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Original Article

Effect of *Vitis gracilis* Wall (gagatan harimau) in the recovery of gastrocnemius muscle cells and cytochrome c expression of *Mus musculus*

[Efecto de *Vitis gracilis* Wall (gagatan harimau) en la recuperación de las células del músculo gastrocnemio y la expresión del citocromo c de *Mus musculus*]

Nila Zusmita Wasnis[1], Syafruddin Ilyas[1*], Salomo Hutahaean[1], Ramlan Silaban[2], Putri C. Situmorang[1]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Sumatera Utara, Medan, Indonesia.
[2]Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Negeri Medan, Medan, Indonesia.
*E-mail: syafruddin6@usu.ac.id

Abstract

Context: Physical exercise with maximum intensity to fatigue can cause impaired immunity. Gastrocnemius muscle damage is found in eccentric activities in sports. *Vitis gracilis* has been proven as a healing agent for degenerative diseases.

Aims: To determine whether the *V. gracilis* Wall extract can recovery of gastrocnemius muscle cells of *Mus musculus* through reduced inflammatory cells and cytochrome c expression.

Methods: The studies turned into performed in 5 groups of remedies withinside the study. The remedy groups consisted of T1: Negative control (no treatment), T2: Positive control (Swim Until the First Sink/SUFS) + Vit. C 0.2 mg/kg BW, T3: SUFS + 50 g/kg BW of *V. gracilis*, T4: SUFS + 75 g/kg BW of *V. gracilis* and T5: SUFS + 100 g/kg BW of *V. gracilis.* Mice were dissected and then the leg were taken for analysis of gastrocnemius muscle cells in immunohistochemistry.

Results: There was a significant difference ($p<0.01$) using the ANOVA one-way test on inflammatory cells in gastronomeus muscle tissue of *Mus musculus* and cytochrome c expression. *V. gracilis* 100 mg/kg BW can repair and reduce inflammatory cells in the gastronomic muscle tissue. The expression of cytochrome c protein becomes weaker when it is increased.

Conclusions: *V. gracilis* can recover gastrocnemius muscle cells of *Mus musculus* through reduced inflammatory cells and cytochrome c expression because of swim exercise.

Keywords: cytochrome c; gastrocnemius; muscle cells; immunohistochemistry; plant extracts.

Resumen

Contexto: El ejercicio físico con máxima intensidad hasta la fatiga puede provocar una alteración de la inmunidad. El daño del músculo gastrocnemio se encuentra en actividades excéntricas en los deportes. Se ha demostrado que la *Vitis gracilis* es un agente curativo de enfermedades degenerativas.

Objetivos: Determinar si el extracto de pared de *V. gracilis* puede recuperar las células del músculo gastrocnemio de *Mus musculus* mediante la reducción de las células inflamatorias y la expresión del citocromo c.

Métodos: Los estudios se realizaron en 5 grupos de remedios dentro del estudio. Los grupos de remedios consistieron en T1: Control negativo (sin tratamiento), T2: Control positivo (Nadar hasta el primer lavabo/SUFS) + Vit. C 0,2 mg/kg BW, T3: SUFS + 50 g/kg BW de *V. gracilis*, T4: SUFS + 75 g/kg BW de *V. gracilis* y T5: SUFS + 100 g/kg BW de *V. gracilis*. Se diseccionaron los ratones y luego se tomaron las patas para analizar las células del músculo gastrocnemio en inmunohistoquímica.

Resultados: Hubo una diferencia significativa ($p<0.01$) usando la prueba ANOVA unidireccional en células inflamatorias en tejido muscular gastronomeo de Mus musculus y expresión del citocromo c. *V. gracilis* 100 mg/kg de peso corporal puede reparar y reducir las células inflamatorias del tejido muscular gastronómico. La expresión de la proteína del citocromo c se debilita cuando esta aumenta.

Conclusiones: *V. gracilis* puede recuperar las células del músculo gastrocnemio de *Mus musculus* mediante la reducción de las células inflamatorias y la expresión del citocromo c debido al ejercicio de natación.

Palabras Clave: células musculares; citocromo c; extractos de plantas; gastrocnemio; inmunohistoquímica.

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AUTHOR INFO
ORCID: 0000-0002-1734-3291

INTRODUCTION

Physical activity can display immunomodulatory effects that affect the immune system and protect against cell damage (Gentile et al., 2019). Stress due to physical exercise can trigger impaired gene expression in cells, thus triggering the immune system such as lymphocytes. Physical activity causes stress involving inflammatory cytokines, increasing levels of NK, neutrophil and lymphocyte antibodies (Alack et al., 2019). Physical exercise with maximum intensity to fatigue, can cause impaired immunity, apoptosis and impaired sexual function and memory. Physical exercise (exercise) can improve cognitive function by increasing brain blood flow and the formation of brain neurotransmitters (Batatinha et al., 2019). Regular physical exercise mediates the promotion of health and longevity involving the SIRT1 regulatory pathway, including antioxidant processes, mechanical mechanisms of macromolecular damage, energy and mitochondrial function, and neuronal plasticity.

The gastrocnemius muscle is located at the back of the lower leg, one of the two main muscles that make up the calf (Carapeto and Aguayo-Mazzucato, 2021). The gastrocnemius muscle functions in the lower leg, connecting behind the knee and the heel (Carapeto and Aguayo-Mazzucato, 2021). The function of the gastrocnemius muscle is to flex the leg at the knee joint and flex the leg at the ankle joint (Nilsson and Tarnopolsky, 2019). The gastrocnemius muscle works primarily in fast leg movements such as racing, jumping, swimming, and, to a lesser extent, walking and standing (Carapeto and Aguayo-Mazzucato, 2021). Skeletal muscle damage is found in eccentric activities in sports. Damage impairs physical performance and prolongs recovery time, so it is necessary to prevent it (Stamati et al., 2011). Prevention of muscle damage is approached through strategies of inhibiting the negative impact and strengthening the positive response of the muscle to eccentric activity (Nilsson and Tarnopolsky, 2019). Cell damage can cause cytochrome c out of the mitochondria and apoptosis occurs through DNA fragmentation. Apoptosis plays an important role in skeletal muscle atrophy (Stamati et al., 2011). Cells undergoing apoptosis will cause nuclear damage that affects another metabolism (Nilsson and Tarnopolsky, 2019). Early denervation of muscle atrophy is associated with increased DNA fragmentation. Denervated muscle will contain more Bax than Bcl-2, which causes cytochrome c activation, indicating susceptibility to apoptosis (Nilsson and Tarnopolsky, 2019).

The use of medicinal plants is environmentally friendly, not dependent, and has harmful side effects. Indonesia is located in the equator and the tropics, so that many plant species can be used as a source of medicinal ingredients in the medical. One of them is *Vitis gracilis* Wall [synonym of *Ampelocissus gracilis* (Wall.) Planch.], the Indonesian people call it "gagatan harimau" (Yamakawa et al., 1983; Ilyas et al., 2019). This plant contains many secondary metabolites such as flavonoids and terpenoids, which act as antioxidants and trigger the repair of organs' histological structure (Aththorick and Berutu, 2018). Especially the content of phenylethanoid glycosides in *V. gracilis* extract acts as a strong antioxidant (Aththorick and Berutu, 2018). Phenylethanoid glycoside, a derivative of phenolic compounds usually found in plants such as tea (*Camelia sinensis*), has been proven as a healing agent for degenerative diseases (Dong et al., 2012). *V. gracilis* is an herbaceous plant belonging to the *Gesneriaceae* family, order *Lamiales*. *V. gracilis* has an epiphytic stature and has a very high species abundance in a karst forest habitat at an altitude of 1300 m above sea level and is found living by sticking to limestone walls (Ilyas et al., 2019; Ogutcen et al., 2021).

This study aims to determine whether the extract of *V. gracilis* can recover gastrocnemius muscle cells and analyze protein cytochrome c expression after administration of this plant. This research is expected to be developed into a potential drug for the recovery of muscle cells.

MATERIAL AND METHODS

Preparation of *V. gracilis* ethanol extract

V. gracilis leaves have been accrued from Tangkahan region is located between two villages, namely: Namo Sialang and Sei Serdang, in Batang Serangan Sub-Disrict, Langkat District, North Sumatra, Indonesia (coordinate points, x = 395 928.28 and y = 408 308.48). The Kalong Cave, which has hot springs, is nearby. This cave borders the Batang Serangan river, which has a depth of 15 m, a width of 1.0 m, and a height of 1.5 m from 1.0 m to 1.5 m in the cave, with a height of 1.5 m to 3.0 m. This cave is muddy smooth and sharp with rocks light brown texture (Rahmawaty et al., 2015). The smell of sulfur removed is not overpowering. The plant was turned into diagnosed and authenticated via way of means of Head of Botany in Universitas Sumatera Utara and deposited into the Medanense Botanical Herbarium.

The dried leaves were mashed using a blender until the leaf powder. It was soaked in ethanol solvent in a ratio of 100 g of leaves dissolved in 1000 mL of 96% ethanol. It was soaked and then filtered, the solvent obtained was concentrated using a rotary evaporator.

The concentrated extract was diluted using carboxymethylcellulose for use in the treatment. The drying process was based on drying of *Rhodomyrtus tomentosa* and *Zanthoxylum acanthopodium* previously used (Situmorang et al., 2019a; 2019b; 2021a; 2021b; 2021c) so that the phytochemical content in this herb was not lost due to heat.

Phytochemical profile

V. gracilis was dissolved in ethanol and dissolved in ethanol and then spotted on a silica gel 60 F254 plate. The plate was observed under 354 and 366 nm ultraviolet light. For flavonoids: The mobile phase comprised chloroform:glacial acetic acid:formic acid:water (64:32:12:8) then sprayed with Lieberman–Bochard solution. For steroids: The mobile phase consisted of n-hexane:ethyl acetate (80:20). For glycosides: the mobile phase consisted of ethyl acetate:methanol:ethanol:water. For saponins: the mobile phase comprised chloroform:glacial acetic acid:formic acid:water (64:32:12:8). For tannins: the mobile phase comprised ethyl acetate:methanol:ethanol:water (81:11:4:8) then sprayed with $FeCl_3$ (Situmorang et al., 2021b).

Animal handling

Animal preparation using 30 male mice (*Mus musculus*) was acclimatized for 14 days. Male mice were aged 8-12 weeks with bodyweight between 20-30 g. Male mice were grouped into 6 groups, wherein a treatment group contained 5 male mice. The treatment of male mice was adapted to the laboratory environment for two weeks and placed in a cage with a constant room temperature (25.0 ± 3.0°C), a humidity of 35-60%. Mice were placed in plastic animal cages (40 × 30 cm) and exposed to the light for 12 hours and the next 12 hours were in the dark. Male mice were fed *ad libitum* with maize and pellets dietary basis and were given free access to water. The research was carried out with permission from the Health Research Ethics Committee of the USU FMIPA Medan (0256/KEPH-FMIPA/2021).

Study design

These studies turned performed the use of a Completely Randomized Design (CRD). This sort of study was an experimental study. The studies turned into performed in 5 groups of remedies withinside the study. The remedy groups consisted of:

- T1: Negative control (no treatment),
- T2: Positive control (Swim Until the First Sink/SUFS) + Vit. C 0.2 mg/kg BW
- T3: SUFS + 50 g/kg BW of *V. gracilis*
- T4: SUFS + 75 g/kg BW of *V. gracilis*
- T5: SUFS + 100 g/kg BW of *V. gracilis*

Mice were dissected by the dislocation method (ketamine injection before), and then the femur until leg were taken for analysis of gastrocnemius muscle cells in immunohistochemistry.

Swim Until the First Sink (SUFS) program

Swimming training program duration until the first sinking for a month, frequency 5×/week at a speed of 7-15 m/min, for 4 weeks. Mice were sacrificed 2 days after the last training session to avoid metabolic effects of the last training session. The legs were quickly cut, weighed, frozen in liquid nitrogen and stored at -80°C, and the next day, paraffin blocks were made for immunohistochemistry (Evangelista et al., 2003).

Immunohistochemistry of cytochrome c

The gastrocnemius muscle tissue in paraffin blocks to a thickness of 5 µm was stained with immunohistochemical methods using a cytochrome c antibody (Ferraresi et al., 2015). Then, deparaffinization and rehydration were carried out—antigen retrieval with the aid of boiling in citrate buffer within the microwave. Endogenous peroxidase turned into blocked the use of H_2O_2 then nonspecific serum-tris buffer (10%) turned into delivered for 20 min at room temperature, then incubated with cytochrome c antibody diluted 1/ a hundred at room temperature for 120 min. The slides have been then incubated with a biotinylated polyvalent secondary antibody and incubated with an avidin-biotin-peroxidase complicated solution (LSAB 2 kit, Dako). The response turned into visualized with the aid of using including 3-diaminobenzidine tetrachloride to the gastrocnemius muscle tissue. Covered with a pitcher cowl and located with a microscope.

Statistical analysis

Statistical analysis for the data was performed using the Statistical Package for Social Science 22.0 (SPSS 22) using ANOVA, while immunohistochemical analysis used the Kruskal Wallis test. Data are represented as mean ± standard deviation. $P<0.05$ was considered statistically significant.

RESULTS

Phytochemical profile of *V. gracilis*

Based on Table 1, the phytochemical test used thin-layer chromatography. Observation under 354 and 366 nm ultraviolet light revealed that this plant

contained alkaloids, glycosides, flavonoids, saponins and tannins. This is in line with Aththorick and Berutu (2018) study, which states that *V. gracilis* contains many secondary metabolites such as flavonoids and terpenoids that act as antioxidants trigger the repair of the histological structure of organs.

Table 1. Phytochemical profile of *V. gracilis*.

No.	Metabolites	Results
1.	Alkaloids	+
2.	Glycoside	+
3.	Flavonoids	+
4.	Saponins	+
5.	Steroids and terpenoids	-
6.	Tannins	+

(+): Positive; (-): Negative.

Analyzing inflammatory cells in the gastrocnemius muscle tissue

There was a significant difference ($p<0.05$) using the ANOVA one-way test on inflammatory cells in gastronomeus muscle tissue of *Mus musculus*. The highest inflammatory cells were found in the SUFS + *V. gracilis* 50 mg/kg BW (T3) group, followed by the SUFS group + *V. gracilis* 75 mg/kg BW (T4). The lowest group was in the Control group (T1) and Swim Until the First Sink (T2). The group with the highest administration of *V. gracilis* (100 mg/kg BW) caused a decrease in inflammatory cells in gastronomes muscle tissue of *Mus musculus*. Based on these data, it is known that *V. gracilis* 100 mg/kg BW can repair and reduce inflammatory cells in the gastronomic muscle tissue.

Cytochrome c analysis on gastrocnemius muscle

There was a significant difference ($p<0.01$) in each group. The highest mean rank value for cytochrome c expression was in the SUFS + *V. gracilis* 50 mg/kg BW (T3) group and the lowest cytochrome c expression was in the control group (T1) and the Swim Until the First Sink/SUFS) + Vitamin C 0.2 mg/kg BW (Table 2 and Fig. 1).

Based on the Mann-Whitney test analysis, the value of cytochrome c expression in each treatment group showed, the higher the dose of *V. gracilis* leaves, the decreased cytochrome c expression in gastronomic muscle tissue. Cytochrome c was expressed strongly in SUFS + *V. gracilis* 50 mg/kg BW (T3) and SUFS + *V. gracilis* 75 mg/kg BW (T4) groups characterized by brown (Fig. 2). The expression of cytochrome c protein becomes weaker when it is increased (yellow arrow).

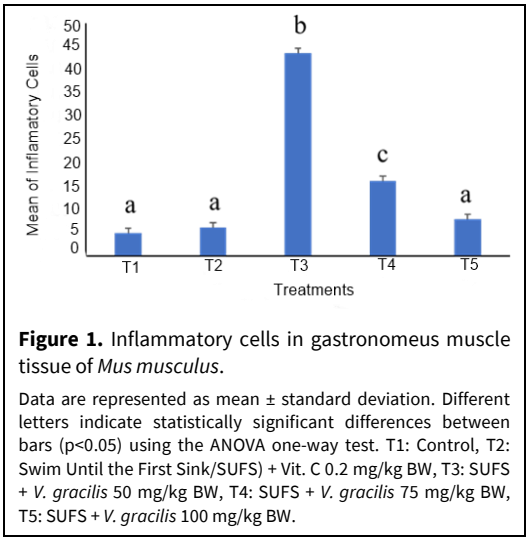

Figure 1. Inflammatory cells in gastronomeus muscle tissue of *Mus musculus*.

Data are represented as mean ± standard deviation. Different letters indicate statistically significant differences between bars ($p<0.05$) using the ANOVA one-way test. T1: Control, T2: Swim Until the First Sink/SUFS) + Vit. C 0.2 mg/kg BW, T3: SUFS + *V. gracilis* 50 mg/kg BW, T4: SUFS + *V. gracilis* 75 mg/kg BW, T5: SUFS + *V. gracilis* 100 mg/kg BW.

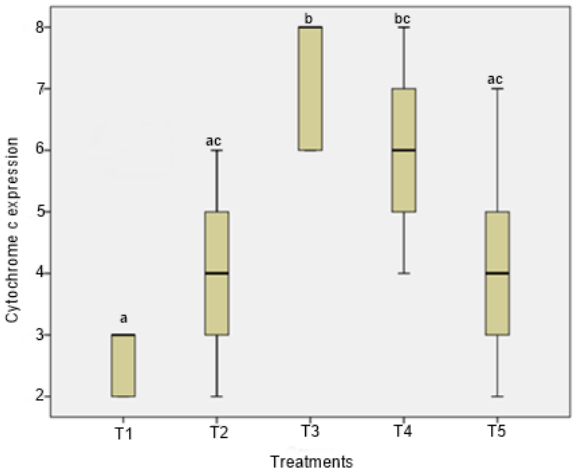

Figure 2. Cytochrome expression of the cells in gastronomeus muscle tissue of *Mus musculus* after treatment of *V. gracilis* extract.

Data are represented as mean ± standard deviation. Different letters indicate statistically significant differences between bars ($p<0.05$) using the ANOVA one-way test. T1: Control, T2: Swim Until the First Sink/SUFS) + Vit. C 0.2 mg/kg BW, T3: SUFS + *V. gracilis* 50 mg/kg BW, T4: SUFS + *V. gracilis* 75 mg/kg BW, T5: SUFS + *V. gracilis* 100 mg/kg BW.

Table 2. Kruskal-Wallis and Mann-Whitney analysis of cytochrome c values in gastrocnemius muscle.

Groups	Mean Rank	Kruskal-Wallis	Mann-Whitney				
			T1	T2	T3	T4	T5
T1	6.60			0.390	0.019*	0.035*	0.035*
T2	9.70				0.025*	0.092	0.669
T3	20.40	0.017				0.278	0.025*
T4	17.10						0.110
T5	11.20						

T1: Control, T2: Swim Until the First Sink/SUFS) + Vit. C 0.2 mg/kg BW, T3: SUFS + *V. gracilis* 50 mg/kg BW, T4: SUFS + *V. gracilis* 75 mg/kg BW, T5: SUFS + *V. gracilis* 100 mg/kg BW (*p<0.05; **p<0.01).

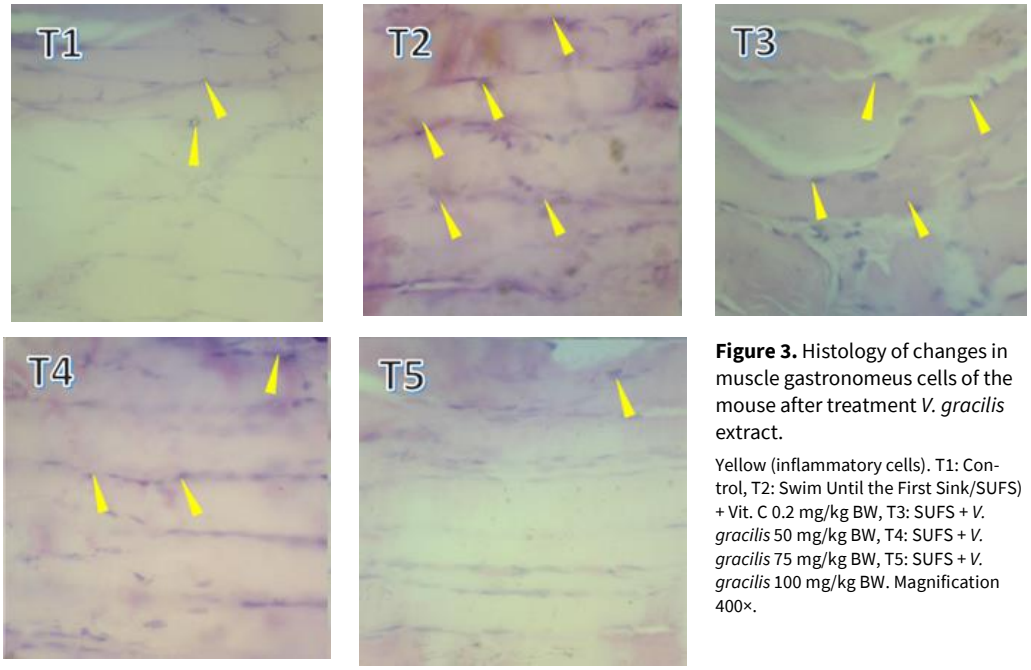

Figure 3. Histology of changes in muscle gastronomeus cells of the mouse after treatment *V. gracilis* extract.

Yellow (inflammatory cells). T1: Control, T2: Swim Until the First Sink/SUFS) + Vit. C 0.2 mg/kg BW, T3: SUFS + *V. gracilis* 50 mg/kg BW, T4: SUFS + *V. gracilis* 75 mg/kg BW, T5: SUFS + *V. gracilis* 100 mg/kg BW. Magnification 400×.

DISCUSSION

V. gracilis contains flavonoids, glycosides, phenols, amino acids, organic acids, and other chemical constituents and can be developed as medicinal ingredients (Table 1). *V. gracilis* is a herbaceous plant belonging to the *Gesneriaceae* family, a family of the order *Lamiales*. *V. gracilis* has an epiphytic stature and has a very high species abundance in a karst forest habitat at an altitude of 1300 m above sea level and is found living by sticking to limestone walls (Ilyas et al., 2019; Ogutcen et al., 2021). This plant is believed to increase the vitality of the lungs. This is reinforced by the presence of flavonoids and terpenoids, which act as antioxidants and phenylethanoid glycosides, which are derivatives of phenolic compounds as disease healing agents (Gong et al., 2019).

Fig. 1 explains that *V. gracilis* 100 mg/kg BW can repair and reduce inflammatory cells in the gastronomic muscle tissue. Phenolic compounds are organic compounds with at least one aromatic ring with one or more functional hydroxyl groups as an antioxidant because they stabilize free radicals. Flavonoids in these plants may affect cognitive abilities with evidence from long-term supplementation in animal models that can modulate synaptic plasticity through neuronal receptor activation, protein signaling, and gene expression (Spencer, 2010).

Inflammation is a dynamic and unstable process, where the process depends on the type of body tissue. Inflammation can occur starting from tissue damage. Under normal circumstances, physiologically cells will produce free radicals as a logical consequence of biochemical reactions in aerobic (Dan et al., 2021). The free radical is considered dangerous because it becomes reactive in trying to find the electron pair. Free radicals will attract electrons to macromolecules such as proteins, fatty acids, and polysaccharides (Zeb, 2020). This reaction will damage the cell membrane, whose components are macromolecules, resulting in cell damage (Zeb, 2020).

Figs. 2 and 3 explain that the expression of cytochrome c protein becomes weaker when it is increased. Two apoptotic pathways, namely extrinsic (receptor pathway) and intrinsic (mitochondrial pathway), lead to the activation of cytochrome c, which cleaves intracellular substrates, resulting in

dramatic morphological and biochemical changes of apoptosis. Overexpression of cytochrome c in tissues such as gastrocnemius muscle tissue can lead to mitochondrial integration after electrochemical potentiation changes in the membrane (Redza-Dutordoir and Averill-Bates, 2016). Responses to lethal stimuli such as hypoxia, oxidative stress, and DNA damage can activate this pathway with the release of cytochrome c as an early sign of apoptosis (Ilyas et al., 2021). This pathway involves mitochondria because it contains pro-apoptotic factors such as cytochrome c and AIF (apoptosis-inducing factors). The apoptotic process in germ cells can also be triggered by exposing particular plant secondary metabolites to specific cells (Situmorang and Ilyas, 2018). *V. gracilis* 100 mg/kg BW can repair and reduce inflammatory cells in the gastronomic muscle tissue. So, it can be concluded that this plant can recover gastrocnemius muscle cells of *Mus musculus* through reduced inflammatory cells and cytochrome c expression because of swim exercise.

CONCLUSION

Vitis gracilis Wall contains flavonoids, glycosides, phenols, amino acids, organic acids, and other chemical constituents and can be developed as medicinal ingredients. This plant can recover gastrocnemius muscle cells of *Mus musculus* through reduced inflammatory cells and cytochrome c expression because of swim exercise.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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REFERENCES

Alack K, Pilat C, Krüger K (2019) Current knowledge and new challenges in exercise immunology. Dtsch Z Sportmed 70: 250–260.

Aththorick TA, Berutu L (2018) Ethnobotanical study and phytochemical screening of medicinal plants on Karonese people from North Sumatra, Indonesia. J Phys Conf Ser 1116: 052008.

Batatinha HAP, Rosa Neto JC, Krüger K (2019) Inflammatory features of obesity and smoke exposure and the immunologic effects of exercise. Exerc Immunol Rev 25: 96-111.

Carapeto PV, Aguayo-Mazzucato C (2021) Effects of exercise on cellular and tissue aging. Aging 13(10): 14522–14543.

Dan L, Zongxian Ding KD, Xiangshi Y, Shixue C, Luciana HA (2021) Reactive oxygen species as a link between antioxidant pathways and autophagy. Oxid Med Cell Longev 2021: 5583215.

Dong F, Yang Z, Baldermann S, Kajitani Y, Ota S, Kasuga H, Imazeki Y, Ohnishi T, Watanabe N (2012) Characterization of l-phenylalanine metabolism to acetophenone and 1-phenylethanol in the flowers of *Camellia sinensis* using stable isotope labeling. J Plant Physiol 169(3): 217–225.

Evangelista FS, Brum PC, Krieger JE (2003) Duration-controlled swimming exercise training induces cardiac hypertrophy in mice. Braz J Med Biol Res 36(12): 1751–1759.

Ferraresi C, Parizotto NA, Pires de Sousa MV, Kaippert B, Huang Y, Koiso T, Bagnato VS, Hamblin, Michael R (2015) Light-emitting diode therapy in exercise-trained mice increases muscle performance, cytochrome c oxidase activity, ATP and cell proliferation. J. Biophotonics 8(9): 740–754.

Gentile A, Musella A, De Vito F, Rizzo FR, Fresegna D, Bullitta S, Mandolesi G (2019) Immunomodulatory effects of exercise in experimental multiple sclerosis. Front Immunol 10: 2197.

Gong X, Xu Y, Ren K, Bai X, Zhang C, Li M (2019) Phenylethanoid glycosides from *Paraboea martinii* protect rat pheochromocytoma (PC12) cells from hydrogen peroxide-induced cell injury. Bioscience Biotech Biochem 83(12): 2202–2212.

Ilyas M, Praptiwi, Wulansari D, Fathoni A, Agusta A (2019) An assemblages of fungal endophytes isolated from medicinal plants collected from Toba and Samosir, North Sumatra. IOP Conf Series: Earth Environ Sci 308: 012070.

Ilyas S, Hutahaean S, Sinaga RSH, Situmorang PC (2021) Apoptosis via cytochrome c in aortic tissue of diabetes mellitus after giving sikkam leaves (*Bischofia javanica* Blume). J Pharm Pharmacogn Res 9(3): 313–323.

Nilsson MI, Tarnopolsky MA (2019) Mitochondria and aging—The role of exercise as a countermeasure. Biology 8(2): 40.

Ogutcen E, Christe C, Nishii K, Salamin N, Möller M, Perret M (2021) Phylogenomics of *Gesneriaceae* using targeted capture of nuclear genes. Mol Phylogenet Evol 157: 107068.

Rahmawaty SEK, Syofyan A, Rauf A (2015) Integrated geographic information system and global positioning system for mapping of forest plants in supporting natural resources protection. Procedia Chem 14: 334–342.

Redza-Dutordoir M, Averill-Bates DA (2016) Activation of apoptosis signalling pathways by reactive oxygen species. Biochim Biophys Acta 1863(12): 2977–2992.

Stamati K, Mudera V, Cheema U (2011) Evolution of oxygen utilization in multicellular organisms and implications

for cell signalling in tissue engineering. J Tissue Eng 2(1): 2041731411432365.

Situmorang PC, Ilyas S, Hutahaean S, Rosidah R (2021a) Histological changes in placental rat apoptosis via FasL and cytochrome c by the nano-herbal *Zanthoxylum acanthopodium*. Saudi J Bio Sci 28(5): 3060–3068.

Situmorang PC, Ilyas S, Hutahaean S, Rosidah R (2021b) Components and acute toxicity of nanoherbal haramonting (*Rhodomyrtus tomentosa*). J HerbMed Pharmacol 10(1): 139–148.

Situmorang PC, Ilyas S, Hutahaean S, Rosidah R (2021c) Effect of nano herbal andaliman (*Zanthoxylum acanthopodium*) fruits in notch1 and hes1 expressions to human placental trophoblasts. Pakistan J Biol Sci 24(1): 165–171.

Situmorang PC, Ilyas S, Hutahaean S (2019a) Study of combination of nanoherbal andaliman (*Zanthoxylum acanthopodium*) and extra virgin olive oil (EVOO) effects in the expression of malondialdehyde (MDA), heat shock protein-70 (HSP70) and placental histology of preeclamptic rats. Pharm Sci 25(3): 205–220.

Situmorang PC, Ilyas S, Hutahaean S (2019b) Effect of combination of nano herbal andaliman (*Zanthoxylum acanthopodium DC*.) and extra virgin olive oil (EVOO) to kidney histology of preeclampsia rats. IOP Conf Series: Earth Environ Sci 305: 01208.

Situmorang PC, Ilyas S (2018) Review: Germinal cell apoptosis by herbal medicine. Asian J Pharm Clin Res 11(9): 24.

Spencer J (2010) The impact of fruit flavonoids on memory and cognition. British J Nut 104(S3): S40–S47.

Yamakawa T, Ishida K, Kato S, Kodama T, Minoda Y (1983) Formation and identification of anthocyanins in cultured cells of *Vitis* sp. Agric Biol Chem 47(5): 997–1001.

Zeb A (2020) Concept, mechanism, and applications of phenolic antioxidants in foods. J Food Biochem 44(9): e13394.

AUTHOR CONTRIBUTION:

Contribution	Wasnis NZ	Ilyas S	Hutahaean R	Silaban R	Situmorang PC
Concepts or ideas	x	x			
Design	x	x			x
Definition of intellectual content		x		x	
Literature search			x		x
Experimental studies		x	x	x	
Data acquisition	x	x			
Data analysis	x	x			
Statistical analysis	x				
Manuscript preparation			x		x
Manuscript editing	x				
Manuscript review	x	x	x	x	x

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