



Protective Effect of *Toraja Robusta* Coffee (*Coffee cane-Nora*) against Muscle Damage Due to Exercise on Balb/C Mice

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ABSTRACT

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Excessive physical activity can increase oxidative stress, and oxidative stress has been identified as the primary cause of exercise-induced muscle damage. Coffee is a natural product that contains antioxidants that can mitigate oxidative stress. The study was aimed to test the protective effects of *Toraja Robusta coffee* against muscle damage, measured through Skeletal muscle Troponin I (sTnI) serum level, muscle histology, and Glutathione peroxidase (GPx) activity. Twenty-four 8-week-old mice with an approximate weight of 20 grams were randomly assigned into four groups: the *Toraja robusta coffee* treatment group and three control groups: normal, placebo, and N-Acetylcysteine (NAC) control groups. The results of this study showed that the sTnI levels in the blood serum of the coffee treatment group were significantly lower compared to the NAC control group ($p = 0.017$) and the placebo control group ($p = 0.024$) but not significantly different from the normal control group ($p = 0.915$) ($p < 0.05$). The activity of GPx was higher in the coffee and NAC-treated groups compared with the placebo group ($p = 0.004$). This study concluded that *Toraja robusta coffee* protects muscles against damage, which is characterised by low serum levels of sTnI. Additional research is needed to elucidate the mechanism(s) of skeletal muscle protection by coffee intake and its potential to prevent other health issues.

Keywords: Antioxidant, Coffee, Glutathione peroxidase, Muscle damage, Physical activity, Skeletal muscle troponin I

Introduction

Engaging in physical activity and exercise offers many health advantages; nevertheless, it is essential to acknowledge the potential risks associated with these activities, including the possibility of sustaining injuries. It is essential to remember that excessive physical activity, particularly when the muscle undergoes eccentric contractions, might result in muscular damage, such as tears in the myofibril.¹ Furthermore, excessive physical activity can also elicit a heightened generation of free radicals.²

Antioxidants are essential components in the functioning of various defensive systems.³ The human body possesses a natural mechanism to safeguard itself against the harmful effects of free radicals using the endogenous antioxidant defence system. Nevertheless, when the intensity and duration of physical activity reach excessive levels, it can significantly increase the presence of free radicals.⁴ Consequently, the human body experiences an imbalance in the production of antioxidants, leading to oxidative stress.⁵

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Increased free radicals such as superoxide and other ROS can lead to the oxidation of cysteine 133 residues exposed to troponin I molecules in skeletal muscles.⁶ As a result, troponin I is released from the troponin/tropomyosin complex. Subsequently, the muscle exhibits an injury distinguished by intense pain and hypercontraction.⁷ This condition has the potential to impede mobility, both in daily life and in sports. Therefore, preventive measures are crucial to accelerate recovery and prevent more severe muscle damage. One of the suggested solutions is an exogenous antioxidant supplement.⁸ To boost the body's antioxidant defence system. The enhancement may lower the factors that put people at risk for these conditions.⁹ Indonesia has a diverse biodiversity, with the number of plant species reaching 30,000, of which 940 are traditional medicinal plants.¹⁰ One of them is *Toraja robusta* coffee, which has become an essential part of the economy of Toraja society.¹¹ It shows how coffee has become an essential commodity in Indonesia and its significant contribution to foreign exchange earnings.¹² It is important to point out that coffee not only exerts a good influence on the economic domain but is also predominantly linked to its numerous health benefits. Prior studies have demonstrated that coffee possesses antioxidant properties crucial in safeguarding against tissue damage, particularly in the liver.¹³ Coffee can act as a free radicals scavenger, contributing hydrogen and electrons, and as a pro-oxidant metal ions chelator. In addition, coffee can also trigger the expression of antioxidant genes in tissues.¹⁴ This study uses *Toraja robusta coffee* as an exogenous anti-oxidant supplement to evaluate its benefits and mechanisms of protection against exercise-induced skeletal muscle damage due to an increase in free radicals measured through sTnI levels, observations of gastrocnemius muscle histopathology and GPx activity in gastrocnemius muscle homogenate.

Materials and Methods

Ethical approval

This research was approved by the Ethics Commission of the Faculty of Medicine at Airlangga University, with ethical Certificate Number 48/EC/KEPK/FKUA/2023.

Preparation of experimental units

The experimental unit of this study is male BALB/C mice, twenty-four 8-week-old with an approximate weight of 20 g. The mice were housed in standard laboratory cages at room temperature (20–24 °C) and allowed to acclimatise for seven days with 12 hours of light and dark cycles.¹⁵

Extraction of *Toraja Robusta* coffee

The coffee used in this study has been tested for its antioxidant strength by a specialist in the testing service unit, Faculty of Pharmacy Airlangga University. The Robusta coffee comes from Tanah Toraja district, South Sulawesi, Indonesia. The coffee was ground using a grinder to produce coffee powder. The resulting powder (15 g) was mixed with 200 mL of water at a temperature of 92°C. The coffee was brewed using the manual brew method, employing a paper filter to produce coffee extract.

Exercise interventions to induce muscle damage

The experimental units K2, K3, and K4 were given maximum running intervention on the treadmill (Colombus). Before starting the core exercise, the experimental units were allowed to warm up as adaptation for 5 minutes on the treadmill. This warming up began with 1 minute of the first 0 cm/sec, 2 minutes of the next 14 cm/sec, then 2 minutes 21 cm/sec. After that, the experimental animals were subjected to core exercise at a speed of 30 cm/sec, and the duration was adjusted to the maximum running ability until fatigue. The exercise used results from modifications based on the training protocol of Davis.¹⁶

Treatment of Experimental Units

Supplementation (*Toraja robusta* coffee) was given immediately after the exercise-induced muscle damage. The experimental unit was supplemented orally using a sonde; K2 received mineral water as a placebo, K3 received *Toraja robusta* coffee, 0.52 mL/20 g per body weight, and K4 received N-acetylcysteine, 0.1 mL/20 g per body weight.

Testing Glutathione peroxidase Activity

The glutathione peroxidase levels were checked through the right leg gastrocnemius muscle wrapped in a cold PBS solution to obtain muscle homogenate. The animals were examined 24 hours post-administration of the extract. The GPx level was measured using the Glutathione Peroxidase Activity ELISA kit (Cat. E-BC-K096-S).

Testing Skeletal muscle Troponin I levels

The examination was done 24 hours after the exercise intervention. Blood (1 mL) was collected through cardiac puncture and dissolved with ketamine. The sample was centrifuged, and the serum was collected. Troponin I level was measured with a troponin I mouse fast skeletal muscle ELISA kit (Cat. E1167Mo).

The Histological examination of the Gastrocnemius muscle

The gastrocnemius muscle of the left leg was removed into a tube containing 10% formalin. Then, the cut muscle specimen was fixed and stained with hematoxylin and eosin. A qualitative examination of the muscle specimens was done under an Obtilab 400x magnification microscope with a special focus on muscle fibre rupture.

Statistical analysis

The statistical analysis was done using the SPSS Statistics 20.0 program (2020). The skeletal muscle Troponin I levels were assessed using a one-way analysis of variance (ANOVA) test, followed by a post-hoc Gomes-Howel test. The glutathione peroxidase activity was evaluated using the Kruskal-Wallis and Mann-Whitney post-hoc tests. The level of significance is set at 5%.

Result and Discussion

Effects of *Toraja Robusta* Coffee on Glutathione Peroxidase Activity

The data analysis of GPx activity of the gastrocnemius muscle homogenate showed the highest rates in the NAC control group, followed by the *Toraja robusta* coffee treatment group, the placebo control group, and then the normal control group. The study showed that *Toraja robusta* coffee supplementation was higher than the placebo control group ($p = 0.228$) and the normal control group ($p = 0.296$). On the other hand, the NAC control group was significantly higher than the coffee treatment group ($p = 0.004$), placebo ($p = 0.016$), and normal control ($p = 0.010$). Based on the measurements, it was found that coffee intake can increase GPx activity. However, the Mann-Whitney test showed no significant difference between coffee treatment and the normal control and placebo groups. However, the NAC control group showed significantly higher results than the other three groups.

According to the results in Table 1, there was an increase in the activity of glutathione peroxidase (GPx) in the group receiving supplementation. Specifically, this increase was observed in the N-acetylcysteine (NAC) and *Toraja robusta* coffee treatment groups. This study showed that taking NAC supplements is better for raising glutathione peroxidase levels than drinking coffee. The primary role of GPx as an endogenous antioxidant enzyme is crucial in regulating cell redox processes.¹⁷ Certain occupations frequently involve excessive physical activity and are associated with a heightened susceptibility to oxidative stress, even in trained athletes.¹⁸ Antioxidant N-acetylcysteine (NAC) can potentially augment glutathione synthesis within the human body.¹⁹ An increase in the concentration of glutathione within the body leads to a corresponding increase in the availability of substrates for glutathione peroxidase (GPx), resulting in elevated levels of GPx.²⁰ Likewise, coffee, renowned for its caffeine concentration, is recognised as a stimulant.²¹ In general, it is observed that green robusta seeds possess about double the amount of caffeine, higher levels of chlorogenic acids, and lower levels of trigonelline compared to arabica seeds on a per-unit weight basis.²² As an illustration, it is noteworthy that Arabica coffee sourced from Laos or Rwanda exhibits a caffeine content of approximately 3.41 percent per dry mass, but Robusta coffee originating from Indonesia has a much higher caffeine concentration of up to 8.16 percent.²³ Coffee has been recognised as a prospective reservoir of antioxidants, which are inherent chemicals that can modulate the synthesis of endogenous antioxidants.²⁴ Coffee activates the Nrf2 antioxidant pathway through the phosphorylation of Erk and Akt kinases, as evidenced by the increased levels of Nrf2 and HO-1 proteins.²⁵ The findings of this experiment supported the above argument by the increase in GPx levels following the administration of coffee supplementation, compared to the control group that did not receive any supplementation. Nevertheless, the observed rise did not demonstrate statistical significance. Viana et al.²⁶ Suggest that a potential explanation for the absence of a significant increase may be attributed to the insufficiency of bioactive chemicals inside coffee to stimulate the antioxidant enzyme GPx effectively. Therefore, to comprehend the function of coffee as a supplementary antioxidant capable of safeguarding muscles against harm, our investigation also examined the quantities of sTnI. The significance of this research is heightened due to the recognition of oxidative stress as a potential factor contributing to skeletal muscle damage.²⁷

Effects of *Toraja Robusta* Coffee on Skeletal Muscle Troponin I Levels

The study of skeletal muscle troponin I (sTnI) data indicated that the N-acetylcysteine (NAC) group had the highest rates, followed by the placebo group, the *Toraja robusta* coffee treatment group, and the normal group. Based on the available evidence, it can be inferred that coffee can protect muscles against damage caused by exercise, surpassing the protective benefits exhibited by N-acetylcysteine (NAC).

Based on the measurement results shown in Table 2, it can be concluded that exercise performed on the provided treadmill significantly caused muscle damage, as evidenced by elevated levels of sTnI in both the placebo and NAC control groups. In contrast, the group treated with *Toraja robusta* coffee exhibited comparable rates of

sTnI levels to those of the normal control group. Skeletal muscle troponin I (sTnI) is a biomarker for early detection of specific muscle injuries caused by exercise.²⁸ The cysteine 133 residue exposed to troponin I in the fast twitch muscle has a thiol group targeted for oxidation.⁶ Troponin I is known to be expressed in three distinct isoforms, namely cardiac, slow skeletal, and fast skeletal muscle.²⁹ Troponin I is an essential constituent of the troponin complex, mainly localised inside the thin filaments of skeletal muscles. Its primary function involves the control of calcium ions, which is essential for the proper contraction and relaxation of muscle fibres.³⁰ The oxidation of sTnI leads to a decrease in the responsiveness of myofibrils to calcium, resulting in a diminished strength of muscular contractions and a disruption in the organisation of myofibrils.⁶ At this point, the muscle experiences an injury, which is detected by an elevation of sTnI levels in the blood serum. From a different point of view, this

study examines qualitatively the state of the skeletal muscle histopathology of the gastrocnemius muscle. Figure 1 displays the observations of muscle fibres in the normal group, revealing a consistent pattern of regular and densely packed fibres. However, there were different patterns in the placebo control group with visible gastrocnemius muscle damage with high rupture conditions.

The occurrence of injury is commonly referred to as an eccentric contraction, which results in a rupture when the muscle is stretched beyond its flexibility limit.¹ Nevertheless, differences can be observed in the *Toraja robusta* coffee treatment group. The exercise appears to cause a tear on some muscle fibres. However, the tear appears to be in better condition than the placebo control and NAC groups. Skeletal muscle troponin I (sTnI) blood levels in the *Toraja robusta* coffee treatment group were also in almost the same condition as the normal control group.

Table 1: The GPx Activity on homogenate muscle gastrocnemius

Group	N	Mean ± SD (U)	Levene test (p)	Kruskal-Wallis (p)
Normal control	6	37.55 ± 17.33 ^a		
Placebo control	6	37.55 ± 20.62 ^a	0.690	0.009
Coffee treatment	6	46.83 ± 14.87 ^a		
NAC control	6	82.27 ± 11.65 ^b		

Note: Different superscripts are used to indicate significant differences in GPx activity. (p < 0.05)

Table 2: The sTnI levels on blood serum

Group	N	Mean ± SD (ng/ml)	Levene test (p)	ANOVA (p)
Normal control	6	3.38 ± 16.22 ^a		
Placebo control	6	3.67 ± 12.21 ^b	0.000	0.000
Coffee treatment	6	3.38 ± 32.73 ^a		
NAC control	6	4.56 ± 65.57 ^b		

Note: Different superscripts are used to indicate significant differences in levels of sTnI (p < 0.05)

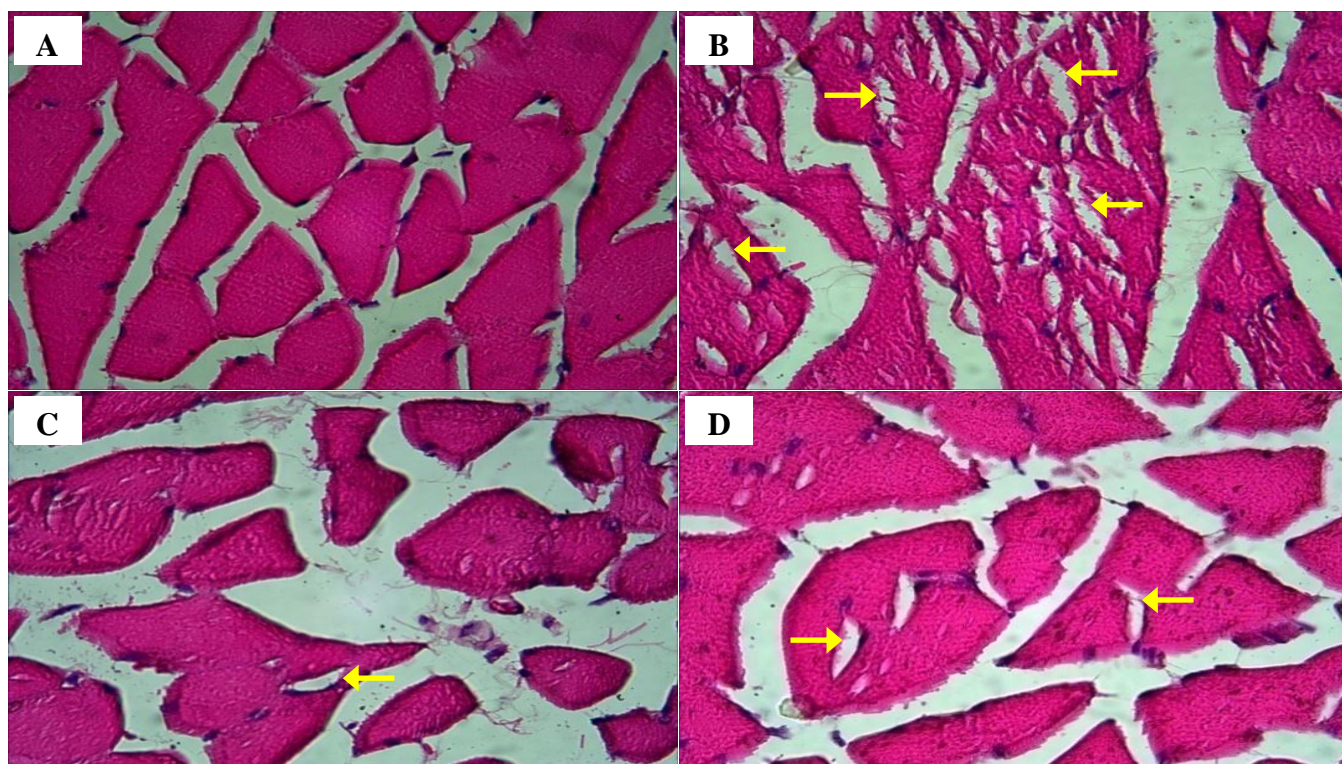


Figure 1: Visualisation of the gastrocnemius muscle using an Obtilab 400x magnification microscope by hematoxylin and eosin to see the tears on muscle fibres qualitatively. (A), normal group (B), placebo group (C), *Toraja Robusta Coffee Treatment* (D), N-Acetylcysteine group. The arrow depicts tears in muscle fibres.

The results suggest that *Toraja robusta* coffee can prevent the release of sTnI in the blood. Previous research has demonstrated that coffee is a beverage that has several beneficial effects on human health.³¹ A lot of bioactive substances are found in coffee, which is one of the most popular drinks in the world.³² Caffeine and chlorogenic acid (CGA) have been identified as active compounds found in coffee that express antioxidant activity and function as scavengers of reactive oxygen species (ROS).²³ The protective effects of *Toraja robusta* coffee on skeletal muscles can be linked to activating the endogenous antioxidant GPx and its activity as a scavenger of ROS. The outcomes of this study present intriguing avenues for additional investigation. According to the data shown in Table 1, there is a significant increase in the GPx activity seen in the NAC group compared to the coffee treatment group. However, based on the measurements of sTnI levels presented in Table 2, it can be seen that N-acetylcysteine (NAC) supplementation failed to cause a decline in sTnI levels. On the other hand, coffee treatment resulted in a non-significant improvement in GPx levels but successfully reduced the sTnI levels. Based on our analysis, the active compound contained in coffee acts not only through antioxidant mechanisms but also through another mechanism, which may be associated with its influence on the effectiveness of vascular function. Because of this, additional research is necessary to fully comprehend this process.

Conclusion

The study results showed that *Toraja robusta* coffee did not significantly increase the levels of the endogenous antioxidant GPx in the gastrocnemius muscle homogenate. Nevertheless, the findings of this study showed a reduction in the levels of skeletal muscle troponin I in the blood serum of the treated animals. Therefore, based on the findings of this study, it was concluded that *Toraja robusta* coffee has protective effects against exercise-induced muscle damage.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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