

# **Original Article**

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# Combined Effects of *Lignosus rhinocerotis* Supplementation and Resistance Training on Isokinetic Muscular Strength and Power, Anaerobic and Aerobic Fitness Level, and Immune Parameters in Young Males

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How to cite this article: Chen CK, Hamdan NF, Ooi FK, Wan Abd Hamid WZ. Combined effects of *lignosus rhinocerotis* supplementation and resistance training on isokinetic muscular strength and power, anaerobic and aerobic fitness level, and immune parameters in young males. Int J Prev Med 2016;7:107.

# ABSTRACT

**Background:** This study investigated the effects of *Lignosus rhinocerotis* (LRS) supplementation and resistance training (RT) on isokinetic muscular strength and power, anaerobic and aerobic fitness, and immune parameters in young males.

**Methods:** Participants were randomly assigned to four groups: Control (C), LRS, RT, and combined RT-LRS (RT-LRS). Participants in the LRS and RT-LRS groups consumed 500 mg of LRS daily for 8 weeks. RT was conducted 3 times/week for 8 weeks for participants in the RT and RT-LRS groups. The following parameters were measured before and after the intervention period: Anthropometric data, isokinetic muscular strength and power, and anaerobic and aerobic fitness. Blood samples were also collected to determine immune parameters.

**Results:** Isokinetic muscular strength and power were increased (P < 0.05) in participants of both RT and RT-LRS groups. RT-LRS group had shown increases (P < 0.05) in shoulder extension peak torque, shoulder flexion and extension average power, knee flexion peak torque, and knee flexion and extension average power. There were also increases (P < 0.05) in anaerobic power and capacity and aerobic fitness in this group. Similarly, RT group had increases (P < 0.05) in shoulder flexion and extension average power. There were also increases (P < 0.05) in anaerobic power and capacity and aerobic fitness in this group. Similarly, RT group had increases (P < 0.05) in shoulder flexion average power. In addition, increases (P < 0.05) in anaerobic power and capacity, aerobic fitness, T lymphocytes (CD3 and CD4), and B lymphocytes (CD19) counts were observed in the RT group.

**Conclusions:** RT elicited increased isokinetic muscular strength and power, anaerobic and aerobic fitness, and immune parameters among young males. However, supplementation with LRS during RT did not provide additive benefits.

**Keywords:** Aerobic fitness, immune parameters, *Lignosus rhinocerotis*, muscular fitness, resistance training

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Quick Response Code:	Website: www.ijpvmjournal.net/www.ijpm.ir
	DOI: 10.4103/2008-7802.190604

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# INTRODUCTION

Lignosus rhinocerotis (LRS), which is also known as the "tiger's milk mushroom," thrives in tropical forests. It is one of the most popular medicinal mushrooms consumed by the indigenous communities of Peninsular Malaysia.<sup>[1]</sup> Medicinal properties of herbs and medicinal mushroom were reported to function as an immune system booster, anticancer, able to treat chronic hepatitis and asthma, lower cholesterol level, and reduce insulin resistance.<sup>[2-6]</sup> 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.<sup>[7]</sup> However, scientific studies regarding the association of medicinal mushroom and sports performance are still lacking in the current literature.

Regular participation in exercise gives positive effects on ideal body composition,<sup>[8]</sup> increased cardiorespiratory fitness<sup>[9,10]</sup> enhanced muscular strength<sup>[11,12]</sup> increased bone mineral density,<sup>[13]</sup> enhanced mental well-being, and can stimulate more positive attitude toward life.<sup>[14]</sup> Health-related benefits of resistance training (RT) have gained the popularity as a modality for health purposes.<sup>[15,16]</sup> Therefore, the American College of Sports Medicine and the American Heart Association have recommended RT for a wide range of population starting from adolescents to elderly and from healthy to clinical populations.<sup>[17]</sup>

To the best of our knowledge, no scientific studies have examined LRS supplementation as a sports performance enhancer to date. In addition, its additional beneficial effects combined with physical training have also not been investigated. The present study, therefore, was proposed to investigate the combined effects of LRS supplementation and RT on isokinetic muscular strength and power, anaerobic and aerobic fitness, and immune parameters in young males.

# METHODS

#### **Participants**

Forty young sedentary but healthy males between 19 and 25 years old were recruited for this double-blind, placebo-controlled study. The participants were randomly assigned to four groups: Control (C), LRS, RT, and the combined RT plus LRS (RT-LRS). Participants in the LRS and RT-LRS groups consumed two capsules of LRS (250 mg/capsule) daily for 8 weeks. Participants in the C and RT groups consumed placebo capsules daily for 8 weeks. The RT program which has ten different types of exercises involving the muscles of the upper and lower limbs was conducted 3 times/week for 8 weeks. Before the experimental period, pretest was carried out to measure anthropometric measurements, isokinetic muscular strength and power, and anaerobic and aerobic fitness. The same tests were carried out after 8 weeks of experimental period. The Human Research Ethics Committee, School of Medical Sciences, Universiti Sains Malaysia approved the study.

# Procedures and variable assessments

Participants' muscular strength and power were determined via an isokinetic dynamometer (System 3 Pro, Biodex Medical Systems, Shirley, New York, USA). Knee extension and flexion peak torque and power were tested at speeds of 60°/s for muscular strength and 300°/s for muscular power.

Participants' anaerobic fitness was determined via a Wingate anaerobic test on a cycle ergometer (Lode Groningen, the Netherlands). During the 30 s test, all the participants were encouraged verbally by the researchers to pedal as fast as they possibly could.

Twenty meter shuttle run test was carried out to estimate the participants' maximum oxygen uptake. Before the shuttle run, participants were given instructions regarding the procedures for this test. The number of shuttles and laps completed were then recorded and subsequently converted to estimated VO<sub>2</sub> max values via the calculator provided in a website (http://www.topendsports.com/ testing/beepcalc.htm).

Full blood count was carried out to determine the count and percentage of red blood cells, white blood cells, and platelets in the samples via automated hematology analyzer (Sysmex XS-800i, USA). Flow cytometer (BD FACSCanto II, Becton Dickinson, USA) with three-color direct immunofluorescence reagent of a commercially available reagent kit (BD Tritest<sup>™</sup>, USA) was used to determine the levels of T lymphocytes (CD3, CD4, and CD8) and B lymphocytes.

Ten stations of resistance exercise were designed using Thera-Band<sup>®</sup> exercise bands and dumbbells as the resistance. These stations included heel raise, side lateral raise, leg curl, biceps curl, leg abduction, frontal raise, knee extension, standing chest fly, half squat, and arm extension. Participants in RT group and combined RT-LRS group were required to perform 3 sets of 10–20 repetitions for each station for 3 times/week for 8 weeks. Between each set, participants were allowed 3–5 min rest. This RT program was not individualized for each participant.

LRS supplements and placebo were provided by LiGNO<sup>M</sup> Biotech Sdn Bhd, Malaysia. The LRS supplements were obtained from its sclerotium which were dried and ground into powder form before being put into capsules. This supplement consists of the substantial amount of carbohydrate, ranging from 90.5% to 98.1% and its protein composition is about 0.67–6.71%.<sup>[18]</sup> In addition, LRS appears to be capable of producing 1,3- $\beta$ - and 1,6- $\beta$ -glucans as well as bioactive proteins such as lectins

and fungal immunomodulatory proteins.<sup>[19]</sup> The color of the capsules was similar and contained the same amount of weight, i.e., 250 mg/capsule.

# **Statistical analysis**

All statistical analyses were performed using SPSS software (version 20.0,SPSS Inc., Chicago, IL, USA). After checking the normality, the data were analyzed by repeated measures ANOVA to determine the significant differences between and within groups. Statistical significance was accepted when P < 0.05. All data are expressed as means  $\pm$  standard deviation.

#### RESULTS

# Physical characteristics of the participants

Thirty-eight male participants (mean age  $20.9 \pm 1.6$  years) completed the present study. Two participants were unable to continue the program due to personal reasons during the experimental period. Table 1 shows the demographic data of the participants at pre- and post-tests. In pretest, participants' mean body height was 169.1  $\pm$  4.5 cm. The mean body weight and percentage of body fat of the subjects were 63.2  $\pm$  10.1 kg and 19.2  $\pm$  6.2%, respectively. There were no statistically significant differences in body weight, percentage of body fat, and body mass index between pre- and post-tests in all the groups.

# Isokinetic muscular peak torque and power

At posttest, peak torque of shoulder extension at 60°/s in the LRS, RT, and combined RT-LRS groups were significantly higher (P < 0.05) compared to the C group [Table 2]. Peak torque of shoulder extension 60°/s in the combined RT-LRS group was also significantly greater (P < 0.05) than LRS and RT groups at posttest. At posttest, peak torque of shoulder flexion at 60°/s was significantly higher (P < 0.05) in LRS, RT, and combined RT-LRS groups compared to the C group [Table 2]. After 8 weeks of intervention, peak torque of shoulder extension at 60°/s significantly increased (P < 0.05) from pretest values in the LRS and RT-LRS groups, whereas peak torque of shoulder flexion at 60°/s significantly increased (P < 0.05) from pre- to post-test in the RT group.

Table '	1:	Demographic	data of	the	participants
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At posttest, average power of shoulder extension at 300% in LRS, RT, and combined RT-LRS groups were significantly higher (P < 0.05) compared to the C group [Table 2]. Average power of shoulder extension at 300°/s in the combined RT-LRS group was also significantly higher (P < 0.05) compared to LRS group. At pretest, no significant differences were found between RT and combined RT-LRS groups compared to C group, respectively [Table 2]. At posttest, average power of shoulder flexion at 300°/s in the LRS, RT, and combined RT-LRS groups was significantly higher (P < 0.05) compared to C group [Table 2]. After 8 weeks of intervention, average power of shoulder extension at 300°/s in the combined RT-LRS group increased (P < 0.05) significantly from pre- to post-test, whereas the average power of shoulder flexion at 300°/s in the LRS, RT, and combined RT-LRS groups increased (P < 0.05) significantly from pre- to post-test.

At posttest, peak torque of isokinetic knee extension at 60°/s was significantly higher (P < 0.05) in combined RT-LRS group compared to C, LRS, and RT groups [Table 3]. The peak torque of knee flexion at 60°/s was also significantly higher (P < 0.05) at posttest in the combined RT-LRS group compared to C, LRS, and RT groups. After 8 weeks of intervention, there was a significant improvement (P < 0.05) in isokinetic knee extension peak torque at 60°/s in the RT group at posttest compared to pretest, whereas peak torque of knee flexion at 60°/s significantly increased (P < 0.05) from pre- to post-test in RT and combined RT-LRS groups.

At posttest, average power of knee extension at 300°/s was significantly higher (P < 0.05) in the RT and combined RT-LRS groups compared to C and LRS groups, respectively [Table 3]. At posttest, average power of knee flexion at 300°/s was significantly higher (P < 0.05) in RT and combined RT-LRS groups compared to C and LRS group, respectively [Table 3]. After 8 weeks of experimental period, average power of knee extension at 300°/s was significantly (P < 0.05) increased between pre- and post-test for both RT and combined RT-LRS groups, whereas average power of knee flexion at 300°/s in the RT and combined RT-LRS groups significantly increased (P < 0.05) compared to pretest.

Groups						
	Body we	eight (kg)	Body	fat (%)	BI	MI
	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest
C	$57.06 \pm 7.55$	56.8±7.18	16.13±3.44	16.65±3.65	20.39±2.40	20.32±2.48
LRS	63.90±11.13	$64.26 \pm 11.78$	$18.61 \pm 5.71$	$19.40 \pm 6.33$	$21.97 \pm 3.48$	$22.10 \pm 3.64$
RT	66.23±11.66	66.42±12.13	$21.31 \pm 8.16$	$21.53 \pm 8.57$	$23.32 \pm 4.19$	$23.28 \pm 4.24$
RT-LRS	$66.05 \pm 8.51$	$65.60 \pm 8.43$	$21.01 \pm 6.36$	$20.22 \pm 6.53$	$27.75 \pm 2.64$	22.48±2.69

RT-LRS=Combined resistance training and Lignosus rhinocerotis group, C=Control group, LRS=Lignosus rhinocerotis group, RT=Resistance training group, BMI=Body mass index, SD=Standard deviation

#### Wingate anaerobic power and capacity

At pre- and post-tests, anaerobic power of the RT group was significantly lower (P < 0.05) compared to the C group [Table 4]. There was no significant difference in LRS and RT-LRS groups compared to the C group, respectively. After 8 weeks of intervention, anaerobic power in the RT and combined RT-LRS groups increased significantly (P < 0.05) compared to their respective pretest values.

Table 2:	Pea	k torque	of shou	ılder	extens	ion and	flex	cion
at 60°/s	and	average	power	of sh	oulder	extens	ion a	and
flexion	at 30	)0°/s						

Groups		Mean±SD					
	Peak toro extension	Peak torque of shoulder extension at 60°/s (Nm)		ower of shoulde n at 300°/s (W)			
	Pretest	Posttest	Pretest	Posttest			
С	58.2±36.9	52.9±10.7	61.9±45.0	64.3±39.4			
LRS	$52.0 \pm 10.8$	64.1±15.7 <sup>#,*</sup>	$81.4 \pm 41.0$	$95.1 \pm 43.8^{\#}$			
RT	$56.7\!\pm\!9.4$	$63.2 \pm 13.5^{\#}$	$63.0 \pm 24.9$	$96.9 \pm 27.4^{\#}$			
RT-LRS	$63.6 \pm 7.7$	$72.2\!\pm\!13.8^{_{\#,\ddagger,+,}*}$	$84.6\!\pm\!25.2$	118.9±33.2 <sup>#,‡,*</sup>			
Crowno		Mee					

	INIGA			
Peak torqu flexion a	Peak torque of shoulder flexion at 60°/s (Nm)		ower of shoulde at 300°/s (W)	
Pretest	Posttest	Pretest	Posttest	
$47.7 \pm 6.2$	$49.0 \pm 5.7$	$68.1 \pm 11.9$	73.73±16.8	
$56.8 \pm 9.9^{\#}$	$60.0 \pm 13.6^{\#}$	$84.8 \pm 19.8^{\#}$	$92.88 \pm 23.8^{\#,*}$	
$53.6 \pm 6.4^{\text{H}}$	$59.4 \pm 10.2^{\#,*}$	$75.7 \pm 24.9$	$96.27 \pm 28.2^{\#,*}$	
$59.0 \pm 8.0^{\text{#,+}}$	$61.1 \pm 10.1^{\#}$	$79.3 \pm 12.7$	98.62±23.6 <sup>#,*</sup>	
	Peak torqu flexion a Pretest 47.7±6.2 56.8±9.9 <sup>#</sup> 53.6±6.4 <sup>#</sup> 59.0±8.0 <sup>#,+</sup>	Peak torque of shoulder flexion at 60°/s (Nm)   Pretest Posttest   47.7±6.2 49.0±5.7   56.8±9.9# 60.0±13.6#   53.6±6.4# 59.4±10.2#.*   59.0±8.0#.+ 61.1±10.1#	Peak torque of shoulder flexion at 60°/s (Nm) Average por flexion at Pretest   Pretest Posttest Pretest   47.7 ± 6.2 49.0 ± 5.7 68.1 ± 11.9   56.8 ± 9.9 <sup>#</sup> 60.0 ± 13.6 <sup>#</sup> 84.8 ± 19.8 <sup>#</sup> 53.6 ± 6.4 <sup>#</sup> 59.4 ± 10.2 <sup>#,*</sup> 75.7 ± 24.9   59.0 ± 8.0 <sup>#,+</sup> 61.1 ± 10.1 <sup>#</sup> 79.3 ± 12.7	

"Significantly different from control group (P<0.05), 'Significantly different from RT group (P<0.05), 'Significantly different from LRS group (P<0.05), 'Significantly different from pretest (P<0.05). C=Control group, LRS=Lignosus thinocerotis group, RT=Resistance training group, RT-LRS=Combined resistance training and Lignosus thinocerotis group, SD=Standard deviation

There were no significant differences in anaerobic capacity between LRS, RT, and combined RT-LRS groups and compared to the C group at pre- and post-tests [Table 4]. After 8 weeks of intervention, anaerobic capacity in the RT and combined RT-LRS groups increased (P < 0.05) significantly from pre- to post-test. There were no significant differences in estimated maximum oxygen consumption (VO<sub>2</sub> max) from the 20 m shuttle run in LRS, RT, and RT-LRS groups compared to C group at pretest [Table 5]. At posttest, estimated VO, max in the combined RT-LRS groups was significantly higher (P < 0.05) compared to the C group. After 8 weeks of intervention, RT and combined RT-LRS groups showed a significant improvement (P < 0.05) in estimated VO<sub>2</sub> max from pre- to post-test.

# **Blood immune parameters**

At pre- and post-tests, there were no significant differences in mean WBC counts, basophil counts, eosinophil counts, and neutrophils counts in LRS, RT, and RT-LRS groups compared to the C group [Table 6]. At posttest, there was no significant difference in lymphocyte counts between LRS, RT, and combined RT-LRS groups and compared to C group [Table 6]. After 8 weeks of intervention, no significant differences were observed in these parameters between pre- and post-test in all the groups, whereas lymphocyte counts increased (P < 0.05) significantly from pre- to post-test in RT group.

No significant difference in T-cell (CD3) counts was found in LRS, RT, and combined RT-LRS groups compared to C group, respectively [Table 7]. At posttest,

Table 3: Peak torque of knee extension and flexion at 60%s and average power of knee extension and flexion at
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Groups		Mean	±SD	
	Peak torque knee of e	Peak torque knee of extension at 60°/s (Nm)		e extension at 300°/s W)
	Pretest	Posttest	Pretest	Posttest
С	138.0±40.2	141.7±17.7	160.6±58.9	175.8±39.3
LRS	149.5±28.9	$152.5 \pm 20.2$	$178.7 \pm 36.8$	193.2±44.8
RT	$143.3 \pm 36.5$	$166.1 \pm 40.8^*$	$171.4 \pm 37.9$	238.5±44.9 <sup>#,‡,*</sup>
RT-LRS	173.4±29.9 <sup>#,‡,+</sup>	187.0±27.2 <sup>#,‡,+</sup>	$189.6 \pm 53.4$	239.9±57.5 <sup>#,‡,*</sup>
Groups		Mean±SD		
	Peak torque of knee	flexion at 60°/s (Nm)	Average power of I (	knee flexion at 300°/s W)
	Pretest	Posttest	Pretest	Posttest
С	64.1±18.4	69.9±18.4	81.3±35.3	94.2±22.9
LRS	63.8±26.2	$72.6 \pm 18.6$	$104.6 \pm 40.3$	$112.3 \pm 40.1$
RT	62.2±8.0	75.0±11.3*	84.1±21.3	131.0±32.9 <sup>#,*</sup>
RT-LRS	77.8±11.2 <sup>#,‡,+</sup>	90.0±8.2 <sup>#,‡,+,*</sup>	110.9±49.1 <sup>#,+</sup>	150.3±33.2 <sup>#,‡,*</sup>

<sup>#</sup>Significantly different from control group (P<0.05), <sup>+</sup>Significantly different from RT group (P<0.05), <sup>+</sup>Significantly different from LRS group (P<0.05), \*Significantly different from pretest (P<0.05). RT-LRS=Combined resistance training and *Lignosus rhinocerotis* group, C=Control group, LRS=*Lignosus rhinocerotis* group, RT=Resistance training group, SD=Standard deviation

there was no significant difference in any of the groups. After 8 weeks of intervention, T-cells (CD3) counts in

Table	4:	Wingate	anaerohic	nower and	anaerobic	canacitv
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Groups		Mean±SD				
	Wingate powe	anaerobic r (W/kg)	Wingate capacit	anaerobic xy (W/kg)		
	Pretest	Posttest	Pretest	Posttest		
С	9.1±2.1	$9.4 \pm 1.5$	7.3±1.8	7.4±1.1		
LRS	$8.7\!\pm\!1.6$	$9.1 \pm 1.9$	$6.9\!\pm\!1.3$	$7.3 \pm 1.2$		
RT	$7.6 \pm 1.5^{\#}$	$8.2 \pm 1.6^{\#,*}$	$6.4\!\pm\!1.4$	$7.1 \pm 1.4^{*}$		
RT-LRS	$8.2 \pm 1.3$	$8.9 \pm 1.8^{*}$	$6.9 \pm 1.0$	7.3±1.2*		

<sup>#</sup>Significantly different from control group (*P*<0.05), \*Significantly different from pretest (*P*<0.05). RT-LRS=Combined resistance training and Lignosus rhinocerotis group, C=Control group, LRS=*Lignosus rhinocerotis* group, RT=Resistance training group, SD=Standard deviation

# Table 5: Aerobic fitness (estimated $VO_2$ max from 20 m shuttle run)

Groups	Mea	n±SD
	Estimated VO <sub>2</sub>	max (mL/kg/min)
	Pretest	Posttest
С	37.1±6.0	$36.0 \pm 4.8$
LRS	$38.0 \pm 6.1$	$39.4 \pm 6.3$
RT	$34.2 \pm 6.9$	$36.8 \pm 5.5^*$
RT-LRS	$36.8 \pm 4.3$	$39.8 \pm 4.0^{\#,*}$

<sup>#</sup>Significantly different from control group (P<0.05), \*Significantly different from pretest (P<0.05). RT-LRS=Combined resistance training and Lignosus rhinocerotis group, C=Control group, LRS=*Lignosus rhinocerotis* group, RT=Resistance training group, SD=Standard deviation

the RT group increased significantly (P < 0.05) from pre- to post-test.

At posttest, T helper/inducer (CD4) counts were not significantly different in LRS, RT, and combined RT-LRS groups compared to the C group [Table 7]. Similarly, there were no significant differences in T cytotoxic/suppressor (CD8) counts between LRS, RT, and combined RT-LRS groups compared to C group at posttest [Table 7]. After 8 weeks of intervention, T helper/inducer (CD4) counts in the RT group increased (P < 0.05) significantly from pre- to post-test, and there was no significant difference in T cytotoxic/suppressor (CD8) count at pre- and post-tests for all groups. B-cell (CD19) counts were significantly higher (P < 0.05) in the RT group compared to C group at posttest [Table 7]. However, no significant difference was found in LRS and RT-LRS groups compared to C group. After 8 weeks intervention, B cell counts increased (P < 0.05)significantly from pre- to post-test (P < 0.05) in the RT group.

# DISCUSSION

One of the notable findings of the present study is that following 8 weeks of intervention, significant positive effects were observed in isokinetic muscular strength and average power in 6 out of the 8 measured parameters in the combined RT-LRS group. These parameters include peak torque of shoulder extension at 60°/s, average

Table 6: White blood cells basophils, eosinophils, neutr	ophils, and lymphocyte counts at pre- and post-tests
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Groups	Mean±SD									
	WBC (10 <sup>3</sup> /µL)		Basophils (10³/µL)		Eosinophils (10 <sup>3</sup> /µL)		Neutrophils (10³/µL)		Lymphocytes (10³/µL)	
	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest
С	$7.5 \pm 1.9$	$7.5 \pm 1.7$	$0.03 \pm 0.01$	$0.03 \pm 0.01$	$0.25 \pm 0.14$	$0.26 \pm 0.17$	$3.86 \pm 1.61$	$3.63 \pm 1.07$	$2.68 \pm 0.80$	$2.86 \pm 0.84$
LRS	$7.6\!\pm\!1.9$	$7.3\pm1.7$	$0.02\!\pm\!0.02$	$0.03\!\pm\!0.02$	$0.26\!\pm\!0.18$	$0.50\!\pm\!0.79$	$4.14 \pm 1.78$	$3.51 \pm 1.29$	$2.59 \pm 0.52$	$2.63\!\pm\!0.68$
RT	$6.9\!\pm\!1.9$	$7.1\pm1.7$	$0.03\!\pm\!0.01$	$0.03\!\pm\!0.01$	$0.35\!\pm\!0.21$	$0.30\!\pm\!0.15$	$3.66 \pm 1.51$	$3.29\!\pm\!1.09$	$2.28\!\pm\!0.50$	$2.88 \pm 0.68^{*}$
RT-LRS	$7.0 \pm 1.3$	$7.1 \pm 1.6$	$0.03\!\pm\!0.01$	$0.02 \pm 0.01$	$0.24 \pm 0.10$	$0.27 \pm 0.12$	$3.35 \pm 1.06$	$3.29 \pm 1.19$	$2.78 \pm 0.61^{+}$	$2.84 \pm 0.68$

<sup>+</sup>Significantly different from RT group (P<0.05), \*Significantly different from pretest (P<0.05). RT-LRS=Combined resistance training and Lignosus rhinocerotis group, WBC=White blood cells, C=Control group, LRS=*Lignosus rhinocerotis group*, RT=Resistance training group, SD=Standard deviation

Table 7: Total T cells (CD3	3), T helper/inducer (CD4),	, T cytotoxic/suppressor	(CD8), and B-cell	(CD19) count at pre-	and
post-tests					

Groups	Mean±SD							
	Total T-cells (CD3) absolute count (10³/µL)		T helper/inducer (CD4) absolute count (10³/µL)		T cytotoxic/suppressor (CD8) absolute count (10³/µL)		B-cell (CD19) absolute count (10³/µL)	
	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest	Pretest	Posttest
С	$1.70 \pm 0.47$	$1.89 \pm 0.56$	$0.81 \pm 0.18$	$0.95 \pm 0.31$	0.77±0.22	0.02±0.24	$0.26 \pm 0.12$	$0.28 \pm 0.06$
LRS	$1.57 \pm 0.59$	$1.76 \pm 0.45$	$0.71 \pm 0.32$	$0.82 \pm 0.15$	$0.74 \pm 0.33$	$0.81 \pm 0.31$	$0.26 \pm 0.09$	$0.32 \pm 0.11$
RT	$1.49 \pm 0.27$	$1.97 \pm 0.58^{*}$	$0.69\!\pm\!0.18$	$0.92 \pm 0.28^{*}$	$0.67 \pm 0.13$	$0.86 {\pm} 0.33$	$0.28 \pm 0.10$	0.39±0.15 <sup>#</sup> ,*
RT-LRS	$1.88 \pm 0.52^{+}$	$1.92 \pm 0.56$	$0.86 \pm 0.27^{+}$	$0.86 \pm 0.28$	$0.84 \pm 0.28^{+}$	$0.81 \pm 0.29$	$0.29 \pm 0.09$	$0.30 \pm 0.11$

\*Significantly different from pretest (P<0.05), \*Significantly different from control group (P<0.05), \*Significantly different from RT group (P<0.05), \*Significantly different from LRS group (P<0.05), RT-LRS=Combined resistance training and Lignosus rhinocerotis group, C=Control group, LRS=Lignosus rhinocerotis group, RT=Resistance training group, SD=Standard deviation

power of shoulder extension and flexion at 300°/s, peak torque of knee flexion at 60°/s, and average power of knee extension and flexion at 300°/s. Similarly, in the RT alone group, significant positive effects were observed in isokinetic muscular strength and average power in the 5 out of the 8 measured parameters following 8 weeks of intervention. These parameters include average power of shoulder flexion at 300°/s, peak torque of knee extension at 60°/s, peak torque of knee flexion at 60°/s, average power of knee extension at 300°/s, and average power of knee flexion at 300°/s.

One of the factors associated with improvement in muscular strength and power from RT was postulated to be muscle hypertrophy. Muscular stress on the components of muscular system triggers the protein signaling to activate and stimulate synthesis muscle protein and lead to increased muscle size.<sup>[17]</sup> The present findings imply that both 8 weeks of combined RT with 500 mg/day of LRS consumption and 8 weeks of RT alone was able to elicit beneficial effects on isokinetic strength and power. These results also showed that combining LRS supplementation with RT may not elicit additional benefits on isokinetic muscular strength and power compared to performing RT alone.

LRS supplements do not seem to cause any toxicity effect as a study has shown that 28-day oral administration of 1000 mg/kg of sclerotial powder had no adverse effect on the growth rate, hematological, and renal and liver function parameters in Sprague-Dawley rats.<sup>[20]</sup> In addition, we found that after 8 weeks of LRS supplementation, there were some minor changes in some of the parameters in the liver and renal function tests of the participants, but they were all within the normal range. Hence, we conclude that there was no adverse effect even in humans, with LRS consumption at the dosage given in this study.

Following 8 weeks of intervention, participants in both RT and combined RT-LRS groups showed significant improvements in Wingate anaerobic power and anaerobic capacity from pre- to post-test. However, no significant changes in Wingate anaerobic parameters were observed in LRS alone group and control group from pre- to post-test. The significant improvement in anaerobic capacity and power with physical training in the present study was in line with the findings of Kraemer *et al.*<sup>[21]</sup> who reported a significant increase in peak power after 4–6 months of RT. It was speculated that the increment in anaerobic capacity and power to be attributed to the intramuscular high-energy phosphates adaptation from the RT and lactate generating capacity<sup>[22]</sup> in the present study.

Consistent with the findings on muscular performance and Wingate anaerobic power and capacity of this study, it also found that the participants' estimated maximum oxygen consumption (VO<sub>2</sub> max), an indicator of aerobic fitness was found to be significantly higher in the posttest compared to pretest in both RT and combined RT-LRS groups.

The present study demonstrated that 8 weeks of combined RT-LRS supplementation, RT alone, and LRS supplementation alone did not give any significant changes in white blood cells, basophil, eosinophil, and neutrophil counts from pre- to post-test. It was speculated that the exercise duration and intensity and the dosage of the LRS supplementation prescribed in the present study may not be adequate to elicit beneficial effects on these few measured immune parameters.

Another notable finding in this study was that there were significant increases in total lymphocytes, B and T lymphocytes counts in RT alone group but not in other experimental groups at postexercise compared to pre-exercise. These findings imply that LRS supplementation alone, and supplementation with LRS during RT may not provide additive benefits on these few immune parameters. The positive effects of RT on lymphocytes of the present study were contrary to the findings of Natale et al.<sup>[23]</sup> who reported no significant effects on total lymphocyte counts in all experimental groups after 8 weeks of three different types of exercise intervention. It was postulated that duration and intensity of training may have contributed to this contradictory findings. The positive findings of the present study in total lymphocytes, B and T lymphocytes counts in RT alone group reflect that the prescribed exercise intensity and duration in the present study were appropriate for enhancing immune functions in the participants.

# CONCLUSIONS

Combined RT with LRS supplementation (500 mg/ day for 8 weeks) and RT alone elicited beneficial effects on isokinetic strength and power. Both combined RT with LRS supplementation and RT alone also showed positive effects in anaerobic capacity, anaerobic power, and aerobic fitness parameters. For immune parameters, only RT alone showed a significant improvement in total lymphocytes, B and T lymphocytes levels but not the other experimental groups. These findings imply that combining LRS supplementation with RT may not elicit additional beneficial effects on isokinetic muscular strength and power, anaerobic and aerobic fitness, and immune parameters compared to RT alone.

# Acknowledgements

We wish to extend our sincere gratitude to all the participants who have participated in this study. Technical assistance provided by Mdm. Jamaayah bt. Meor Osman, Mdm. Norlida bt Azalan, Mdm. Hafizah bt. Hamzah,

Mdm. Parimalah Velo, and Mdm. Nor Aini bt. Sudin, from the Sports Science Unit, Universiti Sains Malaysia, is highly appreciated. Special thanks to Mr. Jamaruddin Mat Asan from the Immunology Department, for his assistance. We would like to thank LiGNO<sup>™</sup> Biotech Sdn Bhd, Malaysia, for providing the *Lignosus rhinocerotis* supplements and placebo.

**Financial support and sponsorship** Nil.

# **Conflicts of interest**

There are no conflicts of interest.

Received: 11 Apr 15 Accepted: 03 Aug 16 Published: 14 Sep 16

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