

BORAGE OIL RICH IN GAMMA LINOLENIC ACID (GLA) REDUCES CARDIOVASCULAR DISEASE (CVD) RISK FACTORS IN HAMSTERS FED IN DIET RICH IN SATURATED FATTY ACIDS (SFAS) AND CHOLESTEROL.

*Mohammad Khalil Alhilal **Ahmad Mufeed Supuh

*** Najeh Hapra

*** *Department of Microbiology, College of Veterinary Medicine, Hamaa University, Syria.

** Department of Animal Production, College of Veterinary Medicine, Hamaa University, Syria.

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ABSTRACT

The experiment was applied on /45/ healthy male of golden Syrian hamsters, were divided into three groups as follows: Control group(C), High Fat-High Cholesterol group(HF-HC) and Borage Oil group (BO). All groups had laboratorial diet freely during/15/ days(first period), after that each of HF-HC and BO groups took diet consisting of 80% laboratorial diet+ 20% fats(13.5% sheep fat+6.5% coconut oil) for /4/ weeks(second period). The treatment of BO group changed after second period and hamsters had laboratorial diet and dosed borage oil 2g/kg of the body weight daily for /4/ weeks(third period). The results showed a significant increase($p=0.0000$) of the total cholesterol(TC), low density lipoprotein cholesterol (LDL-C) and triglycerides(TG) concentrations approximately(163%, 224%, 232%) respectively in each of HF-HC and BO groups in the second period in comparison with first period. Our results showed, in third period, a dramatic significant decrease($P=0.0000$) in the TC, LDL-C and TG concentrations approximately (56%, 69%, 40%) respectively in BO group in comparison with the same group of second period.

INTRODUCTION

Cardiovascular diseases (CVDs) are the leading causes of disability and death in industrialized nations and much of the developing world [1]. Hyperlipidemia, including hypercholesterolemia and hypertriglyceridemia, is a major risk factor for the development of CVD. Lipids in the blood includes low density lipoprotein cholesterol and high density lipoprotein cholesterol (LDL-C and HDL-C) and triglycerides (TG). A review article, with several human studies show that saturated fatty acids (SFAs) of chain length 12:0 –16:0 would increase serum total cholesterol (TC) and LDL-C [2, 3]. Elevated LDL-C is the primary target of cholesterol therapy to reduce CVD risk. Diet is an important cornerstone in the prevention and treatment of CVD [4]. The search for new drugs (eg.: statin derivatives) able to reduce and to regulate serum cholesterol and

TG levels has gained importance over the years, resulting in numerous reports on remarkable activities of natural agents [2]. In the present study, we selected borage oil, which is a great interest because of its medicinal and nutritional properties. The characteristic composition of such oil in fatty acids, particularly high levels of gamma-linolenic acid (GLA) in its seed oil, borage has gained importance. Borage oil consumption is recommended for people suffering from rheumatism and eczema [5]. Borage oil is the best source of GLA, an omega-6 polyunsaturated fatty acid (PUFA) that is not obtained in acceptable amounts in the conventional diet [6], since the fatty acid molecule is comprised of 18 carbon atoms with three double bonds. It is also known as 18:3n-6. In addition, GLA is found naturally in some plants seeds oils. Sources of GLA include evening primrose oil (EPO), black currant oil, and hemp seed oil. GLA is present in EPO at concentrations of 7-14 % of total fatty acids (TFA) [7]. The percentage of GLA in borage oil ranged from 17 to 25% as reported in ref. [8]. Its percentage ranged from 21 to 23% based on TFA [9]. This acid has potential nutritional, pharmaceutical and industrial applications. The percentage of oleic acid (C18:1) = 24.2, Linoleic acid (LA) (C18:2) = 35.4, other fatty acids were found in borage oil at low amounts, which form 4% of TFA: palmitoleic (C16:1), alpha-linolenic (C18:3n-3) and arachidic (C20:0) [5]. Most people don't obtain sufficient quantities of it owing to dietary deficiency, and also because the enzyme in the body governing its metabolism becomes less active with age [10]. It is necessary to clarify that most researches about borage oil were to study the effect of this oil on obesity, asthma, rheumatism, eczema and cancer. Unfortunately, the knowledge about its effects on hyperlipidemia is very scanty. So, our study focused on this property of borage oil. Although EPO has been shown to reduce harmful LDL-C levels in humans [11-14], and animals [15]. This effect has not yet been experimented adequately with borage oil. The aim of this study was to investigate the effect of borage oil rich in GLA on lipids concentrations in blood serum of golden Syrian hamsters fed previously diet rich in SFAs and cholesterol.

MATERIALS AND METHODS

2.1. Animals

Forty five of male hamsters' weight (45–50 g) and (5-7) weeks age were obtained from experimental animals unit (Directorate of Animal Health, Syria, Damascus). One hamster was housed in each cage in an animal room at 22 ± 2 C with a 12/12 h light–dark cycle.

2.2. Oils and fats

Borage oil was obtained from the cold compressing of borage seeds. Fully ripened borage seeds used in this study were collected from the region of Hama (west of Syria) in June 2012. Coconut oil: production of Indian company for Food Industries. Sheep fat was collected from the carcasses of sheep.

2.3. Experimental design

The first period: The hamsters were fed a commercial rodent diet (Laboratorial diet) and water freely for 2 weeks before the treatment to become acclimated. After 2 weeks, food deprived, hamsters were bled and serum TC concentrations were measured. The hamsters were then divided into three groups of (15 each group) based on similar

average serum TC as follows: control group (C), high fat-high cholesterol (HF-HC) group and borage oil (BO) group.

The second period: lasted 4 weeks in which C group stayed as they were in the first period while each of HF-HC and BO groups took diet consisting of 80% Laboratorial diet + 20% fats(13.5% sheep fat+6.5% coconut oil).

The third period: lasted 4 weeks in which C and HF-HC groups left in the same conditions of the second period, while the treatment of BO group changed and became as follows: Hamsters had laboratorial diet and were dosed borage oil 2 g/kg of the body weight daily.

2.4. Serum lipid determinations

Three blood samples were taken (once in the end of each period) from food deprived hamsters (14 h) and collected via the retro-orbital sinus into capillary tubes under anesthesia [16]. Serum was harvested after centrifugation at 3000 x g at room temperature for 10 min and serum TC [17] and TG [18] concentrations were measured enzymatically. Serum very low density lipoprotein cholesterol (vLDL-C) and LDL-C, which we combined, and termed (LDL-C) was precipitated with phosphotungstate reagent [19] and HDL-C was measured in the supernatant. The concentration of LDL-C was calculated as the difference between serum TC and HDL-C[20].

2.5. Statistical analysis

Data were expressed as mean± SD. Data were analyzed using one-way analysis of variance (ANOVA) by using a computerized Statistix program. P<0.05 was considered to be the least limit of significance.

RESULTS AND DISCUSSION

3.1 Animal model used

Hamsters are a good animal model for studying the relationship between fats and lipid metabolism for a number of reasons. Endogenous cholesterol synthesis in the liver of hamsters is similar to that in man [21]. A relatively high proportion of the cholesterol in the blood of hamsters is transported in form of the LDL fraction, more than in other species such as rats or mice [22]. Moreover, mechanisms of LDL-C clearance are the same as those in humans. As in humans approximately two-thirds of the circulating LDL-C was cleared in hamsters by receptor-dependent uptake into the liver [23]. Furthermore, the concentrations of LDL-C in hamsters respond to an altered supply of dietary fats and fatty acids in the same way as in man [24]. In view of these similarities hamsters are frequently used for assessing the effect of lipids on the cholesterol metabolism [25,26]. Golden Syrian hamster were used in the present study, while male hamster has proved a useful model of lipoprotein metabolism. Additionally, its small size and ease of handling, the hamster showed a popular model of dietary effects on lipoprotein metabolism.

3.2 Effect of diet rich in SFAs (Coconut oil) and cholesterol(sheep fat) on lipid concentrations in blood serum.

Coconut oil is an exceptionally rich source of SFAs. Sheep fat is a rich source of cholesterol. SFAs form 91.2% of TFA included in composition of coconut oil [27]. The purpose of feeding hamsters a diet rich in SFAs and cholesterol was to create

hyperlipidemia, LDL-C in particular. This diet caused a significant increase ($P=0.0000$) in the TC, LDL-C and TG concentrations approximately (163%, 224%, 232%) respectively in each of HF-HC and BO groups after 4 weeks of feeding (second period). These results Tables 1-3, were in agreement with previous studies [28-30]. Mammalia have three factors that control cholesterol: 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoA reductase), cholesterol α hydroxylase (CYP7A1) and LDL-receptor. HMG-CoA reductase is the control point for cholesterol biosynthesis [31] while CYP7A1 is the rate-limiting enzyme for cholesterol catabolism/output [32]. Meanwhile LDL-receptor plays an important role in clearance of cholesterol levels from blood [31]. We suppose that this diet created higher levels of HMG-CoA reductase and caused a decrease in LDL-receptors in each of HF-HC and BO groups in second period in comparison with C group. Accordingly, the high concentrations of TC, LDL-C have appeared. Elevated TG levels in our study may result from metabolic abnormalities because of SFAs and cholesterol feeding, which in turn increased serum residence time of chylomicrons and vLDL remnants, most of TG in blood serum are transferred by chylomicrons and vLDL remnants. Hypertriglyceridemia, associated with elevations in vLDL, can be due to overproduction of vLDL particles by the liver, reduced intravascular lipolysis of vLDL-TG, and delayed clearance of small vLDL particles from the plasma [33]. Table 4 showed a significant increase ($P=0.0000$) of HDL-C concentration for HF-HC, BO groups in comparison with C group in the second period, while Table 5 showed a significant decrease ($P=0.0000$) of HDL-C/TC ratio, approximately (30%) for HF-HC, BO groups in comparison with C group in the second period. These results were in agreement with the data reported previously [34]. Hamsters fed the coconut oil diet had higher plasma HDL-C [35]. Previously studies showed that a diet rich in SFA and cholesterol increased HDL-C concentration in comparison with control group [36, 37]. In man dietary SFA increase the concentration of HDL cholesterol [38]. Similar results were found in hyperlipidaemic hamsters [39].

Table 1. Effect of dietary lipids on the serum Total Cholesterol (TC) (mg/dL) of hamsters.

Time Group	TC First Period (Zero week)	TC Second Period (4 weeks)	TC Third Period (8 weeks)
C	b 103.76±6.89 A	a 116.53±5.23 B	a 113.53±5.89 B
HF-HC	c 103.07±7.81 A	b 266.84±10.25 A	a 314.15±4.75 A
BO	c 100.84±9.89 A	a 263.53±7.19 A	b 115.69±2.71 B

Values are expressed as mean±S.D; n=15. Different small letters indicate significant differences in the same row ($P<0.05$), Different large letters indicate significant differences in the same

column ($P<0.05$) C: control group; HF-HC:high fat-high cholesterol group; BO: borage oil group.

Table 2. Effect of dietary lipids on the serum LDL-C (mg/dL) of hamsters.

Time Group	LDL-C First Period (Zero week)	LDL-C Second Period (4 weeks)	LDL-C Third period (8 weeks)
C	b 64.30±6.62 A	a 73.84±4.01 B	a 71.53±4.21 B
HF-HC	c 62±9.32 A	b 198.92±10.96 A	a 249.46±6.47 A
BO	b 61.61±9.18 A	a 198.38±7.87 A	b 61.92±2.56 C

Values are expressed as mean±S.D; n=15. Different small letters indicate significant differences in the same row ($P<0.05$), Different large letters indicate significant differences in the same column ($P<0.05$) C: control group; HF-HC:high fat-high cholesterol group; BO: borage oil group.

Table 3. Effect of dietary lipids on the serum Triglycerides (TG) (mg/dL) of hamsters.

Time Group	TG First Period (Zero week)	TG Second Period (4 weeks)	TG Third period (8 weeks)
C	b 45.30±4.76 A	a 50.53±5.75 B	a 54.61±7.82 C
HF-HC	c 46.23±5.55 A	b 153.30±5.03 A	a 190.46±8.01 A
BO	c 45.23±5.50 A	a 146.38±7.86 A	b 87.38±2.84 B

Values are expressed as mean±S.D; n=15. Different small letters indicate significant differences in the same row ($P<0.05$), Different large letters indicate significant differences in the same column ($P<0.05$) C: control group; HF-HC:high fat-high cholesterol group; BO: borage oil group.

Table 4. Effect of dietary lipids on the serum HDL-C (mg/dL) of hamsters.

Time Group	HDL-C First Period (Zero week)	HDL-C Second Period (4 weeks)	HDL-C Third period (8 weeks)
C	a 39.46±3.71 A	a 42.69±3.70 B	a 42 ±3.93 C
HF-HC	b 41.07±4.45 A	a 67.92±2.49 A	a 64.69±3.30 A
BO	c 39.23±3.41 A	a 65.15±5.01 A	b 53.76±3.51 B

Values are expressed as mean±S.D; n=15. Different small letters indicate significant differences in the same row (P<0.05), Different large letters indicate significant differences in the same column (P<0.05) C: control group; HF-HC:high fat-high cholesterol group; BO: borage oil group.

Table 5. Effect of dietary lipids on the ratio HDL-C/TC of hamsters.

Time Group	HDL-C/TC First Period (Zero week)	HDL-C/TC Second Period (4 weeks)	HDL-C/TC Third period (8 weeks)
C	a 0.381±0.036 A	a 0.366±0.024 A	a 0.369±0.024 B
HF-HC	a 0.401±0.048 A	b 0.254±0.014 B	c 0.206±0.011 C
BO	b 0.391±0.042 A	c 0.247±0.018 B	a 0.464±0.024 A

Values are expressed as mean±S.D; n=15. Different small letters indicate significant differences in the same row (P<0.05), Different large letters indicate significant differences in the same column (P<0.05) C: control group; HF-HC:high fat-high cholesterol group; BO: borage oil group.

3.3 Effect of borage oil on lipid concentrations in blood serum.

It is known that dietary fatty acids play an important role in the development as well as prevention of hypercholesterolemia. In this study, we evaluated the impact of dosed borage oil rich in GLA for a period of 4 weeks on lipid concentrations in blood serum of golden Syrian hamster fed previously a diet rich in SFAs and cholesterol. Our results showed in third period a dramatic significant decrease ($P=0.0000$) in the TC, LDL-C and TG concentrations approximately (56%, 69%, 40%) respectively in BO group in comparison with the same group of second period **Table 1-3**. These results in agreement with the data reported previously [40] which demonstrated that borage oil reduced the elevation of serum TC, LDL-C and TG concentrations of aged rats in the presence of excess cholesterol in the diet. On the contrary, Simoncikova *et al.* [41] reported that borage oil, as a source of GLA, failed to improve lipids levels in the optimal form in male Wistar rats that fed a high fat diet for 3 weeks. Mice being fed a high GLA diet showed a reduction of vLDL-C and LDL-C, as well as a reduction of atherosclerotic lesions [42, 43]. GLA showed lowering in LDL-C and TG levels, while it showed an increase in HDL-C concentration [44, 45]. GLA supplementation reduced TC and TG and dramatically inhibiting oxidation of LDL-C; an important first step in atherosclerosis in rats fed a high-cholesterol diet [46]. Our results in agreement with previously data [7] which reported a dramatically significant reduction in LDL-C concentration after the consumption of GLA in human. The greater effect of borage oil in our study was apparent in third period when it caused a significant increase ($P=0.0000$) in HDL-C/TC ratio, approximately (87 %), in BO group in comparison with the same group of second period **Table 5**. We found, in third period, a significant decrease in HDL-C level in BO group in comparison with HF-HC group **Table 4**, while HDL-C/TC ratio was significantly increased ($P=0.0000$) approximately (125%) for BO group in comparison with HF-HC group **Table 5**. These results were in agreement with previously data [7] That showed a significant increase in HDL-C/TC ratio after the consumption of GLA in human. HDL-C/TC ratio was a better measure of CVD risk than LDL-C [47]. We can ascribe our results to high percentage of GLA in borage oil, particularly that GLA has an approximately 170 times greater cholesterol-lowering ability than LA, indicating that LA is converted to GLA by an enzyme delta-6-desaturase to exert its hypocholesterolemic effects [11]. GLA is further metabolized into dihomogammalinolenic acid (DGLA), arachidonic acid (AA) and eventually into various eicosanoids. The delta-6-desaturase reaction is the rate-limiting (slowest) step in the conversion of LA to long-chain omega 6-fatty acids and is further inhibited by a number of factors including, high intakes of SFAs, stress, aging, gender (males require more essential fatty acids than females), high cholesterol levels, diabetes [6]. By providing dietary GLA, the delta-6-desaturase reaction can be bypassed, resulting in more efficient production of long-chain omega-6 fatty acids and eicosanoids. Treatment with GLA was associated with a beneficial reduction of CVD risk factors as represented by the decrease in arterial blood pressure and plasma TC, LDL-C and the increase in HDL-C. The exogenous supply of GLA increased the levels of higher unsaturated essential fatty acids [48]. Similar changes in lipid parameters were published by other authors as well [49,50]. The decrease in plasma cholesterol may be due to the hypocholesterolemic action of GLA acid and LA [11]. Plasma cholesterol lowering effect of GLA is clinical importance because high cholesterol level inhibits delta-6 desaturase [49] and deepens GLA deficiency with all the consequences on their metabolism. We suppose that the hypolipidemic effects of borage oil in this study associated with its high content of GLA which caused a decrease in HMG-CoA

reductase activity, an increase in lipoprotein lipase (LPL) activity and the up-regulation of plasma cholesterol clearance by increase of LDL-receptor activity and cholesterol catabolism/ output (CYP7A1).

زيت البوراج الغني بالحمض الدسم جاما لينولينيك يُنقص عوامل خطورة الأمراض القلبية الوعائية عند الهمستر المُغذَى على عليقة غنية بالأحماض الدسمة المشبعة والكوليستيرول

* محمد خليل الهلال * أحمد مفيد صبح * * * ناجح هبرة

* قسم الأحياء الدقيقة، كلية الطب البيطري، جامعة حماه، سورية.

* * قسم الإنتاج الحيواني، كلية الطب البيطري، جامعة حماه، سورية.

* * * قسم الأحياء الدقيقة، كلية الطب البيطري، جامعة حماه، سورية.

الخلاصة

أُجريت الدراسة على /45/ ذكراً سليماً من الهمستر السوري الذهبي، قُسمت الحيوانات إلى ثلاث مجموعات كالتالي: المجموعة الشاهدة (C) ومجموعة دهن عالي-كوليستيرول عالي High Fat-High Cholesterol (HF-HC) ومجموعة زيت البوراج (BO) Borage oil، تناولت جميع حيوانات الهمستر العلف المخبري لمدة /15/ يوم (المرحلة الأولى). بعد ذلك تناولت كل من مجموعتي HF-HC و BO عليقة مكوّنة من 80% علف مخبري + 20% دهون (13.5% شحم غنم + 6.5% زيت جوز الهند) لمدة /4/ أسابيع (المرحلة الثانية). تغيّرت مُعاملة مجموعة BO بعد المرحلة الثانية حيث تناولت الحيوانات العلف المخبري فقط و أُعطيت زيت البوراج بجرعة 2غ/كغ من وزن الجسم الحي يومياً لمدة /4/ أسابيع (المرحلة الثالثة). أظهرت النتائج ارتفاعاً معنوياً (P=0.0000) في مستويات TC، LDL-C، TG و بمقدار (163، 224، 232%) على الترتيب لدى كل من مجموعتي BO، HF-HC في المرحلة الثانية وذلك بالمقارنة مع المرحلة الأولى. بيّنت نتائجنا في المرحلة الثالثة انخفاضاً معنوياً مُثيراً (P=0.0000) في مستويات TC، LDL-C، TG و بمقدار (56، 69، 40%) على الترتيب لدى المجموعة BO وذلك بالمقارنة مع نفس المجموعة من المرحلة الثانية.

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