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Nutritional and Biological Assessment of Wheat Biscuits Supplemented by Fenugreek Plant to Improve Diet of Anemic Rats

[1]Nabila Y. Mahmoud, [2]Rabab H. Salem and [2]Amal A. Mater

[1]Nutrition and Food Sciences Department,
[2]Food Sciences and Technology Department,
Faculty of Home Economics, Al Azhar University, Nawag, Tanta, Gharbia, Egypt

Abstract: The present study was carried out to investigate the improvement role of fenugreek (*Trigonella Foenum Graecum*) leaves, seeds (dry and germinated) and wheat flour supplemented with germinated fenugreek powder at 5 to 10% levels on iron deficiency anemia in rats. Results of nutritional characteristics revealed that fenugreek flour is a good source of protein, fat, fiber and minerals (Fe, Ca and Zn). Biscuits supplemented with 10% germinated fenugreek (GF) had the highest content of polyphenols. Supplementation of wheat flour with fenugreek flour at 5 and 10% levels increased the vitamin B_2 and β-carotene contents of biscuits. Wheat flour supplemented by 5 and 10% GF produced acceptable and high nutritive values of biscuits. The biological examination revealed that the supplemented diets with GF, fenugreek leaves (FL), germinated fenugreek biscuits(GFB) and fenugreek seeds(FS) induced the greatest improvement effect on body weight gain and feed efficiency ratio in anemic rats as compared to the other experimental groups. The hematological and biochemical analyses showed that the changes in blood picture, serum total iron binding capacity, serum proteins and minerals (iron and zinc) levels were in favor of supplementation with fenugreek products when compared to the positive control group. It can be concluded that fenugreek products have good nutritive value and positive response on blood picture and serum biochemical parameters in anemic rats. Therefore, this study recommends that intake of fenugreek products may be beneficial for patients who suffer from iron deficiency anemia due to their nutritional and restorative properties.

Key words: Fenugreek · Iron deficiency anemia · Biochemical analysis · Organoleptic testing Hematological examination

INTRODUCTION

Fenugreek (*Trigonella Foenum-Graecum*) found in nature and cultivated in India and Pakistan, is a well-known medicinal plant having properties of reducing blood sugar level [1], anthelmintic, antibacterial [2], anti-inflammatory, antipyretic [3] and antimicrobial [4]. It contains lecithin and choline that helps to dissolve cholesterol and fatty substances, minerals, B. complex, iron, phosphates, (Para-amino benzoic acid) and vitamins A and D. In addition, fenugreek, like other legumes, is a good source of dietary protein for consumption by human and animals; fatty acids from 5-10%, which are predominantly linoleic, linolenic, oleic and palmatic. It had 45-65% total carbohydrates with 15% of galactomannan (a soluble fiber) [5]. Fenugreek leaves and seeds have been extensively used to prepare extracts and powders for medicinal uses [6]. The leaves help in blood formation, while seeds are valuable to treat anemia, being rich in iron [7].

Germination is one of the methods used in elimination of various anti-nutritional factors present in foods. This is the process by which amylase degrade starches into dextrin and maltose [8]. The enzymes convert the stored foods such as insoluble carbohydrates and proteins into soluble components [9].

Biscuits are the most popular bakery items consumed nearly by all sections of the society in Egypt. Some of the reasons for such wide popularity are low cost in comparison with other processed foods, good nutritional

Corresponding Author: Nabila Y. Mahmoud, Nutrition and Food Sciences Department, Faculty of Home Economics, Al Azhar University, Nawag, Tanta, Gharbia, Egypt.

quality and availability in different forms, varied taste and longer shelf life. Bakery products are used as a vehicle for incorporation of different nutritionally rich ingredients [10, 11].

Iron deficiency is defined as decreased total iron body content. Iron deficiency anemia occurs when iron deficiency is sufficiently severe to diminish erythropoiesis and cause the development of anemia. Iron deficiency is the most prevalent single deficiency state on a worldwide basis. It is important economically because it diminishes the capability of individuals who affected to perform physical labor and it diminishes both growth and learning in children [12]. It represents a major problem in developing countries, especially in Egypt [13]. Therefore, the objectives of this study were to assess the nutritional characteristics of fenugreek leaves, dry and germinated seeds and supplemented with germinated fenugreek biscuits as rich source of iron, evaluate their effects on biological, biochemical and hematological parameters in anemic rats.

MATERIALS AND METHODS

Materials: Soft wheat flour (72% extraction) was obtained from South Cairo Mills Company, Giza, Egypt. Fenugreek green leaves, commercial fenugreek seeds (*Trigonella Foenum-Graecum*) and all other materials used in dough preparation wheat flour, sugar, shortening, baking powder, eggs and vanillin were obtained from local market in Tanta, Gharbia governorate, Egypt. Casein, vitamins, minerals and cellulose were purchased from EL-Gomhoryia Company, Cairo, Egypt. Forty-two male albino rats (Sprague Dawely Strain) were obtained from Vaccine and Immunity Organization, Helwan Farm, Cairo, Egypt.

Methods

Soaking: Fenugreek seeds (FS) were first cleaned and freed of broken particles, dust and other foreign materials and then soaked in tap water for 12 hr at ambient temperature (25°C), with seeds to water ratio of 1:5 (w/v). The non-imbibed water was discarded.

Germination: The soaked seeds were separately spread on four wet jute bags, covered with muslin cloth and other wet jute bags. Water was sprinkled on the seeds every 12 hr until the end of germination periods (72hr). The germinated seeds were picked carefully with the sprouts, washed, drained, oven dried at 50°C for 24 hr, milled and stored in name labeled polyethylene bags prior to analysis [14].

Preparation of Blends: Fenugreek germinated seeds powder was blended separately with wheat flour at different levels (0, 5 and 10%).

Preparation of Biscuits: The sweet biscuits were prepared by using the AACC method [15]. Preparation of biscuits was carried out using wheat flour replaced; separately with 5 and 10%, germinated fenugreek seeds flours. The biscuits were baked at 160°C for 20 min. After cooling for 1hr, the organolyptic evaluation was carried out. After baked, the yield of control biscuits was 360 (g) biscuits.

Nutritional Characteristics

Chemical Composition: Fenugreek leaves (FL), fenugreek seeds (FS), germinated fenugreek (GF) and prepared biscuits (CB and GFB) were chemically analyzed for moisture, crude protein, fat, crude fiber and ash according to methods described in [16]. Total carbohydrates were calculated by difference. The energy value was calculated using the Atwater factors of 4, 9 and 4 for protein, fat and carbohydrate, respectively [17].

Total Minerals: Samples were wet acid-digested, using a nitric acid and perchloric acid mixture (HNO_3:$HClO_4$, 5:1 w/v). The total amounts of Ca, Fe, Mn and Zn in the digested samples were determined by atomic absorption spectrophotometry (Thermo–Elmental, Model 300VA, UK) [18].

Anti-nutritional Factors: Phytic acid was determined by the method of Hang and Lantzsch [19]. Total polyphenols were extracted by the method of Singh and Jambunathan [20] and estimated as tannic acid equivalents, according to the Folin–Denis procedure [21].

Vitamin C Analysis: Vitamin C levels were spectrophotometrically (Model No 6300, Designed and manufactured in UK by I en way LTD) analyzed by the method in which 2, 6 -dichlorophenol endophenol dye is reduced by ascorbic acid [22].

Carotene Analysis: Carotenoids were analyzed by reversed phase HPLC using water 600 system equipped with auto sampler injector, degasser, pump and water 996 UV – visible photodiode array detector operating at 450nm. The data were stored and processed by means of Millennium 4.00 software (Waters, Stockholm, Sweden). Absorption spectra were recorded between 250 and 500 nm [23].

Sensory Evaluation of Biscuits: Biscuits produced by using suggested blends were evaluated for their sensory characteristics by ten panelists from the staff of Food Technology Dep., Faculty of Home Economic, Al-Azhar University. The scoring scheme was established as mentioned by Zobik and Hoojjat [24] as followed: Color (20), taste (20), odor (20), texture (20), appearance (20) and overall score 100 degrees.

Experimental Design: Forty two male albino rats (Sprague Dawely Strain) weighing (140±10 g) were used in the study. After the adaptation period of seven days, rats were randomly divided into two main groups, the first group included control (-) group, each of 6 rats. The second group (n= 36 rats), animals were fed on basal diet containing 20g/kg body weight tannic acid to induce iron deficiency anemia [25] for three weeks. One sub group (6 rats) was left as control (+), while the other 5 sub-groups were fed on basal diet supplemented by either FS 5%, GF 5%, FL 5%, control biscuits (CB)20% and GFB (fortified by 5% GF) 20% respectively. Feeding trail continued for three weeks.

Biological Evaluation: Body weight gain (BWG), feed efficiency ratio and organs weight relative to body weight (%) were calculated according to Chapman *et al.* [26].

Biochemical Analysis: At the end of experiment, the rats were fasted overnight. On the morning of the next day, the rats were euthanized by ether anesthesia and one set of blood samples was withdraw from eye plexus of veins into tubes each contains 5 ml Drabkins solution and used for determination of blood picture according to Betk and Savelsberg [27]. After sacrifice of rats, other set of blood samples was collected from the portal hepatic vein for separation of serum, which used for biochemical analysis according to the method described by Drury and Wallington [28]. Blood samples were taken with a micro capillary tube and centrifuged at 5000 rpm for 5 min. The volume of blood cells was measured by using a graded scale, (MCV), (MCH), (MCHC), red blood cell, white blood cells and platelets count were measured according to Fischbach [29]. Serum samples were analyzed for determination of total protein according to Sonnenwirth and Jaret [30] and serum iron according to Wick *et al*. [31]. Serum zinc concentrations were measured by flame atomic absorption technique according to Tietz [32], total iron binding capacity(TIBC) according to Yamanishi *et al.* [33], ferritin according to White *et al*. [34] and transferrin saturation was calculated as (serum iron/TIBC × 100).

Statistical Analysis: Data were presented as means ± standard deviation (SD). Values were statistically analyzed by one-way analysis of variance (ANOVA test) according to Sendecor and Cochran [35] using SPSS 10.1 software package. Differences were considered significant at P values ≤ 0.05.

RESULTS AND DISCUSSION

Nutritional Characteristics

Chemical Composition: Data presented in Table (1) illustrated the chemical composition percentage of raw materials and fenugreek supplemented biscuits. From the obtained data, it could be noticed that fenugreek leaves contained (on dry weight basis) 14.14% dry matter, 4.32% protein, 91.06% carbohydrate and 387.46 kcal energy. These results were nearly similar to the findings obtained by Anon [36]. The effect of germination on the chemical composition of fenugreek seeds showed significant increase ($P \leq 0.05$) of crude protein (on dry weight basis). These results agree with the earlier reports of increased protein content during germination of various cereals, legumes and other seeds [37, 38]. Such increase could be attributed to a net synthesis of enzymic protein (e.g. proteases) by germinating seeds [39]. Other researchers have attributed the increase to the degradation of stored protein and synthesis of new protein. From the same table, there were high significant differences ($P \leq 0.05$) in carbohydrate and total lipid content between fenugreek seed samples on dry weight basis. The observed decrease in the fat content of the germinated seeds might be due to the increased activities of the lipolytic enzymes during germination. The decreased carbohydrate levels of the germinated seeds might be due to increase in α-amylase activity, which breaks down complex carbohydrates to simpler and more absorbable sugars which are utilized by the growing seedlings during the early stages of germination [37, 40].

As shown in Table (1), there were high significant differences ($P \leq 0.05$) among biscuits fortified with GF as compared to the control for crude protein, crude fibers and total carbohydrates. Moisture, crude protein, fat and crude fiber increased by increasing the GF level. Biscuits supplemented with 10% GF had the highest protein content (15.31%), whereas total carbohydrate contents decreased in biscuits fortified with GF (51.11%) compared to the control (70.91%). These results confirm the previous results of Eissa *et al.* [41] and Hooda and Jood [42]. This was also consistent Sharma and Chauhan [43]. Our results indicated that, supplementation of wheat flour with germinated fenugreek flour at 5 to 10% levels increased the protein and fiber contents of biscuits.

Table 1: Chemical composition (%) of raw materials and fenugreek supplemented biscuits (on dry weight basis).

Component	LF	FS	GF	control	W:GFF 95:05:00	W:GFF 90:10
Crude protein	4.32±2.2f	27.99±1.18b	32.04±1.42a	7.53±1.54e	11.50±0.80d	15.31±1.23c
Total lipid	0.66±0.49d	8.39±1.187c	6.78±0.72c	18.87±1.89b	24.41±3.15a	27.24±2.32a
Crude fiber	1.59.±0.54c	9.83±1.37a	10.95±1.19a	2.45±0.35c	3.31±2.13bc	5.46±1.85b
Total ash	2.37±.0.55c	5.88±1.29a	4.48±1.04ab	2.22±1.48c	2.86±1.04bc	2.76±0.98bc
Carbohydrate*	91.06±1.73a	50.06±.2.22d	41.31±1.52e	70.91±3.28b	60.50±.3.69c	51.11±4.41d
Moisture (% fresh base)	85.14	9.65	10.48	3.15	3.36	3.54
Total metabolizable energy (kcal)	387.46±2.57d	387.76±6.59d	361.09±6.89e	489.25±13.51a	496.93±10.76b	460.86±9.22c

* Carbohydrates were calculated by difference. M±SD = means and standard deviation of triplicate trails. Significant at $P < 0.05$ using ANOVA test. (a, b, c, d, insignificantly different between two comparison groups within the same letter and column using Duncan Multiple Range test).

Table 2: Total minerals content of raw materials and fenugreek supplemented biscuits (mg/100 g dry weight basis)

Component	LF	FS	GF	control	W:GFF 95:05:00	W:GFF 90:10
Ca	618.41±12.05^{a}	86.77±7.37^{b}	84.83±6.45^{b}	33.66±3.23^{e}	46.51±5.89^{d}	56.17±3.01^{c}
Fe	111.13±9.54^{a}	54.81±2.08^{c}	89.45±1.17^{b}	3.21±0.87^{e}	8.01±0.12d^{e}	11.95±2.12^{d}
Zn	3.44±0.60^{cd}	7.00±1.49^{b}	10.80±1.67^{a}	2.17±0.73^{e}	2.88±0.36d^{e}	4.36±0.98^{c}
Mn	1.77±.0.13^{c}	6.80±1.05^{a}	3.81±0.41^{b}	0.19±0.03^{d}	0.34±0.22^{d}	0.48±0.07^{d}

Table 3: Phytic acid and polyphenols content of raw materials and fenugreek supplemented biscuits (mg/100 g dry weight basis)

Component	LF	FS	GF	control	W:GFF 95:05:00	W:GFF 90:10
Polyphenol	817.14±10.05^{a}	290.04±4.37^{b}	217.03±4.87^{c}	138.84±3.13^{f}	174.59±4.81^{e}	1196.58±2.51^{d}
Phytic acid	122.88±2.24^{e}	308.92±1.28^{a}	218.02±1.07^{c}	184.51±1.87^{d}	215.06±1.12^{c}	230.58±2.82^{b}

Total Minerals: The results in Table (2) showed the mineral contents of raw materials and fenugreek supplemented biscuits. Such results are partially in agreement with those obtained by Anon [36]. Germinated seeds had the significantly highest iron content 89.45mg/100 g. The increase might be due to the hydrolytic enzymes released more free Fe from its organic complexes. These results were similar with those reported by Echendu *et al*. [44]. The higher Mn for the non-germinated seeds might not be bioavailable as compared with the germinated samples because the Mn may not be released from the organic complexes. Biscuits supplemented with 10% GF had the highest Fe (11.95) and Ca(56.17) mg/100 g. The increase in iron, zinc and calcium of fenugreek-supplemented biscuits can be attributed to the high content of those ingredients in fenugreek. These results are in line with Hussein *et al*. [45]. Previous study [46] suggested that germinated fenugreek flour use could be exploited as functional and nutritional food as well as therapeutic agent.

Anti-Nutritional Factors: Data presented in Table (3) illustrate the phytic acid and polyphenol contents of raw materials and fenugreek supplemented biscuits .It is obvious that, there were significant differences ($P \leq 0.05$) in polyphenol contents between all samples under study. Fenugreek leaves had higher polyphenol contents 817.14mg/100g. Germinated seeds had the lowest polyphenol contents 217.03 mg/100 g. As shown in Table (3), there were significant differences ($P \leq 0.05$) in polyphenols content between biscuits fortified with GF and the control group. Biscuits supplemented with 10% GF had the highest polyphenols content (196.58 mg/100g). The increase in polyphenols content of fenugreek supplemented biscuits can be attributed to the high content in fenugreek. These results were confirmed the results of Sharma and Chauhan [47]. From the obtained data, it was observed that ungerminated seeds had the highest phytic acid content (308.92 mg/100g). The higher phytic acid contents for the ungerminated seeds might not be to the tannin activity during germination. These results agree well with those reported by El-Adawy [48]. Results in Table (3) showed that biscuits supplemented with 10% GF had the highest phytic acid content (230.58 mg/100 g). These findings agreed with those obtained by Sharma and Chauhan [47]. Supplementation of wheat flour with a small percentage of fenugreek flour has been reported to enhance the nutritional quality of biscuits.

Vitamins Composition: The results in Table (4) showed vitamins composition of raw materials and fenugreek supplemented biscuits. It could be noticed that, fenugreek leaves contained (on dry weight basis)

Table 4: Vitamins composition of raw materials and fenugreek supplemented biscuits (mg/100 g dry weight basis).

Component	LF	FS	GF	Control	W:GFF 95:05:00	W:GFF 90:10
β-carotene	9.99±1.90[a]	2.54±1.08[c]	4.78±2.43[b]	1.10±0.13[d]	1.28±0.49[d]	1.61±1.01[d]
B_2	88.80±8.54[a]	34.49±2.38[c]	49.63±4.17[b]	10.63±0.87[f]	16.70±2.12[e]	21.09±2.76[d]
E	3.28±0.80[a]	0.68±0.19[b]	1.35±0.67b	2.36±0.83[a]	2.96±0.26[a]	3.04±1.98[a]
C	284.29±.10.13[a]	84.28±4.05[c]	100.28±9.41[b]	0.44±0.03[d]	0.54±0.22[d]	0.61±0.17[d]

Table 5: Sensory properties of biscuits supplemental with different level of germinated fenugreek flour

Type	Color (20)	Taste (20)	Texture (20)	Appearance (20)	Odor (20)	Overall score (100)	Acceptance
Control	18.99±0.56[a]	18.34±1.00 [a]	18.56±0.84 [a]	18.73±1.04 [a]	19.47±1.31 [a]	94.09±2.05 [a]	VG
W:GFF 95:5	14.63±0.45 [b]	16.60±0.27 [ab]	18.26±0.62 [a]	18.10±0.84 [a]	16.40±1.12 [b]	83.98±2.08 [b]	G
W:GFF 90:10	14.45±0.78[b]	15.54±1.38 [b]	17.33±0.90 [a]	16.44±1.26 [b]	15.06±0.71 [b]	78.81±1.31 [c]	G

90-100 Very Good (VG) 80-89 Good (G) 70-79 satisfactory (S) Less than 70 Questionable (q)

Table 6: Mean values±SD of body weight gain and feed efficiency ratio in rats with iron deficiency anemia and treated groups (n= 6)

Groups	BWG%	BWGT%	FER	FERT
C (-)	11.3±4.5[c]	14.7±3.6[b]	10.10±1.77[b]	16.13±9.09[c]
C (+)	3.4±3.0[a]	6.2±2.0[a]	3.08±3.07[a]	5.21±9.95[a]
FS 5%	3.2±3.8[a]	17.2±4.9[b]	2.45±1.22[a]	10.87±4.80[b]
GF5%	3.6±6.8[b]	21.8±5.2[c]	3.74±3.52[a]	18.13±9.09[d]
FL 5%	3.4±8.1[a]	18.4±5.7[b]	3.08±4.07[a]	17.30±3.99[b]
CB 20%	3.7±2.7[b]	16.3±3.9[b]	2.61±1.14[a]	13.67±4.80[b]
GFB 20%	3.8±2.5[b]	19.6±3.9[b]	2.55±1.41[a]	16.94±6.22[b]

C(-): Control negative C(+): control positive BWGT%: body weight gain of treated groups. FERT: feed efficiency ratio of treated groups.

(9.99mg/100g) β-carotene,(88.80mg/100g) vitamin $B_2$and (284.29mg/100g) vitamin C. In addition, fenugreek leaves had the highest vitamin C content, which is an important dietary antioxidant. These results were in line with the findings obtained by Srinivasan [49]. Germinated seeds had the highest B_2 content 49.63mg/100 g. These results were in line with the findings obtained by Hussein *et al*. [45]. On the other hand**,** there were insignificant differences in vitamin E content between fenugreek seed biscuits samples**.** In the same table, there were high significant differences ($P \leq 0.05$) among biscuits fortified with GF compared to the control for B_2 contents. Biscuits supplemented with 10% GF had the highest B_2 (21.09 mg/100 g). As shown in Table (4), there were insignificant differences among biscuits fortified with GF as compared to the control for β-carotene, vitamin E and vitamin C. Our results indicate that, supplementation of wheat flour with fenugreek flour at 5 and 10% levels increased the vitamin B_2 and β-carotene contents of biscuits.

Organoleptic Characteristics: Data given in Table (5) showed that sensory properties of biscuits supplemental with different levels of germinated fenugreek flour. From the mentioned result, it could be observed that, with the increase in the level of GF flour in the formulation, the sensory scores for color, odor, taste, texture, appearance and overall score of biscuits decreased sharply. The best value was for control biscuits (94.09) followed by biscuits supplemented with 5% GF flour (83.99) and biscuits supplemented with 10% (78.82). From the obtained data, it could be showed that, there were insignificant differences in texture score among the samples, (control or supplemented with different levels of GF flour). These results are in line with the findings obtained by Eissa *et al.* [41] and Sharma and Chauhan [43]. The organoleptic evaluation revealed that wheat flour could be supplemented using 5 and 10% GF flours to produce acceptable and high nutritional value.

Biological Evaluation

Body Weight Gain (BWG) and Feed Efficiency Ratio (*FER g*): As shown in Table (6) the addition tannic acid to basal diet induced significant decrease ($p<0.05$) in body weight gain (BWG%) and FER and the highest significant values were in group treated with GF (21.8±5.2% and 18.13±9.09 g respectively) followed by that treated with FL, GFB, CB and FS as compared with control (+) and control (-)groups. These findings were in agreement with those obtained by Ibrahium and Hegazy [50]. who noted a significant increase in BWG and FER when rats fed on FSF-biscuit diets. These effects are reflected by growth inhibition, negative nitrogen balance, reduced intestinal absorption of sugars and amino acids, reduced immune response and increased liver and protein catabolism.

Table 7: Mean values±SD of blood picture of rats with iron deficiency anemia and treated groups (n= 6)

Groups	Hbg/dL	Hct%	MCVfL(μm^3)	MCHPg	MCHCg/dL	Rbcs10^6/µL	Wbcs10^3/µL	Platelets fL(μm^3)
C (-)	15.6±1.5^c	46±4.7^c	55±3.5^e	18.7±1.1^e	33.9±1.6^d	8.3±0.9^d	3.8±1.5^a	7.7±0.9^d
C (+)	8.7±0.9^a	27±5.4^a	65±3.9^a	21.2±1^a	32±2.1^a	4.1±0.5^a	2.8±0.8a	5.8±0.8^a
FS 5%	12.4±0.6^b	36±1.8^b	63±1.0^{bc}	21.7±1^{ab}	34±2.3^b	5.7±0.3^b	2.6±0.9^a	6.6±0.3^d
GF5%	13.5±0.7^b	43±4.1^c	60±1.8^d	19±0.9^d	31±2.4^c	7.1±0.9^c	3.1±1.4^a	7.5±0.4^{cd}
FL 5%	14.9±1.1^c	45±4.9^c	59±3.6^e	19.6±1.2^e	33±3.1^c	7.6±0.8^b	3.3±1.2^a	7.8±0.7^d
CB 20%	12.9±0.9^b	36±2.3^b	58±1.2^e	20.8±0.9^{bc}	35±1.2^b	6.2±0.2^{bc}	2.8±1.8^a	6.8±0.4^{bc}
GFB 20%	13.1±0.7^b	37±1.9^b	57±1.8^{cd}	20.4±0.8^{cd}	35±1.4^b	6.4±0.4^{bc}	2.9±1.5^a	7.1±0.6^{bcd}

Table 8: Mean values±SD of minerals and iron contents of rats with iron deficiency anemia and treated groups (n= 6)

Groups	Total Iron µg/dL	Zinc µg/dL	T. protein g*/dL*	TIBC µg/dL	Ferritin μg/L	Transferrin Saturation%
C (-)	0.89±0.04^c	3.8±0.2^b	6.3±0.72^{cd}	210±4.1^b	223±9.3^c	0.43±0.63^b
C (+)	0.28±0.03^a	3.5±0.14^a	3.4±0.39^a	396±7.1^c	89±1.2^a	0.14±0.18^a
FS 5%	0.74±0.04^b	3.5±0.02^b	5.9±0.33^{ab}	200±4^{ab}	123±4.8^a	0.49±0.32^b
GF5%	0.85±0.02^{bc}	3.6±0.12^b	6.5±0.34^d	165±1.8^{ab}	196±4.6^{bc}	0.43±0.16^b
FL 5%	0.87±0.03^c	3.8±0.19^b	6.1±0.32^{abc}	155±1.8^a	215±8.3^{bc}	0.44±0.87^b
CB 20%	0.76±0.04^b	3.5±0.12^b	5.6±0.29^b	180±1.7^{ab}	136±5.6^{ab}	0.47±0.61^b
GFB 20%	0.78±0.05^b	3.7±0.15^b	6.1±0.45^{abc}	185±1.8^{ab}	166±6.3^{abc}	0.45±0.27^b

Blood Picture: Data in Table (7) show the concentration of hemoglobin (Hb) content, hematocrit percent (Hct%), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), red blood cells (Rbcs 10^6/µL), white blood cells (Wbcs10^3/µL) and platelets count fL(μm^3) of all groups. The above mentioned hematological parameters were significantly decreased ($p<0.05$) by addition of tannic acid to basal diet. Diets supplemented with fenugreek products caused a significant increase in hemoglobin content. The treated groups with FL showed the highest content hemoglobin, which was close to the normal values of control rat group. and followed by GF, GFB, CB and FS. This result was in agreement with those of Kaosar *et al*. [25] and Rothenbacher and Sherman [51] who reported that eighteen-day old pups of rats fed the iron deficient diet developed an anemia characterized by lower blood hemoglobin and lower packed cell volume levels than rats fed the diet containing 307ppm iron. Ibrahium and Hegazy [50] also, indicated that rats fed on FSF-biscuit diets showed a good hematological response by higher ($p<0.05$) values of blood hemoglobin (Hb) and hematocrite (Hct%), which related to failure of erythropoiesis and increased plasma volume.

In the same table data showed that the control +ve group was significantly decreased ($p<0.05$) Rbcs 10^6/µL as compared with the control negative group. All supplemented diets of anemic rats revealed an enhancement of red blood cells count as compared to the control (+). The best results were obtained for diets supplemented with FL and GF (7.6±0.8 and 7.1±0.9 10^6/µL respectively) as compared with the control negative group. On the other hand, Wbcs10^3/µL did not have any significant changes as compared to the control positive group. These data were in agreement with those of Udayasekhara *et al*. [52] who reported differential WBC counts were not different when studied a short term (90 day) of fenugreek seeds of weanling rats. The platelets count fL(μm^3) showed significant increase ($p<0.05$) in all supplemented diets as compared with the control positive group. These values were actually close to the normal (-) group. These results agrred with those obtained by Ranu *et al.* [53] who found significant protection to bone marrow and erythropoietic cells, which subsequently maintained the normal values of Hb and Hct in peripheral blood and increases the number of RBC by *Trigonella foenum* seeds extract (TFE).

Biochemical Analysis: Data in Table (8) showed serum total iron, zinc (µg/dL), total protein (g /dL), total iron binding capacity, ferritin and transferrin saturation(%) of all normal and anemic treated groups. As shown in this table, the mean values of total iron in all treated groups were significantly higher ($p<0.05$) when compared with control (+) group especially with supplemented rat's groups with FL and GF (0.87±0.03 0.85±0.02 µg/dL respectively). Serum zinc values decreased when rats fed on tannic acid diet compared to control negative group, but treated groups recorded significantly higher ($p<0.05$) values which closed to normal rats group. These results agree with Kaosar *et al.* [25] who demonstrated that more than 10 g tannic acid/kg diet induced anemia by reducing the Fe absorption and due to decreased serum Fe concentration.

In the same table mean values of serum total protein showed a significant decrease ($p<0.05$) by addition of tannic acid to basal diet. The total protein levels in the treated groups with fenugreek products were significantly increased when compared with the control (+) group especially the groups supplemented with GF(6.5±0.34 g/dL) which showed the best result closed to normal group (-) and followed by supplemented groups with FL, GFB, CB and FS. These results were in agreement with those reported by Oler *et al.* [54] who explain the mechanism of the effect of high tannic acid dose by causing degranulation of the rough endoplasmic reticulum, inhibition of protein synthesis and disaggregation of polysomes.

Concerning serum iron content the results illustrated that, the total iron binding capacity (TIBC) elevated in serum level when rats fed on tannic acid diet as compared to control negative group. The treated groups have significantly lower ($p<0.05$) TIBC values. The best result was in the group fed on diet supplemented with FL(155±1.8) and followed by GF, GFB, CB and FS groups. The results of transferring saturation percentage showed significantly higher ($p<0.05$) in all treated groups when compared with control (+) group. Thus serum ferritin recorded significant increased ($p<0.05$) in serum values in treated groups of FL and GF (215±8.3 and 196±4.6 respectively) compared with control (+) group. These results agree with Andrewsp *et al.* [55] who mentioned that, serum ferritin concentration correlates with tissue iron stores. So, serum ferritin is probably the most reliable indicator of total body iron stores in large species.

In conclusion, this nutritional and biological trial proved that supplementation of basal diets with fenugreek leaves, seeds (dry and germinated) and wheat flour supplemented with germinated fenugreek powder at 5 to 10% levels increased the total proteins, fibers, iron, zinc, calcium, vitamin B_2, β-carotene, vitamin E and vitamin C contents. These dietary supplements also improve the blood picture of anemic rats so they have nutritive and restorative properties. The daily use of fenugreek products as a dietary supplement is proved to be safe and healthy. Therefore, this study recommends that intake of fenugreek products may be beneficial for patients who suffer from iron defiency anemia owing to their nutritive and restorative values.

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