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Research Article

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Effect of *Citrullus colocynthis* oil on the lipid profile of obese male rats

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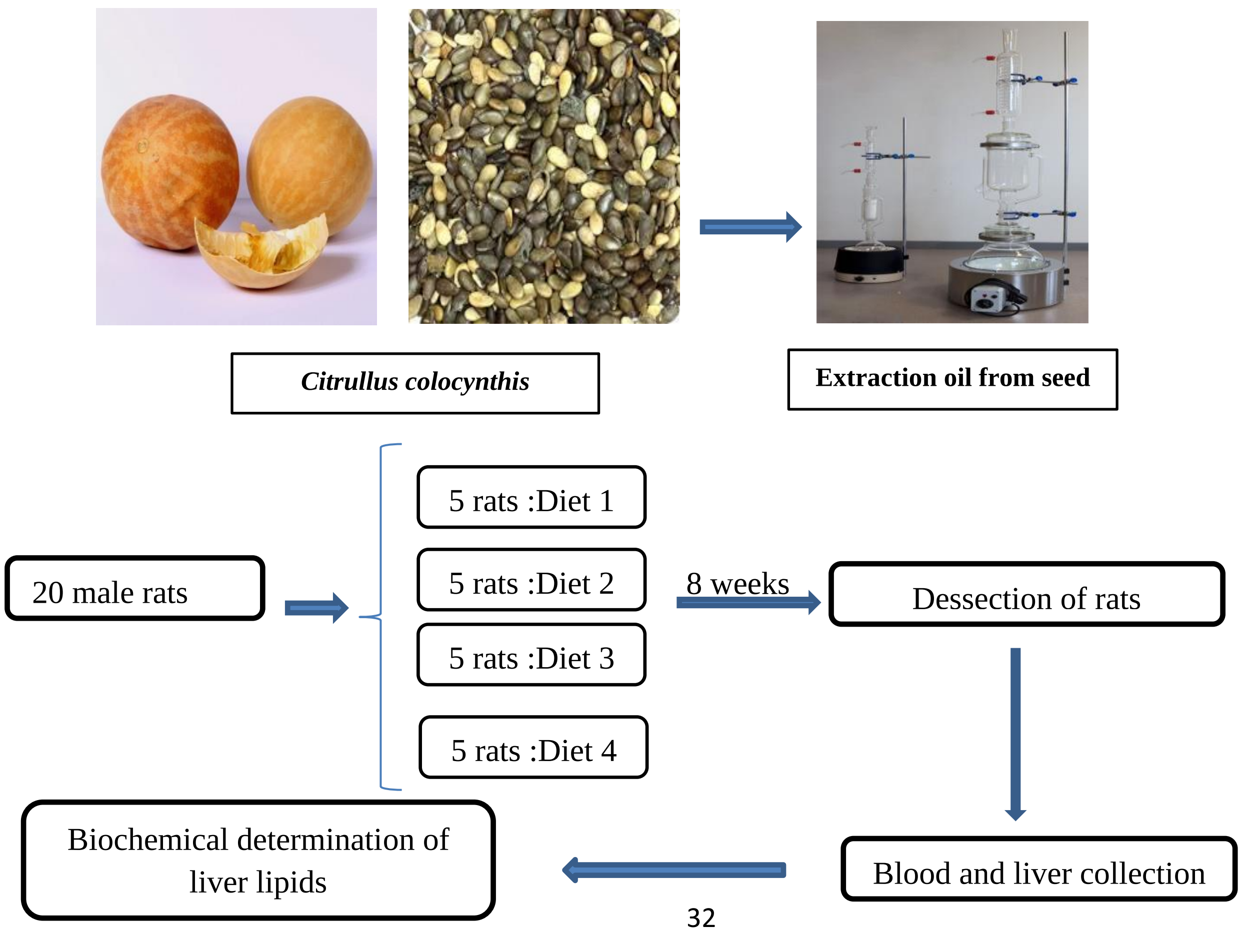
Highlights

- ✓ The high content of omega-6 fatty acids in colocynthis seed oil.
- ✓ Colocynthis seed oil induced decreases in body weight and visceral fat mass.
- ✓ Significantly reduced abdominal fat index, cholesterol, triglycerides and hepatic lipids.
- ✓ Colocynth oil has a positive effect on the reduction of blood and liver lipids.

Graphical Abstract



Abstract

Citrullus colocynthis is a plant of the cucurbitaceae family native to arid soils. It contains around 17% of oil, rich in essential polyunsaturated fatty acids with regulatory powers for certain metabolic pathologies such as hypercholesterolemia. The aim of this work is to test the effect of colocynth oil (CO) on hepatic lipids in *Wistar* rats fed hyper-fatty diets supplemented with (CO), compared with other rats fed control diets based on 8 % sunflower oil (SO). To this end, male *Wistar* rats weighing 80±5g were divided into four batches. The rats were fed different diets (R) for 2 months (D1: 16% casein + 8% SO, D2: 16% casein+ 8% CO, D3: 16% casein+ 30% fat+ 8% SO, D4: 16% casein+ 30% fat+ 8% CO), the weights of the rats and the quantity of food ingested were taken daily, at the end of the diet the rats were dissected and the livers recovered for hepatic lipid assays using the folch method. The results showed a reduction in triglycerides in the liver of rats fed diets containing oil of colocynth (37%) and reduced blood triglyceride (12%) and cholesterol (6%), whereas rats fed high-fat diets had elevated hepatic and blood lipid levels. Colocynth oil has a corrective and/or protective effect on obesity and its complications by preventing the accumulation of fat in the liver, thereby preventing hepatic steatosis.

Key words: Obesity; hepatic steatosis: colocynth oil; liver; PUFAs.

1. Introduction

A diet rich in calories and high in saturated fats contributes to overweight and obesity (Tomé, 1994). All the studies carried out in both industrialised and developing countries indicate a rapid increase in the prevalence of obesity and overweight, this pathology has reached the epidemic threshold and in 2022, 2.5 billion adults (18 years and older) were overweight. Of this total, 890 million were obese (WHO, 2024). Obesity is characterised by the development of several complications (hepatic steatosis, hypertension, hyperlipidemia), the two main ones being insulin resistance and excessive accumulation of visceral adipose tissue (Bray, 2013). These complications make it necessary to look for strategies to treat and/or prevent obesity. Treatment is aimed not only at weight loss, but also at reducing the risk of complications (De Bandt, 2004). Multidisciplinary therapeutic approaches involving doctors, nutritionists, psychiatrists, endocrinologists and gastroenterologists are needed to establish strategies. Comprehensive medical management, advice on diet and physical activity are the necessary prescriptions (Boirie, 2009).

Many studies, research into new remedies is focusing on the natural resources of medicinal plants used as alternatives in traditional medicine (Swantson-Flatt et al., 2004). Efforts are also being made to develop new oilseed crops that could be used for food, industrial and medicinal purposes (Schafferman et al., 1998). The colocynth «Citrullus colocynthis » L. schard belongs to the cucurbitaceae family, originating in tropical Asia and Africa, and is therefore widely distributed in the Saharan-Arabic region of Africa and in the Mediterranean region. The fruit of this species is widely used in natural medicine, and colocynth has a variety of therapeutic properties. It is used in the treatment of urogenital infections, as an anti-inflammatory against haemorrhoids, and as a hypoglycaemic (Schafferman et al., 1998; Adam et al., 2001), hypolipidic and anti-obesity plant (Ali Esmail, 2016). The seeds of this plant are rich in oil (with a yield of around 17 %) and protein (Al-khalifa, 1996).

Colocynth is considered to be an oleaginous plant with medicinal, food and non-food uses. Its oil contains unsaturated fatty acids, oleic acid (14.78%), palmitic acid (9.74%), stearic acid (7.37%) and linoleic acid (66.73%), which is an essential fatty acid that makes this oil medically valuable (Imededdine et al., 2012). Linoleic acid has a cholesterol-lowering effect. More specifically, it tends to lower blood cholesterol levels transported by LDL from the liver to peripheral tissues. n-3 PUFAs have a hypotriglyceridemic and moderating effect on platelet

aggregability in humans and animals (Simopoulos, 2006). Thus, the stability of an n-6: n-3 ratio is essential to maintain the balance of metabolic functions (Dominique, 2010). This composition of polyunsaturated acids in oil of colocynth may provide therapeutic solutions to obesity and its complications.
The aim of this study was to compare the effect of colocynth oil (CO) on weight trends and hepatic and blood lipid profil in obese Wistar rats.

2. Materials and Methods

2.1 Plant materials et seed oil extraction

The fruits used in our work come from the region of Méchria (south-west Algeria); In the laboratory the seeds recovered from the dried fruits are crushed using a grinder until a fine powder is obtained, from this powder the oil is extracted using a Soxhlet apparatus in the presence of an organic solvent "Hexane" for two hours. The oil content of these seeds is 17٪.

2.2 preparation and rearing of rats

This experimental study was carried out on growing male *Wistar* rats, reared in the animal house of the Department of Biology, University of Tlemcen, at an ambient temperature of 25°C. After 21 days from birth, the male rats were weaned and placed in new cages. After one week of weaning, known as the adaptation week, these rats were fed the same standard EL ALEF diet and drank tap water ad libitum until they reached an ideal weight of 80 ± 5g, at which time the rats were divided into 4 batches and each batch received a specific diet (Diet1, Diet 2, Diet 3, Diet 4).

2.3 Diets

Diet1: Balanced diet with 16% casein + 0.3 % methionine + 8% sunflower oil;
Diet2: Diet based on 8% colocynth oil + 16% casein + 0.3 % methionine;
Diet3: Diet has 16% casein + 40% fat (10% butter+ 10% Cheese+ 10% Margarine+8% sunflower oil);
Diet4: Diet based on 16% casein + 40% fat (10% butter+ 10% cheese+ 10% margarine+ 8% Colocynth oil).

2.4 Rat dissection and recovery of blood and organs

After 8 weeks, the rats were anaesthetised by intraperitoneal injection of 10% chloral hydrate (MERCK) and then sacrificed after 12 hours of fasting. Blood was drawn from the abdominal artery and collected in EDTA tubes and centrifuged at 3000 rpm for 15 min at 4°C (refrigerated centrifuge type Eppendorf 5702R). The plasma was recovered for various biochemical assays (glucose, total cholesterol, triglycerides). The liver was also taken to determine hepatic lipid levels.

2.5 Lipids extraction from liver

Lipids are extracted from the liver using the Folch (1957)**.** Technique, Folch extraction process is one of the most popular methods for isolating lipids from biological samples. It takes benefit of the biphasic solvent system consisting of chloroform/methanol/water in a volumetric ratio of 8:4:3 (v/v/v). After recovery of the lipids, cholesterol and total lipid assays are carried out on the recovered lipids using enzyme kits.

2.6. Biochemical analysis

Measurement of liver and plasma lipids (cholesterol and triglycerides cholesterol, triglycerides HDL and LDL cholesterol) was assessed by using commercially available kits, according to the manufacturer’s instructions (Spinreact kit, Spain).

2.7. Statistical analysis

Results are presented as means ± standard errors. After analysis of variance, the comparison of the means between several different groups is carried out by the one-way Anova analysis of variance. This analysis is completed by the Turkey test in order to classify and compare the averages two by two. This analysis is carried out using Minitab version 16 software.

3. Results and discussion

Changes in body weight over the eight weeks of experimentation are shown in (Table 1). All the hyperlipid diets resulted in weight gain compared with the control diet. However, supplementation with colocynth oil (D2 and D4) resulted in a significant reduction in weight compared with the group (D1, D3). The weight of rats fed diet with 4% colocynth oil and even compared with control rats (D1) (Table 1).

Studies have shown that an increase in the PUFA/AGS ratio is associated with an increase in resting energy expenditure, lipid oxidation and post prandial thermogenesis, so dietary intake of polyunsaturated fatty acids could have a protective effect against android obesity compared with saturated fatty acids or monounsaturated fatty acids (Couet et al, 1997, Van Marken et al, 1997). The PUFA/ SFA ratio in CO is (5.14), whereas the ratio in SO is (0.8), so sunflower oil is characterized by a very high quantity of SFA compared with CO, which explains the difference in weight development in rats fed a diet based on oil from the colophony compared with rats fed a diet based on sunflower oil.

Table 1. Body weight, of rats during the experimental period (weeks 0–8);

	Diet 1	Diet 2	Diet3	Diet4
Initial body weight	77±3.15	85±2.54	86.62±5.82	89.71±4.38
Final body weight	249.6±4.49	177.77± 3.62*	305.92±4.31	298.6±3.99

Values represent the mean ± standard error of the mean (SEM) of seven rats per group (n=5)

P < 0.05* *: Significant difference between* ***Diet 3*** *and* ***Diet 4.***

P < 0.05• *: Significant difference between* ***Diet 1****and* ***Diet 2.***

However, the results show an improvement in some lipid parameters in rats fed diets based on CO (Table 2). The latter is rich in minor components such as hydrocarbons, mainly squalenes and phytosterols, known for their beneficial effects. colocynth oil also contains a number of natural antioxidants, such as vitamin E contributes to the prevention of ageing and cardiovascular disease (Delplanque et al., 2002; Sebbagh et al., 2007; Sebbagh et al., 2009). A significant reduction in cholesterol levels was observed in rats fed CO-based diets. These results confirm those obtained by (Chan et al., 1996). According to these authors, a reasonable dietary intake of squalene has a beneficial effect on serum cholesterol levels. It is thought that the amount of squalene contained in CO may lead to a reduction in cholesterol levels, as it is likely that this reduction is due to the inhibition of intestinal absorption of cholesterol. It is indeed likely that this reduction is due to the inhibition of intestinal cholesterol absorption. Furthermore, linoleic acid has a beneficial effect on cholesterol levels provided that it does not exceed 4 to 10% of energy intake (Ruiz-Rodriguez et al., 2010). It should also be noted that myristic acid, present in CO (0.8%) but not in SO (Sebbagh et al., 2007; Sebbagh et al., 2009), occupies a special place and could even play a cholesterol-lowering role in dietary doses, given that it binds to several dozen proteins, giving them their metabolic functions (Walrand et al., 2010).

Table 2. Blood lipid and Glucose parameters.

Parameter	Diet1	Diet2	Diet3	Diet4
Glocose (g/l)	0.80±0.03	2.04 ±0.09	0.92±0.08	1.45±0.05
Total cholesterol	54.04±4.35	62.23 ±3.76*	52.52±2.22	58.46±3.05•
Triglycerides	41.22±3.22	57.78±5.46*	34.91±3.31	44.71±2.71•
HDL (mg/dl)	40.01±2.78	29.73±2.05*	40.10±2.5	35.44±2.59
LDL (mg/dl)	5.78±0.11	20.94±0.43*	5.43±0.16	15.07±0.19•

Values represent the mean ± standard error of the mean (SEM) of seven rats per group (n=5)

P < 0.05* *: Significant difference between* ***Diet 3*** *and* ***Diet 4.***

P < 0.05• *: Significant difference between* ***Diet 1****and* ***Diet 2.***

The results show a significant increase in hepatic lipids, cholesterol and triglycerides in rats fed hyperlipidic diets compared with rats fed isocaloric diets. On the other hand, there was a significant decrease in hepatic cholesterol and triglycerides in rats fed oil-based diets compared with rats fed sunflower oil-based diets (Table 3).

Mean hepatic triglyceride levels remained lower in rats on the Diet 4, Diet 2 diets compared with rats on the Diet 3, Diet 1 diets respectively. Ingestion of linoleic acid reduces hepatic lipid accumulation in some rodent models and hepatic steatosis (Shinji et al., 1996; Fernandez et al., 2005). More recently, it has been shown that PPAR receptors (peroxisome proliferator-activated receptors, regulators of ß-oxidation processes) are also activated by unsaturated fatty acids and certain eicosanoids. These molecules behave both as intercellular mediators and as local hormones that play numerous physiological and pathophysiological roles (Amal, 2006). In rodents, when PPARs are activated, the expression of the gene coding for carnitine palmitoyl transferase (CPT-1, the key enzyme in the penetration of fatty acids into the mitochondria) increases and the expression of the gene coding for acetyl carboxylase (the enzyme that synthesises malonyl CoA, an inhibitor of fatty acid oxidation) decreases (Tsuchida et al., 2005) and, as a result, ß-oxidation processes are accelerated, which is probably reflected in the reduction in hepatic triglycerides.

Table 3. Liver lipids values.

	Diet 1	Diet 2	Diet 3	Diet 4
Total liver Lipids	2.99±0.37	3.37±0.17*	2.47±0.13	2.81±0.17•
Liver Cholésterol	13.93±1.19	32.23±2.53*	10.72±0.73	23.6±1.83•
Liver Triglycérid	111.63±4.34	269.89±5.23*	119.42±3.59	169.05±4.1•

Values represent the mean ± standard error of the mean (SEM) of seven rats per group (n=5)

P < 0.05* *: Significant difference between* ***Diet 3*** *and* ***Diet 4.***

P < 0.05• *: Significant difference between* ***Diet 1****and* ***Diet 2.***

Values represent the mean ± standard error of the mean (SEM) of seven rats per group

A significant reduction in cholesterol levels and hepatic cholesterol was noted in the rats fed the coloquint oil-based diets. These results confirm those obtained by Chan and colleagues. According to these authors, if the diet includes reasonable doses of squalene and phytosterols, there is no harmful effect on serum cholesterol levels. It may be that the dose of squalene in colophony oil leads to a reduction in cholesterol concentration (cholesterolaemia and hepatic cholesterol), and it is probable that this reduction is due to inhibition of intestinal cholesterol absorption (Chan et al., 1996). We know that Hydroxy-Methyl-Glutaryl-CoA (HMG-CoA) reductase, the key enzyme in the cholesterol synthesis pathway, exists in two states: non-phosphorylated (active) and phosphorylated (inactive). The balance between these two forms depends on two enzymes: a phosphatase and a kinase. The action of oxysterols is thought to result from blocking the synthesis of this enzyme and their degree of phosphorylation (Tanaka et al., 1983).

It is clear that the different diets used in this study modify the biochemical parameters in parallel. The results obtained more or less support our team's hypothesis that daily consumption of colocynth oil could modify or correct certain metabolic disorders induced by unbalanced diets.

4. Conclusion

This study has established that high-fat diet causes an increase in body weight and reduces lipid metabolism as clearly seen by the marked rice of liver enzymes and lipid level. However, the survey proves that colocynth oil posses at best anti-obesity and hypolipidemic effects that protect body against the harmful effects of high-fat diet induced obesity. colocynth oil result in lipid lowering effects in obesity-induced rats; they also have weight-reducing ability. Due to the promising effects of colocynth oil in diet-induced obesity, complementary studies are sought in order to determine the active principle from this plant, followed by identifying the probable mechanism of action of colocynth oil in reducing plasma lipid and its weight-maintenance properties.

Author Contribution Statement

Sidi mohammed YAZIT: Experimentation and writing the original manuscript; **Radjaa Kaouther MEZIANE, Fouzia AMAMOU**: Experimentation; **Daoudi CHAABANE SARI**: supervisor work; **Zoubida MAMI, Hammadi LAZZOUNI**: contribution to the writing of the original manuscript.

Conflict of interest

The authors declare no competing conflict of interest.

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