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Artigo

Assessment of anti-hyperlipidemic effect of *Citrullus colocynthis*

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RESUMO: "Avaliação do efeito antihiperlipidêmico de *Citrullus colocynthis*". Hiperlipidemia é um fator de risco bem conhecido para diversas doenças inclusive aterosclerose, doenças cardíacas e derrame cerebral. Na procura de potenciais agentes antihiperlipidêmicos a partir de plantas para prevenir essas doenças, a polpa e as sementes de *Citrullus colocynthis* foram testadas para verificar seus efeitos no perfil lipídico de coelhos Nova Zelândia hiperlipidêmicos. Nos grupos experimentais que receberam a polpa de *C. colocynthis* ou 100 mg/kg das sementes, os perfis lipídicos foram significantemente reduzidos quando comparados ao grupo de controle (P<0,05).

Unitermos: *Citrullus colocynthis*, Cucurbitaceae, hiperlipidemia, coelhos.

ABSTRACT: Hyperlipidemia is a well-known risk factor for several illnesses including atherosclerosis, heart and vascular diseases and stroke. In the search for potential anti-hyperlipidemic agents from plants to prevent these conditions, the pulp and the seeds of *Citrullus colocynthis* were assessed for their effects on the lipid profile of hyperlipidemic New Zealand rabbits. In the experimental groups that received the pulp of *C. colocynthis* or 100 mg/kg of seeds, the lipid profiles were significantly reduced when compared to the control group (P<0.05).

Keywords: *Citrullus colocynthis*, Cucurbitaceae, hyperlipidemia, rabbits.

INTRODUCTION

From the seventeen major causes of human death, cardiovascular diseases rank seventh. Coronary heart disease (CHD) is the cause of mortality in 50% of people around the world (Faergeman, 2000; Sullivan, 2002). Hyperlipidemia is considered to be the most influencial risk factor for CHD (Torres et al., 2000; Nilson et al., 2001; Patel, 2001). Therefore, maintaining low blood lipid profile and blood cholesterol is essential for cardiovascular health. A 20% reduction of blood cholesterol level can decrease about 31% of CHD incidence, and 33% of its mortality rate (Scott, 1997).

Citrullus colocynthis (L.) Schrad. (Cucurbitaceae), commonly known as 'bitter apple', 'colosynth', 'vine-of-Sodom', 'hendavaneye aboojahl', 'tumba' or 'wild gourd', is a tropical plant that grows abundantly in southern Iran, and also in other parts of the world (GRIN Database, 2007). It is described in the first edition of Brazilian Official Pharmacopoeia (Brandão et al., 2006). The usable part of *C. colocynthis* is its fruit about the size of an apple, yellow in color and bitter tasting. Ripe fruits contain white spongy pulp located in the leathern cover with many white or brown ovoid seeds. The fruits are used as a purgative in constipation, anthelmintic, carminative, anti-rheumatic, anti-diabetic and antipyretic (Adam et al., 2001; Madari and Jacobs, 2004; Dr. Duke's Phytochemical and Ethnobotanical Databases, 2007).

The fruits of *C. colocynthis* contain saponins (Montaral and Jersey, 2000; Yoshikawa et al., 2007; ISI Database, 2007). Most saponins form an insoluble complex with 3β-hydroxysteroids, and consequently large micelles with choleic acids and cholesterol. *C. colocynthis* is known to increase peristaltic movements of the gut, and cause diarrhea (Milgate and Roberts, 1995). We now report the effects of this plant on blood lipid profile in hyperlipidemic rabbits.

MATERIAL AND METHODS

Plant material

The pulps and the seeds of *C. colocynthis* were collected during August and September, 2003, from Ahvaz in Khozestan province, Iran. The identity of this plant was confirmed by anatomical examination in comparison with the herbarium specimen retained in

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the School of Pharmacy, Tabriz University of Medical Sciences, Iran. A voucher specimen (TUM-ADA 87) for this collection has been deposited in the herbarium of the Faculty of Pharmacy, Tabriz University of Medical Science, and also in the herbarium of the Plant and Soil Science Department, University of Aberdeen, Scotland (ABD).

Preparation of plant extracts

The ground pulp (500 g) and the seeds (500 g) of *C. colocynthis* were extracted separately by maceration with water:methanol (30:70) for 24 hours and repeated three times. The pooled hydro-methanolic extracts were concentrated by rotary evaporator at 45 ^{o}C.

Animals

Thirty four 1-1.5 month old New Zealand male rabbits, which were suitable models for the examination of anti-cholestrolemic or anti-hyperlipidemic agents (Behr-Roussel et al., 2002; Aguilera et al., 2003; Ishizaka et al., 2003; Hyashi et al., 2004), were purchased from Iran Pasteur Institute (Tehran-Iran), and were housed in the animal farm of Tabriz Medical University for 18 consecutive weeks: 2 weeks and 16 weeks, respectively, for the adaptation and the experimental periods, as described in the literature (Kwon et al., 2000, 2003; Mackness et al., 2000; Zaho and Zhang, 2003) under standard environmental conditions of temperature, humidity and light. Rabbits were kept in aluminium cages and received the standard diet and water *ad libitum* ('free-feeding')(Table1).

Protocol

After 2 weeks, the rabbits were placed in a specific holder and were humanely bled from their marginal ear vein by using xylol to facilitate the process of bleeding. The samples were centrifuged (1500 spin for 15 min) and 5 mL of serum was analyzed for lipid profile (cholesterol, LDL-C, HDL-C and triglyceride) and fasting blood sugar.

After the adaptation period, the rabbits were assigned to 6 groups. Group 1 received a diet containing the standard regimen, plus cholesterol (0.5%) and 100 mg/kg of *Citrullus* pulp extract; group 2 received a diet containing the standard regimen plus cholesterol (0.5%) and 200 mg/kg *Citrullus* pulps extract, group 3 received a diet containing the standard regimen plus cholesterol (0.5%) and 100 mg/kg of *Citrullus* seed extract, group 4 received a diet containing the standard regimen plus cholesterol (0.5%) and 200 mg/kg of *Citrullus* seed extract, group 5 received a diet containing the standard regimen plus cholesterol (0.5%) and group 6 received a diet containing the standard regimen only (Table 2). The cholesterol containing diet was prepared through mixing a solution of cholesterol (dissolved in diethyl ether) with the standard diet.

Rabbits were bled once a week, at random, to confirm hypercholesterolemia or hyperlipidemia. After 12 weeks, rabbits of the intervention groups became hypercholestrolemic or hyperlipidemia. Fasting blood samples were then taken to measure the lipid profile (cholesterol, HDL-C, LDL-C and triglyceride) and the amount of blood sugar. After 18 weeks of the treatment, rabbits were sacrificed and blood samples were collected for serum chemistry analysis and haematological studies. Total amount of cholesterol, LDL-C, HDL-C, serum triglyceride (TG) and blood glucose were measured by a manual kit (Pars azmoon, Iran) following the enzymatic and colorimetric methods (Enzymatic-colorimetric/ CHOD-PAP) available in the literature (Rifai et al., 1991; Artiss and Zak, 1997; Cole et al., 1997; Sacks, 1999). In order to compare the averages, the ANOVA test, Dunkan test, Paired t-test and Student t-test were utilized.

RESULTS AND DISCUSSION

As seeds and pulps generally have different chemical profiles, both extracts were tested for their potential anti-hyperlipidemic effect. The hypercholesterolemic regimen significantly increased the amount of blood cholesterol, LDL-C, HDL-C, triglyceride and blood sugar ($p<0.05$). The hypercholestrolemic control rabbits (group 5) remained hypercholesterolemic throughout the experimental period while serum cholesterol and triglyceride in the first three groups receiving both seeds and pulps extracts of *C. colocynthis* were reduced ($p<0.05$).

The reduction of LDL-C in the groups receiving the pulp extracts and 100 mg/kg seed extract were significant ($p<0.05$). HDL-C reduction was also significant in the first four groups receiving the extract of *C. colocynthis* except at the doses of 200 mg/kg ($p<0.05$). On the other hand, the comparison between the four groups receiving the extract of *C. colocynthis* demonstrated that the average amount of cholesterol, LDL-C, HDL-C, TG and blood sugar were reduced compared to those of the control group. The dose response for the different groups to oral administration of *C. colocynthis* is shown in Tables 3-4.

It was reported that the presence of high amounts of saponins in *C. colocynthis* might contribute to the reduction of cholesterol levels by reducing the absorption of cholesterol, increasing the repel of fecesic estrol, and diarrhea due to increase peristalsis (Milgate and Roberts, 1995). Thus, it is reasonable to assume that the effect of *C. colocynthis* on the blood lipid profile in rabbits might also be owing to the presence of these saponins. In this trial, the use of *C. colocynthis* resulted significant reduction of total serum cholesterol and LDL-C in groups receiving the extracts. However, this

result is different from the findings by Adam et al. (2001) where an oral administration of *C. colocynthis* (0.25 g/kg) in sheep for 12 days had no significant changes on the level of blood cholesterol.

The effect of saponins, natural or synthetic, in lowering the levels of cholesterols has been demonstrated in a number of studies (Morehouse et al., 1999). Jipping et al. (1999) observed that saponins of *Panax quinquefolium*, dose-dependently reduced the level of lipid peroxidation products. Milgate and Roberts (1995) reported that rabbits, which received hypercholesterolemic regimen and 0.6 g/day lucerne saponin for eighteen months, showed no evidence of atherosclerosis.

Table 1. The standard regimen of rabbits.

Materials	Energy proportion (%)
Protein	8
Lysine	6
Metyonine + cystein	7
Calcium	9
Phosphorus	4
Sodium chloride	4
Fiber	12
Fat	12
Carbohydrate	65
Energy	2700 kcal

Table 2. The nutritional regimen of rabbits in each group.

Group	Nutritional regimen	Number	Average of weight (X ± SEM)
1	Base+cholesterol+100mg/kg *Citrullus* pulp's extract	6	1/30 ± 0/03
2	Base+cholesterol+200mg/kg *Citrullus* pulp's extract	6	1/34 ± 0/03
3	Base+cholesterol+100mg/kg *Citrullus* seed's extract	6	1/30 ± 0/03
4	Base+cholesterol+200mg/kg *Citrullus* seed's extract	6	1/266 ± 0/04
5	Base+cholesterol	6	1/32 ± 0/03
6	Base	4	1/01 ± 0/01

Table 3. Comparison of the averages of serum biochemical values, weight and food consumption indexes between the groups receiving cholesterol + 100 mg/kg *Citrullus* pulps, and cholesterol + 200 mg/kg *Citrullus* pulps at the end of the study.

Parameter group	Blood sugar	Triglycerides	HDL-C	LDL-C	Cholesterol
Cholesterol receiver	101.4±9.56	199.4±43.68	33.20±2.33	356.2±22.72	1806±189.98
Cholesterol receiver + 100 mg/kg *Citrullus* pulps extract	97±8.03	90.5±27.63	30.33±3.12	183±25.99	7656.5±154.12
p-value	0.735	0.057	0.497	0.001	0.000
Cholesterol receiver + 200 mg/kg *Citrullus* pulps extract	98±1.81	66.4±14.43	25.8±2.76	162.4±29.13	418.4±119.59
p-value	0.746	0.02	0.075	0.001	0.000

*Units are in mg/dL

Table 4. Comparison of the averages of serum biochemical values, weight and food consumption indexes between the groups receiving cholesterol + 100 mg/kg *Citrullus* seeds, and cholesterol + 200 mg/kg *Citrullus* seeds at the end of the study.

Parameter group	Blood sugar	Triglycerides	HDL-C	LDL-C	Cholesterol
Cholesterol receiver	101.4±9.56	199.4±43.68	33.20±2.33	356.20±22.72	1806.2±189.98
Cholesterol receiver + 100 mg/kg *Citrullus* seed extract	98.8±7.16	133±30.09	27.6±2.22	218.6±29.33	838.2±147.97
p-value	0.834	0.276	0.121	0.006	0.004
Cholesterol receiver + 200 mg/kg *Citrullus* seed extract	89±6.28	83.33±7.46	37.5±5.78	254±25.58	1073.5±236.91
p-value	0.295	0.018	0.539	0.017	0.044

The findings of our present study provide further evidence in support of the previously published reports on the anti-hyperlipidemic properties of saponins or saponin-containing plant extracts in animal models.

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