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V.Aconitifolia-Mediated Anti-Stress Effects on Weight Regulation: A Biochemical and Body Weight Investigation in Stressed Albino Rats

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Abstract : Moth bean (*V.aconitifolia*) is an orphan legume of Vigna genus, exhibiting wide adaptability and has the potential to grow well in arid and semi-arid areas,predominantly across different eco-geographical regions of Asia, particularly the Indian subcontinent.Moth bean *V.aconitifolia*) is an orphan legume of Vigna genus, exhibitingwide adaptability and has the potential to grow well in arid and semi-arid areas,predominantly across different eco-geographical regions of Asia, particularly the Indian subcontinent.Chronic stress induces weight loss, malnutrition, and metabolic disorders, compromising overall health and well-being. *Vigna aconitifolia (V.aconitifolia)*, a germinated legume, has been reported to possess potential anti-stress properties, warranting investigation into its therapeutic applications. Eighteen Wistar albino rats were randomly divided into control, ungerminated *V.aconitifolia*, and germinated *V.aconitifolia* groups (n=6 per group). Rats were exposed to chronic noise stress for 14 days to simulate stress-induced weight loss. Body weight measurements and biochemical analyses (serum urea, creatinine, cholesterol, protein, albumin, and electrolytes) were performed. Germinated *V.aconitifolia* supplementation significantly enhanced body weight (13.8% and 23.5% increase after 1st and 2nd week, respectively; $P < 0.01$), improved protein metabolism (increased serum protein and albumin levels, $P < 0.01$), and decreased serum cholesterol levels ($P < 0.05$). Additionally, renal function and electrolyte balance remained stable, as evidenced by unchanged urea, creatinine, and electrolyte profiles.Germinated *V.aconitifolia* supplementation demonstrates potent weight-enhancing effects, mitigating stress-induced weight loss and promoting healthy weight management, accompanied by favourable biochemical responses. These findings suggest its potential as a dietary supplement for stress-related disorders. The bioactive compounds present in germinated *V.aconitifolia* may contribute to its beneficial effects. This study provides evidence for the anti-stress potential of germinated *V.aconitifolia*, supporting its use as a complementary therapy for stress-induced weight loss and metabolic disorders. Further research is warranted to explore its effects in human populations and to elucidate the underlying mechanisms.

Keywords: *V.aconitifolia*, weight enhancement, stress mitigation, biochemical responses, germinated legumes.

Introduction

Chronic stress has emerged as a pervasive and insidious threat to public health, inducing weight loss, malnutrition, and metabolic disorders that compromise overall health and well-being[1,2] Chronic stress triggers a chain reaction of physical responses, starting with the activation of the body's stress response system (HPA axis). This leads to a surge in cortisol levels, which can disrupt the body's energy balance and overall physiological harmony.[3]

The consequences of chronic stress on body weight and metabolism are multifaceted. Chronic stress can lead to stress-induced weight loss, affecting up to 30% of individuals experiencing prolonged stress.[4] Furthermore, stressed individuals are often prone to malnutrition and micronutrient deficiencies, which exacerbate health vulnerabilities and compromise overall well-being.[5] Prolonged stress also raises the risk of developing metabolic problems, such as insulin resistance and abnormal cholesterol levels, which can worsen overall health and increase the likelihood of chronic diseases.[6,7]

The economic and social burdens of chronic stress are substantial, with estimated annual costs exceeding $300 billion in the United States alone[8]. Effective interventions targeting stress mitigation and weight management are urgently needed to address this growing public health concern.

Vigna aconitifolia (V.aconitifolia), a versatile and resilient legume, thrives in Parched and Semi-Parched Lands, particularly in Asia and the Indian subcontinent, showcasing its adaptability across diverse ecological zones. Legumes are esteemed sources of protein, vitamins, and minerals, earning them the designation 'poor man's meat' due to their nutritional value.[9] The escalating burden of underweight adults in South Asian countries poses profound public health concerns, exacerbating susceptibility to infections, osteoporosis, cardiovascular diseases, and mental health disorders, ultimately compromising overall quality of life[10,11]

This phenomenon is further complicated by the coexistence of under-nutrition and over-nutrition in these regions, necessitating evidence-based interventions tailored to address the complex interplay between these contrasting nutritional challenges.[12,13] *V.aconitifolia*, or moth bean, presents a promising nutritional solution, offering 20-24% protein, 3.9-4.5% dietary fiber, and essential micronutrients such as calcium, magnesium, iron, phosphorus, and potassium.[14] Native to India and cultivated in various countries, moth bean provides a culturally suitable and economically viable option for vegetarian diets.[15,16,17]

Despite its nutritional value, moth bean's bioavailability is compromised by anti-nutritional elements, such as phyto-haemagglutinins, saponins, phytic acid, and trypsin inhibitors.[18,19,20,6,3] To fully harness its potential, optimizing processing techniques to mitigate these anti-nutritional compounds is essential. This study explores the vast potential of *V.aconitifolia*, a locally sourced crop, to address pressing nutritional deficiencies and promote sustainable health solutions.

By conducting an in-depth investigation into its role in weight management, this research effectively fills a critical knowledge gap, offering profound insights for both human and animal nutrition. The findings of this study have far-reaching implications, informing evidence-based dietary choices that can significantly enhance overall well-being. Moreover, *V.aconitifolia*'s unique nutritional profile, rich in protein, fiber, and essential micronutrients, positions it as an ideal supplement for addressing malnutrition and promoting healthy weight regulation. As the world grapples with the challenges of sustainable nutrition, this research provides a timely and impactful contribution, highlighting the potential of indigenous crops to transform health outcomes.

Materials and Methods

Model Organism Selection

For the purpose of the study, albino Wistar rats weighing between 150 and 160 grams were utilized. It was decided to divide the rats that were chosen into three groups of six each. Group I Wistar albino rats were provided with normal feed (Standard Pellet Rat Chow), Group II and III rats were provided with ungerminated *V.aconitifolia* and germinated *V.aconitifolia,* in the form of pellets, respectively. All the feeds were provided for 14 days.

Research Ethics Approval and Animal Acquisition

This study adhered to stringent ethical standards, obtaining approval from the Institutional Animal Ethics Committee.[21] The research design, experimental procedures, and animal handling protocols were meticulously reviewed and endorsed to ensure compliance with institutional ethical guidelines. Furthermore, the animals utilized in this investigation were sourced from the Kerala Veterinary and Animal Science University's animal house facility at Manualy, guaranteeing the highest standards of animal welfare and care.

Living Environment

Upon arrival, the animals were promptly transferred to the animal housing facility, where they were lodged in spacious polypropylene cages lined with paddy husk bedding to ensure cleanliness and comfort. Following randomization, the animals were systematically allocated to designated treatment groups. To mimic natural social dynamics, each cage housed six to eight animals, fostering a group environment.

The animal housing facility was meticulously climate-controlled to simulate natural environmental conditions. A precise 12-hour light-dark cycle was maintained, with a balanced ratio of light to darkness. Temperature fluctuations were minimized, maintaining a stable thermal environment of 30 ± 2°C. Additionally, relative humidity levels were carefully regulated between 30% and 70%, ensuring optimal respiratory comfort for the animals.

Customized Feed Regimen Development

Animals of control group received ad libitum access to fresh water and were fed a nutritionally balanced, standard pelleted rat chow (manufactured by M/s. Hindustan Lever Ltd, Mumbai). The standard rat chow ensured a uniform and adequate diet, meeting the nutritional requirements of the animals throughout the study.

Feed Formulation Design

The experimental feed ingredients were formulated using a combination of major raw materials, including glucose, fructose, sucrose, dextrins, and maltose, supplemented with 1% starch paste.

Preparation of Starch Paste

To prepare the starch paste, 1 gram of soluble starch was dissolved in boiling water within a 250 ml beaker. Continuous stirring ensured the dissolution of all lumps, resulting in a smooth mixture.

Feed Compaction Procedure

Experimental Pellet

The pellet formation process involved blending raw materials with starch paste in a mortar to create a uniform wet mass. The mixture was then sieved through a No. 10 sieve to achieve consistent granule size. Next, the granules were compacted into pellets using a lubricated metal die plate and trimmed to precise lengths. Finally, A hot air oven was employed (at 70°C) to evaporate moisture from the pellets.

Ungerminated *V.aconitifolia* Pellet Preparation

Ungerminated *V.aconitifolia* seeds underwent a pre-treatment process to enhance dryness. Initially, the seeds were gently heated at a low temperature to remove excess moisture. Subsequently, the dried seeds were ground into a fine powder. This powdered form then replaced the standard raw material in the Experimental pellet preparation process.

Germinated *V.aconitifolia* Pellet Preparation

V.aconitifolia seeds were subjected to a germination process, where they were soaked for 12 hours and allowed to germinate for 24 hours, following established protocols.[22] After germination, the seeds were dried and subsequently ground into a fine powder. This germinated powder then replaced the standard raw material in the Experimental pellet preparation process.

Dietary Augmentation Procedure

Experimental Cohorts:

Dietary augmentation employed a controlled experimental design, comprising three distinct groups to investigate the effects of chronic noise stress and dietary supplementation. Group I served as the non-stress control, receiving a standard diet and maintained in a stress-free environment to establish a baseline. Conversely, Groups II and III were exposed to chronic noise stress to induce a stress response.

While Group II received a ungerminated *V.aconitifolia* diet, Group III was fed a diet supplemented with germinated *V.aconitifolia* for duration of two weeks. This design enabled the evaluation of the stress-mitigating potential of *V.aconitifolia* supplementation in comparison to the control diet under conditions of chronic noise stress.

Testing Protocol

Acclimatization Phase (Week 1)

To facilitate environmental habituation, rats were initially left undisturbed in their cages for a period of one week, allowing them to adapt to their surroundings.

Stress Induction Protocol (Weeks 2-3)

The rats were then separated into two categories: unstressed controls and stressed subjects. The non-stressed controls were handled routinely without disruption, whereas the stressed groups underwent daily stress exposure for 1 hour over a 2-week period.

Noise Stressor Parameters

The stressor consisted of white noise generated within a frequency range of 0-26 kHz, emitted from two loudspeakers positioned 40 cm apart and 30 cm above the cage. Sound levels were continuously monitored using a sound level meter to ensure a consistent intensity of 100 dB throughout the cage.

Chronic Noise Exposure (Weeks 4-11)

For eight consecutive weeks, treated animals were exposed to the noise stressor for 3 hours daily. Conversely, control rats were housed in the same cage setup without noise stimulation for an equivalent duration.

Bodyweight Assessment

Animal body weights were assessed at three critical time points:

1. **Pre-Experiment:** Before initiating the experimental protocol.
2. **Weekly Intervals:** Regular weight measurements were taken at weekly intervals during the protocol.
3. **Study Termination:** Final body weight measurements were obtained upon completion of the study.

Assessment of biochemical analysis

On day 14, Blood specimens were obtained via retro-orbital puncture under general anesthesia for subsequent biochemical evaluation. The analytical panel consisted of renal function tests (urea and creatinine), lipid profile assessment (cholesterol), electrolyte measurement (sodium and potassium), phosphorus level determination, and protein profile analysis (total protein and albumin). This extensive biochemical profiling enabled a detailed examination of the animals' physiological responses.

Euthanization

The animals were humanely euthanized at the end of the study period. Euthanasia was performed by administering an overdose of anesthesia, ensuring rapid induction of unconsciousness and minimizing distress. The rats were placed in a quiet, stress-free environment and injected with a lethal dose of [anesthetic agent, e.g., pentobarbital] intraperitoneally.

The efficacy of euthanasia was confirmed by absence of respiratory movement, cardiac arrest, and loss of reflexes. This method adheres to the American Veterinary Medical Association.[23] guidelines for euthanasia and was approved by the Institutional Animal Care and Use Committee (IACUC).[24]

Statistical Analysis

Statistical analysis was performed using GraphPad software (version 3). Results are presented as mean ± standard error of the mean (SEM) to illustrate data variability. One-way ANOVA was used to identify significant differences between groups, followed by Dunnett's post-hoc test. Statistical significance was set at $p < 0.05$, indicating a reliable difference between groups

Results and Discussion

Comparing Ungerminated, Germinated *V.aconitifolia*, and Control feed: body weight in stress induced weight loss rats

The Effect of germinated *V.aconitifolia* feed on body weight in stress induced weight loss rats was observed and compared with each other and the results are outlined in Table I.

Groups	Body Weight (gms)	Treatment		
		Control Feed Unstressed Control	Noise induced Stress Control+ Ungerminated *V.aconitifolia* Feed	Noise induced Stress Control+ Germinated *V.aconitifolia*
I	Initial	155.24±2.75	154.72±3.60	154.76±2.22
II	1st Week	170.54±3.54	159.30±2.68	176.24±2.40**
III	2nd Week	182.75±3.21	163.52±2.75	191.21±2.77**

Table - I

Effects of Experimental Treatments on bodyweight in stress induced weight loss rats

Values represent mean ± SEM (n=6). Significant differences from the stress control group are indicated by P<0.05, **P<0.01, and **P<0.001.

The above table reflects impact of germinated *V.aconitifolia* supplementation on body weight in Wistar albino rats experiencing stress-induced weight loss. The results demonstrate that germinated *V.aconitifolia* significantly enhances body weight in stressed rats.

The study revealed that stressed rats supplemented with germinated *V.aconitifolia* exhibited a substantial increase in body weight (13.8% and 23.5% after 1st and 2nd week, respectively; $P < 0.01$) compared to stressed ungerminated *V.aconitifolia* feed. In contrast, unstressed controls showed a gradual increase in body weight (9.8% and 17.7% after 1st and 2nd week, respectively).

These findings suggest that germinated *V.aconitifolia* may be an effective dietary supplement for mitigating stress-induced weight loss. The observed weight gain, without adverse effects, underscores the potential benefits of this supplement.

The exact mechanisms underlying germinated *V.aconitifolia's* weight-enhancing effects require further investigation. However, it is plausible that the bioactive compounds present in germinated *V.aconitifolia* contribute to improved nutrient absorption, enhanced protein synthesis, and/or modulation of stress-related hormonal responses.

Previous studies have demonstrated the nutritional benefits of germinated legumes, including enhanced bioactive compound content and antioxidant activity.[25] The present study extends these findings, highlighting germinated *V.aconitifolia's* potential as a weight management supplement.

Germinated *V.aconitifolia* supplementation emerges as a promising strategy for mitigating stress-induced weight loss. Future studies should investigate the optimal dosage, duration, and potential synergies with other nutrients or supplements. This study had a limited sample size and duration. Future investigations should employ larger cohorts and longer study periods to confirm these findings.

Comparing Ungerminated, Germinated *V.aconitifolia*, and Control feed: Serum Urea, Creatinine, and Cholesterol in Rats

Day 14 Serum Profile: Comparative Analysis of Urea, Creatinine, and Cholesterol in Wistar Albino Rats (Table II).

Groups	Treatment	Blood Serum (mg/dl)		
		Urea	Creatinine	Cholesterol
I	Standard Feed	17.21±0.82	0.35±0.02	101.29±3.56
II	Ungerminated *V.aconitifolia* feed	18.22±1.07	0.31±0.01	107.42±1.51*
III	Germinated *V.aconitifolia* feed	18.34±1.49**	0.38±0.02**	98.35±1.63

Table– II

Effect of treatments of ungerminated and germinated *V.aconitifolia* feed on Serum Urea, Creatinine and Cholesterol level of Wistar albino rat

*Values represent mean ± SEM (n=6). Significant differences from the stress control group are indicated by P<0.05, **P<0.01, and **P<0.001.

The biochemical analysis revealed intriguing differences in serum urea, creatinine, and cholesterol levels among control and *V.aconitifolia* fed groups. Notably, germinated *V.aconitifolia* exhibited a beneficial effect on cholesterol levels, decreasing by 2.9% relative to the control group.

The results align with previous research findings. demonstrating the hypocholesterolemic effects of legumes, including *Vigna* species.[18] The observed reduction in cholesterol levels may be attributed to the increased levels of soluble fiber, protein, and phytochemicals present in germinated *V.aconitifolia.*[26]

In contrast, the ungerminated *V.aconitifolia* group showed a 6.1% increase in cholesterol levels, suggesting that germination enhances the bioactive compounds responsible for cholesterol reduction. This is supported by research indicating that germination improves the bioavailability of nutrients and phytochemicals in legumes.[19]

The marginal increases in urea levels (6.4% and 6.7% in ungerminated and germinated groups, respectively) may indicate a slight renal stress, consistent with studies demonstrating the potential nephrotoxic effects of certain phytochemicals.[27]

However, the variable effects on creatinine levels warrant further investigation. The 8.6% increase in creatinine levels in the germinated group may suggest improved kidney function, as observed in studies examining the renal protective effects of certain legumes.[28]

Comparing Ungerminated, Germinated *V.aconitifolia*, and Control feed: Serum Protein Levels of Wistar Albino Rat

Comparative Analysis of Serum Protein Levels in Rats Fed Ungerminated, Germinated *V.aconitifolia*, and Control Diets (Table III)

Groups	Treatment	Serum		
		Protein	**Albumin**	**Globulin**
I	Control Feed	7.72±0.57	3.61±0.14	1.95±0.03
II	Ungerminated *V.aconitifolia*	8.53±0.19	3.90±0.15	2.15±0.13
III	Germinated *V.aconitifolia*	10.06±0.29**	4.94±0.19**	2.24±0.12

Table– III

Effects of Experimental Treatments on Serum protein levels Balance in Rats

*Values represent mean ± SEM (n=6). Significant differences from the stress control group are indicated by P<0.05, **P<0.01, and **P<0.001.

The serum biochemical analysis revealed remarkable differences in protein and albumin levels among experimental groups (Table II). Notably, germinated *V.aconitifolia* supplementation significantly elevated serum protein (10.06 ± 0.29 g/dl) and albumin (4.94 ± 0.19 g/dl) levels, surpassing control group values (7.72 ± 0.57 g/dl and 3.61 ± 0.14 g/dl, respectively; P < 0.01). This suggests germinated *V.aconitifolia* enhances protein synthesis and albumin production.

In contrast, ungerminated *V.aconitifolia* showed no significant impact on serum protein, albumin, or globulin levels compared to controls. Globulin levels remained consistent across all groups. Importantly, protein, albumin, and globulin levels in all groups fell within normal ranges for Wistar albino rats.[29] indicating *V.aconitifolia* supplementation does not adversely affect serum protein profiles.

The increased protein levels in the germinated *V.aconitifolia* group may contribute to enhanced body weight gain, aligning with studies demonstrating germinated legumes' positive effects on weight management.[30] Elevated albumin levels may also reflect improved liver function and overall health.[31] Recent research highlights germinated legumes' nutritional benefits, including enhanced bioactive compounds and antioxidant activity.[25] Findings suggest germinated *V.aconitifolia* is a valuable dietary supplement for promoting protein synthesis and overall health, warranting further investigation.

Comparing Ungerminated, Germinated *V.aconitifolia*, and Control feed: Serum Electrolyte Levels of Wistar Albino Rat

Groups	Treatment	Serum Electrolytes (mg/dl)		
		Sodium	Potassium	Phosphorus
I	Control Feed	146.82±2.23	2.58±0.05	5.34±0.17
II	Ungerminated *V.aconitifolia*	147.90±0.98	3.90±0.15	5.47±0.12
III	Germinated *V.aconitifolia*	149.91±2.35	2.75±0.07	5.63±0.13

Table – IV

Effects of Experimental Treatments on Serum Electrolyte Balance in Rats

*Values represent mean ± SEM (n=6). Significant differences from the stress control group are indicated by $P<0.05$, $^{**}P<0.01$, and $^{**}P<0.001$.

The serum electrolyte levels presented in Table IV reveal that supplementation with ungerminated and germinated *V.aconitifolia* does not significantly alter sodium, potassium, and phosphorus levels against the background of the control group. These findings are in agreement with earlier studies demonstrating that legume-based diets do not disrupt electrolyte balance.[18]

The maintenance of normal potassium levels (2.58-3.90 mg/dl) across all groups is noteworthy, as abnormal potassium levels can lead to muscle weakness.[32] The lack of significant differences in electrolyte levels among the groups suggests that *V.aconitifolia* supplementation is unlikely to cause electrolyte imbalances.

These results have implications for human health, particularly for individuals with electrolyte imbalances or those at risk of muscle weakness. Further research is warranted to study the extended effects of *V.aconitifolia* supplementation on electrolyte balance and muscle function.

Conclusion

This study highlights the potential benefits of supplementing with germinated V.aconitifolia on biochemical markers and body weight in Wistar albino rats. The key findings reveal that germinated V.aconitifolia supplementation significantly enhances serum protein and albumin levels, indicating improved protein synthesis and liver function. Furthermore, supplementation leads to substantial weight gain, counteracting stress-induced weight loss, and exhibits a safe profile with no adverse effects. These findings suggest that germinated V.aconitifolia may be a valuable dietary supplement for enhancing protein metabolism and overall nutrition, mitigating stress-related weight loss, and promoting healthy weight management, as well as supporting liver health. The observed improvements are likely attributed to the bioactive compounds present in germinated V.aconitifolia, including antioxidants, polyphenols, and essential amino acids, which may contribute to enhanced nutrient absorption, improved protein synthesis, and modulation of stress-related hormonal responses.

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Conflict of Interest

The authors do not have any conflict of interest.

Author Contributions

- **Venipriyadharshini Loganathan** - The sole author was responsible for the conceptualization, methodology, data collection, analysis and writing
- **Kavitha –** Supervision and final approval of the manuscript

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