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# Oxidative stress, inflammation and biochemical parameters in rats on high fat diet and experimentally induced diabetes mellitus after the administration of *Cornus mas* L. extract

**Remus Moldovan[1], Daniela-Rodica Mitrea[1], Irina-Camelia Hărăguș[1], Luminița David[2], Bianca Elena Moldovan[2], Laura Elena Mureșan[3], Șoimița Suciu[1], Simona Clichici[1]**

[1] *Department of Physiology, Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, Romania*

[2] *Research Center for Advanced Chemical Analysis, Instrumentation and Chemometrics, Faculty of Chemistry and Chemical Engineering, Babes-Bolyai University, Cluj-Napoca, Romania*

[3] *Raluca Ripan Institute of Research in Chemistry, Babes-Bolyai University, Cluj-Napoca, Romania*

**Abstract**

*Background. Cornus ma*s is a plant rich in flavonoids, anthocyanins, vitamins and other important chemicals with beneficial effects in several pathological situations such as inflammation, hyperglycemia or noxious localized processes. Numerous studies have shown gold nanoparticles (AuNPs) to be effective drug transporters to the target tissues.

*Aims.* The present study evaluated the effects of *Cornus mas* L. extract administration, as a simple solution or as a functionalizing agent for AuNPs, on the oxidative stress and biochemical parameters in rats with experimentally diabetes mellitus.

*Methods.* Streptozocin-induced diabetes mellitus in adult Sprague Dawley female rats was performed after 8 months of high fat diet that ensured the animal's body weight increase (600 ± 20g). The animals were randomly allocated in the following groups: *CMC group* received carboxymethylcellulose (CMC); *Insulin group* with Insulin administration; *Pioglitazone group* received pioglitazone in CMC solution; *AuNPsCM group* was treated with solution of gold nanoparticles functionalized with *Cornus mas* L. extract (AuNPsCM) and *Cornus mas group* received solution with extract of *Cornus mas*. The parameters of the oxidative stress were evaluated in serum, heart and aorta wall. In addition, biochemical parameters were investigated from the serum of all rats.

*Results. Cornus mas* L. extract simple solution produced significant modifications: in aorta wall decreased significantly malondialdehyde, endothelin 1 and TNF-α; in heart homogenate decreased malondialdehyde, TNF-α and increased GSH/GSSG ratio; in serum increased GSH/GSSG ratio and decreased C-reactive protein (CRP). AuNPsCM administration recorded significant modifications: iNOS increases and TNF-α decreases in aorta wall; malondialdehyde, TNF-α decreases and iNOS increases in heart homogenate; in serum, glucose, HDL and CRP decreases.

*Conclusions. Cornus mas* L. extract simple solution presented beneficial effects in aorta and heart tissues, decreasing the oxidative stress and the inflammation that were generated in rats on high fat diet and experimentally induced diabetes mellitus. AuNPsCM administration showed conflicting inflammatory effects in aorta and heart homogenates but decreased the glucose levels in serum of rats.

**Keywords:** *Cornus mas*, oxidative stress, aorta, heart, gold nanoparticles.

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*Address for correspondence:* Dept. of Functional Science, Physiology, Iuliu Hatieganu University of Medicine and Pharmacy, 1-3 Clinicilor Street, 400006, Cluj-Napoca, Romania

*E-mail:* rdmitrea@yahoo.co.uk

*Corresponding author:* Daniela-Rodica Mitrea; rdmitrea@yahoo.co.uk

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## Introduction

Obesity, hypertension and diabetes mellitus is a triad well known among the pathological mechanisms related to human metabolism. Diabetes mellitus alters the endothelium, diabetic nephropathy triggers the hypertension (Leon & Maddox, 2015), while overweight and obesity are linked to diabetes mellitus (Chobot et al., 2018). Diabetic cardiomyopathy represents a complication of diabetes mellitus with an increasing prevalence in population, the heart structure and function being altered independently of the vascular impairment, modifications that are represented by oxidative stress and inflammation (Ritchie & Dale Abel, 2020).

*Cornus mas* L. is a plant that may provide natural compounds like vitamins, carotenoids, flavonoids or anthocyanins with beneficial effects on metabolic and cardiovascular diseases (Dinda et al., 2016). *Cornus mas* L. extract was used as simple solution or as a natural compound for functionalization of gold nanoparticles in several studies that investigated its effects in numerous pathological or physiological conditions (Popovic et al., 2012; Soltani et al., 2015; Perde-Schrepler et al., 2016; Lietava et al., 2019).

Among the delivery systems, gold nanoparticles (AuNPs) were considered safe and efficient for numerous drug transportation toward the target cells (Zugravu Pop et al., 2020). AuNPs synthesized with natural extracts present specific bio-distribution that is determined by physiochemical characteristics (Tiwari et al., 2011), being able to diffuse from the administration area and to influence the tissues functionality (Volkov et al., 2019). Gold nanoparticles may be cytotoxic (Bhamidipati & Fabris, 2017) and may produce reactive oxygen species (Jawaid et al., 2020).

The study was intended to investigate the oxidative stress and inflammation in aorta wall and heart tissue, and the biochemical modifications in the serum of rats with high fat diet and diabetes mellitus, after the administration of *Cornus mas* L. solution or AuNPsCM solution. As positive controls, the authors used insulin, the only hormone that may decrease the glucose level, and pioglitazone, a synthetic drug from thiazolidinediones class that increases the insulin sensitivity.

## Hypothesis

The study was based on the *Cornus mas* L. extract beneficial effects and started from the hypothesis that both solution types (simple or AuNPsCM) of this natural compound may re-establish the impaired antioxidant capacity of the cardiovascular system that occurs in diabetes mellitus.

## Material and methods

*Research protocol*

a) *Place, period, materials used in the research*

The study took place in Physiology Department of Iuliu Hatieganu University of Medicine and Pharmacy, Cluj-Napoca, it was approved by the Ethics Committee of the University (no. 158/11.03.2019) and observed the Directive 86/609/EEC. The authors used Sprague Dawley female rats that received for 9 months high fat diet and during the last month of the study, diabetes mellitus was induced and the treatment was administered: solution of *Cornus mas* L. extract or gold nanoparticles functionalized with *Cornus mas* L extract. Pioglitazone, insulin and carboxymethylcellulose (CMC) were used as positive controls.

Since the present study is a part of a much more complex experiment during which the same solutions were used, the preparation of gold nanoparticles functionalized with *Cornus mas* L. extract (AuNPsCM) and of the *Cornus mas* L. extract solution could be found in details in our previous published research (Moldovan et al., 2022). Carboxymethylcellulose and pioglitazone were purchased from Merck Darmstadt, Germany and insulin retard was bought from Cluj-Napoca drug store.

b) *Subjects and groups*

For the study, 35 Sprague Dawley adult female rats were used, ages of 5-6 months, weight 300 ± 10 g. The animals were obtained from Cantacuzino National Medico-Military Institute for Research and Development, Bucharest, Romania. The female rats were randomly allocated into 5 groups (n = 7), kept at standard temperature (21 ± 2 °C), relative humidity (55% ± 5) and fed for 9 months by gavage with standardized fat rich diet, 20g /100gbw/day. The diet, sourced from Cantacuzino National Medico-Military Institute for Research and Development, Bucharest, Romania (ROB0001), offered 4.75 kcal/g.

Before the induction of diabetes mellitus, blood samples were taken from the rats for glycemia investigation and the results showed that all of the animals had normal levels.

During the last 3 days of the 8$^{th}$ month of experiment, in all rats, diabetes mellitus was induced in the following manner: streptozotocin was injected intraperitoneally, 30 mg/kg, two times, one day apart. The glycemia levels were also investigated after streptozotocin administration, all the rats presented values higher than 250 mg% and were used for the experiment.

The diabetic rats were treated daily, between 8 a.m. and 9 a.m., for one month (the 9$^{th}$ month of the experiment), as follows: *group CMC* -0.6 mL/day of 1% carboxymethylcellulose solution, through gavage; *group Insulin* -0.1 mg/kg of insulin, subcutaneous injection; *Pioglitazone group* -0.6 mL/day of pioglitazone solution, 10 mg/kg, through gavage; *AuNPsCM group* -0.6 mL/day of gold nanoparticles functionalized with *Cornus mas* L. extract (260 μg AuNPs/kg/day), by gavage; *Cornus mas group* -0.6 mL/day of *Cornus mas* L. extract solution (0.158 mg/mL polyphenols), through gavage.

In the last day of the experiment, under mild anesthesia, the blood samples were collected and later on, under deep anesthesia, euthanasia of the animals was performed, aorta and heart were taken. The serum biochemical parameters and the oxidative stress from serum, aorta and heart were analyzed.

c) *Applied tests*

*Oxidative stress investigation*

The serum parameters of the oxidative stress were evaluated as follows: lipid peroxidation through malondialdehyde (MDA) by using the Conti's method (Conti M, 1991), reduced glutathione (GSH) with the Hu's

method (Hu, 1994) and glutathione disulfide through Vats' method (Vats P, 2008). The ratio GSH/GSSG was obtained to evaluate the antioxidant activity. Inducible Nitric Oxide Synthase (iNOS) was investigated in aorta and heart with spectrometer-based ELISA readers (absorbance 450 nm, 37ºC, Magellan data Analysis software).

*Tumor Necrosis Factor-alpha (TNF-α), endothelin 1*

From the heart and the aorta wall, using spectrometer-based ELISA readers (absorbance 450 nm, 37ºC, Magellan data Analysis software), the inflammatory factors were established.

*Serum biochemical parameters*

BioSystems A15 analyzer, utilizing specific reagent for every blood element, was used to investigate several serum parameters: glucose, glycosylated hemoglobin (HbA1c), cholesterol, triglycerides, high density lipoprotein (HDL), low density lipoprotein (LDL), gamma GT, C-reactive protein (CRP).

d) *Statistical processing*

GraphPad Prism version 5.03 for Windows, GraphPad Software, (San Diego California USA) was used to evaluate the modification significance of the measured parameters, One-way ANOVA followed by the Post-test Tukey. The threshold significance level was considered at $p<0.05$.

## Results

*Gold nanoparticles tissue distribution* was investigated and the presence of AuNPsCM was discovered: in serum 0.074±0.006 mg/L and in the investigated tissues (aorta wall, heart) 0.020±0.002 mg/g.

The *aorta* response to the administered treatment was investigated through malondialdehyde (MDA), iNOS, endothelin 1 and TNF-α.

Malondialdehyde was significantly increased by insulin administration ($p<0.01$) and significantly decreased by *Cornus mas* ($p<0.05$), compared to control group (CMC group). When compared to Insulin group, the administration of the other treatments produced significant decreases of lipid peroxidation ($p<0.001$) (Fig. 1A).

The levels of inducible nitric oxide synthase were increased significantly by the gold nanoparticles functionalized with *Cornus mas* L. extract, in comparison with CMC ($p<0.001$), Insulin ($p<0.05$) and Pioglitazone ($p<0.05$) groups. Compared to AuNPsCM group, *Cornus mas* L. solution decreased significantly the iNOS level ($p<0.001$) (Fig. 1 B).

Endothelin 1 levels were decreased significantly by pioglitazone and *Cornus mas* L. solution ($p<0.001$),

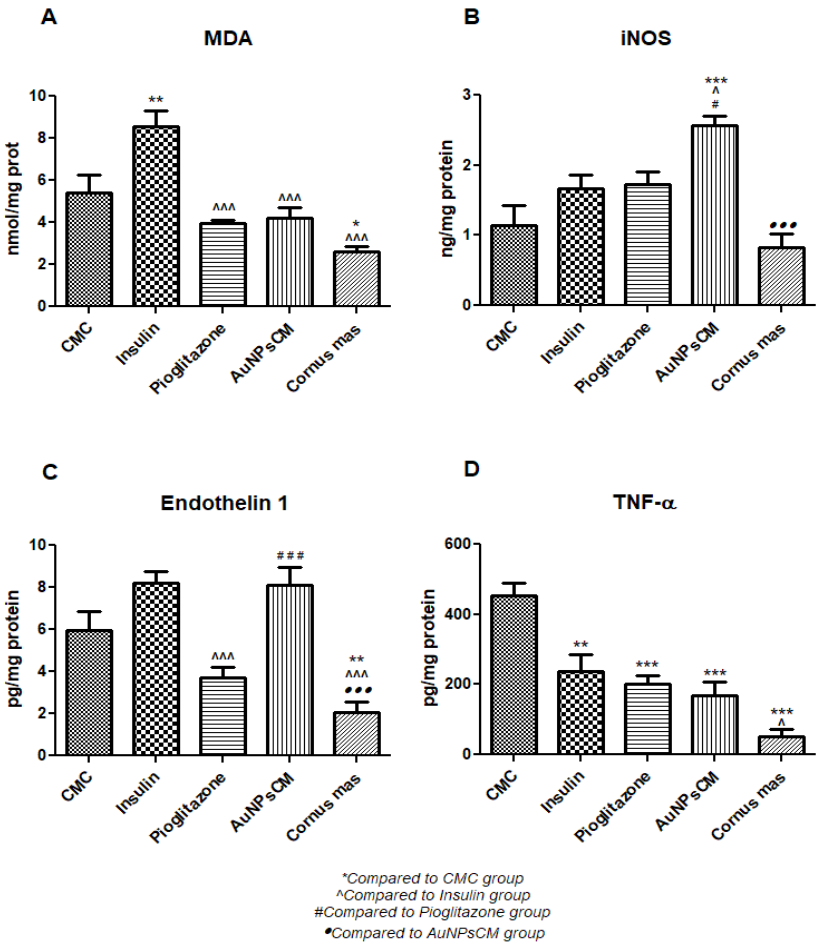


**Fig.1** – The parameters investigated in aorta wall: MDA, iNOS, Endothelin 1 and TNF-α in Sprague Dawley adult female rats with high fat diet and diabetes mellitus treated for one month with insulin, pioglitazone, gold nanoparticles functionalized with *Cornus mas* L. extract (AuNPsCM) or simple *Cornus mas* L. extract solution, compared to carboxymethylcellulose (CMC) administration.

compared to those recorded in Insulin group. AuNPsCM administration increased significantly ($p<0.001$) the endothelin 1 levels, compared to pioglitazone treatment. The greatest reduction in endothelin 1 level was recorded in Cornus mas group, compared to CMC ($p<0.01$), Insulin and AuNPsCM groups ($p<0.001$) (Fig. 1C).

The levels of TNF-α, compared to CMC group, were decreased by all treatment types: insulin ($p<0.01$), pioglitazone, AuNPsCM and *Cornus mas* L. solution ($p<0.001$). The greatest reduction was obtained in Cornus mas group, even when compared to Insulin group ($p<0.05$) (Fig. 1D).

The effects of the *Cornus mas* L. extract administration, as simple solution or in functionalization of gold nanoparticles, were also investigated in the ***heart*** of rats. Compared to control group (CMC), malondialdehyde level was significantly increased in Insulin group ($p<0.001$) and significantly decreased in Pioglitazone ($p<0.001$), AuNPsCM ($p<0.05$) and Cornus mas ($p<0.001$) groups. Insulin administration produced lipid peroxidation, even when compared with the other medication types ($p<0.001$). The lowest level of MDA was recorded in Cornus mas group, significant decreases being seen also when compared to pioglitazone ($p<0.01$) or with AuNPsCM ($p<0.001$) administration (Fig. 2A).

The best antioxidant protection was produced by *Cornus mas* L. solution, the ratio GSH/GSSG being increased significantly when compared to all the other groups: CMC, Pioglitazone and AuNPsCM ($p<0.001$) or Insulin ($p<0.01$). The administration of insulin produced significant increases, compared to CMC group ($p<0.05$) and in comparison with Pioglitazone group ($p<0.01$) (Fig. 2B).

iNOS levels were increased in a significant manner by pioglitazone ($p<0.05$) and AuNPsCM ($p<0.001$), in comparison with insulin. AuNPsCM solution produced the highest increase of iNOS levels, even compared to control (CMC) group ($p<0.01$). *Cornus mas* L. solution reduced the iNOS levels when compared to all the other groups, but significantly in comparison with Pioglitazone ($p<0.01$) and with AuNPsCM ($p<0.001$) groups (Fig. 2C).

The levels of TNF-α, compared to CMC group, were decreased significantly in Insulin ($p<0.01$), AuNPsCM ($p<0.05$) and Cornus mas ($p<0.001$) groups. Pioglitazone administration increased significantly the level of TNF-α, compared to CMC ($p<0.01$), Insulin, AuNPsCM and Cornus mas groups ($p<0.001$) (Fig. 2D).

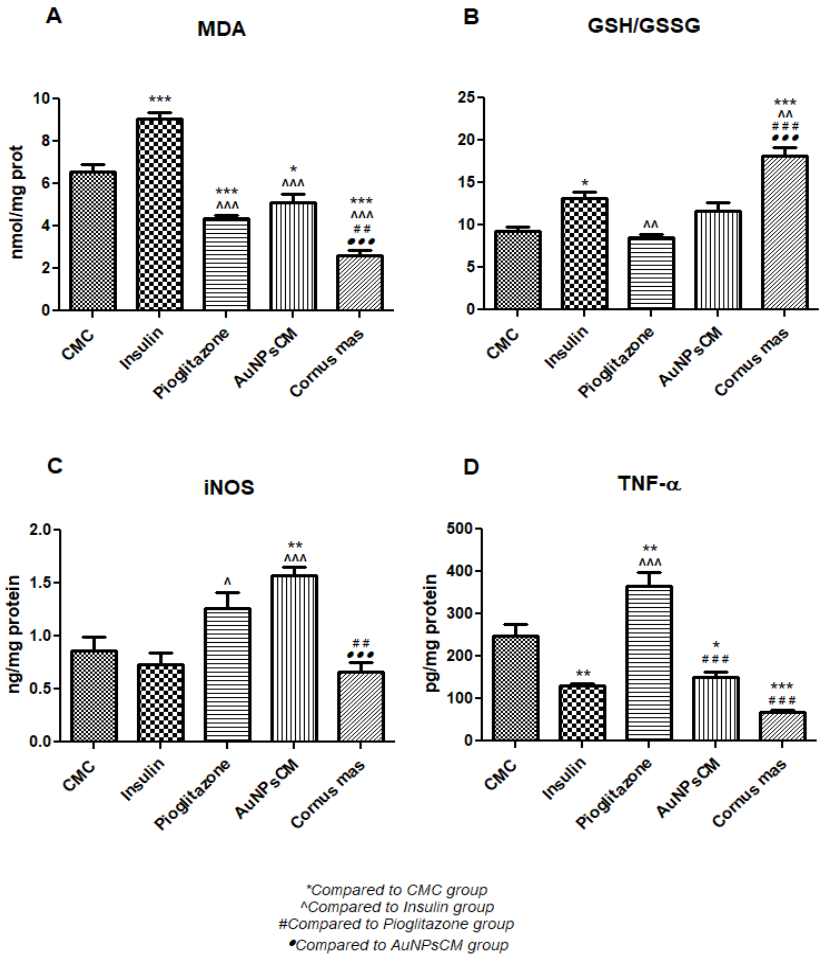


**Fig. 2** – The parameters investigated in rats' heart: MDA, GSH/GSSG, iNOS, and TNF-α in Sprague Dawley adult female rats with high fat diet and diabetes mellitus treated for one month with insulin, pioglitazone, gold nanoparticles functionalized with *Cornus mas* L. extract (AuNPsCM) or simple *Cornus mas* L. extract solution, compared to carboxymethylcellulose (CMC) administration.

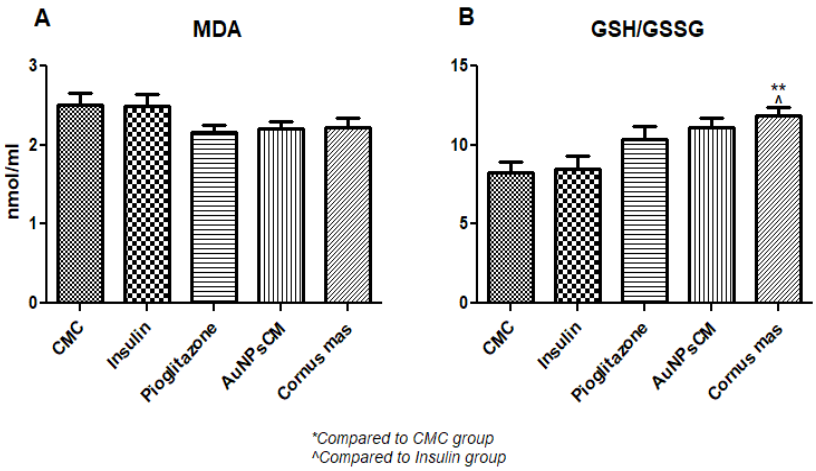


**Fig. 3** – Oxidative stress parameters in serum of rats: MDA and GSH/GSSG in Sprague Dawley adult female rats with high fat diet and diabetes mellitus treated for one month with insulin, pioglitazone, gold nanoparticles functionalized with *Cornus mas* L. extract (AuNPsCM) or simple *Cornus mas* L. extract solution, compared to carboxymethylcellulose (CMC) administration.

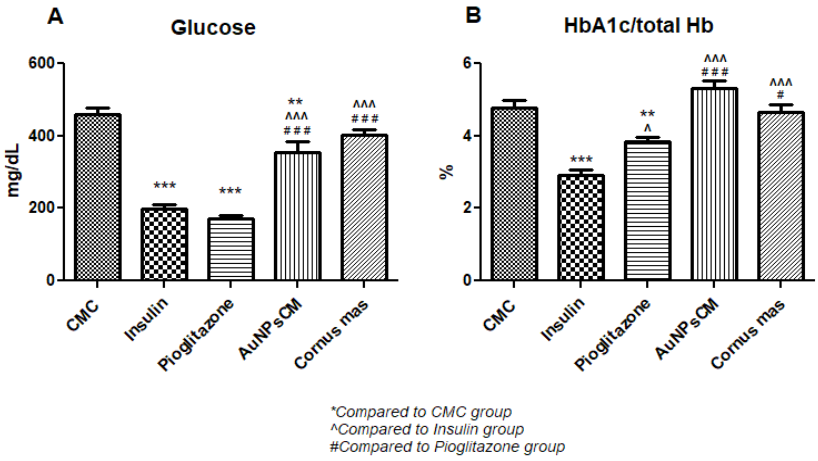


**Fig. 4** – Glycemia and glycated hemoglobin in serum of rats with high fat diet and diabetes mellitus treated for one month with insulin, pioglitazone, gold nanoparticles functionalized with *Cornus mas* L. extract (AuNPsCM) or simple *Cornus mas* L. extract solution, compared to carboxymethylcellulose (CMC) administration.

The oxidative stress was investigated in the serum of rats through MDA and the ratio GSH/GSSG. Lipid peroxidation (MDA level) was not modified in a significant manner (Fig. 3A). Significant increases of the antioxidant protection (GSH/GSSG) were seen only in Cornus mas group, compared to control (CMC) group ($p<0.01$) and to Insulin group ($p<0.05$) (Fig. 3B).

Some biochemical parameters were determined from rats' blood, to investigate the effects of the administrated solutions to the diabetic rats on high fat diet.

The glucose levels were decreased significantly in Insulin and Pioglitazone groups, compared to CMC, AuNPsCM and Cornus mas groups ($p<0.001$). The administration of gold nanoparticles funtionalized with *Cornus mas* L. extract decreased significantly the glycemia in rats, compared to CMC group ($p<0.01$) (Fig. 4A).

Glycosylated hemoglobin (HbA1c) percentage from total hemoglobin was reduced significantly in Insulin group ($p<0.001$) in comparison with CMC, AuNPsCM and Cornus mas groups, and when compared to Pioglitazone group ($p<0.05$). Decreases of this ratio (HbA1c/total Hb) were recorded also in Pioglitazone group in comparison with CMC ($p<0.01$), AuNPsCM ($p<0.001$) and Cornus mas ($p<0.05$) groups (Fig 4B).

The lipid profile was also investigated, to analyze the effects of *Cornus mas* L. extract administration.

Cholesterol level was significantly increased by simple solution of *Cornus mas* L. extract administration, compared to pioglitazone treatment ($p<0.05$) (Fig. 5A).

Triglycerides concentration was evaluated and significant decreases were found in Insulin group ($p<0.01$) compared to CMC group but the greatest reduction was recorded in Pioglitazone group, compared to CMC ($p<0.001$), Insulin ($p<0.05$), AuNPsCM ($p<0.001$) and Cornus mas ($p<0.001$) groups (Fig. 5B).

The levels of HDL were reduced significantly by pioglitazone compared to CMC ($p<0.01$), insulin

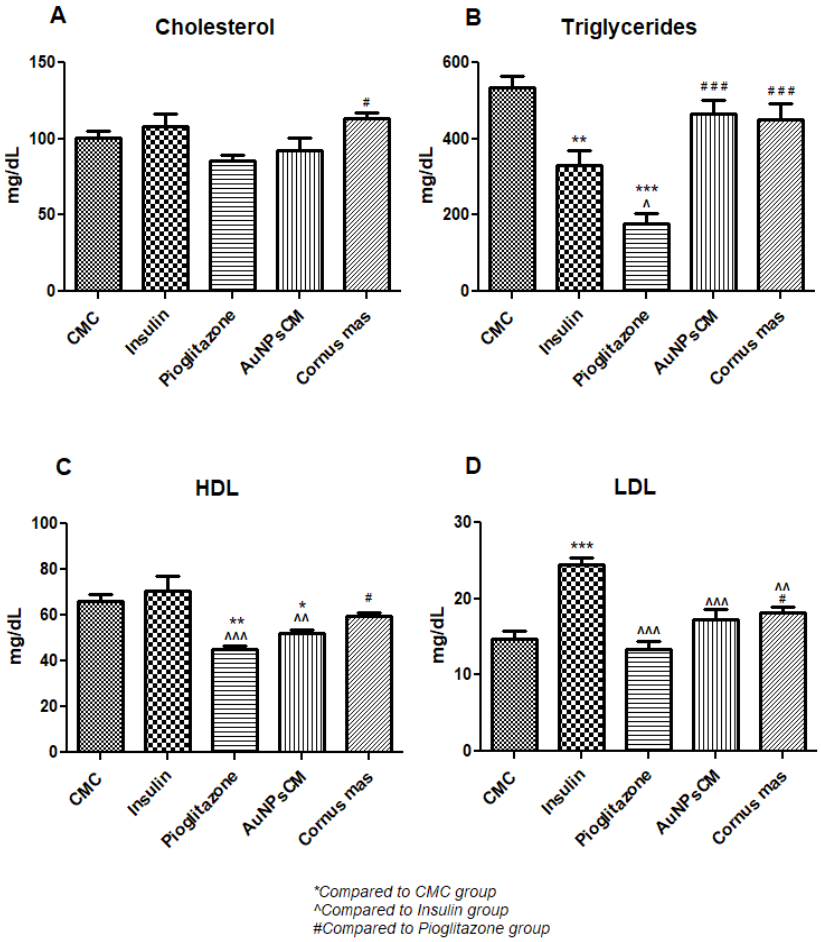


**Fig. 5** – Lipid profile of rats' blood: cholesterol, triglycerides, HDL and LDL in Sprague Dawley adult female rats with high fat diet and diabetes mellitus treated for one month with insulin, pioglitazone, gold nanoparticles functionalized with *Cornus mas* L. extract (AuNPsCM) or simple *Cornus mas* L. extract solution, compared to carboxymethylcellulose (CMC) administration.

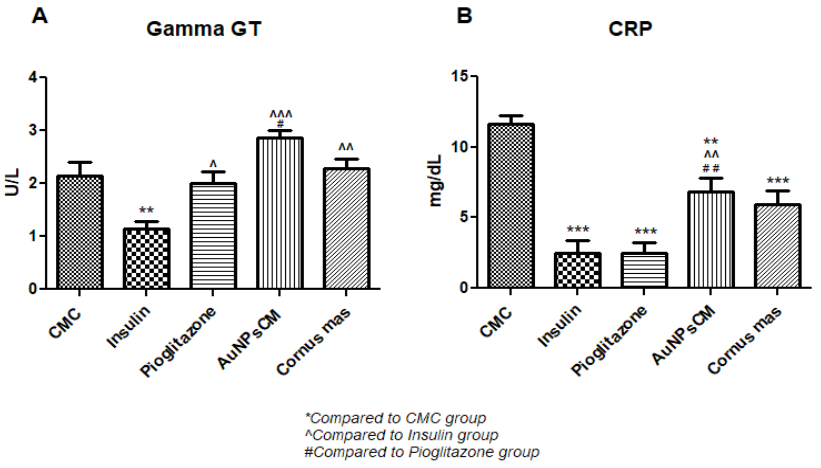


**Fig. 6** – Gamma-glutamyl transferase and C-rective protein levels in the serum of rats with high fat diet and diabetes mellitus treated for one month with insulin, pioglitazone, gold nanoparticles functionalized with *Cornus mas* L. extract (AuNPsCM) or simple *Cornus mas* L. extract solution, compared to carboxymethylcellulose (CMC) administration.

($p<0.001$) and *Cornus mas* L. extract ($p<0.05$) administration. Gold nanoparticles functionalized with *Cornus mas* L. produced significant decreases compared to CMC ($p<0.05$) and Insulin ($p<0.01$) groups (Fig. 5C).

LDL evaluation showed increased levels in Insulin group ($p<0.001$) compared to CMC, Pioglitazone and AuNPsCM groups, but also when compared to Cornus mas group ($p<0.01$). In comparison with pioglitazone administration, the treatment with *Cornus mas* L. solution increased significantly ($p<0.05$) the LDL levels (Fig. 5D).

Gamma-glutamyl transferase (gamma GT; GGT) was analysed in serum of rats and significant decreases were recorded in Insulin group compared to CMC ($p<0.01$), Pioglitazone ($p<0.05$), AuNPsCM ($p<0.001$) and Cornus mas ($p<0.01$) groups. The highest levels were recorded in AuNPsCM administration, significant increases being noticed when compared to insulin ($p<0.001$) and pioglitazone ($p<0.05$) administration (Fig. 6A).

C-reactive protein (CRP) concentration was reduced in all treated groups, compared to control (CMC) group: Insulin, Pioglitazone and Cornus mas groups ($p<0.001$); AuNPsCM group ($p<0.01$). The administration of AuNPsCM presented, among the treated groups, the highest levels of CRP, significant increases in comparison with insulin and pioglitazone treatments ($p<0.01$), these two medications producing the greatest reduction of this parameter (Fig. 6B).

## Discussion

*Cornus mas* L. extract administration produced important modifications in the aorta wall, heart and serum of rats.

In the *aorta wall*, lipid peroxidation was increased significantly by insulin administration, results that are in concordance with those presented by Quiñones-Galvan et al. in their study that showed the effects of hyperinsulinemia on lipids oxidation (Quiñones-Galvan et al., 1999). *Cornus mas* L. extract administration reduced significantly the lipid peroxidation inside the aorta wall, effect that is similar with those presented by Haghi et al. in their study performed in mice with $CCl_4$ administration, study that showed the obtained decreased MDA levels in kidneys when *Cornus mas* L. extract was given as prophylactic medication or as a treatment (Haghi et al., 2014). The levels of iNOS in the aorta wall were increased significantly by the AuNPsCM administration, the same effect being recorded by the authors in a previous study realized in rats with only hyperlipid diet (Moldovan et al., 2022). Endothelin 1, a peptide secreted by endothelial cells in hyperlipid diet (Idris et al., 2019), atherosclerosis (Fan et al., 2006) or in inflammations (Tinazzi et al., 2015), was decreased significantly by the administration of the simple solution of *Cornus mas* L. extract, result that is in concordance with the effects of the anthocyanins (from *Cornus mas*) on the vessel walls, effects related by Mozos et al. in their review (Mozos et al., 2021). The insulin administration produced non-significant increases of endothelin 1 levels compared to control group (CMC), effects that are concordant to those presented by Ferri et al. in their study realized in type II diabetic patients on endothelin 1 levels, measured in plasma and urine (Ferri et al., 1995). In the present study, the diabetic rats on high fat diet presented significant decreased levels of TNF-α in all treated groups, the lowest level being produced by the administration of the simple solution of *Cornus mas* L. extract. Insulin administration decreased significantly the level of this pro-inflammatory cytokine, result that is similar with the insulin role in the decrease of TNF-α expression, presented by Sun et al. in their review (Sun et al., 2014). In our study, the level of TNF-α was decreased efficiently by pioglitazone administration, effect that is similar to that obtained by Li et al. in rabbits with high cholesterol diet (Li et al., 2012). *Cornus mas* L. extract administration produced significant decreases of this pro-inflammatory cytokine, the best reduction of TNF-α being realized by the simple solution, effects that are concordant to those presented by Sozanski et al. in their study performed in rabbits with hyperlipid diet (Sozanski et al., 2014).

In the *heart*, lipid peroxidation was increased significantly by the insulin administration, outcome that is concordant with the result obtained by Kaefer at al. in their research performed in diabetic patients that presented increased levels of MDA in the individuals treated with insulin (Kaefer et al., 2012). Pioglitazone administration decreased significantly the lipid peroxidation, protective effect that is similar to that obtained by Al-Mufazar et al. in their study performed in rats with high lipid-carbohydrates diet for 4 months, the last month with oral administration of pioglitazone 17.5 mg/kg/day (Al-Muzafar et al., 2021). Both solution types of *Cornus mas* L. extract decreased significantly the level of MDA compared to the control group, the simple solution producing the best effect similarly to the results noticed by Abbasi et al. in their research realized in rats with cisplatin-induced toxicity (Abbasi et al., 2020). The ratio GSH/GSSG was increased significantly by the administration of insulin and *Cornus mas* L. simple solution. Bravi et al. already demonstrated, in the plasma of type II diabetic patients, the beneficial effect of insulin administration on GSH/GSSG ratio (Bravi et al., 2006) and the present study completed their findings by showing the favourable impact of this medication on the antioxidant activity in the heart tissue. Similarly to the results presented by Nowak et al. in liver homogenates of ovariectomized female rats where *Cornus mas* L. extract administration increased the GSH/GSSG ratio (Nowak et al., 2022), the present study recorded the same favourable effect of this natural extract but in the heart tissue. The levels of iNOS in the heart homogenate increased significantly after AuNPsCM administration, compared to the control group (CMC), showing the heart impairment (Wilmes et al., 2020) that is connected with the aorta modifications recorded in the present study and is concordant to the results presented by the authors in the preliminary research realized in rats on a high fat diet (Moldovan et al., 2022) or in the study performed in healthy rats with normal diet (Moldovan et al., 2021). The level of TNF-α was increased significantly by pioglitazone administration, result that is concordant to that presented by Sinha and Ghosal in their meta-analysis focused on pioglitazone needs in type II diabetic patients (Sinha & Ghosal, 2020). Insulin, AuNPsCM and *Cornus mas* L. extract simple solution produced significant decreases of TNF-α in heart homogenates. Insulin administration reduced the TNF-α level, effect that is concordant to that presented by Sun et al. in their review based on insulin effects on inflammatory mediators (Sun, 2014). Gold nanoparticles functionalized with *Cornus mas* L. extract produced an inhibition of TNF-α production in the heart of the rats with hyperlipid diet and diabetes mellitus, effect that is similar with that noticed by Crisan et al. in their research made *in vivo* with AuNPsCM in humans with

inflamed psoriasis skin (Crisan et al., 2018). The best reduction of the TNF-α level was recorded in the heart of rats that received simple solution of *Cornus mas* L. extract, result that is concordant with the anti-inflammatory effects of this natural compound presented by Szczepaniak et al. in their review (Szczepaniak et al., 2019).

In the *serum* of diabetic rats with hyperlipid diet, the lipid peroxidation was not significantly modified but the antioxidant protection was increased by the oral administration of simple *Cornus mas* L. extract solution. The increased serum antioxidant protection is similar with that presented by Celep et al. in their experiment with *Cornus mas* L. leaves extract performed in $CCl_4$-treated rats (Celep et al., 2013).

Glycemia and glycated hemoglobin levels were decreased significantly by the insulin and pioglitazone administration, the only hormone that decreases the glycemia (Woerle & Gerich, 2004) and the insulin sensitizer drug, respectively (Alim et al., 2010). As Makris and Spanou presented in their study, the relationship between glycemia and glycated hemoglobin may be influenced by undetermined factors (Makris & Spanou, 2011), situation that was also recorded in the present study, variations among the treated groups showing only certain similarities. Barathmanikanth et al. presented in their study, performed in diabetic mice treated with AuNPs, significant decreases of glycemia (Barathmanikanth et al., 2010), effects that were also recorded in our study in the diabetic rats with oral administration of gold nanoparticles functionalized with *Cornus mas* L. extract. Insulin administration affected the lipid profile of diabetic rats with high fat diet: decreased significantly the triglycerides level and increased significantly the LDL level, compared to control group (CMC), effects that were mentioned by Sadur and Eckel in their study, indicating these types of lipid modifications in obese hyperinsulinemic patients (Sadur & Eckel, 1983). Pioglitazone decreased significantly the triglycerides and non-significantly the cholesterol and LDL levels, compared to control group (CMC), effects that were mentioned by Nesti et al. in their review that was focused on this drug mechanisms in human body (Nesti et al., 2021). In our experiment, pioglitazone decreased significantly the HDL, result that is not concordant with the literature data. AuNPsCM administration in diabetic rats with high fat diet decreased significantly the level of HDL, an opposite effect to the one found in our preliminary study performed in rats with only high fat diet (Moldovan et al., 2022). *Cornus mas* L. extract simple solution did not modified significantly the lipid profile, result that is in contradiction with the literature data.

Increased serum gamma GT levels are related to an increased risk of metabolic syndrome and cardiovascular problems (Lee et al., 2007), levels that in the present study were significantly reduced by the insulin administration, showing the protective effects of the insulin (Haidara et al., 2018). *Cornus mas* L. extract administration did not produce significant modifications compared to control group (CMC), results that are similar with those presented by Abbasi et al. in their study performed in Wistar rats with or without cisplatin administration where the hydro-methanolic extract of this natural compound produced non-significant increases of gamma GT levels (Abbasi et al., 2020).

C-reactive protein was decreased significantly by all treatment types, compared to the control group (CMC). Insulin administration produced significant reduction of the CRP level, showing its anti-inflammatory effect in diabetic rats with high fat diet, C-reactive protein being increased in these specific conditions, as Stanimirovic et al. presented in their review (Stanimirovic et al., 2022). Pioglitazone treatment decreased significantly the level of C-reactive protein, effect that is in concordance with that presented by Hanefeld et al. in their trial performed in humans (Hanefeld et al., 2007). Both solution types of *Cornus mas* L. extract produced significant reduction of the CRP levels, effects that are similar with those recorded in our previous research performed in rats on high fat diet (Moldovan et al., 2022).

Diabetes mellitus activates mechanisms that trigger the oxidative stress and inflammation (Francés et al., 2013) and in our study, these two processes were enhanced by high fat diet (Tan & Norhaizan, 2019). *Cornus mas* L. extract simple solution produced favorable effects: decreased oxidative stress, increased antioxidant protection and reduced the inflammation in aorta wall, heart and serum.

The treatment with AuNPsCM solution produced, in aorta wall and heart tissue, conflicted results: reduced the TNF-α, a pro-inflammatory cytokine but increased iNOS level, enzyme that synthesizes nitric oxide from L-arginine and is expressed in inflammatory processes. This could be explained through the inefficiency of these gold nanoparticles functionalized with *Cornus mas* L. extract on antioxidant protection that we recorded in the present study, being known the role of GSH in the regulation of iNOS expression (Miralles et al., 2000).

Compared to *Cornus mas* L. solution, AuNPsCM administration showed a much more potent effect on glycemia, decreasing the glucose level. The lipid profile of the rats that received AuNPsCM solution presented decreased HDL levels, results that may be explained through the consumption of this protective cholesterol type in the reaction with the recorded high levels of iNOS (Keul et al., 2019).

## Conclusions

1. The simple solution of *Cornus mas* L. extract had better effects than AuNPsCM, presenting beneficial outcomes in all investigated compartments (aorta wall, heart tissue and serum): antioxidant and anti-inflammatory effects.

2. Related to the glucose level, only AuNPsCM reduced glycemia.

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**Conflict of interests**
None declared.

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