A study of some physiological aspects in mature male rabbits after oral administration of *Citrullus colocynthis*

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Summary

The goal of this study is to determine the effect of *Citrullus colocynthis* (CC) fruit (bitter apple) on some physiological aspects; the body weight, hematological parameters and the function of thyroid gland of male rabbits as a model for mammals. Two experiments were accomplished on sixty mature male rabbits that equally (30 animals in each experiment) and randomly divided into six groups each one contains five animals. In the experiment No.1 and experiment No.2 , independently, three groups were considered as treated groups in each experiment (represented as T1, T2 and T3 groups) and daily orally administrated low dose of CC (4.8mg/Kg/day, experiment No.1) for three groups in experiment No.1 and double dose of CC extract (9.6mg /Kg /day , experiment No.2) for 2, 4 and 8 weeks for the three groups in experimentNo.2,respectively. The other three groups in each experiment were considered as control (C1, C2 and C3 groups, respectively) groups were given orally distilled water (DW) at the same periods of treated groups. Different hematological parameters and thyroid hormones were studied. There was a significant ($P<0.05$) increase in the body weight gaining, with no significant changes in RBCs, WBCs, and Hb concentration through the first two weeks in all the treated rabbits compared to before treatment and control groups. Results of this study revealed that all the groups showed a significant ($P<0.05$) increased in the serum triiodothyronine hormone level and a decrease in the serum thyroxin hormone level of the thyroid gland when both doses were orally administrated through different periods of treatment compared to control groups. It is concluded that low dose of CC treatment has an advantageous physiological effect on the body weight and triiodothyronine hormone with no changes in certain hematological parameters through first two weeks .The significant differences that found following 4 and 8 weeks of CC treatment were within physiological normal level, and accordingly with the dose and treatment periods in rabbits.

Keywords: *Citrullus colocynthis*, Physiological aspects, male rabbits, thyroid gland, rabbits.

دراسة بعض الصفات الوظيفية لذكور الارانب البالغة بعد التجريع الفموي بالحنظل

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الخلاصة

هدفت الدراسة الحالية لتحديد تاثير نبات الحنظل فى بعض الصفات الفسلجية كوزن الجسم ، بعض معايير الدم ووظيفية الغدة الدرقية لذكور الارانب . انجزت تجربتين لستين ذكر ارنب بالغ و قسمت بشكل متساوي وعشوائي الى 30 ارنب في كل تجربة . تحوي كل منها على ثلاث مجاميع معالجة وثلاث مجاميع سيطرة وكل مجموعة ضمت 5 ارانب . في التجربة الاولى والثانية وبشكل منفصل , اعدت الارانب المعالجة(T1, T2 and T3 groups) وجرعت في التجربة الاولى بجرعة منخفضة من مستخلص الحنظل والبالغة 4.8 ملغم/ كغم من وزن الجسم/ يوميا وفي التجربة الثانية جرعت الارانب بجرعة مضاعفة بلغت 9.6 ملغم/كغم من وزن الجسم/ يوميا للفترات اسبوعيين واربعة اسابيع وثمان اسابيع، في حين جرعت حيوانات السيطرة(C1, C2 and C3 groups) في كل تجربة ماء مقطر وللفترات ذاتها .تم دراسة وزن الجسم وبعض معايير الدم الرئيسية وهرمونات الغدة الدرقية. اظهرت النتائج زيادة معنوية في وزن

الجسم ولم يلاحظ اي اختلاف معنوي في عدد كريات الدم الحمر و وخلايا الدم البيض و تركيز خضاب الدم وحجم الخلايا المرصوصة بعد اسبوعيين من التجريع الفموي للحنظل باستعمال الجرعة المنخفضة (4.8mg/Kg/day) او الجرعة المضاعفة (9.6mg /Kg /day) ما عدا الزيادة في حجم الخلايا المضغوط باستعمال الجرعة المضاعفة عند المقارنة مع مجموعة السيطرة . اظهرت النتائج وجود زيادة معنوية في مستوى هرمون triiodothyronine وانخفاض معنوي في مستوى هرمون thyroxine عند استعمال الجرعتين وعند الفحص ضمن الفترات الثلاث المذكورة عند مقارنتها مع مجموعة السيطرة . استنتجت الدراسة بان لاعطاء نبات الحنظل ذو الجرعة المنخفضة تاثير ايجابي على وزن الجسم مع زيادة في مستوى هرمون الغدة الدرقية triiodothyronine دون حدوث تاثير على معايير الدم خلال اسبوعيين من التجريع. وان التغيرات المعنوية التي لوحظت في بعض معايير الدم بعد اربع وثمان اسابيع من التجريع كانت زيادة ضمن المعدلات الطبيعية .

الكلمات المفتاحية: الحنظل, صفات وظيفية , ذكور الارانب ,الغدة الدرقية للارانب , هورمونات الغده الدرقيه.

Introduction

Herbal remedies are widely used for the treatment and prevention of various diseases and often contain highly active pharmacological compounds (1). Interest in the exploitation of medicinal plants as pharmaceuticals, herbal remedies, flavorings, perfumes, cosmetics and other natural products has greatly increased in the recent years. It is important to note that in the traditional utilization of medicinal plants, many toxic plants were used at a low dose and/or treated before being used in order to eliminate the poisonous components of the plant (by cooking, decoction, etc.) (2).

Before 1994, the herbal medicines were regulated as food and were evaluated for safety before being launched into the market for consumer purchase. In 1994, Dietary Supplement Health and Education Act (DSHEA) was allowed the distribution herbal supplements without testing their efficacy and toxicity. Currently, there are many agencies which are working on the regulation of these products (3).

Citrullus colocynthis is a well known medicinal plant that grows naturally in the western part of Iraqi desert, north of Iraq-kurdistan ,and in many other tropical and subtropical countries (4). The bitter apple of CC has been recommended for indigestion and diabetic people in traditional medicine. *Citrullus colocynthis* is used as a purge for man and animals in Mauritania (5). There are very few studies regarding the therapeutic effect of CC on several body systems. Therefore, the aim of this study is to examine the medical effect of the oral administration of crude extract of *Citrullus colocynthis* fruit on some physiological aspects.

Materials and Methods

Sixty adult healthy local breed of male rabbits were used in this study through September 2008 until April 2009 at the College of Veterinary Medicine-Al-Sulaimanyia University. The age of rabbits was 6 – 8 months old, weighing 1– 1.5 Kg, reared at an optimal room temperature ranged between 18 – 24 °C. The animals were kept two weeks for adaptation and through this period physical and laboratory examinations were done, (6). The rabbits were fed on compressed prepared ration ad *libtium* , in addition to green vegetable food that was added to the ration(7). The weights of all animal groups were recorded at beginning of the experiment and at the end of each different period of treatment and for each experimental group using electric digital balance (Mettler Toledo, Switzerland). The fresh fruits Plant preparation of *Citrullus colocynthis* were purchased from the village of Bakrbaef-darbandekhan district in Kurdistan, Iraq. The crude powder extract was prepared as follows. The shade-dried fruit was crushed into small pieces and then grinded until it became a powder by special electric grinder. The crude powder extract (1gm) was mixed with distilled water (1Litter) in order to prepare stock solution (0.1%) in a manner that lower concentrations were prepared by serial dilution (1).

Sixty adult male rabbits were equally divided into 2 experiments (30 animals each) and randomly the animals of each experiment were sub divided into six groups, each included five

rabbits. In the experiment No.1 and experiment No2 ,independently, three groups were considered as treated groups in each experiment(T1 ,T2,and T3).Rabbits of Exp.1 were orally administered with low dose (4.8mg/Kg/day) of CC crude fruit powder extract once a day in intervals 2,4 and 8weeks by using stainless steel gavages needle .The three rabbits groups of Exp.2(T1,T2,andT3) were treated with high dose(9.6mg/kg/day) of CC once a day for 2,4 and 8weeks .

The controlled rabbits of the two independent experiments (C1, C2 and, C3 for experiments No.1 and No2.) were divided into six sub groups similar to that of treated rabbits and were gavages DW through the same periods.

Dosage Preparation as mentioned earlier, the concentration was prepared for each two experiments by preparing stock solution of the required amount of CC with the appropriate amount of vehicle (distilled water) in a manner that:

1- Each 4.8ml of stock solution contained 4.8mg of CC crude powder for experiment No.1.

2- Each 9.6 ml of stock solution contained 9.6mg of CC crude powder for experiment No.2.

Smaller amounts of blood (up to 5 ml) were safely being sampled from the auricular marginal vein, with a 23-25-gauge needle. Blood samples for serum separation were collected in plastic test tubes without anticoagulant.

Hematological testes including red blood cells count, white blood cells count, hemoglobin concentration and packed cell volume were routinely performed with an auto analyzer (HyCell ,Pejohesh-Co.,Sweden) for all rabbits throughout the treatment period in the two experiments. Thyroid hormones including: triiodothyronine (T_3) and thyroxine (T_4) were measured and recorded by standard assays on a biochemical analyzer (ELISA, contains a specific T4 and T3 antibodies, Pejohesh -Co., Sweden) for each different experimental groups and periods.

Data were shown as the mean ± SE. When a significant interaction between major factors was identified by ANOVA. The Duncan's new multiple range test was used post-ANOVA to identify significant differences between mean values, and a probability level of 5% ($P < 0.05$) was taken as significant (8).

Results and Discussion

Animals Body Weight and Thyroid hormones

The effect of CC crude extract treatment on the body weight in rabbit groups administrated orally a dose of 4.8 and 9.6 mg/kg/day during different experimental periods is shown in the table 1. All rabbit groups showed weight gaining after treatment with 4.8mg/Kg/day and 9.6mg/Kg/day of CC at the end of each different periods (2 weeks, 4 weeks and 8 weeks) in comparison with their corresponding groups before treatment .Following 8weeks of treatment the weight gaining was significantly ($p<0, 05$) increase in rabbits treated with low dose (4.8mg/Kg/day) compared to control group (Table 1).

During the study, all treated male rabbit groups that were ingested the crude extract of CC with daily doses of 4.8 mg/kg and 9.6 mg/Kg resulted in a statistically significant ($P<0.05$) elevation of triiodothyronine (T_3) hormone (Table2) and decrease in the thyroxin hormone (T_4) level (Table 3) after different intervals of treatment which become more obvious after eight weeks in comparison with unchangeable value of their control groups.

Table 1: Effect of orally administering a low dose (4.8 mg/kg/day) and double dose (9.6 mg/kg/day) of *Citrullus colocynthis* on the body weight of rabbits in different periods.

Group	Period of treatment	Body weight (kg)							
		low dose (4.8 mg/kg/day)				double dose (9.6 mg/kg/day)			
		Treatment N=5		Control N=5		Treatment N=5		Control N=5	
		Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
T1	2 weeks	1.346±0.92 a	1.422±0.86 b	1.345±0.92 a	1.427±0.86 b	1.473±0.86 a	1.535±0.86 a	1.473±0.82 a	1.540±0.92 b
T2	4 weeks	1.340±0.52 a	1.497±0.80 b	1.345±0.84 a	1.597±0.88 b	1.470±0.86 a	1.578±0.85 b	1.470±0.70 a	1.619±0.92 b
T3	8 weeks	1.343±0.85 a	1.747±0.76 b	1.341±0.67 a	1.643±0.66 b	1.475±0.92 a	1.776±0.92 b	1.476±0.89 a	1.700±0.86 b

Values are expressed as means ± SE. **Different letters mean a significant difference at P < 0.05.**

Table 2: Effect of orally administering a low dose (4.8mg/kg/day) and double dose (9.6mg/Kg/day) of Citrullus *colocynthis* on triiodothyronine hormone levels (µg/dl) of male rabbits in different periods.

Group	Period of treatment	Triiodothyronine (µg/dl) T3							
		(4.8mg/kg/day)				(9.6mg/Kg/day)			
		Treatment (n=5)		Control (n=5)		Treatment (n=5)		Control (n=5)	
		Before	After	Before	After	Before	After	Before	After
T1	2 weeks	1.260±0.71 a	2.240±0.18 b	1.240±0.17 a	1.240±0.70 a	1.260±0. 12 a	1.640±0.7 0 b	1.200±0. 11 a	1.200±0.5 2 a
T2	4 weeks	1.240±0.15 a	2.120±0.16 b	1.240±0.15 a	1.220±0.51 a	1.270±0.1 4 a	2.200±0.4 2 c	1.220±0. 10 a	1.250±0.1 1 a
T3	8 weeks	1.220±0.17 a	2.320±0.60 b	1.250±0.11 a	1.245±0.51 a	1.240±0.1 2 a	2.220±0.3 1 c	1.245±0. 13 a	1.255±0.1 0 a

Values are expressed as means ± SE. **Different letters mean a significant difference at P < 0.05.**

Table 3: Effect of orally administering a low dose (4.8mg/kg/day) and double dose (9.6mg/Kg/day)of *Citrullus colocynthis* on thyroxine hormone levels (µg/dl) of male rabbits in different periods.

Group	Period of treatment	Thyroxine (µg/dl) T4							
		(4.8mg/kg/day)				(9.6mg/Kg/day)			
		Treatment (n=5)		Control (n=5)		Treatment (n=5)		Control (n=5)	
		Before	After	Before	After	Before	After	Before	After
T1	2 weeks	6.540±0.40 a	5.640±0.2 1 b	6.500±0.50 a	6.560±0.50 a	6.420±0.6 9 a	5.220±0.58 b	6.220±0.80 a	6.520±0 .10 a
T2	4 weeks	6.540±0.71 a	5.400±0.4 1b	6.540±0.45 a	6.540±0.40 a	6.530±0.4 5 a	5.160±0.38 b	6.450±0.63 a	6.440±0 .41 a
T3	8 weeks	6.560±0.90 a	5.360±0.3 0 b	6.540±0.47 a	6.540±0.59 a	6.500±0.2 9 a	4.980±0.30 b	6.520±0.46 a	6.420±0 3.6 a

Values are expressed as means ± SE. **Different letters mean a significant difference at P < 0.05.**

The results of treated groups showed, no lethality happened for rabbits feeding CC extract. This finding may be because CC contains different constituents such as oil, protein, fiber, ash, and carbohydrate. They are good sources of essential amino acids, especially arginine, tryptophan, methionine, vitamins B1, B2, niacin and minerals such as selinum (Se), calcium (Ca), magnesium (Mg), manganese (Mn), potassium (K), phosphorous (P), Fe^{+}, and Zinc (Zn). It contains oil mostly oleic and linoleic acids (9). The body required these vitamins and minerals to remain fit and healthy, leading to increase the body weight .At the same time ,the increase in the level of T3 and decrease in the serum T4 level indicate that CC may increase the basal metabolic rate leading to increase in body weight .This result is comparable with the study reported by Ojiako and Igwe, (10) who reported that feeding of male rabbits with cucurbitaceous plant for 4 weeks have a positive effect on the body weight gaining.

It is well known that CC has a proposed mechanisms such as insulin-like effects (11), stimulation of pancreatic insulin secretion (12), decreased hepatic gluconeogenesis, increased hepatic glycogen synthesis and increased peripheral glucose oxidation (13) which then lead to an increase in stimulation of fat and protein synthesis which may aid in building of body cells and fat tissue of the rabbits in the present study.

The elevation in T3 hormone may be due to the enzymes activation (14) such as deiodinase enzyme which is responsible for conversion of T4 to T3 after formation of thyroid hormones by the follicular cells of the thyroid gland (15).The activation of deiodinase enzyme may be triggered by the CC effects. Thus ,an increase in T3 concentration with significant decline in T4 concentration by low and high doses of the plant extract suggest that CC may increase the basal metabolic rate and protein synthesis(16) causing the increase in body weight.

Effect of low and double doses of *Citrullus colocynthis* on the hematological parameters of the rabbits

The results of treatment with low (4.8 mg/kg/day) and double (9.6 mg/kg/day) doses of CC showed no significant difference in RBCs count, WBCs count and Hb- concentration. The mean of packed cell volume of rabbits treated with low dose showed no significant ($P>0.05$) changes after 2 weeks of treatment compared to before treatment group and the control group. While after 4 and 8 weeks of treatment, the results of treated groups revealed statistically significant ($P<0.05$) increment in RBCs , WBCs count and Hb- concentration compared to those of control groups (Tables 4,5 and 6).

Table 4: Effect of orally administering a low dose (4.8 mg/kg/day) of *Citrullus colocynthis* on the red and white blood cells count of male rabbits in different periods.

Group	Period of treatment	Red blood cell count RBCs x 10^6 / µl				White blood cell count WBCs x 10^3 / µl			
		Treatment N=5		Control N=5		Treatment N=5		Control N=5	
		Before	After	Before	After	Before	After	Before	After
T1	2 weeks	5.34±0.35 a	5.53±0.43 a	5.35±0.40 a	5.35±0.30 a	5.42±0.91 a	6.00±0.46 a	5.46±0.10 a	5.47±0.80 a
T2	4 weeks	5.46±0.22 a	6.14±0.38 b	5.45±0.35 a	5.45±0.50 a	5.00±0.56 a	7.84±0.35 b	5.54±0.32 a	5.57±0.30 a
T3	8 weeks	5.44±0.22 a	6.34±0.16 b	5.45±0.22 a	5.45±0.50 a	5.56±0.30 a	8.68±0.42 c	5.58±0.22 a	5.60±0.50 a

Values are expressed as means ± SE. **Different letters mean a significant difference at $P < 0.05$.**

Table 5: Effect of orally administering a double dose (9.6mg/kg/day) of *Citrullus colocynthis* on the red and white blood cells count of rabbits in different periods.

Group	Period of treatment	Red blood cell count RBCs x 10^6 / µl				White blood cell count WBCs x 10^3 / µl			
		Treatment N=5		Control N=5		Treatment N=5		Control N=5	
		Before	After	Before	After	Before	After	Before	After
T1	2 weeks	5.43±0.45 a	5.53±0.40 a	5.44±0.30 a	5.44±0.35 a	5.53±0.35 a	5.73±0.43 a	5.34±0.35 a	5.34±0.35 a
T2	4 weeks	5.53±0.25 a	6.55±0.50 b	5.48±0.26 a	5.50±0.20 a	5.73±0.65 a	6.14±0.38 b	5.46±0.22 a	5.56±0.32 a
T3	8 weeks	5.48±0.37 a	6.96±0.46 b	5.44±0.60 a	5.48±0.10 a	5.84±0.75 a	6.34±0.16 b	5.44±0.22 a	5.48±0.52 a

Values are expressed as means ± SE. **Different letters mean a significant difference at P < 0.05.**

Table 6: Effect of orally administering a low dose (4.8 mg/kg/day) of *Citrullus colocynthis* on hemoglobin concentration and packed cell volume of rabbits .

Group	Period of treatment	Hemoglobin concentration gm/dl				Packed cell volume (%)			
		Treatment (n=5)		Control (n=5)		Treatment (n=5)		Control (n=5)	
		Before	After	Before	After	Before	After	Before	After
T1	2 weeks	11.23±0.1 1 a	11.98 ±0.17 a	11.22 ±0.24 a	11.22 ± 0.41 a	37.15±1.6 a	37.44 ±4.8a	37.02±1 .6a	37.02±1. 9 a
T2	4 weeks	11.20 ±0.34 a	12.84 ±0.26 b	11.24±0.1 0 a	11.24 ±0.14 a	37.12±1.5 a	39.76±8. 9b	37.12±4 .1a	37.12±4. 8 a
T3	8 weeks	11.56 ±0.14 a	14.18 ±0.49 c	11.28 ±0.27a	11.28 ±0.29 a	37.15±1.5 a	42.92±4. 3c	37.36±2 .6a	37.36 ±2.8 a

Values are expressed as means ± SE. **Different letters mean a significant difference at P < 0.05.**

There was a significant (P<0.05) increase in the mean of PCV of treated rabbits given 9.6 mg/kg/day CC for 2 weeks(T1), 4 weeks(T2) and 8 weeks(T3) compared to the control groups at the same periods (C1=2weeks, C2=4 weeks, and C3=8 weeks). Moreover, Table 7 revealed that there was a significant (P<0.05) difference in the values of PCV between the three periods of treated groups.

Table 7: Effect of orally administering a double dose (9.6 mg/kg/day) of Citrullus colocynthis on hemoglobin concentration and packed cell volume of rabbits .

Group	Period of treatment	Hemoglobin concentration gm/dl				Packed cell volume (%)			
		Treatment (n=5)		Control (n=5)		Treatment (n=5)		Control (n=5)	
		Before	After	Before	After	Before	After	Before	After
T1	2 weeks	11.12±0.33 a	11.82±0.75 a	11.10±0.2 5 a	11.10 ±0.35a	37.15±0.1 6a	38.46±0. 20b	37.06 ±0.17 a	37.02±0. 19 a
T2	4 weeks	11.18 ±0.65 a	12.98±0.38 b	11.30 ±0.40a	11.30±0. 55a	37.12±0.1 5a	39.30±0. 18c	37.07±0.53 a	37.14± 0.48a
T3	8 weeks	11.12±0.24 a	13.78±0.22 c	11.15±0.1 0a	11.15±0. 25a	37.15±0.1 5a	40.90±0. 29d	37.14±0.32 a	37.36±0. 28a

Values are expressed as means ± SE. **Different letters mean a significant difference at P < 0.05.**

In this study, the dose and time dependent increases were observed in certain hematological parameters of rabbits given 4.8 mg/kg/day and 9.6mg/kg/day of CC extract particularly after four and eight weeks of treatment(Tables 4-7). The physiological increases in the blood cells could be related to the chemical composition of the plant. The chemical composition of this plant includes protein, fat, carbohydrate, calcium, iron and vitamins (17). Most of these constituents are well known factors that have direct influence on the production of blood from the bone marrow (16).

These results are in accordance with those reported by Elawad (18) who shows that hematological changes in 10 sheep administered orally high dose of *Citrullus colocynthis* fruit indicated the development of hemoconcentration. Furthermore, increase in WBCs count was observed at the same time. The stimulating effects of medicinal plants on immune system associated with chemical components of saponins and flavonoids showed that plant extract flavonoids can stimulate the production of both antibodies and macrophages of the immune system. Flavonoids are agonists of adenosine receptors in the brain (19) which are highly expressed in the brain and GABA nergic neurons (20). Several adaptogenic plants have been shown to abolish suppression of bone marrow erythropoiesis and increased blood cells. Erythropoiesis is regulated by serotonin, norepinephrine and acetylcholine. The presence of these compounds in *CC* is also based on leucocytosis activity of this plant.

The finding of this study is comparable with the results of Shafaei (21) in 30 male rabbits gavage with seed extract from CC fruit for 4 weeks and it showed a potentiating immune stimulant effect of the extract by a significant increase of lymphocytes in comparison with control groups.

Cucurbitacin E from CC has also been shown to have immune modularly effects on peripheral human lymphocytes (22).Present study found increase in RBCs count, HB concentration, PCV and WBCs count of treated groups with CC. The same observation was recorded by Dina (23) who demonstrated that rabbits gavages by cucurbitaceous plant for 4 weeks at a dose rate of 4mg/ml possesses haemopoietic properties.

It is concluded from this study that orally administration 4.8mg/Kg/day of CC is safer than double dose and have positive effects on the body weight and certain blood parameters. .More researches are recommended to found out CC effects on production and reproduction in large animals.

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