Health, Sports & Rehabilitation Medicine
Vol. 23, no. 1, January-March 2022, 4–13

ORIGINAL STUDIES

The effects of ellagic acid-coated gold nanoparticles on oxidative stress in experimentally induced inflammation

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Abstract

Background. Oxidative stress, the result of the oxidant/antioxidant imbalance, is involved in the pathogenesis of different diseases associated with inflammation.

Aims. The study aimed to investigate the effects of orally administered gold nanoparticles phytoreduced with ellagic acid (AuNPs-EA) on oxidative stress in carrageenan-induced paw oedema, in Wistar rats.

Methods. Paw oedema and oxidative stress parameters were investigated in rats after intraplantar carrageenan injection (0.2 ml 1% solution). Prior to this, the animals, randomly allocated into four groups, received, for 15 days, vehicle (1% carboxymethyl cellulose –CMC) (group C), 1 mg/kg/day indomethacin (group I), 40 mg/kg/day ellagic acid (group II) or 0.3 mg/kg/day AuNPs-EA (group III). The paw oedema was evaluated through plethysmometry before and at 2, 6 and 24 hours after carrageenan injection and blood samples, paw tissues and kidneys fragments were collected for oxidative stress investigation.

Results. AuNPs-EA and indomethacin administration produced significant decreases of the paw oedema, compared to the control group. Lipid peroxidation was reduced significantly by the tested medication in the paw tissues, but it was increased significantly by AuNPs-EA in the kidneys. The antioxidant protection, evaluated through the GSH/GSSG ratio, was amplified significantly in all rats that received AuNPs-EA and indomethacin, in paw tissues and in serum. Ellagic acid provided antioxidant protection only in the serum. In the kidneys, only indomethacin administration increased significantly GSH/GSSG ratio.

Conclusions. AuNPs-EA reduced the paw oedema and oxidative stress in inflamed tissue and in serum, in carrageenan-induced inflammation model.

Keywords: ellagic acid, gold nanoparticles, oxidative stress, inflammation, antioxidants, carrageenan

Abbreviations: EA – ellagic acid, AuNPs-EA – gold nanoparticles phytoreduced with ellagic acid, GSH – reduced glutathione, GSSG – oxidized glutathione, MDA – malondialdehyde, ROS – reactive oxygen species.

Introduction

Ellagic acid (EA) is a natural phenol found in fruits, nuts and wine (Derosa et al., 2016; Seeram et al., 2008; Larrosa et al., 2006), with potent antioxidant, anti-inflammatory and antitumor activities, through the inhibition of IL-6, TNF-α, and γ interferon secretion and down-regulation of NF-kB expression. Additionally, EA reduced the activity of cyclooxygenase-2 (COX-2) and of the inducible nitric oxide synthase (iNOS) (Derosa et al., 2016; Marin at al., 2013) in different *in vitro* and *in vivo* experimental models. Ellagic acid, a highly thermo-stable molecule, slightly soluble in water and alcohol but soluble in potassium hydroxide (Bala et al., 2006), can be easily bio-transformed or degraded in physiological environment, its pharmacokinetic characterization showing low blood levels, short peak time and rapid clearance (Dandan et al., 2019). Accordingly, there have been numerous attempts to coat different nanocarriers with EA, with the purpose of increasing the tissue availability and efficiency.

Carrageenan, seaweed derived polysaccharide, frequently utilized in food industry (Weiner, 2014), has

Received: 2022, January 17; *Accepted for publication:* 2022, January 25
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https://doi.org/10.26659/pm3.2022.23.1.4

been used for many years to induce inflammation in various experimental models. The inflammatory response develops in three phases that involve the following chemical mediators: histamine and serotonin, then bradykinin and in the last phase, prostaglandins (Morris et al., 2003). The pro-inflammatory cytokines (TNF-α, IL-1, IL-6, IL-8) trigger local and systemic inflammation (Borthakur et al., 2007; Necas & Bartosikova, 2013) and enhance the local neutrophils infiltration that amplifies the production of oxygen reactive species (ROS), nitrogen reactive species (RNS) and in this way, the increase of the oxidative stress (Mittal et al., 2014).

Previous studies with ellagic acid on carrageenan-induced inflammation in rodents demonstrated its beneficial effects, revealed through the significant decreases of the oxidative stress markers, including malondialdehyde (MDA) in the liver, and also through the leucocytes migration inhibition into the inflammatory area (Dandan et al., 2019).

Non steroid anti-inflammatory drugs (NSAIDs) have beneficial effects on the inflammation by inhibiting the COX-2 activity and thereby inhibiting the prostaglandin production (Goldstein & Cryer, 2015). Indomethacin represents a prototype of NSAID (Necas & Bartosikova, 2013), which, used in low doses, does not produce gastric alterations but preserves its anti-inflammatory effects, as it has previously been demonstrated (Ucar et al., 1998).

Nanoparticles are nanosize metallic, ceramic, organic, hybrid particles (Thota et al., 2017) with a large surface area that gives them a high ability to bind, adsorb and carry other compounds such as drugs, proteins etc. (De Jong & Borm, 2008). They can be engulfed by macrophages, accumulated in the tissues, making them potent drugs or contrast agents' carriers that can be used in different diseases (Kiessling et al., 2014; Chen et al., 2018a).

Over recent years, gold nanoparticles, easy to be synthesized, have been used for a wide range of biomedical applications as a result of their characteristics: colloidal stability, easy manipulation, low toxicity (Zugravu et al., 2021; Gharatape & Salehi, 2017; Guo et al., 2017). Despite their therapeutic potential, their widely use recommendations remain a concern because of the controversial side effects reported by some authors, such as increased ROS production, mitochondrial and lysosomal damages, inflammation, DNA damages, apoptosis and necrosis (Bhamidipati & Fabris, 2017; Sultana et al., 2015; Dreaden et al., 2012; Hornos Carneiro & Barbosa, 2016; Singh et al., 2018; Enea et al., 2020).

In the present study, the authors investigated the effects of EA and AuNPs-EA on the paw edema and several oxidative stress parameters, in an experimental model of carrageenan-induced inflammation in rats, in comparison with indomethacin administration.

Hypothesis

Based on the hypothesis that EA exerts anti-inflammatory and antioxidant effects, this experimental study was designed to assess the effects of the nanoparticles coated with EA on paw edema and on the oxidative stress induced by carrageenan administration.

Material and methods

Research protocol

a) Place of research

The study was performed in the Physiology Department of Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, the procedures were approved by the Ethical Committee of Iuliu Hatieganu U.M.Ph. Cluj-Napoca (Nr. 197/17.01.2020) and respected the Directive 86/609/EEC.

Albino Wistar male adult rats were placed in cages, in room with environmental standard conditions (temperature: 22 ± 2º C; relative humidity: 45-50%; day-night cycle of 12 h), with 14 days before the experiments, for their acclimatization.

b) Chemicals

Ellagic acid, indomethacin, type IV lambda carrageenan, chloroauric acid (HAuCL4), o-phthalaldehyde, 2-thiobarbituric acid, Bradford reagent, and carboxymethyl cellulose were purchased from Sigma-Aldrich GmbH (Germany). All the reagents were of high-grade purity.

Synthesis of EA-coated gold nanoparticles

A hot solution of 0.1 mM EA was added, under stirring, to a boiling solution of 1mM $HAuCl_4$ (ratio 2:1 v/v). After about 10 minutes, the solution changed its color from yellow to purple red. The UV-Vis spectrum of the obtained solution exhibits an absorption peak at λ_{max} = 545 nm (Fig. 1), which is the characteristic wavelength of colloidal gold, confirming the reduction of Au^{3+} ions by the EA and the obtaining of gold nanoparticles. To use them for biological determinations, the nanoparticles thus obtained were purified by repeated washings and centrifugations (1).

The diameter of AuNPs-EA was estimated using the λ_{max} value and was around 66 nm.

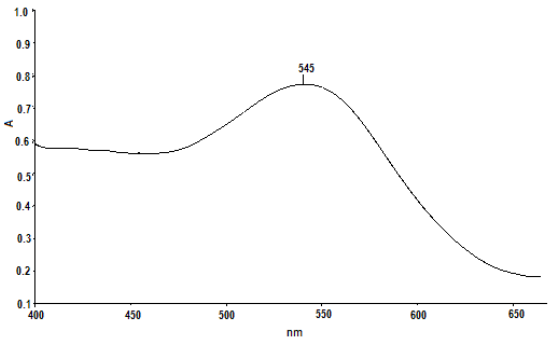

Fig. 1 – UV-Vis spectrum of gold nanoparticles phytoreduced with ellagic acid.

c) Subjects and groups

Twenty-eight Wistar adult male rats (150-180 grams) were randomly allocated into 4 groups that received for 15 days, between 7:00 am and 8:00 am, 0.5 mL/day solution by oral gavage, the vehicle or the treatment dissolved in vehicle: group C -received 1% carboxymethyl cellulose (vehicle), group I -received 1 mg/kg/day Indomethacin, group II -received 40 mg/kg/day ellagic acid and group III -0.3 mg/kg/day gold nanoparticles phytoreduced with ellagic acid (AuNPs-EA). The doses of EA and AuNPs-EA were chosen according to the literature (Chen et al., 2018b).

On the 15th day of the treatment, the rats received the

daily medication, their posterior paws were measured and, at 30 minutes from these procedures, intraplantar injection in the posterior right paw was performed, with 0.2 mL of carrageenan 1%, to produce the inflammation. After the paws' measurements post-carrageenan injection, using the general anesthesia, the animals were humanly euthanized then blood, paw tissue and kidneys were collected.

d) Applied tests

Plethysmometry, with UGO BASILE North America 7140 plethysmometer, was used to evaluate the edema induced by carrageenan injection.

The paws measurements were done before the carrageenan injection and also at 2, 6 and at 24 hours after it. The modification of rat paw volume was calculated with the following formula:

$dv\ (mL) = v_i\ (mL) - v_b\ (mL)$

where dv represents the difference between the paws' volumes, v_i - the inflamed paw volume after carrageenan injection and v_b – the basal volume of the same paw before the carrageenan administration.

The anti-edematous activity (AE) is proportional with efficacy of the treatment and it was evaluated using the following formula:

$$\%AE = [(\text{average } dv_{\text{control group}} - \text{average } dv_{\text{test group}})/ \text{average } dv_{\text{control group}}] \times 100$$

The efficacy of the treatment was considered zero if the difference between average $dv_{\text{control group}}$ and average $dv_{\text{test group}}$ was negative (Stepanovic-Petrovic et al., 2012).

e) Biochemical determination

Oxidative stress parameters were evaluated using the spectrofluorimetric method. Malondialdehyde (MDA) was investigated by Conti method (Conti et al., 1991), reduced glutathione (GSH) using the method of Hu (Hu, 1994) and oxidized glutathione (GSSG) with the method described by Vats (Vats et al., 2008). GSH/GSSG ratio was also calculated.

f) Statistical processing

The parameters were statistically analyzed using GraphPad Prism version 5.03 for Windows, GraphPad Software (San Diego California USA) and One-way ANOVA followed by the post-test Tukey. The paws edema evolution was observed over 24 hours and the recorded values at 2, 6 and 24 hours were used for statistical analysis using Two-way ANOVA followed by the Bonferroni post-test. The significance level was established to $p<0.05$ ($p<0.001$ marked with ***; $p<0.01$ marked with **; $p<0.05$ marked with *).

Results

a) Anti-edematous activity of EA and AuNPs-EA

The effects of EA, AuNPs-EA and indomethacin on rats paws edema was evaluated through plethysmometry.

At 2 hours after carrageenan injection, AuNPs-EA and indomethacin groups showed significant decreases of the paw volume, compared to the control group ($p<0.001$). The animals treated with AuNPs-EA presented a significant decrease of paw volumes when compared to the animals that received only EA ($p<0.01$). The rats that received ellagic acid presented significant increases of paws volume, compared to indomethacin, at 2 hours post-carrageenan injection ($p<0.001$).

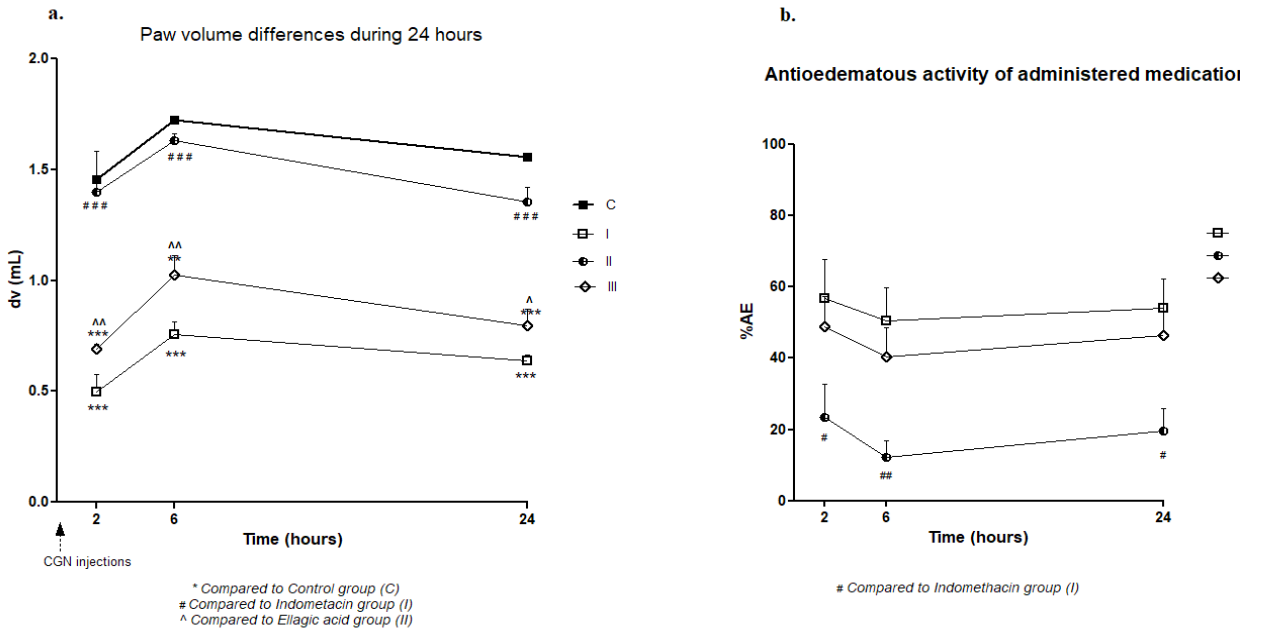

Fig. 2 – The dynamics of paw edema in rats with carrageenan-induced inflammation and treated with EA, AuNPs-EA and indomethcin
a. Paw volume differences (dv) between inflamed and basal paw volume (volume of the same paw before carrageenan injection). The moment of injection is indicated by arrow.
b. Dynamics of the local anti-edematous activity of EA, AuNPs-EA and indomethacin.
Post therapy, the paw volumes were measured at 2, 6 and at 24 hours after carrageenan-induced inflammation. Two-way ANOVA followed by the Bonferroni post-test was used for statistical analysis and the significance level was established to $p<0.05$ (***$p<0.001$ **$p<0.01$ *$p<0.05$).

After 6 hours, the volumes of the inflamed paws were significantly reduced in the groups that received indomethacin ($p<0.001$) and AuNPs-EA ($p<0.01$), compared to the control group. Also, a significant inhibition of paws edema was observed after treatment with AuNPs-EA compared to EA ($p<0.01$). The paw volume of EA-treated animals was maintained increased compared to those of the animals that received indomethacin ($p<0.001$).

After 24 hours, the paws volumes of AuNPs-EA and indomethacin groups, were significantly reduced compared to the control group ($p<0.001$). A significant difference between AuNPs-EA and EA was maintained also after 24 h ($p<0.05$) (Fig. 2a).

Regarding the anti-edematous activity, the administration of AuNPs-EA exerted a comparable effect with that of indomethacin. EA administration had a mild anti-edematous activity, compared to indomethacin at 2, 6 and 24 hours ($p<0.05$, $p<0.01$, $p<0.05$), suggesting a minimal effect of EA on paws edema (Fig. 2b).

b) Oxidative stress evaluation

In *inflamed tissue,* compared to the control group, MDA levels decreased significantly in all treated groups (group I and II, $p<0.01$; group III, $p<0.05$), compared to the control group. EA inhibited lipid peroxidation with a comparable efficacy to that of indomethacin. Treatment with AuNPs-EA reduced significantly the lipid peroxidation but lesser than indomethacin or EA treatment (Fig. 3a).

Compared to the control group, all the rats that received treatment with EA, AuNPs-EA or indomethacin presented significantly increased levels of reduced glutathione (group I, $p<0.001$; group II $p<0.05$ and group III, $p<0.001$).

When comparing the effects of AuNPs-EA with those of EA, the antioxidant defense was significantly increased in the group treated with AuNPs-EA, compared to the ellagic acid group ($p<0.05$) (Fig. 3b). There were significant decreases of glutathione disulphide concentrations in all examined animals (group I, $p<0.001$; group II, $p<0.05$ and group III, $p<0.01$), compared to the control group. Indomethacin had the best lowering effect on GSSG levels, proving its beneficial impact on local inflammation. Compared to indomethacin group, EA presented significant increased levels of GSSG ($p<0.05$) (Fig. 3c). Indomethacin and AuNPs-EA treated groups presented a significant increased antioxidant protection based on the GSH/GSSG ratio (group I, $p<0.001$; group III, $p<0.05$), compared to the control group, and ellagic acid group showed a lower defence activity compared to that of indomethacin ($p<0.05$) (Fig. 3d).

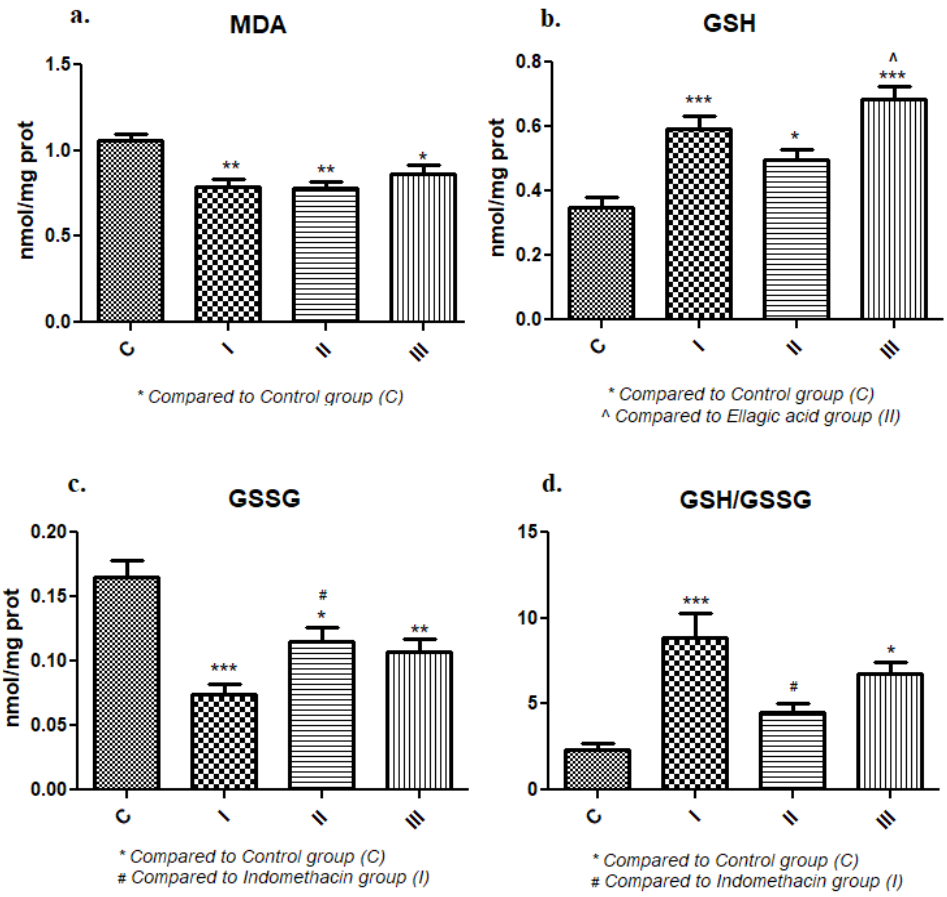

Fig. 3 – Oxidative stress parameters in inflamed rat paw tissues of animals treated with EA, AuNPs-EA and indomethacin.
The levels of malondialdehyde (a), reduced glutathione (b), oxidized glutathione (c) and GSH/GSSG ratio (d), in paws tissue homogenates.
One-way ANOVA followed by the post-test Tukey was used for statistical analysis. The data are expressed as mean ± SEM (n=7); *$p<0.05$; **$p<0.01$ and ***$p<0.001$ vs. Control; #$p<0.05$ vs. indomethacin group; ^$p<0.05$ vs. EA group.

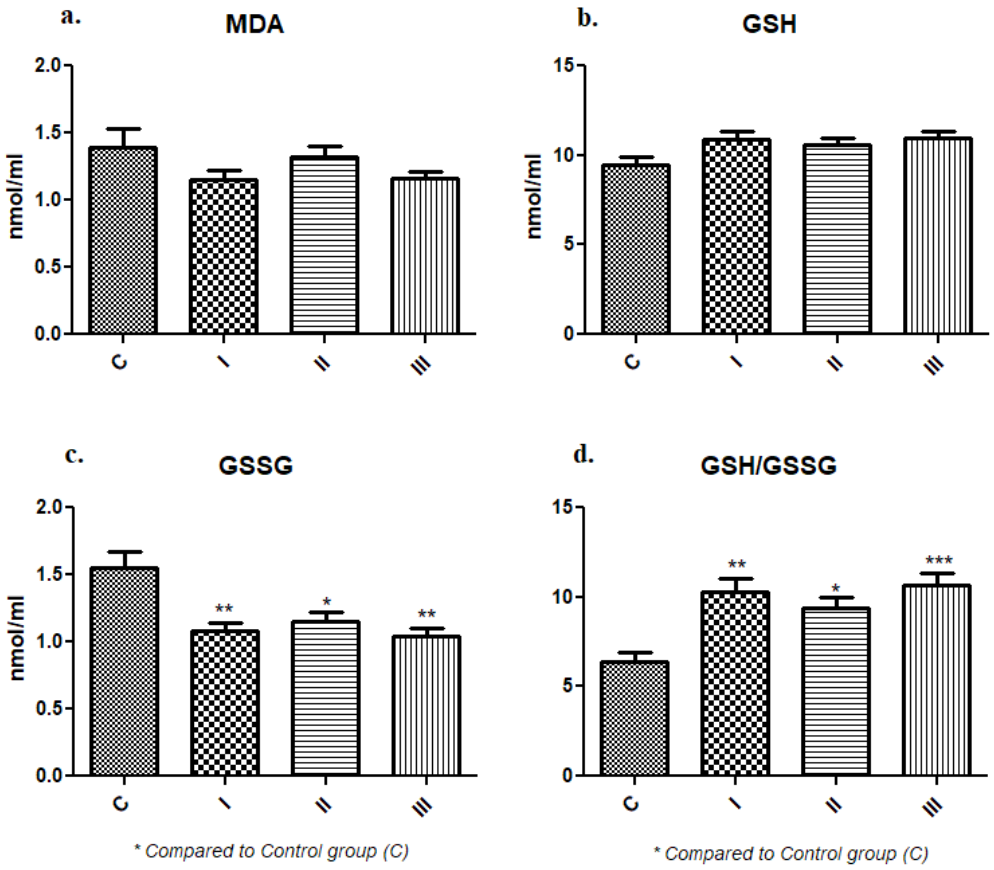

Fig. 4 – Oxidative stress parameters in the serum of rats with carrageenan-induced paw inflammation and treated with AE, AuNPs-EA and indomethacin.
Malondialdehyde (a), reduced glutathione (b), oxidized glutathione (c) and GSH/GSSG ratio (d) in the paws tissue homogenates.
One-way ANOVA followed by the post-test Tukey was used for statistical analysis. The data are expressed as mean ± SEM (n=7); *p<0.05; **p<0.01 and ***p<0.001 vs. control.

In the *serum*, all groups showed reduced levels of MDA without significant differences between them ($p>0.05$) (Fig. 4a). Reduced glutathione levels presented comparable values in the serum in all groups (Fig. 4b), while GSSG levels decreased significantly after the treatments, compared to the control group (group I, $p<0.01$; group II, $p<0.05$ and group III, $p<0.01$) (Fig. 4c). Significant increases of the GSH/GSSG ratio were also recorded for all evaluated groups, compared to the control (group I, $p<0.01$; group II $p<0.05$ and group III, $p<0.001$). The best antioxidant protection in the serum was provided by AuNPs-EA treatment (Fig. 4d).

In the *kidneys*, compared to the control group, indomethacin reduced significantly the lipid peroxidation ($p<0.05$). In comparison with the rats treated with indomethacin, the therapy with AuNPs-EA triggered high levels of MDA ($p<0.05$) (Fig. 5a). The reduced glutathione and oxidized glutathione levels did not differ significantly among the groups (Fig. 5b, Fig. 5c). Indomethacin produced the best antioxidant protection, compared to control group ($p<0.05$), the lowest protection being provided by AuNPs-EA, with significant differences when compared to indomethacin group (Fig. 5d).

Discussion

The study brings evidence that the oral administration of AuNPs-EA has protective effects against carrageenan-induced inflammation. These beneficial effects were confirmed by plethysmometry and by oxidative stress evaluation from paws tissue homogenates.

Carrageenan, a natural compound, is used in different experimental models of drug-induced inflammation to produce local and general signs of inflammation like edema, excessive generation of oxygen reactive species and proinflammatory cytokines (Di Rosa et al., 1971).

In our study, indomethacin, 1 mg/kg/day orally administrated, was used as positive control and CMC as the negative control. At this dose, indomethacin provided anti-inflammatory effects and decreased the paw edema, even in prolonged administration, without side effects (Ucar et al., 1998).

Several previous studies demonstrated the beneficial effects of polyphenols administration in carrageenan-induced inflammation (Mitrea et al., 2020a; Mitrea et al., 2020b). Ellagic acid (EA), a powerful natural polyphenol, was used in our experiment because of its potent antioxidant activity (Whitley et al., 2005). It is an efficient

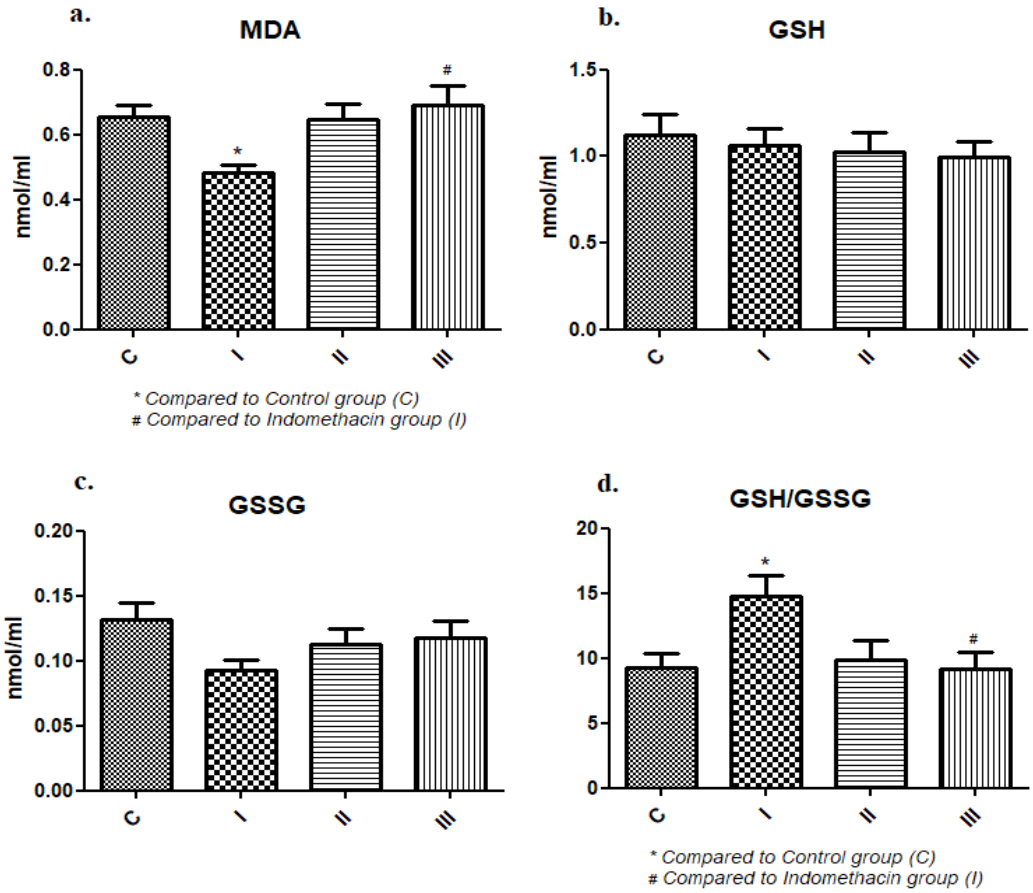

Fig. 5 – Oxidative stress parameters in the kidneys of rats with carrageenan-induced paw inflammation and treated with EA, AuNPs-EA and indomethacin.
Levels of malondialdehyde (a), reduced glutathione (b), oxidized glutathione (c) and GSH/GSSG ratio in the kidney homogenates (d). One-way ANOVA followed by the post-test Tukey was used for statistical analysis. The data are expressed as mean ± SEM (n=7); *$p<0.05$ vs. control and $^{\#}p<0.05$ vs. indomethacin group.

free radicals scavenger (Ateşşahin et al., 2007; El-Shitany et al., 2019), presenting antitumour (Whitley et al., 2003) and anti-inflammatory (Chen et al., 2018b; Marin et al., 2013) activities.

We chose to test gold nanoparticles phytoreduced with EA, because of the nanoparticles proven benefits concerning bioavailability, good tissue penetration and reduced side effects (De Jong et al., 2008; Kiessling et al., 2014; Chen et al., 2018a). Thus, we analyzed the anti-edematous effects of gold nanoparticles coated with EA, comparing them with those produced by EA in CMC. The results revealed protective mechanisms against redox imbalance in paw inflamed tissues and against paw edema, only in the groups of animals that received AuNPs-EA.

The paw volume measurements, performed with plethysmometer, recorded significant decreases only in rats treated with indomethacin or AuNPs-EA, compared to the control group, while EA did not induce significant changes of the inflamed paws' volume. The inhibitory effect of AuNPs-EA was significant at 2, 6 and 24 hours after carrageenan injection. It is known that clinical use of EA was limited by the poor water solubility and low pharmacokinetic profile, limited absorption rate and plasma half-life <1 hour after ingestion of pomegranate juice, properties related to the chemical nature of the organic heterotetracyclic compound (Ceci et al., 2020). The low oral bioavailability of EA depends on the chemical structure, free molecule or compounds derived from its metabolism. In the blood, were previously identified besides free EA molecules, derivatives of EA like ellagitannins and metabolized forms as urolithins (Landete et al., 2011; Kang et al., 2016). Several authors stated that EA induces an irreversible binding to cellular DNA and proteins (Murugan et al., 2009; Sharma et al., 2007).

Experimental animal studies confirmed that EA, orally administered, was poorly absorbed and its retention in the body was low and hence therapeutic effects could not be achieved (Smart et al., 1986; Lei et al., 2003). In our study, probably the reduced efficacy of EA may be explained by its low bioavailability to the target area.

Therefore, there is a strong need to develop an effective delivery carrier for EA, to achieve considerable therapeutic output. Little has been reported on efficient strategies to enhance EA poor oral bioavailability, including chemical structure modifications, encapsulation within nano-microspheres as carriers, and molecular dispersion in

polymer matrices (Ceci et al., 2020). Encapsulation into nanogels is another tested method, in which EA may retain its radical scavenging ability (Behl et al., 2011).

Nanoparticles represent a reliable option for the delivery of low water soluble drugs (Bala et al., 2005) to the target site. Considering their potential toxicity and the purpose to improve their safety and biocompatibility, the "green" synthesis with polyphenols from plants or fruits was used for nanocarriers fabrication (Elbagory et al., 2021). Gold nanoparticles synthesized using plant extracts with biological activity have gained attention in recent years for their use in multiple biomedical applications. The structure of the nanoparticles affects their colloidal stability in different environments, and leads to changes in the physico-chemical properties including size and surface charge and can modify the biological activity. All these properties may influence the expected therapeutic/ diagnostic efficacy and toxicological outcome (Nambiar et al., 2018).

The concentration of polyphenols was found to be a critical factor for the preparation of AuNPs, and the synergistic competition between phenolic hydroxyl and carbonyl groups caused by the oxidation of polyphenols could affect the particle size and morphology of AuNPs (Liu et al., 2020). Perde Schrepler et al. showed that gold nanoparticles synthesized with a polyphenols-rich extract from *Cornus mas* fruits were up-taken by skin cells where exerted low toxicity and induced minimal ROS production, with no additional DNA damages or inflammatory cytokines production (Perde Schrepler et al., 2016).

The efficacy of AuNPs-EA may be related to their moderate size, being known that NPs with large dimensions were found to be less toxic (Alkilany & Murphy, 2010), and their exocytosis is realized at a slower rate, compared to small nanoparticles (Chithrani & Chan, 2007). These properties of our gold nanoparticles can explain the results.

Indomethacin, the non-steroidal anti-inflammatory drug, considered among the most efficient medication against edema development, decreased significantly the carrageenan-injected paws volume, a result that is in accordance with literature data (Ferreira & Vane, 1974).

Carrageenan injection induces acute local inflammation by the release of several chemical mediators, including reactive oxygen and nitrogen species. At the inflammation site, neutrophils, attracted by the chemokines, may increase the local reactions through the ROS release (Morris et al., 2003). In our study, the oxidative stress was investigated in the inflamed paw, in the serum and in the kidney.

In inflamed tissue, the lipid peroxidation was decreased significantly by indomethacin, EA or AuNPs-EA in comparison with the group treated with vehicle. These results are in agreement with those recorded by Mansouri et al. (2015) in their experiment, performed in rats, with 10 or 30 mg/kg of EA administrated 30 minutes before the carrageenan intraplantar injection that presented the decreased MDA levels. Gold nanoparticles may increase the MDA level, as Li at al. showed in their *in vitro* study, on MRC-5 lung fibroblasts (Li et al., 2010). In our study, AuNPs-EA decreased significantly the paws MDA formation and increased GSH levels and GSH/GSSG ratio suggesting the beneficial effect on local inflammation

In the present study, EA, AuNPs-EA and indomethacin promoted the GSH synthesis in inflamed skin. Even if the bioavailability of the free EA is low, at the inflammation zone it stimulated the synthesis of GSH. Similar effects on GSH synthesis were found by El-Shitany et al. in their experiments performed with EA on a carrageenan-induced paw model (El-Shitany et al., 2014).

In our experiments, in inflamed tissues, GSSG, the oxidized form of glutathione, decreased significantly after administration of indomethacin, EA and AuNPs-EA compared to the control group, results that are similar with those presented by Majid et al. that showed that EA used in drinking water in mice, protected against the oxidative stress, increasing the activity of glutathione-disulfide reductase (Majid et al., 1991).

GSH/GSSG ratio was significantly increased in the inflamed area in rats treated with indomethacin or AuNPs-EA, suggesting the protection against oxidative stress. The results are consistent with those found in the literature. Geng et al. demonstrated that pre-treatment with indomethacin diminished the cytokines release in rats subjected to cholangio-pancreatography (Geng et al., 2020). The antioxidant and anti-inflammatory effects of EA were demonstrated also by Rahimi et al. in their review: EA increased significantly GSH levels, decreased the leukocytes' recruitment at the inflammation site and reduced the inflammatory cytokines release in a dose-dependent manner (Rahimi et al., 2020).

In our study, EA increased in serum the antioxidant activity without affecting the lipid peroxidation. GSSG decreased in the groups that received indomethacin, EA, or AuNPs-EA, results that confirmed the antioxidant effect of tested compounds, as the disulphide-bond formation in the endoplasmic reticulum of both mammalian and yeast cells can lead to the formation of ROS (Harding et al., 2003; Haynes et al., 2004). GSH/GSSG ratio increased significantly in the serum after indomethacin, EA and AuNPs-EA administration. These results are concordant with other data in the literature, regarding the effect of EA in serum (Ghoochani et al., 2016).

In the kidneys, AuNPs-EA induced lipid peroxidation, probably because of the ineffective local antioxidant activity. It is known that gold nanoparticles (AuNPs) can generate ROS after interaction with the cellular material, especially because of their physicochemical properties including surface reactivity, particle size, and surface charge (Manke et al., 2013). Cellular uptake of AuNPs is dose, time and cell type dependent, as Patra et al. found, and their toxicity is related to the used cell lines types (Patra et al., 2007). Trono et al. also concluded that a better uptake of AuNPs into cells depends on appropriate size of NPs in combination with optimum incubation time and suitable concentration (Trono et al., 2011). The antioxidant defense in kidney was not influenced by EA or AuNPs-EA administration, results that are in contradiction to those reported by several authors that revealed the beneficial effects of EA against oxidative damages and inflammation by decreasing the lipid peroxidation and improving the glutathione level and catalase enzyme activity (Aslan et al., 2020; Ahad et al., 2014). Bozkurt et al. presented in their study performed in rats with renal ischemia the

protective effects of a single dose of EA administration, 30 minutes before the surgery (Bozkurt et al., 2012). In our study, EA did not increase the antioxidant defencs in kidney, probably because of the special pharmacokinetic properties in the renal tissues. Further studies are necessary to identify the best concentration and the best method of EA administration, to ensure an efficient concentration in the target tissue.

Conclusions

1. AuNPs-EA and indomethacin oral administration decreased significantly the paw edema, compared to the control group.

2. Lipid peroxidation was inhibited significantly in paw tissues in the groups treated with EA and AuNPs-EA, while in the kidney, the MDA levels increased, especially after AuNPs-EA treatment.

3. The antioxidant defense, evaluated by GSH/GSSG ratio, was amplified significantly in all rats that received AuNPs-EA, in paw tissues and in serum. EA provided antioxidant effects only in the serum, while indomethacin administration increased significantly GSH/GSSG ratio in all investigated compartments.

Conflict of interests

The authors declare that they have no conflict of interest.

Acknowledgments

This work was carried out using the researchers' own funds.

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