



## ***Cinnamomum burmannii* Supplementation Regulation of the Expression of IFN- $\gamma$ and TNF- $\alpha$ as Markers of Circulating Cytotoxic T Lymphocytes that Recognize Inflammatory Responses due to High-Intensity Exercise**

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<b>Article History</b>	<b>Abstract</b>
Received: 16 July 2023 Revised: 15 Sept 2023 Accepted: 26 Oct 2023	<p><i>This study aims to determine the role of <i>Cinnamomum burmannii</i> supplementation in increasing CD8<sup>+</sup> T cells or cytotoxic T cells. It employed the posttest-only control group design. The experimental units in this study were 41 male mouse (<i>Mus musculus</i>) of the BALB/c strain, which were randomly divided into four groups. The first group of 10 mice was a control group that received no treatment. The second group was treated with 200 mg/kg BW of cinnamon extract without exercise. The third group consisted of 10 mice undergoing a high-intensity exercise regimen and receiving a placebo treatment. The fourth group both received the cinnamon supplementation at 200 mg/kg BW and underwent an exercise regimen. The exercise regimen took the form of a regular 7-day-long high-intensity downhill treadmill training with a load of 80-90% of maximum capacity and an inclination angle of -15°. On the 8<sup>th</sup> day, twelve hours after exercise, the spleen of the mice was taken for a flow cytometric examination to count the numbers of CD8<sup>+</sup> T cells and CD8<sup>+</sup> T cells expressing IFN-<math>\gamma</math> and TNF-<math>\alpha</math>. The counts of CD8<sup>+</sup> T cells were significantly different among the groups (<math>p = 0.004</math>), with the median value of the control group being <math>5.79 \pm 2.19</math>, in comparison to that of the fourth group (<math>5.23 \pm 1.84</math>). There were significant differences in the counts of CD8<sup>+</sup> T cells expressing IFN-<math>\gamma</math> among the groups (<math>p = 0.027</math>), with the median value of the control group being <math>18.73 \pm 11.94</math>, in comparison to that of the fourth group (<math>10.58 \pm 3.78</math>). However, the counts of CD8<sup>+</sup> T cells expressing TNF-<math>\alpha</math> lacked significant differences among the groups (<math>p = 0.857</math>), with the median value of the control group being <math>11.61 \pm 10.27</math>, in comparison to that of the fourth group (<math>8.86 \pm 3.50</math>). In summary, cinnamon supplementation to high-intensity exercise led to significant increases in the relative counts of CD8<sup>+</sup> T cells (<math>p = 0.004</math>) and CD8<sup>+</sup> T cells expressing IFN-<math>\gamma</math> (<math>p = 0.027</math>), but it did not show any significant difference in the relative count of CD8<sup>+</sup> T cells expressing TNF-<math>\alpha</math>.</i></p>
CC License CC-BY-NC-SA 4.0	<b>Keywords:</b> <i>Cinnamomum burmannii</i> , high-intensity exercise, CD8 <sup>+</sup> , TNF- $\alpha$ , IFN- $\gamma$

### **1. Introduction**

High stress due to a strenuous exercise program can cause failure of adaptation, which results in decreased physical performance called overtraining syndrome (OTS). Excessive training in a short period of time has been shown to reduce physical performance. However, overreaching usually occurs for a short time and is followed by a decrease in training load, so that supercompensation, and consequently a slight increase in training, can be achieved<sup>1</sup>. Excess achievement in a short period of time is required in the training program for supercompensation to occur, which will cause the athlete to achieve better performance in the upcoming competition<sup>2</sup>. Conversely, overtraining is a training with an exceedingly heavy load that will cause a permanent decrease in performance. It should be noted that overreaching can turn to overtraining if the training is performed in a prolonged time. Tissue damage due to strenuous exercise can lead to the production of pro-inflammatory cytokines, which are responsible for cases of infection and chronic fatigue that often occur in athletes<sup>3</sup>. The relationship of exercise intensity and susceptibility to upper respiratory tract infections has been depicted in a “J” curve.

This model explains that moderate-intensity exercise can improve immune function compared to inactivity, while high-intensity exercise can ironically increase upper respiratory tract infections<sup>4</sup>. Several previous studies showed that the incidence of respiratory tract infections increases in athletes who perform heavy-intensity exercise. For instance, seven retrospective and prospective longitudinal studies showed that the incidence of upper respiratory tract infections in elite athletes increases during competition<sup>5</sup>. Walsh and Oliver (2016) reported a 58% increase in cases of upper respiratory infections and allergies in 208 running athletes after facing a marathon in London compared to a control group<sup>6</sup>.

The incidence of infections that occur in athletes during competition cannot be separated from the work of the immune system in the athletes' bodies. T cells that are still developing will be introduced to all self-antigens that represent all the components that exist in the individual. At this stage, prospective T cells are directly exposed to the peptides bound to MHC class I and MHC class II molecules. T cells that tend to interact with MHC class I molecules will develop into CD8<sup>+</sup> T cells, while those that tend to interact with MHC class II molecules will develop into CD4<sup>+</sup> T cells. CD4<sup>+</sup> T cells are known as helper T cells, while CD8<sup>+</sup> T cells are known as killer or cytotoxic T cells. Helper T cells have the potential to produce proinflammatory cytokines such as TNF- $\alpha$  and IFN- $\gamma$ <sup>7</sup>. In a study, cinnamon ethanol extract supplementation showed an immunostimulatory effect which was marked by an increase in CD4<sup>+</sup> T cells and CD8<sup>+</sup> T cells in mice infected with *S. enteritidis*. The amounts of B220 and immunoglobulin G were found to be increasing in *S. enteritidis*-infected mice. The number of CD4<sup>+</sup> T cells expressing IFN- $\gamma$  increased; the greater the dose of cinnamon ethanol extract, the higher the numbers of CD4<sup>+</sup> T cells and CD4<sup>+</sup> T cells expressing IFN- $\gamma$ . Thus, it can be concluded that the ethanol extract of cinnamon (*C. burmannii*) has an immunostimulatory effect<sup>8</sup>.

## 2. Materials And Methods

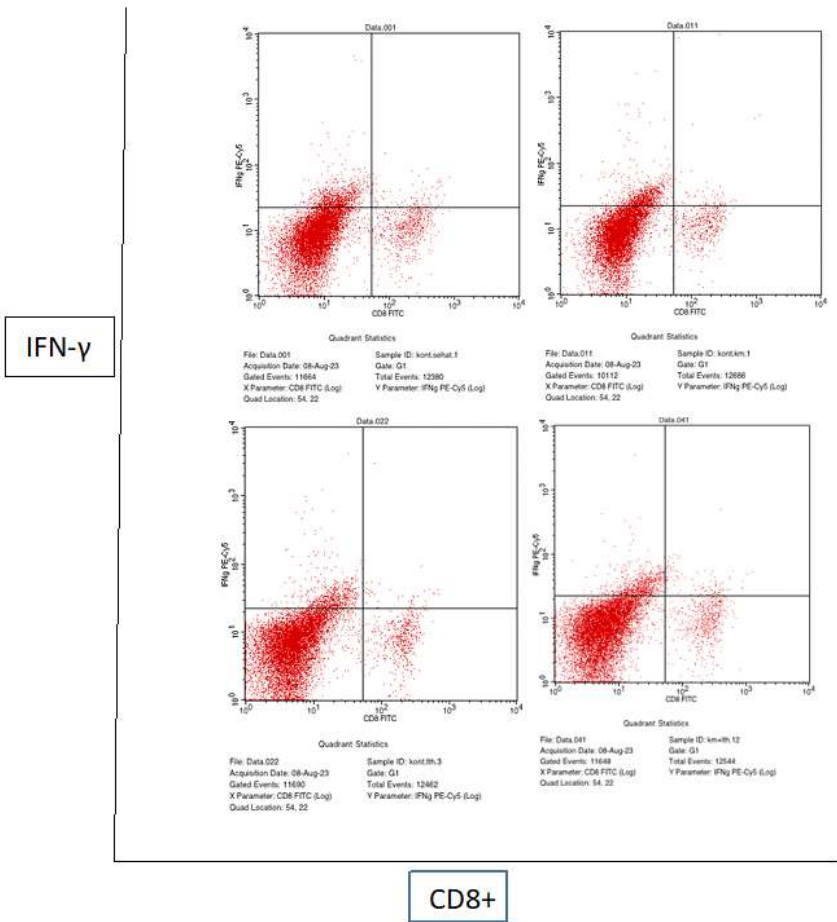
This The study implemented the randomized posttest-only control group design. The samples used in this study were male mice (*Mus musculus*) of the BALB/c strain. The sample size was calculated pursuant to Steel and Torrie. A total of 41 mice were used, consisting of 10 mice in the control group, 10 mice in the group with high-intensity exercise and placebo, 9 mice in the group given cinnamon supplementation without exercise, and 12 mice in the group with both high-intensity exercise and cinnamon supplementation. Eight replications were obtained for each group. Efforts to find supplements for athletes who receive high-intensity training, which often results in OTS due to muscle damage that has the potential to suppress immune system function, must be carried out because to maintain athletic performance, preventive measures must be taken, so that athletes will neither be easily infected nor have diminished performance. Cinnamon supplementation can be used as an alternative considering that cinnamon is an herbal product that is often used by Indonesian people to treat various cases of infection and inflammation<sup>9</sup>. Cinnamon supplementation, which is a mixture of cinnamon extract, 0.05% CMCNa, and 0.4 ml of aquadest, was given orally for 7 days half an hour before training. In this experiment, a dose of 200 mg/kg BW/0.4 mL/mice/day was applied to groups 2 and 4<sup>10 11</sup>.

High-intensity training in this study is a dynamic training where speed and endurance are trained with a load of 80–90% of maximum capacity. The exercise used in this study was downhill treadmill training with an angle inclination of -15° for 23 minutes, consisting of 5 minutes of running adaptation and 18 minutes of actual running load at a speed of 30 cm/second for 7 days. Before the actual training, the mice were given 5 minutes to adapt to downhill running, which was done gradually, including the following stages: the first 1 minute at a speed of 0 cm/second, the next 2 minutes at a speed of 14 cm/sec, and the next 2 minutes at a speed of 21 cm/second. On the 8<sup>th</sup> day, termination was carried out to collect the spleens of the mice, which were then processed by the flow cytometric method to observe the percentage of CD8<sup>+</sup> T cells that expressed TNF- $\alpha$  and IFN- $\gamma$ . The antibodies used were conjugated FITC anti-mouse CD8<sup>+</sup> antibody, PE anti-mouse IFN- $\gamma$  antibody, and PE anti-mouse TNF- $\alpha$  antibody<sup>12</sup>.

## 3. Results and Discussion

The mice spleen flow cytometry results on the counts of CD8<sup>+</sup> T cells and CD8<sup>+</sup> T cells expressing IFN- $\gamma$  and TNF- $\alpha$  are provided in Table 1 and Figs. 1 and 2.

This study aims to determine the effect of cinnamon supplementation at a dose of 200 mg/kg BW half an hour before high-intensity eccentric exercise of 80% VO<sub>2</sub>max on the immune response. Based on the flow cytometry results, the mean counts of CD8<sup>+</sup> T cells of the control, cinnamon supplementation, exercise, and cinnamon supplementation + exercise groups were 5.795  $\pm$  2.190, 4.971  $\pm$  1.919, 4.928  $\pm$  1.265, and 5.225  $\pm$  1.797, respectively.



**Fig. 1:** Relative counts of CD8<sup>+</sup> T cells and CD8<sup>+</sup> T cells expressing IFN- $\gamma$  and TNF- $\alpha$  based on the flow cytometry

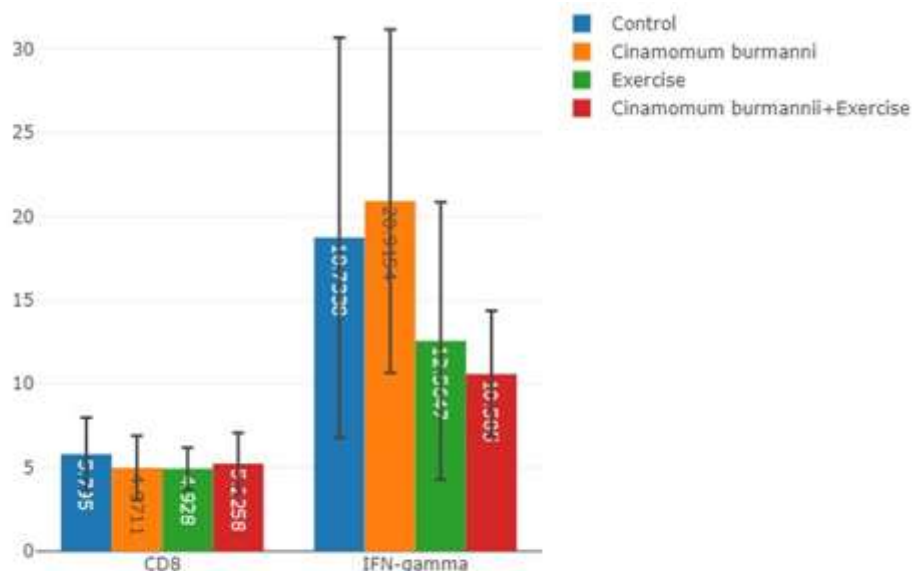
Descriptive Statistics				
	Group	Mean	Std. Deviation	N
CD8 <sup>+</sup>	control	5.7950	2.19090	10
	Cinamomum burmannii	4.9711	1.91991	9
	exercise	4.9280	1.26485	10
	Cb+Exercise	5.2258	1.84352	12
	Total	5.2361	1.79706	41
			18.733	11.94093
IFN-gamma	control	8		8
	Cinamomum burmannii	20.915	10.25436	9
	exercise	12.564	8.28711	10
	Cb+Exercise	10.585	3.78388	12
	Total	15.323	9.54377	41
			0	

**Table 1:** Relative counts of CD8<sup>+</sup> T cells and CD8<sup>+</sup> T cells expressing IFN- $\gamma$

The mean counts of CD8<sup>+</sup> T cells expressing IFN- $\gamma$  of the control, cinnamon supplementation, exercise, and cinnamon supplementation + exercise groups were  $18.733 \pm 11.940$ ,  $20.915 \pm 10.254$ ,  $12.564 \pm 8.287$ , and  $10.585 \pm 3.783$ , respectively.

IFN- $\gamma$  affects a variety of intracellular events in CD8<sup>+</sup> T cells via the IFN $\gamma$ R. It induces IL-12R expression<sup>19</sup>, and in murine models of infection, CTL proliferation and immunodominance appear to rely on IFN- $\gamma$ . However, it may also directly increase T cell apoptosis and reduce proliferation. Thus, reports on the actions of IFN- $\gamma$  on CD8<sup>+</sup> T cells vary. In the skin, IFN- $\gamma$  appears to be essential to

promoting T cell migration to sites of inflammation, even in sterile conditions. IFN- $\gamma$  is suppressive of CD8<sup>+</sup> T cell function when other antigens are expressed<sup>13</sup>.



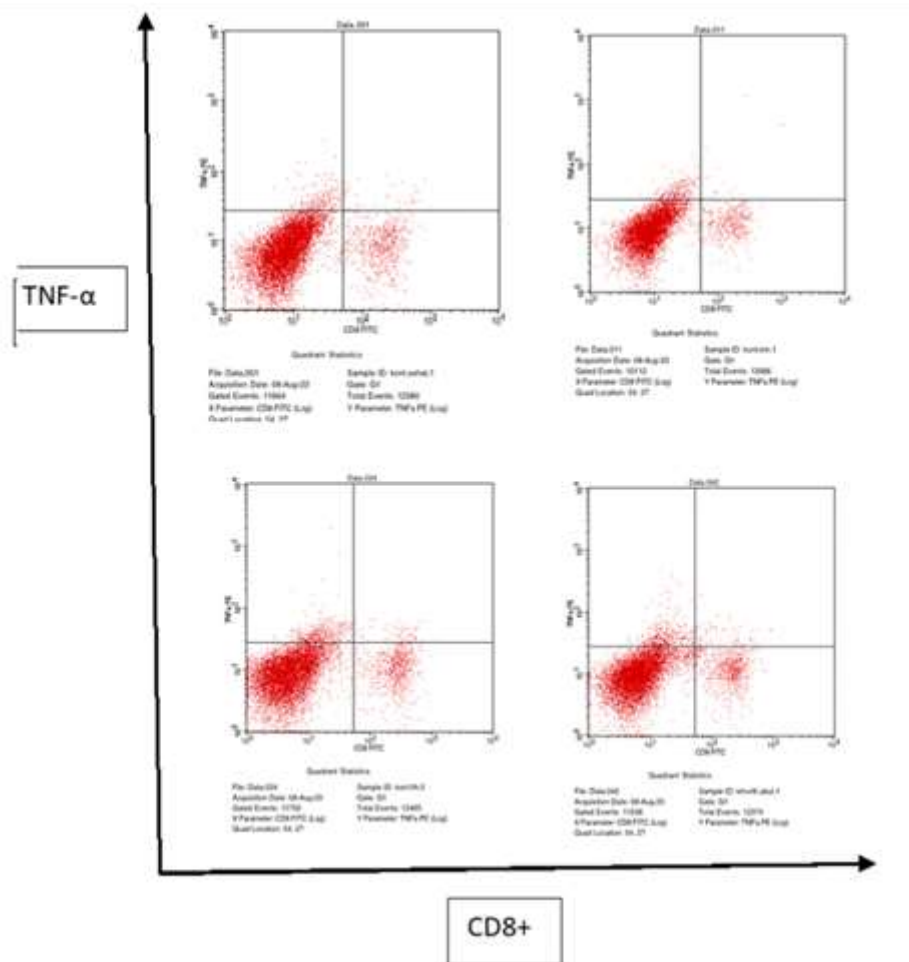
**Fig. 2:** Relative counts of CD8<sup>+</sup>T cells and CD8<sup>+</sup> T cells expressing IFN- $\gamma$  of the control, exercise + placebo, *Cinnamomum burmannii* without exercise, and *Cinnamomum burmannii* + exercise groups

Univariate Tests						
Dependent Variable		Sum of Squares	df	Mean Square	F	Sig.
CD8	Contrast	160.272	3	53.424	5.271	.004
	Error	375.033	37	10.136		
IFNgama	Contrast	3.984	3	1.328	3.417	.027
	Error	14.380	37	.389		

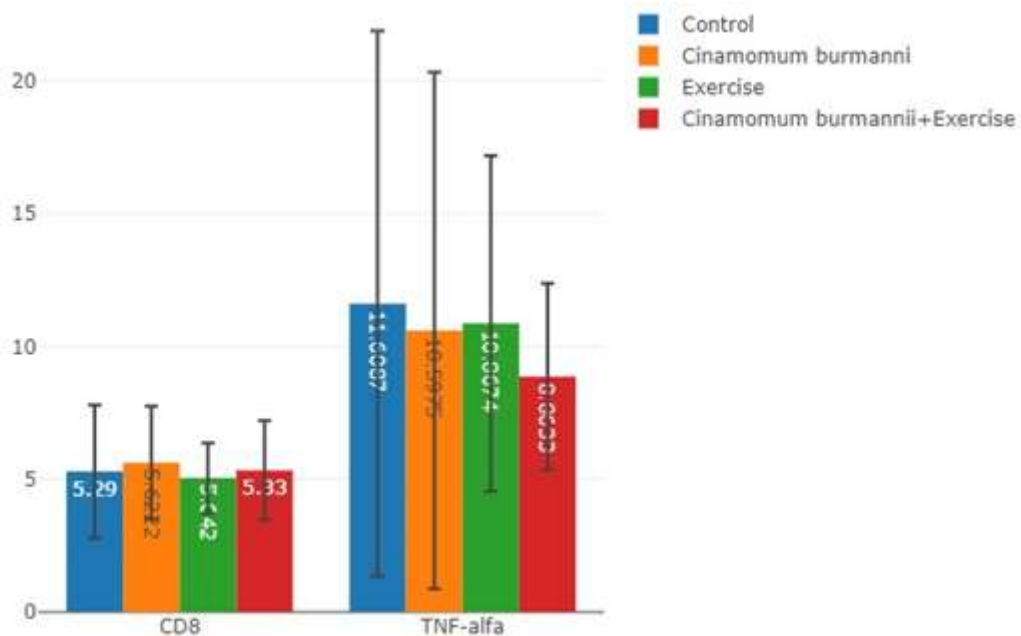
The F tests the effect of kelompok. This test is based on the linearly independent pairwise comparisons among the estimated marginal means.

**Table 3.** Univariate tests of CD8<sup>+</sup> and CD<sup>+</sup> expressing IFN- $\gamma$

Based on Table 3, cinnamon supplementation to exercise had a significant effect on the relative counts of CD8<sup>+</sup> T cells ( $p = 0.004$ ) and CD8<sup>+</sup> T cells expressing IFN- $\gamma$  ( $p = 0.027$ )



**Fig. 3:** Relative counts of CD8<sup>+</sup> T cells and CD8<sup>+</sup> T cells expressing TNF- $\alpha$  based on the flow cytometry



**Fig. 3:** Relative counts of CD8<sup>+</sup> T cells and CD8<sup>+</sup> T cells expressing TNF- $\alpha$  of the control, exercise + placebo, *Cinnamomum burmannii* without exercise, and *Cinnamomum burmannii* + exercise groups

**Table 4.** Relative counts of CD8<sup>+</sup> T cells and CD8<sup>+</sup> T cells expressing TNF- $\alpha$

Descriptive Statistics				
	Group	Mean	Std. Deviation	N
CD8	control	5.2900	2.49907	10
	Cinamomum burmannii	5.6222	2.12624	9
	exercise	5.0420	1.32794	10

	Cb+Exercise	5.3300	1.87687	12
	Total	5.3141	1.92743	41
	control	11.6087	10.27096	10
TNF- $\alpha$	Cinamomum burmannii	10.5975	9.72832	9
	exercise	10.8674	6.31904	10
	Cb+Exercise	8.8633	3.50543	12
	Total	10.4024	7.49430	41

**Table 5.** Results of Between-Subjects Effects Test

Source	Dependent Variable	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	CD8	1.604 <sup>a</sup>	3	.535	.135	.939
	TNF- $\alpha$	45.483 <sup>b</sup>	3	15.161	.255	.857
Intercept	CD8	1148.497	1	1148.497	289.087	.000
	TNF- $\alpha$	4458.681	1	4458.681	74.950	.000
Group	CD8	1.604	3	.535	.135	.939
	TNF- $\alpha$	45.483	3	15.161	.255	.857
Error	CD8	146.995	37	3.973		
	TNF- $\alpha$	2201.096	37	59.489		
Total	CD8	1306.445	41			
	TNF- $\alpha$	6683.175	41			
Corrected Total	CD8	148.599	40			
	TNF- $\alpha$	2246.579	40			

a. R Squared = .011 (Adjusted R Squared = -.069)

b. R Squared = .020 (Adjusted R Squared = -.059)

It was figured out that plasma IL-12 increased significantly after exercise. IL-12 was discovered to generate an IL-2 response, further enhancing the activity of CD8<sup>+</sup> T cells and CD56<sup>+</sup> T cells<sup>14,15</sup>.

Helper T cells expressing the CD4 phenotype recognize the antigenic epitope in major histocompatibility complex (MHC) class 1 molecules on the cell surface, while killer T cells or CD8<sup>+</sup> T cells recognize foreign antigens on transformed or virus-infected MHC class I molecules<sup>16</sup>.

Additionally, Nieman and Wentz showed an increase in the number of NK cells after exercise as soon as the exercise intensity was increased, presumably owing to an increase in catecholamine secretion and cell demarcation due to intravascular shear stress after the exercise intensity was increased.

#### 4. Conclusion

In sum, cinnamon supplementation to high-intensity exercise led to a significant increase ( $p = 0.04$ ) in the relative counts of CD8<sup>+</sup> T cells and CD8<sup>+</sup> T cells expressing IFN- $\gamma$  ( $p = 0.027$ ) but did not show any significant difference in the relative counts of CD8<sup>+</sup> T cells expressing TNF- $\alpha$ .

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