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# Moth Bean (Vigna aconitifolia) as Potential Supplement to Evaluate the Weight Gain in Wistar Albino Rats (*Rattus norvegicus*)

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Abstract: Vigna aconitifolia is an essential crop in Indian agriculture, predominantly cultivated in India. It is acknowledged for its significant nutritional value and its affordability, making it a valuable dietary choice for individuals facing economic challenges. This study investigates the potential of ungerminated and germinated Vigna aconitifolia seeds as a dietary supplement to evaluate their effects on weight growth and general health in Wistar Albino Rats. The Wistar Albino Rats were categorized into three groups viz., Group I (Control), Group II (Fed with ungerminated Vigna aconitifolia) and Group III (Fed with germinated Vigna aconitifolia). The rats were given unrestricted access to water and food for a duration of 28 days. Water and food consumption and changes in body weight were observed on days 1, 7, 14, 21 and 28. On day 29, animals were killed and their kidneys and intestines histopathologically examined. Although the ungerminated and germinated groups consumed similar amounts of water, both consumed more than the control group. On the 28<sup>th</sup> day, ungerminated Vigna aconitifolia had significantly lower food intake compared to germinated and regular feed groups (P<0.05). Rats given both ungerminated and germinated seeds gained more weight. All groups had normal kidney histology, with no tubular epithelial cell degeneration or congestion. Rats administered ungerminated and germinated Vigna aconitifolia had villi, mucus-secreting epithelium and glands in their ileums but no hyperplasia or edema. The study found that Wistar Albino Rats gain weight when supplemented with germinated Vigna aconitifolia. This shows it could be a growth-promoting nutritional supplement. Further research is needed to determine the fundamental processes behind the claimed effects.

## Introduction

Adults who are underweight may have underlying health issues or poor nutrition. Adulthood's under-weight condition can have serious negative health repercussions (Ghimire et al., 2017; Hore and Tanti, 2023). Global epidemiological change documented the has simultaneous development of under-nutrition and overnutrition across numerous nations (Bhadra et al., 2018; Panicker, 2021). South Asian nations are dealing with this public health risk in an extraordinary way. According to a recent systematic analysis, low body mass index (BMI) frequencies were a little greater than that of

high BMI globally, and the Asian and African regions had the highest incidence of underweight people (Senthilkumar, 2020).

Phaseolus aconitifolius Jacq. (2n=2x=22) It is also known as Moth Bean [Vigna aconitifolia (Jacq.)], which is a tiny grain legume that is underutilized. Vigna aconitifolia is grown mostly in India (De Candolle, 1986). Rajasthan, the most arid state in India, has the highest contribution in terms of both production and area at the national level (Gupta et al., 2016; Viswanatha et al., 2016). It is also grown in Sri Lanka, Myanmar, China, Pakistan, Malaysia, Africa, and also in the Southwestern

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USA (Jain and Mehra, 1980; Brink and Jansen, 2006; Kochhar, 2016).

In terms of nutrition, moth beans are regarded as a decent and affordable source of protein in vegetarian diets based on cereal among those in developing countries. Moth bean seeds contain between 20 and 24% of their weight in protein, 52 to 68% of their total weight in carbohydrates, 1.1 to 3.9% in fat, and 3.9 to 4.5% in crude fiber. It is also discovered to be rich in vitamins like niacin, thiamine, and riboflavin as well as minerals like calcium, magnesium, iron, phosphorus, and potassium (Kanishka et al., 2023). Like other pulses, moth bean includes flatulence-causing oligosaccharides such as raffinose, stachyose, and verbascose. Like other legumes, moth bean contains anti-nutritional and phytonutrient components that affect nutrient absorption, palatability, and digestibility. These elements comprise trypsin inhibitors, phytic acid, saponins, and phytohaemagglutinins (lectins). Trypsin and chymotrypsin inhibitors reduce protein digestion and cause pancreatic hypertrophy (Liener, 1976). Saponins irritate the stomach, while phytic acid inhibits proteolytic enzymes and amylases, reducing mineral absorption (Erdman, 1979; Singh and Krikorian, 1982; Pugalenthi et al., 2005; Bouchenak and Lamri-Senhadji, 2013). Soaking, sprouting, boiling, pressure cooking, and fermenting moth beans improve flavor and nutritional bioavailability. These approaches eliminate anti-nutritional elements, making moth bean starch and protein digestion easier.

In this present study, when Group I and Group II were compared to their respective controls, histopathological analysis of a few organs-the liver, kidney, and pancreas-as well as testicles or ovaries and female mammary glands indicated histological changes. Eissa et al. (2019) also found similar observation. Considering the economic importance of Vigna aconitifolia and the increasing interest in sustainable and cost-effective nutritional supplements, the results of this study could not only enhance our understanding of the potential health advantages of moth bean but also contribute to the wider discussion on using locally accessible crops to improve nutritional outcomes. The results of this investigation might have significant consequences for both animal and human nutrition, providing opportunities for more research and perhaps impacting dietary habits to enhance overall health and well-being. Very few research studies have coded about the role of Vigna aconitifolia in weight management and hence, the study focused on delivering a clear view of the impact of weight when it is supplemented.

## Materials and Methods Selection and Grouping of Animal Model

For the purpose of the study, albino Wistar rats weighing between 60 and 65 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 Chaw), Group II and III rats were provided with ungerminated *Vigna aconitifolia* and germinated *Vigna aconitifolia*, in the form of pellets, respectively. All the feeds were provided for 28 days.

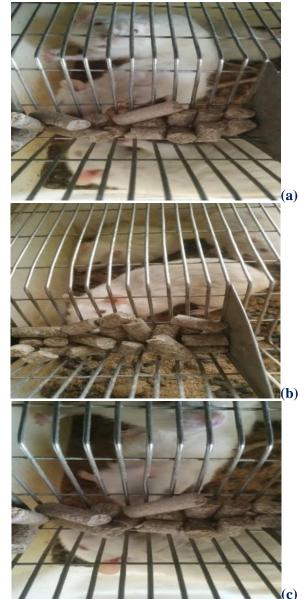


Figure 1. Wistar Albino Rats (a) Group I – Control Feed (b) Group II - ungerminated Vigna aconitifolia Feed (c) Group III - germinated Vigna aconitifolia Feed

## **Ethical Clearance and Purchase of Animals**

The use of animals, experimental procedures and protocols used in this study were reviewed and approved (509/PO/Re/S/01/CPCSEA) by the Institutional Animal Ethics Committee and were in accordance with the Institutional ethical guidelines. The animals were

obtained from an animal house at Kerala Veterinary and Animal Science University, Mannuthy.

#### **Bedding and Atmosphere**

As soon as they arrived, the animals were moved to the animal houses, where they were placed in polypropylene cages with paddy husk as bedding. Additionally, they were randomly assigned to treatment groups. Keeping the animals in a group of six to eight in each cage, the typical environmental condition of photoperiod (12:12 hours of dark and light cycle) was maintained. The temperature was kept at  $27 \pm 2^{\circ}$ C and the relative humidity ranged from thirty to seventy percent as well.

#### **Diet Fixation and Feeding**

Every single animal was provided with unrestricted access to water and was fed a commercially available pelleted rat chaw that was of the standard variety (M/s. Hindustan Lever Ltd, Mumbai).

## **Formulation and Development of Experimental Feed Ingredients**

Raw material: major form - glucose, fructose, sucrose, dextrins, and maltose with 1% starch paste.

#### **Starch paste preparation**

1 gm soluble starch was taken in a 250 ml beaker containing boiling water and stirred

Continuously until all lumps have dissolved and form a smooth mixture.

## **Procedure for Pellet Formation**

#### **Control pellet preparation**

Raw material was taken in a mortar and a sufficient quantity of starch paste was added to make a wet mass. Then, the wet mass passed through sieve number 10 to get the uniform size of granules. Then, granules were filled into the lubricated metal die plate to form compact pellets and then cut into predetermined sizes. The pellets were dried in a hot air oven at 70°C.

#### **Ungerminated** Vigna aconitifolia

Ungerminated Vigna aconitifolia was pre-heated for a few minutes in a pan with low temperature to remove the moisture content and then made into powdered form. It was then kept as an alternative for the raw material and the above procedure was followed to make as pellet

## Germinated Vigna aconitifolia Feed

The Vigna aconitifolia was soaked for 12 hours and allowed to germinate for 24 hours (Venipriyadharshini and Kavitha, 2022). The germinated Vigna aconitifolia

was then dried and ground into fine powder, further made as an alternative to ungerminated feed formation.

## **Supplementation Process**

The control pellet, ungerminated Vigna aconitifolia and germinated Vigna aconitifolia feeds were supplemented with free access for the period of 28 days under observation. Group - I (Normal feed), Group II (ungerminated feed) & Group III (germinated feed).

## **Assessment of Parameters**

After the observation period, by 29<sup>th</sup> day, the animals were euthanized with excess anesthesia; organs like Kidneys & Intestine were removed for histopathological studies.

#### **Histopathological Studies**

Histopathological studies of the organs of the kidney & intestine of all three groups were experimented. The gross and microscopic appearance of specimens of the organs were assessed and compared with one another to examine the shreds of evidence for any significant pathology or cell degeneration/ congestion.

## **Statistical Analysis**

Mean ± standard error of the mean was used to express the results. Using GraphPad version 3, the data were analyzed using a one-way analysis of variance (ANOVA), which was then followed by Dunnet's t-test. P values under 0.05 were deemed to be statistically significant.

#### **Results and Discussion**

Table 1 (Chart 1) shows the effect on water consumption in rats given ungerminated and germinated Vigna aconitifolia. Up to 14 days of observation, no discernible variation was seen when comparing the water intake of animals given ungerminated Vigna aconitifolia to groups that were fed normally. On 21<sup>st</sup> (P<0.05) and 26<sup>th</sup> (P<0.001) day of observation, there was a significant increase in water intake compared to normal fed animals. The animals were provided with germinated Vigna aconitifolia. On 7<sup>th</sup> day onwards, there was a significant increase in water intake compared to normal-fed animals. Compared to ungerminated and germinated Vigna aconitifolia, a more significant increase in water intake was noted on 7<sup>th</sup> to 21<sup>st</sup> day observations.

 Table 1. Effect of Vigna aconitifoliaon water intake in Wistar albino rats

Groups	Drug Treatment	Water Intake (ml)					
		1 <sup>st</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	21 <sup>st</sup> Day	28 <sup>th</sup> Day	
Ι	Normal Feed	42.75±2.4 6	43.62±1.5 8	44.24±2.21	44.76±3.2 4	46.44±2.85	
II	Ungerminated Vigna aconitifolia	40.24±1.8 8	45.67±2.3 0	48.43±3.19	50.23±2.2 5*	54.76±3.17 ***	
III	Germinated Vigna aconitifolia	41.76±3.4 1	50.83±3.3 8*	52.74±2.15 **	51.95±3.1 1***	58.18±4.63 ***	

Values are in mean  $\pm$  SEM (n=6); \*P<0.05, The \*\*P<0.01 & \*\*\*P<0.001 Vs Normal Feed normal

The change in body weight in rats provided with normal feed and ungerminated and germinated *Vigna* 

Table 2. Effect of Vigna aconitifolia on food intake in Wistar albino rats

Groups	Drug Treatment	Food Intake (gm)					
Groups		1 <sup>st</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	21 <sup>st</sup> Day	28 <sup>th</sup> Day	
Ι	Normal Feed	30.45±2.25	32.75±2.11	35.42±2.42	38.16±3. 50	42.55±2.67	
II	Ungerminated Vigna aconitifolia Feed	28.24±1.15	31.25±3.15	32.24±2.05	35.62±3. 08	36.17±1.44 *	
III	Germinated Vigna aconitifolia Feed	30.05±1.74	31.22±2.95	35.76±1.22	37.25±2. 62	41.54±2.75	

Values are in mean ± SEM (n=6); \*P<0.05, \*\*P<0.01 & \*\*\*P<0.001 Vs Normal Feed

Table 2 shows the food intake of rats given a regular diet and ungerminated and germinated *Vigna aconitifolia*. There was no significant change in food intake among the ungerminated and germinated *Vigna aconitifolia* compared to normal feed animals. On 28<sup>th</sup> day, the groups administered with ungerminated *Vigna aconitifolia* showed a significant (P<0.05) decrease in food intake compared to normal control animals. The rat took less feed of ungerminated green gram when compared with the standard (Bhadkaria, 2021).

*aconitifolia* feed are given in Table 3. There was no significant change in body weight in both ungerminated and germinated *Vigna aconitifolia* compared to normal feed animals up to 7<sup>th</sup> day. There was a significant (P<0.05) increase in body weight of animals treated with Ungerminated *Vigna aconitifolia* on  $21^{st}$  day and Germinated *Vigna aconitifolia* on  $14^{th}$  day of observation. On 28<sup>th</sup> day of observation, there was normal feed (47.85g), ungerminated (53.39g) and germinated (70.01g) *Vigna aconitifolia* feed significantly (P<0.01) increased its body weight from day 1 and hence the study revealed that there was a higher difference in the weight enhancement of the Wistar albino rat fed with germinated (70.01g) *Vigna aconitifolia* feed when compared with the

Table 3. Effect of	f Vigna aconitif	olia on body we	eight in Wista	r albino rat
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Groups	Drug Treatment	Body weight (gm)					
		1 <sup>st</sup> Day	7 <sup>th</sup> Day	14 <sup>th</sup> Day	21 <sup>st</sup> Day	28 <sup>th</sup> Day	
Ι	Normal Feed	62.65±3.17	66.31±3.95	73.85±3.26	92.35±1.26*	110.50±4.30**	
п	Ungerminated Vigna aconitifolia Feed	59.45±3.94	65.64±4.24	72.94±4.55*	95.72±5.08	112.84±3.80**	
III	Germinated Vigna aconitifolia Feed	58.23±1.67	65.75±3.74	82.50±3.05	102.62±4.70	128.24±4.05	

Values are in mean  $\pm$  SEM (n=6); \*P<0.05, \*\*P<0.01 other. & \*\*\*P<0.001 Vs Normal Feed

In comparison to lower-protein diets, higher-protein diets that contain between 1.2 and 1.6 g of protein and possibly include meal-specific protein quantities of at least 25-30 g/meal, which improved appetite, body weight management, and/or cardio metabolic risk factors. Although greater satiety, weight loss, fat mass loss, and/or the preservation of lean mass are frequently observed with increased protein consumption in controlled feeding studies, it is difficult to confirm a sustained protein effect over the long term in free-living adults due to dietary noncompliance with recommended diets (Verma, 2021; Chandora, 2023).

#### **Histopathological Studies**

Specimen:Kidneys Group –I: Normal Feed

## **Gross Appearance**

Specimen of kidneys with the measurement of  $1.2 \times 0.6 \times 0.4$  cms and  $1.0 \times 0.5 \times 0.4$  cms was noted. PE: Two bits – One block.

#### **Microscopic Appearance**

A section of the kidney showed both the cortex and medulla. Glomeruli showed no significant pathology. Tubules, interstitium and blood vessels showed no significant pathology. The section showed normal histology and no evidence of tubular epithelial cell degeneration was observed.

## Specimen :Kidneys

**Group –II:** Ungerminated *Vigna aconitifolia* Feed **Gross Appearance** 

Specimen of kidneys with the measurement of  $1.5 \times 0.5 \times 0.5$  cm and  $1.2 \times 0.4 \times 0.5$  cm was noted.

PE: Two bits – One block.

#### **Microscopic Appearance**

A section of the kidney showed both the cortex and medulla. Glomeruli showed no significant pathology. Tubules, interstitium and blood vessels showed no significant pathology. The section remains normal histology and no evidence of tubular epithelial cell degeneration/ congestion was observed.

Specimen:Kidneys

Group –III: Germinated Vigna aconitifolia Feed

# **Gross Appearance:**

Specimen of kidneys with the measurement of  $1.2 \times 0.5 \times 0.4$  cm and  $1.2 \times 0.4 \times 0.5$  cm was noted.

PE: Two bits - One block.

#### **Microscopic Appearance**

A section of the kidney showed both the cortex and medulla. Glomeruli showed no significant pathology. Tubules, interstitial and blood vessels showed no significant pathology. The section showed normal

ein histology and no evidence of tubular epithelial cell and degeneration/congestion was observed.

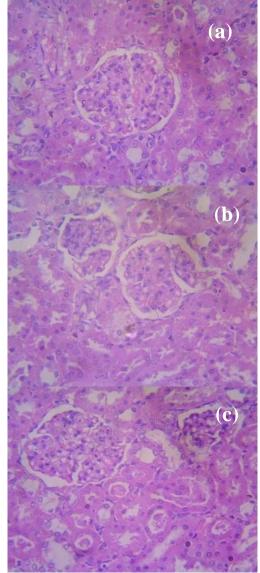


Figure 2. Microscopic appearance of kidney of Wistar albino rat (a) Group I - Control Feed (b) Group II ungerminated *Vigna aconitifolia* Feed (c) Group III germinated *Vigna aconitifolia* Feed

In the histological evolution of the kidney after 28 days fed with ungerminated *Vigna aconitifolia* and germinated *Vigna aconitifolia*, it was found that there was no significant change in cellular arrangements. Normal fed showed the section of the kidney with normal rat pallet fed showed no pathological changes in glomeruli. The tubules, interstitium and blood vessels appear normal and there is no evidence of congestion. The section shows normal histology and no evidence of tubular epithelial cell degeneration.

Rats fed with ungerminated *Vigna aconitifolia* showed a kidney section with a fixed normal cortex and medulla. Glomeruli seem normal, and the tubules, interstitium, and blood vessels appear normal, with no evidence of congestion. The kidney section remained normal histology; no evidence of tubular epithelial cell degeneration/ congestion was observed.

Rats fed with germinated *Vigna aconitifolia* showed a section of the kidney with a fixed normal cortex and medulla. No significant pathological changes in Glomeruli and it appears normal. The tubules, interstitium and blood vessels appear normal and no evidence of congestion. The section shows normal histology and no evidence of tubular epithelial cell degeneration/congestion was noted. Hence it is clear that the both the forms of feed (ungerminated and germinated) from *Vigna aconitifolia* do not having any side effects or complications in the kidney during or after the supplementation process.

Specimen:Intestine

Group –I: Control Feed

## **Gross Appearance**

Specimen of intestinal tissue measuring 1.4  $\times$  1.0  $\times$  0.7 cm was noted.

PE: One bit - One block.

#### **Microscopic Appearance**

Section from intestine showed ileum. The section showed a normal histological pattern with a prominent villus.

#### Specimen:Intestine

Group –II: Ungerminated *Vigna aconitifolia* Feed Gross Appearance

Specimen of intestinal tissue measuring  $1.2 \times 1.2 \times 0.6$  cms was noted.

PE: One bit – One block.

#### **Microscopic Appearance**

Section from intestine showed ileum. The section shows a normal histological pattern with villus and mucus-secreting epithelium and glands. No evidence of hyperplasia and edema was observed.

### Specimen:Intestine

Group –III: Germinated Vigna aconitifolia Feed

# **Gross Appearance**

Specimen of intestinal tissue measuring  $1.2 \times 0.8 \times 0.6$ cms was noted.

PE: One bit – One block

#### **Microscopic Appearance**

Section from intestine showed ileum. The section showed a normal histological pattern with villus and mucus-secreting epithelial cells. No evidence of hyperplasia and edema was observed.



Figure 3. Microscopic appearance of ileum of Wistar albino rat (a) Group I - Control Feed (b) Group II ungerminated Vigna aconitifolia Feed (c) Group III germinated Vigna aconitifolia Feed

After being given both ungerminated and germinated *Vigna aconitifolia* for 28 days, the histological development of the gut was observed. It was found that, no marked changes in histological architecture in the section of ileum. Normal fed showed the section of ileum in the intestine with no pathological changes in villus.

Rats fed with ungerminated and germinated *Vigna aconitifolia* showed sections of ileum showing normal histological patterns with villus and mucus-secreting epithelium and glands. No evidence of hyperplasia and edema noted. Hence, it is clear that both the forms of feed (ungerminated and germinated) from *Vigna aconitifolia* do not have any side effects or complications in the illium during or after the supplementation process, so it is affordable and fit for consumption.

#### Conclusion

performed research has illuminated The the substantial nutritional potential and cost-effectiveness of Vigna aconitifolia, a vital crop in Indian agriculture. The study aimed to evaluate the effects of including both ungerminated and germinated Vigna aconitifolia seeds into the diet of Wistar Albino Rats, specifically in terms of weight gain and overall health outcomes. The study shows by supplementation process, there was a constant increase in normal feed (110.50g), ungerminated (112.84g )and germinated (128.24g) Vigna aconitifolia feed in its body weight from day 1 as its weight of normal feed (62.65g), ungerminated (59.45g )and germinated (58.23g) Vigna aconitifolia feed in an average respectively and hence the results revealed that there was a higher difference in the weight enhancement of the Wistar albino rat fed with germinated (70.01g) Vigna aconitifolia feed when compared with the other two groups (I & II). Histological analyses demonstrated that kidney slices in all groups had normal morphology, with no evidence of degeneration or congestion in tubular epithelial cells. The histological analysis of the ileum sections from rats administered with ungerminated and germinated Vigna aconitifolia revealed a characteristic pattern, demonstrating the existence of villi, mucussecreting epithelium, and glands, without any signs of hyperplasia or edema. Compared to normal and ungerminated feed, germinated feed can much more aid standard progression in body weight increase. Hence, the study recommends human supplementation for the assurance of body weight enhancement. Nevertheless, it is advisable to do more research to clarify the fundamental mechanisms contributing to these outcomes. The study suggests that supplementing with germinated *Vigna aconitifolia* can help raise body weight, promoting consistent development in weight gain.

#### **Conflict of Interest**

The researcher declares no conflict of interest.

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