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Abstract:

This study aims to determine the hypolipidemic properties of white cheese supplemented with olive leaves extracts on blood lipids of rats. Olive leaves extract (OLE) at two concentrations (1% and 2%) were used in producing white cheese. Twenty-four male Sprague rats (100-120g) were housed individually in cages and were fed standard diet for 7 days, then randomly allocated to two groups; normal group (NC, 6 rats), and experimental group (18 rats). The NC continued on standard diet along the experiment, while the experimental group was fed hyperlipidemic diet (HL-diet) for 2 weeks. After that, the experimental rats were divided into 3 equal groups; hyperlipidemic control group (HC), fed HL-diet only; 1% olive leaves group (1OL), fed HL-diet plus 10% of cheese supplemented with 1% OLE; and 2% olive leaves group (2OL) fed HL-diet plus 10% of cheese supplemented with 2% OLE. After 30 days, all rats were fasted, then anesthetized and blood samples were collected from hepatic portal vein. Serum was analyzed for determination of cholesterol, HDL, triglyceride, LDLc, total protein, albumin and globulins. All obtained data was statistically analyzed and presented as mean±SD. The HPLC analysis showed that OLE contains huge amounts of total phenols (262.0 mg/100g). Moreover, the radical scavenging activity of OLE was very high and represented 93.70%. Obviously, there was a significant increase ($P<0.05$) in the levels of TC, TG and LDLc and a decrease in level of HDLc in HC and 1% OLE groups when compared to NC and 2% OLE groups. Meanwhile, rats fed with cheese supplemented with 2% of olive leave extract, had significantly the lowest levels of total cholesterol, triglycerides, and LDL-c and highest levels of HDLc. In conclusion, the higher the concentration of OLE the higher the positive effect on blood lipids, and cheese may enhanced the favorable effects of OLE.

Key words: Olive leaves, cheese, cholesterol, phenolic compounds, rats.

Background

Many studies showed that natural antioxidants, as flavonoids and other phenolic phytochemical present in plants are associated with reduced chronic disease risk **(Bandyopadhyay *et al.*, 2008**). Moreover, the type of phenolic compounds has been demonstrated to inhibit lipid peroxidation of human low-density lipoprotein in vitro (**Goncalves *et al.*, 2004**). Olive mill and olive processing residues are attractive sources of such natural antioxidants. Olive leaves extract has been reported to have anti-oxidative, antimicrobial, anti-HIV, vasodilator, and hypoglycemic properties **(Erbay and Icer, 2010; Lalas *et al.*, 2011; Mujic *et al.*, 2011 and Theodora *et al.*, 2013)**.

Olive leaves are obtained as by- product during oil extraction, and has been used traditionally in Mediterranean countries as a rich source of nutrients for animals **(Martın Garcıa *et al.*, 2003)**. The chemical analysis showed that leaves are save and poor in nitrogen and tannins, whilst it was rich in crude fat and fiber. Several studies found that olive leaves has a variety of other compounds, e.g. uropein and flavonoids **(Briante *et al.*, 2002)**. Moreover, it contains high amounts of secoiridoiduropein-bioactive components that constitute up to 6–9% of the dried leaves. Olive leaves contain other bioactive components like secoiridoids, flavonoids, and triterpenes. Nevertheless, Australian researchers found that olive leaves have up to 40 times more antioxidants than virgin olive oils (**http://www.olea.com.au/benefits /antioxidant-power**).

Historically, Ancient Egyptians used olive leaves for mummification and as a remedy against several diseases. Old British used it to treat Malaria in the 1800s. Because of it contains several bioactive components that might have anti-inflammatory effects, olive leaves have been used in the human diet as an extract, herbal tea, or a powder (**Bitler *et al.*, 2005**), olive leaves may also be anti-thrombic, preventing LDL oxidation **(Carluccio *et al.*, 2003)**, lowering blood glucose, blood cholesterol, triglycerides, **(Mousa *et al.*, 2014)** decrease lipidemia (**Andreadou *et al.*, 2006**), antioxidant and antihypertensive **(Sedef, Sibel Karakaya, 2009)**.

All of these interesting findings encourage the authores to carry out this study and tried to use most popular foods to be as a vehicle for olive leaves extracts. So that the researchers have chosen the white cheese to

supplement it with olive leaves where it is popular, cheap, nutritious, and easy to reach.

Soft cheese is one of the most appreciated cheeses in Middle East countries. This type of cheese is produced either by enzymatic or acidic coagulation of fresh milk (buffalos' or cows' milk) or reconstituted skim milk powder with oils **(Ramadan *et al.*, 2014)**. It also has been made with or without the addition of starter cultures to cheese milk. Starter cultures govern the flavor and texture of the cheese, and help to suppress the growth of spoilage bacteria.

The ultimate goal of this study was to find out the effect of white cheese supplemented with different concentration of aqueous extracts of olive leaves on blood lipids of experimental rats.

Methodology

Preparation of olive leaves extract:

Green olive leaves were collected, dried and stored until use. A hot water extract of olive leaves was prepared according the methods described in **(Mousa *et al.*, 2014)**. Olive leaves were cut into small pieces and boiled for 10 minutes in distilled water. The mixture was filtered twice: first through cheese cloth (50% cotton/50% polyester), and then through filter paper (Whatman No.2). The solutions obtained were preserved in sterile dark bottles in a cool environment (4°C) until use.

Determination of total phenolic content:

The concentration of total phenols in rice ran oil was measured by a UV spectrophotometer **(Jenway-UV–VIS Spectrophotometer)**, based on a colorimetric oxidation/reduction reaction, as described by **Škerget *et al.* (2005)**. The oxidizing reagent used was Folin-Ciocalteu reagent **(AOAC, 1990).**

Identification of phenolic compounds by HPLC:

The phenolic and flavonoid compounds of the olive leaves extracts were identified according to the method described by **Goupy *et al.*, 1999 and Mattila *et al.*, 2000)**.

Radical scavenging activity (Scavenging DPPH):

The electron donation ability of the obtained extracts was measured by bleaching of the purple colored solution of the 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) according to the method of Hanato et al. 1988 and modified method by **Gulcin *et al.* (2004)**.

Cheese processing:

Soft cheese were made by the conventional method used in processing of Domiati cheese (**Fahmi and Sharara, 1950**). All ingredients used in processing of white cheese were obtained from authorized sources, either locally or internationally. Fresh bulk buffalo's milk containing 3% fat was pasteurized at 63°C for 30 min; calcium chloride and sodium chloride were added at the ratios of 0.02% and 4% (W/V) respectively. The treated milk was divided into 3 parts; 1st part was left without any additives and used as a control (C); 2nd and 3rd parts were supplemented with olive leaves extract (1% and 2% respectively). All milk treatments were reneging and yielded cheese were kept in plastic containers with formerly boiled saline (4% salt) and stored at refrigerator temperature (5-7 °C) for four weeks and sampled for analysis after processing.

Chemical analysis:

Total solids, fat, total protein contents of soft cheese, and acid value of extracted lipids were determined according to **AOAC (2007**). Peroxide value (PV) of extracted lipids was determined according to the method described by **Egan *et al.* (1981)**.

Sensory properties of cheese:

The sensory properties of produced cheese were assessed by 10 panel members of the Dairy Sci., Dept., Fac. Agric., Zagazig, Univ. for flavor, body and texture, and appearance **(Pappas *et al.*, 1996)**.

Animals:

Twenty-four male Sprague Dawely rats weighing 100 – 120 grams were purchased from Animal Unit, Helwan Farm, Ministry of Health, Cairo, Egypt. The rats were housed individually in cages, and were kept at 22 C°, 56% humidity (40 to 70%) and in a 12-h: 12-h light: dark cycle and were allowed free access to food and tap water. The experiment was conducted in the animal lab at Faculty of Agriculture, Zagazig University, Egypt. After 7 days of acclimatization, rats were weighed and randomly allocated to two main groups; normal group (6 rats), and experimental group (18 rats).

Diet:

Standard diet:

The standard diet used in this study was obtained from The Central Animal House of the National Research Center, Dokki, Giza Governorate, Egypt. The diet composed of carbs 64.0%; crude protein 22.0%; crude fat 3.5%; crude fiber 3.5%; ash 2.4% **(AIN, 1993)**.

Hyperlipidemia diet:

The hyperlipidemic diet used in this study was based on standard diet with adding tallow and cholesterol powder. The diet composed of; carbs 46.0%; crude protein 22.0%; crude fat 3.5%; animal fat 17.0%, cholesterol powder 1%, bile acid 0.25%; crude fiber 3.5% and ash 2.4%.

Diet supplemented with cheese:

The standard diet was fed to rats plus 10% of produced cheese – either supplemented with 1% or 2% olive leaves extracts.

Experimental Design:

After 7 days of acclimation, the normal control group was continued on standard diet for another 14 days, while the experimental group was fed hyperlipidemic diet for 14 days. After this period the rats were weighed and distributed in the following experimental groups:

Normal control group: composed of 6 rats and fed standard diet only.

Hyperlipidemic control group: composed of 6 rats and fed hyperlipidemic diet only.

1% olive leaves group: composed of 6 rats and fed hyperlipidemic diet plus 10% of cheese supplemented with 1% olive leaves extract.

2% olive leaves group: composed of 6 rats and fed hyperlipidemic diet plus 10% of cheese supplemented with 2% olive leaves extract.The intervention trial continued for 30 days.

Blood Sampling and Laboratory Analysis:

After 30 days, blood samples were collected from control and experimental groups after 8 hours fasting, and the rats were scarified under ether anesthetized. Blood samples were collected from hepatic portal vein into tubes then centrifuged immediately for 10 minutes at 3000 rpm to separate the serum. Serum was carefully aspirated and transferred into clean tubes and stored frozen at-20 C for analysis. All serum samples were analyzed for determination of total cholesterol, HDL and triglyceride **(Devi and Sharma, 2004).** LDL and VLDL were calculated according to the method given by **Johnson *et al*. (1997).** Total serum protein, and serum albumin were determined according to **(Henry, 1964 and Doumas *et al*., 1971**, respectively).

Statistical Analysis

All obtained data was statistically analyzed and presented as mean±SD, also the significant differences between different between groups were calculated by ANOVA and LSD at confidence interval 95%. Differences between treatments for the same group were calculated by paired-sample t-test at confidence interval 95%.

Results and Discussion

Olive leave extract was determined for total phenols (Table 1). The data showed that olive leave extract contains huge amounts of total phenols.

These results agreed with that obtained by (**Mohan *et al.*, 2013)**. Therefore, leaves of olive might be an excellent and promising source of bioactive compounds which in turn have a high antioxidative properties. Moreover, the results showed that radical scavenging activity (Table 1) of olive leaves extract was also high and represented 93.70%, which may be due to phenolic compounds (mainly flavonoids and tannins) that found in the plant and this compounds may be act as primary antioxidants or free radical scavengers as evidenced by several recent studies (**Erbay and Icer, 2010; Lalas *et al.*, 2011; Mujic *et al.*, 2011 and Theodora *et al.*, 2013**). However, the researcheres tried to find out the exact active phenolic compound soccurred in olive leaves extract (Table 1). In accordance with (**Herrero *et al.*, 2011)**, the extract contains huge amounts of phenolic compounds especially benzoic acid (176.22 mg/100g) and averages amounts from catechin (25.72 mg/100g)ferulic (24.10 mg/100g),and catechol (20.73 mg/100g).

Table (1): Phenolic compounds and radical scavenging activity of olive leave extract.

Variable	**Amount**
Total phenolic compound (mg/g)	262.0mg/100g
Radical scavenging activity (RSA)%	93.70%
Phenolic Compounds Determined by HPLC (mg/100 g)	
Gallic	1.87
Cinnamic	2.87
Protocatechuic	3.89
Caffien	4.21
Catechol	20.73
Ferulic	24.10
Catechin	25.72
Benzoic	176.22

The average, score points given for appearance body characteristics and flavor of white soft cheese as affected by adding natural antioxidants are illustrated in Table (2), these results showed that there were

significant differences between the control and the two treatments. Cheese supplemented with OLE showed the highest scores in sensory evaluation, especially cheese supplemented with 2% of OLE. Also, results showed that control cheese recorded the highest score for appearance.All produced cheese had a remarkable acceptance by panelists.

Table (2): Organoleptic properties of white soft cheese supplemented with olive leave extracts.

Samples	Appearance (10)	Flavor (50)	Body& texture (40)	Total (100)
Control cheese	9	46	36	91
Cheese supplemented with 1% OLE	8	48	37	93
Cheese supplemented with 2% OLE	8	49	38	95

As shown in table (3), there was a significant increase ($P<0.05$) in the levels of serum total cholesterol (TC), triglyceride (TG) and LDL-c and a decrease in level of HDL-c in HC and 1% OLE groups when compared to NC and 2% OLE groups. In excellent agreement with Pankajet al., 2010 and **Mousa *et al.*, 2014**, the rats fed on cheese supplemented with 2% of olive leave extract, had significantly the lowest levels of total cholesterol, triglycerides, and LDL-c and highest levels of HDLc. Similar improvement on feeding high doses of OLE were reported by**(Eidi *et. al.*, 2009**), who found that the oral administration of the olive leaves extract for 14 days significantly decreased the serum glucose, total cholesterol, triglycerides, urea, uric acid, creatinine, AST and ALT. The author believe and hypothesized that these beneficial effect of high dose of OLE may be due to the higher concentration of phenolic compounds which naturally occurred in olive leaves, and concluded that the higher the concentration of OLE the higher the positive effect on blood lipids. Also, the author suggest that cheese may enhanced the favorable effects of OLE.

Table (3): Effect of cheese supplemented with olive leave extract on lipid profile in hyperlipidemic rats (mean ± SD).

Groups	TC (mg/dl)	TG (mg/dl)	VLDL (mg/dl)	LDL (mg/dl)	HDL (mg/dl)
NC	92.4±4.7[a]	132.2±18.7[a]	26.4±4.6[a]	26.2.0±1.2[a]	39.8±9.0[a]
HC	158.6±12.2[b]	302.4±33.2[b]	60.5±6.8[b]	67.8±18.1[b]	30.3±2.1[b]
1% OLE	168.4±13.6[b]	308.8±35.2[b]	61.8±6.9[b]	78.2±18.8[c]	28.3±4.3[b]
2% OLE	88.6±3.5[a]	127.6±18.2[a]	25.5±3.7[a]	24.5±4.1[a]	38.6±2.7[a]

NC: Normal control group; HC: Hyperlipidemic control group; 1% OLE: group fed cheese supplemented with 1% OLE; and 2% OLE: group fed cheese supplemented with 2% OLE. SD= Standard deviation. Mean values subscribed with different letters in the same column shows significant differences between these values as calculated by one-way ANOVA and LSD at $P<0.05$.

It is evident that hyperlipidemic state accompanied by an increment in total serum protein and albumin. Serum total protein and albumin of HC group are significantly the lowest when compared with other three groups. Similar results are obtained by (**Mousa *et al*., 2014)** and **(Eidi *et al*., 2009),** who found an improvement in the levels of total proteins and albumin among rats fed OLE in different concentrations.

Table (4): Effect of cheese diets supplemented with olive leaves extract on serum total protein and albumin (mean ± SD).

Groups	Total protein (mg/dl)	Albumin(mg/dl)
NC	8.4±0.9[a]	5.1±0.8[a]
HC	7.34 ±0.3[b]	4.4±0.4[b]
1% OLE	7.98 ±1.1[a]	4.80 ±0.9[a]
2% OLE	8.14 ±1.4[a]	4.9 ±0.9[a]

NC: Normal control group; HC: Hyperlipidemic control group; 1% OLE: group fed cheese supplemented with 1% OLE; and 2% OLE: group fed cheese supplemented with 2% OLE. SD= Standard deviation. Mean values subscribed with different letters in the same column shows

significant differences between these values as calculated by one-way ANOVA and LSD at P<0.05.

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تأثير الجبن المدعم بمستخلص أوراق الزيتون على الفئران المصابة بارتفاع دهون الدم

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الملخص

يهدف هذه البحث إلي دراسة تأثير الجبن المدعم بمستخلص أوراق الزيتون كمخفض لدهون الدم فى فئران التجارب, تم إجراء التجربه بإستخدام تركيزين من مستخلص أوراق الزيتون (1%, 2%) في عمل الجبن الأبيض وتم تطبيق التجربه علي 24 فأرا من فئران التجارب الذكور والذي يتراوح متوسط أوزانهم بين (100 – 120 جم), وتم تغذيتهم على الوجبة االإعتياديـة لمدة 7 أيام وبعد ذلك قسموا إلى مجموعتين على النحو التالى :1- المجموعة الضابطة (6 فئران).2- المجموعة التي تمت عليها التجربة (18 فأرا). وقد إستمرت المجموعة الضابطة في تناول الوجبة الإعتيادية طوال فترة التجربة, بينما تغذت المجموعة الأخرى على وجبة عالية الدهون لمدة أسبوعين ثم تم تقسيمهم إلى 3 مجموعات: أ- مجموعه تتغذى على وجبات عالية الدهون فقط. ب- مجموعة تتغذى على وجبات عالية الدهون + 10% جبن مدعم بـ 1% من مستخلص أوراق الزيتون. ج – مجموعة تتغذى على وجبات عالية الدهون + 10% جبن مدعم بـ 2% من مستخلص أوراق الزيتون. وبعد مرور30 يوما تم ذبح الفئران وأخذ عينات دم وتحليلها لتقدير مستويات دهون الدم والبروتين الكلي وبعد ذلك تم تحليل النتائج إحصائيا باستخدام برنامج Anova. وقد أظهر تحليل كروماتوجرفي عالي الكفاءة (HPLC) أن مستخلص أوراق الزيتون تحتوي على عدد هائل من المركبات الفينولية بمعدل (262 ملج/100ج), بالإضافة إلى إرتفاع نسبة مضادات الأكسدة وكانت موجودة بنسبة (93.7%). كما أظهرت النتائج أن مجموعة الفئران التي تغذت على الجبن الأبيض المدعم بـ 2% من مستخلص أوراق الزيتون كان أكثر تأثيرافي تقليل مستويات

دهون الدم وتقليل الآثار المترتبة على إرتفاعها مقارنة بمجموعة الفئران التي تغذت على الجبن الأبيض المدعم بـ 1% من مستخلص أوراق الزيتون والمجموعة الضابطة.