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Protective Effect of *Laurus nobilis* Extract against Hypercholesterolemia Damage in Male Rats

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ABSTRACT

Laurus nobilis (Bay leaf) has been shown to possess various biological activities such as antioxidant activity, anti-inflammatory activity and prevention of cardiovascular diseases. The present study aims to investigate the effectiveness of bay leaf extract (BLE) in protection against high cholesterol diet (HCD) induced hypercholesterolemia. About thirteen compounds of essential oils were identified by GC/MS analysis including 1.8-cineole, Linalool and sabinene which represent the main volatile oils of bay leaf. Supplementation of BLE with HCD for 10 weeks resulted in reduction in the levels of some lipid contents (total cholesterol, triglycerides, Low-density lipoprotein-cholesterol and very Low-density lipoprotein-cholesterol), tumor necrotic factor-alpha and interleukin-6 as well as reduced activity of hepatic and cardiac enzymes. Also, feeding rats on HCD with BLE enhance the antioxidant status in liver and heart tissues with indicated inhibition of lipid peroxidation by reducing the level of malondialdehyde compared to HCD-fed rats. The results concluded that BLE may have an effective role in reducing high cholesterol levels.

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ANE performed animal experiments. ANAA and MHMAM performed the biological study and collected blood samples. ANE, ANAA and MHMAM wrote the manuscript

Key words
***Laurus nobilis*, High cholesterol diet, Tumor necrotic factor-alpha, Interleukin-6, Antioxidant activity**

INTRODUCTION

Hypercholesterolemia is understood as elevation of total cholesterol levels within the blood which may have occurred as a result of inherited diseases, obesity, unbalanced diet or different diseases like diabetes (Makkos *et al*., 2020). It is one among metabolic disorders that plays an important role within the occurrence of atherosclerosis and may be a risk factor for coronary heart diseases (Pluijmert *et al*., 2019). Hypercholesterolemia can impair left ventricular (LV) function by decreasing coronary blood flow reserve and capillary density with induction of apoptosis (Yao *et al*., 2020). Additionally, hypercholesterolemia induces oxidative and nitrative stress that plays a role in several cardiac disfunction (Pluijmert *et al*., 2019). Therefore, modulation of hypercholesterolemia appears to be mandatory approach to avoid hypercholesterolemic myocardium complications (Csonka *et al*., 2016) but there are some of unexpected side effects could be occurred due to using of anti-cholesterol chemical drugs in a long-term (Hartanti *et al*., 2019). Thus, there is an obvious need for more efficacious and alternative treatment options such as using of herbal plants that contain different components characterised by their pharmacological effectiveness without any complications.

Laurus nobilis (Bay leaf) is an aromatic herb that belonging to the Lauraceae family and widely used as a condiment and spice (Mohammed *et al*., 2021). The tea of bay leaves is used traditionally as a therapy against diarrhea, for rheumatic pains and treatment of asthma, and cardiac diseases (Mohammed *et al*., 2021). This herbal plant possesses several types of metabolites that characterized by their antioxidant properties, anti-inflammatory actions, inhibition of oxidative enzymes (Hartanti *et al*., 2019). Also, the bioactive components in bay leaves such as saponin, terpenoid, flavonoid, polyphenol, alkaloid, and essential oil have been shown to have effects on insulin sensitivity and can lower cholesterol levels by inhibiting the action of HMG-CoA Reductase (Hartanti *et al*., 2019). Therefore, this study aims to investigate the possible hypocholesterolaemic effect of *Laurus nobilis* leave extract in male rats fed a high-cholesterol diet (HCD) to avoid the side effects induced by anti-cholesterol chemical drugs.

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MATERIALS AND METHODS

All experiments were carried out during 2021 at the Egyptian atomic energy authority, food irradiation department. Chemicals and reagents were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Essential oil components of Laurus nobilis

Fresh Bay leaf leaves (*Laurus nobilis*) were purchased from local market (Cairo, Egypt). Separation and identification of essential oil components were performed by using Gas chromatography instrument, Model Hewlett-Packard- MS (5970) series II Condition analysis as follow: Column: 30m hp Methyl silicon 0.1mm; Temperature: Initial 60 ºC; Rate: 3 ºC/ min up to 240 ºC; Carrier gas: Helium 1.0 ml/min; Injection port; Temperature: 250 ºC; Detector temperature: 270ºC; Integration: By using HP software Data; Injection volume: 0.3ml. The isolated peaks were identified by matching with data from the library of mass spectra and compared to those of authentic compounds and published data (Adams, 1995). Quantitative determination was carried out based on peak area integration.

Preparation of Bay leaves extract (BLE)

Bay leaves (BL) were cleaned, washed and dried at room temperature. Then, leaves were grounded for 2 min by electrical grinder.20 g of the leaves were soaked in 200 mL distilled water and was heated at 70 °C in 10 min. by a heater-stirrer (500 r/min). Finally, all the plant residues were removed by filtration and centrifugation of the extract.

Experimental animal

Male albino rats Sprague Dawley (170 to 200g body weight (B. wt.)) were purchased from the Egyptian Holding Company for Biological Products and Vaccines (Cairo, Egypt) and used for the different investigations carried out in the present study. Rats were acclimated to controlled laboratory conditions for two weeks. Rats were maintained on rodent diet and tap water ad libitum. All animals' procedures were carried out in accordance with the Ethics Committee of the National Research Centre conformed to the Guide for the Care and Use of Laboratory Animals, published by the US National Institutes of Health (NIH Publication No. 85-23, 1996). The high-cholesterol diet (HCD) contained 100 g cholesterol, 30 g propyl thio-uracil, and 100 g cholic acid in 1 L of peanut oil (Inoue *et al*., 1990).

Experimental design

The study was performed on the adult male rats divided in four groups, each of 7 rats: Group 1 (Control group): was rats fed with normal pellet diet for 10 weeks, group 2 (HCD group): was fed with HCD for 10 weeks, group 3 (BLE group): was given 200 mg kg^{-1} of BLE (Mohammed *et al*.,2021) that administered every day orally using intragastric tube for 10^{th} weeks during the examination and group 4 (HCD and BLE): was fed with HCD plus BLE (200 mg/kg B.wt./day/10 weeks) by using intragastric tube.

At the end of 10^{th} week, rats were fasted for 24 h and anaesthetized with diethyl ether. Blood samples were collected through heart puncture and allowed to coagulate and centrifuged for to obtain serum for biochemical analysis. Also, liver and heart tissues were removed for biochemical investigation.

Biochemical analysis

Total cholesterol (TC), triglycerides (TG) and high-density lipoprotein-cholesterol (HDL-C) were determined according to procedure described by Allain *et al*. (1974), Fossati and Prencipe (1982) and Demacker *et al*. (1980), respectively. Low-density lipoprotein-cholesterol and very low-density lipoprotein-cholesterol were evaluated according to Friedwald's formula (Friedwald *et al*., 1972) by the following equations: LDL-C (mg/dl) = TC-(TG/5+HDL-C), vLDL (mg/dl) = TG/5. The levels of lactate dehydrogenase (LDH) and creatine phosphokinase (CPK) were determined by the method of King (1965). Creatinine kinase-MB (CK-MB) and cardiac troponin I (cTnI) were performed by ELISA technique (BioSource International, Camarillo, CA, USA) according to the manufacturer's instructions. The activity of serum aspartate transaminase (AST) and alanine transaminase (ALT) was estimated according to Reitman and Frankel (1957), serum γ-glutamyl transferase (GGT) was assessed according to Rosalki (1975) and serum alkaline phosphatase activity (ALP) was assessed according to Kind and King (1954). Detection of serum tumour necrotic factor-alpha (TNF-α) and interleukin-6 (IL-6) was performed by ELISA technique (BioSource International, Camarillo, CA, USA) according to the manufacturer's instructions.

Hepatic and cardiac tissues (100 mg tissue/ml buffer) were homogenized in 50 mM phosphate buffer (pH 7.2; St. Louis, MO, USA); the homogenates were then centrifuged at 1,200 x g for 15 min and the supernatant was used for determination of the concentration of malondialdehyde (MDA)was according to Yoshioka *et al*. (1979), GSH content by Beutler *et al*. (1963), superoxide dismutase activity (SOD) by the method of Minami and Yoshikawa (1979) and catalase activity (CAT) by Johansson and Borg (1988).

Statistical analysis

Results were presented as mean ± SE (n= 6). Experimental data were analysed using one way analysis of variance (ANOVA). Duncan's multiple range test was used to determine significant differences between means. Data were statistically analysed by the aid of Statistical Package of the Social Sciences, SPSS version 25 (copyrighted by IBM SPSS software, USA). Differences between means were considered significant at $P < 0.05$.

RESULTS

Table I shows the essential oil contents extracted from bay leaves. 1.8-cineole represents the main volatile oil with value 36.42g/100 g, followed by Linalool 15.17 g/100 g and sabinene 9.39 g/100 g.

As shown in the results under the influence of HCD a significant increase was observed in the level of lipid profile contents (TC, TG, LDL-C, vLDL-C), inflammatory factors (TNF-α and IL-6), liver and cardiac markers (ALP, γGT, ALT, AST, LDH, CK-MB and cTnI) and the level of hepatic and cardiac MDA accompanied by reduction in the level of HDL-C, GSH and the activity of SOD and CAT compared to control group (Tables II, III).

Supplementation of HCD-rats with BLE caused significant reduction in the levels of TC, TG, LDL-C, vLDL-C, TNF-α, IL-6, ALP, γGT, ALT, AST, LDH, CK-MB, cTnI and MDA associated with significant elevation in the level of HDL-C, GSH and the activity of antioxidant enzymes (SOD and CAT) compared to rats fed with HCD.

Table I. Essential oil contents extracted from *Laurus nobilis.*

S. No	Compound	Rt. (min)	g/100 g
1	α-Thujene	15.38	0.37
2	α-Pinene	15.48	3.11
3	Sabinene	16.17	9.39
4	β -Pinene	16.20	2.86
5	β -Myrcene	16.50	1.24
6	1.8-Cineole	17.13	36.42
7	γ-Terpinene	17.60	0.61
8	α -Terpinolene	18.10	0.26
9	β -Terpineol	18.32	0.32
10	Linalool	18.37	15.17
11	Terpinen-4-ol	19.60	1.03
12	α - Terpinyl acetate	22.48	10.05
13	α -Farnesene	24.98	2.62
	Total	-	83.45
	Total unknown	-	16.55

Table II. Effect of bay leaves extract (BLE) on lipid profile, liver function, heart function, TNF-α and interleukin-6 in high cholesterol diet (HCD) induced hypercholesteraemic rats.

Parameters	C	BLE	HCD	HCD & BLE
TC (mg/dl)	146.18±6.92^{c}	140.73±6.43^{c}	285.46±7.62^{a}	192.27±6.37^{b}
TG (mg/dl)	109.45±5.83^{c}	102.54±5.12^{c}	207.32±5.71^{a}	146.34±4.92^{b}
HDL-C (mg/dl)	41.58±2.64^{a}	43.92±2.45^{a}	30.68 ±1.29^{c}	37.83±1.46^{b}
LDL-C (mg/dl)	82.71±4.16^{c}	76.30±4.24^{c}	213.32±5.68^{a}	125.18±6.13^{b}
vLDL-C(mg/dl)	21.89±1.48^{c}	20.50±1.56^{c}	41.46±1.85^{a}	29.26±1.44^{b}
AST (U/ml)	31.52±1.57^{c}	30.42±1.38^{c}	54.24±2.31^{a}	42.58±1.73^{b}
ALT (U/ml)	28.65±1.42^{c}	26.47±0.92^{c}	49.52±1.76^{a}	37.65±0.93^{b}
ALP(U/100ml)	6.58±0.64^{c}	5.87±0.71^{c}	21.23±0.81^{a}	15.24±0.67^{b}
γGT (U/ml)	5.12±0.48^{c}	4.86±0.42^{c}	13.25±0.63^{a}	8.29±0.61^{b}
LDH (U/ml)	229.31±16.21^{c}	223.61±18.65^{c}	539.43±19.27^{a}	345.31±17.12^{b}
CPK (U/L)	259.65±7.59^{c}	256.71±9.82^{c}	464.29±10.10^{a}	308.34±9.95^{b}
CK-MB(ng/mL)	3.15 ±0.69^{c}	3.11±0.76^{c}	7.29±1.22^{a}	4.97 ±0.97^{b}
CTnI (ng/mL)	24.63±1.57^{c}	23.59±1.34^{c}	62.74±2.86^{a}	36.17±1.48^{b}
TNF--α (pg/mL)	621.34± 24.51^{c}	609.37 ± 21.72^{c}	908.23±37.15^{a}	702.43 ± 32.11^{b}
IL-6 (pg/mL)	321.25 ± 22.41^{c}	315.37± 21.13^{c}	492.38±26.97^{a}	371.21± 23.16^{b}

TC, total cholesterol; TG, triglycerides; HDL-C, high-density lipoprotein-cholesterol; LDL-C, Low-density lipoprotein-cholesterol; vLDL-C, very Low-density lipoprotein-cholesterol. ALT, alanine transaminase; AST, aspartate transaminase; ALP, alkaline phosphatase; γGT, γ-glutamyl transferase; LDH, lactate dehydrogenase; CPK, creatine phosphokinase; CK-MB, creatinine kinase-MB; cTnI, cardiac troponin I; TNF-α, tumour necrotic factor-alpha; IL-6; interleukin-6. Values are expressed as means ± S.E. (n=7). Values in the same row with different superscript are significantly different at $P<0.05$.

Table III. Effect of BLE on hepatic and cardiac lipid peroxidation and antioxidant status in high cholesterol diet (HCD) induced hypercholesteraemic rats.

Parameters		C	BLE	HCD	HCD and BLE
MDA (n mol/g tissue)	Liver	223.12±4.17^{c}	216.42±5.20^{c}	386.12±7.14^{a}	259.36±6.12^{b}
	Heart	136.25±4.31^{c}	137.42±4.51^{c}	241.63±5.36^{a}	172.40±4.22^{b}
GSH (mg/g tissue)	Liver	34.32±0.61^{a}	36.56±0.92^{a}	16.92±0.57^{c}	28.91±0.92^{b}
	Heart	5.39 ± 0.26^{a}	5.89 ± 0.25^{a}	3.05 ± 0.21^{c}	4.81 ± 0.24^{b}
SOD (U/mg protein)	Liver	45.71±1.23^{a}	47.35±1.17^{a}	26.35±1.30^{c}	39.21±1.19^{b}
	Heart	28.57±1.34^{a}	29.32±1.28^{a}	17.24±1.16^{c}	21.96±1.19^{b}
CAT (U/mg protein)	Liver	49.83±1.61^{a}	50.91±1.72^{a}	31.76±1.52^{c}	44.15±1.35^{b}
	Heart	42.95±1.35^{a}	44.18±1.30^{a}	21.62±1.23^{c}	35.29±1.27^{b}

MDA, malondialdehyde; GSH, glutathione; SOD, superoxide dismutase; CAT, Catalase. Values are expressed as means ± S.E. (n=7). Values in the same row with different superscript are significantly different at $P<0.05$.

DISCUSSION

Feeding high-cholesterol diet (HCD) on a long term is one of risk factors that can cause arteriosclerosis, thrombosis and infarction (Csonka *et al*., 2016). Bay leaves represent a good source of bioactive components that help to elevate the total antioxidant status and protect against lipid peroxidation induced by oxidative stress (Casamassima *et al*., 2017).

The results of essential oil composition revealed that bay leaf possesses a lot of effective volatile oils that characterized by their antioxidant properties such as 1.8-cineole, Linalool and sabinene. These results agree with the results of Da Silveira *et al*. (2014) and Flamini *et al*. (2007). The composition of the essential oil is highly affected by genotype of the plant species, seasonality, and geographic and weather conditions (Da Silveira *et al*., 2014).

The obtained results revealed that there is a significant increase in lipid profile in HCD- group compared to control group. Li *et al*. (2011) concluded that long-lasting high-fat diet leads to lipid metabolism disturbance and hyper-lipidemia by reducing lipid metabolic enzymes such as hepatic lipase and lipoprotein lipase. The study of Fungwe *et al*. (1994) demonstrated that severe hypercholesterolemia resulted in inhibition of rate-limiting enzyme in cholesterol catabolism cholesterol 7a-hydroxylase, enhancement of hepatic TG synthesis and the reduction of fatty acid beta-oxidation. The increased serum levels of LDL-C and vLDL-C in this study indicate that more cholesterol and triglyceride are being transported from the liver to the extra-hepatic tissues to be taken up by those tissues (Adekunle *et al*., 2013). Additionally, feeding HCD found to increase the level of LDL-C which could be attributed to the ability of cholesterol and saturated fatty acids included in the diet to induce down regulation in LDL receptors (Mustad *et al*., 1997). The elevation of LDL-C, total cholesterol, triglycerides, and reduction of HDL can lead to the development and progression of atherosclerosis (Adams, 2005).

Also, the results showed that HCD induced over production of free fatty acids into blood stream resulted in excessive release of pro-inflammatory adipocytokines such as IL-6 and TNFα (Soto-Vaca *et al*., 2013) with marked elevation in the level of hepatic and cardiac MDA and reduction of GSH content and the activity of antioxidant enzymes (SOD and CAT) when compared with control group. The inflammation and oxidation induced by feeding HCD can cause cellular damage, loss of functional integrity, and/ or permeability of cell membrane that can induce the leakage of LDH, CPK, AST, ALT (Dikshit *et al*., 1995) and CK-MB into the plasma (Mitani *et al*., 2003). The release of these enzymes from the damaged hepatic or myocardial membranes indicates hypercholesterolemia-induced hepatic and myocardial necrosis (Fouad, 2020).

A significant reduction in the group of rats integrated with HCD and BLE has been observed in the level of lipid contents, enzymatic parameters of liver and heart function, Ro-inflammatory factors (IL-6 and TNF-α) and MDA. While a marked elevation in the same group has been observed in the level of HDL-C, GSH content and the activity of antioxidant enzymes (SOD and CAT) when compared with the hypercholesterolemic group. The results agreed with Casamassima *et al*. (2016) who showed the bay leaf recovering lipid profile in hyperlipidaemic rabbits. Asadi-Samani *et al*. (2014) reported that medicinal plants such as bay leaf may reduce hyperlipidaemia, supress atherosclerosis and vascular endothelium damage. The effect of BLE could be attributed to its content of essential oil, flavonoids and phenolic compounds that induced improvements in insulin sensitivity which would lead to improvements in the level of glucose and blood

lipids (Al-Samarrai *et al*., 2017). Aljamal (2010) suggested that there is a positive effect of bay leaves consumption in preventing atherosclerosis by increasing the level of HDL that can prevent the accumulation of lipid peroxides on LD. Gasparyan *et al*. (2015) found that injection of ethanol extract of dried bay leaves in carbon tetrachloride-mice improved liver function by reducing the level of ALT, AST, ALP and gamma-GT. The antioxidant effect of BLE could be related to Bay leaves' scavenger activity of essential oil and its main components that can reduce superoxide and hydroxyl radicals (Basak and Candan, 2013). In addition, Casamassima *et al*. (2016) indicated that the antioxidant activity of bay leaves could be attributed to the ability of its phenol compounds to act as donors of hydrogen, metal chelators and radical scavenger of peroxides and superoxides.

CONCLUSION

The results of present research underline that the treatment of hypercholesterolemia with bay leaf leaves extract is highly effective in lowering hyperlipidaemia. Also, the results obtain that BLE has high potential effect in improving liver function and preventing atherosclerosis and controlling the oxidative status. The effective role of BLE could be attributed to the presence of several type of essential oil (1.8-cineole, Linalool and sabinene) that have been identified in this study by using Gas chromatography instrument.

Statement of conflict of interest

The authors have declared no conflict of interest.

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