Effects of gold nanoparticles phytoreduced with *Cornus mas* L. extract on the aorta wall function in rats with hyperlipid diet – a study on isolated aortic rings

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Abstract

Background. Vascular reactivity may be influenced by the dysfunction of the perivascular adipose tissue (PVAT) that occurs after a prolonged high fat diet (HFD).

Aim. The aim of this study was to investigate the vascular responses in rats with prolonged HFD after the administration of *Cornus mas* L. extract as a simple solution or as a reducing agent for gold nanoparticles (AuNPs).

Methods. Sprague-Dawley adult female rats (21 animals) were randomly allocated into three groups (n=7) and received for 9 months hyperlipid diet, the last month with treatment administered through oral gavage, 0.5 mL/day of solution as follows: HFD group - 0.9% saline solution, HFD+CM group - *Cornus mas* L. extract (0.158 mg/mL polyphenols), HFD+AuNPsCM group - gold nanoparticles phytoreduced with *Cornus mas* L. extract (AuNPsCM, 260 µg Au/kg/day). The Control group of rats (n=7) was fed with standard diet and in the last month received 0.9% saline solution as treatment. At the end of the experiment, the rats' descending aortas were collected and were used to investigate the aorta wall responses to vasoconstrictor (phenylephrine) and vasodilator (acetylcholine) substances added in tissue bath.

Results. AuNPsCM administration, compared to Control and HFD groups, increased the contraction and reduced the relaxation in aorta rings of rats with prolonged high-fat diet. The simple solution of *Cornus mas* L. extract produced contractile responses similar to those recorded in the Control group, at lower levels than in HFD group, and relaxation responses significantly decreased in comparison with Control group and significant increased when compared to HFD group.

Conclusions. *Cornus mas* L. extract administered as simple solution improved the aorta functions, while AuNPsCM solution enhanced the existed aorta wall modifications occurred after prolonged HFD, altering the vessel wall responses.

Keywords: aorta, hyperlipid diet, *Cornus mas*, tissue bath

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Background and aims

Vascular reactivity represents the response of the blood vessels to stimuli, a response that may be physiological or pathological and which is realized through vasodilation or vasoconstriction. The vascular reactivity impairment is associated with cardiovascular diseases and represents an important morbidity and mortality factor [1], sometimes being a precocious sign of a pathological situation. Endothelial cells, smooth muscle layer, fibroblasts and inflammatory cells intervene in the vascular response [2]. Endothelium responds to stimuli through platelets function regulation, inflammation, effects on smooth muscle layer or through vessel tone modulation. Tunica media is controlled by the autonomic nervous system; but the endogenous substances (hormones, peptides, reactive oxygen species, etc.) can also modulate the smooth muscle contractility, a function involved in vessel tone maintenance. Nitric oxide

(NO) has important role in cardiovascular system producing vasodilation, inhibition of platelet aggregation, blood flow control and proliferation of the smooth muscle cells. NO is synthesized under the action of nitric oxide synthase (NOS) that is found as three types: neuronal -nNOS or NOS1; inducible-inflammation-related, iNOS or NOS2; endothelial -eNOS or NOS3 which is the major regulator of vascular function [3]. Acetylcholine may activate eNOS that will increase the nitric oxide production [4], NO being involved also in vascular tone maintenance [5].

Prolonged high-fat diet (HFD) may lead to obesity, a disorder that influences the perivascular adipose tissue (PVAT) functionality, producing vascular abnormal responses to different stimuli [6].

Cornus mas is part of over 60 species of Cornaceae family and contains vitamin C, monoterpenes, organic acids (citric acid, malic acid, oxalic acid, etc.), flavonols (quercetin, hyperoside, rutin, etc.), cinnamic acids (caffeic acid, chlorogenic acid, ferulic acid) [7]. Beneficial effects of *Cornus mas* were demonstrated in different studies that showed its antioxidant, anti-inflammatory, hypolipemiant, anti-hyperglycaemic, hepatoprotective, antimicrobial and antitumor effects [8].

The advance of nanotechnology and its applications in medicine require numerous in-depth studies on the effects of different administered nanoparticles on physiological and pathological mechanisms. The gold nanoparticles are considered appropriate for medical usage as delivery systems for natural and synthetic medication to the target organs [9] but several studies demonstrated their noxious effects on different tissues [10-12].

The authors have previously tested the oxidative stress and the aorta ultra-structure in rats with prolonged hyperlipid diet (9 months), treated in the last month of the experiment with gold nanoparticles phytoreduced with *Cornus mas* L. extract or with simple solution of *Cornus mas* L. extract [13], the obtained results fostering new studies for a better understanding of the mechanisms that are involved in the aorta function.

The aim of this study was to evaluate, inside the organ bath, the aorta wall reactivity of these rats.

Methods

Since the chemicals and the techniques were explained in detail in a previous study in rats with prolonged hyperlipid diet [13], the methods related to the *Cornus mas* L. extract and nanoparticles synthesis are only briefly described.

Preparation of *Cornus mas* L. extract

To prepare the fruit extract, Cornelian cherries were bought in August 2021 from the Central Market of Cluj-Napoca, and the necessary chemicals from Merck (Darmstadt, Germany). The crushed fruits of *Cornus mas* were mixed with acetone, then they were vacuum-filtrated and, using a rotary evaporator, the extract was obtained without acetone. This concentrated fruit extract was used for gold nanoparticles phytoreduction. The phenolic concentration of the *Cornus mas* L. extract was expressed in grams of gallic acid equivalents per litre (GAE/L).

Synthesis of gold nanoparticles

Gold nanoparticles (AuNPs) were synthesized through a green method, using as a source the tetrachloroauric acid, and the obtained gold ions were reduced with the concentrated *Cornus mas* L. extract, leading to the colloidal solution. For AuNPs characterization, the following methods were used: UV-Vis spectroscopy for surface plasmon resonance, transmission electron microscopy to determine their shape, size, morphology and microelectrophoresis for zeta potential.

Animals

Adult female rats, Sprague-Dawley breed, 3 months old with body weight 300 ± 10 g were purchased from Cantacuzino National Medico-Military Institute for Research and Development, Bucharest, Romania. They were kept in polysulfone cages, in standard environment (temperature 21 ± 3ºC, relative humidity 60 ± 5%) with ad libitum access to water but also to standard diet or to standardized lipid-rich food, depending on the experimental group. The study had the approval of ANSVSA and of the Ethics Committee of the Iuliu Hatieganu University of Medicine and Pharmacy (no. 158/11.03.2019; 464/19.12.2018) and respected Directive 86/609/EEC.

High-Fat Diet (HFD)

For the entire experiment, a standardized lipid-rich diet was administrated by gavage. The hyperlipid diet (identification no ROB0001) was bought from Cantacuzino National Medico-Military Institute for Research and Development, Bucharest, Romania and provided an additional 45% level of energy.

Experimental design

For the 9 months of experiment, four groups of rats were used: three with hyperlipid diet and one with standard diet. Twenty one rats were randomly allocated in three groups (n=7) that received for 8 months standardized hyperlipid diet. A control group of rats was used (n=7), fed with standard diet. During the 9th month of the experiment, the rats with high-fat diet (HFD) (600 ± 10 g) and those with standard diet were treated with 0.5 mL/day of different solutions, by oral gavage, as follows: Control group 0.9% saline solution; HFD group received 0.9% saline solution; HFD+CM group received *Cornus mas* L. extract (0.158 mg/mL polyphenols); HFD+AuNPsCM group was treated with gold nanoparticles phytoreduced with *Cornus mas* L. extract (AuNPsCM, 260 µg Au/kg/day). After 30 days of treatment, deep anesthesia was induced (ketamine 10%, 5 mg/100gbw; xylazine hydroxychloride 2%, 100 mg/100gbw) and the descending thoracic aortas were collected.

Tissue bath

Aorta wall function was investigated through the

method that is described in the following part.

To determine the aorta wall function, an electro-physiologic device was used to transform the mechanical action into an electric signal that was recorded as a graph of isometric contraction and relaxation. BIOPAC MP 150 was equipped with three modular tissue baths of 20 mL volume and with linear transducers DA 100C, Ugo Basile, Trappe, PA, USA.

Preparation of aortic tissue and measurement of isometric force

Fragments of thoracic descending aortas were taken and immersed in modified Krebs–Henseleit solution (KHS), at 4-5º C and sparged with mixture of gases (95% CO_2, 5% O_2). The modified KHS consisted of (in mM): 120.0 NaCl; 26.0 $NaHCO_3$; 4.9 KCl; 1.5 $CaCl_2$; 1.3 KH_2PO_4; 1.1 $MgSO_4$ and 11.9 Glucose. Each aorta fragment was denudated, segmented into three parts of 2.5-3 mm length and then they were placed inside the tissue bath that contained 20 mL of KHS at 37ºC.

Isolated aortic rings were used to record the vessels reactivity at cumulative doses of contractile and dilator substances [14]. The aorta rings were kept for 30 minutes at a resting tension of 1.6 - 1.8 g (the optimum tension achieved after several tests realized in healthy rats). The solution was replaced at every 30 minutes.

The viability of the smooth muscle wall of the thoracic descending aorta was evaluated after 30 minutes of resting pretension through the maximum contractile response obtained at KCl solution, 85 mM/L. The obtained maximum contraction was considered the standard maximum contraction.

The function of the aorta wall was studied using phenylephrine (PE) for contractile responses that must be 80-100% from KCl-induced contraction, and for dilator responses using acetylcholine (Ach) that must be above 10-15% from KCl-induced contraction.

Statistical data analysis

Contractile response to PE was expressed as dose-response curve and was calculated as percentage of the standard maximum KCl-induced contraction (logarithmic scale). The relaxation Ach-induced was evaluated at every concentration as the percentage of maximum PE-induced contraction (logarithmic scale). Two-way ANOVA followed by the Post-test Bonferroni was used to analyze the obtained data. The significance threshold was set at $p<0.05$.

Results

Contractile responses of rats' aorta rings to phenylephrine (PE)

Hyperlipid diet (HFD group), compared to standard diet (Control group) increased the aorta rings contractile responses, with significant values at $3x10^{-6}$ M ($p<0.001$) PE concentration.

The administration of AuNPsCM solution produced a significant increase of contractile responses at 10^{-7} M, $3x10^{-7}$ M of PE ($p<0.05$) compared to control group. AuNPsCM administration induced significant increases of aorta rings contraction at $3x10^{-8}$ M ($p<0.05$), 10^{-7} M ($p<0.01$) and at $3x10^{-6}$ M ($p<0.05$) of PE in comparison with *Cornus mas* L. simple solution (HFD+CM), but when compared to untreated HFD rats, at $3x10^{-6}$ of PE, the AuNPsCM administration decreased significantly ($p<0.001$) the aorta muscle contraction.

The administration of *Cornus mas* extract solution decreased significantly the aortic rings contraction at $3x10^{-6}$ M ($p<0.001$) PE concentration, compared to HFD group. Non-significant modifications were recorded in HFD+CM group compared to control group (Figure 1).

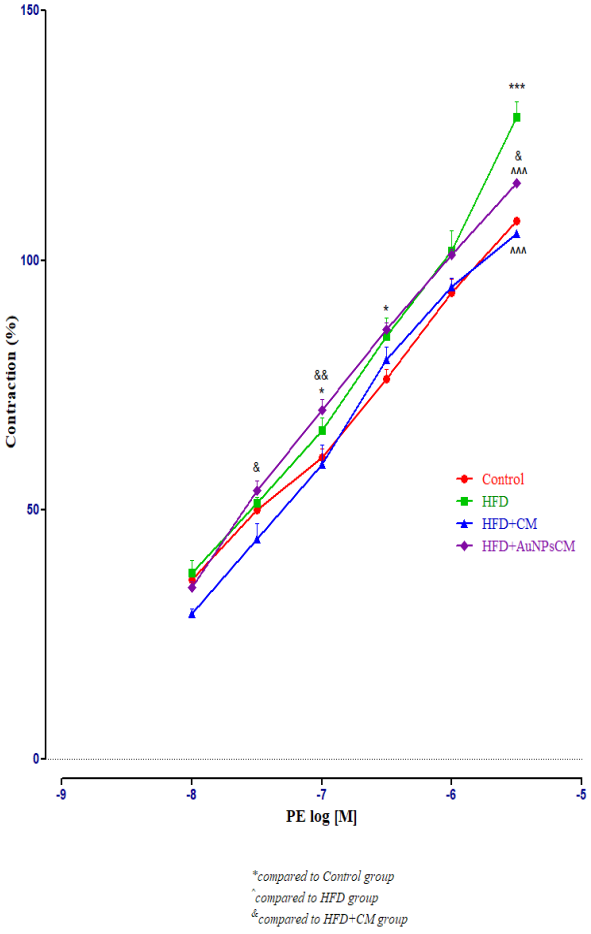

Figure 1. Contractile responses of the aorta rings to cumulative doses of phenylephrine (PE) (10^{-8} – $3x10^{-6}$ M) in rats with HFD treated with solution of *Cornus mas* L. extract (CM) or with gold nanoparticles phytoreduced with *Cornus mas* L. extract (AuNPsCM group), compared to rats with standard diet (Control group) or with rats with high-fat diet (HFD group). Contraction was expressed as % of maximum KCl contraction. Values are expressed as mean ± SEM.

Relaxation responses of rats' aorta rings to acetylcholine (Ach)

Prolonged hyperlipid diet (HFD group), compared to standard diet (Control group), reduced the aorta relaxation to acetylcholine, with significant modifications between $3x10^{-7}$-10^{-5} M ($p<0.001$) Ach concentration.

The aorta rings relaxation responses to acetylcholine showed in rats with HFD and AuNPsCM administration (HFD+AuNPsCM group) a significant increase in vascular tension at $3x10^{-8}$ M ($p<0.01$) and between 10^{-7}-10^{-5} M ($p<0.001$) compared to control group, and when compared to rats that received simple solution of *Cornus mas*, significant increased tension at $3x10^{-6}$ M ($p<0.05$) and at 10^{-5} M ($p<0.01$) was recorded. Non-significant modifications were recorded in HFD+AuNPsCM group compared to HFD group.

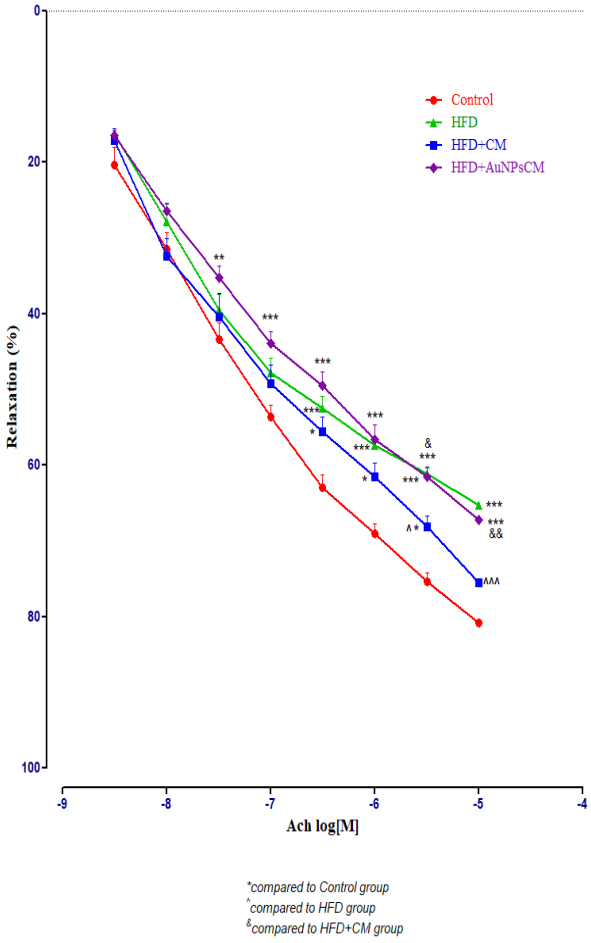

Figure 2. The relaxation responses of rats' aorta rings to cumulative concentrations of acetylcholine (Ach) ($3x10^{-9}$–10^{-5} M) in rats with HFD treated with solution of *Cornus mas* L. extract (CM) or with gold nanoparticles phytoreduced with *Cornus mas* L. extract (AuNPsCM group), compared to rats with standard diet (Control group) or with rats with high-fat diet (HFD group). The relaxation responses are expressed as percentages of maximum PE-induced contraction. Values are expressed as mean ± SEM.

The administration of *Cornus mas* extract solution (HFD+CM group) diminished the aorta wall relaxation between $3x10^{-7}$-$3x10^{-6}$ M ($p<0.05$) Ach concentration, compared to control group and, in comparison with rats with only HFD, the aorta wall relaxation was significantly improved at $3x10^{-6}$ M ($p<0.05$) and at 10^{-5} M ($p<0.001$) concentration of acetylcholine (Figure 2).

Discussion

Endothelium, a barrier between the blood and the other layers of the vessels, controls the vascular homeostasis through the diverse substances that it produces. The different cells of the vessel layers have a complex communication [15] and among them, the cells of the perivascular adipose tissue (PVAT) present an important role because they may control the vascular responses through numerous biologically active substances [16]. PVAT represents a specific type of adipose tissue that surrounds most of the blood vessels, with the exception of brain vessels and some of the microvessels [17], and it is involved in intravessel thermoregulation [18], vessel inflammation [19], atherosclerosis development [20], hypertension [21], being able to release adipokines (leptin, adiponectin, etc.), cytokines (IL-6, TNF-α, etc.), angiotensin II, nitric oxide, reactive oxygen species (hydrogen peroxide, superoxide) and many other molecules (ICAM-1, VCAM-1, STAT4, etc.) [22,23] that may have a pivotal role in vascular dysfunction.

The aim of the present study was to evaluate the responses of the aorta wall of rats with prolonged fat diet to phenylephrine (PE) and acetylcholine (Ach). Xia and Li presented in their review the important role of PVAT in vascular dysfunction occurred in obesity, focusing on the oxidative stress, hypoxia and inflammation that were produced in perivascular adipose tissue, important mechanisms that led to the loss of the anti-contractile vessel function [6]. In the present study, prolonged HFD (9 months) altered the aorta wall responses showing significant decreases of relaxation and increases of contraction, results that are concordant with the notions presented by Xia and Li in their review. The investigated aortas were taken from the rats that were part of a complex study from which the results of the oxidative stress, the aortas diameter and the histological and transmission electron microscopy structures were published [13]. In the present study, all the aorta walls responses to vasodilator or vasoconstrictor substances that were added in tissue baths correlate with our previous published work. Prolonged HFD produced oxidative stress and inflammation in the aorta wall, the macrophages and foam cells being noticed in the subendothelial connective layer, important results that make connections indirectly with the PVAT responses to subsequent inflammation occurred after the high lipid diet, as Xia and Li noticed [6]. The increase of iNOS

levels in aorta of rats with HFD [13] may be the result of macrophages activation in aortic PVAT [24] and may show the level of the aorta wall inflammation [25] that leaded to increased contractility [26].

AuNPsCM administration worsened the aorta responses to PE and Ach in rats with prolonged HFD, results concordant with our previous determinations in aorta wall of these rats that showed: severe alterations of endothelium; disorganization of subendothelial connective layer with lipid inclusions that may be also the effects of PVAT oxidative stress and inflammation; increased levels of investigated parameters [13]. The inflammation and the oxidative stress produced by the AuNPsCM administration were added to the already existed pathological alterations occurred after prolonged HFD. The reduced dimensions of the administered AuNPsCM (19 nm) gave them the possibility to accumulate in aorta wall where they aggravated the oxidative stress and inflammation [13], effects that were also mentioned by the De Berardis et al. in their review focussed on noxious effects of inhaled AuNPs [27]. The increased inflammation in aorta wall of rats with prolonged HFD and AuNPsCM oral administration triggered the release of biological active substances like endothelin-1 and iNOS [13] that are involved in vascular dysfunction, increasing the aorta wall contractile responses, effects that are in concordance with the data presented by Guzik and Cosentino in their review [28].

The administration of *Cornus mas* L. extract solution produced the best results on the aorta wall of rats with HFD. Concordant with literature data, we found in our previous work on the same rats that one month of daily oral administration of *Cornus mas* L. extract in rats with prolonged HFD produced beneficial effects: aorta inflammation was decreased, serum antioxidant protection was increased, triglycerides level in serum was reduced [13] and, in the present study: the aorta rings responses enhanced till being similar to those of the Control group (standard diet). The anti-contractile effects of PVAT are realized through a relaxing factor that stimulates the endothelium to release NO which produces smooth muscle cells hyperpolarization [29] but also through hydrogen peroxide that diffuses toward media where acts as a K^+ channels activator [30], response that is H_2O_2-dose dependent [31]. *Cornus mas* L. extract administered as solution in rats with HFD decreased the inflammation in aorta wall. The natural extract solution could have had effects on the PVAT, this important tissue reducing its influences on the aorta layers as a result of a reduced release of molecules produced during a diminished tissue alteration (pro-inflammatory chemokines, etc) and re-balancing the release of NO from endothelial cells, so when Ach was added to the tissue bath, similar responses to those recorded in rats on a standard diet were recorded.

The present study showed in rats with prolonged hyperlipid diet the beneficial effects of oral treatment for 30 days with a solution of *Cornus mas* L. extract on the contractile and relaxation responses, while the administration of AuNPsCM solution altered the vessel wall responses.

Conclusions

Prolonged high-fat diet reduced the contractile and the relaxation responses of the aorta rings to phenylephrine and acetylcholine respectively.

The administration of gold nanoparticles phytoreduced with *Cornus mas* L. extract showed the highest contractile and the lowest relaxation responses in aorta rings of rats with high fat diet.

Cornus mas L. extract solution improved the contractile responses of aorta rings of rats with high fat diet to phenylephrine, showing similar results with those recorded in aortas of rats with standard diet. The relaxation responses to acetylcholine in the aorta rings of rats treated with *Cornus mas* L. solution were significantly decreased in comparison with the Control group and significantly increased when compared to the HFD group.

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