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AMELIORATIVE POTENTIALS OF *Trigonella foenum-graecum* (FENUGREEK) SEEDS ON PROTEIN-ENERGY MALNOURISHED RATS

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ABSTRACT

Malnutrition especially Protein-Energy Malnutrition (PEM) is one of the major challenges that brings about both short and long term health issues as well as weaken immune system and necrosis. It accounts for 45% of child deaths in both low and middle income countries. *Trigonella foenum-graecum*, a leguminous plant known for its antiviral, antimicrobial, anti-inflammatory and antioxidant was evaluated for its potential as diet supplement in the body of a protein-energy malnourished individual using wistar rats as a model. 74 female wistar rats weighing between 50 and 70g used for the study were malnourished by feeding them with low protein iso-caloric diet for 4 weeks. The rat samples were grouped namely as untreated group (malnourished rats that were not treated), commercial-fed group (malnourished rats treated with commercial feed), 25 g/kg, 50 g/kg and 100 g/kg supplemented diets groups (malnourished rats treated with 25 g/kg, 50 g/kg and 100 g/kg fenugreek seeds supplemented diets respectively) and were treated for 4 weeks after which they were sacrificed. The proximate analysis of the feeds were carried out, the anthropometric parameters, hematological parameters, total protein and lipid profile of the experimental animals were also assessed. There were significant ($p<0.05$) difference in the observed parameters of malnourished rats when compared with the control which were reversed when treated with fenugreek seed supplemented diets. Hence, 25 g/kg, 50 g/kg and 100 g/kg fenugreek seed supplemented diets improves the nutritional status of malnourished rats.

Keywords: Malnutrition, Fenugreek, Hematology, Supplement diet

INTRODUCTION

Malnutrition is a common health issue that affects individuals of all ages and races (Morteza *et al*., 2019). It is characterized by an imbalance intake of nutrient and can manifests either as under- or overnutrition (Zhang *et al*., 2022). Protein-Energy Malnutrition (PEM) is a global health concern which arises due to inadequate intake of protein and energy, it particularly affects children and elderly as well as individuals with chronic illnesses (Tim, 2020). A malnourished individual may experience stunted growth, weak immune response, body weakness and fatigue (Tim, 2020). Globally approximately 147.7 million cases of PEM was reported in the year 2019 with associated death of 212, 242 and it can be predicted that over 160 million cases would arise by 2044 (Jiang *et al.* 2023). Due to the challenges posed by PEM worldwide, innovative dietary interventions are being explored to enhance nutritional status, and prevent malnutrition. One of such intervention is the supplementation of diets with fenugreek (*Trigonella foenum-graecum*) seeds. Fenugreek seeds is a plant of fabaceae family which contains essential nutrients including proteins, dietary fibres, vitamins and minerals. It is known for its antimicrobial, anti-inflammatory and antioxidant properties (Singh *et al.*, 2023). The seed has been traditionally used as spice, baking, treatment of diabetes and skin infections, also to boost milk productions in lactating mothers (Wani and Kumar, 2018, Khan *et al*., 2018, Singh *et al.*, 2023). Researches have also shown that fenugreek seed supplemented diets improved muscle performance and regulated blood sugar levels in obese rats (Konopelnyuk *et al.* 2015). Fenugreek seed is also rich in bioactive compounds such as saponins, alkaloids, and flavonoids which contributes to its therapeutic effects which helps to restore nutrient availability and prevent malnutrition (Singh *et al.* 2022). Another research also suggests that fenugreek seeds can improve nitrogen retention and protein synthesis, making them effective in boosting muscle mass and general nutrition in malnourished individuals, also, the seed has been proved to have hypoglycemic and lipid lowering effects which could enhance the metabolic health of a malnourished individual by providing energy and protein, and restore normal body function (Mehram *et al.* 2022, Kumar *et al.*, 2021). Hence, this study evaluates the effects of fenugreek seed supplemented diets on protein-energy malnourished rats by examining the physiological and biochemical changes in malnourished rats treated with the supplemented diets. The study provides information about the roles of fenugreek seeds in improving nutrient availability in the body and prevent some diet related diseases. Understanding the mechanism and potential role of fenugreek seed supplementation of diet can provide a promising avenue for enhancing nutritional intake and addressing the issue of PEM especially in developing countries. It could also pave way for innovative strategies aimed at improving public health outcomes and enhancing food security.

MATERIALS AND METHODS

Experimental Animals

A total of Seventy-four (74) female wistar rats weighing between 50 and 70 g were obtained from the Animal Holding Unit of Fulcrum Laboratory, Ilorin, Nigeria. The animals were kept in plastic cages, placed in a well-ventilated environment and were allowed to acclimatize for a period of 7 days. During the period, they were fed with top feed growers mash feed and clean water.

Feed Materials and Formulation

Corn starch, corn chaff, soybean, Dangote refined sugar, and Golden Penny soybean oil were obtained at Oja'gbo, Ogbomosho, Oyo State. DL-methionine and Vitamin/Mineral mix (Miavit GmbH Germany) were obtained at Olufunmilayo Farms Limited, Offa Garrage, Ilorin, Kwara State. Fenugreek seed was bought at Mandate market, Ilorin, Kwara State, Nigeria. Five different feeds were prepared; control feed, low protein iso-caloric feed and 3 different levels of

Trigonella foenum-graecum seed supplemented diet (25 g/kg, 50 g/kg & 100 g/kg).

Corn starch was prepared by soaking yellow corn in water for 3 days, washed thoroughly, grinded and sieved using muslin cloth. The filtrate was drained in a sack under heavy weight for 6 hours and then dried to constant weight. Corn chaff was used as a source of cellulose and was prepared by sun drying corn chaff for 2 days and grinded using commercial grinder at Mandate market. Soybean grain flour was prepared by removing the bean coat, dry and then grinded into powder. These ingredients were thoroughly mixed together in proportion shown in table 1. Water was slowly added to form a paste and then pelletized using a wire mesh and dried to a constant weight.

Table 1: Feed Formulations

Ingredients (g/kg)	Control (25% protein)	Low-protein iso caloric diet (4% protein)	Supplemented diet		
			25 g/kg	50 g/kg	100 g/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
DL Methionine	4	4	4	4	4
Trigonella foenum-graecum	-	-	25	50	100

Source: Lambe and Bewaji, 2021

Animal Grouping

Seventy four (74) female wistar rats were used for this study, they were allowed to acclimatize for a period of one week during when they were fed with commercial feed and water. After which they were randomly selected and grouped into 6 groups of 12 rats each. Table 2 shows Animal Grouping and their Treatment:

Table 2: Animal Grouping and Treatment

Groups	Doses Administration (g/kg body weight)
Control	Rats that were fed 25% protein
Untreated	Malnourished rats that were not treated
Commercial-fed	Malnourished rats that were treated with Commercial Feed
25 g/kg Supplement	Malnourished rats that were treated with 25 g/kg supplemented diet
50 g/kg Supplement	Malnourished rats that were treated with 50 g/kg supplemented diet
100 g/kg Supplement	Malnourished rats that were treated with 100 g/kg supplemented diet

Induction of Malnutrition

Malnutrition induction experiment was carried out using the method of Nadia *et al.* (1999) modified by Lambe and Bewaji, (2021). Sixty-two female rats were malnourished by feeding them with low protein iso-caloric diet for 4 weeks. During this period, body weight, food intake as well as appearance and behavior of the animals were observed, to further confirm the induction of malnutrition, two (2) animals were sacrificed and Biochemical assays including total protein levels were assessed.

Sacrifice of animals and Preparation of serum

The sacrifice of the animals and collection of serum was done by adopting the procedure described by Yakubu *et al.* (2009). Summarily, the rats were anaesthetized in diethyl ether fumes, following loss of consciousness, the jugular veins were cut, and 3 ml of blood was collected into plain heparin sample bottles. Clear serum was collected after centrifuging the clotted blood samples at 1252 x g for 10minutes and kept frozen for 12 hours before being used for further analysis.

Preparation of Tissue Supernatants

The tissue supernatants were prepared as described by Yakubu *et al.* (2009). Briefly, 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.

Proximate Analysis of the feed composed

Proximate analysis of the feeds was carried out according to the AOAC (2009) method.

Anthropometric Parameters

The weight, height, and Body-Mass Index of the rats were measured weekly throughout the experiment using triple beam balance according to the method described by Scoccia *et al.* (2001).

Hematological Parameters

The hematological parameters including Hemoglobin (HGB), Packed Cell Volume (PCV), White Blood Cells (WBC), Red Blood Cells (RBC), and Mean Corpuscular Hemoglobin (MCH) were determined using the Sysmex KX 21 N hematology analyzer as described by Dacie and Lewis (1991).

Biochemical Assays

Total Serum Protein

The concentration of total serum protein of the animals was assayed, using the Biuret reagent (Gornall *et al.,* 1949).

Lipid Profile

The concentrations of serum total cholesterol were determined by the method described by Fredrickson *et al.* (1967), Triacylglycerol as described by Hainline *et al.* (1980), and High Density lipoprotein as described by Albers *et al.* (1978).

Statistical Analysis

The data generated from the study was presented as the Mean ± Standard Error of the Mean 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).

RESULTS AND DISCUSSION

Proximate Composition of the Feeds

Iso-caloric diet contains significantly lower (p<0.05) protein, lipid, ash, fibre, and carbohydrate when compared with the control, the commercial feed has same significant level of lipid, fibre, and carbohydrates, lower significant level of protein but higher ash when compared with the control. However, supplemented diets contain same significant level of protein and ash, higher significant level of lipid, and fibre but lower significant level of carbohydrates when compared with the control. All the diets contain same significant level of moisture as the control (Table 3).

Table 3: Proximate Composition of the feeds (%)

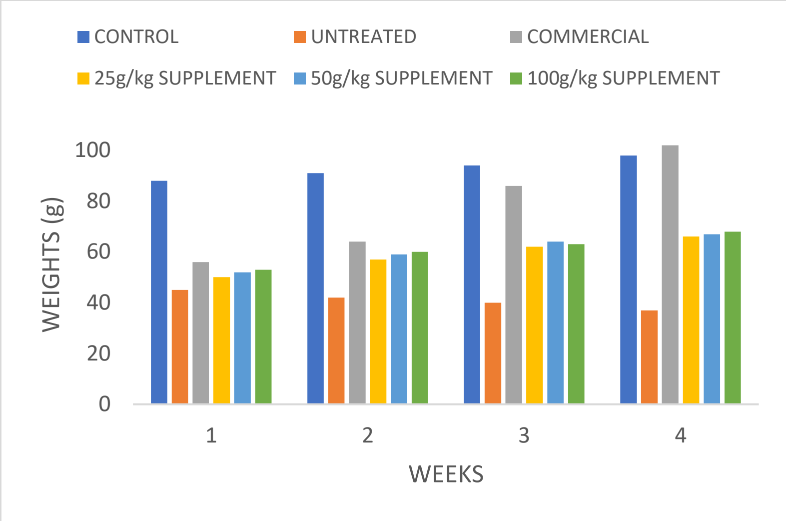
Feeds	Crude Protein	Crude Lipid	Moisture	Total Ash	Crude Fibre	Carbohydrate
Control	11.98 ± 0.45^a	14.34 ± 0.11^a	6.19 ± 0.02^a	4.03 ± 0.21^a	15.04 ± 0.02^a	47.61 ± 0.00^a
Isocaloric	2.71 ± 0.05^b	7.92 ± 0.02^b	7.88 ± 0.12^a	1.02 ± 0.02^b	37.99 ± 0.33^b	41.28 ± 0.10^b
Commercial	6.49 ± 0.01^c	12.67 ± 0.31^a	7.86 ± 0.01^a	7.10 ± 0.00^c	16.86 ± 0.14^a	48.24 ± 0.50^a
25 g/Kg Supplement	11.96 ± 0.14^a	18.95 ± 0.32^c	7.29 ± 0.08^a	3.09 ± 0.54^a	16.38 ± 0.49^a	46.33 ± 0.74^a
50 g/Kg Supplement	12.98 ± 0.11^a	19.66 ± 0.00^c	7.15 ± 0.14^a	3.90 ± 0.10^a	19.04 ± 0.00^c	39.27 ± 0.57^b
100 g/Kg Supplement	12.54 ± 0.17^a	20.74 ± 0.11^c	7.10 ± 0.07^a	3.01 ± 0.30^a	24.93 ± 0.62^d	31.68 ± 0.50^c

Data are a mean of two replicates with standard error of mean (SEM)
Groups with superscripts different from the control down the rows are significantly different ($p < 0.05$).

Anthropometric Parameters

The weights of the malnourished rats significantly reduced while that of control rats significantly increased (Figure 1a). During treatment, the weight of the untreated rats continuously decreased, while there was significant increase in the weight of all treated rats with the highest significant weight observed in those treated with commercial feed, however the weights of those treated with fenugreek supplemented diets is significantly lower than the control (Figure 1b).

(a)



(b)

Figure 1: Mean Weight of rats during (a) induction of malnutrition and (b) treatment

Body Mass Index During Induction of Malnutrition and Treatment

The body mass of the malnourished rats was significantly reduced ($p<0.05$) at week 3 and 4 of malnutrition induction (Table 4). However, upon treatment with the three levels of fenugreek seed supplemented diets, the body mass index was significantly increased ($p>0.05$) at a rate lower than the control (Table 5).

Table 4: Body Mass Index during Induction of Malnutrition

Group/Week	**1**	**2**	**3**	**4**
Control	0.43 ± 0.01^{a}	0.45 ± 0.02^{a}	0.46 ± 0.03^{a}	0.46 ± 0.02^{a}
Malnourished	0.42 ± 0.02^{a}	0.44 ± 0.00^{a}	0.43± 0.02^{b}	0.40± 0.01^{b}

Each data is the mean of five replicates with standard error of mean

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

Table 5: Body Mass Index during Treatment

Group/Week	**1**	**2**	**3**	**4**
Control	0.45 ± 0.01^{a}	0.47 ± 0.05^{a}	0.47 ± 0.02^{a}	0.49 ± 0.00^{a}
Untreated	0.39 ± 0.02^{b}	0.39 ± 0.04^{b}	0.38 ± 0.01^{b}	0.36 ± 0.03^{b}
Commercial	0.46 ± 0.00^{a}	0.48 ± 0.01^{a}	0.51 ± 0.01^{c}	0.56± 0.02^{c}
25 g/kg Supplement	0.42 ± 0.01^{a}	0.43 ± 0.02^{c}	0.44 ± 0.01^{d}	0.45 ± 0.00^{d}
50 g/kg Supplement	0.43 ± 0.01^{a}	0.44 ± 0.03^{c}	0.44 ± 0.01^{d}	0.45± 0.01^{d}
100 g/kg Supplement	0.44 ± 0.04^{a}	0.44 ± 0.00^{c}	0.43± 0.01^{d}	0.44± 0.01^{d}

Each data is the mean of five replicates with standard error of mean

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

Hematological Parameters

The hematology parameters including white blood cell, red blood cell, and hemoglobin significantly reduced ($p<0.05$) in the untreated group, but were significantly increased when treated with fenugreek supplemented diets compared to the control (Table 6)

Table 6: Hematological Parameters of Malnourished and Treated rats

	Control	**Untreated**	**Commercial-feed**	**Supplement**		
				25 g/Kg	**50 g/Kg**	**100 g/Kg**
WBC×10^{9}/L	6.10 ± 0.50^{a}	3.89 ± 0.58^{b}	4.98 ± 0.47^{b}	5.80 ± 0.56^{a}	7.17 ± 0.32^{c}	7.99 ± 0.52^{c}
RBC×10^{12}/L	5.01 ± 0.01^{a}	4.00 ± 0.32^{b}	4.87 ± 0.11^{a}	5.13 ± 0.03^{a}	5.84 ± 0.07^{c}	6.01 ± 0.34^{c}
HB (g/dl)	10.01 ± 1.33^{a}	6.34 ± 0.45^{b}	9.93 ± 0.24^{a}	10.59 ± 0.46^{a}	12.38 ± 0.13^{c}	13.00 ± 0.35^{c}
PCV (%)	31.03 ± 0.00^{a}	25.00 ± 0.03^{b}	32.50 ± 1.08^{a}	34.05 ± 051^{c}	35.50 ± 0.82^{c}	36.25 ± 0.11^{c}
MCV (fl)	59.99 ± 1.23^{a}	43.00 ± 0.03^{b}	58.08 ± 0.01^{a}	63.20 ± 1.25^{c}	64.54 ± 2.00^{c}	65.22 ± 1.25^{c}
MCH (Pg)	20.15 ± 0.32^{a}	15.00 ± 0.45^{b}	20.01 ± 0.02^{a}	20.70 ± 0.40^{a}	21.40 ± 0.51^{a}	21.95 ± 0.09^{a}
MCHC (g/d)	33.03 ± 0.36^{a}	30.07 ± 1.08^{b}	30.52 ± 0.47^{b}	32.46 ± 0.01^{a}	37.05 ± 0.13^{c}	38.04 ± 0.22^{c}

Each data is the mean of five replicates with standard error of mean

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

Total Serum Protein

The total serum protein in the untreated rats significantly reduced, however, it was increased when treated with commercial and supplemented diets, the 50 g/kg and 100 g/kg supplement groups showed a significant increase higher than the control. (Figure 2)

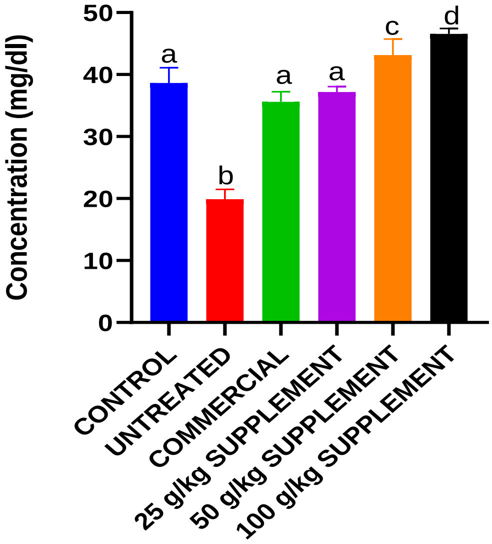


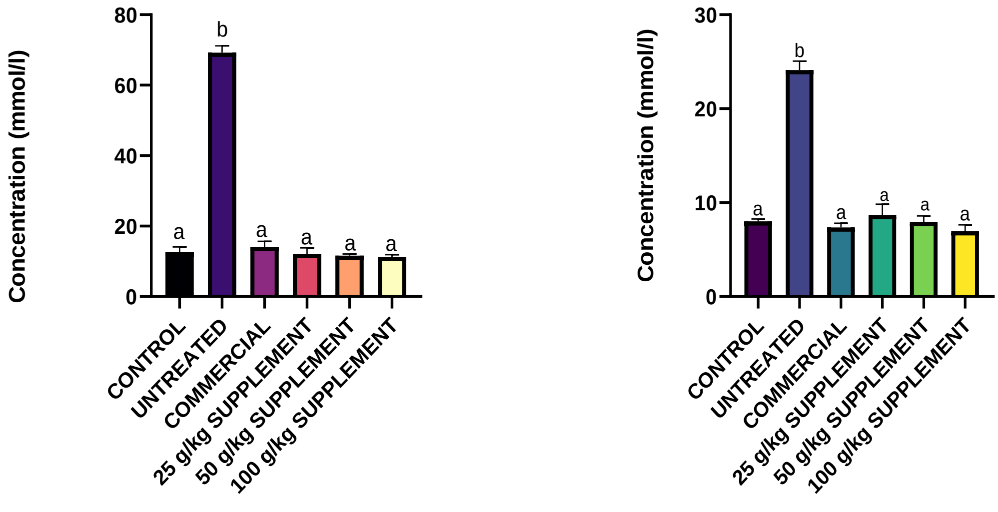
Figure 2: Effect of fenugreek seed supplemented diet on total serum protein of malnourished rats

Each data is the mean of five replicates with standard error of mean

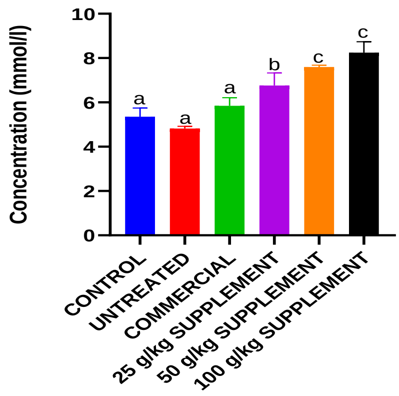
Groups with superscripts different from the control are significantly different ($p < 0.05$).

Lipid Profile

The concentration of total serum cholesterol and triglycerides increased while high density lipoprotein decreased in the untreated rats, however, these concentrations were reversed in the supplemented diets fed rats (4 a, b, and c).



(a) (b)



(c)

Figure 3: Effect of fenugreek seed supplemented diet on Total Serum Cholesterol (b) Serum Triglycerides (TAG) (c) High Density Lipoprotein (HDL) of Malnourished Rats
Each data is the mean of five replicates with standard error of mean
Groups with superscripts different from the control are significantly different ($p< 0.05$).

Discussion

Fenugreek seed has been reported to be an indispensable herb due to its high phytochemical and nutritional content responsible for its antioxidant, antimicrobial, and anti-inflammatory properties as supported by the proximate analysis carried out on the supplemented diets. Similar result was also reported by Abdel-Azeem (2006) and Raju (2004). The seed therefore can be used as a substitute for high protein rich food when it is supplememnted in a diet. The loss of weight experienced by malnourished rats could be attributed to increased catabolic activities such as glycogenolysis and proteolysis which was due to inadequate supply of nutrients, fenugreek seed supplemented diets significantly increased the weight at a lower rate compared to the control because of its high fibre content which promotes feelings of fullness and promotes weight loss. This supports the report made by Zenhom and Ibrahim (2020) who stated that the dietary addition of 20% fenugreek seed by-produced meal enhanced the growth performance of common carp. However, Paneru *et al*. (2022) and Duru *et al*. (2013) reported no significant increase in the weight of broilers when fed with fenugreek seed supplemented diet. Hence, the seed can be used to maintain a healthy weight as it regulates Body Mass Index and Organ Body Mass Ratio due to its galactomannan fiber content as reported by Konopelnyuk *et al.* (2015).

Trigonella foenum-graecum seed also improved the hematological parameters of malnourished rats which was altered as a result of nutrient deficiencies causing impaired blood cell production. This can be due to the high protein with essential amino acids, iron, ascorbate, and folate content of the seed. It was also reported by Doshi *et al.,* (2012) that fenugreek seed improved the hemoglobin level in females of childbearing age, Abdel-Azeem (2006) also supported improved hematological parameters in rats when fed with 7.5% fenugreek seed. This results suggest that fenugreek seed can enhance body immune response and prevent some blood health related problem.

The special amino acid content of T*rigonella foenum-graecum* known as 4-hydroxyisoleucine as well as lycine, valine, and phenylalanine play essential roles in protein synthesis, which aids protein synthesis that was altered in malnourished rats due to inadequate protein diet. Similar result was observed by Singh *et al*. (2020) who reported that fenugreek has a high nutritional profile including amino acids, hence, it promotes protein metabolism.

The accumulation of serum total cholesterol and triglycerides and reduced high density lipoprotein observed in malnourished rats can be attributed to alteration in lipid metabolism caused by endocrine adaptation that occurs in response to inadequate nutrition. The lipid profile was improved by fenugreek seed due to the galactomanan content and antioxidant property of fenugreek seed. Similar result was reported by Mehram *et al.* (2022) who observed that water extracts of fenugreek seed improved the serum lipid content, when malnourished rats are fed with 2ml of the water extract of fenugreek seed per rat per day for 8 weeks even better than olive and chicory leaves. Fedacko *et al*. (2016) also reported that it reduced high cholesterol in hypercholesterolemia patients. In addition, Yousefi *et al.* (2017) reported significantly reduced total cholesterol, triglycerides and low density lipoprotein when conducted a double-blind randomized placebo-controlled trial on 56 patients suffering from borderline hyperlipidemia to evaluate the effect of *Trigonella* seeds extract for 8 weeks. Singh *et al.* (2016) after his research, also reported that *Trigonella* seed extract monotherapy is a more potent agent in controlling lipid content as compared to the glipizide treatment and their combined therapy. Hence, fenugreek seed supplement diet can be used to treat hyperlipidemia, prevents obesity, improve lipid profile in the body due to the presence of 4-hydroxyisoleucine which improve lipid metabolism and enhance insulin sensitivity consequently reduce fat storage.

CONCLUSION

Fenugreek seed is a promising seed with high nutritional profile that can be used as a food supplement. Its supplementation in diet can be used to improve nutrient availability, maintain a healthy body weight, improve hematological parameters, and reduce body cholesterol. This can help in restoring abnormalities caused by protein-energy malnutrition and prevent nutrient related diseases. The 50 g/kg and 100 g/kg fenugreek seeds are best suitable to improve a malnourished condition.

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