The effect of Laurus nobilis on the blood and lenses antioxidant activity in rabbit under fat-enriched diet.

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Running title: Protective role of *Laurus nobilis*

Summary

Fat-enriched diet is strongly associated with cataract development. *Laurus nobilis* shows antioxidant activity. Herein we evaluated the effect of *Laurus nobilis* oral administration on the blood and lenses antioxidant activity in rabbits under fat-enriched-diet. Sixty rabbits divided into 4 groups were used. One group represented the control (N-CTR). The second group (P-CTR) fed a diet supplemented with 2.5% of pig fat; the third group (EXP1) received a diet supplemented with 2.5% of pig fat and 1 g/kg of dried-bay leaves; the fourth group (EXP2) was treated with dried-bay leaves at the rate of 1 g/kg of feed. At baseline and at the end of the study (56 days) the following blood parameters were determined: thiobarbituric acid reactive substances (TBARS), reactive oxygen metabolites (ROMs), total phenols, superoxide dismutase (SOD), oxygen radical absorbance capacity ($ORAC_{pca}$), ferric ion reducing antioxidant power (FRAP), retinol and alfa-tocopherol. At the end of the follow-up, the eyes were enucleated and the antioxidant profile, such as total antioxidant activity (TAC), TBARS, retinol and alfa-tocopherol of lenses was evaluated. Plasma ROMs and TBARS levels were statistically lower in the groups receiving bay leaves integration. A significant increase of plasma retinol, FRAP and $ORAC_{pca}$ levels was found in EXP1 and EXP2 groups, whereas plasma alfa-tocopherol resulted statistically higher only in EXP2 group. Bay leaves supplementation enhanced TAC, retinol and alfa-tocopherol in rabbit lens, particularly in EXP2 group; whereas lenses TBARS levels significantly decreased in both treated groups. These findings demonstrate that *Laurus nobilis* oral administration exerts a protective effect on the risk of cataract development in rabbits under fat-enriched diet.

Keywords: bay leaves, antioxidant, lens, cataract, fat enriched diet.

Introduction

Oxidative stress plays a key role in the pathogenesis of cataract both in experimental animal models and humans (Nita and Grzybowski 2016). Reactive oxygen species (ROS) are normally produced in aerobic organisms, in fact oxidative stress can be minimized but cannot be eliminated. However, a certain level of ROS is necessary as mediators in several cellular processes and signalling networks (Mittler *et al.* 2011), i.e. cell signalling, cell adhesion, cellular immune response, apoptosis and cell survival (Zhu *et al.* 2012). When free radicals accumulate in the lens, the polyunsaturated fatty acids are easily oxidized, leading to the initiation of cataract. Malondialdehyde, one of the end products of lipid peroxidation, is itself toxic, due to its high cross-linking ability. Lens opacity was detected also in dyslipidemic patients (Heydari *et al.* 2012), with or without ischemic heart disease (Leino *et al.* 1992; Nucci and Mets 1990).

Since oxidative stress is a common trigger of many age related conditions, including cataract, dietary natural extract-based approach to delay the onset or the progression of cataract has been widely investigated (Libondi *et al.* 1991). However, in literature several findings on this topic are inconsistent, where no evidence was reported from dietary supplementation with antioxidant to prevent or slow the progression of cataract. (Chiu and Taylor 2007; Mathew *et al.* 2012). Clinical evidences reported little benefit deriving from vitamin supplementation either in prevention of age-related cataract or in reducing its progression (Chiu and Taylor 2007; Milton *et al.* 2006). Diet rich in fruits, vegetables, fish, pulses and starchy foods may exert a protective role against cataractogenesis (Theodoropoulou *et al.* 2014), whereas a strong association with cataract development and ocular degenerative diseases was found with hypercholesterolemia, hypertriglyceridemia, high LDL (low density lipoprotein) cholesterol, high intake of saturated fat and high fasting glucose (Heydari *et al.* 2012).

Although the correlation between antioxidants intake and age-related cataract is not well comprehended, clinical evidence suggest that dietary total antioxidant capacity is inversely associated with the risk for age-related cataract (Mares 2015). Thus, it is possible that dietary changes and antioxidants intake may reduce the risk for age related cataract. Most of these antioxidants are reducing agents, such as polyphenols, that are able to interrupt the free radical based chain reactions. Plant phytochemicals have shown preventive activities in models of oxidative stress. Ellagic acid inhibited the formation of cataracts induced by selenite in Wistar rats (Sakthivel *et al.* 2008) and prevented alterations in lens proteins (Sakthivel *et al.* 2011). The polyphenols in *Moringa oleifera* also prevented cataract formation in selenite-treated rat pups (Sasikala *et al.* 2010).

Flavonoids, phenolic acids, carotenoids, vitamins and lactoferrin are natural antioxidant molecules with anti-cataract activity (Sunkireddya *et al.* 2013). In traditional societies, it is more acceptable, accessible and affordable to have antioxidant substances in indigenous, user friendly and user accessible forms than administer them as pills, drugs or capsules.

Laurus nobilis is an evergreen plant or small tree, belonging to the family of *Lauraceae* in the genus *Laurus*. It is thought to have origin in Asia Minor region, from where it distributed to all over the Mediterranean region and other parts of Asia. It grows in many warm regions of the world, particularly in Southern Europe and around the Mediterranean Sea area (Chmit *et al.* 2014). *Laurus nobilis* presented high levels of nutritional support due to the content of proteins, free sugars, organic acids, PUFA and tocopherols together with antioxidant activity, such as scavenging activity, reducing power and lipid peroxidation inhibition (Dias *et al.* 2014). *Laurus nobilis* leaves showed to improve insulin function in *in vitro* study, whereas in *in vivo* human-trial exhibited a significant decrease of fasting serum glucose, serum LDL cholesterol and triglycerides, together with an increase in HDL (high density lipoprotein) cholesterol, after the intake of 1 and 3 g per day (Khan *et al.* 2009).

These findings prompted us to verify whether the oral administration of bay meal dried-leaves is associated with increased anti-oxidant activity in blood plasma and lenses of rabbit fed a fat enriched diet. To the best of our knowledge, the findings described herein represent the first report on the effect of *Laurus nobilis* on cataract prevention.

Materials and methods

Animals and diet

The study lasted 56 days and was carried out on 60 New Zealand white male rabbits weaned at 35 ± 2 days and divided into 4 groups of 15 animals each, matched for age and body weight. The first group, negative control (N-CTR), received *ad libitum* a standard diet (Agrizoo, Miranda, Isernia, Italy); the second group, positive control (P-CTR) group, received a diet supplemented with 2.5% of pig fat; the third group (EXP1) fed a diet supplement with 2.5% of pig fat and 1 g/kg of dried-bay leaves (*Laurus nobilis*); lastly, the fourth group (EXP2) received dried-bay leaves at the rate of 1 g/kg in feed. Bay leaves were purchased by the Herboristeria Erbamea (San Giustino, Perugia, Italy). Rabbits were housed under conventional conditions and exposed to light-dark cycle of 12 h with free access to water and feed (daily recorded). All procedures were performed in accordance with the guidelines n. 86/609/EEC stipulated by Committee and the European Union Guidelines for animal experimentation. At the end of the experiment (56d), animals were sacrificed by gas

embolism, the eyes were quickly enucleated and the lenses were microscopically removed. The study was conducted in accordance with the Association for Research in Vision & Ophthalmology Statement for the use of animals in Ophthalmology and Vision Research.

Blood collection

At the beginning (0d) and the end of the study (56d), blood samples were collected from the *vena auricularis marginalis*, using a vacutainer method (Venoject, Terumo Europe N.V., Leuven, Belgium) with lithium-heparin tubes to produce plasma. Blood samples were centrifuged for 20 minutes at 3000 rpm and the following plasma parameters were determined: thiobarbituric acid reactive substances (TBARS), reactive oxygen metabolites (ROMs), total phenols, superoxide dismutase (SOD), oxygen radical absorbance capacity ($ORAC_{pca}$), ferric ion reducing antioxidant power (FRAP), retinol and alfa-tocoferol were performed. ROMs values were spectrophotometrically determined with the method of Cesarone *et al.* (1999), at a wavelength of 505 nm using a specific commercial kit (Diacron, Grosseto, Italy). Results were expressed in Carr units (1 U/Carr corresponds to 0.024 mmol/l of H_2O_2). The determination of TBARS was performed according to the method of Esterbauer and Zollner (1989), using a standard curve with the 1,1,3,3 tetramethoxypropane (Sigma Aldrich, St. Louis). Results were expressed in μmol of malondialdehyde (MDA) per l of plasma. Retinol and alfa-tocoferol were extracted from plasma samples with chloroform, according to the method of Zhao *et al.* (2004); results were expressed inμg/ml of plasma. The determination of total phenols was done with the method of Folin-Ciocalteau reaction (Swain and Hills 1959), and results were expressed in mg of Trolox equivalents per ml of plasma (mg TE/ml). SOD was determined using a colorimetric assay (Zhou and Prognon 2006); SOD activity was expressed in units per milligram of protein (U/mg). $ORAC_{pca}$ test was performed in accordance with the study of Ou *et al.* (2002); results were expressed in μmol of Trolox equivalents per l of plasma (μmol TE/l). FRAP test, expressed in mmol/ml, indicates the number of moles of ferric ion (FeIII) reduced to ferrous ion (FeII) from one mol of tested antioxidants (Benzie and Strain 1996).

Preparation of lenses supernatant

Two lenses of each rabbit, pooled together to give one sample, were washed with normal physiological saline solution and then processed. Each sample was homogenized for 60” in equal volume of 50 mM phosphate buffer (pH 7.2) and centrifuged at 12,000×g for 15 min at 4°C. The obtained supernatant was used for analysis.

TBARS assay

TBARS were determined in the lenses homogenates with the use of a spectrophotometric method based on the 2-thiobarbituric acid reaction. Sample was mixed with 2 volumes of cold 10% (w/v) trichloroacetic acid (TCA) to precipitate protein. The pellet was removed, and an aliquot of the supernatant reacted with an equal volume of 0.67% (w/v) thiobarbituric acid in a boiling water bath for 10 min. After cooling, TBARS absorbance was detected at 532 nm, using a spectrophotometer Varian Cary 100 UV-VIS (Varian, Australia). Results were expressed in μmol/g eye wet weight.

TAC assay

The total antioxidant capacity (TAC) of lenses was measured on sample lenses supernatant by 2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonate) (ABTS) radical cation decolourization assay, according to the method of Re *et al.* (1999). The $ABTS^{+}$ radical was generated by chemical reaction with potassium persulfate. For these propose, 25 ml of ABTS (7 mM) was spiked with 440 μl of potassium persulfate (140 mM) and allowed to stand in darkness at room temperature for 12-16 h (time required for the formation of the radical). Trolox was used as standard and the total antioxidant capacity of samples was defined as the concentration of Trolox having equivalent activity as μmol/g eye wet weight.

Alpha-tocopherol and retinol assay

Alpha-tocopherol and retinol in lenses sample were determined using a procedure of Zhao *et al.* (2004) modified. Samples were analysed by an HPLC system (Kontron Instruments, Milan, Italy) consisting of an autosampler (HPLC autosampler 360, Kontron Instruments, Milan, Italy) with a loop of 20 μL, a high-pressure pump and a C18 column 5 μm, 250 x 4.60mm (Phenomenex, Torrance, CA, USA). The mobile phase consisted of acetonitrile and methanol (75:25 v/v), and a flow rate of 1 mL min^{-1} was used. Alpha-tocopherol and retinol were identified using a fluorimeter detector and comparing the samples retention time with the pure standards (97 %) purchased from Sigma Aldrich (St. Louis, USA). The quantification was carried out using the Geminyx system (version 1.91) comparing the area sample peak with that of the reference standards curve. Results were expressed as μmol/mg of eye wet weight.

Statistical analysis

Statistical analyses were performed with SPSS 19 for Windows (2010). Blood parameters were assessed using repeated measures assay, including the dietary treatment as main effect between-groups (N-CTR, P-CTR, EXP1 and EXP2), while within-group the sampling time (Time) and

dietary treatment x time (Diet x Time) was considered as main effect. Lenses were analyzed with one-way ANOVA test using the dietary treatment as independent variable. Post-hoc Tukey's t-test was used to compare the groups. Relationships between blood and lenses considered parameters were assessed by Pearson correlation coefficients. The results are presented as mean values and pooled standard error of mean (SEM). Differences were considered statistically significant at a level of $P<0.05$.

Results

During the whole experiment the welfare and the body condition of animals was considered good and before sacrifice an ophthalmologist observed at a slit lamp lenses of *in vivo* animals and they were all normal and clear. At the end of the experiment, no effect due to the dietary treatment was recorded on the feed intake between the controls and experimental groups (average 137.48 g/day).

Plasma oxidative parameters

Plasma oxidative status is reported in Table 1. At the end of the follow-up ROMs levels were significantly lower ($p<0.01$) in EXP1 and EXP2 groups (280.1 *vs* 193.0 U/Carr, respectively) compared to those recorded in P-CTR group (352.7 U/Carr). Moreover, the difference of ROMs levels between EXP2 group and N-CTR was also significant (193.0 *vs* 227.4 U/Carr, respectively). Bay leaves integration significantly reduced ROMs values in all treated groups (EXP1 and 2; $p<0.01$), whereas in the fat diet enriched group the ROMs values were significantly increased (P-CTR 352.7 U/Carr, $p<0.001$).

TBARS values were found to be lower in the groups receiving bay leaves (EXP1, 2.83 μmol/l; EXP2, 2.65 μmol/l; $p<0.05$), compared to the control groups (N-CTR, 3.07 μmol/l; P-CTR, 3.50 μmol/l). In fact, EXP2 group showed the TBARS values lower than those recorded in control animals. TBARS values significantly increased in fat enriched diet group without integration of bay leaves compared to controls.

A marked decrease of retinol levels ($p<0.01$) was recorded in the P-CTR group (0.191 μg/ml). Bay leaves administration maintained the retinol levels within the normal range (EXP2, 0.316 μg/ml). However, retinol values in groups receiving bay leaves supplementation were higher than those recorded in the control group (N-CTR, 0.262 μg/ml).

Fat enriched diet induced a significant diminution of plasma alfa-tocopherol levels (P-CTR, 2.01 μg/ml; EXP1, 2.41 μg/ml; $p<0.05$). Bay leaves oral administration did not affect this parameter in the treated group (EXP2, 3.13 *vs* N-CTR, 2.99 μg/ml; not significant).

Total phenols showed a significant decrease secondary to fat enriched diet (P-CTR, 58.3 mg TE/ml; $p<0.05$); *Laurus nobilis* administration counteracted the fat effects (EXP1, 62.8 mg TE/ml); in EXP2 group a significant increase of total phenols was recorded (75.1 mg TE/ml), compared to control (N-CTR, 62.7 mg TE/ml; $p<0.05$).

The plasma SOD values recorded at the end of the study showed a trend similar to the total phenols. In fact, plasma SOD content showed a decrease in the group fed a fat enriched diet but not significant (P-CTR, 37.3 U/mg; $p>0.05$); bay leaves administration attenuated the fat effects (EXP1, 42.3 U/mg); in EXP2 group a significant increase of SOD values was recorded (61.3 U/mg), compared to control (N-CTR, 42.5 U/mg; $p<0.05$).

Plasma $ORAC_{pca}$ levels were affected only in the P-CTR group (631.9 µmol TE/l). Bay leaves administration maintained the ORAC levels within the normal range (EXP1, 788.4 µmol TE/l).

A marked decrease of FRAP levels was recorded in the P-CTR group (383.7 mmol/ml; $p<0.01$). Bay leaves administration maintained the FRAP levels within the normal range (EXP2, 566.6 mmol/ml).

For all parameters of plasma oxidative status a significant interaction effect between dietary supplementation and time (Diet x Time) was also recorded (Table 1).

Antioxidant markers in rabbit lenses

In table 2 are summarized the TAC level recorded in rabbit lens. Animals fed a fat enriched diet showed a significant decrease of this parameter compared to the negative control group. Contrarily, bay leaves administration increased the ability to counteract oxidative stress (EXP1, 146.69; EXP2, 160.02 µmol TE/g; $p<0.001$). The group receiving *Laurus nobilis* only exhibited TCA values statistically higher than those found in negative control group (N-CTR, 149.22 µmol TE/g; $p<0.001$).

TBARS levels markedly increased in both fat enriched diet groups (P-CTR, 6.58; EXP1, 5.64 µmol/g; $p<0.001$) compared to the negative control group (N-CTR, 5.10 µmol/g). Bay leaves administration significantly affected the TBARS levels in EXP1 group respect to P-CTR group.

Fat enriched diet significantly reduced the retinol levels in both groups (P-CTR, 22.79 µmol/mg; EXP1, 23.53 µmol/mg; $p<0.001$). The bay leaves administration did not affect the retinol content both in EXP 1 and in EXP 2 groups (23.53 *vs* 37.34 µmol/mg; not significant).

Alpha-tocopherol levels were significantly affected in animals under a fat enriched diet. P-CTR group showed a significant decrease of this parameter compared to the control values (0.08 *vs* 0.19 µmol/mg, respectively; $p<0.001$). Contrarily, bay leaves administration increased the alpha-tocopherol levels in EXP1 (0.17 µmol/mg), exhibiting a protective effect towards the diet induced

lipid oxidation. Lastly, in the group receiving only *Laurus nobilis,* alfa-tocopherol content did not differ from the values recorded in control group (0.20 *vs* 0.19 μmol/mg, respectively; not significant).

Coefficient correlation analysis between the antioxidant parameters and the oxidative markers provided different results in blood and in lens. In particular, a positive and significant ($p<0.05$) correlation was found between the antioxidant lens parameters (TAC, alpha-tocopherol and retinol) and the antioxidant blood content ($ORAC_{pca}$, FRAP, total phenol, alpha-tocopherol and SOD). Lens MDA levels were negatively correlated ($p<0.001$) with all the antioxidant markers tested, being positively correlated ($p<0.001$) to blood ROMs values only. Antioxidant blood parameters ($ORAC_{pca}$, FRAP, total phenol, retinol, alpha-tocopherol) were negatively correlated ($p<0.001$) with blood ROMs and MDA values, whereas blood oxidative markers (ROMs and MDA) were positively correlated ($p<0.001$) between them. All correlations were performed on data collected at the end of the experiments.

Discussion

Our findings demonstrate that oral administration of bay leaves was associated with increased antioxidant activity in plasma and lenses of rabbit. Fraga (2003) reported that sesquiterpene lactones, extracted from bay leaves, exhibited a biological and pharmacological antioxidant activity on *in vitro* cultured cells. This datum was further confirmed by Elmastas *et al.* (2006), who suggested that the antioxidant activity exerted by bay leaves could be attributed to the ability of phenol compounds which act as donors of hydrogen, metal chelators and radical scavenger of peroxides and superoxides. This antioxidant effect delays the free radicals-induced proteins cross-linking and aggregation in the lens, events known to lead to cataract formation (Tan *et al.* 2008).

Laurus nobilis leaves represent a good source of antioxidant components that help to increase the overall antioxidant capacity of lens and protect it against lipid peroxidation induced by oxidative stress. Oral administration of *Laurus nobilis* leaves significantly increases retinol levels in the lens and decreases those of MDA, compared to the levels recorded in the fat-stress induced group. This effect could be due to its high carotenoid content (Yahyaa *et al.* 2015). Beta-carotene is a fat-soluble compound of the carotenoids, which are considered pro-vitamins, since they can be converted to active retinol. It is a strong antioxidant and is the best quencher of singlet oxygen. Contrary, when beta-carotene, ascorbic acid, folic acid, iron, phytate and polyphenols levels are insufficient, oxidative stress in blood and lens increases (Tarwadi *et al.* 2008). The lowest MDA levels in the lens of treated animals, also confirms the protective effects against membrane lipid

peroxidation exerted by *Laurus nobilis* intake. These findings agree with those reported by Gupta *et al.* (2010) who investigated the anti-cataracts effects of *Trigonella foenum-graecum* (Fenugreek) and found that the incidence of cataract in rats receiving fenugreek was lower than that observed in the untreated animals, by the inhibition of lipid peroxidation; biochemical parameters were modified according to the antioxidant property of the diet treatment. Also onions juice application, due to the high levels of flavonoids, counteracts cataract development in a model of selenite-induced cataract, as demonstrated by Javadzadeh *et al.* (2009). Lens GSH, SOD and GPX levels were higher in the onion-injected group than in the selenite-induced group, highlighting the additional support of bioactive compounds to the antioxidant agents. Curcumin significantly decreased the oxidative stress, responsible of cataract formation, in selenite-induced rat pups. These effects indicate that the consumption of curcumin in food can help to prevent the onset of cataract also in humans (Manikandan *et al.* 2010).

During the entire life, the lens is exposed to biochemical, physiological, and functional changes, as result of the natural process of aging. Senile cataract becomes progressively more severe and frequent in over-50y people and represents the 48% of worldwide blindness. Protein damaging stress, fiber cell-membranes damage, deficit of glutathione, oxidative damage, calcium high level, abnormal lens epithelial cell migration, are several specific mechanisms responsible for senile cataract (Gupta *et al.* 2014). Surgery remains the only available treatment for cataract, and although all surgical procedures are effective for treatment, it is still under discussion the post-operative complications, the cost of surgery, and high number of people requiring surgery. However, the latter explained problems inspire researchers to find out alternative strategy for the treatment of cataract.

The lens has substantial supplies of antioxidant reserves, antioxidant enzymes and secondary defences, to prevent cataract formation. The production of radical species is encouraged when eyes are exposed to environmental stress, such as UV light, smoking and oxygen, which damage lens proteins. Superoxide and hydroxyl radicals cause damage to cell membrane lipids and proteins, which deposit on the surface of the lens causing opacities.

Dietary antioxidants play an important role in helping endogenous antioxidant system for the neutralization of oxidative stress. Their deficiency is one of the causes of numerous chronic and degenerative pathologies. When the oxygen reactive species are in low or moderate concentrations, they are necessary for the maturation process of cellular structures and may act as tool for the host defense system. Since antioxidants block the oxidation process that produces free radicals, several evidences indicate that nutritional intervention may offer a way to diminish the risk of cataract (Mittler *et al.* 2011; Angelo *et al.* 2015).

Recently, Theodoropoulou *et al* (2014), in a case-control study to assess the association between diet and risk of cataract in a Caucasian population, have found that cataract was positively associated with meat consumption and high intake of total fat, cholesterol and carbohydrates, whereas diet rich in fruits, vegetables, fish, pulses and starchy foods protect against cataract. On the other hand, Varma (2016) has ascertained that antioxidant nutrients are highly effective in inhibiting the formation of cataracts both in animals and in human epidemiological researches.

Laurus is a common component in European and North American dishes, such as soups, stews, meat, seafood and other vegetable dishes. Dried bay leaves have a pleasant odour, and its taste characteristically strong, pungent and aromatic greatly helps in digestion; in fact, it is used as a natural remedy in a wide range of digestive disorders. In high fat dishes its use as ingredient could reduce fat adsorption leading health benefits (Nurbas and Bal 2005).

In conclusion, the intake of bay leaves was associated with an improvement of blood and lenses antioxidant markers, highlighting a protective activity at the cellular level counteracting free radicals. These findings of ours suggest that the antioxidant activity of *Laurus nobilis* may exert a protective role on the risk of cataract development, secondary to a fat enriched diet.

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Conflict of interest

The authors declare that they do not have conflict of interests (political, personal, religious, ideological, academic, intellectual, commercial, or otherwise) regarding the publication of the paper.

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444

445 **Table 1.** Plasma oxidative status markers in rabbit. Results are expressed as mean values and pooled SEM (n=15).

Parameters	Time (d)	Diet†				SEM	p-value		
		N-CTR	P-CTR	EXP1	EXP2		Diet	Time	Diet x Time
ROMs (U/Carr)	0	225.1_{a}	221.3_{a}	221.4_{a}	220.8_{a}	2.80			
	56	227.4_{1b}	352.7_{2b}	280.1_{3b}	193.0_{4b}	13.99	0.001	0.001	0.001
TBARS (μmol/l)	0	2.90	2.80_{a}	2.71	2.91	0.05			
	56	3.07_{1}	3.50_{2b}	$2.831_{1,3}$	2.65_{3}	0.09	0.001	0.015	0.001
Retinol (μg/ml)	0	0.266	0.277_{a}	0.300_{a}	0.276_{a}	0.01			
	56	0.262_{1}	0.191_{1b}	0.330_{2b}	0.316_{2b}	0.01	0.001	0.048	0.005
Alfa-tocopherol (μg/ml)	0	3.11	2.88_{a}	3.07_{a}	3.05	0.03			
	56	2.99_{1}	2.01_{2b}	2.41_{2b}	3.13_{1}	0.10	0.041	0.001	0.003
Total phenol (mg TE/ml)	0	63.1	66.2_{a}	63.7	64.8_{a}	0.52			
	56	62.7_{1}	58.3_{1b}	62.8_{1}	75.1_{2b}	1.46	0.028	0.047	0.004
SOD (U/mg)	0	41.4	42.7_{a}	40.9	41.8_{a}	0.73			
	56	42.5_{1}	37.3_{1b}	42.3_{1}	61.3_{2b}	2.13	0.011	0.001	0.001
$ORAC_{pca}$ (μmol TE/l)	0	754.7	764.2_{a}	774.7	765.9	5.29			
	56	755.9_{1}	631.9_{2b}	788.4_{1}	787.1_{1}	15.07	0.001	0.001	0.011
FRAP (mmol/ml)	0	582.1	591.6_{a}	583.2	587.8	1.84			
	56	581.7_{1}	383.7_{2b}	622.5_{3}	566.6_{1}	20.25	0.001	0.001	0.022

446 †N-CTR:negative control group without fat integration; P-CTR: positive control group with pig-fat integration; EXP1:
447 experimental group with pig-fat and meal dried-bay leaves integration; EXP2: experimental group with meal dried-bay
448 leaves integration.

449 [1,2,3,4]Different numbers within the same row indicate significant differences ($p < 0.05$).

450 [a,b] Different letters within the same column indicate significant differences ($p < 0.05$).

451

452 **Table 2.** Lens oxidative status markers in rabbit. Results are expressed as mean values and pooled SEM (n=15).

Parameters	Diet[†]				SEM	
	N-CTR	P-CTR	EXP1	EXP2		Diet
TAC (μmolTE/g)	149.22_{1}	106.78_{2}	146.69_{1}	160.02_{3}	4.51	0.001
TBARS (μmol/g)	5.10_{1}	6.58_{4}	$5.64_{1,2}$	$4.87_{1,3}$	0.16	0.001
Retinol (μmol/mg)	37.93_{1}	22.79_{2}	23.53_{2}	37.34_{1}	1.68	0.001
Alfa-tocopherol (μmol/mg)	0.19_{1}	0.08_{2}	0.17_{1}	0.20_{1}	0.01	0.001

453 [†]N-CTR:negative control group without fat integration; P-CTR: positive control group with pig-fat integration;
454 EXP1: experimental group with pig-fat and meal dried-bay leaves integration; EXP2: experimental group with
455 meal dried-bay leaves integration.

456 [1,2,3,4]Different numbers within the same row indicate significant differences ($p < 0.05$).