

The therapeutic role of alcoholic extract of fenugreek seeds on hypothyroidism state induced by thiourea and some blood parameters in adult male rabbits

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Summary

This study was carried out to investigate the effect of the alcoholic extract of fenugreek seed (*Trigonella foenum graecum*) on thyroid gland function in adult male rabbits exposed to thiourea that induce hypothyroidism state. Thirty adult male rabbits weighing 1500-2000 gm were used, and randomly divided into five equal groups (6 rabbits/group) and treated daily for 42 days as follows: First group (control), (T1) group were treated with alcoholic extract of fenugreek seed (200mg/ kg B.W. orally), (T2) group were treated with thiourea (10 mg/kg. B.W. orally), (T3) group were treated once thiourea daily by (10 mg/ kg. B.W) and after (3) hours given alcoholic extract of fenugreek 200 mg/kg B.W., and (T4) group were treated once thiourea daily by (10mg /kg B.W) and after (3) hour given levothyroxine (10 ug/kg B.W). Blood samples were collected at (0, 14, 28 and 42) days of the experiment for measuring the concentration of hematological parameters. Blood parameters (HB, RBC, WBC, and PCV) were analyzed in this study. The results showed significant increase in (HB, RBC, WBC and PCV) in (T1) group significantly decreased in (T2) groups, while (T3 and T4) groups showed a significant increase of these parameters and their values appeared closely to control group. In conclusion supplementation of fenugreek seeds extract and levothyroxine have therapeutic effects against harmful effect of thiourea.

Keywords: Fenugreek seeds, Thiourea, Hypothyroidism.

Introduction

Fenugreek (*Trigonella foenum graecum*) is annual herb that belongs to the family Leguminosae widely grown in Pakistan, India, Egypt, and Middle Eastern countries (1). Fenugreek seeds (El Helba) it used for thousands of years to reduce blood sugar, increase lactation, stomach ulcers, appetite loss, fever, catarrh of the respiratory tract, bronchitis, pellagra and eczema (2). The biological and pharmacological actions of fenugreek are attributed to the variety of its constituents, namely: steroids compounds, polyphenolic substances, volatile constituents, amino acids (3). Fenugreek seeds are rich source of the polysaccharide (galactomannan). They are also a source of saponins such as gitogenin, tigogenin and neotigogens. Other bioactive constituents of fenugreek include mucilage, volatile oils and alkaloids such as choline and trigonelline, sotolone and pyrazines. Bitterness of fenugreek seeds is mainly due to the oil, steroidal saponins and alkaloids which are all nontoxic on consumption (4). Fenugreek is one of the most medicinal plants which are widely used in folk medicine. It has a diuretic, uterine and

cardiotonic, hypotensive, hypolipidemic, hypoglycemic, hyperinsulinemic, ant diuretic effects, antinociceptive and anti-inflammatory (5 and 6). Also type II diabetes is a disease characterized by chronic hyperglycemia and oxidative stress (7). Thiourea type goitrogen that interferes with the organic binding of iodine with tyrosine in the thyroid gland consequently results in malfunctioning of the thyroid gland and exhibits antagonistic effects (hypothyroidism) (8). There have been sporadic reports on hypothyroidism vis-à-vis productive and reproductive performances in ruminants, mainly cattle (9). Levothyroxine is one of the thirteen most commonly prescribed medications. It given either as physiologic replacement therapy in patients with hypothyroidism or as interventional therapy to suppress TSH secretion in patients with nodular thyroid disease or thyroid cancer (10). Aims of the study: Furthermore, there is little information about the effect therapeutic role of fenugreek seeds on male thyroid gland against the hypothyroidism state. The aim of the present study is evaluation the therapeutic role of fenugreek seeds extract comparatively with levothyroxin against hypothyroidism induced

by thiourea and measurement hematological parameters involve RBC, WBC, PCV and HB.

Materials and Methods

The fenugreek seeds were collected from local market and air dried in the shade, grounded into a fine powder by use coffee grinder and weighing 100 gm and then put it in a volumetric conical flask. Then 1000 ml of 70% ethyl alcohol was added to the powder which made the ratio 1/10 W/V. After that the mixture was shaken by using magnetic stirrer apparatus for 24 hr, the mixture was filtered by using 4 layers of medical gauze and then was filtered again using No.1 filter paper. The filtrated mixture was concentrated by using incubator on 40°C for 72 hr, to obtain crude extract. This extract was stored in a dark sterile screw bottle in (4°C) until use (11).

Six to nine months old male rabbits (1500-2000) gm were used randomly divided in to five groups each group consist a six rabbits and handled as follows for 42 days: Group C (Control), group (T1) were treated orally with 200 mg/kg. B.W. of alcoholic extract of fenugreek seeds (12), group (T2) rabbits were treated orally with 10 mg/kg B.W. thiourea (13), group (T3) rabbits were treated orally with thiourea 10 mg/kg B.W. and with alcoholic extract of fenugreek seeds 200 mg/kg and group (T4) rabbits were treated orally daily of thiourea 10 mg/kg B.W. and with levothyroxine 10 ug/100 g B.W. (14). Fasting blood samples were collected at 0, 2, 4 and 6 weeks of the experiment by cardiac puncture technique for hematological studies (15).

A Total Erythrocyte Count (Cell X 10⁶/L): was determined by using hemocytometer with neubauer slide (16) as the following equation:

$$T.E.C = \text{No. of cell in 5 medium squares} / 80 \times 400 \times 200 \times 10 = \text{RBC} / \text{mm}^3$$

Total leukocyte counts (cell x 10³/L): Estimation of total leukocyte counts (17) through the following equation:

$$T.L.C = \text{No. in 4 large squares} / 4 \times 20 \times 10 = \text{cell} / \text{mm}^3$$

Hemoglobin concentration, Hb: The Hb (g/d) evaluation was determined by whereas usina cyanomethemoglobin methodod by using Drabk, n's reagent (18) as the following equation:

$$Hb \text{ (g/dL)} = Hb/1000 \times 251 \times \text{Sample} / \text{Standard Hb}$$

Packed cell volume Estimation, PCV (%): Furthermore, the micro-haematocrit method was used to determine the percentage of packed blood cell volume (19 and 20).

Results and Discussion

Table (1) showed a general trend for the hemoglobin value to increase in the alcoholic extract of fenugreek seeds treated group (T1) (200mg/kg B.W) as compared to that of control and reach the significance level at last week. While thiourea treated group (T₂) showed a significant decrease in the level of (P<0.05) at all period treatment. Alcoholic extract of fenugreek seed and levothyroxine (T3 and T4) groups caused a significant increase in the level of (P<0.05) in HB concentration as compared with T2 and control groups at the last period of experimentation.

Table, 1: Effect of alcoholic extract of fenugreek seeds, thiourea and levothyroxine on hemoglobin (HB) concentration (g/dl) in adult male rabbits.

Groups	Control	T1 Group alcoholic extract of Fenugreek seeds (200 mg /kg B.W)	T2 Group Thiourea (10 mg/kg B.W)	T3 Group Thiourea and alcoholic extract of Fengreek seeds	T4 Group Thiourea and Levothyroxine (10mg/kg B.W.)
Zero day	11.61 ± 0.29 A a	11.23 ± 0.43 A b	11.45 ± 0.56 A a	11.51 ± 0.28 A a	10.85 ± 0.66 A b
2 weeks	11.65±0.31 A a	11.68 ± 0.27 A ab	10.51 ± 0.21 B a	10.03 ± 0.44 B a	11.21 ± 0.54 B a
4 weeks	11.62 ± 0.20 A a	12.11 ± 0.26 A ab	10.22 ± 0.70 B a	11.85 ± 0.37 A a	12.08 ± 0.48 A a
6 weeks	11.60 ± 0.19 A a	12.87 ± 0.15 C ab	10.15 ± 0.82 B a	12.55 ± 0.39 C a	12.01 ± 0.48 A a

Different small letters means significant (P ≤ 0.05) results between groups.
Different capital letters means significant (P ≤ 0.05) results between periods.

Depending on the results in (Table, 2), there was significant increase ($P < 0.05$) in PCV% of the T1 group (alcoholic extract of fenugreek seed group) at 4 and 6 weeks interval as compared with the control group. While thiourea treated group (T2) appears a significant decrease ($P < 0.05$) in PCV% compared with T1 and control groups. The

results have also shown that a treatment dose of alcoholic extract of fenugreek seed and levothyroxine (T3 and T4) caused a significant increase ($P < 0.05$) of a PCV % at 4 and 6 weeks of the experimental period as compared with thiourea treated group (T2) also T4 group showed that PCV % reach the same level as in the control group.

Table, 2: Effect of alcoholic extract of fenugreek seeds, thiourea and levothyroxine on PCV% in adult male rabbits

Groups Time	Control	T1 Group alcoholic extract of Fenugreek seeds (200 mg /kg B.W)	T2 Group Thiourea (10 mg/kg B.W)	T3 Group Thiourea and alcoholic extract of Fengreek seeds	T4 Group Thiourea and Levothyroxine (10mg/kg B.W.)
Zero day	40.16 ± 0.98 A a	40.00 ± 2.25 A a	40.68 ± 0.81 A a	40.00 ± 0.57 A a	40.68 ± 0.73 A a
2 weeks	41.37 ± 1.13 A a	44.66 ± 1.22 A a	36.66 ± 2.02 C a	39.66 ± 1.40 C a	39.33 ± 0.61 C a
4 weeks	41.30 ± 1.77 A a	45.53 ± 0.80 B a	37.00 ± 2.62 C a	42.23 ± 0.85 B b	40.41 ± 0.87 A a
6 weeks	40.00 ± 1.39 A a	45.16 ± 0.83 B a	32.00 ± 1.31 C b	43.53 ± 1.30 B a	41.45 ± 0.35 A a

Different small letters means significant ($P \leq 0.05$) results between groups.
Different capital letters means significant ($P \leq 0.05$) results between periods.

The RBC count of control and treated groups are presented in (Table, 3). There were significant increases in RBC counts of (T1) group at 2, 4 and 6 weeks interval as compared with the control group. While thiourea treated group (T2) caused a significant decrease in

RBC counts at 4 and 6 weeks as compared with (T1) and control groups. The results of T3 and T4 groups showed significant increase of RBC counts at 4 and 6 weeks of the treatment period as compared with (T2) and control group.

Table, 3: Effect of alcoholic extract of fenugreek seeds, thiourea and levothyroxine on (RBC) concentration ($N \times 10^6$ cells/L) in adult male rabbit.

Groups Time	Control	T1 Group alcoholic extract of Fenugreek seeds (200 mg /kg B.W)	T2 Group Thiourea (10 mg/kg B.W)	T3 Group Thiourea and alcoholic extract of Fengreek seeds	T4 Group Thiourea and Levothyroxine (10mg/kg B.W.)
Zero day	4.35 ± 0.27 A a	4.39 ± 0.14 A b	4.80 ± 0.17 A a	4.51 ± 0.13 A ab	4.55 ± 0.28 A ab
2 weeks	4.11 ± 0.22 A a	4.75 ± 0.14 B ab	4.40 ± 0.22 A a	4.70 ± 0.17 A a	4.10 ± 0.14 A b
4 weeks	4.12 ± 0.29 A b	5.15 ± 0.16 D a	3.20 ± 0.17 C b	4.83 ± 0.22 B ab	4.88 ± 0.23 B a
6 weeks	4.00 ± 0.10 A b	5.61 ± 0.094 D a	3.11 ± 0.079 C c	5.26 ± 0.14 D a	4.60 ± 0.22 B ab

Different small letters means significant ($P \leq 0.05$) results between groups.
Different capital letters means significant ($P \leq 0.05$) results between periods.

Table (4) illustrates the mean value of WBC counts in the control and treated groups through the experimental period. It showed a significant decrease of WBC counts in rabbits that consumed thiourea (T2) group as compared with the control group at all periods. While (T1) group didn't cause any significant

differences in WBC counts as compared with the control group. On the other hand (T3) showed a significant increase in WBC counts compared with thiourea treated group, while treatment of thiourea with thyroxin (T4) did not cause any significant differences as compared with the same group.

Table, 4: Effect of alcoholic extract of fenugreek seeds, thiourea and levothyroxine on (WBC) concentration (Cell x 10³/L) in adult male rabbits.

Groups Time	Control	T1 Group alcoholic extract of Fenugreek seeds (200 mg /kg B.W)	T2 Group Thiourea (10 mg/kg B.W)	T3 Group Thiourea and alcoholic extract of Fenugreek seeds	T4 Group Thiourea and Levothyroxine (10mg/kg B.W.)
Zero day	4.11 ± 0.079 A a	4.55 ± 0.099 A a	4.03 ± 0.20 A a	4.07 ± 0.35 A a	4.62 ± 0.17 A a
2 weeks	4.21 ± 0.35 A a	4.35 ± 0.84 A a	3.39 ± 0.11 B a	3.88 ± 0.59 B a	3.43 ± 0.69 B b
4 weeks	4.36 ± 1.08 A a	± 0.844.21 A a	3.68 ± 0.48 B a	4.13 ± 0.59 A a	3.63 ± 0.69 B ab
6 weeks	4.31 ± 0.20 A a	4.59 ± 0.47 AB a	3.38 ± 0.15 B a	4.65 ± 0.11 A a	4.17 ± 0.22 AB b

Different small letters means significant ($P \leq 0.05$) results between groups.

Different capital letters means significant ($p \leq 0.05$) results between periods.

Effect on Hemoglobin, PCV, Erythrocyte Counts and Leukocyte Counts caused as anemia, leukopenia and granulocytosis has been occurring as results of administering thiouracil and thiourea these results could be due to hypothyroidism induce by thiourea (21). Evidence the thyroid plays a part in the conversion of carotene to Hemoglobin, erythrocyte Counts and leukocyte Counts. A spot check was made on a limited number of lambs administered thiouracil and thiourea in the feed at the close of the experiment and periodic checks were on the sheep administered thiouracil in capsules for blood hemoglobin, erythrocyte counts and leukocyte counts.

The results showed that these blood components were not affected by thiouracil intakes of 0.18 to 3.0 gms. And thiourea intakes of 0.02 to 0.07 gins. per lamb daily for periods of approximately ~2 weeks (22). Hypothyroidism can cause various forms of anemia (normocytic - normochromic, hypochromic-microcytic or macrocytic) through reducing the oxygen metabolism. Microcytic anemia generally attribute to mal absorption of Iron and loss of Iron by menorrhagia, whereas, macrocytic anemia causes or induces mal absorption of vitamin B12, foliate, pernicious anemia and insufficient nutrition (23), hypothyroidism have a decreased erythrocyte mass due to reduction of plasma volume and may undetectable by routine measurement such as hemoglobin concentration, (23) reported that RBC, HB and HCT in patients with hyperthyroidism were significantly higher than

control groups while RBC and HB were decreased in hypothyroidism, but HCT was increased. They also showed that MCH and MCHC were lower in both groups in comparison with control group and MCV was increased in two groups of hypothyroidism and hyperthyroidism (24). Disorders of the hematopoietic system have also been described and could be accounted for in part by a hemolytic effect of thiourea. When repeated administration of high doses of thiourea in the diet, drinking water or by intraperitoneal injection resulted in reduced osmotic resistance of the erythrocytes, congestion, haemosiderosis and atrophy of the spleen, anemia, leukocytopenia, granulocytopenia, increased erythropoiesis in the bone marrow, reduced clotting times and increased phospholipid levels in blood (25).

The increasing in RBC count is reported to be due to the antioxidant effect of fenugreek seed (26). The fenugreek seeds contain a certain amount of vitamin C which is a good anti-oxidant. It protects the RBC membrane from oxidative process and then it declines the hemolysis of RBC (27). The fenugreek seed may produce its effect by increasing T₄ level (28). The thyroxin hormone act as hematopoietic stimulator which lead to increase RBC formation from bone marrow (29 and 30). Another possibility is that fenugreek seeds contain a considerable amount of iron in an organic form, which may be readily absorbed and seems to facilitate hematopoietic stimulation in bone marrow which in turn leads to increase synthesis of RBC from stem cells (31). The Hb

concentration showed a significant increase in the 3rd week of experiment dose, but there was a significant increase in fenugreek received groups as compared with control. This increase may be due to the anti-oxidative effect of fenugreek seeds which reduce the oxidative damage of Hb by the free radicals (32). It has been reported that vitamin C which found in these seeds act on reduction of Cu^{+2} into Cu^{+1} (33). And that lead to stimulation of Hb synthesis and hence increasing Hb concentration (34 and 35). It has been reported that fenugreek seeds increase thymus weight accompanied by increase in its cells count, and that leads to increase lymphocyte% (36). The fenugreek seeds have an important anti-oxidative role because it contains vitamin C, which protects the WBC from oxidative process and that lead to increase in their count (37) Another explanation that fenugreek seeds cause an increase in T_4 level (28). This hormone has been found to be associated with increase in Lymphocytes percentage (38). The increase in total WBC count is mainly due to the immune stimulatory effect of fenugreek seeds. While the treatment dose of levothyroxine treated groups (T4) caused to significant increase in (Hb, RBC, PCV) and non significant increase in WBC counts. Other researcher (39) was reported when administration of given exogenous thyroxin in drinking water at dosage of 50 mg/100 ml of water/pair/day with restricted feed significantly increased the (hemoglobin, PCV, RBC) values in both treated male and female rats, but decreased the WBC values in both male and female. Thyroid hormones may regulate haematopoiesis in the bone marrow. The association of thyroid disorders and abnormalities in hematological parameters is well known (40).

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التأثير العلاجي للمستخلص الكحولي لبذور الحلبة على نقص الغدة الدرقية استحدثت تجريباً بواسطة الثايويوريا و على بعض المعايير الدموية في ذكور الأرانب البالغة

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الخلاصة

صممت هذه الدراسة لمعرفة التأثير الدوائي للمستخلص الكحولي لبذور الحلبة (*Trigonella foenum graecum*) في وظيفة الغدة الدرقية في ذكور الأرانب البالغة المعرضة لمادة الثايويوريا المستحدثت لحالة قصور الغدة الدرقية (Hypothyroidism state). تمت دراسة المعايير الدموية (مستوى الخضاب، كريات الدم الحمراء، كريات الدم البيضاء الكلي وحجم الكريات الحمر المرصوصة). استخدمت (30) من ذكور الأرانب البالغة تراوح معدل أوزانها (1500-2000) غم، قسمت عشوائياً إلى خمسة مجاميع وبواقع (6) حيوانات / مجموعة وعولمت يومياً ولمدة (42) يوماً: حيوانات المجموعة الأولى أعطيت (1) مل من الماء المقطر واعتبرت مجموعة سيطرة (C)، أما حيوانات المجموعة الثانية فقد أعطيت المستخلص الكحولي لبذور الحلبة بجرعة (200 ملغم/كغم من وزن الجسم فمويًا) (T1)، بينما حيوانات المجموعة الثالثة فأعطيت الثايويوريا (10 ملغم/كغم من وزن الجسم فمويًا) (T2)، بينما المجموعة الرابعة فقد أعطيت الثايويوريا (10 ملغم/كغم من وزن الجسم فمويًا) وبعد (3) ساعات أعطيت المستخلص الكحولي لبذور الحلبة (200 ملغم/كغم من وزن الجسم فمويًا) (T3). أما حيوانات المجموعة الخامسة فقد أعطيت الثايويوريا (10 ملغم/كغم من وزن الجسم فمويًا) وبعد (3) ساعات أعطيت الثايروكسين (levothyroxine) (10 نانوغرام/كغم من وزن الجسم فمويًا). تم سحب عينات الدم في الأيام (0 و 14 و 28 و 42) قبل وبعد المعاملة لغرض قياس تركيز المعايير الدموية. أظهرت النتائج وجود ارتفاع معنوي في مستويات المعايير الدموية عدد الكريات الحمراء الكلي، حجم الكريات الحمر المرصوصة مستوى الخضاب وعدد الكريات بيضاء الكلي في مجموعة (T1) غير ان معدلاتها قد انخفضت معنوياً في مستوى المعدلات في مجموعة (T2) مقارنة مع السيطرة. أما بالنسبة لمجموعتي العلاج (T3 و T4) فقد أظهرت النتائج زيادة معنوية في هذه المستويات وقد وصلت الى معدلات مقارنة لما موجود في مجموعة بالسيطرة. مشيرة الى دور المستخلص الكحولي لبذور الحلبة و الثايروكسين في إزالة التأثيرات الضارة للثايويوريا .

الكلمات المفتاحية: بذور الحلبة، الثايويوريا، قصور الغدة الدرقية.