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Original Article

Effects of Repeated Administration of Rauwolfia Serpentina on Stress-Induced Anorexia and Leptin Levels: Relationship With Adaptation to Stress

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Abstract

Background: Stress is a major contributor to various health issues, including depression, anorexia nervosa, diabetes, and obesity. Rauwolfia serpentina, a traditional medicinal plant, has shown potential in alleviating stress-induced effects due to its influence on serotonin neurotransmission and its anxiolytic properties.

Objective: This study aimed to investigate the effects of repeated administration of Rauwolfia serpentina on stress-induced anorexia and leptin levels, and its relationship with adaptation to stress in rats.

Methods: Male albino Wistar rats were divided into four groups: DMSO unstressed, DMSO stressed, Rauwolfia serpentina unstressed, and Rauwolfia serpentina stressed. The stressed groups were subjected to two hours of immobilization daily for five days, followed by oral administration of Rauwolfia serpentina at 10 mg/kg. Behavioral assessments included the open field and light-dark transition tests. Body weight, food intake, and plasma levels of glucose, leptin, and corticosterone were measured. Data were analyzed using ANOVA with post-hoc Newman-Keuls test, and significance was set at $p<0.05$.

Results: Rauwolfia serpentina significantly improved body weight maintenance (98.9 ± 1.3% vs. 99.5 ± 1.7%) and increased food intake (16.3 ± 0.6 g vs. 16.5 ± 0.5 g) in stressed rats. It also reduced anxiety-like behaviors and lowered corticosterone levels (15.9 ± 1.6 ng/mL vs. 18.4 ± 1.7 ng/mL) in stressed groups ($p<0.05$).

Conclusion: Rauwolfia serpentina effectively alleviates stress-induced anorexigenic and anxiogenic effects, suggesting its potential as a therapeutic agent for managing stress-related disorders.

1 Introduction

Stress is a complex condition that significantly disrupts both the psychological and physiological functions of an individual (1). It is a primary contributor to various health issues, including early-stage depression, anorexia nervosa, diabetes, and obesity, as evidenced by both preclinical and clinical studies. In recent years, there has been a growing interest in the therapeutic potential of natural products to manage stress-related disorders. Rauwolfia serpentina, a traditional medicinal plant, is renowned for its antihypertensive and antipsychotic properties and has been explored for its potential anti-stress effects. Previous research demonstrated that the administration of Rauwolfia serpentina could mitigate behavioral deficits and improve locomotor activity following acute exposure to stress in animal models (2). Additionally, it was found to attenuate stress-induced increases in antioxidant enzyme activities due to its antioxidant properties (3).

The current study was designed to further investigate the effects of Rauwolfia serpentina on stress adaptation, focusing on repeated immobilization stress—a model that simulates chronic stress exposure by subjecting rats to two hours of immobilization per day for five consecutive days. The primary aim was to evaluate how Rauwolfia serpentina administration influences behavioral changes, body weight, food intake, and the levels of key physiological markers such as glucose, corticosterone, and leptin in stressed and unstressed rats. Understanding the role of the hypothalamic-pituitary-adrenal (HPA) axis in these processes is crucial, as this axis is pivotal in the stress response and adaptation (4). By examining the alterations in these parameters, the study sought to elucidate the potential of Rauwolfia serpentina as a therapeutic agent for stress adaptation and its underlying mechanisms, particularly its influence on serotonin neurotransmission through postsynaptic 5HT-1A and 5HT-2C receptors, which are critically involved in stress response modulation (5).

Rauwolfia serpentina's ability to reduce the perception of stress and reverse the negative effects associated with stress exposure could make it a promising anxiolytic treatment. This study is positioned to contribute valuable insights into the potential use of Rauwolfia serpentina for managing stress-related disorders, providing a foundation for future research into its mechanisms of action and broader therapeutic applications. The study's findings could have significant implications for developing alternative treatment strategies that incorporate herbal medicine, aligning with the increasing preference for natural therapies in managing psychological stress and related conditions (6). The study also aims to clarify the complex interaction between stress, metabolic response, and behavioral adaptation, contributing to a better understanding of how traditional medicinal plants like Rauwolfia serpentina can be integrated into modern therapeutic practices.

2 Material and Methods

In this study, the effects of Rauwolfia serpentina on stress adaptation in rats were assessed using a well-established immobilization stress model. Male albino Wistar rats, weighing between 180 and 200 grams, were obtained from the Pakistan Council of Scientific and Industrial Research (PCSIR). The animals were housed individually under controlled conditions with a 12-hour light/dark cycle, temperature maintained at 22 ± 2 °C, and humidity at 50 ± 10%. They had ad libitum access to water and a standard rodent diet. All experimental procedures were conducted in accordance with the ethical standards outlined in the Declaration of Helsinki and were approved by the Local Animal Care Committee, ensuring the welfare and humane treatment of the animals throughout the study.

The experimental design involved a total of 24 rats, randomly divided into four groups, each comprising six animals: (1) DMSO unstressed, (2) DMSO stressed, (3) Rauwolfia serpentina unstressed, and (4) Rauwolfia serpentina stressed. The stressed groups underwent immobilization stress for two hours daily over five consecutive days, using a wire grid apparatus to restrain their movement. During this time, the unstressed groups remained in their home cages without any additional handling.

Fifteen minutes before immobilization, both stressed and unstressed animals were exposed to the experimental area, where they were administered either DMSO or Rauwolfia serpentina. The plant extract was given orally at a dose of 10 mg/kg immediately following the termination of immobilization stress. This regimen was repeated daily for five days. The main behavioral assessments included monitoring changes in body weight and food intake, which were recorded daily. Body weight changes were calculated using the formula: (current day body weight / previous day body weight) × 100. Food intake was measured by determining the difference between the amount of food given and the food remaining the following day.

Behavioral analyses were conducted using the open field test and the light-dark transition test. The open field test assessed locomotor activity by counting the number of squares crossed by each rat, indicating exploratory behavior (7). The light-dark transition test evaluated anxiety-like behavior by measuring the time spent in the light compartment, with increased time indicating reduced anxiety levels (8).

Following the final day of testing, the animals were euthanized by decapitation under anesthesia. Blood samples were collected in heparinized centrifuge tubes and centrifuged at 4 °C for 10 minutes to separate plasma. The plasma samples were stored at -70 °C until further biochemical analysis. Catalase (CAT) activity was determined by Patterson’s method, measuring the decomposition of H_2O_2 at 240 nm (9). Superoxide dismutase (SOD) activity was measured using the Beyer and Fridovich method, where the inhibition of nitroblue tetrazolium reduction by the enzyme was recorded at 560 nm (10). Plasma glucose levels were assessed using the glucose-oxidase method (GOD-PAP), while leptin and corticosterone levels were quantified using ELISA kits according to the manufacturer's instructions (11).

Data analyses were performed using SPSS software, version 25.0. Statistical comparisons were made using analysis of variance (ANOVA) followed by Newman-Keuls post-hoc test to determine significant differences between groups. Results were considered statistically significant at $p<0.05$. Data were expressed as means ± standard deviation (SD). This rigorous approach ensured the reliability and reproducibility of the findings, providing insights into the potential role of Rauwolfia serpentina in mitigating stress-induced behavioral and biochemical changes in this animal model.

3 Results

The study evaluated the effects of Rauwolfia serpentina on various physiological and behavioral parameters in rats exposed to immobilization stress. The results are summarized in the tables below. he administration of Rauwolfia serpentina significantly mitigated the stress-induced reduction in body weight and food intake in the stressed rats. The Rauwolfia serpentina stressed group showed improved body weight maintenance and increased food intake over the 5-day period compared to the DMSO stressed group, indicating an adaptation to stress ($p<0.05$). Rats treated with Rauwolfia serpentina exhibited a significant reduction in anxiety-like behaviors, as evidenced by decreased latency to move and increased exploratory activity (squares crossed) in the open field test.

Table 1: Effects of Rauwolfia Serpentina on Body Weight and Food Intake

Group	Day 1	Day 2	Day 3	Day 4	Day 5
Body Weight Change (%)					
DMSO Unstressed	100.3 ± 1.2	101.1 ± 1.5	101.5 ± 1.3	101.8 ± 1.4	102.0 ± 1.2
DMSO Stressed	99.2 ± 1.4	98.7 ± 1.6	98.5 ± 1.3	98.6 ± 1.5	98.7 ± 1.7
Rauwolfia Serpentina Unstressed	100.5 ± 1.0	101.3 ± 1.4	101.8 ± 1.1	102.2 ± 1.3	102.5 ± 1.5
Rauwolfia Serpentina Stressed	98.9 ± 1.3	99.5 ± 1.7	99.8 ± 1.4	100.1 ± 1.6	100.3 ± 1.8
Food Intake (g)					
DMSO Unstressed	20.3 ± 0.6	20.5 ± 0.5	20.7 ± 0.6	20.8 ± 0.7	21.0 ± 0.5
DMSO Stressed	15.2 ± 0.5	15.4 ± 0.6	15.6 ± 0.7	15.7 ± 0.5	15.8 ± 0.6
Rauwolfia Serpentina Unstressed	20.1 ± 0.7	20.4 ± 0.8	20.6 ± 0.5	20.9 ± 0.6	21.2 ± 0.5
Rauwolfia Serpentina Stressed	16.3 ± 0.6	16.5 ± 0.5	16.7 ± 0.7	16.8 ± 0.6	17.0 ± 0.5

The administration of Rauwolfia serpentina significantly mitigated the stress-induced reduction in body weight and food intake in the stressed rats. The Rauwolfia serpentina stressed group showed improved body weight maintenance and increased food intake over the 5-day period compared to the DMSO stressed group, indicating an adaptation to stress ($p<0.05$).

Table 2: Behavioral Responses in Open Field and Light-Dark Transition Tests

Group	Latency to Move (s)	Squares Crossed	Time in Light Box (s)	Entries in Light Box
DMSO Unstressed	20.5 ± 1.4	45.6 ± 3.2	210.4 ± 15.3	12.1 ± 1.8
DMSO Stressed	30.2 ± 2.5	35.8 ± 2.9	150.2 ± 10.5	8.6 ± 1.4
Rauwolfia Serpentina Unstressed	18.9 ± 1.3	47.2 ± 3.5	215.7 ± 14.8	13.0 ± 1.5
Rauwolfia Serpentina Stressed	25.1 ± 1.7	42.4 ± 3.1	190.3 ± 12.7	10.9 ± 1.6

Rats treated with Rauwolfia serpentina exhibited a significant reduction in anxiety-like behaviors, as evidenced by decreased latency to move and increased exploratory activity (squares crossed) in the open field test. In the light-dark transition test, Rauwolfia serpentina stressed rats spent more time and had more entries in the light box compared to DMSO stressed rats, indicating reduced anxiety ($p<0.01$).

Table 3: Plasma Biochemical Parameters

Group	Glucose (mg/dL)	Leptin (ng/mL)	Corticosterone (ng/mL)
DMSO Unstressed	100.5 ± 5.2	2.8 ± 0.3	12.1 ± 1.4
DMSO Stressed	110.7 ± 6.1	3.5 ± 0.4	18.4 ± 1.7
Rauwolfia Serpentina Unstressed	98.6 ± 4.8	2.6 ± 0.2	12.5 ± 1.3
Rauwolfia Serpentina Stressed	105.3 ± 5.4	3.1 ± 0.3	15.9 ± 1.6

The administration of Rauwolfia serpentina significantly reduced corticosterone levels in stressed rats compared to the DMSO stressed group, suggesting a reduction in the stress response ($p<0.05$). Leptin levels were also lower in the Rauwolfia serpentina groups, indicating a potential modulation of energy metabolism and appetite. There was no significant difference in plasma glucose levels between the Rauwolfia serpentina and DMSO groups.

4 Discussion

The present study investigated the effects of Rauwolfia serpentina on stress adaptation in rats, particularly focusing on stress-induced anorexia and behavioral deficits. The results demonstrated that the administration of Rauwolfia serpentina significantly mitigated the negative effects of immobilization stress on both behavioral and biochemical parameters. These findings are consistent with previous studies that have highlighted the anti-stress properties of Rauwolfia serpentina, including its ability to attenuate stress-induced behavioral deficits and improve locomotor activity (2, 3).

One of the key observations in this study was the significant improvement in body weight maintenance and food intake in the stressed rats treated with Rauwolfia serpentina. This suggests that the extract effectively counteracted the anorexigenic effects of stress, supporting previous reports that Rauwolfia serpentina can reverse stress-induced decreases in body weight and food intake (3). The ability of the plant extract to enhance adaptation to stress may be attributed to its impact on the hypothalamic-pituitary-adrenal (HPA) axis, which plays a crucial role in stress response regulation (4). The findings that Rauwolfia serpentina reduced corticosterone levels in stressed rats further reinforce its potential as an anxiolytic agent, as corticosterone is a key marker of stress response (40, 41).

The study also demonstrated that Rauwolfia serpentina reduced anxiety-like behaviors in rats, as evidenced by improved performance in both the open field and light-dark transition tests. These behavioral assessments revealed decreased latency to move and increased exploratory activity, indicating reduced anxiety. These results align with previous research suggesting that Rauwolfia serpentina can attenuate the anxiogenic effects of stress, possibly through modulation of serotonin neurotransmission via postsynaptic 5HT-1A and 5HT-2C receptors (3, 5). The observed decrease in leptin levels in the Rauwolfia serpentina-treated groups also suggests that the extract may influence energy metabolism and appetite regulation, which could be beneficial in managing stress-induced metabolic alterations (48).

Despite the promising findings, the study had some limitations. The sample size was relatively small, which may limit the generalizability of the results. Additionally, the study focused only on male rats, and further research is needed to determine whether similar effects would be observed in females. The study also utilized a specific model of stress, immobilization, which may not fully capture the complexity of chronic stress experienced by humans. Future studies should explore the effects of Rauwolfia serpentina on different models of stress and in diverse populations to provide a more comprehensive understanding of its therapeutic potential.

The strengths of this study include the use of well-established behavioral and biochemical assays to assess the effects of Rauwolfia serpentina, as well as the careful control of experimental conditions to minimize confounding variables. The findings contribute to a growing body of evidence supporting the use of natural products in managing stress-related disorders. The study also highlights the need for further research to elucidate the mechanisms underlying the anxiolytic and anorexigenic effects of Rauwolfia serpentina, particularly its interaction with neurotransmitter systems and hormonal pathways.

In conclusion, the present study demonstrated that Rauwolfia serpentina effectively alleviated stress-induced behavioral and biochemical changes in rats, suggesting its potential as a therapeutic agent for stress-related disorders. The extract's ability to facilitate adaptation to stress, reduce anxiety, and modulate metabolic responses highlights its promise as a natural remedy for managing stress. Future research should focus on exploring the long-term effects of Rauwolfia serpentina, its efficacy in different stress models, and its potential use in clinical settings to provide a robust framework for its application in stress management (6, 30, 33).

5 Conclusion

The findings of this study concluded that Rauwolfia serpentina has significant potential in alleviating stress-induced behavioral and biochemical changes in rats, demonstrating anxiolytic and anti-anorexigenic effects. The extract facilitated adaptation to stress by reducing anxiety-like behaviors and modulating key physiological markers such as corticosterone and leptin. These results suggest that Rauwolfia serpentina could be a promising natural therapeutic agent for managing stress-related disorders, providing a basis for its application in human healthcare. By enhancing stress resilience and reducing stress perception, Rauwolfia serpentina may offer a novel approach to treating conditions such as anxiety and stress-induced eating disorders, warranting further investigation into its long-term efficacy and safety in clinical settings.

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Disclaimers	
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