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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 hall 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 porgative 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 hypoglycimic 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 insuline secretion (Nmila *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 streptozotocin-induces 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.

Component	Control (g %)	Atherogenic diet (g %)
Protein	20	15
Carbohydrate	65	60
Sucrose	3	3
Fat	5	15
Salts	4	4
Vitamin	1	1
Fiber	2	2

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: *Citrullus colocynthis* 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 Nmila *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 + *Citrullus colocynthis* L (70% EtOH) 1.2 g kg^{-1} body weight/day (120-150 days).
Group D: Atherodiet + cholesterol feeding (120 days, Atherodiet withdrawn + *Citrullus colocynthis* 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 (Burnstein *et al.*, 1970).

Determination of LD_{50} in mice: Graded doses of the aqueous extract of *Citrullus colocynthis* 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 Poly, 1970).

RESULTS

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

Citrullus colocynthis 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 ($p \leq 0.001$). In addition a significant reduction in the blood serum cholesterol was recorded in both *Citrullus colocynthis* L. treatment group (Group C and D). Serum triglyceride increased significantly ($p \leq 0.001$)

Table 1: Change in body, liver and heart weight after cholesterol/*Citrullus colocynthis* L. (70% EtOH) extract feeding in rabbits (8 animals per treatment)

	Body weight (kg)		Body weight (%)	
Treatments	Initial	Final	Liver	Heart
Group A	1.76±0.14	1.81±0.16	2.23±0.22	0.21±0.22
Group B	1.67±0.04	1.52±0.11	4.37±0.50	0.24±0.50
Group C	1.73±0.12	1.55±0.21	2.28±0.61	0.22±0.61
Group D	1.68±0.15	1.53±0.15	2.18±0.42	0.21±0.42

Table 2: Change in tissue lipids after cholesterol/*Citrullus colocynthis* L. (70% EtOH) extract feeding in rabbits (8 animals per treatment)

	Cholesterol (mg g^{-1})		Triglycerides (mg g^{-1})		Phospholipids (mg g^{-1})	
Treatments	Liver	Heart	Liver (g)	Heart (g)	Liver	Heart muscles
Group A	11.2±0.16	7.2±0.30	4.2±0.07	4.9±0.17	7.90±0.20	10.30±0.03
Group B	17.1±0.34[c]	8.3±0.40[c]	6.1±0.13[c]	13.3±0.18[c]	12.90±0.12[c]	11.02±0.05[c]
Group C	12.3±0.7[c]	11.3±0.3[c]	5.9±0.10[b]	5.9±0.37[d]	9.78±0.35[c]	9.41±0.02[c]
Group D	11.6±0.6[c]	9.2±0.2[c]	4.8±0.33[c]	4.8±0.23[c]	8.70±6.05[c]	8.90±0.03

[b]$p \leq 0.01$ [c]$p \leq 0.001$, [d]NS = Non-significant

Table 3: Change in Serum analysis after cholesterol/ *Citrullus colocynthis* L (70% EtOH) extract feeding in rabbits (8 animals per treatment)

Treatments	Total cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	Phospholipids (mg dL^{-1})	HDL cholesterol (mg dL^{-1})
Group A	99.30±9.0	66.30±3.1	175.60±9.1	37.30±2.47
Group B	824.19±18.3[c]	268.30±11.7[c]	367.50±11.4[c]	261.11±13.4[c]
Group C	208.70±13.5[b]	92.34±14.3[c]	181.30±13.6[b]	72.50±3.4[c]
Group D	106.50±14.6[c]	76.50±9.5[c]	117.33±15.3[c]	43.66±2.3[c]

[b]$p \leq 0.01$, [c]$p \leq 0.001$

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 Trails (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 (Azzarito *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

Abdel-Hassan, I.A., J. Ahmed, A. Barry and S.T. Mohammeda, 2000. The hypoglycaemic and antihyperglycaemic effect of *Citrullus colocynthis* fruit aqueous extract in normal and alloxan diabetic rabbits. J. Ethnopharmacol., 71: 325-330.

Adam, S.E.I., A.H. Al-Farhan and A. Al-Yahya, 2000. Effect of combined *Citrullus colocynthis* and *Rhazya stricta* use in Najdi sheep. Am. J. Clin. Med., 28: 385-390.

Ageel, A.M., J.S. Mossa, M.A. Al-Yahya, M. Tariq and M.S. Al-Said, 1987. Plants used in Saudi folk medicine. King Saud University Press, Riydah.

Al-Ghaithi, F., M.R. El-Ridi, E. Adeghate and M.H. Amiri, 2004. Biochemical effects of *Citrullus colocynthis* in normal and diabetic rats. Mol. Cell Biochem., 261: 143-149.

Azzarito, C., L. Boiardi, W. Vergoni, M. Zini and I. Portioli, 1996. Testicular function in hypercholesteromic male patients during prolonged simvastatine Treatment. Horm. Metab. Res., 28: 193-198.

Bakhiet, A.O. and S.E.I. Adam, 1995. An estimation of *Citrullus colocynthis* toxicity for chicks. Vet. Hum. Toxicol., 37: 356-359.

Brattsand, R., 1975. Actions of vitamins A and E and some nicotinic acid derivatives on plasma lipids and on lipid infiltration of aorta in cholesterol-fed rabbits. Atherosclerosis, 22: 47-61.

Burnstein, M., H.R. Scholnic and R. Morfin, 1970. Rapid method of isolation of lipoprotein from human serum by precipitation with polyanion. J. Lipid. Res., 11: 583-587.

Carlson, L.A. and L.E. Bottiger, 1985. Risk factors for ischaemic heart disease in men and women: Results of the 19 year follow-up of the Stockholm Prospective Study. Acta Med. Scand., 218: 207-211.

Choi, J.S., T. Yokozawa and H. Oura, 1991. Improvement of hyperglycemia and hyperlipemia in streptozotocin-diabetic rats by a methanolic extract of Prunus davidiana stems and its main component, prunin. Planta Med., 57: 208-211.

Elawad, A.A., E.M. Abdel Bari, O.M. Mahmoud and S.E. Adam, 1984. The effect of *Citrullus colocynthis* on sheep. Vat. Hum. Toxicol., 26: 481-485.

Gottfried, S.P. and B. Rosenberg, 1973. Improved manual spectrophotometric procedure for determination of serum triglycerides. Clin. Chem., 19: 1077-1078.

Ipstein, J. and F. Poly, 1970. In: Banchroft`s introduction to biostatics 2nd Edn. Harper International, pp: 44-64.

Litchfield, J.T. and F.A. Wilcoxon, 1949. A simplified method of evaluating dose-effect experiments. J. Pharmacol. Exp. Ther., 96: 99-113.

Lev, E. and Z. Amar, 2002. Ethnopharmacological survey of traditional drugs sold in the Kingdom of Jordan. J. Ethnopharmacol., 82: 131-145.

Miller, C. and N.C. Miller, 1975. Plasma-high-density-lipoprosein concentration and development of ischaemic heart-disease. Lancet, 1: 16-19.

Nmila, R., R. Gross, H. Rchid, M. Roye, M. Manteghetti, P. Petit, M. Tijane, G. Ribes and Y. Sauvaire, 2000. Insulinotropic effect of *Citrullus colocynthis* fruits extract. Planta Med., 66: 418-423.

Sherlock, S., 1998. Overview of chronic cholestatic conditions in adults: Terminology and definitions. Clin. Liver Dis., 2: 217-233.

Watt, J.M. and M.G. Breyer-Brandwijk, 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. Being an Account of Their Medicinal and Other Uses, Chemical Composition, Pharmacological Effects and Toxicology in Man and Animal. 2nd Edn., Edinburgh: E and S Livingstone Ltd.

Zilversmit, D.B. and A.K. Davis, 1950. Microdetermination of plasma phospholipids by trichloroacetic acid precipitation. J. Lab. Clin. Med., 35: 155-160.

Zlatkis, A., B. Zak and A.J. Boyle, 1953. A new method for the direct determination of serum cholesterol. J. Lab. Clin. Med., 41: 486-492.