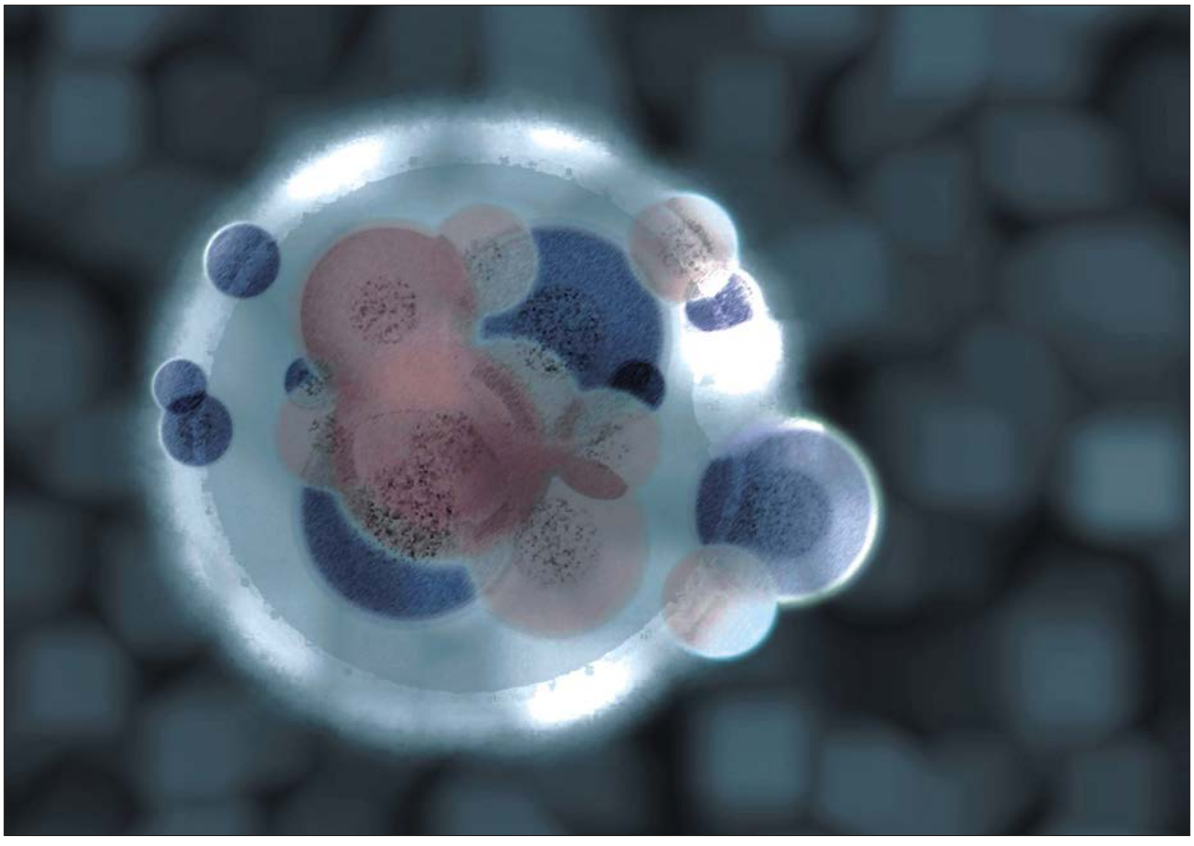

Journal of Biological Sciences

ISSN 1727-3048

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Journal of Biological Sciences 9 (1): 88-91, 2009
ISSN 1727-3048

Hypolipidaemic Effects of *Euphorbia prostrata* in Rabbits

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Abstract: The aim of this research was to study the effect of 70% ethanol extract of *Euphorbia prostrata* on lipid profile in rabbits. The plant extract was orally administered to the atherogenic rabbits (atherogenic diet + cholesterol powder supplement at 400 mg/kg/body weight/ day dissolved in 5 mL coconut oil) at dose of 0.0012 kg body weight/day. During the hall period of the experiment blood samples were collected and serum was analyzed for lipid profile. At the end of the experiment the animals were sacrificed; the heart and the liver were collected and stored at -20°C until assayed. Biochemical analysis of blood serum and tissue (liver and heart muscle) were performed 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 *Euphorbia prostrata* extract was studied in hyperlipidaemic rabbits. The increased cholesterol levels were brought to normal by administration of *Euphorbia prostrata.* 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 reduced. The tissues lipids profiles of liver and heart muscle showed similar changes in those noticed in serum lipids. We can conclude from these results that *Euphorbia prostrata* possesses active hypolipidaemic constituents. The results suggest the validity of *Euphorbia prostrata* clinical use in hypolipidaemic control, after their toxicological investigation.

Key words: Hyperlipidaemic, atherosclerosis, HDL-cholesterol, *Euphorbia prostrate*

INTRODUCTION

Desert plants in Jordan and their medicinal usage have been studied over thousands of years. 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.

The genus *Euphorbia* is the largest in the plant family Euphorbiaceae, comprising about 2000 known species and ranging from annuals to trees. All contain latex and have unique flower structures. A significant percentage, mostly those originating in Africa and Madagascar, are succulent (Zargari, 1993).

The plants of the family Euphorbiaceae contain the well-known skin irritating and tumor-promoting diterpenoids, which have tigliane, ingenane and daphnane skeletons (Kanokwiroon *et al*., 2008).

Some of the species are used in folk medicines to cure skin diseases, gonorrhea, migraines, intestinal parasites and warts (Singla and Pathak, 1990). In addition, several macrocyclic diterpenoids with antibacterial, anticancer, PGE2-inhibitory, anti-multidrug-resistant, prolyl endopeptidase inhibitory, antifeedant, anti-HIV and analgesic activity have recently been isolated from different *Euphorbia* species. *Euphorbia* species include jatrophane, ingol and myrsinane diterpenoids (Abdelgaleil *et al*., 2001; Ravikanth *et al*., 2002; Wang *et al*., 2002).

The most important constituents of *Euphorbia prostrata* are gallic acid, corilagin, 1,2,3- tri-O-galloyl-D-glucose, geraniin, tellimagradin I, II, rugosin A, rugosin E, rugosin D and rugosin G (Chen *et al*., 1992). *Euphorbia prostrata* is traditionally used in many parts of the world for the treatment of many diseases, it live in warm month in mountain and hill.

This plant was suggested to exert anti-diabetics (Akhtar *et al*., 1984; Alarcon-Aguilara *et al*., 1998) , anti-inflammatory properties (Singla and Pathak, 1989, 1990), anti dysentery (Kamgang *et al*., 2007). In this study, the aim is to determine the effect of oral administration of *Euphorbia prostrata* plant extract on the cholesterol fed albino Rabbits.

MATERIALS AND METHODS

Animals: Adult healthy albino rabbits weighing 1.6-1.7 kg were housed individually in metallic cages in an air-

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conditioned room (26±2°C) and were fed control diet (standard pellets). This diet was supplemented with green leafy vegetables and water *ads 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 as shown in Table 1.

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

Plant and treatment: Aerial parts of *Euphorbia prostrata* plants were collected from Zoubia area (West-north of Jordan) during summer of 2007. The aerial parts were dried and grinded into powder in Al-Zaytoonah Private University, Jordan. Each 500 g of dried and ground *Euphorbia prostrata* was 6 then refluxed in (2 L) 70% ethanol at 50°C for 36 h in continuous extraction using soxhlet apparatus. Ethanol extract was filtered and concentrated under reduce pressure at 50°C using a rotary evaporator. The net yield was 30 g kg^{-1}. 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).

Determination of LD_{50} in mice: Determination of LD_{50} in mice was conducted to determine the dose to be given to rabbits. Graded doses of the aqueous extract of *Euphorbia prostrata* in 0.2 distilled water were administered intraperitonealy to six 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 1 h (Hruskova *et al*., 1961; Litchfield and Wilcoxon, 1949).

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

Group A: Vehicle (5 mL normal saline) treated control (120 days)

Group B: Atherodiet + cholesterol feeding (120 days, 400 mg cholesterol/kg body 3 weight/day in 5 mL coconut oil)

Table 1: Foods supplement to animal groups (8 animals per treatment)

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

Group C: Atherodiet + cholesterol feeding (120 days, atherodiet withdrawn + *Euphorbia prostrata* (70% EtOH) 1.2 g kg^{-1} body weight/day (120-150 days)

Group D: Atherodiet + cholesterol feeding (120 days, Atherodiet withdrawn + *Euphorbia prostrata* (70% EtOH) extract 1.2 g kg^{-1} body weight/day (120-180 days)

At the end of the experiment all the animals were sacrificed and the heart, the aorta and the liver were removed, cleaned from the fat and adhering connective tissue and stored 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 and Davis, 1950) and triglyceride (Gottfried and Rosenberg, 1973). In addition blood serum was analyzed further for HDL-cholesterol (Burnstein *et al*., 1970).

Statistical analysis: Data were expressed as Mean±SD [statistical package for social sciences (SPSS, version 11.5)]. Differences between control and *Euphorbia prostrata* exposed groups were analyzed using either the Chi-square test, t-test or nonparametric (Sheskin, 2004), when applicable. A p-value of <0.05 was considered significant (Ipsen and Feigl, 1970).

RESULTS

A non-significant reduction in the body weights was noticed in rabbits fed with cholesterol diet and later treated with *Euphorbia prostrata* extract (Groups C and D) in comparison with the initial body weights. A non-significant change in hear weight of cholesterol fed rabbits. Liver weight was significantly increased in cholesterol fed rabbits (Table 2).

Euphorbia prostrata (70% EtOH) extract feeding (Groups C and D) resulted in a significant lowering of

Table 2: Change in body, liver and heart weight after cholesterol/ *Euphorbia prostrata* (70% EtOH) extract feeding in rabbits (8 animals per treatment)

Treatment group	Body weight (kg)		Body weight (%)	
	Initial	Final	Liver	Heart
A	1.68±0.11	1.75±0.73	2.17±0.27	0.22±0.60
B	1.58±0.07	1.46±0.89	4.19±0.16	0.25±0.38
C	1.65±0.32	1.50±0.33	2.21±0.31	0.23±0.34
D	1.60±0.11	1.47±0.27	2.13±0.37	0.20±0.17

Group A: Vehicle (5 mL normal saline) treated control (120 days). Group B: Atherodiet + cholesterol feeding (120 days, 400 mg cholesterol/kg body 3 weight/day in 5 mL coconut oil). Group C: Atherodiet + cholesterol feeding (120 days, atherodiet withdrawn + *Euphorbia prostrata* (70% EtOH) 1.2 g kg^{-1} body weight/ day (120-150 days). Group D: Atherodiet + cholesterol feeding (120 days, Atherodiet withdrawn + *Euphorbia prostrata* (70% EtOH) extract 1.2 g kg^{-1} body weight/day (120-180 days)

Table 3: Change in tissue lipids after cholesterol/ Euphorbia prostrata (70% EtOH) extract feeding in rabbits (8 animals per treatment)

Treatment group	Cholesterol		Triglycerides (mg g^{-1})		Phospholipids (mg g^{-1})	
	Liver	Heart	Liver (g)	Heart (g)	Liver	Heart muscles
A	9.40±0.16	6.80±0.22	3.80±0.14	4.30±0.21	7.72±0.56	9.19±0.09
B	17.10±0.70^c	8.40±0.60^c	5.50±0.31^c	12.60±0.37^c	12.80±0.56^c	9.98±0.07^c
C	10.09±0.50^c	10.30±0.40^c	5.60±0.17^b	4.33±0.86^d	9.90±0.84^c	8.45±0.02^c
D	9.81±0.17^c	8.20±0.90^c	4.02±0.88^c	3.99±0.48^c	8.43±0.27^c	8.04±0.05^c

^{a}p≤0.05, ^{b}p≤0.01, ^{c}p≤0.001

Table 4: Change in serum analysis after cholesterol/*Euphorbia prostrata* (70% EtOH) extract feeding in rabbits (8 animals per treatment)

Treatment group	Total cholesterol (mg dL^{-1})	Triglycerides (mg dL^{-1})	Phospholipids (mg dL^{-1})	HDL cholesterol (mg dL^{-1})
A	99.3±3.3	65.30±2.66	172.00±6.3	27.30±1.9
B	810.2±6.8^c	263.50±6.87^c	257.33±7.66^c	249.00±4.3^c
C	207.6±7.8^b	94.90±9.7^c	178.47±5.22^b	70.20±1.44^c
D	105.2±8.84^c	78.21±4.11^c	116.80±7.67^c	44.54±1.26^c

^{a}p≤0.05, ^{b}p≤0.01, ^{c}p≤0.001

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 3).

An 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 *Euphorbia prostrata* treatment group (C and D). Serum triglyceride increased significantly (p≤0.001) after cholesterol feeding but was subsequently reduced after *Euphorbia prostrata* extract treatment. An increased in phospholipids and HDL cholesterol followed by cholesterol diet could be corrected by *Euphorbia prostrata* extract feeding (Table 4).

The LD_{50} of the aqueous extract of *Euphorbia prostrata* was 4.14 g kg^{-1} body weight (according to the Litchfield and Wilcoxon, 1949) method which represents 20.54 g of 15 crude powdered plant material 1 kg body weights.

DISCUSSION

The present study was designed to investigate the hypolipidemic effects of *Euphorbia prostrata* (70% EtOH) extract on lipid profile on rabbits. Results of this study demonstrated that hypolipidaemic nature of *Euphorbia prostrata*. The increased cholesterol levels were brought to normal by adding of *Euphorbia prostrata.* Serum cholesterol levels dropped significantly 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.

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 *Euphorbia prostrata* feeding indicates a risk reduction action.

Plasma triglycerides and cholesterol carry the highest risk for ischemic heart disease (McBride, 2008). HDL and LDL cholesterol are significant variables and indicator for 11 coronary heart disease (Miller and Miller, 1975). It is reported that HDL is inversely related to total body cholesterol. Treatment with *Euphorbia prostrata* extract reduces serum cholesterol and triglyceride by 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 *Euphorbia prostrata* L. in hyperlipidaemic subject.

The possible mechanism of lipid alteration might be cholestatic effect of *Euphorbia prostrata* 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 *Euphorbia prostrata* possesses active hypolipidaemic constituents. Further chemical and pharmacological investigations are in progress.

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