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Alcoholic extract effect of *Withania somnifera* roots on cholesterol diet induced hyperlipidemia in male rabbits

Layla Abd-Al-Sattar Sadiq Laylani¹, Ahmed Hamad Saleh^{*2}

¹Community Health Department .Instituted Kirkuk Technical . Northern Technical University, Iraq.

²Medical Laboratory Technology Department, Colloge Al-Qalam University, Kirkuk, Iraq

Abstract

The current study was used 20 male rabbits that divided into four groups (consist of five rabbits in each group), The first group was considered as control and fed normal diet and given tap water, The second group was consisted of rabbits that fed on high cholesterol diet for six weeks, The third group was consisted of rabbits that fed on high cholesterol diet and 50mg root extract for six weeks, The forth group was consisted of rabbits that fed on high cholesterol diet and 100mg root extract for six weeks. The male rabbits that feeding on high cholesterol diet showed significant increased ($P < 0.05$) the levels of cholesterol, triglyceride, LDL, VLDL, MDA and HMG-CoA reductase while HDL and GSH levels were significantly decreased ($P < 0.05$) compared with to control group. It was concluded from this study that root extract has amply good effective role on hyperlipidemia of male rabbits.

Keywords: *W. somnifera*, hyperlipidemia, HMG-CoA reductase, cholesterol.

تأثير المستخلص الكحولي لجذور نبات الونذنية (*Withania somnifera*) ضد فرط الدهون المستحدث بوساطة الكوليسترول في ذكور الارانب

ليلى عبد الستار¹، احمد حمد^{*2}

¹قسم الصحة المجتمعية، معهد كركوك التقنية، الجامعة التقنية الشمالية ، العراق.

²قسم تكنولوجيا المختبرات الطبية ، جامعه القلم ، كركوك ، العراق

الخلاصة

استخدمت الدراسة الحالية ٢٠ ذكر ارنب ووزعت على ٤ مجموعات (كل مجموعة تحتوي ٥ ارناب) مجموعة السيطرة والتي تغذت على غذاء وماء اعتيادي، مجموعة الارانب التي تغذت على غذاء عالي الكوليسترول لمدة ٦ اسابيع، مجموعة الارانب التي تغذت على حمية غذائية عالية الكوليسترول مع تجريع مستخلص الجذور 50ملغم/كغم لمدة 6 اسابيع، مجموعة الارانب التي تغذت على حمية غذائية عالية الكوليسترول مع تجريع مستخلص الجذور 100 ملغم/كغم لمدة 6 اسابيع. الارانب الذكور التي تغذت على حمية غذائية عالية الكوليسترول اظهرت ارتفاع مستويات كل من الكوليستيرول والشحوم الثلاثية والبروتينات الدهنية واطئة الكثافة والبروتينات الدهنية واطئة الكثافة جدا و MDA وانزيم HMG-CoA reductase مع انخفاض معنوي في مستويات البروتينات الدهنية عالية الكثافة والكلوتاثيون. يستنتج من هذه الدراسة ان مستخلص جذور نبات الونذنية دور فعال ضد فرط الدهون في ذكور الارانب.

Introduction

Hyperlipidemia has been implicated in atherosclerosis, which is the primary cause of heart disease and stroke [1, 2]. There are several mechanisms which could play a role in the pathogenesis of vascular complications and most of them are triggered by hyperglycemia and hyperglycemia-induced oxidative stress [3]. Atherosclerosis develops from low-density lipoprotein molecules (LDL) becoming oxidized (LDL-OX) by free radicals, particularly oxygen free radicals (ROS). When oxidized LDL comes in contact with an artery, uptake of oxidized LDL by macrophages, smooth muscle cell migration and proliferation and deposition of collagen. Plaque ruptures lead to platelet activation and thrombosis [4, 5].

Withania somnifera belongs to the family solanaceae. It is an evergreen shrub, also known as winter cherry and Ashwagandha, has been an important property as herbal medicine [6]. *W. somnifera* is used in medicine, for antimicrobial activity, anti-fungal, antioxidant, anti-inflammatory, anticancer and other different diseases [7]. The roots of *W. somnifera* contain different type of alkaloids, flavonoids, anolides, reducing sugars, amino acids, steroids, volatile oil, starch, glycosides, hentriacontane, dulcitol and withaniol [8-9]. So the aim is to study the effect of *Withania somnifera* roots extract on hyperlipidemia in male rabbits.

Materials & methods

Animal model

Twenty adult male rabbits, (wt 1.5-2 kg & age: 6-8 Mon.) collected from Kirkuk city, and fed on standard pellet diet and given tap water for two month to be sure all animals without any diseases.

Plant collection and extraction

W. somnifera were collected from several places in Kirkuk during April 2016. The roots were cleaned and washed and then crushed by an electric grinder. The powder of roots was extracted with 70% ethyl alcohol, filtered through filter paper. The filtrate was evaporated to dryness in a vacuum oven. The extract was kept in freezer (0 °C) until use [6].

Experimental design

Twenty adult male rats were used and divided to four groups (each group consist five rats) as follow:

- A. Control group (CG): rabbits feeding on normal diet for six weeks.
- B. Hyperlipidemia group (HG): rabbits feeding on high cholesterol diet for six weeks.
- C. Hyperlipidemia and 50mg/kg extract group (HEG 50mg/kg): rabbits feeding on high cholesterol diet and 50mg root extract for six weeks.
- D. Hyperlipidemia and 100mg/kg extract group (HEG 100mg/kg): rabbits feeding on high cholesterol diet and 100mg root extract for six weeks.

Blood samples

The blood samples were collected by cardiac puncture under anesthesia (ketamine and xylazine) and put in test tubs. Then, the tubes (after clotting) were centrifugation 5000 cycle/min for 10 min to obtain sera. Sera were kept in freezer (0°C) until use.

Blood measurements

The reagents of lipid profile (total cholesterol, triglyceride, high lipoprotein density (HDL), low lipoprotein density (LDL) and very low lipoprotein density (VLDL)) were supplied by Randox/China, while reagent of HMG-CoA reductase was supplied by Biosource/USA and measurements done according to the procedure of kits. Malonedialdehydied (MDA) was measured in the liver extract by thiobarbituric acid (TBA) according to method [10], with Glutathione (GSH) was measured in the liver extract by using DTNB according to method [11].

Statistical analysis

The statistical analysis of data was basis of one way analysis of variance (ANOVA) using significant level at (P<0.05), and using a statistical Minitab program.

Results & Discussion

Lipid profile and HMG-CoA reductase

Total cholesterol level in HG group (305± 28.82 mg/dl) show high significant increased (P≤0.05) compare to control group (117±16.42 mg/dl). While, in treated groups (HGE 50 mg/kg and HGE 100 mg/kg), (138.19±21.54 mg/dl; 121±13.72 mg/dl respectively), there are Non-significant changes compare to control group. Triglyceride level in group HG (218± 26.15 mg/dl) shows high significant increased compare to control group (98±12.39 mg/dl). While, in treated groups (HGE 50 mg/kg and

HGE 100 mg/kg), (109.48±18.11 mg/dl; 92±15.2 mg/dl respectively), there are Non-significant difference compare to control group. HDL level in group HG (30± 4.49 mg/dl) shows high significant decrease compare to control group (41±7.37 mg/dl). While, in treated groups (HGE 50 mg/kg and HGE 100 mg/kg), (39.54±5.14; 40±4.67 respectively), there are Non-significant changes compare to control group as shown in Table-1.

LDL level in group HG (231.69±34.46 mg/dl) show high significant increased ($P \leq 0.05$) compare to control group (56.4±11.07 mg/dl). While, in treated groups (HGE 50 mg/kg and HGE 100 mg/kg), (76.79±12.82 mg/dl; 62.59±9.61 mg/dl respectively), there are Non-significant changes compare to control group. VLDL level in group HG (43.31± 6.18 mg/dl) show high significant increased compare to control group (19.6±3.17). While, in treated groups (HGE 50 mg/kg and HGE 100 mg/kg), (21.86±4.05 mg/dl; 18.41±3.2 mg/dl respectively), there are Non-significant changes ($P \leq 0.05$) compare to control group. HMG-CoA reductase level in group HG (2.67± 0.085 ng/ml) show high significant increased ($P \leq 0.05$) compare to control group (2.79±0.062 ng/ml). While, in treated groups (HGE 50 mg/kg and HGE 100 mg/kg), (2.69±0.058 ng/ml; 2.73±0.072 ng/ml respectively), there are Non-significant changes compare to control group as shown in Table-2.

Table 1- Levels of total cholesterol, triglyceride and HDL in experimental groups.

Parameters Groups	Total cholesterol mg/dl	Triglyceride mg/dl	HDL mg/dl
CG	117±16.42 ^b	98±12.39 ^b	41±7.37 ^a
HG	305± 28.82 ^a	218± 26.15 ^a	30± 4.49 ^b
HGE 50 mg/kg	138.19±21.54 ^b	109.48±18.11 ^b	39.54±5.14 ^a
HGE 100 mg/kg	121±13.72 ^b	92±15.2 ^b	40±4.67 ^a

Note: same letters mean there is non-significant difference while different letters mean there is non-significant difference.

Table 2- levels of total LDL, VLDL and HMG-CoA reductase in experimental groups.

Parameters Groups	LDL mg/dl	VLDL mg/dl	HMG-CoA reductase ng/ml
CG	56.4±11.07 ^b	19.6±3.17 ^b	2.79±0.062 ^a
HG	231.69±34.46 ^a	43.31± 6.18 ^a	2.67± 0.085 ^a
HGE 50 mg/kg	76.79±12.82 ^b	21.86±4.05 ^b	2.69±0.058 ^a
HGE 100 mg/kg	62.59±9.61 ^b	18.41±3.2 ^b	2.73±0.072 ^a

Note: same letters mean there is non-significant difference while different letters mean there is non-significant difference.

On the other hand, the present study shows high significant increased ($P < 0.05$) in MDA levels in group HG compare to control group. While, GSH levels show significant decreased in group HG compared to control group. after using root extract, HGE 50 mg/kg and HGE 100 mg/kg groups show no significant changes ($P < 0.05$) compare to control group as shown in Table-3.

Table 3- levels of MDA and GSH reductase in experimental groups.

Parameters Groups	MDA (mmol/l)	GSH (mol/l)
CG	1.27 ± 0.03 ^b	0.73 ± 0.043 ^a
HG	2.09 ± 0.036 ^a	0.23 ± 0.031 ^c
HGE 50 mg/kg	1.38 ± 0.29 ^b	0.62 ± 0.055 ^a
HGE 100 mg/kg	1.28 ± 0.036 ^b	0.71 ± 0.62 ^a

Note: same letters mean there is non-significant difference while different letters mean there is non-significant difference.

The results of present study show the potential role against the hyperlipidemia that inducing in rats and the results is in agreement with [12] who referred to the role of *W. somnifera* root extract as antihyperlipidemia. Where, after increased the levels of total cholesterol (190.42 ± 16.20 mg/dl), triglyceride (114.82 ± 10.17 mg/dl), LDL (75.03 ± 4.55 mg/dl), VLDL (22.96 ± 2.03 mg/dl) and decreased HDL (16.82 ± 1.59 mg/dl). They using *W. somnifera* root extract in treatment and found that the levels of total cholesterol (102.46 ± 6.92 mg/dl), triglyceride (86.43 ± 7.65 mg/dl), LDL (47.83 ± 4.81 mg/dl), VLDL (17.29 ± 1.53 mg/dl) and HDL (22.31 ± 1.76 mg/dl) return to normal

ranges. They suggest that the reason of this potential back to flavonoids that found in roots. Also, [13] referred to the role of *W. somnifera* root extract as antihyperlipidemia and lead to decrease levels of total cholesterol, triglyceride and increase levels of HDL. On the other hand, the present study show decreased in HMG-CoA reductase but its non-significant decreased compare with control group. This reduction may be related to the role of flavonoids that affect lipid metabolism through inhibition of acyl coenzyme A: cholesterol O-acyltransferase and 3-hydroxy-3-methylglutaryl-coenzyme A (HMG CoA) reductase in rats [14, 15].

The results of present study is in agreement with [16] that referred to role of *W. somnifera* extract against oxidative stress that induced in mice. The study suggest that the effect of *W. somnifera* extract to reduce MDA and increased GSH levels due to anti-oxidant activity of plant. The antioxidant activity of *W. somnifera* extract due to phenols [17].

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