



Comparative study among aqueous, hexane extracts of sweet almond (*Prunus amygdalus*) with Atorvastatin for treating hyperlipidemia induced in mice

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Abstract:

This research was developed to find out the treatment impact of aqueous and hexane extracts of sweet almond (*Prunus amygdalus*) on some biological indicators related to hyperlipidemia that induced in mice and comparative with Atorvastatin which was dried and grinded by an electrical grinder to form fine crude powder that extracted by two ways: by using 95% hexane and water by using the distilled water with Soxhlet apparatus, (40) of mature mice were randomly separated into 8 categories (5 mouse per group) and treated every day for 60 days, the first group was fed and drank normally and regarded as a negative control group (NC1), a second group was given polypropylene glycol offered as negative control group (NC2), third group was given normal water containing 0.5% of hydrogen peroxide and 1% of cholesterol in the feed for 60 days for induction of hyperlipidemia and offered as positive control group (PC), hyperlipidemia was induced in the other five categories as in the third group. Treated hyperlipidemia by hexane extract at a dose of 500 mg/ kg of body weight and aqueous extract of sweet almond with three different doses (500, 750 and 1000) mg/ kg of bodyweight and compared with the other groups that treated with atorvastatin (Lipitor)[®] 20 mg/ kg B.W. as antihyperlipidemic drug. The outcomes discovered that oral dosing with extracts of sweet almond and medication therapy led to acquire beneficial changes in the parameters, which were showed incident of a significant decrease ($P < 0.05$) at a concentration of malondialdehyde (MDA), total cholesterol (TC), triacylglycerol (TAG), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and atherogenic index (AI) in serum, moreover to a significant improved ($P < 0.05$) in the concentration of high density lipoprotein cholesterol (HDL-C) and serum reduced glutathione (GSH) compared with untreated group and has been proven that a dose of 1000 mg/kg of aqueous extracts of sweet almond was the best in therapy.

Key word: sweet almond extracts, Atorvastatin, lipid profile, MDA, GSH.

دراسة مقارنة بين المستخلص المائي ومستخلص الهكسان للوز الحلو مع الأتورفاستاتين لعلاج فرط الدهنية المستحدث في الفئران

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الخلاصة:

صممت هذه الدراسة لمعرفة التأثير العلاجي للمستخلص المائي ومستخلص الهكسان للوز الحلو (*Prunus amygdalus*) على بعض المعايير البيولوجية المتعلقة بفرط الدهنية في الدم والمستحدثه في الفئران ومقارنتها مع دواء الأتورفاستاتين. تم تجفيف وطحن اللوز بالمطحنة الكهربائية للحصول على المسحوق الخام للنبات والذي تمت عملية إستخلاصه بطريقتين: باستخدام الهكسان (95% hexane) ومائيا باستخدام الماء المقطر وباستخدام جهاز السوكسليت. استخدام (40) من الفئران البالغة وقسمت عشوائيا الى (8) مجاميع متساوية (5 حيوان لكل مجموعة) تم معاملتها يوميا لمدة 60 يوم بحيث تم اعطاء المجموعة الاولى العلف الطبيعي والماء العادي وعدت كمجموعة سيطرة سالبة وتم اعطاء المجموعة الثانية ايضا الماء والعلف الطبيعي مع التجريع الفموي للبروبيلين كلاكول وعدت كمجموعة سيطرة سالبة في حين تم اعطاء المجموعة الثالثة ماء الشرب المحتوي على 0.5% بيروكسيد الهيدروجين و 1% كولستيرول في العلف لمدة 60 يوم لأحداث فرط الدهنية في الدم وعدت كمجموعة سيطرة موجبة. أما المجاميع الخمس الباقية فقد استحدثت فيها حالة فرط الدهنية في الدم كما في المجموعة الثالثة. وتم معالجة الحيوانات المصابة بفرط الدهنية بمستخلص الهكسان بجرعه 500ملغم/كغم من وزن الجسم والمستخلص المائي للوز الحلو بثلاث جرعات مختلفة (500, 750, 1000) ملغم/كغم من وزن الجسم وتم مقارنتها مع المجموعة المتبقية المعاملة بالأتورفاستاتين (لوبيتور)[®] 20ملغم/كغم من وزن الجسم وهو من الادوية المخفضة للدهون. تبيننت النتائج ان التجريع الفموي بمستخلصات اللوز الحلو والعلاج بالادويه ادت الى حصول تغيرات ايجابية في المعايير والتي تمثلت بحدوث انخفاض معنوي ($P<0.05$) في تركيز MDA, TC, TAG, LDL-C, VLDL-C و AI في مصل الدم بالاضافه الى حدوث ارتفاع معنوي ($P<0.05$) في تركيز HDL-C و GSH مقارنة مع المجاميع المصابة، وقد تبين ان الجرعة 1000ملغم/كغم للمستخلص المائي للوز الحلو هي الافضل في العلاج.

Introduction:

Cardiovascular diseases are one of the leading causes of death all over the world (1). The development of these diseases has been linked to several factors such as high calorie diet intake, lack of exercise, smoking, age, alcohol consumption and genetic disposition (2). These factors ultimately result in disorders of lipid and lipoprotein metabolism including lipoprotein overproduction and deficiency (3) and aside from genetic factors, which are regarded as the primary cause of hypercholesterolemia, food patterns also contribute to its prevalence. Hyperlipidemia illness has affected humankind since antiquity. The treasure house of plant kingdom has a number of vegetation to cure this condition. World Health Organization has recognized that therapeutic

vegetation are good resources of drugs and therefore, should be investigated in order to understand their properties, safety and efficacy (4).

Atorvastatin are the first line treatment for decreasing lipid levels. Treatment of hyperlipidemia with statins has become a fundamental element of control of vascular diseases (5). Such impact is linked to statins major mechanism of action, such as cholesterol decreasing property. The aim of study was to do a comparative study among aqueous, hexane extracts of sweet almond (*Prunus amygdalus*) with Atorvastatin for treating hyperlipidemia induced in mice.

Material and methods:

Sweet almond seeds were collected during October, 2013 from a

local market in the Erbil/ Kurdistan region-Iraq, the whole almond seeds sliced and dried in an oven in about temperature 25°C until the almonds become free of moisture (6). The extraction was carried out by Soxhlet apparatus using two methods hexane extract (7) and watery extract (8) that considered as a very effective in extracting the active ingredients of the almond. After that different doses given daily to mice orally by using gavages needle which divided into eight groups: group (1) negative control no induction of hyperlipidemia and given normal feed and distal water only, group (2) negative control no induction of hyperlipidemia and given normal feed, distal water and propylene glycol, group (3) positive control that induction of hyperlipidemia by addition of 1% cholesterol in diet (9) and H₂O₂ 0.5% in drinking water (10) for two months and not treated, group (4) induction of hyperlipidemia and treated with hexane extract of sweet almond at a dose of 500 mg/kg B.W. for two months (11), groups (5, 6 and 7) induction of hyperlipidemia and treated with watery extract of sweet almond at a dose of (500 or 750 or 1000) mg/kg B.W. for two months and group (8) induction of hyperlipidemia and treated with atorvastatin (Lipitor)[®]

at a recommended dose 0.3 mg/kg B.W. for two months (12).

At the end of the period of experiment, mice were fasted over night before sacrificed by anesthesia. Blood was collected through cardiac puncture into EDTA containing tubes and serum was isolated by centrifugation 2500 rpm for 10 minute to determination of lipid profile (TC, TAG, LDL-C, VLDL-C and HDL-C), GSH and MDA. The serum samples were analyzed spectrophotometrically for TC, TG, HDL-C, LDL and VLDL by using suitable assay kits (Liner chemical) (13). In addition to measurement of reduce glutathione concentration (GSH) (14) and Malondialdehyde concentration (MDA) (15).

Results:

The results of the lipid profile showed significant increase in serum concentration of TC, TG, LDL and VLDL during induction of hyperlipidemia in 60 days, while HDL serum concentration was decrease significantly compared with normal control group and after treatment with extracts and atorvastatin a significant decrease in serum concentration of TC, TG, LDL and VLDL in 60 days of treatment, while HDL serum concentration was increase significantly compared with (NC1, NC2 and PC) as illustrated in table (1).

Table (1): The lipid profile test parameters of different groups control, treated with aqueous and hexane extracts of sweet almond compared with atorvastatin drug (Lipitor)[®].

Parameter Groups	TC Mg/dl	TG Mg/dl	HDL-C Mg/dl	LDL-C Mg/dl	VLDL-C Mg/dl
NC1 (-ve control) treated with distilled water.	121.56±6.39 G	83.66±4.05 D	58.58±1.04 AB	46.24±6.64 G	16.73±0.81 D
NC2 (-ve control) treated with propylene glycol.	119.66±3.10 G	80.12±4.98 D	56.58±0.95 B	47.05±3.01 G	16.02±0.99 D
PC (+ve control) induced hyperlipidemia and not treated.	315.02±10.16 A	190.02±5.67 A	40.62±0.97 E	236.39±9.99 A	38±1.13 A
T1 treated with aqueous extract of sweet almond 500mg/kg B.W	281.40±10.86 B	160.28±7.77 B	48.92±1.18 D	200.42±10.50 B	32.05±1.55 B
T2 treated with aqueous extract of sweet almond 750mg/kg B.W	229.94±9.88 D	129.06±6.07 C	52.68±1.34 C	151.44±7.40 D	25.81±1.21 C
T3 treated with aqueous extract of sweet almond 1000mg/kg B.W	155.92±15.79 F	87.38±6.21 D	59.96±0.88 A	78.48±15.51 F	17.47±1.24 D
T4 treated with hexane extract of sweet almond 500mg/kg B.W	251.84±9.94 C	119.44±5.51 C	53.90±1.20 C	174.05±10.04 C	23.88±1.10 B
T5 treated with atorvastatin (Lipitor) [®] 20mg/kg B.W	198.80±4.23 E	114.4±5.48 C	57.96±0.90 A	117.96±4.03 E	22.88±1.09 B

❖ n=5 for each group, the values represent Mean± SE, different capital letters showed significant (p<0.05) among groups.

Atherogenic index (AI) and serum malondialdehyde (MDA) concentration showed highest significant (p<0.05) elevation in untreated groups in 60 days comparing to control groups. On the other hand, significant (p<0.05) reduction in AI and MDA were observed during 60 day after treated with sweet almond extracts and atorvastatin compared with PC group, in addition to a significant (p<0.05) decrease in mean value of

serum GSH concentration were detected at 60 days in PC comparing to NC groups. The protective effect of sweet almond extracts and drug on the antioxidant status of mice were clarified after 60 days of treatment, where significant elevation in serum GSH concentration were observed in T1, T2, T3, T4 and T5 comparing to untreated groups (NC1, NC2 and PC) as showed in table (2).

Table (2): Effect of aqueous, hexane Extracts of Sweet Almond and Atorvastatin drug on atherogenic index (AI), serum malondialdehyde concentration (MDA) and reduced glutathione concentration (GSH).

GROUPS \ TIMES	Atherogenic index	Malondialdehyde	Reduced Glutathione
NC1 (-ve control) treated with distilled water.	1.08±0.14 E	0.29±0.01 B	37±0.94 BC
NC2 (-ve control) treated with Propylene glycol.	1.11±0.08 E	0.28±0.008 B	36±1.14 BC
PC induced hyperlipidemia and not treated (+ve control).	6.79±0.42 A	0.47±0.01 A	23.4±1.43 D
T1 treated with aqueous extract of sweet almond 500mg/kg B.W	4.78±0.36 B	0.28±0.01 B	35±1.22 C
T2 treated with aqueous extract of sweet almond 750mg/kg B.W	3.38±0.25 C	0.25±0.01 C	41±1.51 B
T3 treated with aqueous extract of sweet almond 1000mg/kg B.W	1.61±0.30 E	0.22±0.01 D	49±0.94 A
T4 treated with hexane extract of sweet almond 500 mg/kg B.W	3.69±0.28 C	0.26±0.01 BC	38±1.18 BC
T5 treated with atorvastatin (Lipitor) [®] 20 mg/kg B.W	2.43±0.12 D	0.24±0.008 C	46±1.41 A

❖ n=5 for each group, the values represent Mean± SE, different capital letters showed significant ($p < 0.05$) among groups.

Discussion:

In present study, oral administration of atherogenic diet 1% (w/w) cholesterol and 0.5% H₂O₂ in drinking water to mice in all groups except control group for 60 days caused a case of hypercholesterolemia and hypertriglyceridemia induced oxidative stress and this results accordance with (16). The observed increase in MDA level and decrease in GSH level in mice fed high cholesterol diet is consistent with several experimental studies like (17). These outcomes are in agreement with past research (18) this indicated that H₂O₂ is regarding as one of the reactive oxygen species which has direct effect on the level of plasma cholesterol, TG and atherogenic lipoproteins. These results suggest that may be due to the fact that there was a dynamic alteration in the process of absorption and exertion of

steroid or there was reduction in bowel bile salts. This results were in accordance with the result of other workers, who reported significant depletion of GSH level in hyperlipidemia groups may possibly attributed to the role of H₂O₂ in increasing O₂ production in the stomach followed by state of tissue hyperoxia which in turn lead to excessive formation of free radicals which lead to deterioration of biological molecules (19).

Treatment with sweet almond extracts lowered TC, LDL-C and TG levels, as well as increase HDL-C (20). watery extract of sweet almond gives good results in decrease of hyperlipidemia due to the almonds are low in saturated fatty acids and rich in unsaturated fatty acids and contain

fiber, protein, α -tocopherol, arginine, magnesium, copper, manganese, calcium and potassium, and also considered sources of phytosterols and other phytochemical compounds such as polyphenols and ellagic acid, with possible serum cholesterol-modulating effects (21). Water extract of sweet almond at a doses (1000 mg/kg B.W.) considered the best in treatment of hyperlipidemia, according to results that agreement with (22) shown no detrimental effects on blood lipids were observed when consumed a small amount of almonds relative to control group.

The current data revealed that significant decrease in serum level of TC, TG, LDL and VLDL and significant improve in serum amount of HDL by atorvastatin treatment and was in agreement with (23). The mechanism included is the most likely linked to the capability of atorvastatin to damage cholesterol synthesis via inhibiting the enzyme HMG-CoA reductase, which is the rate limiting step in cholesterol biosynthesis. These results in both, reduce distributing lipoproteins and increase their uptake by up regulating hepatic LDL-C receptors. The overall fat lowering effect consist of improve usage and deterioration of LDL-C, inhibition of LDL-C oxidation decrease in cholesterol build up and esterification and reduces lipoprotein release and cholesterol synthesis (24).

Present data accordance with other researchers have determined that overproduction of reactive oxygen species (ROS) performs a critical part in the oxidation of LDL molecules, which get accumulated in the layers of blood vessels, in addition to (25) who reported decreased total antioxidant capacity in a group of hyperlipidemia. The result showed that given hexane and watery extracts sweet almond

reduce from oxidative stress and were in agreement with other studies showed that the anti-oxidant effects of sweet almond against oxidative damage might develop from phytochemicals in its content that have powerful free radical scavenging ability (26).

Sweet almond contain chemical compounds exhibiting antioxidant properties and demonstrating such protective effects (27). These benefits are mainly linked to their lipid profile, fibers, arginine and vitamin E as well as to other substances with antioxidants such as polyphenols (28). Atorvastatin has antioxidants by reducing fat peroxidation and ROS producing. It decreases the susceptibility of lipoproteins to oxidation, they decrease the LDL oxidation (29). The most likely explanation for the rise of GSH and decrease of MDA by atorvastatin was linked to the anti-oxidant mediated impact of atorvastatin which results from inhibition of mevalonate process (30). In conclusion: The using of sweet almond extracts for treating hyperlipidemia may be near to the antihyperlipidemic drugs to treat this case.

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