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(RESEARCH ARTICLE)

Antifatigue activity of hydroethanolic extract from *Landolphia myrtifolia* Bojer (Apocynaceae) roots

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Abstract

Landolphia myrtifolia is a plant utilized traditionally to treat fatigue in Madagascar. The objective of this study was to evaluate the antifatigue activity of *Landolphia myrtifolia* extract in mice subjected to both short- and long-duration physical exertion. A total of 20.2 g of defatted ethanolic extract was obtained from 500 g of *Landolphia myrtifolia* root powder by maceration in an ethanol-water mixture (80:20), yielding 4.04%. Phytochemical screening revealed a high content of flavonoids, tannins, and leucoanthocyanidins, with lower amounts of heterosides and polyphenols. In the hanging test, mice treated with the extract at doses of 100, 200, and 400 mg/kg showed significantly increased suspension times of 41.11 ± 0.42, 60.88 ± 0.51 and 87.55 ± 0.55 seconds, respectively, compared to 30 ± 1.41 seconds in the negative control group. The reference group exhibited a suspension time of 51.33 ± 0.55 seconds. In the forced swimming test, treated mice swam for 21.46 ± 0.40, 28.45 ± 0.84, and 54.62 ± 2.36 minutes at doses of 100, VBC200, and 400 mg/kg, respectively, significantly longer than the control group (8.62 ± 0.28 minutes, $P < 0.05$). The reference drug STIMOL induced a swimming time of 14.33 ± 0.37 minutes. Additionally, the distance traveled on the rotarod increased dose-dependently in treated mice (7.47 ± 0.19, 9.19 ± 0.27, and 17.47 ± 0.41 cm) compared to controls (2.69 ± 0.18 cm), while the reference group covered 4.358 ± 1.201 cm. These results indicate that *Landolphia myrtifolia* extract, supporting its potential as a natural antifatigue agent.

Keywords: *Landolphia myrtifolia*; Apocynaceae; Antifatigue; Mice; Physical effort

1. Introduction

Fatigue is a common physiological phenomenon resulting from prolonged or intense physical activity, characterized by a decline in the ability to perform muscular work. It represents a significant limiting factor in both physical performance and overall health [1]. Exploring natural products with potential antifatigue properties has become an important focus of biomedical research due to their promising therapeutic benefits and relatively low side effects [2].

Landolphia myrtifolia, belonging to the Apocynaceae family, are traditionally used in various African regions for their medicinal properties, including anti-inflammatory, antioxidant, and tonic effects [3]. Despite its ethnopharmacological uses, scientific studies investigating the antifatigue potential of *Landolphia myrtifolia* are limited.

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The objective of this study was to evaluate the antifatigue activity of a defatted ethanolic extract of *Landolphia myrtifolia* in mice subjected to both short-term, intense effort and long-term endurance exercise models. These models, namely the hanging test and forced swimming test, are well-established paradigms for assessing muscular fatigue and endurance capacity in rodents [4], [5].

2. Materials and Methods

2.1. Chemical study: Extraction and phytochemical screening

Five hundred grams (500g) of powdered root of *Landolphia myrtifolia* was made using the cold maceration technique with in an ethanol-water mixture (80:20) during 7 days. The extract was filtered and the filtrate was evaporated to dryness. The extract was stored in a desiccator until used for the phytochemical screening and biologically tests [4], [5], [6].

2.2. Biological study

2.2.1. Experimental Animals

Male Swiss mice, aged 1 to 3 months and weighing between 20 and 38 grams, were used in this study. They were purchased from IMVAVET and housed in the Pharmacodynamics Laboratory animal facility under a 12-hour light/dark cycle at room temperature. The mice were fed LFL 14-20 pellet diet and had free access to water.

Prior to the experiment, the mice were fasted for 18 hours with free access to water ad libitum.

2.2.2. Tested Products

The plant extract used in the tests included the defatted ethanolic extract at doses of 100, 200, and 400 mg/kg, a reference product, STIMOL, at a dose of 10 mg/kg, and distilled water.

2.2.3. Study of the effect of the extract on fatigue induced by short-duration effort on the hanging bar

The hanging bar test was used to induce an intense and brief muscular effort to provoke muscle fatigue in mice [7]. Prior to the experiment, a preliminary test was conducted to select suitable mice. The hanging bar apparatus consisted of two metal supports, each approximately 1 cm in circumference, connected by an iron rod with a diameter of approximately 2 to 3 mm, stretched over 15 cm in length and positioned 20 cm above the ground. The mice's hind limbs were tied to prevent any support or grip on the bar. Mice able to remain suspended from the bar for 20 to 30 seconds were selected.

The selected mice were divided into five groups of three animals each. The control group received distilled water orally. The treated groups received the extract orally at doses of 100, 200, and 400 mg/kg. The reference group was treated orally with STIMOL at 10 mg/kg [8]. Thirty minutes after oral administration of the products at a volume of 10 ml/kg, mice were suspended by their forelimbs from the hanging bar, with their hind limbs tied [9]. When fatigued, the mice could no longer grip the bar and fell. The suspension time of each mouse until exhaustion was recorded.

An increase in suspension time indicates that the extract has an antifatigue effect.

2.2.4. Effect of the extract on fatigue induced by prolonged muscular effort

Study of the effect of the extract on fatigue induced by forced swimming

The forced swimming test was used to subject mice to endurance exercise in order to evaluate the effect of the extract on their physical performance, employing a Morris-type swimming pool [10].

The experimental apparatus consisted of a glass container with a height of 37 cm and a diameter of 15 cm, filled with water to a depth of 21 cm, allowing the mouse to float without touching the bottom.

The water temperature was maintained at ambient temperature throughout the experiment. Before the experiment, the mice were divided into five groups of three animals each. The control group received distilled water orally. The treated groups received the extract orally at doses of 100, 200, and 400 mg/kg. The reference group was treated orally with STIMOL at 10 mg/kg [8]. Thirty minutes after oral administration of the substances at a dose of 10 mL/kg, the mice were placed in the pool and allowed to swim freely until exhaustion [9]. A mouse was considered fatigued when it remained immobile and floated for more than 7 seconds. Swimming time was recorded with a stopwatch.

An increase in swimming time indicates that the extract possesses antifatigue properties.

Study of the effect of the extract on fatigue induced by walking on a rotarod

To assess the effect of the extract on physical endurance and, consequently, the ability to maintain prolonged effort in mice, the forced walking test was performed using a rotarod apparatus [11].

The experimental device consisted of an electric motor attached to a wooden rod approximately 30 cm long, 3 cm in diameter, and 10 cm in circumference, held horizontally. The rod was divided into 5 compartments and rotated at a speed of 20 revolutions per minute.

Before the experiment, mice were divided into 5 groups of 3 animals each: a control group, a reference group, and three treatment groups receiving the extract. Control group animals received distilled water, the treatment groups received 100, 200, and 400 mg/kg of the extract, respectively and the reference group received 10 mg/kg of STIMOL [8].

Thirty minutes after oral administration of the substances at a dose of 10 mL/kg, animals were placed on the rotarod rod [9]. The time each mouse remained on the rotating rod before falling was recorded. The distance traveled by each mouse was calculated using the following formula:

Distance traveled (revolutions/minutes) = speed (revolutions) /times duration of walking (minutes)

An increase in the distance traveled by mice treated with the extract compared to controls indicates that the extract has aerobic-type antifatigue muscular activity.

2.2.5. Expression and Analysis of Results

Results are expressed as mean ± standard error of the mean (SEM). Comparisons between means were performed using Student's t-test. Differences were considered statistically significant when p values were less than 0.05 ($p < 0.05$).

3. Results

3.1. Chemical Analysis

3.1.1. Extraction yield

Twenty point two grams (20.2 g) of extract were obtained after maceration of 500 grams of powdered root of *Landolphia myrtifolia* in an ethanol-water mixture (80:20), corresponding to a yield of 4.04%.

3.1.2. Phytochemical screening results

Phytochemical screening of the extract revealed a high content of flavonoids, tannins, and leucoanthocyanidins, and a low content of heterosides and polyphenols.

3.2. Biological Study

3.2.1. Effect of the extract on fatigue induced by short and intense effort

The hanging time of mice treated with the extract at doses of 100, 200, and 400 mg/kg was 41.11 ± 0.42, 60.88 ± 0.51 and 87.55 ± 0.55 seconds, respectively; in contrast, the negative control group exhibited a hanging time of 30 ± 1.41 seconds (Figure 1). The hanging time increased dose-dependently with the administered extract. The reference group showed a hanging time of 51.33 ± 0.55 seconds.

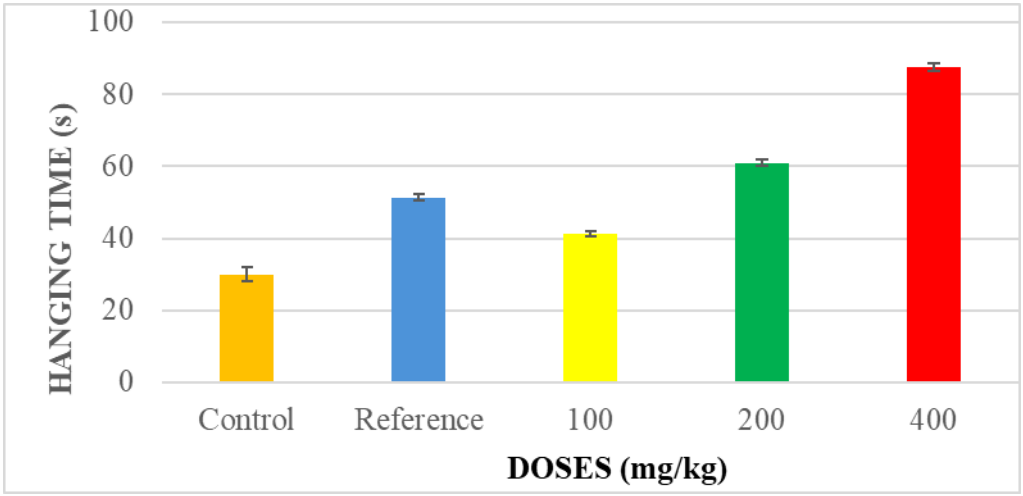

Figure 1 Variation of the hanging time in the hanging bar by mice control group (.), reference group (.) and mice treated with the extract orally at doses 100 (.), 200(.) et 400(.) mg/kg (± ; n= 3; P < 0,05)

3.2.2. Effect of the extract on fatigue induced by prolonged muscular effort

Study of the effect of the extract on fatigue induced by forced swimming

Mice that received distilled water swam for 8.62 ± 0.28 minutes, whereas mice treated with the extract at doses of 100, 200, and 400 mg/kg swam for 21.46 ± 0.40, 28.45 ± 0.84, and 54.62 ± 2.36 minutes, respectively (P < 0.05) (Figure 2). Swimming time increased dose-dependently in treated mice. The reference group treated with STIMOL swam for 14.33 ± 0.37 minutes.

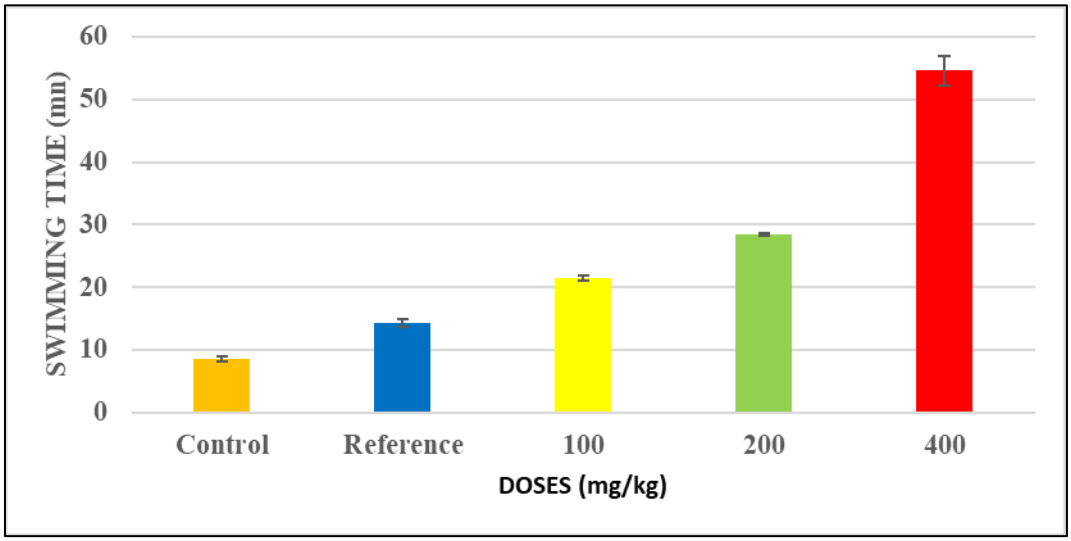

Figure 2 Variation of swimming time in the swimming pool by mice control group (.), reference group (.) and mice treated with the extract orally at doses 100 (.), 200(.) et 400(.) mg/kg (± ; n= 3; P < 0,05)

Study of the effect of the extract on fatigue induced by forced walking

The distance traveled on the rotarod by mice treated with the extract at doses of 100, 200, and 400 mg/kg (7.47 ± 0.19, 9.19 ± 0.27, and 17.47 ± 0.41 cm, respectively) was significantly greater than that of the control group (2.69 ± 0.18 cm) (Figure 3). The distance traveled increased dose-dependently with the administered extract. The reference group covered a distance of 4.47 ± 0.16 cm.

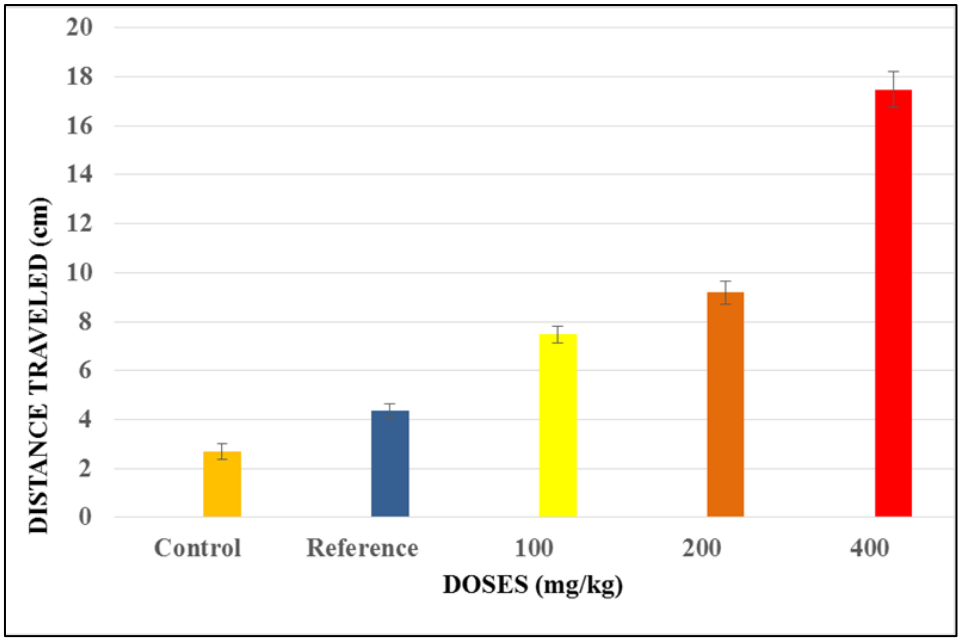

Figure 3 Variation of the distance travelled on the rotarod by mice control group (_), reference group (_) and mice treated with the extract orally at doses 100 (_),200(_) et 400(_) mg/kg (± ; n= 3 ; P < 0,05)

4. Discussion

The present study aimed to evaluate the antifatigue activity of a extract from *Landolphia myrtifolia* in mice subjected to both short- and long-duration physical efforts. The extraction process yielded 20.2 g of extract from 500 g of *Landolphia myrtifolia* root powder using an ethanol-water mixture (80:20), corresponding to a 4.04% yield. This yield is consistent with extraction efficiencies reported for similar medicinal plants [12].

Phytochemical screening revealed that the extract contains high levels of flavonoids, tannins, and leucoanthocyanidins, along with lower amounts of heterosides and polyphenols. The presence of these bioactive compounds is notable given their well-documented antioxidant and muscle-protective properties, which could contribute to the antifatigue effects observed [13].

In the hanging test, mice treated with the extract exhibited a dose-dependent increase in suspension time compared to the negative control group, with times of 41.11 ± 0.42, 60.88 ± 0.51 and 87.55 ± 0.55 seconds at 100, 200, and 400 mg/kg respectively, compared to 30 ±1.41 seconds in controls. The reference group showed an intermediate duration of 51.33 ± 0.55 seconds. This suggests that the extract enhances short-term muscular endurance, possibly by improving neuromuscular coordination or delaying the onset of muscle fatigue [14].

Similarly, in the forced swimming test, treated mice demonstrated significantly longer swimming times than controls, with a clear dose-response relationship. The maximum swimming time observed at 400 mg/kg was 54.62 ± 2.36 minutes, compared to 8.62 ± 0.28 minutes in the control group ($P < 0.05$). The reference drug, STIMOL, produced a swimming time of 14.33 ± 0.37 minutes. These results indicate that *Landolphia myrtifolia* extract can substantially improve endurance capacity, potentially through enhanced energy metabolism or antioxidative mechanisms reducing exercise-induced oxidative stress [4], [5].

The rotarod test further confirmed these findings, with treated mice traveling significantly greater distances at all doses (7.47 ± 0.19, 9.19 ± 0.27 and 17.47 ± 0.41 cm) compared to controls (2.69 ±0.18 cm). The reference group covered 4.47 ± 0.16 cm. The increased distance reflects improved motor coordination and resistance to fatigue during prolonged effort, aligning with previous studies on natural extracts with similar phytochemical profiles [15].

Overall, the dose-dependent antifatigue activity demonstrated by *Landolphia myrtifolia* extract across multiple physical endurance models supports its potential as a natural ergogenic aid. The bioactive flavonoids and tannins are likely key contributors to these effects, possibly through antioxidant activity and enhancement of mitochondrial function [16]. Further studies are warranted to isolate specific active compounds and elucidate the underlying molecular mechanisms.

5. Conclusion

Extract from *Landolphia myrtifolia roots* significantly and dose-dependently improved physical performance in established models. Teated mice exhibited increased hanging time, prolonged swimming endurance, and enhanced rotarod performance compared with controls, with higher doses producing effects comparable to or greater than the reference compound.

Taken together, these findings demonstrate that the defatted ethanolic extract of *Landolphia myrtifolia* effectively enhances resistance to both acute and prolonged physical fatigue. The antifatigue effect is likely mediated by polyphenolic constituents through antioxidant and neuromuscular mechanisms. This study provides experimental support for the traditional use of this plant and highlights its potential as a natural ergogenic agent, warranting further mechanistic and translational investigations.

Compliance with ethical standards

Disclosure of conflict of interest

The authors declared no conflict of interest.

Statement of ethical approval

This study approval from the Scientific and Ethical Committee of the Sciences faculty of the University of Antananarivo, Madagascar. The animals were treated with care throughout the study period and were kept in a well-controlled area according to the National Guidelines for the Care and Use of Laboratory Animals.

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