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Flavonoids from artichoke (*Cynara scolymus L.*) upregulate eNOS gene expression in human endothelial cells

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Running title: eNOS upregulation by artichoke flavonoids

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- **8** Figures;
- **39** References;
- **244** words in *Abstract*;
- **413** words in *Introduction and*
- **798** words in *Discussion*.

ABBREVIATIONS:

ALE, artichoke leaf extract; ASF, aqueous sub-fraction from ALE; BH_4, (6R)-5,6,7,8-tetrahydro-L-biopterin; DMEM, Dulbecco's modified Eagle's medium; DRB, 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole; HUVEC, human umbilical vein endothelial cells; LDL, low-density lipoprotein; L-NAME, N^G-nitro-L-arginine methyl ester; NO, nitric oxide; NOS, NO synthase; eNOS, endothelial-type NOS; OSF, organic sub-fraction from ALE; SNAP, S-Nitroso-N-penicillamine

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ABSTRACT

Nitric oxide (NO) produced by endothelial NO synthase (eNOS) represents an antithrombotic and anti-atherosclerotic principle in the vasculature. Hence, an enhanced expression of eNOS in response to pharmacological interventions could provide protection against cardiovascular diseases. In EA.hy 926 cells, a cell line derived from human umbilical vein endothelial cells (HUVEC), an artichoke leaf extract (ALE) increased the activity of the human eNOS promoter (determined by luciferase reporter gene assay). An organic sub-fraction from ALE was more potent in this respect than the crude extract, whereas an aqueous sub-fraction of ALE was without effect. ALE and the organic sub-fraction thereof also increased eNOS mRNA expression (measured by RNase protection assay) and eNOS protein expression (determined by Western blot) both in EA.hy 926 cells and in native HUVEC. NO production (measured by NO-ozone chemiluminescence) was increased by both extracts. In organ chamber experiments, *ex vivo* incubation (18 hours) of rat aortic rings with the organic sub-fraction of ALE enhanced the NO-mediated vasodilator response to acetylcholine, indicating that the upregulated eNOS remained functional. Caffeoylquinic acids and flavonoids are two major groups of constituents of ALE. Interestingly, the flavonoids luteolin and cynaroside increased eNOS promoter activity and eNOS mRNA expression, whereas the caffeoylquinic acids cynarin and chlorogenic acid were without effect. Thus, in addition to the lipid-lowering and anti-oxidant properties of artichoke, an increase in eNOS gene transcription may also contribute to its beneficial cardiovascular profile. Artichoke flavonoids are likely to represent the active ingredients mediating eNOS upregulation.

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Nitric oxide (NO) produced by endothelial-type NO synthase (eNOS) plays a protective physiological role in the vasculature (Li and Förstermann, 2000a). NO is a potent vasodilator and contributes to blood pressure control. Blockade of NO synthesis with pharmacological NOS inhibitors causes significant peripheral vasoconstriction and elevation of blood pressure (Rees et al., 1989). Similarly, mice with a disrupted eNOS gene are hypertensive and lack endothelium-dependent, NO-mediated vasodilation (Huang et al., 1995).

Besides its vasodilator effects, NO also protects blood vessels from thrombosis by inhibiting platelet aggregation and adhesion. In addition, endothelial NO possesses multiple anti-atherosclerotic properties, which include (i) prevention of leukocyte adhesion to vascular endothelium and leukocyte migration into the vascular wall; (ii) decreased endothelial permeability, reduced influx of lipoproteins into the vascular wall and inhibition of low-density lipoprotein (LDL) oxidation; and (iii) inhibition of DNA synthesis, mitogenesis, and proliferation of vascular smooth muscle cells (Li and Förstermann, 2000a). In agreement with these protective effects of endothelial NO, pharmacological inhibition of eNOS caused accelerated atherosclerosis in rabbits (Cayatte et al., 1994). Based on these antihypertensive and anti-atherosclerotic effects, the enhancement of endothelial NO production could be of prophylactic or therapeutic interest.

Artichoke (*Cynara scolymus L.*) is one of the world's oldest medicinal plants. It has been known by the ancient Egyptians, and the ancient Greeks and Romans used it as a digestive aid. Clinical trials have shown antidyspeptic (Fintelmann, 1996; Marakis et al., 2002; Holtmann et al., 2003) and lipid-lowering effects (Fintelmann, 1996; Englisch et al., 2000) of artichoke leaf extract (ALE). Oral administration of ALE increased bile flow in rats (Saenz-Rodriguez et al., 2002). Such a choleretic action has also been documented in human (Kirchhoff et al., 1994). In primarily cultured rat hepatocytes (Gebhardt, 1998) as well as in HepG2 cells (Gebhardt, 2002), artichoke extracts inhibited cholesterol biosynthesis, likely due to an indirect inhibition of HMG-CoA reductase activity (Gebhardt, 1998). Interestingly, ALE also possesses antioxidant properties. It protected cultured rat hepatocytes against hydroperoxide-induced oxidative stress (Gebhardt, 1997). ALE also inhibited LDL oxidation

(Brown and Rice-Evans, 1998) and reduced the production of intracellular reactive oxygen species by oxidized LDL in cultured endothelial cells and monocytes (Zapolska-Downar et al., 2002).

Besides its lipid-lowering and antioxidant activities (Brown and Rice-Evans, 1998; Gebhardt, 1998; Zapolska-Downar et al., 2002), preliminary evidence from our laboratory suggested that artichoke may also stimulate vascular NO production. Therefore, the current study was designed to investigate the effect of artichoke on the vascular NO system and to identify the active constituents.

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Methods

Materials.

Extracts from artichoke leaves were provided by Lichtwer Pharma AG (Berlin, Germany). Three types of extracts were used (Table 1): ALE was the dried supernatant of an aqueous extraction of artichoke leaves (commercially produced as LI220, Suprasern®). An aqueous solution of ALE was further extracted with a mixture of ethylacetate and n-butanol (2:1, v/v) resulting in an organic and an aqueous phase. The two phases were separated and the solvents were evaporated. This yielded two extracts, the organic sub-fraction (OSF) and the aqueous sub-fraction (ASF), respectively (Table 1).

Cynarin (1,3-dicaffeoylquinic acid) and cynaroside (luteolin-7-O-glucopyranoside) were from AppliChem (Darmstadt, Germany), chlorogenic acid (5-O-caffeoylquinic acid) was from Cayman (Ann Arbor, MI). Luteolin was from Sigma (Taufkirchen, Germany). S-Nitroso-N-penicillamine (SNAP) and 5,6-dichloro-1-β-D-ribofuranosylbenzimidazole (DRB) were from Merck (Darmstadt, Germany).

Cell culture.

Human umbilical vein endothelial cells (HUVEC) were isolated by collagenase digestion. HUVEC were cultured in endothelial cell growth medium (PromoCell, Heidelberg, Germany). HUVECs from passages 3 to 5 were used in the experiments. HUVEC-derived EA.hy 926 endothelial cells were kindly provided by Dr. Cora-Jean Edgell (Chapel Hill, NC). EA.hy 926 endothelial cells were grown under 10% CO_2 in Dulbecco's modified Eagle's medium (DMEM, Sigma) supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin, 100 µg/ml streptomycin, and 1x HAT (hypoxanthine, amethopterin/methotrexate and thymin) (Invitrogen, Karlsruhe, Germany) (Li and Förstermann, 2000b).

Analysis of eNOS promoter activity by stable transfection of EA.hy 926 cells.

A stable EA.hy 926 cell line was generated by transfection of EA.hy 926 cells with pGL_3-eNOS-Hu-3500-neo, which contains a neomycin-resistance gene and a 3.5 kb promoter

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fragment of human eNOS driving the luciferase reporter gene (Li et al., 1998). Stable EA.hy 926 cells were cultured in medium containing 1 mg/ml compound G418. For analysis of eNOS promoter activity, the stably transfected cells were incubated with artichoke extracts for 18 hours. Then, cells lysates were prepared and luciferase activities were determined as described (Li et al., 1998). The luciferase activity, normalized for protein concentration of cell lysates, was used as a determinant of eNOS promoter activity.

RNase protection assay for eNOS mRNA analyses.

Confluent HUVEC and EA.hy 926 cells were incubated with artichoke extracts for 18 hours and total RNA was isolated. The expression of eNOS mRNA was analyzed by RNase protection assay as described previously (Li et al., 1998; Li and Förstermann, 2000b).

Real-Time RT-PCR for eNOS mRNA analyses.

In some experiments, eNOS mRNA expression was analyzed with quantitative Real-Time RT-PCR using an iCycler™ iQ System (Bio-Rad Laboratories, Munich, Germany). Confluent HUVEC and EA.hy 926 cells were incubated with cynarin, chlorogenic acid, luteolin or cynaroside for 6 hours and total RNA was isolated. 0.5 µg of total RNA was used for Real-Time RT-PCR analysis with the QuantiTect™ Probe RT-PCR kit (Qiagen, Hilden, Germany). Sequences of used primers were GTGGCTGTCTGCATGGACCT (forward) and CCACGATGGTGACTTTGGCT (reverse). The sequence of the dual-labeled TaqMan probe was AGTGGAAATCAACGTGGCCGTGCTGC.

Western blot for eNOS protein analyses.

Confluent EA.hy 926 cells were incubated with artichoke extracts for 18 hours and total protein was isolated. Western blotting was performed using 50 µg of protein and a monoclonal anti-eNOS antibody (BD Biosciences Pharmingen, San Diego, CA), as described previously (Li and Förstermann, 2000b). Immunocomplexes were developed using an enhanced horseradish peroxidase/luminol chemiluminescence reagent (PerkinElmer Life Sciences, Boston, MA) according to the manufacturer's instructions.

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Determination of NO synthesis.

EA.hy 926 cells were treated with artichoke extracts for 18 hours and then stimulated with 10 µM calcium ionophore A23187 for 1 hour. The supernatants were collected and the oxidation products of NO, nitrite and nitrate, were assayed as a measure of NO synthesis. After reduction of nitrate with nitrate reductase, total nitrite was determined by NO-ozone chemiluminescence using a NOA^{TM} 280 NO Analyzer (Sievers, Boulder, CO). Total protein content of the cells was determined (Bradford), and nitrite levels were normalized for protein (Li et al., 2002a; Li et al., 2003).

Organ chamber experiment using rat aorta.

Aortas were isolated from male Sprague-Dawley rats (250 - 300g) and cut into rings of 3 mm width. The rings were washed twice with penicillin/streptomycin (100 U/ml, 100 µg/ml)-containing PBS and then maintained in cell culture incubators in DMEM (supplemented with 10% fetal bovine serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 100 U/ml penicillin and 100 µg/ml streptomycin) for 18 hours, with or without cynara OSF (100 µg/ml), or, in other experiments, for 8 hours with or without 30 µM cynaroside. After the *ex vivo* incubation, rings were mounted into organ chambers and isometric tension was measured. Concentration-response curves to norepinephrine (1 nM to 1 µM) were generated (a contraction induced by 80 mM KCl was set 100%). After washout, the rings was contracted again using 100 nM norepinephrine and relaxation concentration-response curves were generated with acetylcholine in the absence or present of the NOS inhibitor N^{G}-nitro-L-arginine methyl ester (L-NAME, 1 mM). Vasodilator response to the NO donor SNAP was achieved after precontraction with 100 nM norepinephrine in the presence of L-NAME.

Statistics

Statistical differences between mean values were determined by analysis of variance (ANOVA) followed by Fisher's protected least-significant-difference test for comparison of different means.

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Results

eNOS promoter activity in human EA.hy 926 endothelial cells.

An 18 hour ALE treatment of EA.hy 926 cells stably transfected with a 3.5 kb human eNOS promoter fragment resulted in a concentration-dependent increase in eNOS promoter activity (Fig. 1). The maximal increase was seen with 100 µg/ml ALE. The cynara OSF was more potent than ALE. The ASF, however, was without any effect.

eNOS mRNA expression in human endothelial cells.

Treatment of HUVEC and EA.hy 926 cells for 18 hours with 100 µg/ml ALE or OSF resulted in a significant increase in eNOS mRNA expression, as analyzed with RNase protection assay (Fig. 2).

eNOS protein expression in EA.hy 926 cells.

As analyzed with Western blot, both ALE and OSF increased eNOS protein in EA.hy 926 cells after an 18-hour treatment (Fig. 3). Densitometric analyses of the three blots demonstrated an average increase to 185% of control after 100 µg/ml ALE and to 197% of control after 100 µg/ml OSF.

NO production in EA.hy 926 cells.

Both ALE and OSF increased NO synthesis in EA.hy 926 cells after an 18-hour treatment, as determined by the NO-ozone chemiluminescence assay (Fig. 4). OSF was more efficacious than ALE in stimulating endothelial NO production.

In contrast to the long-term effects on eNOS expression (and thus activity), ALE and OSF had no *acute* effect on eNOS activity. Incubation of EA.hy 926 cells for up to 30 min with ALE or OSF (up to 100 µg/ml), or with the flavonoid cynaroside (up to 30 µM) did not increased NO production.

Effects of artichoke flavonoids and caffeoylquinic acids.

ALE is known to contain large amounts of polyphenolic compounds, with caffeoylquinic acids and flavonoids being major constituents. We therefore tested 4

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commercially available compounds known to be present in ALE: two caffeoylquinic acids (cynarin and chlorogenic acid) and two flavonoids (luteolin and cynaroside). As shown in Fig. 5, luteolin and cynaroside, but not cynarin or chlorogenic acid, increased eNOS promoter activity in a concentration-dependent manner. In parallel, the two artichoke flavonoids also increased eNOS mRNA expression both in HUVEC and EA.hy 926 cells (Fig. 6). Cynarin and chlorogenic acid did not increase eNOS mRNA expression (three experiments).

eNOS mRNA stability.

In order to study eNOS mRNA stability, EA.hy 926 cells were treated either with 100 µg/ml OSF for 18 hours, or with 30 µM cynaroside for 8 hours. After the pretreatment, transcription was stopped by adding 60 µM DRB, an inhibitor of RNA polymerase II transcription (Cai et al., 2001), to the culture medium. eNOS mRNA levels were determined with quantitative Real-Time RT-PCR at 0, 6, 12 and 24 hours thereafter. As shown in Fig. 7, OSF and cynaroside had no significant effect on eNOS mRNA stability.

Vasomotor responses of rat aorta *ex vivo*.

In order to investigate functional consequences of an eNOS upregulation, we studied the effects of OSF and cynaroside on vasomotion. In organ chamber experiments, rat aortic rings pretreated with OSF for 18 hours showed a decreased vasoconstriction in response to norepinephrine (Fig. 8A). The NOS inhibitor L-NAME (1 mM) did not change basal vascular tone. However, the norepinephrine-induced vasoconstriction was significantly enhanced by L–NAME, both in control- and OSF-pretreated rings, although the OSF-exposed rings did not reach the same level of contraction as control vessels (Fig. 8A).

The vasodilator response to acetylcholine was significantly enhanced by OSF-pretreatment (Fig. 8B). No significant relaxation was observed in the presence of the NOS inhibitor L-NAME (Fig. 8B). The vasodilator response to the NO donor SNAP was not changed by OSF (Fig. 8C).

Pretreatment of aortic rings with the flavonoid cynaroside had similar effect on vasomotion as OSF. Cynaroside-pretreated rings showed decreased constriction response to

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norepinephrine (similar to Fig. 8A, three experiments). Also the vasodilator response to acetylcholine was enhanced (similar to Fig 8B, three experiments).

Additional experiments using aortic rings without OSF/cynaroside-pretreatment showed that OSF and cynaroside has no *acute* effects on vasomotion. In the absence of acetylcholine, no relaxation to OSF (10 - 300 µg/ml) or cynaroside (1 – 100 µM) was observed within 10 minutes in rings precontracted with 100 nM norepinephrine (three experiments).

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Discussion

In the present study, we investigated the effects of artichoke on the synthesis of endothelial NO. Long-term incubation with a crude extract from artichoke leaves or its organic sub-fraction increased eNOS promoter activity, eNOS mRNA expression, eNOS protein expression and NO production in cultured human vascular endothelial cells. Furthermore, long-term e*x vivo* incubation of aortic rings with the organic sub-fraction enhanced the endothelium-dependent, NO-mediated vasodilator response to acetylcholine.

NO synthesis can be modulated by eNOS activity and/or eNOS gene expression (Li et al., 2002b; Li et al., 2002c). As short-term incubation with the artichoke extract had no effect on NO production in EA.hy 926 cells and did not change vascular tone of aortic rings, the effects of artichoke on endothelial NO synthesis seem to results mainly or exclusively from upregulation of eNOS gene expression. Due to the antithrombotic, anti-atherosclerotic and antihypertensive properties of endothelial NO, the eNOS enzyme could be an interesting target for the prevention or therapy of cardiovascular diseases. *In vivo* upregulation of eNOS gene expression seems to be a reasonable and realistic strategy.

There is precedent for this strategy in the form of the 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors (statins). Statins possess vasoprotective properties independent of their cholesterol lowering effect, which include the upregulation of eNOS expression in platelets and endothelial cells. This eNOS upregulation was found to be associated with a decrease in platelet activation (Laufs et al., 2000b), an improvement of endothelial function in hypercholesterolemia (Wilson et al., 2001), an augmentation of cerebral blood flow (Endres et al., 1998; Laufs et al., 2000a), and a protection from stroke (Endres et al., 1998; Laufs et al., 2000a; Amin-Hanjani et al., 2001). Upregulation of eNOS gene expression seems to be the predominant if not the only mechanism, because the protective effects are absent in eNOS-deficient mice.

However, one recent study has questioned the beneficial effects of eNOS upregulation *in vivo* (Ozaki et al., 2002). In this study, a transgenic mouse strain (eNOS-Tg) was interbred with atherogenic apoE-deficient (apoE-KO) mice resulting in apoE-KO/eNOS-Tg mice. These mice expressed about 11-fold more eNOS than the wild-type mice, and, unexpectedly,

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developed larger atherosclerotic lesions than apoE-KO mice (Ozaki et al., 2002). As discussed by the authors themselves, "uncoupling" of eNOS seems to occur under these circumstances. It is established that under certain pathological conditions, eNOS can generate superoxide rather than NO by dissociation of the ferrous-dioxygen complex (Vasquez-Vivar et al., 1998; Xia et al., 1998). Superoxide produced by the uncoupled eNOS may enhance the preexisting oxidative stress. The molecular mechanisms underlying eNOS uncoupling have not been completely understood. A relative lack of (6*R*)-5,6,7,8-tetrahydro-L-biopterin (BH_4), an eNOS cofactor, seems to play a crucial role in many cases (Stuehr et al., 2001; Vasquez-Vivar et al., 2003).

In contrast to the extreme overexpression of eNOS mentioned above, the eNOS upregulation by pharmacological compounds like statins is usually moderate (< 3-fold). At these levels, the upregulated eNOS seems to remain functional. In addition, a novel compound from Aventis (Cpd2431), which also moderately upregulates eNOS expression, has been shown to reduce experimental atherosclerosis in apoE-KO mice (Wohlfart et al., 2002). In the present study, artichoke extracts increased eNOS gene expression to a similar extent. Results from the organ bath experiments indicate that the upregulated eNOS remained functional.

The active constituents responsible for this eNOS-upregulating action of artichoke are present in the organic sub-fraction OSF. The OSF is rich in polyphenolic compounds, with caffeoylquinic acids and flavonoids as the major chemical components. Examples are cynarin and chlorogenic acid for caffeic acid derivatives, and luteolinas well as apigenin for flavonoids (Rechner et al., 2001; Llorach et al., 2002; Wang et al., 2003). The aqueous sub-fraction contains sesquiterpenes (cynaropicrin, aguerin B, and grosheimin) and sesquiterpene glycosides (cynarascolosides A, B, and C) (Shimoda et al., 2003).

Polyphenolic compounds, such as resveratrol (present in red wine and grapes), can increase eNOS gene expression, as recently reported by our group (Wallerath et al., 2002; Wallerath et al., 2003).

The artichoke extract OSF enriched in flavonoids and caffeoylquinic acids was more efficacious in stimulating eNOS expression than the crude extract. The ASF, in contrast,

contains only small amounts of these compounds and was without effect on eNOS expression (Table 1). Results from our experiments with cynarin, chlorogenic acid, luteolin and cynaroside suggest that artichoke flavonoids represent one group of the compounds that are responsible for the eNOS upregulation caused by cynara extracts.

In conclusion, the present study demonstrates that artichoke leaf extracts increase eNOS gene expression and NO production in cultured human vascular endothelial cells. Artichoke leaf extract also enhances endothelium-dependent vasodilation in rat aorta. This novel action of the plant seems to be mediated, at least in part, by artichoke flavonoids. Based on the beneficial effects of endothelial NO, vasoprotective and anti-atherosclerotic effects are likely to ensue also *in vivo*.

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Footnotes:

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Figure legends

Fig. 1. Effects of artichoke extracts on human eNOS promoter activity. Human EA.hy 926 endothelial cells were stably transfected with a 3.5 kb human eNOS promoter fragment driving a luciferase reporter gene. Artichoke leaf extract (ALE) is the crude aqueous extract from artichoke leaves; OSF and ASF are the organic and aqueous sub-fractions of ALE, respectively. The EA.hy 926 cells were treated with artichoke extracts for 18 hours and then luciferase activity was analyzed as a determinant of eNOS promoter activity. Symbols represent mean ± SEM of three experiments (**P<0.01, *** P<0.001 vs. untreated cells).

Fig. 2. Artichoke extracts increase eNOS mRNA expression in human endothelial cells. Human umbilical vein endothelial cells (HUVEC) and EA.hy 926 cells were treated for 18 hours with artichoke leaf extract (ALE, 100 µg/ml) or the organic sub-fraction (OSF, 100 µg/ml) thereof. Then, eNOS mRNA expression was analyzed with RNase protection assays. Panel **A** demonstrates an original gel of an RNase protection assay (performed in triplicate). The gel shows the protected bands for eNOS (top) and for ß-actin (bottom; used for normalization). Panels **B** and **C** illustrate the results of densitometric analyses of three different gels; columns represent mean ± SEM of the three experiments (**p < 0.01; ***p < 0.001 compared with control).

Fig. 3. Artichoke extracts enhance eNOS protein expression. EA.hy 926 cells were treated for 18 hours with artichoke leaf extract (ALE, 100 µg/ml) or the organic sub-fraction (OSF, 100 µg/ml) thereof and eNOS protein expression was analyzed with Western blot using a monoclonal anti-eNOS antibody. The blot shown is representative of three independent experiments with similar results.

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Fig. 4. Artichoke extracts augment endothelial NO production. EA.hy 926 cells were treated with artichoke extracts for 18 hours and then stimulated with 10 μM calcium ionophore A23187 for 1 hour. The supernatant was collected and the oxidation products of NO, nitrite and nitrate, were assayed NO-ozone chemiluminescence using a NO Analyzer. Symbols represent mean ± SEM of three experiments (*$p < 0.05$, ***$p < 0.01$).

Fig. 5. Effects of artichoke polyphenolic compounds on human eNOS promoter activity. Human EA.hy 926 endothelial cells were stably transfected with a 3.5 kb human eNOS promoter fragment driving a luciferase reporter gene. Cells were treated with caffeoylquinic acids (cynarin and chlorogenic acid) or flavonoids (luteolin and cynaroside) and then luciferase activity was analyzed as a determinant of eNOS promoter activity. Symbols represent mean ± SEM of three experiments (*P<0.05, *** P<0.001 vs. untreated cells).

Fig. 6. Artichoke flavonoids increase eNOS mRNA expression in human endothelial cells. Human umbilical vein endothelial cells (HUVEC, panel **A**) and EA.hy 926 cells (panel **B**) were treated with artichoke flavonoids (luteolin and cynaroside) and eNOS mRNA expression was analyzed with Real-Time RT-PCR. Columns represent mean ± SEM of three experiments (*P<0.05, **$p < 0.01$ compared with control).

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Fig. 7. Artichoke extract and flavonoid had no effect on eNOS mRNA stability. Human EA.hy 926 endothelial cells were pretreated with vehicle (Co), OSF (100 µg/ml) or cynaroside (30 µM) and then gene transcription was terminated by DRB (60 µM). eNOS mRNA was analyzed with Real-Time RT-PCR at indicated time points after adding DRB. eNOS levels at time 0 hour of all three groups were set 100%. Symbols represent mean ± SEM of three experiments.

Fig. 8. Effects of artichoke extract on vasomotion of rat aorta. Isolated rat aortic rings were incubated *ex vivo* with control (Co) or OSF (100 µg/ml) for 18 hours and then mounted into organ chambers. Constriction response was achieved with norepinephrine (NE, panel **A**). Vasodilator response to acetylcholine or to the NO donor S-Nitroso-N-penicillamine (SNAP) was carried out after precontraction with 100 nM norepinephrine, in the absence or presence of the NOS inhibitor L-NAME (1 mM) (panels **B** and **C**). Symbols represent mean ± SEM of three to four experiments (*$p < 0.05$, vs. Co; #$p < 0.05$, vs. without L-NAME).

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Table 1. Artichoke extracts used.

Extract	Description of extraction	Drug:extract ratio	Caffeoylquinic acids content (%, w/w)	Flavonoids content (%, w/w)
ALE	Aqueous extract of artichoke leaves	4-6:1	10.10	2.17
OSF	Organic sub-fraction of ALE	20-30:1	26.30	13.29
ASF	Aqueous sub-fraction of ALE	5-7.5:1	6.30	0.76

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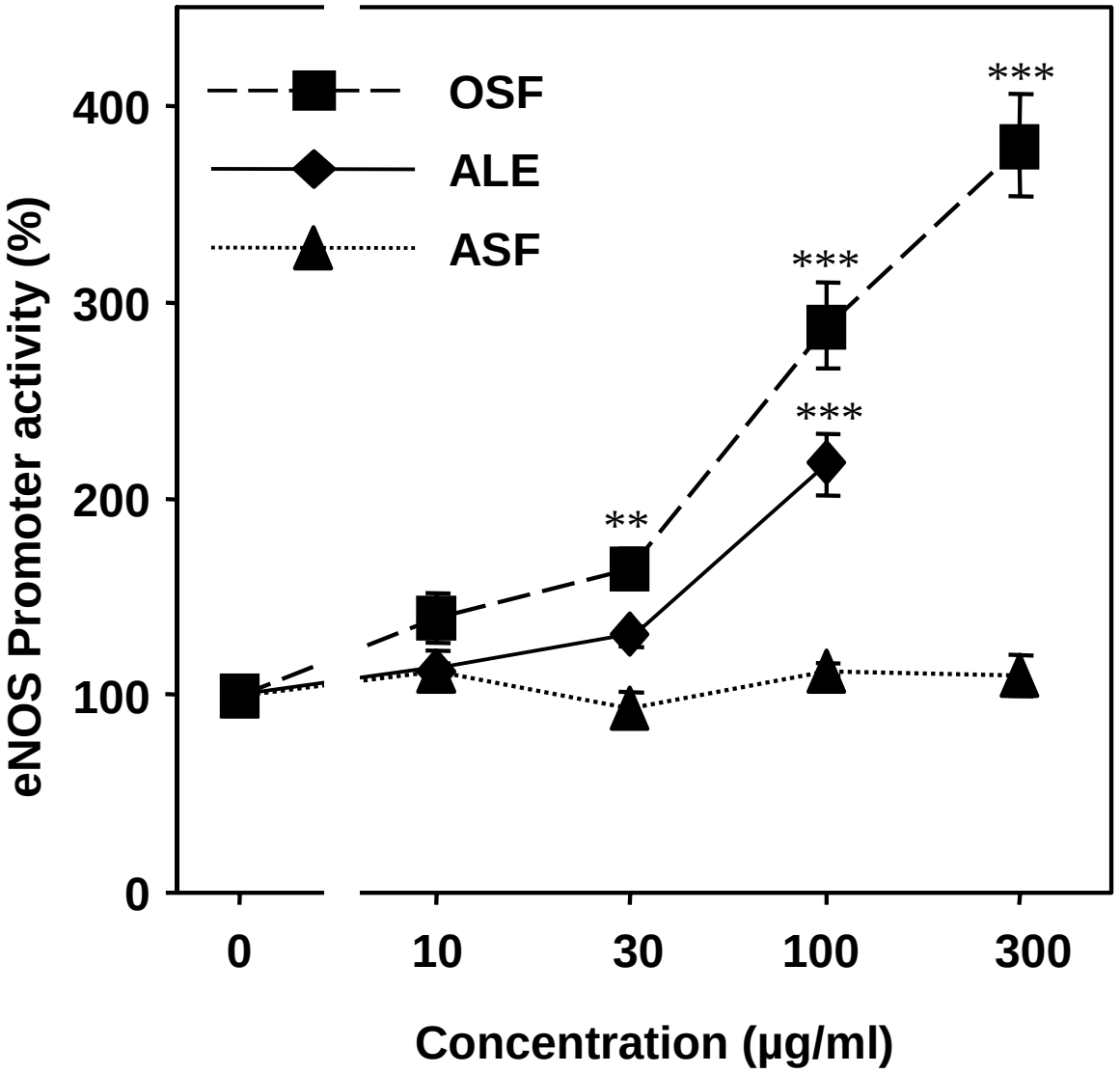
Li et al. Fig. 1



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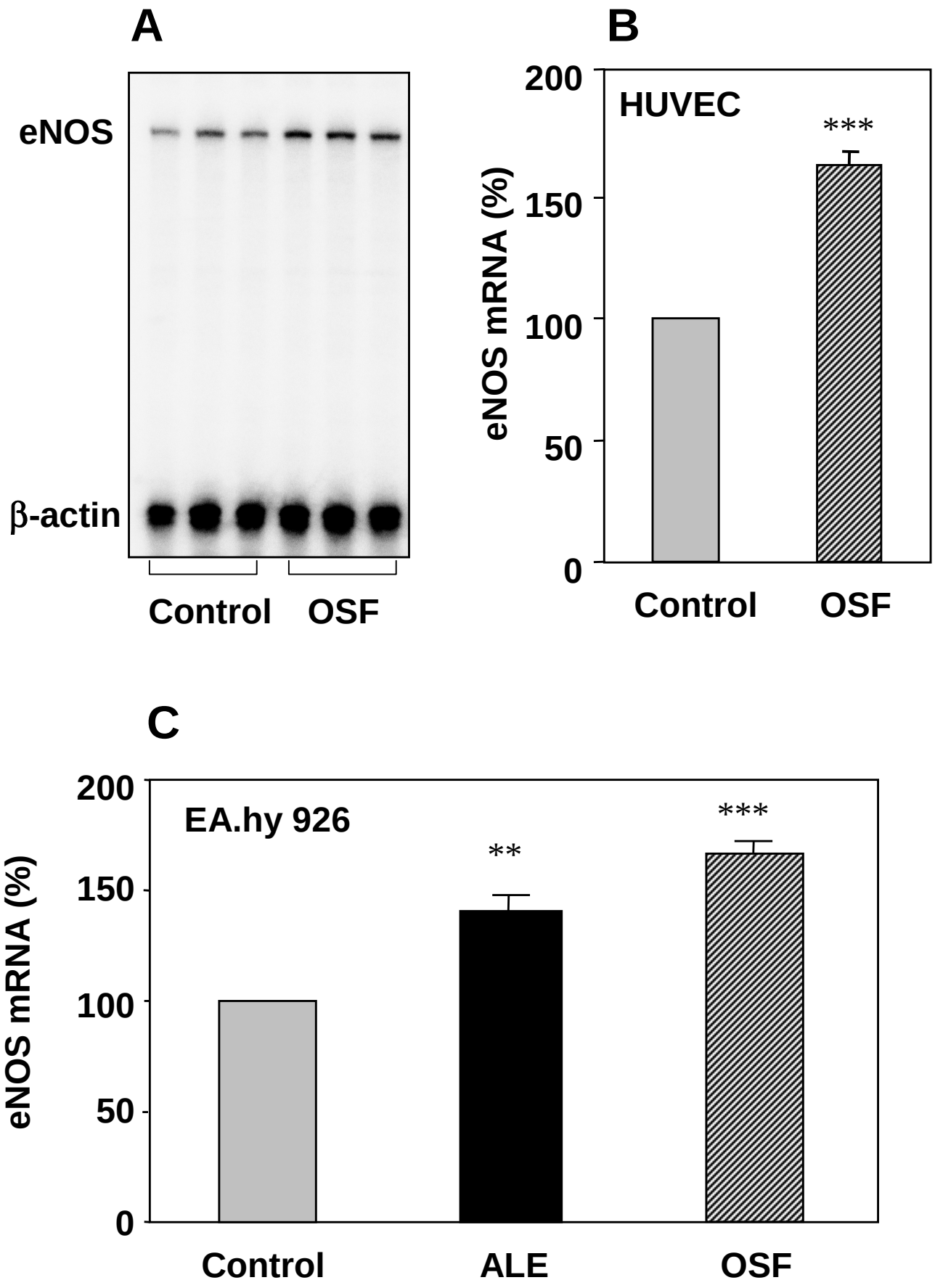
Li et al. Fig. 2



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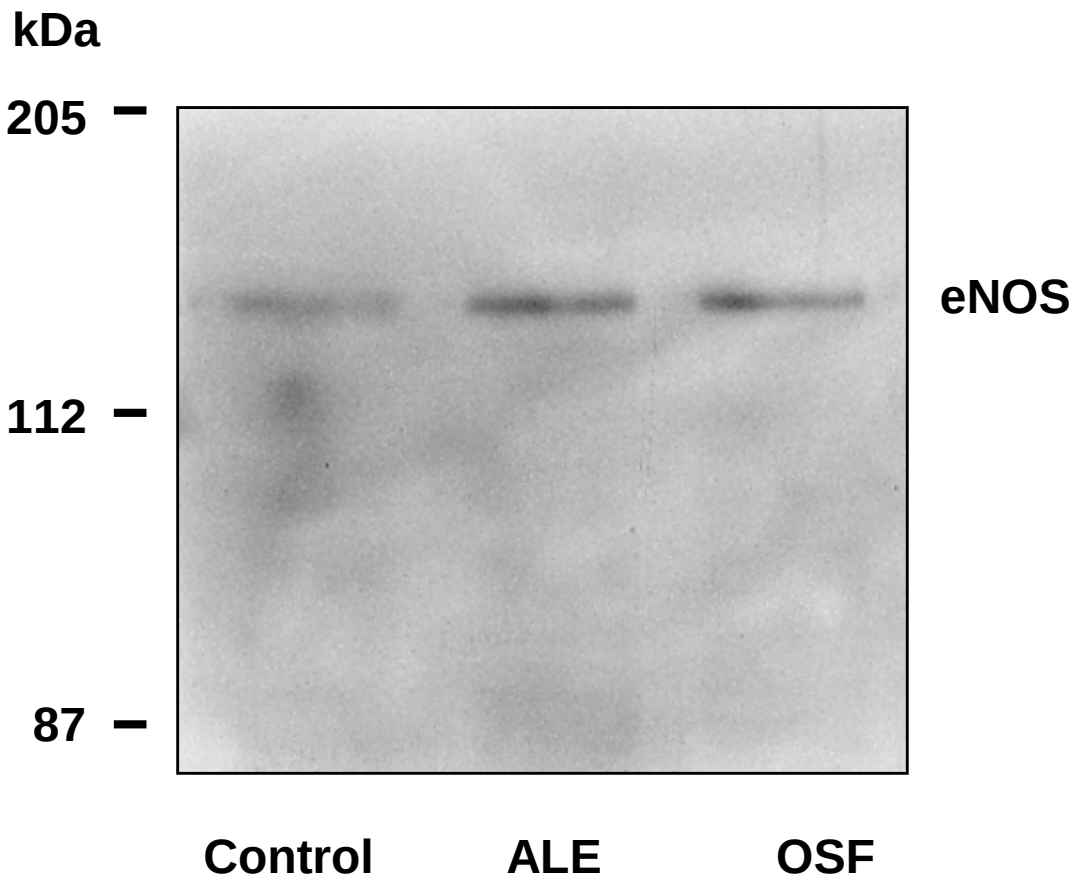
Li et al. Fig. 3



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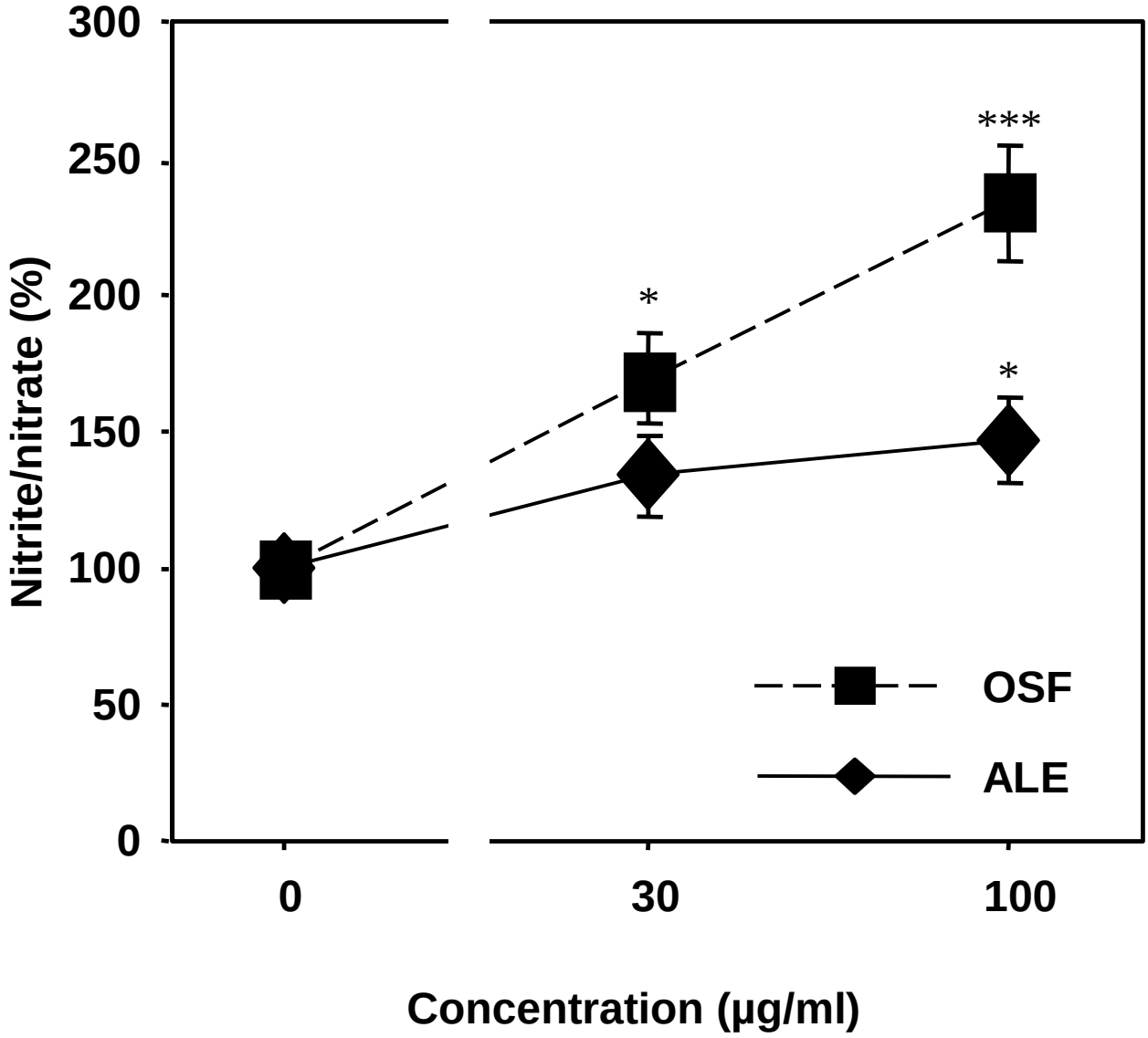
Li et al. Fig. 4



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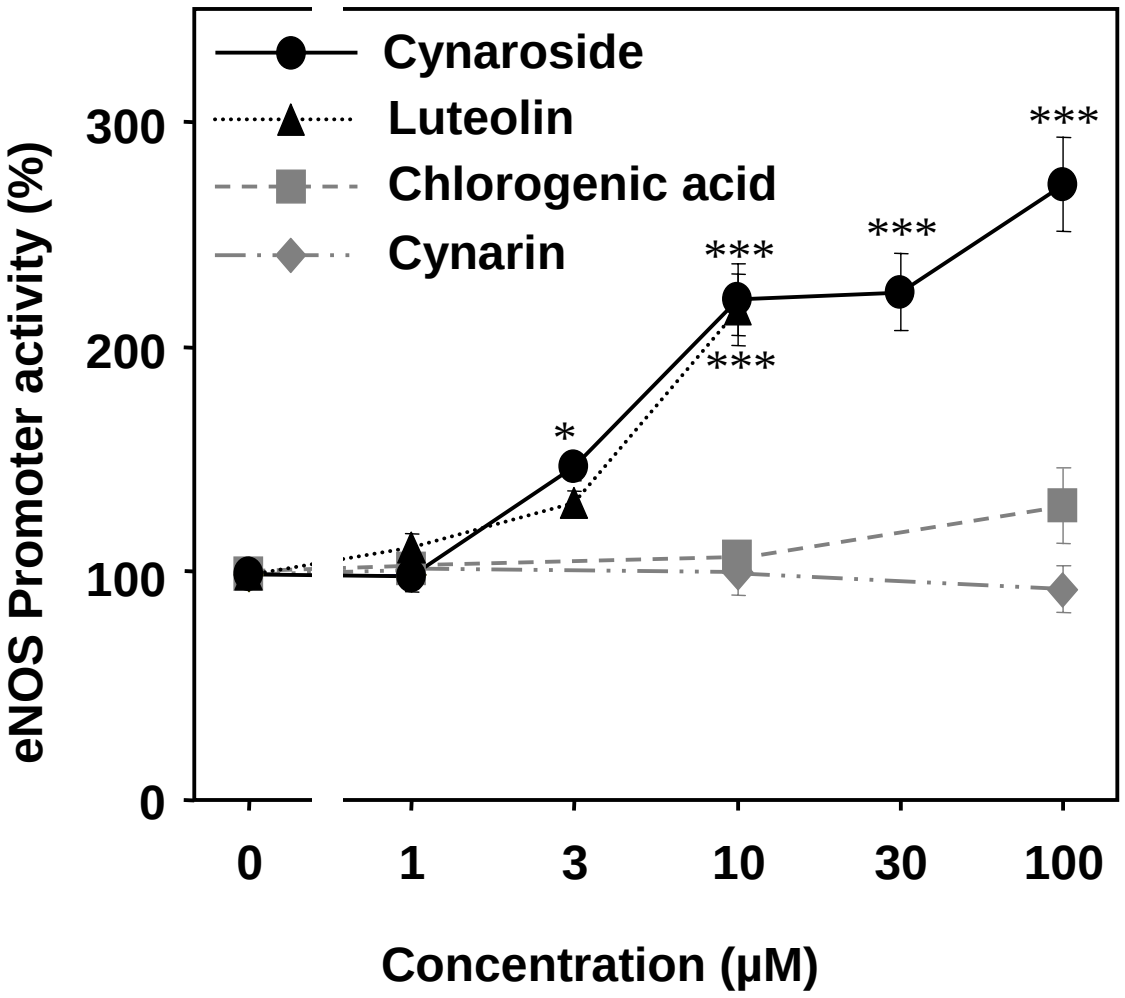
Li et al. Fig. 5



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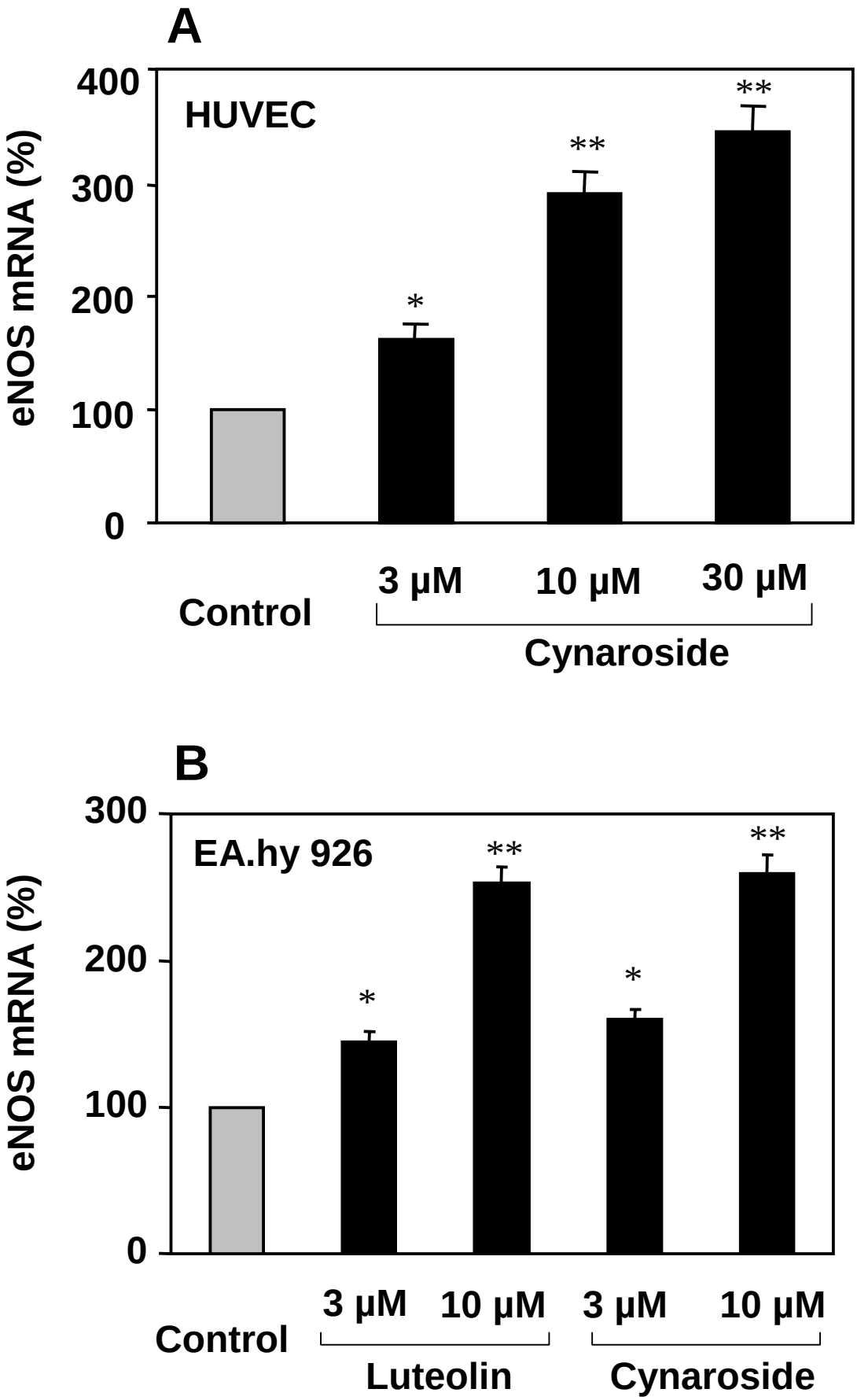
Li et al. Fig. 6



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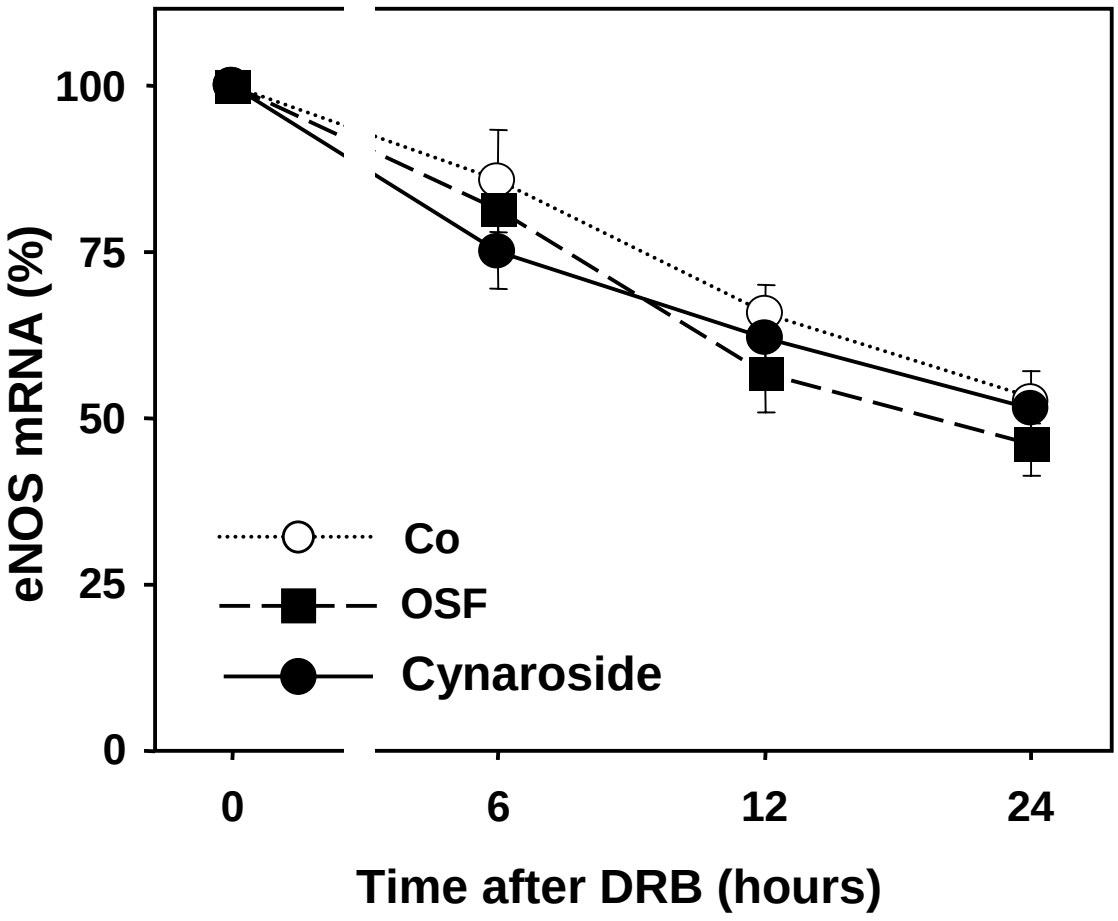
Li et al. Fig. 7



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Li et al. Fig. 8