J.Agric.Chem.and Biotechn., Mansoura Univ.Vol. 8(2): 21 - 26, 2017

Assessment of Licorice (*Glycyrrhiza glabra l.*) Aqueous Extract on Lipid Profile in Hypercholestermic Rats

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ABSTRACT

This study was undertaken to assess the effect of *Glycyrrhiza glabra* root extract (GGE) on the plasma lipid profile of rats. Thirty male albino rats weighing between 150 g and 170 g were used for this investigation. Rats fed 150, 250 and 400 mg/kg (GGE) for a period of 60 days showed significantly ($p < 0:05$) decreased the levels of total cholesterol (TC),total low density lipoprotein (LDL) and total triglycerides (TG) cholesterol on the other hand increased the level of high density lipoprotein (HDL) cholesterol in compared with positive control fed high cholesterol diets HCD.

Keywords: *Glycyrrhiza glabra*, Antioxidants, hypercolesterolemic, lipid profile.

INTRODUCTION

Hyperlipidemia refers to elevated lipid levels in blood (Owens *et al.,* 2014). The condition is also called hyperlipemia, or lipidemia, and includes elevated total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and total triglyceride (TG) levels in the blood (Nelson, 2013). Hyperlipidemia is an important agent for cardiovascular and cerebrovascular disease (Malloy and Kane, 2012 and Tietge, 2014). Hyperlipidemia can induce atherosclerosis, which can lead coronary heart disease, stroke, and myocardial infarction (Tietge, 2014 and Schmitz and Orsó, 2015). Many adult population in the United States has elevated cholesterol levels, and millions of people world-wide are affected (Go *et al.,* 2013).The incidence of hyperlipidemia is gradually increasing, and may worsen with an aging population. Therefore, the prevention and management of hyperlipidemia is important (Dixon *et al.,* 2015). However, almost all of the main lipid-lowering drugs cause side effects. For instance, statins cause myopathy in approximately 10% of patients receiving treatment (Harper and Jacobson 2007). An estimated 20% of patients are statin-resistant or intolerant (Maningat and Breslow 2011) novel treatments are therefore required.

In several countries, herbs have been widely used in the treatment of hyperlipidemia. The etiology and pathogenesis of hyperlipidemia in Traditional Chinese Medicine (TCM) is pathogenic dampness, a TCM symptom pattern identified in terms of TCM's theory. Because of body fluid metabolic disturbances, excessive phlegm-dampness stays within the body, affecting blood circulation and causing hyperlipidemia (Yang, 2005 and Liang, 2012).

Iritani, 1992 and Duval *et al.,* 2007 reported that *Glycyrrhiza glabra* roots, its known as sweet root or licorice, this plant used for many thousands years as medicinal plant. Root and many other parts of *Glycyrrhiza glabra* were used by the Greek, Indian, Chinese, Roman, and Egyptian as a carminative and expectorant. Many pharmacological actions, like antidiuretic, antiviral, antiallergic, antiepileptic, anti-inflammatory, antiulcer and antioxidant activity have been refer to the bioactive compounds glycyrrhizic acid and glycyrrhizin (Nassiri and Hosseinzadeh, 2008 and Visavadiya *et al.,* 2009). Nakagawa *et al.,* 2004 reported that *Glycyrrhiza glabra* oil smash visceral fat aggregation by organized the average of enzyme activities linked with fatty acid synthesis and oxidation in the liver. Extract of *Glycyrrhiza glabra* and glycyrrhiza are widely used in United States and recognized as safe foods by the United States (Nakagawa *et al.,* 2008 a, b).

Aoki *et al.,* 2007 reported that Clinical studies reported that *Glycyrrhiza glabra* flavonoid had no any serious side effects happen in daily use for about 3 months, also many studies confirmed the good effect of *Glycyrrhiza glabra* extracts by using it orally.Haraguchi *et al.,* 1998 resulted that isoliquiritigenin is the great aromatic ketone in *Glycyrrhiza glabra,* many pharmacological effects like anti-tumor, anti-inflammatory, antioxidant,anti-peptic ulcer actions, and antiplatelet.Choi *et al.,* 2010 resulted that isoliquiritigenin has agood effects on mitochondrial cells versus polyunsaturated omega-6 fatty acid 20:4 (ω-6) and Fe induced oxidative stress.Lee *et al.,* 2008 reported that isoliquiritigenin is a safe phenolic compound by using it orally.Therefor, this study was carried out to investigate the effect of *Glycyrrhiza glabra* root extract (GGE) on the plasma lipid profile of rats.

MATERIALS AND METHODS

Material

Roots of *Glycyrrhiza glabra* L. plant was obtain from local market of Zagazig city,Sharkia governorate, Egypt and identified by botanical members of the Department of Botany, Faculty of Agriculture, Zagazig University. (DPPH, approximately 90%) 1,1-diphenyl-2-picrylhydrazyl was procured from Sigma (Saint Louis County, Missouri, United States) other chemicals and reagents were of the highest purity available.

Methods

Preparing aqueous extract

The plant roots were cleaned several times with distilled water to remove dirt particles. The wetted plant roots were air dried at room temperature and then oven dried. The dried plant roots were ground into a powder with blender (Philips, Japan). About 500g of the ground roots were soaked in distilled water for seventy two h. and the residue was separated. Finaly the aqueous extract was lyophilized and the final yield was 50 g. The

extract was kept in a clean vial and cooled in a refrigerator until use (Nwangwa and Ekhoye, 2013).

Total phenolics determination

Total phenolic contents were determined by the following method:one mile roots extract to ten mile of distilled water and one mile Folin's phenol reagent. After five minute, two mile of 20% sodium carbonate was added to the mixture. The solution was kept in dark place, and the absorbance was analyzed by spectrophotometer at 750 nm. standard prepared using gallic acid for the construction of the calibration curve, total phenolic were calculated as mg gallic acid/g DW. Ghasemzadeh *et al.,* (2010).

Determination of total flavonoids:

The total flavonoid compounds were determined as following : half mile of 2% aluminum chloride prepared in ethanol solution was added to half mile of sample or standard. After one h. at room temperature then measured at 420 nm. standard prepared using quercetin for the construction of the calibration curve (Ahn *et al.,* 2007).

Radical scavenging activity (RSA) of aqueous extract

The free radical scavenging activity of the extract was determined as following : half mile of samples with three concentrations (250,500 and 1000 µg/mile) were mixed with three mile of a methanol solution of DPPH (final concentration of DPPH was 0.2 mM). The mixture was incubated at room temperature in dark place after half hour scavenging activity on the DPPH radical was determined by measuring the absorbance at 517 nm. Sarikurkcu *et al.,* (2008) . The inhibition activity was determined by using following equation:

% inhibition = [(absorbance of control – absorbance of test sample)/ absorbance of control] x 100.

Biological assay

Experimental

Aqueous extracts of *Glycyrrhiza glabra* L. (root) were administered to the animals groups 1,2 and 3 in the doses (150, 250 and 400 mg/kg) daily, by means of an orogastric cannula plus the experimental diets which were either high in cholesterol (HCD) or cholesterol free diet (CFD) for group 5. The full compositions of diets were established in table 1. Basal diet compositions was as described in table 1 group 5 (A.O.A.C., 2000).

Table 1. Compositions of the experimental diets (g)/100 g.

Ingredients	**HCD + aqueous extract of *Glycyrrhiza glabra L.* (root)**			**HCD positive (control)**	**CFD negative (control)**
	1	2	3	4	5
Casein	15	15	15	15	15
Starch	63.75	63.75	63.75	63.75	65
Salt mixture [a]	4	4	4	4	4
Vitamin mixture [b]	1	1	1	1	1
Cellulose	5	5	5	5	5
Colic acid	0.25	0.25	0.25	0.25	-
Cholesterol	1	1	1	1	-
Cheep tail fat	10	10	10	10	-
aqueous extract of *Glycyrrhiza glabra L.* (root) (mg/kg)	150	250	400	-	-

[a]*Salt mixture* contained % in the mix K_2HPO_4, 1.62; NaCl,0.5; KI,.013; $MgSO_4$, 0.325; $CaHPO_4$,0.30; $CaCO_3$,1.49; $CuSO_4$,0.0015; $FeSO_4$,0.126; $ZnSO_4$,0.00961and $MnSO_4$,0.011;

[b]*Vitamin mixture contained (mg):* Inositol,10; Vitamin K,0.5; Ca pantothenate,4; Niacin,4; Thiamin HCL,0.5; Riboflavin,0.8; Pyridoxine, 0.5; Biotin,0.04; Folic acid,0.2; Choline chloride, 200; Vitamin B_{12},0.003; beta-carotene ,2000(iu); Cholecalciferol,200(iu) ;4- amino benzoic acid,10 and tocopherol , (iu).starch was aded to each of salt and vitamin mixture to make the mixtures 100% as reported by A.O.A.C. (2000).

Animal experiment

Thirty male albino rats (weight about 150 g and 170 g) tested in this experiment. The experiment established for 60 days and the rats were stayed in cages with twelve hour light and twelve hour dark rotation. Over the all period of experiment water and diets were available . All groups were fed basal diet for ten days as a period of adaptation after that the rats were divided into 5 groups every one included 6 rats. The groups (4and5) perform as positive and negative control groups,negative or normal control CFD was group 5 which fed basal diet according to A.O.A.C (2000), on the other hand positive control group (4) was received high cholesterol diet (HCD) to the end of the experiment. Three groups (1, 2, and 3) were allowed to feed HCD with aqueous extracts of *Glycyrrhiza glabra* L. (root).

Blood sampling

From the plexuses of eye in the present of diethyl ether anaesthesia, the samples of blood were taken after 15, 30, 45 and 60 days from the beginning into tubes with heparin as anti-coagulant and then centrifuged at 3000 rpm for about 25 min.

Biochemical analysis

The method of determined Total cholesterol was described by Richmond (1973). Low-density lipoprotein (LDL) was determined as described by Demacker *et al.,* (1984).

Fossati and Prencipe, 1982 described the method of analyse triglycerides. High-density lipoproteins (HDL) (mg /dl) were calculated according to Friedewald *et al.*, (1972) by the following equation:

HDL - cholesterol concentration (mg/dl) = Total cholesterol (mg / dl) – [LDL - cholesterol concentration (mg /dl) + (Triglyceride /5)]

Statistical Analysis

All studied data were statistically analyzed using SPSS Computer Program (Co- Stat Software Computer Program) using analyses of variance ANOVA "two way" **.**

RESULTS AND DISCUSSION

Total phenolic and flavonoid content

Rice-Evans *et al.,* 1996 and Mattei *et al.,* 1998 reported that in the last thirty years the phenolic compounds have been widely studied, phenolic compounds have at least one aromatic ring which can carry the hydroxyl groups which can work as reducing agents. The natural antioxidants such as phenolics and flavonoids compounds have wide spectrum pharmacological effects like antibacterial, anti-allergic, neuroprotective activities, anti-inflammatory and anticancer, also protect plants from the attack of pathogenic microbial. Roth 2004 reviewed that the *Glycyrrhiza glabra* L. root is a great source of bioactive compounds like flavanoid and phenolic .Therefore, the total flavonoid and phenolic in the *Glycyrrhiza glabra* L. roots are very important to discover the effect of root extract. (Jaberian 2013) reported that the plant roots contain high amount of flavonoid and phenolic compounds .This work resulted that the phenolic contents was (188.36 ± 0.5 mg/ gm). Pratibha *et al.*, (2012), resulted that the phenolic content was (73.51 mg GAE/g) of *Glycyrrhiza glabra* L. dichloromethane extract .In this work the flavonoid level was 85 mg QE/g extract. The ethanol and aqueous extracts of *Glycyrrhiza glabra* L. root had a good content of flavonoid (4.2 and 5.1 µg QE/mg, respectively) on the other hand dichloromethane extract was higher than n-hexane extract Tohma and Gulcin (2010). Cakmak *et al.,* (2012) resulted that the content of flavonoid in methanolic extract of root was 116.54 mg RE/g. Li *et al.,* (2011) reported that the content of flavonoid in ethyl acetate, chloroform, hexane , n-butanol and water extracts of *Glycyrrhiza glabra* L. root and resulted that it varied between 3.601 and 66.546 mg RE/g.

Radical scavenging activity (RSA) of *Glycyrrhiza glabra* water extract

Many studies in the last ten years interested in the theory of free radical disease causation, especially in certain forms of cancer and vascular diseases. Because of the developments in the free radical field have guided us to the consideration on dietary agents, the natural antioxidant (especially vitamins E, A, and C), in a possible prophylactic and the role of the disease process. A free radical is a chemical species that has unpaired electrons Pryor *et al.,* (2006). These electrons, which made free radicals very reactive and take section in chemical reactions with other components in cell such as proteins, complex carbohydrates, nucleic acids and lipids) in the body Kohen and Nyska.(2002). In the biological systems, free radicals are referred to reactive oxygen species (ROS), as the most biologically significant free radicals. ROS produced in cells include hydroxyl radical (•OH), hydrogen peroxide (H_2O_2), and superoxide anion (O^{-2}) Pryor *et al.,* (2006). Many studies start to make a great case for the presence of a relationship between high levels of antioxidant in blood and a lowered incidence of disease. The tests expressing antioxidant potency can be classified into two groups: assays that test the ability to inhibit lipid oxidation under accelerated conditions and assays for radical scavenging ability. Schwarz, *et al.* (2000) reported that the scavenging stable free radicals model is a widely used model for assessment the antioxidant activity in a short time vs. other methods. Antiradical properties of the *Glycyrrhiza glabra* water extract determiend and resulted in Figure 1.data Show that the changes in absorbance of DPPH scavenging activity was detected from 250 µg/ml to 1000 µg/ml, *Glycyrrhiza glabra* water extract recorded a higher scavenging activity (93.66%).

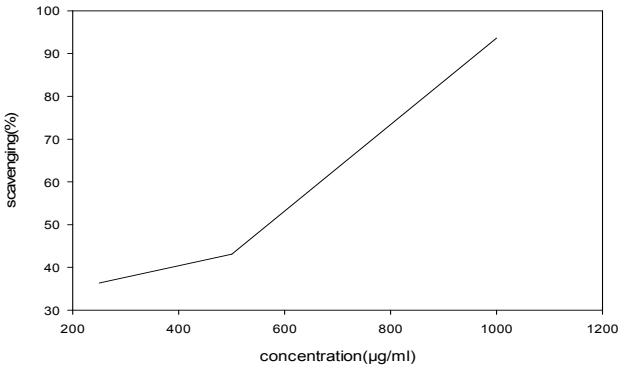

Fig. 1. DPPH scavenging activity of *Glycyrrhiza glabra* root extracts

Impact of feeding *Glycyrrhiza glabra* water extract on the plasma lipid profile

The total cholesterol (TC) and plasma triacylglycerols (TAG) levels in rats blood during different periods recorded in table 2. A high reduce in levels of total cholesterol and triacylglycerols was spotted in rats treated with *Glycyrrhiza glabra* water extract. After 60 days, the *Glycyrrhiza glabra* water extract significantly reduced plasma triacylglycerols (74.5 , 82.82 and 120.85 vs. 195.36) as calculated in mg/dl (table 2) for HCD /400 mg *Glycyrrhiza glabra* water extract ,HCD /250 mg *Glycyrrhiza glabra* water extract and 150 mg *Glycyrrhiza glabra* water extract compared with the positive control, respectively. Also, high decrease was spotted in total cholesterol level at the final of the work (table 2), as resulted in mg/dl (88.7, 102.6, and 124.6 vs. 222.2) for HCD /400mg *Glycyrrhiza glabra* water extract , HCD /250 mg *Glycyrrhiza glabra* water extract and 150 mg *Glycyrrhiza glabra* water extract compared with the positive control, respectively. As resulted, *Glycyrrhiza glabra* water extract significantly lowered total cholesterol and plasma triacylglycerols , which may be back to the good amount of bioactive compounds in *Glycyrrhiza glabra* water extract.

The increased in high-density lipoproteins (HDL) and decreased low-density lipoprotein (LDL) in blood may be helpful in many diseases. Increase of low-density lipoprotein play a strong role in atherosclerotic plaque. Level of low-density lipoprotein reduced on the

hand high-density lipoproteins was high in the investigated groups treatment with *Glycyrrhiza glabra* water extract (table 3) , at the end of the experiment (60 days), the *Glycyrrhiza glabra* water extract significantly increased the concentration of high-density lipoproteins (45.03, 45.52, and 52.3 vs. 28.82) in mg/dl for HCD /400 mg *Glycyrrhiza glabra* water extract , HCD /250 mg *Glycyrrhiza glabra* water extract and 150 mg *Glycyrrhiza glabra* water extract compared with the control /group, respectively. Data given in table 3, the greatest level of high-density lipoproteins was in the group that treatment with 150 mg *Glycyrrhiza glabra* water extract .On the other hand a decrease in the levels of low-density lipoprotein at the final of the work (table 3), asresulted in mg/dl (28.75, 40.56, and 48.1 vs. 139.28) for HCD /400 mg *Glycyrrhiza glabra* water extract ,HCD /250 mg *Glycyrrhiza glabra* water extract and 150 mg *Glycyrrhiza glabra* water extract compared with the control /group, respectively.

Glycyrrhizic acid a constituent of *Glycyrrhiza glabra*,reduces plasma cholesterol by down-regulating hepatic HMG COA reductase (HMGR) mRNA expression in hamster fed a high fructose - fat diet. Glycyrrhizic acid treatment significantly decrease apolipoprotein B (APO B), lipase a (LP a) and cholesteroliester - transport protein (CETP) concentrations but increased apolipoprotein A - 1 levels and apolipoprotein A-1/apolipoprotein A-2 ratio. Maurya and Srivastava, (2011) reported that The level of triglyceride and cholesterol in hepatic tissue were significantly lower in the glycyrrhizic acid group than in the control group. Yoke Yin *et al.,* (2010) reported that glycyrrhizic acid improved insulin sensitivity and lipid profiles and induced up-regulation of total Peroxisome proliferator activated receptor gamma and lipoprptein lipase expression level in rats and decrease in total cholesterol , triacylglycerol and a highness in HDL cholesterol .

Licochalcone A(LA) a constituent of *Glycyrrhiza glabra*, suppressed hepatic triglyceride accumulation through modification of AMP-SREBP pathway. Licochalcone A restrained lipogenesis via supperession of sterol regulatory element-binding protein1 (SREP1C) and target enzymes (glycerol-3-phosphate acyltransferase, stearoyl-COA desaturase 1 and fatty acid synthase) transcription.Quan *et al.,* (2013) reported that licochalcone A upregulated gene expression of proteins such as Peroxisome proliferator activated receptor alpha and fatty acid transport (FAT/CD36) which are responsible for lipolysis and fatty acid transport. Chalcones of glycyrrhiza glabra roots decrease the levels of plasma total cholesterol and triglyceride. Chalcones showed strong inhibition against pancreatic lipase (Rahul *et al.,* 2011).

Glabrol from *Glycyrrhiza glabra* roots act as antihypercholesterolemic agent.Glabrol inhibited acyl-coenzyme A cholesterol acyltransferase(ACAT) and decreased cholesterol ester formation (Jung *et al.,* 2007). Beta-sitosterol from glycyrrhiza glabra roots reduced intracellular levels of triglyceride and cholesterol in L6 cells.Benefical effects of beta-sitosterol on lipid metabolismIn L6 myotube cells are mediated by AM-activated protein kinase (Seung *et al.,* 2008).Glabirdin from *Glycyrrhiza glabra* roots showed inhibitory effect on adipogenesis in a dose dependent manner.The inhibitory effects of glabirdin resulted from inhibiting the induction of transcription factor CAAAT enhancer binding protein alpha and PPAR gamma (Jiyun *et al*., 2013).

Table 2. **Impact of feeding different (GGE) on the levels of triglycerides and total cholesterol.**

Groups	Triglycerides mg/dl				Total cholesterol mg/dl			
	15	30	45	60	15	30	45	60
Group 1	88.54 ^{de} ±12.67	91.07^{de} ±13.85	102.28^{ef} ±18.34	120.85^{fg} ±5.49	96.9^{abcde} ±8.90	112.6^{ef} ±3.50	113.4^{ef} ±11.12	124.6 ^{f} ±1.39
Group 2	65 ^{abc} ±12.11	71.46^{abcd} ±13.22	85.5^{cde} ±4.42	82.82^{bce} ±2.87	96.9^{abcde} ±10.59	105.7^{cdef} ±2.60	107.4^{def} ±4.11	102.6^{bcdf} ±0.59
Group 3	69.3^{abcd} ±14.78	70.64^{abcd} ±4.52	83.27^{bcde} ±5.31	74.5^{abcd} ±3.28	83.6^{abcd} ±6.92	89.8^{abcde} ±3.80	98.5^{abcde} ±5.69	88.7^{abcde} ±5.88
Group 4	87.87^{cde} ±19.14	97.53^{e} ±16.51	135.93^{g} ±14.44	195.36^{h} ±6.31	125.2^{f} ±9.10	151.7 ^{g} ±2.92	191.5 ^{h} ±35.15	222.2^{i} ±36.63
Group 5	54.89^{a} ±1.12	62.32^{ab} ±7.47	69.23^{abcd} ±14.86	61.58^{ab} ±18.46	74.1^{a} ±6.36	79^{ab} ±3.91	82.2^{abc} ±3.43	75.2^{a} ±9.05

Values with different letters in the same column or row are significantly different (P< 0.05)
Group 1 = HCD + (GGE) 150 mg/kg **Group 2 = HCD + (GGE) 250 mg/kg**
Group 3 = HCD + (GGE) 400 mg/kg **Group 4 = Hypercholesterolemic group** **Group 5 = Normal control**

Table 3. **Effect of feeding different (GGE) on the levels of LDL- cholesterol, and HDL- cholesterol**

Groups	HDL- Cholesterol mg/dl				LDL- Cholesterol mg/dl			
	15	30	45	60	15	30	45	60
Group 1	38.98^{abc} ±8.04	48.87^{bc} ±7.14	43.59^{abc} ±19.74	52.3^{bc} ±5.94	40.19^{abcd} ±3.15	45.53^{bcd} ±9.39	49.32 ^{d} ±10.60	48.1^{cd} ±3.58
Group 2	46.66^{abc} ±14.62	56.12 ^{c} ±6.35	56.53^{c} ±9.40	45.52^{abc} ±1.75	37.21^{abcd} ±6.44	35.28^{abc} ±7.51	33.8^{ab} ±8.56	40.56^{abcd} ±2.12
Group 3	41.14^{abc} ±3.26	43.47^{abc} ±5.97	48.11^{bc} ±13.63	45.03^{abc} ±4.98	28.6^{a} ±2.63	32.24^{ab} ±9.56	33.72 ^{ab}±7.56	28.75^{a} ±11.37
Group 4	42.41^{abc} ±11.81	47.03^{abc} ±9.88	23.8^{a} ±27.64	43.89^{abc} ±33.80	65.22^{e} ±1.22	85.2^{f} ±5.47	122.79 ^{g} ±10.86	139.28^{h} ±5.10
Group 5	35.02^{abc} ±35.02	29.51^{ab} ±29.51	38.67^{abc} ±3.52	28.82^{ab} ±10.53	28.08^{a} ±8.51	37.07^{abcd} ±2.01	29.64 ^{a} ±5.40	34.09^{ab} ±10.33

Values with different letters in the same column or row are significantly different (P< 0.05)
Group 1 = HCD + (GGE) 150 mg/kg **Group 2 = HCD + (GGE) 250 mg/kg**
Group 3 = HCD + (GGE) 400 mg/kg **Group 4 = Hypercholesterolemic group** **Group 5 = Normal control**

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تقيم تأثير المستخلص المائي لنبات العرق سوس علي حالة الدهون في الفئران المصابة بارتفاع الكوليسترول

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أجري هذا البحث لدراسة التأثير الناتج عن استخدام المستخلص المائي لنبات العرق سوس علي حالة الدهون في بلازما الفئران، وقد تم أجراء هذة الدراسة علي ثلاثين فأر جميعهم ذكور يزن كل منهم مابين ١٧٠ - ١٥٠ جرام ، وقد أستغرق هذا البحث شهرين وقد تم تقسيم الفئران الي خمس مجاميع كل مجموعة ستة فئران، وقد أعتبرت المجموعة الخامسة الكنترول السالب والتي غذيت علي وجبات أساسية طوال فترة الدراسة، والمجموعة الرابعة التي غذيت علي علائق عالية الدهن (١% كوليسترول + ٠.٢٥ % حمض الكوليك) بينما أعطيت باقي المجاميع وجبات عالية الدهن ١% كوليسترول + ٠.٢٥ % حمض الكوليك بالأضافة الي المستخلص المائي لنبات العرق سوس بتركيزات ١٥٠و٢٥٠و٤٠٠ مج/لكل كيلو جرام، وقد أوضحت النتائج أن مستخلص نبات العرق سوس بتركيزاتة المختلفه أعطت نقص في الكوليسترول والجليسريدات الثلاثية والدهون منخفضة الكثافة كما أعطت زيادة في الدهون مرتفعة الكثافة وذلك مقارنة بالكنترول الموجب والكنترول السالب .