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Effect of Bay leaf (*Laurus nobilis* L.) and its isolated (flavonoids and glycosides) on the lipids profile in the local Iraqi female rabbits

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Abstract
The present study aimed to explain the effect of Bay leaf and its isolated flavonoids and glycosides on the levels of TC, TG, HDL-C, LDL-C and VLDL-C in the local Iraqi female rabbits. Isolation of flavonoids and glycosides from Bay leaf were carried out, the study design included four groups (n=6): Control group-C fed with standard pellet diet; group1-G1 orally administrated daily dose 100 mg/ml/kg of Bay leaf crude for 30 days; group2-G2 orally administrated daily dose 50 mg/ml/kg of isolated flavonoids for 30 days; group3-G3 orally administrated daily dose 12.5 mg/ml/kg of isolated glycosides for 30 days. TC, TG and HDL-C were determined by enzymatic colorimetric method. The results showed that oral administration of Bay leaf and its isolated flavonoids and glycosides reduced levels of TC, TG, LDL-C and VLDL-C compared to control, therefore Bay leaf useful agent in reducing hyperlipidemia.

Keywords: Bay leaf, *Laurus nobilis* L, flavonoids, glycosides, lipids profile.

Introduction
Laurus nobilis L. (Lauraceae family) is a perfumed evergreen tree or large bush with dark-green, smooth leaves, native to the Mediterranean region countries and Europe [1]. It known in English as Bay leaf, bay laurel, Turkish laurel, and known AL Ghar in Arabic island [2]. Bay leaf is one of the oldest known spices, broadly utilized as a condiment and spice as a spicy fragrance and flavour in traditional meat dishes, rice, stews and soups [3,4]. Bay leaf contains many types of flavonoids and glycosides, such as kaempferol, quercetin, apigenin, luteolin, quercetin 3-O-α-L-rhamnopyranoside, kaempferol-3-O-β-glucopyranoside, quercetin-3'-O-β-glucopyranoside, quercetin-3-O-β-galactoside, isorhamnetin-3-O-β-glucopyranoside, isorhamnetin-3-O-β-galactopyranoside, quercetin-3-O-rutinoside, kaempferol-3-O-rutinoside, isorhamnetin-3-O-rutinoside and isorhamnetin [1,5]. Bay leaf has a broad range of biological properties including, anti-microbial [6], anti-inflammatory [2], anti-fungal [7], improves blood lipids profile [8], improves liver function [9] and having antioxidant properties [10]. The aim of the present study is to characterize the effect of Bay leaf and its isolated flavonoids and glycosides on the levels of total cholesterol (TC), triglycerides (TG), high density lipoproteins-cholesterol (HDL-C), low density lipoproteins-cholesterol (LDL-C) and very low density lipoproteins-cholesterol (VLDL-C) in the local Iraqi female rabbits.

Materials and Methods
Kits used for the determination of TC, TG and HDL-C were supplied from Biolabo Co. France, and solvents of analytical grade were products of Sigma and BDH chemicals Co. Bay leaf was purchased from the market in Samarra city in Salahaddin-Iraq. Dried in hot air oven at 40 °C for 1 hr. The dried plant was then coarsely powdered using a mixer grinder and stored in an airtight container.

Isolation of flavonoids and glycosides
Flavonoids isolation: 100 ml of 70 % ethanol was added to 10 g of the sample. The mixture was heated to the boiling water bath with stirring for 2 hr., then filtered hot solution and the solvent was evaporated to dryness to obtain a precipitate [11].

Glycosides isolation: 100 ml of 80 % ethanol was added to 10 g of the Bay leaf; leave the mixture for 24 hr. The solution was filtered and focused in half by a rotary evaporator, and added to 50 ml of ether and 5 ml of lead acetate solution 0.3 M, concluded in a separating funnel. Dried water layer at 30 °C until completely dry [11].

Animals
Local female rabbits (900-1600 g weight) were used at (3-5) month average age. Groups of rabbits were housed at room temperature. Animals had free access to a standard pellet diet and tap water during the entire study. The experimental period was carried out for 4 weeks, from April to May 2016.

Experimental design
A total of 24 rabbits randomly was divided into four groups (6 animals in each group). Control group-C fed with standard pellet diet; group1-G1 orally administrated daily dose 100 mg/ml/kg of Bay leaf crude; group2-G2 orally administrated daily dose 50 mg/ml/kg of isolated flavonoids; group3-G3 orally administrated daily dose 12.5 mg/ml/kg of isolated glycosides for 30 days.

Collection of Blood Samples
After the end of the dosing period, animals fasted for 12 hr., and the blood samples were obtained by the heart puncture (5 ml). The blood was put in gel tube and serum was collected by centrifuge the blood at 3000 rpm for 15 min., then was divided into 4 parts in eppendorf tube and stored at -20 °C until biochemical analyzed.

Determination of lipids
TC, TG and HDL-C were determined by enzymatic colorimetric method. VLDL-C was calculated from following equation: VLDL=TG/5. LDL-C was calculated from following equation: LDL-C = TC – (HDL-C+VLDL-C).

Statistical analysis

The results were expressed as mean ± SD for all groups. Data were analysed for significant difference using Duncan's multiple range test (P<0.05) by Minitab program.

Results and Discussion

Table 1 and figure 1 summarize the levels of TC, TG, HDL-C, LDL-C and VLDL-C in the sera of adult all female rabbits of control and experimental animals treated with Bay leaf and its isolated flavonoids and glycosides.

Table 1: Effect of Bay leaf and its isolated flavonoids and glycosides on the lipids profile in different groups

Groups	TC mg/dl	TG mg/dl	HDL-C mg/dl	LDL-C mg/dl	VLDL-C mg/dl
	Mean ± SD, n = 6, p<0.05				
C	68.251±1.662 [a]	93.573±3.566 [a]	32.394±3.032 [a]	17.142±3.055 [b]	18.715±0.713 [a]
G1	37.674±0.801 [c]	53.268±2.703 [b]	23.695±0.644 [c]	3.326±0.511 [c]	10.654±0.541 [b]
G2	39.607±0.560 [c]	42.593±2.084 [c]	27.244±0.811 [b]	3.844±0.466 [c]	8.519±0.417 [c]
G3	53.359±4.716 [b]	54.684±6.014 [b]	15.553±2.788 [d]	26.868±4.132 [a]	10.936±1.203 [b]
C: Control, G1: Bay leaf crude, G2: Isolated flavonoids, G3: Isolated glycosides.					

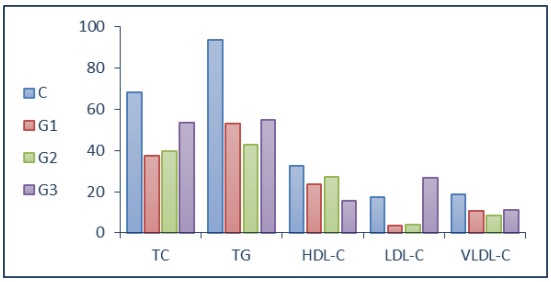

Figure 1: Effect of Bay leaf and its isolated flavonoids and glycosides on the lipids profile in different groups

The mean ± SD of cholesterol in C group and the three groups G1, G2, G3 were (68.251±1.662) mg/dl, (37.674±0.801) mg/dl, (39.607±0.560) mg/dl and (53.359±4.716) mg/dl respectively. The results indicate that the levels of total cholesterol were significantly decreased (p ≤0.05) in G1, G2 and G3 groups as compared with C group, with significantly decreased (p≤0.05) in G1 and G2 groups as compared with G3 group, but no-significant between G1 and G2 groups. The mean ± SD of TG in C group and three groups G1, G2, G3 were (93.573±3.566) mg/dl, (53.268±2.703) mg/dl, (42.593±2.084) mg/dl and (54.684±6.014) mg/dl respectively. The result showed that the level of TG was significantly decreased (p ≤0.05) in all three groups G1, G2 and G3 as compared with C group, but no-significant change in G1 and G3, and significantly lower in G2 as compared with G1 and G3. The mean ± SD of HDL-C in C group and three groups G1, G2, G3 were (32.394±3.032) mg/dl, (23.695±0.644) mg/dl, (27.244±0.811) mg/dl and (15.553±2.788) mg/dl respectively. The result showed that the level of HDL-C were significantly decreased (p≤0.05) in all three groups G1, G2 and G3 as compared with C group, with a significant change between them. The mean ± SD of LDL-C levels in C group and three groups G1, G2, G3 were (17.142±3.055) mg/dl, (3.326±0.511) mg/dl, (3.844±0.466) mg/dl and (26.868±4.132)mg/dl respectively. The result showed that the level of LDL-C was significantly decreased (p≤0.05) in two groups G1 and G2 compared with C group, but no-significant change between them, and significantly increased (p≤0.05) in G3 as compared with C group. The mean ± SD of VLDL-C levels in C group and three groups G1, G2, G3 were (18.715±0.713) mg/dl, (10.654±0.541) mg/dl, (8.519±0.417) mg/dl and (10.936±1.203) mg/dl respectively. The result showed that the level of VLDL-C was significantly decreased [p≤0.05] in all three groups G1, G2 and G3 compared with C group, but no-significant change between G1 and G3 groups.

This study revealed that Bay leaf improving lipid profile by decreasing the levels of TC, TG, LDL-C and VLDL-C in serum. This conclusion are in agreement with Gasparyan *et al.* who mentioned that extract of Bay leaf leading to decrease serum TC and TG in male Wistar rats undergoes induced toxicity liver [9]. Also, agreed with Casamassima *et al*. who showed the Bay leaf recovering lipid profile in hyperlipidemic rabbits [12]. Additionally, agreed with Ravindran *et al.* result [13].

Medicinal plants may lower hyperlipidemia, inhibiting atherosclerosis and vascular endothelium injury [14,15]. Hyperlipidemia increases free radicals production, which in turn increases oxidative stress and LDL-C oxidation. During this process, LDL-C converts to OxLDL-C, which plays a critical role in the formation of atherosclerosis, cardiovascular diseases, neurological disorders and diabetes[16,17]. Antioxidants are substances that act to remove, prevent or delay oxidative harm to a target molecule, deficient levels of antioxidants or inhibition of the antioxidant enzymes, cause oxidative stress that may harm all constituents of the cell. Therefore, an antioxidant may act to control the free radicals level to neutralize oxidative damage [17,18].

The result of this study indicated decrease the levels of TC and TG in three groups, decrease the level of LDL-C in G1 and G2**,** this may be due to flavonoids and derivatives of Bay leaf. Flavonoids play important role in enhance lipids profile, it founds that treatment rats with quercetin caused a decrease in the level of LDL-C and TG [19]. Moreover, treatment mice with naringenin lead to decreased the levels of TC and TG, the improvement in plasma lipids was

due to a significant reduction in hepatic TG secretion into plasma [20]. Additionally, rutin, luteolin-7-O-glucoside and apigenin-7-O-glucoside has been found to reduce TC and TG [21]. Moreover, it founds that treatment rats with anthocyanin reduced the levels of TC and TG [22].

The result of this study showed the level of HDL-C was decreased in three groups, and LDL-C was increased in G3, this may be due to side effect of some polyphenols. Polyphenols in medicinal plants can act as either antioxidants or pro-oxidants, depending on many conditions, such as concentration of extract and oxygen or transition metals are present or not. Therefore, some extracts of plants that may possibly become pro-oxidant and stimulate oxidative stress by unknown mechanisms [17].

Conclusion

The result showed the Bay leaf and its isolated flavonoids and glycosides reduced TC, TG, LDL-C and VLDL-C, therefore Bay leaf useful agent in reducing hyperlipidemia.

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تأثير ورق الغار (*Laurus nobilis* L.) والفلافونيدات والكلايكوسيدات المعزولة منه على وظائف الدهون في إناث الأرانب المحلية العراقية

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الملخص

هدفت هذه الدراسة إلى توضيح تأثير ورق الغار والفلافونيدات والكلايكوسيدات المعزولة منه على مستويات الكوليسترول، الكليسريدات الثلاثية، البروتينات الدهنية عالية الكثافة، البروتينات الدهنية واطئة الكثافة والبروتينات الدهنية واطئة الكثافة جداً في إناث الأرانب المحلية العراقية. تضمنت الدراسة عزل الفلافونيدات والكلايكوسيدات من ورق الغار، وشمل تصميم الدراسة أربع مجاميع (n=6): مجموعة السيطرة–C غُذيت نظام غذائي قياسي، مجموعة أولى–G1 جُرعت فموياً 100 ملغم/ مل/ كغم من ورق الغار الخام لمدة 30 يوماً، مجموعة ثانية–G2 جُرعت فموياً 50 ملغم/ مل/ كغم من الفلافونويدات المعزولة لمدة 30 يوماً، مجموعة ثالثة–G3 جُرعت فموياً 12.5 ملغم/ مل/ كغم من الكلايكوسيدات المعزولة لمدة 30 يوماً. قيست الدهون بواسطة الطريقة الانزيمية اللونية. أظهرت النتائج أن ورق الغار، الفلافونيدات والكلايكوسيدات المعزولة أدت الى انخفاض مستويات الكوليسترول، الكليسريدات الثلاثية، البروتينات الدهنية واطئة الكثافة والبروتينات الدهنية واطئة الكثافة جداً مقارنة مع مجموعة السيطرة، ولذلك يمكن استعمال الغار كمخفض للدهون.