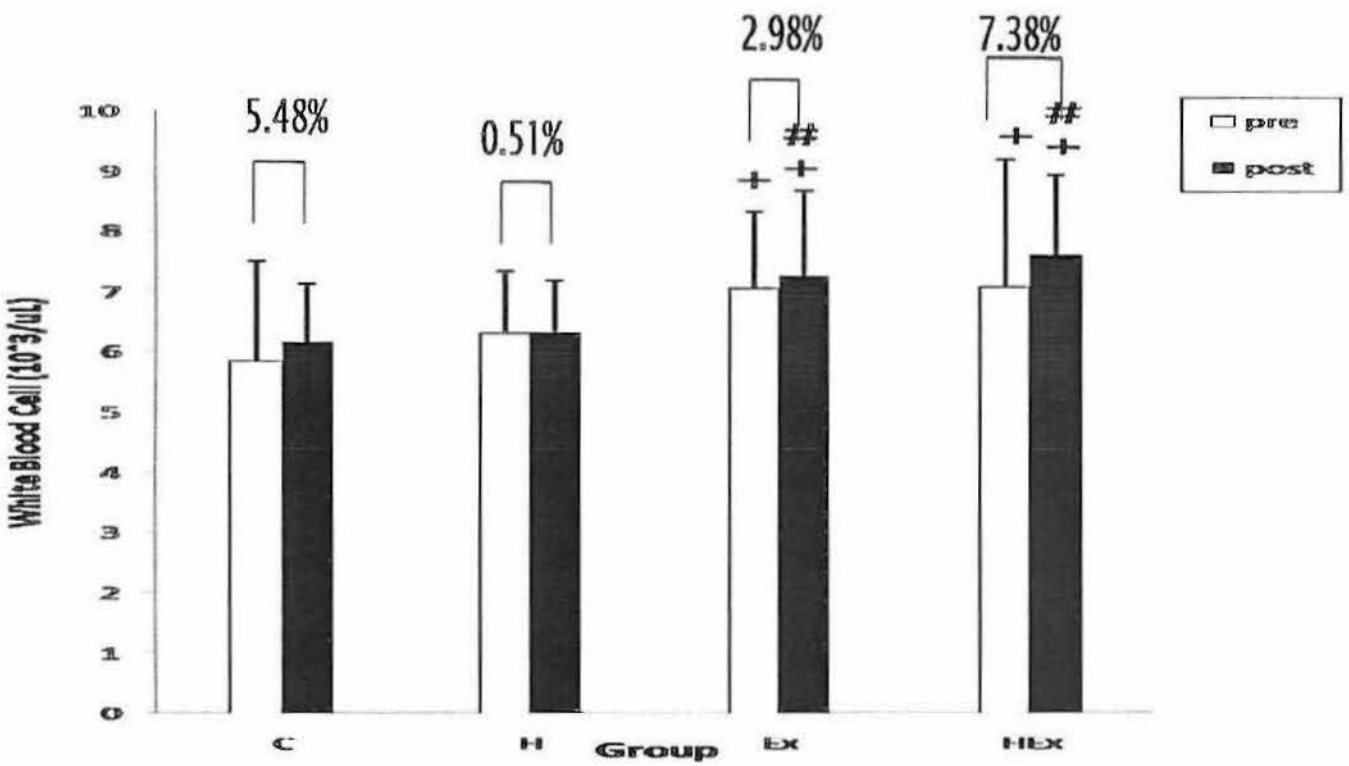

Figure 4.1: White blood cells at pre and post tests (Mean±SD)

+, significantly different from respective control group ($p<0.05$)

#, significantly different from respective honey group ($p<0.05$)

4.2.2 Lymphocytes

Results of lymphocytes in all the groups at pre and post tests are presented in Table 4.3 and Figure 4.2. In pre test, there was significant difference ($p<0.05$) between exercise group (Ex) and control group (C). However, no significant differences were observed in H and HEx compared to respective C group respectively. In post test, there was significant difference ($p<0.05$) between Ex and C group. However, no significant differences were observed in H and HEx compared to respective C group respectively. After 6 weeks, no significant differences were observed between pre and post test in lymphocytes in all the groups.

Table 4.3: Lymphocytes of the subjects at pre and post test (Mean±SD)

Lymphocytes (10^3/uL)				
Groups	Pre test	Post test	Mean difference between pre- and post test (±SD)	Percent difference compared to pre-test (%)
Control (C)	2.10±0.82	2.55±0.43	0.45±0.71	21.51
Honey (H)	2.13±0.67	2.23±0.58	0.10±0.40	4.60
Exercise (Ex)	2.71±0.50+,#	3.03±0.79+,#	0.32±0.75	11.77
Combined Honey and Exercise (HEx)	2.40±0.42	2.72±0.77#	0.32±0.97	13.39

+, significantly different from respective control group ($p<0.05$)

#, significantly different from respective honey group ($p<0.05$)

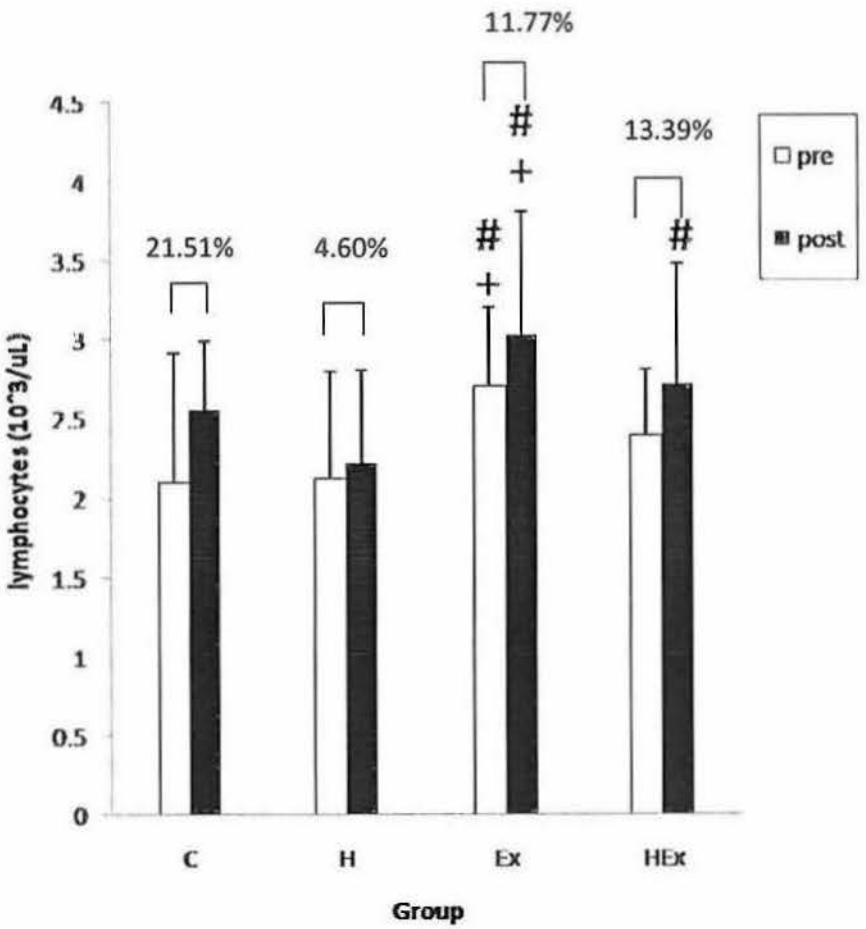

Figure 4.2 : Lymphocytes at pre and post tests (Mean±SD)

+, significantly different from respective control group ($p<0.05$)

#, significantly different from respective honey group ($p<0.05$)