COMBINED EFFECTS OF *LIGNOSUS RHINOCERUS* (TIGER MILK MUSHROOM) SUPPLEMENTATION AND RESISTANCE TRAINING ON ISOKINETIC MUSCULAR STRENGTH AND POWER, ANAEROBIC AND AEROBIC FITNESS LEVEL, AND IMMUNE FUNCTIONS AMONG YOUNG MALES

by

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Introduction: Although exercise and dietary supplementations were postulated to be beneficial for health and physical fitness, the issue of combined effects of resistance training and Tiger Milk Mushroom supplementation is still lacking in the literature and not well documented.

Objective: This study was carried out to investigate the beneficial combined effects of 8 weeks of *Lignosus Rhinocerus* (Tiger Milk Mushroom) supplementation and resistance training on isokinetic muscular strength and power, anaerobic and aerobic fitness, and immune functions among young males.

Method: In this double blind placebo-controlled trial, 38 young males (19-25 years old) were assigned into four groups with 9 t0 10 participants per group: Control (C) group, Tiger Milk Mushroom (TMM) group, resistance training (RT) group and combined resistance training and Tiger Milk

Mushroom (RT&TMM) group. Participants in TMM group and RT&TMM group consumed two capsules of TMM (250mg per capsule) 7 days per week for 8 weeks. Participants in the control (C) group and RT group participants were consumed placebo capsules for 7 days per week for 8 weeks. Resistance training programme consisted of 10 different types of exercise which involved muscle of the upper and lower limbs. It was conducted three times per week for 8 weeks. Prior to the intervention period, pre-test was carried out to measure anthropometric measurements, isokinetic muscular strength and power, anaerobic and aerobic fitness. Blood samples were also withdrawn in order to determine immune function parameters through full blood counts and immunophenotyping. After 8 weeks of intervention period, all these parameters were measured again during post test.

Result: Positive effects observed in isokinetic muscular strength and power in combined RT&TMM group were peak torque of shoulder extension at $60^{o}.s^{-1}$, average power of shoulder flexion and extension at $300^{o}.s^{-1}$, peak torque of knee flexion at $60^{o}.s^{-1}$, average power of knee flexion and extension at $300^{o}.s^{-1}$. In addition, there were also increases in anaerobic power and capacity and estimated maximum oxygen consumption. Similarly, positive effects in RT alone group, were observed in the isokinetic muscular strength and power parameters such as average power of shoulder flexion at $300^{o}.s^{-1}$,peak torque of knee flexion and extension at $60^{o}.s^{-1}$, average power of knee flexion and extension at $300^{o}.s^{-1}$. Additionally, increases in anaerobic power and capacity, aerobic fitness, T lymphocytes (CD3 and CD4) and B lymphocytes (CD19) counts were observed in RT alone group.

Conclusion: Resistance training with dumbbells and elastic bands elicited increased isokinetic muscular strength and power, anaerobic and aerobic fitness and immune functions among young males. However, combining resistance training with Tiger Milk Mushroom did not result in additional benefits.

Dr. Chen Chee Keong: Supervisor

Dr. Ooi Foong Kiew: Co-Supervisor

Dr: Wan Zuraidah binti Wan Abd Hamid: Co-Supervisor

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ABSTRAK

KESAN GABUNGAN LATIHAN RINTANGAN DAN PENGAMBILAN CENDAWAN SUSU RIMAU KEATAS KEKUATAN DAN DAYA OTOT, KECERGASAN ANAEROBIK DAN AEROBIK SERTA FUNGSI IMUN DALAM KALANGAN LELAKI MUDA

Walaupun senaman dan pengambilan makanan tambahan diandaikan memberi kesan baik terhadap kesihatan dan kecergasan fizikal, namun pengetahuan tentang kesan gabungan latihan rintangan dan pengambilan suplemen Cendawan Susu Rimau masih kurang dalam bahan bacaan dan masih kurang didokumenkan. Kajian ini dijalankan untuk menyiasat kebaikan kesan gabungan latihan rintangan dan pengambilan suplemen Cendawan Susu Rimau terhadap kekuatan isokinetik dan kuasa otot, kecergasan anaerobik dan aerobik serta fungsi imun dalam kalangan lelaki muda. Dalam kajian cubaan kawalan-rabun-berganda ini, 38 orang peserta yang terdiri daripada lelaki berumur lingkungan 19-25 tahun dibahagikan kedalam 4 kumpulan yang berbeza dengan bilangan peserta diantara 9 hingga 10 orang setiap kumpulan; kumpulan kawalan (C), kumpulan latihan rintangan (RT), kumpulan Cendawan Susu Rimau (TMM) dan kumpulan gabungan latihan rintangan dan Cendawan Susu Rimau (RT&TMM). Peserta dalam kumpulan TMM dan RT&TMM mengambil 2 kapsul Cendawan Susu Rimau (250mg setiap kapsul) 7 hari seminggu selama 8 mingggu. Manakala para peserta dalam kumpulan kawalan dan RT mengambil kapsul placebo sepanjang 7 hari semiggu selama 8 minggu. Program latihan rintangan terdiri daripada 10 jenis senaman yang melibatkan kumpulan otot kaki dan lengan. Latihan diadakan sebanyak tiga kali seminggu

selama 8 minggu. Sebelum program latihan ini, parameter yang diukur terdiri daripada antropometri, kekuatan dan kuasa otot isokinetik, kecergasanan aerobik dan aerobik. Sampel darah juga diambil untuk menentukan fungsi imun melalui pengiraan jumlah darah dan fenotip imun. Selepas 8 minggu, kesemua parameter ini diukur semula dalam pasca ujian. Kesan-kesan positif yang diperolehi oleh kumpulan kombinasi RT&TMM dari segi kekuatan dan purata kuasa otot isokinetik seperti tork tertinggi ekstensi bahu pada $60^{o}.s^{-1}$; purata daya ekstensi dan fleksi bahu pada $300^{o}.s^{-1}$; tork tertinggi fleksi lutut pada $60^{o}.s^{-1}$; purata daya ekstensi dan fleksi lutut pada $300^{o}.s^{-1}$. Tambahan pula, kecergasan anaerobik dan aerobik juga meningkat dalam kumpulan ini. Manakala bagi kumpulan RT, kesan-kesan positif dikenalpasti dalam kekuatan dan kuasa otot isokinetik seperti purata daya fleksi bahu pada $300^{o}.s^{-1}$; tork tertinggi ekstensi dan fleksi lutut pada $60^{o}.s^{-1}$; purata daya ekstensi dan fleksi lutut pada $300^{o}.s^{-1}$. Di samping itu, kuasa dan keupayaan anaerobik, kecergasan aerobik dan bilangan T limfosit (CD3 and CD4) and B limfosit (CD19) juga meningkat dalam kumpulan RT. Latihan rintangan dengan menggunakan dumbbell dan pita elastik dapat meningkatkan kekuatan dan kuasa otot isokinetik, kecergasan anaerobik dan aerobik, serta fungsi imun dalam kalangan lelaki muda. Walaubagaimanapun, gabungan latihan rintangan dengan pengambilan Cendawan Susu Rimau tidak menghasilkan manfaat tambahan.

ABSTRACT

COMBINED EFFECTS OF *LIGNOSUS RHINOCERUS* (TIGER MILK MUSHROOM) SUPPLEMENTATION AND RESISTANCE TRAINING ON ISOKINETIC MUSCULAR STRENGTH AND POWER, ANAEROBIC AND AEROBIC FITNESS LEVEL AND IMMUNE FUNCTIONS AMONG YOUNG MALES

Although exercise and dietary supplementations were postulated to be beneficial for health and physical fitness, the issue of combined effects of resistance training and Tiger Milk Mushroom supplementation is still lacking in the literature and not well documented. This study was carried outto investigate the beneficial combined effects of 8 weeks of *Lignosus Rhinocerus* (Tiger Milk Mushroom) supplementation and resistance training on isokinetic muscular strength and power, anaerobic and aerobic fitness, and immune functions among young males. In this double blind placebo-controlled trial, 38 young males (19-25 years old) were assigned into four groups with 9 t0 10 participants per group: Control (C) group, Tiger Milk Mushroom (TMM) group, resistance training (RT) group and combined resistance training and Tiger Milk Mushroom (RT&TMM) group. Participants in TMM group and RT&TMM group consumed two capsules of TMM (250mg per capsule) 7 days per week for 8 weeks. Participants in the control (C) group and RT group participants were consumed placebo capsules for 7 days per week for 8 weeks. Resistance training programme consisted of 10 different types of exercise which involved muscle of the upper and lower limbs. It was conducted three times per week for 8 weeks.

Prior to the intervention period, pre-test was carried out to measure anthropometric measurements, isokinetic muscular strength and power, anaerobic and aerobic fitness. Blood samples were also withdrawn in order to determine immune function parameters through full blood counts and immunophenotyping. After 8 weeks of intervention period, all these parameters were measured again during post test. Positive effects observed in isokinetic muscular strength and power in combined RT&TMM group were peak torque of shoulder extension at $60^{o}.s^{-1}$, average power of shoulder flexion and extension at $300^{o}.s^{-1}$, peak torque of knee flexion at $60^{o}.s^{-1}$, average power of knee flexion and extension at $300^{o}.s^{-1}$. In addition, there were also increases in anaerobic power and capacity and estimated maximum oxygen consumption. Similarly, positive effects in RT alone group, were observed in the isokinetic muscular strength and power parameters such as average power of shoulder flexion at $300^{o}.s^{-1}$,peak torque of knee flexion and extension at $60^{o}.s^{-1}$, average power of knee flexion and extension at $300^{o}.s^{-1}$. Additionally, increases in anaerobic power and capacity, aerobic fitness, T lymphocytes (CD3 and CD4) and B lymphocytes (CD19) counts were observed in RT alone group. Resistance training with dumbbells and elastic bands elicited increased isokinetic muscular strength and power, anaerobic and aerobic fitness and immune functions among young males. However, combining resistance training with Tiger Milk Mushroom did not result in additional benefits.

CHAPTER 1

INTRODUCTION

The production of energy can be termed as ergogenesis. Ergogenic aids are substances or devices used to improve energy production and in turn, improve human performance. They can be classified into several categories including nutritional, psychological, pharmacological, physiological and mechanical aids. A large body of data concerning the effects of ergogenic aids on exercise performance can be gather from various integrated disciplines such as sport nutrition, biochemistry, pharmacology, exercise physiology, and sport psychology (Bucci, 1993).

Previous studies (Bucci, 1993; Manore and Thompson, 2000; McArdle *et al.*, 2009) had reported the mechanisms and research results on ergogenic aids specifically in nutrition discipline. Theoretically, there are thousands of substances that are potentially involved in ergogenesis. Bucci (1993) categorised nutritional ergogenic aids into five major categories. There were macronutrients, micronutrients supplementation, metabolic intermediates, minerals and amino acid ergogenic aids.

Macronutrients ergogenic aids are basically involved in the manipulation of specific amount and specific time of carbohydrate, protein and fat consumption that should be taken by athletes individually to store energy level for sports specific events. One of the most applied concepts of macronutrient ergogenic aid

is glycogen super compensation or carbo-loading by using Astrand regimen or Sherman method (Bucci, 1993).

Micronutrients ergogenic aids are related to the consumption of vitamin B, C and four other fat soluble vitamins; A,D,E and K, while metabolic intermediate ergogenic aids put more concern on substance such as bicarbonate, antioxidant, aspartate salts, carnitine, creatine and co-enzyme Q_{10} (Bucci, 1993). These two categories of ergogenic aids were closely related to dietary supplement (Ervin *et al.*, 2004). According to the third National Health and Nutrition Examination Survey from 1988 to 1994 (NHANES III), dietary supplements were classified into six broadly defined categories. There were single vitamins, multiple vitamins, multiple minerals, combination vitamins and mineral and other dietary supplements like herbal and botanical supplements (Ervin *et al.*, 2004).

People take dietary supplementation for some reasons such as making up for nutrients missing in food supply, to reduce susceptibility to disease, to lower severity of disease, to promote weight loss, to improve self appearance, to increase energy and to improve performance (Ervin *et al.*, 2004). In addition, a prevalence study entitled, “Vitamin-mineral supplements use and associated factors among young Malaysians” reported that out of 105 participants, 43% used vitamin-mineral supplementation. The main reasons were to maintain good health and to ensure adequate nutrition (Al-Naggar and Chen, 2011).

Besides vitamins and minerals, natural or botanical supplements are becoming popular dietary supplements and have been used as medicine

throughout history (Williams, 2006; Tan *et al.*, 2012). According to botanist, botanical products can be defined as plants that contain numerous phytochemical which can be derived from various parts such as leaves, bark, berries, roots, gum, seeds, stems and flowers of the plants (Chen *et al.*, 2012). Based on this definition, herbs and medicinal mushroom are included in botanical products. Medicinal mushroom that has gained interest is Lignosus Rhinocerus (Tiger Milk Mushroom). Tiger Milk Mushroom (TMM) is an important medicinal mushroom found in Southeast Asia and one of the most popular medicinal mushrooms used by indigenous communities of Peninsular Malaysia (Tan *et al.*, 2012).

Medicinal properties of herbs and medicinal mushroom were reported to function as immune system booster, anticancer, chronic hepatitis, lowering cholesterol level, reducing insulin resistance and asthma treatment (Davis-Searles *et al.*, 2005; Lee *et al.*, 2012a; Lee *et al.*, 2012b; Deng *et al.*, 2013; Petiwala *et al.*, 2013). Recently studies on herbs as a performance enhancer became a topic of interest. (Williams, 2006; Senchina *et al.*, 2009; Chen *et al.*, 2012).Herbs have been used to enhance physical performance on the basis of increasing mental alertness and stimulating fat-burning (Bucci, 2000). Several herbs have been studied for their contribution of ergogenic potential such as Chinese, Korean and American ginsengs, Siberian ginseng, Ma Huang or Chinese Ephedra. The used of herbs as ergogenic aids in exercise and sport is not a new trend (Chen *et al.*, 2012). However, the scientific study regarding

association of medicinal mushroom and sport performance is still lacking in literature (Aziz *et al.*, 2006).

In contrast, uses of dietary supplements can also be a risk rather than benefits to some individuals. Theoretically, when substance of a good thing is being used over the optimum level, it might turn to a bad consequence (Pendleton *et al.*, 2012; Kim *et al.*, 2013). The overused of dietary supplement can lead to medical complications including neurologic disturbances, gastrointestinal symptoms, liver toxicity, birth defects and homeostasis interferences (Hathcock, 1997; Rader and Pawar, 2012). According to Moughan *et al*., high intake of some dietary supplements on a long-term basis might be harmful especially when it poses metallic elements such as zinc and iron. (Maughan *et al.,* 2004) Thus, the dependency on dietary supplement alone without putting an effort on physical activity to gain various health benefits is not fully recommended.

Mounting evidence postulated the benefits of exercise in order to promote and motivate people to have a healthy lifestyle. The regular participation in exercise gives positive effects on ideal body composition (Hicks *et al.*, 2011), increased cardiorespiratory fitness (Hill *et al.*, 2007; Dassios *et al.*, 2013) enhancing muscular strength (Sukalinggam *et al.*, 2012; Evaggelos *et al.*, 2013) increased bone mineral density (Ooi *et al.*, 2009), enhance mental well-being and stimulate more positive attitude towards a lifetime (Gerber *et al.*, 2012). However, the prevalence of obesity in Malaysian seems to increase year by year (Ismail *et al.*, 2002; Rampal *et al.*, 2007).

In exercise or training, the main objectives are to stimulate structural and functional adaptation in order to improve physical performance (McArdle *et al.*, 2007). These adaptations require a strict planned programmes based on selective fitness goals. The attention should focus more on frequency and length of workouts, type of exercise or training, intensity, repetition, duration and rest intervals between activities. Health related benefits of resistance training have recently been gained the popularity as a modality for health purposes (Kang *et al.*, 2004; Kraemer and Ratamess, 2004). American College of Sports Medicine (ACSM) and American Heart Association (AHA) were recommended resistance training for wide range of population starting from adolescents to elderly and from healthy to clinical populations (Feigenbaum and Pollock, 1999).

Understanding the concept and relationship between botanical product supplementation, resistance exercise, physical fitness and immune functions has potential benefits for public health. To our knowledge, to date no scientific studies have examined Tiger Milk Mushroom supplementation as a performance enhancer except for positive effects of immune functions (Lee *et al.*, 2012a; Lee *et al.*, 2012b). The present study therefore investigated the combined effects of Tiger Milk Mushroom supplementation and resistance exercise on muscular strength and power, anaerobic and aerobic fitness, and immune functions in young males with age ranging from 19 to 25 years old.

1.1 OBJECTIVES OF THE STUDY

i. To investigate the combined effects of 8 weeks of *Lignosus Rhinocerus* (Tiger Milk Mushroom) supplementation and resistance training on isokinetic muscular strength and power among young males.
ii. To investigate the combined effects of 8 weeks of *Lignosus Rhinocerus* (Tiger Milk Mushroom) supplementation and resistance training on anaerobic & aerobic fitness among young males.
iii. To investigate the combined effects of 8 weeks of *Lignosus Rhinocerus* (Tiger Milk Mushroom) supplementation and resistance training on immune functions among young males.

1.3 HYPOTHESES

i. Ha: There are significant differences in isokinetic muscular strength and power in combined resistance training and Tiger Milk Mushroom supplementation group compared to sedentary without supplementation control, Tiger Milk Mushroom supplementation group or resistance training group.
ii. Ha: There are significant differences in anaerobic fitness in combined resistance training and Tiger Milk Mushroom supplementation group compared to sedentary without supplementation control, Tiger Milk Mushroom supplementation group or resistance training group.
iii. Ha: There are significant differences in aerobic fitness in combined resistance training and Tiger Milk Mushroom supplementation group compared to sedentary

without supplementation control, Tiger Milk Mushroom supplementation group or resistance training group.

iv. Ha: There are significant differences in immune functions in combined resistance training and Tiger Milk Mushroom supplementation group compared to sedentary without supplementation control, Tiger Milk Mushroom supplementation group or resistance training group.

1.4 SIGNIFICANCE OF THE STUDY

Since the combined effects of resistance training with Tiger Milk Mushroom supplementation on muscular strength and power, anaerobic and aerobic fitness and immune functions have not been established and the literature regarding this issue is still lacking, the present study will give some insight on the possible beneficial effects of combination of resistance training and Tiger Milk Mushroom supplementation. It is hope that the result obtain from this finding can be used for developing and promoting exercise especially to young males by formulating guidelines for the enhancement and maintenance of muscular strength and power, anaerobic and aerobic fitness and immune functions.

1.5 OPERATIONAL DEFINITIONS

Resistance Training: The resistance training consists of 10 different exercises working on upper and lower body muscles. The training sessions were carried out for 3 times per week for 8 weeks of intervention period. The subjects were required to perform 3 sets of 10 to 20 repetitions per station.

Supplementation: Tiger Milk Mushroom and placebo capsules were randomly given to the participants. Participants in TMM group and RT&TMM group consumed two capsules of TMM (500mg; 250mg per capsule) 7 days per week for 8 weeks. Participants in the control (C) group and RT group consumed placebo for 7 days per week for 8 weeks.

Muscular Strength and Power: Measurement of dominant upper and lower limb peak torque and average power of the participants was carried out by using isokinetic dynamometer (Biodex multi-joint dynamometer System 3 Pro, New York) with the set of angular velocity at $60^{o}.s^{-1}$ for strength and $300^{o}.s^{-1}$ for power.

Anaerobic Fitness: Measurement of anaerobic power and anaerobic capacity was performed by using Wingate anaerobic test on a cycle ergometer (Lode Groningen, Netherlands).

Aerobic Fitness: Prediction of participants' maximum oxygen uptake (VO_2 max) was carried out by using 20 meters shuttle run test. The test involved running continuously between two points that were 20m apart in and synchronised with a pre-recorded audio tape which has the 'beep' sound at set intervals.

Immune Function: Measurements of blood parameters such as full blood counts: white blood cells, neutrophil, and lymphocyte counts by using Automated Hematology Analyzer (Sysmex XS-800i, USA).

Immunophenotyping measurement for serum T lymphocytes (CD3, CD4, and CD8) and B lymphocytes (CD19) by using Flow Cytometer (BD FACS Canto II, Becton, USA).

CHAPTER 2

LITERATURE REVIEW

2.1 Overview of Dietary Supplementation of Plant Origin

With the influenced of technology and the increasing appearance of synthetic food, people nowadays are turning towards food and medicines including dietary supplements of plant origin as they seem to be more natural and safe (Kaptchuk and Eisenberg, 1998). This growing uses of dietary supplements from plant origin is due to health concern and easily access to complementary and alternative medical practices (Eisenberg *et al.*, 1998). Previous study found that only products intended for ingestion in the form of a capsule, powder, soft gel or gel cap and not represented as a conventional food as a sole item of a meal or a diet can be marketed as dietary supplements (Marriott, 2003). A prevalence study estimated that 30-35% of American population consumed dietary supplement on a regular basis (Aarts, 1998).

The diet supplement intake has also become an alarming issue in Malaysia. The prevalence however has only been reported in a few publications and is still lacking in the literature (Teng *et al.*, 2008; Aziz and Tey, 2009). Teng *et al.,* (2008) reported that 71.9% out of 882 young adults in Malaysia used dietary supplements daily. This group was classified into 3 categories and found that 32.8% were vitamins, 12.6% were dietary supplements and the rest 30.4% were mineral dietary supplement consumers. In addition, Aziz and Tey (2009) reported a prevalence study on herbal usage among Malaysians. The study

demonstrated that 33.9% out of 542 respondents used herbal medicine in the previous 12 months. Moreover, Al-Naggar and Chen (2011) reported that 43% of out of 105 young Malaysians used vitamin-mineral supplements.

Factors that contribute to dietary supplementation among Malaysians were to maintain good health, to ensure adequate nutrition, to increase energy, to improve self appearance, affordable, and easily assessed (Teng *et al.*, 2008; Aziz and Tey, 2009; Al-Naggar and Chen, 2011).

2.1.1 Dietary Supplements from Plant Origin on Health and Physical Performance

One of the most popular herbal dietary supplements worldwide is ginseng (Bahrke and Morgan, 2000). Belonging to the plant family Araliaceae, ginseng has two major forms of species that are Chinese, Korean or Asian ginseng which belong to the genus *Panax*; and Siberian or Russian ginseng which belongs to the genus *Eleutherococcu* (Vogler *et al*., 1999). In *Panax ginseng,* the biologically active complex mixture constitute of triterpene saponins known as ginsenosides. Siberian, or Russian ginseng consists of the dried roots and rhizome of *Eleutherococcus senticosis*, which contains phenolics, polysaccharides, and eleutherosides. In China, *Eleutherococcus senticosus* is known as *wujiaseng* or C*iwujia*, and ciwujianosides is proposed as active ingredients (Cheuvront, 1999).

Research findings relative to the effect of ginseng on endurance performance are consistent (Williams, 2006). Asian ginseng found to improve in

exercise performance with use of standardized extracts, long duration of supplementation, large numbers of subjects, and elderly subjects (Bucci, 2000a). Ergogenic effect of ginseng is attributed to *Ginsenosides*, *eleutherosides,* and *ciwujianosides*. Ginseng is theorised to reduce the catabolic effects of the stress hormone, cortisol by influencing the hypothalamic-pituitary-adrenal cortex (HPA) axis. This theory of anti-stress effects proposed that ginseng supplementation could enhance sports performance by allowing athletes to train more intensely or to induce an ant fatiguing effect and increase stamina during competition (Williams and Branch, 2002). Given these postulates, much of the research involving the ergogenicity of ginseng supplementation has focused on cardiovascular or aerobic endurance performance, with some emphasis on psychomotor performance (Bucci, 2000b; Williams and Branch, 2002).

In line with these claims, a study was done to investigate the effects of 1350mg dose of Panax notoginseng supplementations on aerobic capacity, endurance and mean blood pressure (MAP) in young adults (Liang *et. al*, 2005). Participants aged 20-35 years were randomly assigned into experimental group (EXP, n =13) and control group (CON, n=16). After 30 days of intervention period, it was found that EXP group improved their endurance time by 7 minutes, lower maximal MAP from 113 ± 12 to 109 ± 14 mmHg, and VO_2 at the 24^{th} minute from 32.5±8ml.kg^{-1}.min^{-1} to 27.6±8ml.kg^{-1}.min^{-1} during endurance cycle exercise. These researchers concluded that endurance time to exhaustion was improved and MAP and VO_2 were lowered during endurance exercise after consuming 1350 mg per day of PNG supplement for 30 days (Liang *et al.*, 2005).

Besides ergogenicity on physical and physiological performance, herbs also reported possess anti-stress properties. While comparing the effectiveness of *Gingko biloba* and *Panax ginseng* on stress-induced disorders in rat, Rai *et al.* (2003) found that *G.biloba* (30mg/kg body weight) was more effective in acute stress, where as *P.ginseng* (100mg/kg body weight) was beneficial for chronic stress. *G. biloba* reduced the acute stress determinant factors such as in ulcer index (UI), adrenal gland weight (AGW), plasma level (GL), creatine kinase activity (CK), and serum corticosterone (CORT). However, it did not produce any significant effect on chronic stress alteration. In addition, *P. ginseng* reduced the UI, AGW, GL, Triglycerides (TG), CK activity, and CORT level significantly plus attenuates the hypothalamic-pituitary- adrenal axis (HPA) by different pathways during chronis stress, which is remains inactive during acute stress (Rai *et el.*, 2003).

Besides ginseng, Chen *et al.* (2012) investigated the effects of *Eurycoma longifolia Jack (ElJ)* or Tongkat Ali on endurance cycling and running performance. These researchers reported that ElJ supplementation has no significant improvement in either cycling or running endurance and suggested that more studies should be conducted to evaluate and substantiate the potential effects of this herb on sports and exercise with additional factors such as dosage, supplementation period and a larger sample size should be taken into consideration.

Another popular dietary herb is Ganoderma lucidum (*G.lucidum*) or Reshi Mushroom. Ganoderma lucidum also known as ling zhi in China and they grow

wildly in decaying logs and stumps. The red variety is most commonly used and commercially cultivated in East Asia and North America. In traditional China medicine, *G.lucidum* has been used to treat liver disorder, hypertension, fatigue, athma, insomnia, cough, arthritis and other disease. Ancient Chinese and Japanese believed that Ganoderma lucidum could maintain calmness during meditation (Ulbricht *et al.*, 2010).

Ganoderma lucidum had been studied for it's the ergonegic effects (Aziz *et al.*, 2006) and health contribution (Zhang *et al.*, 2008). A double-blind, placebo-controlled experimental design was carried out to investigate the effects of this medicinal mushroom on aerobic and anaerobic and strength parameters in 19 hockey players (Aziz *et al.*, 2006). The subjects were randomly divided into control and experimental groups. 440mg extract of *G.lucidum* was given to experimental group for 30 days while control group consumed placebo. The investigators reported that the results were no significant improvement on the measured parameters and it has been postulated that the result were due to small number of subjects, and low dosage of supplementation (Aziz *et al.*, 2006).

Zhang *et al*. (2008) reported the effects of *Ganoderma Lucidum* supplementation on T lymphocyte subsets. This study involved 40 football players randomly assigned to four experimental groups of control (living at sea level) and living high and training low groups (LHTL1, LHTL2, and LHTL3). Subjects in three groups stayed in normobaric hypoxic room for 28 days. These four groups were trained together at sea level. The LTHL 1, LTHL2, and LTHL3 were provided with placebo, *G. Lucidum* 10 capsules per day and 20 capsules

per day respectively for 6 weeks (2 weeks for baseline supplementation, followed by 4 weeks of treatment protocol). Finding from this study concluded that the ingestion of *G.Lucidum* in LTHL3 could help to improve the T lymphocytes due to its main active components of polysaccharides (Zhang *et al.*, 2008).

2.2 Overview of *Lignosus Rhinocerus* (Tiger Milk Mushroom)

Lignosus Rhinocerus or Tiger Milk Mushroom has been selected as one of the unique national treasure in Malaysia. Tiger Milk Mushroom (TMM) is only found in a small geographic region in Malaysia, Thailand, Philippines, Papua New Guinea, New Zealand, and Australia. TMM is called as "*Cendawan Susu Rimau*" in the Malay language was early purposed by a Pahang resident, Tuan Haji Mat Yusop back in 1970s and Chinese recognised TMM as "*Hurulingzhi*" (Huang, 1999; Tan, 2009).

According to Tan *et. al.* (2012), 400 years ago, this important medicinal product was given to a European government agent. It was recorded as "*Lac Tygridis*" which means tiger's milk in the Diary of John Evelyn in 1664 and was discovered in the Malaysian tropical rain forest. TMM possesses such quite a number of names which mean that Tiger Milk Mushroom was well recognised previously. The scientific name and taxonomy of TMM has recently been identified and confirmed by morphology characterisation as *P.Rhinocerus Cooke* or *Lignosus Rhinoceros* Cooke Rvy because it was successfully cultivated. Thus, taxonomy of *Lignosus Rhinoceros* belongs to Eumycota, Basidiomycotina,

Hymenomycetes, Aphllophorales, and Polyporaceae (Tan *et al.,* 2010; Tan *et al.,* 2012)

TMM has been described as a fungus that is used by local communities to treat diseases. In addition, Sir Henry Nicholas Ridley, the pioneer in Malay rubber industry mentioned the medicinal uses of TMM (Tan *et al.*, 2010a). Malaysia former Prime Minister, Tun Dr. Mahathir Mohamed during opening speech in Biomalaysia 2002 stated that his chronic cough had been cured after taking this medicinal mushroom (Tan *et al.*, 2012a). In Hong Kong, China, *Hurulingzhi* was used by chinese physicians to treat liver cancer, hepatitis, and gastric ulcer (Huang, 1999). While aboriginal people in South East Asia used "*Cendawan Susu Rimau*" as an antipruritic (itching reliever) and antipyretic (pertaining to fever) general tonic, starve off hunger, healing cold, cough, cancer, food poisoning, and swollen breasts (Tan, 2009).

The most valuable part of TMM is its sclerotium. The main functions of sclerotia in medicinal mushrooms are to provide reserves for the fungus to germinate and allow the fungus to survive under unfavorable condition (Willtes and Bullock, 1992). The sclerotium of the TMM is located under the ground with spherical, oval and irregular in shape about 4-5 cm in diameter. The rough and wrinkly surface or called as "rind" of the sclerotia is brownish white in color while its fruiting-bodies are oval shaped, ciliated and depressed in the center and in tea brown in color. TMM's internal structure is a bit powdery and white color (Huang, 1999; Wong and Cheung, 2008a). The existence of TMM in Malaysia's rain forest

can only be noticed when the cap (fruiting body) sprouts out from the ground (Wong and Cheung, 2008a).

The rare species of TMM can still be collected from various forest areas of Cameron Highland, Sungai Perak, Grik, Hulu Langat and Raub, Malaysia. Tan *et. al.,* (2010) started to focus on genetic marker of TMM in order to authenticate the collected specimen. The spores and tissue from its sclerotium, stem, and pileus were then grown in special formulated culture media. 6 months is required for cultivation process of sclerotia. However, formation of stem and pileus takes 2 to 3 years to cultivate. By using this formula, sclerotia parts of TMM have been successfully cultivated at about 300kg per month in environmentally controlled 1000 square feet culture room (Tan *et al.*, 2010b). In order to qualify as functional ingredient, cultivated TMM was submitted to National Pharmaceutical Control Bureau (NPCB).

The document on safety, history of use and bioactivities of TMM had been accepted to be listed in NPCB Traditional Medicine Active Ingredients List. TMM then are eligible to be used in any registered product. In April 2011, the first product of 250mg of 100% cultivated TMM sclerotic was approved by NPCB as traditional medicine (Tan *et al.*, 2010b).

2.2.1 Medicinal Value of Tiger Milk Mushroom

The sclerotial powder has high carbohydrate but low fat content. According to Yap *et al.* (2013), the cultivated strain contains higher amounts of protein and water-soluble substances than the wild type. Phenolic content of hot-water, cold-water, and methanol extracts of the sclerotial powders ranged from 19.32 mg Gallic Acid equivalents (GAE) g^{-1} extract to 29.42 mg.GAE.g^{-1} extract, while the ferric reducing antioxidant power values ranged from 0.006 mmol.min^{-1}.g^{-1}.extract to 0.016 mmol.min^{-1}.g^{-1}.extract. The $DPPH^{\bullet}$, $ABTS^{\bullet+}$, and superoxide anion radical scavenging activities of the extracts ranged from 0.52 to 1.12, 0.05 to 0.20, and -0.98 to 11.23 mmol Trolox equivalents (TE) g^{-1} extract, respectively. Both strains exhibited strong superoxide anion radical scavenging activity comparable to rutin. The cold-water extracts exhibited anti-proliferative activity against human breast carcinoma (MCF-7) cells, with IC_{50} values of 206µg.mL^{-1} and 90µg.mL^{-1} for the wild type and cultivated strains, respectively (Yap *et al.,* 2013)

A research done in 2008 has found that the composition of sclerotia part of *Lignosus rhinocerus* consists of substantial amount of carbohydrate ranging from 90.5% to 98.1% dry matter (DM). The lipid content was very low ranging from 0.02% to 0.14% DM while protein composition was about 0.67% – 6.71% DM (Wong and Cheung, 2008b). Several studies on the pharmaco properties of TMM were carried out to produce evidence-based to ethno-botanical claims of TMM's medicinal properties. Previous studies had examined the anti-proliferative effect against leukemic cells, anti-inflammatory effects of *L. rhinoceros*, and

immune-modulating in animal studies (Lai *et al.*, 2008; Wong *et al.*, 2009; Wong *et al.*, 2010).

Lai *et al*. (2008) examined the antiproliferative effects of hot water (PR-HW) and cold alkaline extract (PR-CA) of sclerotia polysaccharides form *P.rhinocerus* on three different kinds of leukemic cells that were human acute promyelocytic leukemic cells HL—60 (CCL-240, ATCC); chronic myologenous leukemia cells— K562 (CCL-243, ATCC); and acute monocytic leukemia— THP-1 (TIB-202, ATCC). The hot water and cold alkaline extract were said to have active compounds, polysaccharide-protein complex and a glucan respectively (Lai *et al.*, 2008). This study found that hot water extract (PR-HW) shown significant growth inhibition of human acute promyelocytic leukemic cells (HL-60), chronic myologenous leukemia cell (K562), and acute monocytic leukemic (THP-1) *in vitro* PR-CA showed no inhibition. Immunophenotyping analysis revealed that the antiproliferative effects of PR-HW contains polysaccharide-protein complex on human acute promyelocytic leukemic cells (HL-60) was mediated by cells cycle arrest at G_1 phase, which subsequently led to apoptosis (Lai *et al.*, 2008).

Sclerotia polysaccharides of *P.rhinocerus* has also been noted to stimulate innate immune cells (Wong *et al.*, 2009). Polysaccharide-protein complexes and ß-glucan water extract of *P.rhinocerus* were incubated with human innate immune cells. The extracellular cytokines released by the immune cells to the culture medium was analysed by using the Raybio® human cytokine antibody array. From this study, results showed that glucan in *P.rhinocerus*

significant stimulated the proliferation of NK-92MI (Natural Killer) cells and increased in the expression of cytokines IL-2 and IL-309. Researchers suggested that sclerotia of *P.Rhinocerus* could stimulate innate immune cells in an *in vitro* medium (Wong *et al.*, 2009).

Lee *et al.* (2011) investigated the potential effects of aqueous extract of sclerotium part of *L. rhinoceros* on neurite outgrowth activity by using PC-12Adh cell line (adherent variant). The PC-12 cell line which derived from transplantable rat pheochromocytoma was a rare tumor of adrenal gland tissue. This cell line has been widely used as neuronal model because it proliferates in growth medium and stop proliferating then differentiate into neuron-like cells as they respond to Nerve Growth Factor (NGF) (Ohnuma *et al.*, 2006). The researchers had chosen PC-12Adh for this study because it is NGF-dependent and does not synthesise epinephrine. The results showed that aqueous sclerotium *L.Rhinocerus* extract induced neurite outgrowths by double the length of cells diameter (Lee *et al.*, 2011). The doses were found not causes toxicity effects as appointed a study that high doses of orally intake Tiger Milk Mushroom up to 1000mg did not induce any pathological changes in liver, kidney, heart, spleen and lung of Sprague Dawley rat (Lee *et al.*, 2011).

Recently, Lee *et al*, (2012) reported that cold water sclerotium extract of *L.rhinocerus* elicited significant anti-proliferative activity against breast cancer cell MCF-7 and lung cancer A549. From this study, researchers also found that the cold water extract was not cytotoxic against the normal breast and lung cells. The cytotoxicity action is due to a high molecular weight fraction isolated from cold

water extract and this cytotoxic action is mediated via apoptosis. The anti-proliferative actions against MCF-7 provide better understanding and rational scientific basis for the traditional used of TMM for breast cancer treatment by the Malaysian aboriginal tribe (Lee *et al.*, 2012).

2.3 Overview of Resistance Training

In the past two decades, resistance training has gained popularity in the form of exercise for enhancing physical fitness and performance (Kraemer and Ratamess, 2004). Resistance training programme is a composite of variables including muscle action used, resistance used, volume, exercises selected structure, sequence of exercise performance, rest interval between sets, and training frequency (Fleck and Kreamer, 2004). Resistance training is widely recognised as a programme that can offer a great value, not only for athletes, but also for all those interested in optimising health and fitness. The correct prescription of resistance training is a key factor for success of the programme. The programme design should be conducted with proper instruction such as technique and breathing (Kraemer and Ratamess, 2004).

A repetition is one complete movement of an exercise which consists of two muscle actions that are concentric and eccentric muscle action. A group of repetition performed continuously without resting is called a set. For example, a set of resistance training composed a range of 1 to 15 repetitions. Repetition maximum (RM) is the maximal number of repetition per set can be performed with proper technique using a given resistance to complete 1 repetition. For example, resistance that is completed with 10 repetitions with proper technique is

10RM. Strength is the maximal amount of force a muscle group can generate in a specified velocity of movement. Power can be defined as rate of performing work (Fleck and Kreamer, 2004).

2.3.1 Principles of Resistance Training

In a resistance training programme, most primary muscle actions are concentric and eccentric whereas isometric contractions play a secondary role. While compared to concentric muscle action, eccentic action involved less motor unit activation per unit of muscle activation, requires less energy per level of force, critical for optimal muscle hypertrophy and may result in more delay muscle soreness (Ebbeling and Clarkson, 1989). In resistance exercise selection, there are two general types of selected resistance training which are effective for increasing muscular strength in the targeted muscle groups; single and multiple joints exercise. Single-joint exercises pose less risk of injury due to the reduced level of skill and technique involved examples of single joint exercise are leg extension and leg curl. Multiple-joint exercises such as bench press, squat, hang pulls, and power clean involved the larger muscle mass and coordination. These exercises have been regarded as the most effective exercises for increasing muscular strength and power (Fleck and Kreamer, 2004).

In selection of exercise order and workout structure, the sequencing of exercises and number of muscle groups trained during a workout significantly affects the acute expression of muscular strength (Sforzo, 1996). There are three

basic workout structures: 1) total-body workouts, 2) upper/lower body split workouts, and 3) muscle group split routines. Firstly, the most common among general fitness is the total-body workouts which involve performance of exercises stressing all major muscle groups. The upper/lower body split workouts involve performance of upper-body exercises during one workout and lower-body exercises during another. Muscle group split routines involve performance of exercises for specific muscle groups during the same workout (e.g., a "chest/triceps" workout where all exercises for the chest are performed then all exercises for the triceps are performed. All three workout structures are effective for improving muscular fitness (Kraemer and Ratamess, 2004).

When training all major muscle groups in a workout, it has been advised to perform large muscle group exercise first before small muscle group, performing multiple-joint before single joint exercises and perform total-body exercises before basic exercise like squat or bench press for strength and power training in order to minimise injury (Kraemer and Ratamess, 2004). Loading describes the amount of weight lifted or the resistance one exercises with. Loading is highly dependent upon other variables such as exercise order, volume, frequency, muscle action, repetition speed, and rest interval length (Kraemer and Ratamess, 2004).

Load prescription depends upon individual training status and goals. For untrained individuals, light loads of approximately 45–50% of 1 RM may increase dynamic muscular strength. (Kearney and Anderson, 1982). This initial phase of lifting is important to improved motor learning and coordination (Rutherford and

Jones, 1986). Heavy loads are not required to increase strength at this level of training while the individual is learning correct form and technique. Progressively, greater loading is needed to increase gradually to advanced levels of training (Fleck and Kreamer, 2004).

2.3.2 Benefits of Resistance Training

The adaptations of resistance training are related to improving performance and biomotor abilities (Aaberg, 2007). Movement improvement can be expressed through increased speed of movement, enhanced force production, greater active range of motion and improved control and coordination. These types of adaptations lead to increased performance. Aaberg (2007) categorised 7 factors of biomotor abilities that were linked to performance improvement into two hierarchy. For training priorities known as base abilities, they consists of strength, endurance, stability and mobility; and a need for proper progression as known as performance abilities which comprised of speed, power and agility.

Properly designed resistance training leads to morphological adaptations such as muscular strength and power and muscle hypertrophy (Izquierdo *et al.*, 2005); enhanced flexibility and improved maximal aerobic capacity (Takeshima *et al.*, 2004). Strength is important for joint mobility because any muscle weakness or imbalance will limit joint range of motion. Its combination with endurance will produce greater force production which in turn increases movement efficiency. For example, circuit-style routine of resistance training is

considered as an anaerobic activity which will increase blood and oxygen flow to the muscles resulting in prolong elevated heart rate and prolong movement with less demand on the muscular and cardiorespiratory systems (Aaberg, 2007).

Endurance was often thought to be only an adaptation of the cardiorspiratory systems as it is associated primarily with increases of maximal oxygen consumption (VO_2 max). This misconception has led to training protocols that ignore the possible benefits of strength training. Resistance training is valuable for development of local muscular endurance such as delaying onset of blood lactate accumulation (OBLA) which is the beginning of anaerobic threshold. The development of lactic acid tolerance will prolong the muscles to perform optimally (Aaberg, 2007). Another potential benefit of resistance training is increasing a person's ability to gain in hemoglobin and myoglobin content which are crucial for endurance.

Power is expressed as a combination of strength and speed considered as ability to generate appropriate force within the appropriate amount of time. Activities that require crucial power are quick and explosive movements like jumping and throwing. These movements integrated the use of stretch reflex and reliance on the development of person's level of explosive strength. Plyometric with resistance exercise is more appropriate for power improvement and should be trained progressively.

Resistance training also involves neurological adaptations. Neural adaptations are the ability of the muscle to produce force that is modulated by motor unit recruitment, and the rate of motor unit firing. The combination of these