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Dose-Dependent Amelioration of Protein-Energy Malnutrition in Rats by *Trigonella foenum-graecum* Seed Supplementation

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Abstract

Protein-Energy Malnutrition (PEM) is most prevalent in low- and middle-income countries, where 45% of child deaths were attributed to under-nutrition. Trigonella foenum-graecum (fenugreek) seed is known for its important phytonutrients. It is planted and mostly found in northern Nigeria, where there is a higher prevalence of malnutrition. This study demonstrates the potential of graded fenugreek seed supplementation, a locally available resource, for mitigating PEM-associated biochemical dysregulation. Sixty-three (63) female rats weighing between 50 and 70g were randomly grouped into six groups of ten (10) animals each. Group 1 was the control while all others were malnourished with low protein (4%) iso-caloric diet for four weeks, thereafter randomly grouped into group 2 which were untreated, group 3 which were fed withstandard commercial feed, while Groups 4, 5, and 6were fed with 25g/kg, 50g/kg and 100g/kg fenugreek seeds supplemented diets respectively for 4 weeks. There was significant (p<0.05) reduction in all hematological parameters (which includes White Blood Cell (WBC), neutrophils, lymphocytes, Packed Cell Volume, and Mean Corpuscular Volume) of malnourished untreated rats whereas, alltreated groups significantly (p<0.05) increased after treatment with fenugreek supplemented diets with the highest effect observed in the 100g/kg FG. Also, the serum protein was significantly (p<0.05) reduced in malnourished rats by 43% when compared with the control, but was significantly increased after treatment with 100g/kg FG. In addition, the significant (p<0.05) increase in Alanine transaminase (ALT), and aspartate aminotransferase (AST), as well as the significant (p<0.05) decrease in alkaline phosphatase (ALP) observed in the serum of untreated rats were significantly (p<0.05) reversed in the groups treated with fenugreek seed supplemented diets. Hence, a diet supplemented with fenugreek seeds was concluded to improve the biochemical parameters of malnourished rats in adose-dependentt manner.

Keywords: fenugreek, protein-energymalnutrition, iso-caloric diet, supplementdiets

INTRODUCTION

An important aspect of life that involves the study and analysis of food and nutrients consumed, their absorption, assimilation and function in relation to health and proper body functions is nutrition (Tim, 2020). It also relates food to disease control and prevention (Tim, 2020). Various nutrients obtained from foods serve different purposes, including the provision of energy in the form of Adenosine TriPhosphate (ATP) that is required for daily life activities, building and repairing damaged body tissues, as well as prevention and control of certain diseases (Rattan and Kaur, 2022). New Health Scotland (2023) reported that, for a living organism to be healthy and able to prevent certain diseases (diet-related diseases), a well-balanced diet consisting of both macro and micronutrients in the right proportion is required. To reduce the risk of diseases, nutritionists have employed several techniques to formulate healthy diet combinations (Tim, 2020). Malnutrition is one of the most important health issues in developing countries (Morteza *et al.*, 2019). It occurs as a result of an imbalance between the nutrients required by the body and the nutrients it gets (Larson-Nath and Goday, 2019). An undernourished person is deficient in some important body nutrients such as protein, they experience stunted growth and

development and are prone to illnesses (Govender *et al.*, 2021). Malnutrition can lead to short- and long-term health issues such as scurvy, eye problems, marasmus, and kwashiorkor (Rattan and Kaur, 2022). As reported by the World Health Organisation (2023), 34% of the populace in Nigeria experiences malnutrition. Globally, in 2020, 149 million children were estimated to be undernourished, while 38.9 million were overweighed, and 45% of child deaths were attributed to undernutrition. According to Khaliq *et al.* (2022), for the body to obtain enough essential nutrients and prevent certain diseases, diets can be supplemented with important plant-based rich nutrients.

Fenugreek (*Trigonella foenum-graticum*) is a plant of the Fabaceae or Leguminoseae family that is cultivated in various parts of the world, especially Asia and Africa. It is the third largest family of flowering plants with more than 20,000 species (Singh *et al.*, 2022). Fenugreek seed is known for its antiviral, antimicrobial, anti-inflammatory, galactagogue, and antioxidant activities (Tewari *et al.*, 2020). Due to the high nutritional value of fenugreek seeds, it is used as wheat and maize flour supplement for bread making (Mehrafarin *et al.*, 2011). The Seeds have also been traditionally used for the treatment of diabetes, leg weakness, and to boost milk production in lactating mothers (Ahmad et al., 2016). While fenugreek's nutritional value is recognized, its efficacy in reversing established PEM, particularly across defined dosage regimens and its impact on comprehensive hematological, hepatic, and protein metabolic parameters, remains underexplored. This study therefore, investigates the dose-dependent ameliorative potential of T. *foenum-graecum* seed supplementation (25g/kg, 50g/kg, and 100g/kg) on key biochemical markers in a rat model of PEM.

MATERIALS AND METHODS

Experimental Animals

Sixty-three female Wister rats weighing between 50 and 70g were obtained from the Animal Holding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Kwara State, Nigeria. The animals were kept in cages and placed in a well-ventilated environment. All the animals were handled strictly according to the Instructions, Guidelines on Care and Use of Laboratory Animals (National institute of Health, 2023).

Feed Materials and Formulation

The feed materials are fenugreek seeds, cornstarch, distilled water,cornchaff, soybean, Dangote refined sugar and Golden penny soybean oil were obtained from Mandate market, Ilorin. Also DL-methionine and Vitamin/Mineral mix (Miavit GmbH Germany) were obtained at Aromokeyestore, opposite A division, Ilorin.

Fenugreek seed was identified and authenticated at the University of Ilorin herbarium and a voucher number of UILH/001/1799/2024 was deposited. Five different feeds were prepared: control feed, low protein iso-caloric feed, and three levels of Trigonella foenum-graecum seed-supplemented diet (25g/kg, 50g/kg, and 100g/kg). The components of the feeds are as shown on Table 1.

Table1:Components of Feed Formulations

In gredients (g/kg)	Control (25% protein)	Low protein iso-caloric Diet(4% protein)	Supplemented Diet 25g/kg	50g/kg	100g/kg
Corn starch	516	100	520	491	441
Cellulose	40	400	40	40	40
Sucrose	100	366	100	100	100
Soybean	250	40	225	225	225
Soybean oil	40	40	40	40	40
Vitamin/Mineral Mix	50	50	50	50	50
DLMethionine	4	4	4	4	4
Trigonellafoenum-graecum	-	-	25	50	100

Induction of Malnutrition

Animals were malnourished using the method described by Lambe and Bewaji (2021). Fifty-three female rats were malnourished by feeding them with 4% low-protein iso-caloric diet for 4 weeks. After 4 weeks of feeding and observation of body weight, food intake as well as appearance and behaviour, 3 animals were sacrificed to evaluate their biochemical Parameters to confirm induction of PEM.

Animal Grouping and Treatment

Sixty-three female rats were used for this study. They were allowed to acclimatize for one week during which they were fed with commercial feed and water. After acclimatization, they were randomly selected and grouped into 6 groups of 10 rats each. Table 2 shows Animal Grouping and their Treatment:

Table 2: Animal Grouping and Treatment

Groups	Description of treatment
ControlGroup	Rats that were given diet containing 25% protein
UntreatedGroup	Malnourished rats that were not treated
ReferenceGroup	Malnourished that were given regular commercial feed
25g/kg FG	Malnourished rats that were treated with 25g/kg supplemented diet
50g/kg FG	Malnourished rats that were treated with 50g/kg supplemented diet
100g/kg FG	Malnourished rats that were treated with100g/kg supplemented diet

Preparation of Tissue Homogenate and Serum

Rats were anaesthetized with diethyl ether fumes. Following the unconsciousness, the jugular veins were cut, and 5ml of blood was collected into a clean and dried EDTA bottle to prevent coagulation. Clear serum was collected after centrifuging at 1252 x g for 10 min. The sera were frozen in a refrigerator before various biochemical assays. The tissue the liver and kidney were blotted with blotting paper, cut very thinly, and homogenized in an ice-cold 0.25M sucrose (1:5 w/v). The homogenates were then centrifuged at 894 x g for 15 min, and the supernatants were frozen in refrigerator before biochemical assays were carried out (Sulaiman *et al.*, 2021).

Proximate Analysis of the Feed Composed

Proximate analysis of the feeds, such as protein, carbohydrates, crude lipids, crude fibre and moisture content, were carried out according to the methods described by AOAC (2023).

Anthropometric Parameters

The weight, and height of the rats were measured weekly throughout the experiment using the method described by Scoccia *et al.* (2001).

Hematological Parameters

The method described by Sulaiman *et al.* (2021) was used to evaluate the hematological parameters such as Hemoglobin (HGB), Packed Cell Volume (PCV), White Blood Cells (WBC), Red Blood Cells (RBC), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Mean Corpuscular Volume (MCV), Neutrophils, and Lymphocytes using the Sysmex KX 21 N hematology analyzer.

Total Protein

The total protein concentration in the liver, kidney and serum of the animals was assayed, using Biuret reagent as described by Gornall *et al* (1949)

Liver Function Indices

The method described by Reitman and Frankel (1957) was used to assay for the activity of alanine aminotransferase and aspartate aminotransferase, while alkaline phosphatase (ALP) was assayed using the method described by Wright et al. (1972).

Statistical Analysis

The data generated from the study was presented as the mean standard error of five replicates and subjected to a one-way analysis of variance (ANOVA). The data was considered statistically different at ($p < 0.05$) using Graph Pad Prism version 8.01 (Graph Pad Software, Inc., San Diego, California, United States).

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RESULTS

ProximateAnalysis

Table 3: Proximate Analysis of Feeds (%)

FEEDS	CRUDE PROTEIN	CRUDE LIPID	MOISTURE	TOTAL ASH	CRUDE FIBRE	CARBOHYDRATE
CONTROL	12.58±0.55^{a}	17.38±0.13^{a}	7.98±0.02^{a}	4.03±0.17^{a}	16.85±0.00^{a}	41.19±0.00^{a}
ISOCALORIC	2.63±0.00^{b}	7.25±0.00^{b}	8.79±0.15^{a}	1.92±0.02^{b}	39.19±0.36^{b}	40.22±0.10^{a}
REFERENCE	7.44±0.00^{c}	11.38±0.13^{c}	8.58±0.06^{a}	6.91±0.05^{c}	17.64±0.14^{a}	48.05±0.50^{b}
25g/kg FG	8.86±0.11^{c}	10.88±0.88^{c}	7.73±0.06^{a}	3.16±0.25^{a}	16.38±0.49^{a}	52.99±0.74^{b}
50g/kg FG	6.89±0.13^{c}	15.38±1.00^{a}	7.84±0.29^{a}	4.80±0.01^{a}	19.04±0.00^{a}	46.05±0.57^{b}
100g/kg FG	8.91±0.22^{c}	15.38±0.13^{a}	7.61±0.08^{a}	4.10±0.20^{a}	24.93±0.62^{C}	39.07±0.50^{a}

Data are a mean of 3 replicates with standard error of mean (SEM)
Groups with superscripts different from the control for each parameter along the rows are significantly different ($p < 0.05$).

The proximate analysis of the feeds (Table 3) showed that the reference and supplemented diets contain the same significant level of crude protein, which is lower than the control. The reference and 25g/kg FG contain a lower significant level ($p<0.05$), while 50g/kg FG and 100g/kg FG contain the same significant level of crude lipid compared to the control. The fenugreek seed-supplemented diets contain the same significant level of ash, a higher significant level in the reference diet, and a lower significant level in the iso-caloric diet when compared with the control. Reference, 25g/kg FG, and 50g/kg FG contain the same significant level of crude fibre, a higher significant level in 100g/kg, and the highest significant level in an iso-caloric diet when compared with the control. The iso-caloric and 100g/kg FG diets contain the same significant level of carbohydrate as the control, while the reference, 25g/kg FG, and 50g/kg FG diets contain a higher significant level when compared with the control. However, the moisture content in all the feeds is significantly the same as the control.

Anthropometric Parameters

The weight of the malnourished rats significantly($p< 0.05$) reduced due to the intake of an unbalanced diet (iso-caloric diet)**,** while that of the control rats significantly increased as they were fed with **an** adequate protein diet (Figure 1).

After the treatment of malnourished rats with fenugreek seed supplemented diets, there was significant ($p< 0.05$) increase in the weight of the rats treated with the supplemented diet at a lower significant rate when compared with the control, however, the group treated with standard feed showed the highest weight. (Figure 2).

Hematological Parameters

As shown on Table 4, the hematological parameters such as White Blood Cell (WBC), neutrophils, lymphocytes, PCV, MCV, and MHC are significantly ($p<0.05$) altered in the malnourished rats but when treated with Fenugreek seeds supplemented diets, the conditions were reversed with the highest effect recorded in the 100g/kg FG. However, there was no significant difference in the MCHC concentration in both treated and untreated groups of rats, but it was increased in the groups treated with 50g/kg FG and 100g/kg FG.

Total Serum Protein

The concentration of serum protein was significantly decreased ($p<0.05$) in the untreated

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rats compared with the control, but increased significantly ($p<0.05$) after treatment with reference diet, 25g/kg FG, 50g/kg FG, at the same significant level as the control. However, 100g/kg FG was significantly ($p< 0.05$) higher than the control (Figure 3).

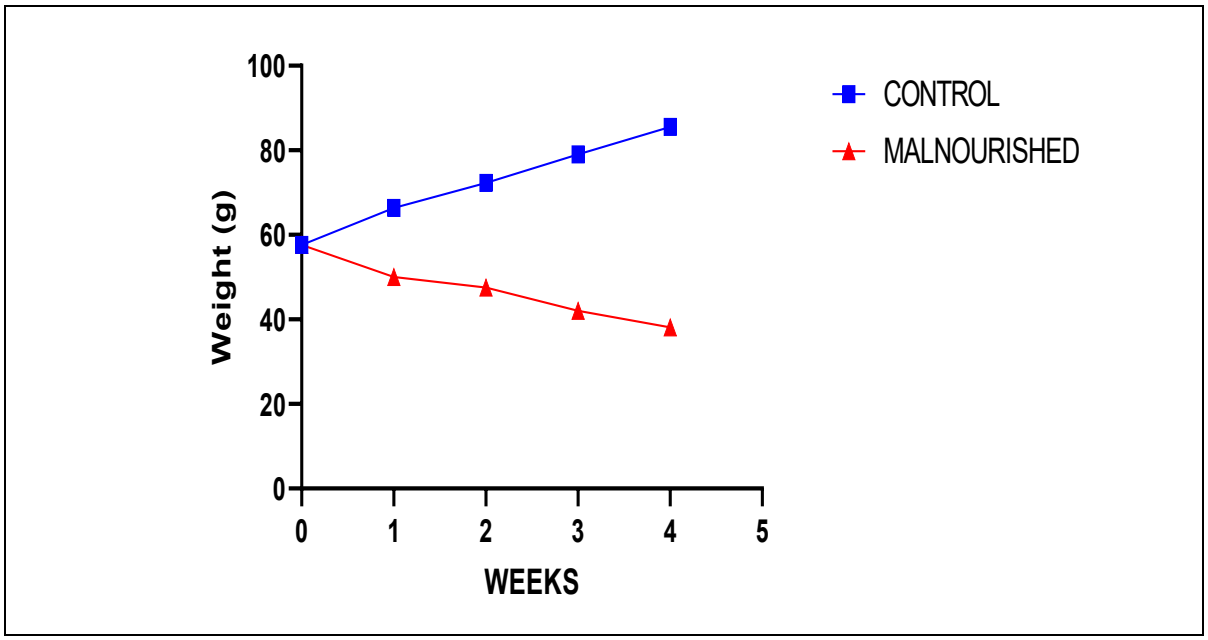

Figure 1: Weight of Experimental Rats during the weeks of malnutrition induction
Each data is a mean of 5 replicates

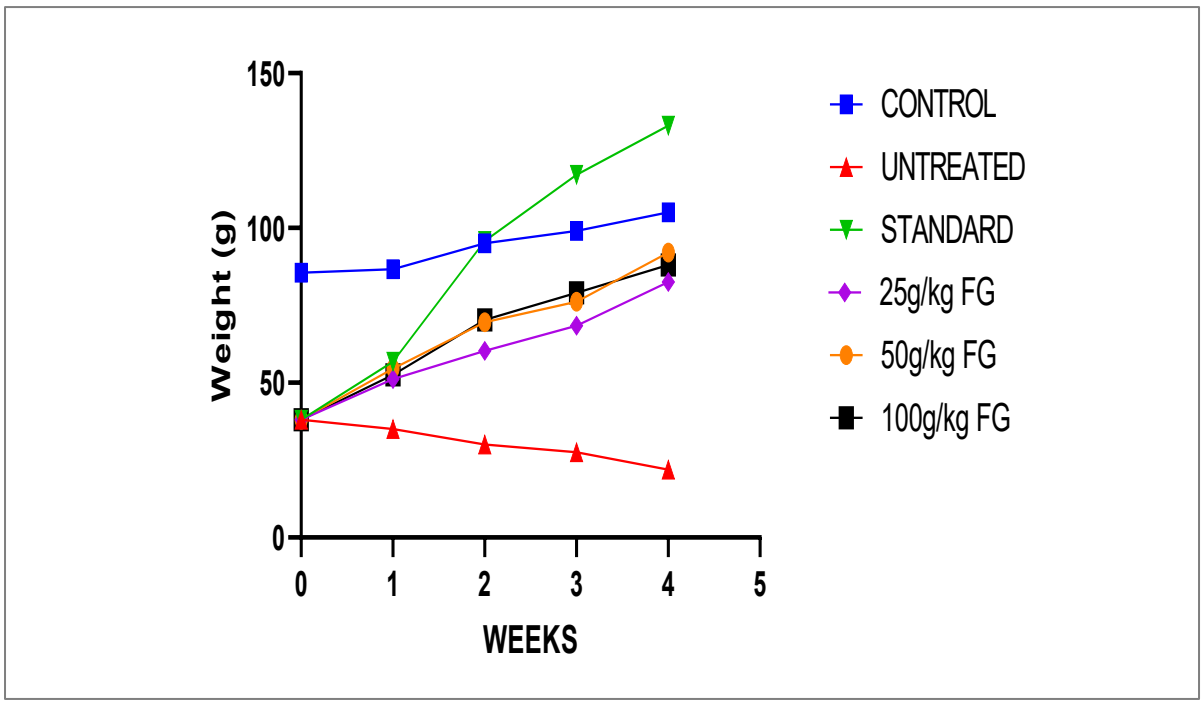

Figure 2: Mean Weight of Experimental Rats during the Weeks of Treatment
Each data is a mean of 5 replicates

Liver Function Indices

The specific activity of Aspartate aminotransferase (AST), as shown in Figure 4, indicated a significant ($p< 0.05$) increase in the specific activity of AST, which was reversed on treatment with fenugreek seed-supplemented diets, but not significantly ($p< 0.05$) different from the control.

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The concentration of ALP significantly ($p< 0.05$) reduced in untreated rats when compared with the controlbutthiswas significantly increased when treated with fenugreek seeds supplemented diets (Figure 5).

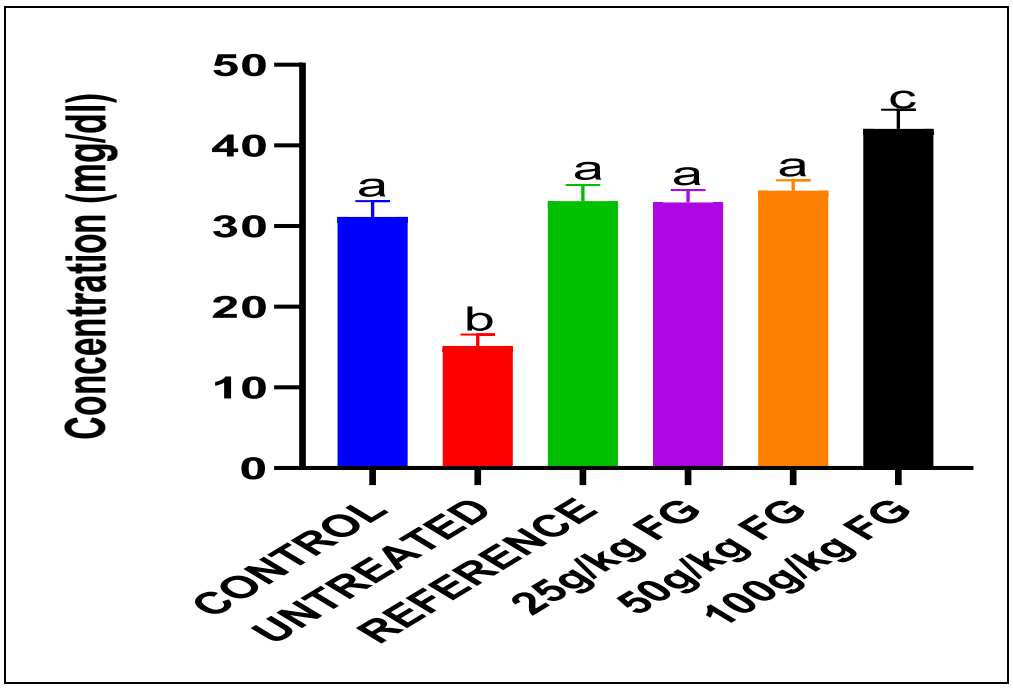

Figure 3: Effects of fenugreek seed-supplemented diet on total serum protein of malnourished Rats.
Each data is **the** mean of 5 replicates
Groups with superscripts different from the control are significantly different (p<0.05).

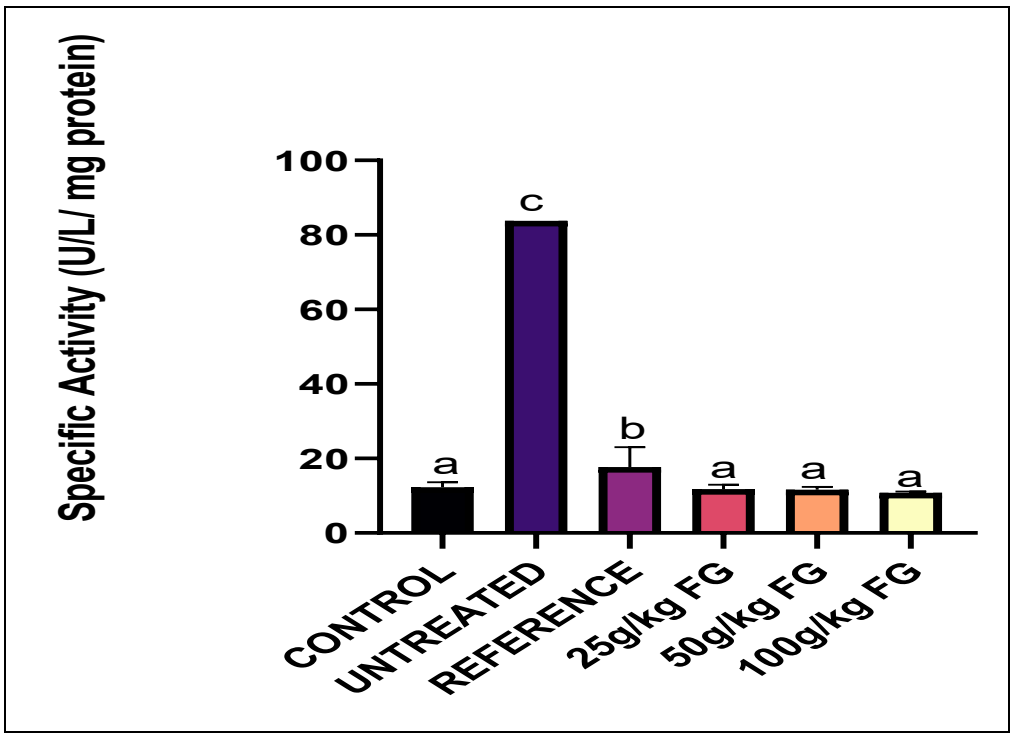

Figure 4: Effects of fenugreek seed supplemented diet on Specific Activity of Aspartate Transaminase of Malnourished Rats.
Each data is **the** mean of 5 replicates.
Groups with superscripts different from the control are significantly different (p<0.05).

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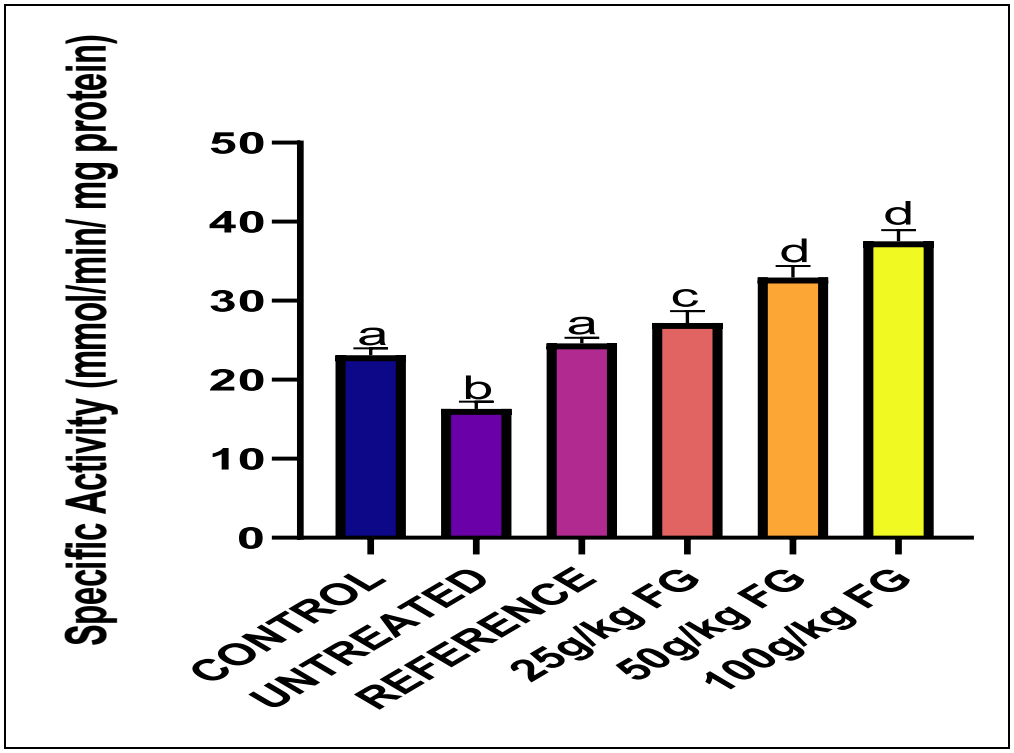

Figure 5: Effects of Fenugreek seed-supplemented diet on Specific activity of Alkaline Phosphatase of Malnourished Rats.
Each data is the mean of 5 replicates
Groups with superscripts different from the control are significantly different ($p<0.05$).

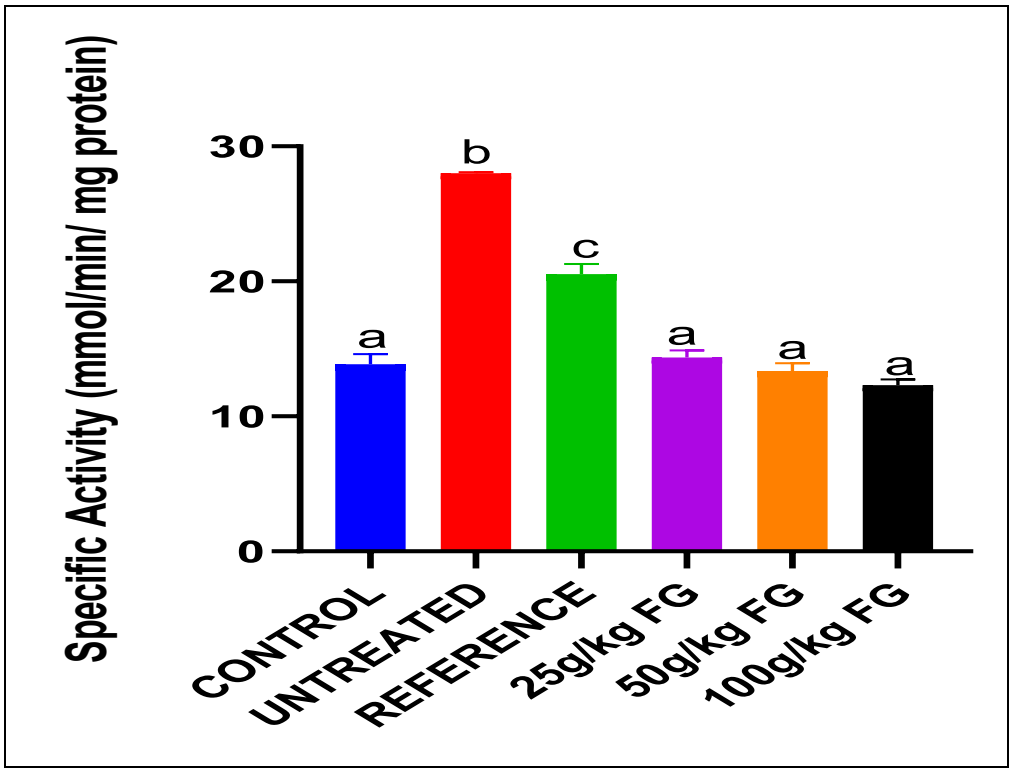

Figure 6: Effects of Fenugreek seed-supplemented diet on Specific activity of Alaninev Amino Transferase of Malnourished Rats.
Each data is **the** mean of 5 replicates
Groups with superscripts different from the control are significantly different ($p<0.05$).

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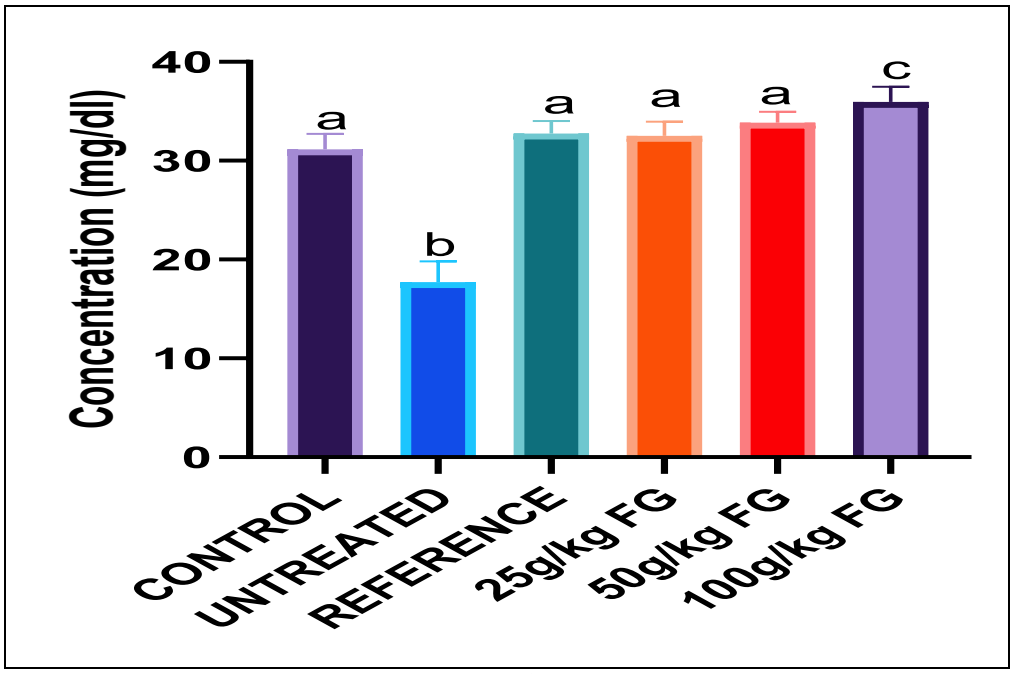

Figure7:Effects of Fenugreek seed-supplemented diet on the Concentration of Albumin of Malnourished Rats.

Each data is the mean of 5 replicates

Groups with superscripts different from the control are significantly different (p<0.05).

There was a significant ($p< 0.05$) increase in theserum specific activity of ALT of malnourished rats when compared with the control (Figure 6), however, this was reversed when the rats were treated with fenugreek seed supplemented diets (25g/kg, 50g/kg, and 100g/kg) with no significantdifference($p< 0.05$) when compared with the control.

There was significant decrease *(p<0.05)* in the albumin concentration of the malnourished untreated rats when compared with the control group of rats, treatment with the referencefeed, 25g/Kg and 50g/Kgfenugreek seed supplements showed same significant increase *(p<0.05)*as the control while 100g/Kg FG showed higher significant *(p<0.05)* increase when compared with the control (Figure 7).

DISCUSSION

Fenugreek seed has been reported to be rich in important phytochemicals, which are responsible for its various biochemical actions. The result of the proximate analysis carried out on the fenugreek supplemented feed is similar to the one reported by Raju *et al.* (2004) who claimed that fenugreek seed contains high carbohydrates (mucilaginous fibre and galactomannan) and proteins which were reported to be 20-30% high in tryptophan and lysine, free amino acids such as 4-hydroxyisoleucine, arginine, lysine and histidine. Abdel-Azeem (2006) also reported similar results for crude protein, moisture, and ash contents. Hence, a diet supplemented with fenugreek seeds can be used as a nutritional supplement due to their lysine content and as a substitute for soybean. It can also be used in the treatment of digestive disorders and weight management.

Fenugreek-supplemented seeds showed a significant increase in the weight of the malnourished rats, although this was lower than the control. This might be because they contain some bioactive compounds, such as 4-hydroxyisoleucine, diosgenine, and stigmasterol, which contribute to their ability to maintain a healthy weight. In addition, the high-fibre and low-calorie content of fenugreek seed promotes feelings of fullness, which aids in weight loss. This is in line with the work of Zenhom and Ibrahim (2020), who reported that the dietary addition of 20% fenugreek seed by-produced meal enhanced the growth performance of common carp. However, Knott *et al.* (2017) and Paner et al. (2022) reported that fenugreek seed did not significantly increase the weight of rats and broilers when fed a fenugreek seed-supplemented diet.

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Table 4: Hematological Parameters of Malnourished and Treated Rats

	CONTROL	UNTREATED	REFERENCE FEED	25g/kg FG	50g/kg FG	100g/kg FG
WBC×10^9/L	5.30 ± 0.50^a	4.00 ± 0.58^b	4.33 ± 0.57^b	4.80 ± 0.58^a	5.65 ± 0.38^a	8.20 ± 0.35^c
NEU (%)	67.00 ± 2.80^a	56.00 ± 2.31^b	62.75 ± 2.36^a	65.50 ± 3.93^a	74.00 ± 1.16^c	77.25 ± 3.59^c
LYMPH (%)	33.00 ± 2.78^a	44.00 ± 2.31^b	37.25 ± 2.36^a	34.50 ± 2.75^a	26.00 ± 0.95^a	22.75 ± 3.59^a
RBC×10^{12}/L	5.20 ± 0.03^a	4.06 ± 0.35b	4.92 ± 0.15^a	5.42 ± 0.05^c	5.59 ± 0.08^c	5.98 ± 0.21^d
HB (g/dl)	9.63 ± 1.37^a	7.20 ± 0.65^b	9.84 ± 0.44^a	10.67 ± 0.58^c	11.01 ± 0.19^c	12.12 ± 0.55^c
PCV (%)	30.00 ± 0.00^a	22.00 ± 1.03^b	33.25 ± 2.78^a	33.00 ± 0.41^a	34.00 ± 0.82^a	36.75 ± 1.11^c
MCV (fl)	61.22 ± 3.33^a	42.68 ± 0.04^b	58.99 ± 0.01^a	62.30 ± 1.75^a	63.48 ± 2.03^a	64.74 ± 1.32^a
MCH (Pg)	20.05 ± 0.41^a	15.40 ± 0.58^b	20.00 ± 0.00^a	20.50 ± 0.50^a	20.70 ± 0.75^a	21.90 ± 0.29^a
MCHC (g/d)	33.33 ± 0.40^a	30.28 ± 2.88^a	29.46 ± 0.47^a	32.20 ± 0.01^a	36.35±0.25^b	37.53±0.28^b

Values with different superscripts along the column are considered significantly ($p<0.05$) different from the control across the row.
Each data is the mean of 5 replicates

In addition, it was observed that *Trigonella foenum-graecum seed improved some haematological parameters of malnourished rats*, including its ability to increase the number of White Blood Cell (WBC) and Packed Cell Volume (PCV). This can be supported by a study by Abdel-Hamid (2019), which reported that there was no significant effect on White Blood Cells when rats were fed with 2.5% fenugreek seed for 4 and 8 weeks, but increased when fed with 7.5% fenugreek seed. This may be because fenugreek seed is rich in protein, essential amino acids, iron, ascorbate, and folate. Doshi *et al.* (2012) reported that fenugreek seed improved the haemoglobin level in females of childbearing age. Generally, it can be obtained from the result that supplementing a diet with fenugreek seed in a dose-dependent manner can boost the body's immune system and prevent certain blood-related diseases such as anaemia, as suggested by Abdel-Azeem (2006).

Data obtained from total protein tests showed that fenugreek seed contains certain compounds that increased the serum and liver proteins of malnourished rats, the malnourished rats treated with 100g/kg FG showed the highest concentration of protein in the serum and liver which were significantly different from the control, however, the malnourished rats treated with 25g/kg and 50g/kg FG showed no significant improvement in serum and liver protein when compared with the rats in the control group. This confirmed that 100g/kg of fenugreek seed in a diet can be used to increase the protein content in the serum and liver of malnourished rats. In addition, the kidney protein of malnourished rats fed a fenugreek-supplemented diet showed increased protein in a dose-dependent manner. The 50g/kg FG showed significant improvement compared to the control group, while the rats fed 100g/kg FG showed the highest concentration. Elmandi and El-Bahr (2015) reported no significant increase in serum total protein and albumin in treatment with non-diabetic rats, while Zewali *et al.* (2015) reported a significant increase. These variations may be due to differences in dose, plant preparation, route of administration, and duration of exposure or state of health of the rats used. It has been established that fenugreek seed is rich in amino acids such as leucine, valine, lysine, phenylalanine, and glutamic acid. These amino acids play essential roles in protein synthesis and metabolism, which may be the reason why it was able to improve the total protein in malnourished rats.

In the malnourished rats, the specific activity of liver enzymes (AST and ALT) was increased. This is likely a result of the liver's adaptive response

to protein deficiency, oxidative stress, and altered metabolism (Grzeszczak et al., 2023; Karajiban et al., 2021; AbdElmonem et *al.*, 2023).This was reduced when the rats were fed with fenugreek seed-supplemented diets. Hence, fenugreek seeds at 25g/kg, 50g/kg, and 100g/kg are considered safe for consumption without liver damage. However, the specific activity of ALP was found to be reduced in malnourished rats compared to ALT and AST. This may be due to protein synthesis impairment in the liver. This was also reported by Karajibani *et al.*(2021).

Conclusively, fenugreek seeds, due to their high antioxidant and phytochemical content, can improve certain nutritional disorders and enhance blood haematology, which can help to boost immunity. Doses 25g/kg, 50g/kg, and 100g/kg are safe for consumption and does not harm the liver.

CONFLICT OF INTEREST

There was no any form of conflict of interest

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