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***J. Nat. Prod. Plant Resour.*, 2011, 1 (3): 1-7**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Citrullus colocynthis: a desert plant native in Algeria, effects of fixed oil on blood homeostasis in Wistar rat

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ABSTRACT

Citrullus colocynthis is a species belonging to the botanical family of Cucurbitaecea; it is a medicinal plant known for their various healing properties. We investigated the effects of the fixed oil extracted from the seeds in rats by monitoring blood homeostasis and body weight as well as toxicity. Animals were given daily 4% of dietary regimen of the C. colocynthis oil for 08 weeks showed significant slowdown of the body weight evolution comparatively to the animal control group received 4% of sunflower oil. Furthermore, Colocynth oil treatment had a tendency to increase significantly food intake feces output, and lipid feces. In parallel, the serum cholesterol, triglycerides, ALP levels and the count of erythrocytes and haematocrit level decreased significantly by 15.38, 22.22,46.29, 14.97 and 14.17%, compared to control values, respectively; while AST level increased significantly by 21.71%. These results support the suggestion of using C. colocynthis oil as a treatment for dyslipidemia and hyperglycemia, and related abnormalities. The chronic toxicity and the mode of action of this oil must be studied.

INTRODUCTION

Citrullus colocynthis (L.) Schrad. (Cucurbitaceae), commonly known as « bitter-apple », « colocynth », or « wild-gourd », is a tropical plant that grows abundantly in many place in the world **[1]**. Originally from Tropical Asia and Africa, it is now widely distributed in the Saharo-Arabian phytogeographic region in Africa and the Mediteranean region. The stems are angular and rough; the leaves are rough, 5–10 cm in length, deeply 3–7 lobed; solitary pale yellow blooms. Each plant produces 15–30 round fruits, about 7–10 cm in diameter, green with undulate yellow stripes, becoming yellow all over when dry. Seeds are small (6 mm in length), smooth and brownish when ripe **[2]**. Seeds containing represent 75% of the weight of fruit **[12]**. In Algeria, *C.colocynthis* occurs in many places from the north to the hot desert it's known as « Handal », « Hdejj » « Tijjeltl », « Tabarka » or « Tifersite ». The fruits are widely used medicinally as an anti-inflammatory, purgative in constipation, anti-rheumatic and anti-diabetic in Mediterranean countries **[3, 4, 6]**. The seeds of *C.colocynthis* have been subjected to a range of pharmacological, phytochemical and nutritional investigations in recent years. It has been

shown to contain 17% of a fixed oil with high proportion of unsaturated fatty acids, mainly linoleic acid (60-70%), oleic acid (11.7-15%) and a very low n-3 poly-unsaturated FA level (0.5%). It is also rich in antioxidant (eg. tocopherol, polyphenol and plant sterol) **[3, 5]**. This study was undertaken to determinate the effects of this oil on blood biochemical and hematological parameters in rat.

MATERIALS AND METHODS

Plant materials

C. colocynthis fruits were collected from Mechria (western Algeria), divided in half and the seeds removed by hand. Mature black seeds were selected, dried and they were powdered mechanically. The lipid fraction was extracted with petroleum ether (40-60°c) in a soxhlet apparatus for to hours by the Natural products laboratory in Tlemcen, Algeria. The solvent was evaporated and the lipid fraction was weighed. Oil content in seeds was 17%.

Sunflower oil is commercial local products (Fleurial plus, Cevital, Algeria). Oils fatty acids compositions were analyzed by gaze chromatography and are shown in Table2.

Animals

The experimental protocol was approved by the Animal Care and Use Committee of the University of Tlemcen. Twenty male Wistar rat 4-weeks old (80±5 g weight) were placed in stainless-steel cages and maintained under controlled conditions; 12-h light and 12-h dark cycle, and a constant temperature of 22±3°c. Food and water were freely available in the home cages.

Diets

Two different diets were prepared:
Diet1: 16% casein and 4% sunflower oil (control group)
Diet2: 16% casein and 4% Colocynth oil
The composition of each diet is presented in Table1.

Experimental Procedures

Animals were divided into two groups (10 rats per cage); each group was fed with one of the two diets for two months. Body weight, food consumption, fecal output and fecal lipid contents were measured through the study. The feces from each group of rats were pooled and dried to constant weight. The fecal lipids were extracted by soxhlet.

At the end of the experiment, all the rats were given a lethal intraperitoneal dose of hydro-chloral 10% (300µl/100g bw). Blood was immediately collected from the abdominal aorta. Plasma obtained by low speed (2000 rpm) centrifugation for biochemical analysis. Blood glucose was determined with the glucometer type (Acku check Active, Roche, Mannheim, Germany). Total cholesterol, triacylglycerol, urea, creatinin, uric acid, ALP, AST and ALT concentrations, assays were done by enzymatic kits (Biosystem, Barcelona, Spain). Haematological parameters were determined automatically by.....

Statistical analysis

All values are expressed as mean± standard error (SEM). Independent Student's test was applied to analyze the significance of differences between mean values and critical P-values were considered to be significant at 0.05.

Table1: Overall composition of dietary regimens

Constituents (g/100g diet)	S-rats (diet 1)	Cc-rats (diet 2)
Casein	16	16
Methionin	0.3	0.3
Starch	60.33	24.33
Saccharose	05	05
Cellulose	05	05
Mineral mix	7.37	7.37
Vitamin mix	02	02
Oil	04So	O4Co
Total	100	100
Energetic values (Kcal/100g)	362.52	362.52

S-rats: show rats group; Cc-rats: C. colocynthis treated rats group; So: sunflower oil; Co: Colocynth oil.

Mineral mix provided the following nutrient (g/100g of dry diet): Ca,4; K,2.4; Na,1.6; Mg,0.4 Fe,0.12; elements (traces): Mn,0.032; Cu, 0.05; Zn,0.018. Vitamin mix provided the following nutrient (mg/1Kg of dry diet): retinol,1.8;Cholicalciferol,0.019; thiamine,6;riboflavin,4.5; pantothenic acid,21; inositol;45 ; ascorbic acid,240; α tocopherol,51; nicotinic acid, 30;folic acid, 1.5; biotin, 0.09 .

Table2: Percentage composition of the main fatty acids of dietary oils

Fatty acids(g/100g)	Sun flower oil	Colocynthis oil
SFA	10	15
MUFA	30	7.8
18:2(n-6)	54	76.4
20:4(n-6)	5.8	0.3
Total n-6	59.8	76.7
Total n-3	0.2	0.5
Total PUFA	60	77.2
P/S	06	5.14
n-6/n-3	299	153.7

SFA: Saturated fatty acids, MUFA: Monounsaturated fatty acids, PUFA: Polyunsaturated fatty acids

Table3: Effect of C. colocynthis fixed oil on plasma parameters

Parameter (unit)	S-rats (n=10)	Cc-rats (n=10)
Glycaemia (Mm/l)	5.17±0.14	5.29±0.20
Triglycerides(g/l)	0.45±0.04	0.35±0.05*
Cholesterol (g/l)	0.39±0.02	0.33±0.03*
Urea (g/l)	0.43±0.01	0,44± 0,03
Creatinin (mg/l)	7.54±0.25	7.29±0,35
Uric acid(mg/l)	15.8±5.8	15.5±5.13
AST (U/l)	142.5±39	173±44*
ALT (U/l)	42.40±5.91	48.34±9.1
ALP (U/l)	193.66±38.34	104±25.75*

*S-rats: show rats group; Cc-rats: C. colocynthis treated rats group. AST: aspartate aminotransferase; ALT: alanine aminotransferase, ALP:alkaline phosphatase Values are means ± SEM. Data were analyzed by Student test, *Significantly different from S-rats versus Cc-rats by Student's test, $p<0.05$.*

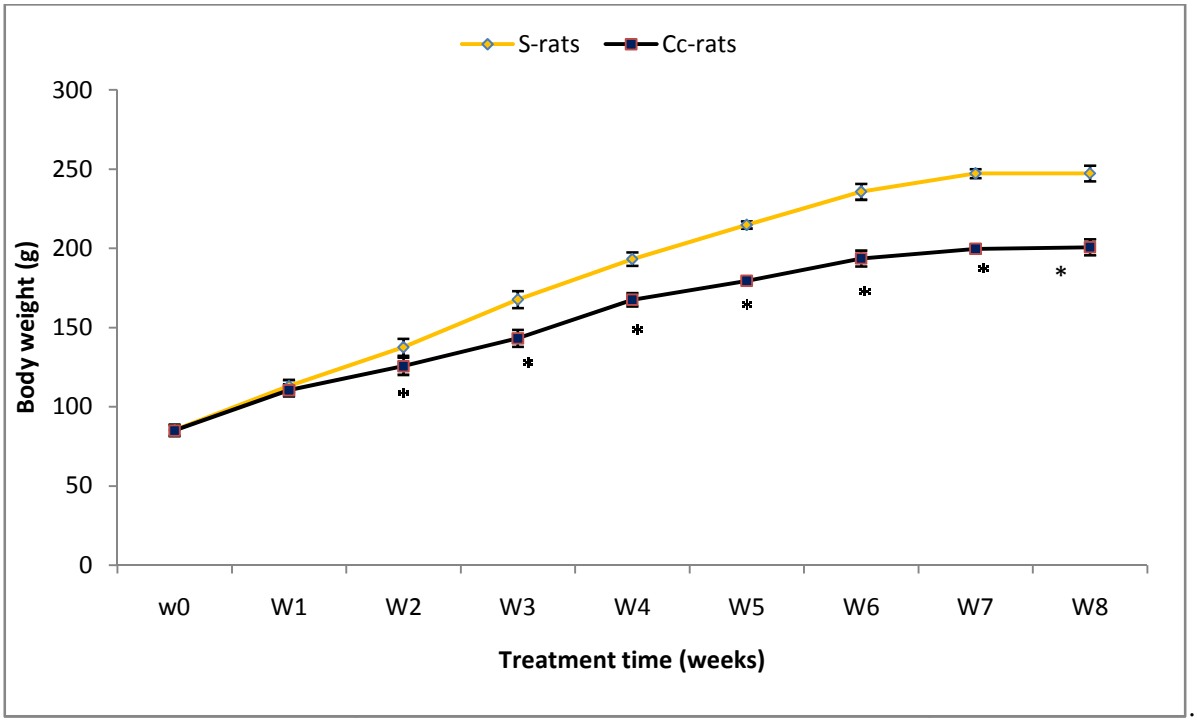

Fig. 1. Effect of C.colocynthis fixed oil on body weight evolution. S-rats: show rats group; Cc-rats: C. colocynthis treated rats group.

*Values are means ± SEM. Data were analyzed by Student test, *significantly different from S-rats versus Cc-rats by Student's test, p<0.05.*

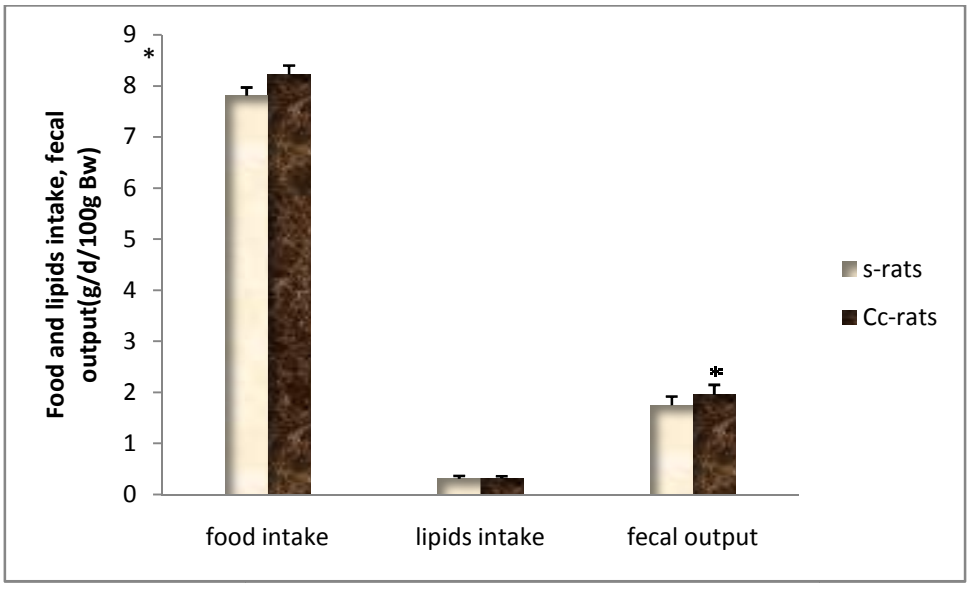

Fig. 2. Effect of C.colocynthis fixed oil on food and lipids intake and fecal output. S-rats: show rats group; Cc-rats: C. colocynthis treated rats group.

*Values are means ± SEM. Data were analyzed by Student test, *Significantly different from S-rats versus Cc-rats by Student's test, p<0.05.*

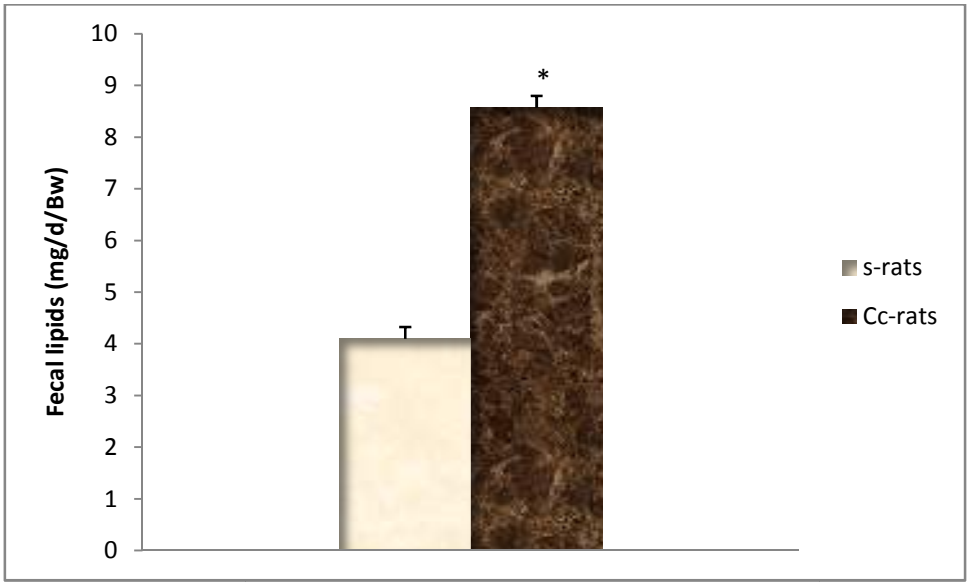

Fig. 3. Effect of C.colocynthis fixed oil on fecal lipids. S-rats: show rats group; Cc-rats: C. colocynthis treated rats group.

*Values are means ± SEM. Data were analyzed by Student test, *Significantly different from S-rats versus Cc-rats by Student's test, p<0.05.*

Table4: Effect of C. colocynthis fixed oil on haematological parameters.

Parameter (unit)	S-rats (n=10)	Cc-rats (n=10)
Erythrocytes (10^6/mm³)	8.08±0.14	6.87±0.47*
Leucocytes (10^6/mm³)	4.68±0.25	4.18±0.22
Platelets (10^6/mm³)	484± 59,5	508.67± 18,2
Haematocrit (%)	39.85± 0,75	34.2± 2,1 *
hemoglobin(g/dl)	12.95± 0,025	11.95±0,68
MGV (µm³)	49.5± 0,25	49.67±0,51
MCCH1 (pg)	16.05± 0,75	17.5±0.41
MCCH2 (g/dl)	32.45±0.41	34.97±1.34

*S-rats: show rats group; Cc-rats: C. colocynthis; MGV, mean globular volume; MCCH1, mean corpuscular content of hemoglobin; MCCH2, mean corpuscular concentration of hemoglobin. Values are means ± SEM. Data were analyzed by Student test, *Significantly different from S-rats versus Cc-rats by Student's test, p<0.05.*

RESULTS

Effects of dietary oils on body weights, food/lipids intake and lipid feces

As shown in Figure 1, body weights in C. colocynthis treated rats were significantly lower than that of control diet group during the experiment. Furthermore, Colocynth oil treatment had a tendency to increase significantly food intake feces output, and lipid feces (figure2, 3).

Effects of dietary oils on plasma parameters

Table 3 shows that plasma Triglycerides total cholesterol and ALP levels were decreased significantly by C. colocynthis oil treatment when compared to the show values while AST level was increased. By contrast, urea, and creatinin in rats as a function of treatment time did not increase significantly compared to the control values.

Effects of dietary oils on hematological parameters

Table 4 illustrates the hematological parameters in C. colocynthis treated rats. After 8 weeks of treatment, the erythrocytes and hematocrit decreased significantly when compared to the control values ($p<0.05$).

DISCUSSION

The results obtained in the present study clearly show that C. colocynthis fixed oil treatment was effective in influencing blood homeostasis and body weight in rat. This later witch decreased significantly from the second week of experiment. By contrast food intake, fecal output and fecal lipids were increased by C. colocynthis treatment. These effects can be explained by the presence of some inhibitory factors of digestibility in colocynth oil decreasing intestinal absorption of lipids and thereby promoting there fecal elimination. Other explanations of reduction of body weight are also possible, like a toxic effect. Moreover the mean levels of plasma lipids remained relatively low in rats fed diets with colocynth oil, which is rich in minor components such as hydrocarbons, mainly squalenes, α-tocopherol and phytosterols [5]. Many and various studies have shown that these substances exert beneficial effects [7]. This effect could be due also to the inhibition of intestinal absorption of lipids by this oil.

In parallel, AST, ALT and ALP are the commonly employed biological markers for hepatic injury and efficacy of hepatoprotective interventions. The present study revealed that colocynth oil treatment increases the levels of AST witch could be due to a toxic effect. In fact, an other study demonstrated that rats fed with 10% of C. colocynthis ripe fruit for 6 weeks had increased levels of AST, ALT and ALP [8].

On the other hand our result shows a decreasing on erythrocytes and hematocrit by colocynth oil treatment. This could be explain by the presence of phytosterols in this oil witch could replace cholesterol in cell membrane in erythrocytes and change physics proprieties of erythrocytes membrane and reduced there flexibility [9, 10]. The reduction of flexibility of erythrocytes membrane can short cell life [11].

In conclusion, our data support the suggestion of using C. colocynthis oil and its derived products as a treatment for dyslipidemia and hyperglycemia, and related abnormalities. The chronic toxicity and the mode of action of this oil must be studied.

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