Hypolipidaemic Effects of *Citrullus colocynthis* L. in Rabbits

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Abstract: Aim of this research to study the effect of *Citrullus colocynthis* (70% EtOH) extract on lipid profile on Rabbits. The plant extract was orally administered to the atherogenic rabbits (atherogenic diet + cholesterol powder supplement 400 mg/kg/body weight/day dissolved in 5 mL coconut oil) at dose of 1.2 g kg⁻¹ body weight/day. During the half period of the experiment blood was collected and serum was analyzed for lipid profile. Animals were sacrificed, the heart and the liver were collected and kept at -20°C until assayed. Biochemical analysis of blood serum and tissue (liver and heart muscle) level were made for cholesterol, phospholipids and triglycerides. In addition blood serum was analyzed further for HDL -Cholesterol. All the results were statistically analyzed using students t-test. Hypolipidaemic nature of *Citrullus colocynthis* (70% EtOH) extract was studied in hyperlipidaemic Rabbits. The increased cholesterol levels were brought to normal by administration of *Citrullus colocynthis*. Serum cholesterol levels dropped from 940.7 to 230.41 (75.55%) and further to 119.2 (87.32%) by the end of the experiment. Similarly, phospholipids and triglycerides levels were observed to be also reduced. The tissues lipids profiles of liver and heart muscle showed similar changes in those noticed in serum lipids. *Citrullus colocynthis* possesses active hypolipidaemic constituents.

Key words: Hyperlipidaemic, atherosclerosis, HDL-cholesterol, *Citrullus colocynthis*

INTRODUCTION

Desert plants in Jordan and their medicinal usage have been studied over thousands of years. Between 1998-1999, Lev and Amar (2002) conducted a survey study in the Kingdom of Jordan that enclosed selected markets dealing with traditional medicinal substances of ethnic communities throughout the kingdom. This survey included diversified medicinal plants used in the Kingdom and their healing characteristics.

In Jordan, *Citrullus colocynthis* L. (Cucurbitaceae), locally known as Handal is a well recognized plant in the traditional medicine and was used by people in rural areas as a purgative, antidiabetic and insecticide (Ageel et al., 1987). Mediterranean Handal was also known as effective medicine and was used as traditional medicine by both the old Greeks and Romans. Powder generated from the ripped fruit pulp has been used as purgative acting directly on the gastrointestinal tract a fact demonstrated by Elawad et al. (1984). This plant contains a number of chemical compounds including cucurbitacins A, B, C, D and α-elaterin attributed to its purgative effect (Watt and Breyer-Brandwijk, 1962; Bakhiet and Adam, 1995). Furthermore, components such as saponin and glycoside were also found in this plant possessing a hypoglycemic effect on rabbits (Abdel-Hassan, 2000). Recently, pharmacological research performed with this plant confirmed its effectiveness in the treatment of induced diabetes mellitus in rats through a significant stimulation of insulin secretion (Nimila et al., 2000). Toxic effect of this plant seeds and leaves extracts was demonstrated in sheep and found to be attributed to the administration of higher doses which could eventually lead to death (Adam et al., 2000). Moreover, Al-Ghaithi et al. (2004) reported that oral administration of this plant aqueous extract reduces certain biochemical parameters such as AST and LDH, eliminating the toxic effect of streptozocin-inuces diabetes in rats.

In this study, the aim is to determine the effect of oral administration of this plant extract on the cholesterol fed albino rabbits.

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MATERIALS AND METHODS

Animals: Adult healthy albino rabbits weighing 1.6-1.7 kg were housed individually in metallic cages in an air-conditioned room (26±2°C) were fed control diet (standard pellets). This diet was supplemented with green leafy vegetables and water ad libitum. The average consumption of diet was calculated 200 g day⁻¹.

Atherogenic diet was prepared by mixing wheat flour, milk powder, dried egg yolk, hydrogenated fat, butter, salt, jaggery and vitamins in the given below proportion.

<table>
<thead>
<tr>
<th>Component</th>
<th>Control (g%)</th>
<th>Atherogenic diet (g%)</th>
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<tbody>
<tr>
<td>Protein</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>65</td>
<td>60</td>
</tr>
<tr>
<td>Sucrose</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>Fat</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>Salts</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Vitamin</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Fiber</td>
<td>2</td>
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</tr>
</tbody>
</table>

In addition to the atherogenic diet, the rabbits were fed with cholesterol (400 mg kg⁻¹ body weight/day) dissolved in 5 mL coconut oil.

Plant and treatment: Citrus colycynthis L. plants were collected from Aqaba area, mature black seeds were separated manually from the pulp of the fruits and then the pulp was dried and grinded into powder. Powder was extracted by water-ethanol mixture (70/30 V/V) for 6 h following the instructions published by Nimla et al. (2000). This step was repeated three times then the filtrate was pooled and concentrated under vacuum keeping a temperature less than 50°C. The concentrate was dissolved in a normal saline and used. The extract, 1.2 g kg⁻¹, was administered orally to rats using animal feeding intubation's needles (Popper and Sons, New York).

Experimental design: Rabbits were divided in the following groups of six animals each:

- Group A: Vehicle (5 mL distilled water) treated control (120 days).
- Group B: Atherodiet + cholesterol feeding (120 days, 400 mg cholesterol/kg body weight/day in 5 mL coconut oil).
- Group C: Atherodiet + cholesterol feeding (120 days, atherodiet withdrawn + Citrus colycynthis L (70% EtOH) 1.2 g kg⁻¹ body weight/day (120-150 days).
- Group D: Atherodiet + cholesterol feeding (120 days, Atherodiet withdrawn + Citrus colycynthis L (70% EtOH) extract 1.2 g kg⁻¹ body weight/day (120-180 days).

All the animals were sacrificed and the heart, the aorta and the liver were removed, cleaned from the fat and adhering connective tissue and kept at -20°C until assayed. Biochemical analysis of blood serum and tissue (liver and heart muscle) were made for cholesterol (Zlatkis et al., 1953), Phospholipids (Zilversmit et al., 1950) and triglyceride (Gottfried and Rosenberg, 1973). In addition blood serum was analyzed further for HDL cholesterol (Burrstein et al., 1970).

Determination of LD₅₀ in mice: Graded doses of the aqueous extract of Citrus colycynthis L. in 0.2 normal saline were administered intraperitoneally to nine groups of six non fasted male albino mice (25-30 g each). They were housed in transparent plastic cages at 24°C. Mortality was noted after 24 h (Litchfield and Wilcoxon, 1949).

Statistical analysis: Student’s t-test was used to determine the significance of the differences between various groups. The results were expressed as mean±SE (Ipstein and Pol, 1970).

RESULTS

A non-significant reduction in the body weights was noticed in rats fed with cholesterol diet and later treated with Citrus colycynthis L. extract (Groups C and D) in comparison with the initial body weights. A non-significant change in heart weight of heart was. Liver weight was significantly increased in cholesterol fed rabbits (Table 1).

Citrus colycynthis L. (70% EtOH) extract feeding (Groups C and D) resulted in a significant lowering of total cholesterol, triglycerides and phospholipids of liver and ventricular heart muscles in comparison with cholesterol fed rabbits. In group D the reduction was on higher side (Table 2).

A eight-fold increase was observed in serum cholesterol in treated rabbits fed with atherogenic diet (p<0.001). In addition a significant reduction in the blood serum cholesterol was recorded in both Citrus colycynthis L. treatment group (Group C and D). Serum triglyceride increased significantly (p<0.001)

<table>
<thead>
<tr>
<th>Table 1: Change in body, liver and heart weight after cholesterol Citrus colycynthis L. (70% EtOH) extract feeding in rabbits (8 animals per treatment)</th>
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<tbody>
<tr>
<td>Body weight (kg)</td>
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<tr>
<td>Initial</td>
</tr>
<tr>
<td>Group A</td>
</tr>
<tr>
<td>Group B</td>
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<td>Group C</td>
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<td>Group D</td>
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after cholesterol feeding but was subsequently reduced after Citrullus colocynthis L. extract treatment. An increased in phospholipids and HDL cholesterol followed by cholesterol diet could be corrected by Citrullus colocynthis L. extract feeding (Table 3).

The LD$_{50}$ of the aqueous extract of Citrullus colocynthis L. was 4.14 g kg$^{-1}$ body weight (Litchfield and Wilcoxon method, 1949) which represents 20.54 g of crude powdered plant material 1 kg body weight.

**DISCUSSION**

A positive correlation between cholesterol plasma concentration and the risk of coronary heart disease has been widely demonstrated by the lipid research Clinics Primary Prevention Trials (Choi et al., 1991). In order to find good means to decrease plasma cholesterol level with minimal toxicity.

The level of cholesterol in lipoprotein fractions has been shown to be a good indicator of atherosclerosis risk in rabbits (Azzaron et al., 1996). Significant lowering of cholesterol after Citrullus colocynthis L. feeding indicates a risk reduction action.

Plasma triglycerides and cholesterol carry the highest risk for ischemic heart disease (Carlson and Bottiger, 1985), HDL and LDL cholesterol are significant variables and indicator for coronary heart disease (Miller and Miller, 1975). It is reported that HDL is inversely related to total body cholesterol. Treatment with Citrullus colocynthis L. extract reduces serum cholesterol and triglyceride by 7.8 and 3.5 times, respectively. HDL alters the balance of unesterified cholesterol between plasma and cell by increasing its utilization in the lecithin cholesterol acyl transferase (LCAT) system to form cholesterol ester which moves rapidly into the cells. Decreased total cholesterol and phospholipid after Curcuma longa extract feeding indicate the anti-atherogenic or hypolipidaemic nature of the plant product. Further reduction in total cholesterol, triglyceride and phospholipids of liver and ventricular heart muscle may be suggestive of a beneficial role of Citrullus colocynthis L. in hyperlipidaemic subject.

The possible mechanism of lipid alteration might be cholestatic effect of Citrullus colocynthis L. in liver enhanced removal or catabolism of lipoproteins (Brattsand, 1975) and/or inhibition of lysosomal lipid hydrolytic enzymes secreted by the liver (Sherlock, 1998). In conclusion Citrullus colocynthis L. possesses active hypolipidaemic constituents. Further chemical and pharmacological investigations are in progress.

**REFERENCES**


