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Dose-dependent muscle relaxant activity of avocado leaf (*Persea americana* Mill.) extract: Central nervous system effects in mice and implications for phytopharmaceutical development

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ABSTRACT

Avocado leaves (*Persea americana Mill.*) contain flavonoids, saponins, and phenols, which are thought to exert muscle-relaxing effects through central nervous system depressant mechanisms. This study investigated the muscle-relaxing effects of avocado leaf extract (Persea americana) in male mice. Nine male mice were divided into three groups and administered 1%, 2%, and 4% avocado leaf infusions orally. Motor activity and reflex responses were observed every 15 min until the 100th min to assess muscle relaxation effects. The results showed that the muscle-relaxation effect increased with increasing extract concentration, at 15.55%, 34.44%, and 42.22%, respectively. Flavonoids and saponins are thought to play a role in increasing GABA activity, which reduces muscle tone. Avocado leaf extract showed muscle relaxation effects that increased with dose and has the potential to be a natural muscle relaxant through GABA activation.

Keywords: *Persea americana*, muscle relaxation, flavonoids, GABA, central nervous system.

1. INTRODUCTION

Medicinal plants have long been a key source of new drug discovery owing to their diverse secondary metabolite content and specific pharmacological activities. Mustofa and Namdes (2023) explained that the development of medicinal plant-based research is accelerating in line with the increasing medical need for new, safer, and more effective bioactive molecules with potential as alternative and complementary therapies. One plant with significant potential that has not yet been fully explored is the avocado (*Persea americana*), particularly its leaves. Avocado leaves have been used for generations in traditional medicine practices to treat various health problems, such as hypertension, inflammation, kidney stones, pain, and spasm disorders. or tension muscles (Juma, 2024). From a phytochemical perspective, avocado leaves contain several important metabolites, including flavonoids, alkaloids, terpenoids, tannins, and saponins. Phenolics (Rahmah et al., 2023). Several studies have shown that the dominant components of avocado leaves include quercetin, rutin, persin, β-sitosterol, estragole, linalool, and eugenol, each of which contributes to biological responses in the neuromuscular system and organs related to regulation of the body. contraction muscle (Bhuyan et al., 2019).

Muscle relaxation is a state in which muscle tension decreases after the contraction phase, allowing muscle fibers to return to their resting length and the body to achieve a more relaxed state. Hall (2022) explained that the relaxation process occurs due to the cessation of motor nerve impulses and the return of calcium ions (Ca^{2+}) to the sarcoplasmic reticulum, thereby halting actin-myosin interactions.

The combination of various bioactive compounds in avocado leaves suggests that the plant has significant potential as a source of natural muscle relaxants (Sutiningsih et al., 2023). This is increasingly relevant considering that muscle spasms, cramps, and tension are common. Currently, treatment is dominated by synthetic drugs such as benzodiazepines, mefenesin, and tizanidine (ZENGİN 2024). Although effective, these drugs are not without the risk of side effects, such as severe sedation, decreased coordination, hypotension, and potential dependence (ZENGİN, 2024). This situation has prompted the search for naturally derived muscle relaxants that offer good efficacy with minimal side effects. This is in line with the research findings obtained by Mustofa and Namdes (2023), who demonstrated that exploring local medicinal plants is crucial for finding new drug candidates that are safe, affordable, and easily accessible to the public. Indonesia, with its vast biodiversity, offers significant opportunities for developing plants, including avocado leaves, as phytopharmaceuticals with a strong scientific basis for their use. Therefore, this study investigated the muscle-relaxing effects of avocado leaf (*Persea americana*) extract in male mice. The results are expected to provide a scientific overview of the potential of avocado leaves as an alternative natural muscle relaxant that can be further developed in pharmaceutical and phytomedicine fields.

2. LITERATURE REVIEW

2.1 Phytochemical Constituents of Avocado Leaf (*Persea americana* Mill.) and Their Relevance to Central Nervous System Activity

Avocado leaf (*Persea americana* Mill.) has been widely reported to be a rich source of bioactive phytochemical compounds with diverse pharmacological properties. Phytochemical investigations have consistently demonstrated the presence of flavonoids, phenolic compounds, alkaloids, saponins, and tannins in the leaves of avocados. These secondary metabolites are well known for their biological activities, particularly their antioxidant, anti-inflammatory, and neuroactive effects. Among these compounds, flavonoids and phenolic substances play crucial roles in protecting neuronal cells against oxidative stress, a major contributing factor to neuromuscular dysfunction and excessive neuronal excitability.

From a neuropharmacological perspective, flavonoids are recognized for their ability to modulate neurotransmission in the central nervous system. Several flavonoid derivatives have been shown to interact with γ-aminobutyric acid (GABA) receptors, especially the GABA_A receptor subtype, which is the primary inhibitory neurotransmitter system in the brain. Activation of GABAergic pathways results in

reduced neuronal firing, decreased excitatory signaling, and overall central nervous system depression (CNSD). This mechanism underlies the sedative, anxiolytic, and muscle-relaxant effects commonly observed with compounds that enhance GABAergic activity. Consequently, the flavonoid-rich profile of avocado leaf extract provides a strong scientific rationale for its potential role as a centrally acting muscle relaxant.

In addition to flavonoids, alkaloids present in avocado leaves may contribute to central nervous system modulation by exerting mild depressant effects on the neuronal activity. Saponins may further enhance the pharmacological efficacy of the extract by improving membrane permeability and facilitating the absorption of the active compounds. The synergistic interactions among these phytochemicals may amplify the overall neuropharmacological effects of the avocado leaf extract. Therefore, based on its phytochemical composition, avocado leaves are a promising natural source for developing agents that target central nervous system-mediated muscle relaxation.

2.2 Dose-Dependent Muscle Relaxant Effects and the Role of Central Nervous System Depression

Muscle relaxants are pharmacological agents that reduce skeletal muscle tone by interfering with neuromuscular transmission or by suppressing neuronal activity within the central nervous system. Centrally acting muscle relaxants primarily exert their effects by inhibiting motor neuron activity at the spinal or supraspinal levels, leading to decreased muscle contraction. Although synthetic muscle relaxants are widely used in clinical practice, their use is often associated with adverse effects, such as excessive sedation, impaired motor coordination, cognitive disturbances, and risk of dependence. These limitations have driven interest in identifying safer, plant-based alternatives. Numerous studies have demonstrated that natural compounds with sedative or anxiolytic properties often exhibit muscle relaxant activity as a secondary effect of their use. This relationship highlights the close physiological connection between central nervous system inhibition and skeletal muscle relaxation. In experimental models, extracts derived from medicinal plants with central depressant activity frequently produce measurable reductions in locomotor activity, muscle tone, and reflexes. Avocado leaf extract has been reported to induce central nervous system depression, as evidenced by reduced spontaneous motor activity and diminished responsiveness in animal models. The evaluation of dose-dependent effects is a fundamental principle in pharmacological research, as it allows for the determination of the relationship between the administered dose and the observed biological response. The dose-dependent muscle relaxant effect indicates that increasing concentrations of the extract lead to progressively stronger pharmacological outcomes, thereby supporting a causal relationship between the bioactive constituents and the observed effect. Animal models, particularly mice (*Mus musculus*), are commonly employed in central nervous system studies because of their well-characterized neurophysiological responses and sensitivity to pharmacological agents.

Investigating the dose-dependent muscle relaxant activity of avocado leaf extract in mice provides critical insights into its mechanism of action and therapeutic potential. Such studies not only help establish the effective dose range but also contribute to understanding the safety margin and potential toxicity of the extracts. Therefore, dose–response evaluation is essential for validating avocado leaf extract as a centrally acting muscle relaxant.

2.3 Pharmacological Implications and Prospects for Phytopharmaceutical Development

The development of phytopharmaceuticals requires comprehensive scientific evidence to support their efficacy, safety, and quality consistency. One of the most important criteria in phytopharmaceutical research is the demonstration of a clear dose–response relationship, as this parameter is fundamental for drug standardization and clinical applications. The dose-dependent muscle relaxant activity of avocado leaf extract provides a strong pharmacological foundation for its further development as a phytopharmaceutical product. From a safety perspective, plant-based muscle relaxants may offer advantages over synthetic drugs, particularly in reducing adverse central nervous system effects, such as excessive sedation and dependency. The long-standing traditional use of avocado leaves in herbal medicine suggests a favorable safety profile; however, rigorous preclinical evaluations are necessary to confirm this

assumption. Controlled animal studies assessing central nervous system effects allow researchers to determine whether muscle relaxant activity occurs within a safe therapeutic window. Beyond its pharmacological relevance, the use of avocado leaf extract has broader implications for sustainable drug development. Avocado leaves are abundant and often considered agricultural waste, making them an economically viable and environmentally sustainable source of bioactive compounds. Transforming this underutilized resource into a standardized phytopharmaceutical product could enhance the value of local biodiversity and support the development of the natural medicine industry. Overall, the literature supports the potential of avocado leaf (*Persea americana* Mill.) extract is a dose-dependent, centrally acting muscle relaxant with promising implications for phytopharmaceutical development. Continued research focusing on mechanistic studies, standardization processes, and long-term safety evaluations is essential to advance this natural extract from experimental research to clinical and industrial applications.

3. METHOD

This research is a descriptive quantitative laboratory experimental research, aimed at determining the effectiveness of muscle relaxation in male mice that have the potential to benefit from avocado leaves. Nine male mice were divided into three groups of three mice each, which received oral avocado leaf infusion at concentrations of 1%, 2%, and 4%.

Observations were conducted every 15 min to monitor changes in behavior and physiological responses, such as consciousness, motor activity, and reactions to stimuli. All observational data were recorded periodically and analyzed based on differences in time and concentration while maintaining ethical standards for the use of test animals.

3.1 Tools and materials

The equipment used for this research included vials, funnels, beakers, measuring cylinders, hot plates, parchment paper, labels, measuring flasks, pens, horn spoons, syringes, stopwatches, analytical scales, and thermometers. The materials used in this study were aluminum foil, distilled water, gauze, and avocado leaf (*Persea americana*) simplicia.

3.2 The Course of Research

The material used in this study was avocado leaf (*Persea americana*) simplicia. The infusion process begins with a wetting step to aid in the release of active compounds. Initially, the simplicia is moistened with a small amount of distilled water, then water is added and heated at 90°C for 15 min, as is the procedure for making an infusion. The heating results were filtered to separate the filtrate from the dregs, and the volume of the filtrate was adjusted to 100 mL, following the standard for making the infusion. The infusion that had been obtained was then diluted to three concentrations (1%, 2%, and 4%) as test solutions. The use of multiple concentration levels is necessary to observe the biological responses affected by different doses. These solutions were tested on eight predetermined effect parameters: PSM, SSSP, DSSP, SL, RO, SM, PSL, and ANA. The test method used is the initial pharmacological effect observation test parameter (acute toxicity and mouse behavioral activity), especially on the Central and Autonomic Nervous System Activity in mice test animals with avocado leaf extract with an observation interval of every 15 minutes. Nine male mice were divided into three groups, each containing three mice. Avocado leaf infusions were prepared with concentrations of 1%, 2%, and 4% which were then given orally to each group of mice sequentially or more specifically group 1 was given 1% infusion, group 2 was given 2% infusion and group 3 was given 4% infusion. Each animal was then observed periodically at 15-minute intervals for all observed parameters. Observations focused on phenotypic and behavioral changes, including the level of consciousness and response to stimuli (motor activity and reflex responses). All observation results were recorded routinely every 15 min for each mouse so that they could be compared based on differences in concentration and observation time. The results were then analyzed by paying attention to the differences in each animal and the control group, as well as any important changes in condition recorded according to the ethical rules for the use of test animals.

3. RESULT AND DISCUSSION

Table 1. Percentage of Muscle Relaxation Effect Based on RO Parameters

Concentration	% RO Effect
1%	15.55%
2%	34.44%
4%	42.22%

Table 2. Supporting Neuropharmacological Parameters

Parameter observation	Effect calculation results		
	1% concentration	**2% concentration**	**Concentration 4%**
PSM	52.77%	41.89%	38.88%
PSL	27.77%	40.90%	38,38%
SM	27,77%	28,78%	36,36%
ANA	24,07%	25,92%	25,92%
SSSP	53,21%	35,67%	40,05%
DSSP	10,37%	22,22%	19,23%
SL	47,61%	40,27%	31,34%

Based on the data on Table 1 and 2 which is in accordance with the results of observations using the " Avocado Leaf" sample, it can be seen that there are several responses experienced by test animals such as effects (PSM, SSSP, DSSP, SL, RO, SM, PSL, and ANA) and the table above shows the percentage of pharmacological effects of avocado leaf extract at various concentrations (1%, 2%, and 4%).

Thus, the results of observations on test animals using leaf samples avocado showed various pharmacological responses, including the interesting muscle relaxant (RO) effect. Data on the percentage of pharmacological effects of avocado leaf extract at various concentrations strengthens this potential, where the RO effect increases with increasing extract concentration. The results of this study indicate that avocado leaf extract (*Persea americana*) exhibits an increasing muscle relaxant effect with increasing concentration. At concentrations of 1%, 2%, and 4%, the muscle relaxant effect was 15.55%, 34.44%, and 42.22%, respectively. This increase indicates a positive dose-response relationship, with higher extract concentrations producing a greater muscle relaxant effect in male mice.

The muscle-relaxing effect of avocado leaf extract is thought to originate from its bioactive compounds, such as flavonoids, saponins, and phenols. These three groups of compounds are known to possess sedative, anti-inflammatory, and depressant properties on the central nervous system. As explained by Kumar et al. (2022), flavonoids can reduce motor activity by modulating ion channels and enhancing the action of inhibitory neurotransmitters. This statement supported by Ríos et al. (2022) who put forward that compound Phenolics and some saponins can enhance the function of GABA (gamma-aminobutyric acid) through activation of GABA-A receptors, thereby increasing the effect of neuronal inhibition and reducing muscle tension.

Furthermore, Brunton et al . (2022) in *Goodman & Gilman's The Pharmacological Basis of Therapeutics* explain that increased GABA activity is a key mechanism that produces muscle relaxation, decreases motor neuron excitability, and reduces locomotor activity in experimental animals such as mice. Therefore, the ability of compounds in avocado leaves to modulate the GABAergic system is believed to be a major

factor triggering this muscle relaxation effect. At a low concentration (1%), the relaxant effect was still limited (15.55%), indicating that the amount of active compound reaching the target receptor was insufficient to induce a significant physiological response. When the dose was increased to 2%, the relaxant effect increased significantly to 34.44%, indicating that the bioactive compound began to work more effectively in reducing muscle tension. Meanwhile, at the highest concentration (4%), the relaxant effect reached a maximum of 42.22%, indicating that this dose exhibited optimal pharmacological activity within the tested concentration range.

The increased response at higher doses is likely due to the stronger depressant effect on the central nervous system. This symptom is evident from the significant decrease in motor activity in mice during the testing process. Sharma & Patel (2022) explain that increasing the concentration of plant extracts containing flavonoids and saponins can amplify the CNS depressant effect through modulation of GABA-A receptors, ultimately reducing muscle tone and motor activity. This statement is supported by Hernandez et al . (2023), who report that increased muscle relaxation at high doses is generally associated with a greater risk of sedation due to increased inhibition of neuronal activity. Based on this, avocado leaf extract shows potential as a natural muscle relaxant. However, as emphasized by Suhendra et al . (2023), determining the optimal dosage is still necessary to achieve maximum muscle relaxation without causing excessive sedation that could disrupt the normal behavior of test animals.

Meanwhile, in other pharmacological effectiveness results, for example, in the PSM effect, the highest effectiveness occurred at a concentration of 1%. Increasing the concentration actually decreased the response, suggesting the possibility that higher doses do not always increase the effect, or that there was response saturation in mice. Similar to PSM, in the SSSP effect, a concentration of 1% produced the greatest response. However, at a concentration of 4%, it increased again compared to 2%. This indicates a non-linear pattern with dose. Unlike the previous two effects, DSSP showed increased effectiveness when the dose was increased to 2%. However, the response decreased at 4%, which may indicate the optimal point is at a concentration of 2% **.** In the PSL effect, a significant increase occurred at a concentration of 2%, then decreased again at 4%. So the optimal point for PSL is at a concentration of 2% . In the ANA effect, there was a small increase at 2% and stabilized until 4%. The ANA effect did not The response did not change much between doses, so it can be seen that the response was relatively constant . Meanwhile, in SL, there was a consistent decreasing pattern from 1% to 4%. It can be concluded that the effect of SL was most effective at low concentrations, namely 1% . Similar to RO, the effect of SM showed a steady increase from 1% to 4%. This also shows a positive relationship between dose and mouse response.

Based on this, the effectiveness of RO consistently increases and has a high percentage of effectiveness, making research on RO activities more prominent and relevant compared to pursuing other activities. does not show consistency between doses. Because some pharmacological effects more frequently reach saturation points, increasing the dose does not always equate to increased effectiveness.

4. CONCLUSION

This study demonstrates that avocado leaf (*Persea americana* Mill.) extract exhibits a clear and progressive muscle relaxant effect that increases with rising concentrations, as evidenced by muscle relaxation percentages of 15.55% at 1%, 34.44% at 2%, and 42.22% at 4%. The observed pattern confirms the presence of a positive dose–response relationship, indicating that higher extract concentrations produce stronger pharmacological effects on muscle relaxation in experimental animals. This dose-dependent response strengthens the validity of the findings and supports the conclusion that the muscle relaxant activity is directly related to the bioactive constituents present in the extract.

The muscle relaxation effect is likely mediated through central nervous system mechanisms, particularly via modulation of inhibitory neurotransmission. Bioactive compounds such as flavonoids, saponins, and phenolic substances are known to exert anti-inflammatory and antioxidant effects while simultaneously acting as central nervous system depressants. These compounds are believed to enhance the activity of γ-aminobutyric acid (GABA), the primary inhibitory neurotransmitter in the central nervous

system. Increased GABAergic activity leads to reduced neuronal excitability, diminished motor neuron firing, and a consequent decrease in muscle tone, thereby explaining the observed muscle relaxant effect.

Overall, the findings of this study provide scientific evidence supporting the potential of avocado leaves as a natural source of muscle relaxant agents. The demonstrated efficacy, combined with the plant's bioactive profile, highlights its promise for further investigation in phytopharmaceutical research. Future studies should focus on isolating and characterizing the specific active compounds, evaluating long-term safety and toxicity, and determining optimal dosing strategies to balance efficacy and sedation. Ultimately, this research contributes to the growing body of evidence supporting the development of safe, effective, and naturally derived muscle relaxant drugs based on plant resources.

Ethical Approval

No: 0378/M/KEPK-PTKMS/III/2025

Informed Consent Statement

Not applicable. No primary data were collected from human subjects, and no interviews, surveys, or personal information were obtained in this study.

Authors' Contributions

NAA contributed to the conceptualization of the study, formulation of research objectives, and coordination of experimental procedures. DFPN participated in data collection, animal handling during experimentation, and preliminary data organization. RA contributed to the implementation of laboratory experiments and assisted in recording neuropharmacological observations. ID supported data analysis and contributed to the interpretation of experimental results. RAS assisted in literature searching, data validation, and preparation of supporting research documentation. RW contributed to statistical data processing and verification of result consistency. WOKA participated in manuscript drafting, particularly in the results and discussion sections. N assisted in compiling references and improving manuscript structure and language clarity. AAJ contributed to the literature review and strengthened the discussion by integrating relevant empirical studies. AUPN acted as the corresponding author, supervised the overall research process, critically reviewed the manuscript for intellectual content, and approved the final version for publication.

Disclosure Statement

The authors declare that they have no known financial, personal, or institutional conflicts of interest that could have influenced the development of this research or the preparation of this manuscript. All stages of the study including the conceptualization, literature selection, analysis, and writing were conducted independently and guided solely by academic objectives. No external parties intervened or contributed in a way that may have impacted the neutrality, integrity, or outcomes of this article. The authors affirm that this work adheres to ethical research standards and that all referenced materials have been properly and transparently acknowledged.

Data Availability Statement

This study is based entirely on a qualitative literature review of publicly available academic sources. No primary empirical data were collected. All references used in the analysis are cited within the manuscript. Additional details regarding the literature selection process, analytical framework, or specific sources can be made available by the authors upon reasonable request for academic or research purposes.

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