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Abstract:

The aim of the study was to evaluate the potential protective role of sildenafil and tadalafil in contrast-induced nephropathy (CIN) by modulating oxidative stress. Thirty Wistar male rats were equally assigned into five groups: sham, CIN, CIN+sildenafil (10 mg/kg bw/day), CIN+tadalafil (5 mg/kg bw/day) and CIN+N-Acetyl Cysteine (NAC) (100 mg/kg bw/day) as a positive control. CIN was induced by 12 h dehydration and administration of indomethacin (10 mg/kg bw), N-ω-nitro-L-arginine methyl ester (10 mg/kg bw), and iopromide (3 g/kg bw iodine). Blood was drawn prior to and 24 h after CIN induction for evaluating renal function and oxidative stress. In the CIN group, total antioxidant capacity (TAC), reduced glutathione (GSH) and catalase (CAT) levels were significantly decreased; and protein carbonyl (PROTC) and thiobarbituric reactive species (TBARS) were significantly increased compared to the sham group. Pre- Sildenafil and tadalafil pre-treatment reduced CIN risk and reversed oxidative stress almost to the sham group levels. These results suggest that PDE5Is can be good candidates for preventing CIN based on their ability to modulate the oxidant/antioxidant balance.
**PRE-TREATMENT FOR 7 DAYS**

- PBS
  - SHAM (n=6)
  - CIN (n=6)
  - CIN+SIL (n=6)
  - CIN+TAD (n=6)
  - CIN+NAC (n=6)

- SIL 10 mg/kgbw/day
- TAD 5 mg/kgbw/day
- NAC 100 mg/kgbw/day

12h dehydration + PBS + saline + saline

**CIN INDUCTION**

12h dehydration + indomethacin (10 mg/kgbw) + L-NAME (10 mg/kgbw) + lopromide (3 g/kgbw iodine)

**OXIDATIVE STRESS EVALUATION**

CAT, GSH, PROTC, TBARS, TAC

- CIN (n=6)
  - ↓Anti-oxidant activity: ↓TAC, ↓GSH, ↓CAT
  - ↑Pro-oxidant effects: ↑TBARS, ↑PROTC

- CIN+SIL/CIN+TAD (n=6)
  - ↑Anti-oxidant activity: ↑TAC, ↑GSH, ↑CAT
  - ↓Pro-oxidant effects: ↓TBARS, ↓PROTC
1. Introduction

Contrast-induced nephropathy (CIN), is a serious iatrogenic form of acute kidney injury, occurring 24-72 h after the administration of iodinated contrast media (CM) during angiographic or other radiological procedures (Mamoulakis et al., 2017; Tsarouhas et al., 2018). In the last years, due to the increasing use of CM, CIN has become a big concern due to its high morbidity, hospital stay and mortality (Parfrey, 2005). CIN is an acute manifestation that appears two to three days after CM administration, lasts up to seven days and is not attributed to other causes of renal failure. Usually, the serum creatinine (CRE) levels return to normal within 14 days (Iordache et al., 2019).

CIN incidence is low (0.6-2.0%) in the general population (Caixeta et al., 2009) but it increases up to almost 15-24% in high risk patients such as older patients, and those suffering from chronic renal insufficiency, diabetes mellitus, and cardiovascular conditions (heart failure, hypertension, myocardial infarction) (Faucon et al., 2019; Lameire and Kellum, 2013; Mamoulakis et al., 2017). The route of administration and the type of procedure for which CM is used can influence CIN risk. CIN rate is increased after arterial CM administration compared to intravenous administration (Morabito et al., 2012).

There is no consensus on CIN definition, prevention, and treatment. Data are conflicting, depending on the definition used, on the biomarkers used for the evaluation of renal function, and on the timing of measurements. Consequently, the reported incidence of the condition varies among studies (Mamoulakis et al., 2017). CRE has been widely used for CIN diagnosis, either as 25% to 50% increase of the baseline levels and/or an absolute elevation of 0.5 to 2.0 mg/dL from baseline (ACR Committee on Drugs and Contrast Media, 2016). According to the criteria developed by the Acute Kidney Network consensus group (Mehta et al., 2007), acute kidney injury is diagnosed if within 48 h after a nephrotoxic event (e.g.,
intravascular iodinated CM exposure), one of the following is observed: i) absolute CRE increase ≥0.3mg/dL (26.4 µmol/L); ii) % increase in CRE ≥ 50% (≥1.5-fold above baseline); iii) urine output reduced to ≤0.5 mL/kg/h for at least 6 h.

The exact pathophysiology of CIN is not fully elucidated, and the identification of novel biomarkers that may more accurately detect renal function changes; reflect kidney damage; assist monitoring; and elucidate pathophysiology have attracted considerable scientific attention nowadays (Mamoulakis et al., 2019). Different theories and multiple mechanisms appear to be involved (Mamoulakis et al., 2017). The nephrotoxic potential of CM differs among various agent classes and is influenced by their osmolality, molecular structure and viscosity (Barrett and Carlisle, 1993; Tungjai et al., 2018). CM may exert a direct cytotoxic effect on the proximal tubular renal cells characterized by vacuolization, interstitial inflammation, and apoptosis due to the production of reactive oxygen species (ROS) and increase outflow resistance. Another mechanism is related to the ability of CM to increase renal vasoconstriction.

Hydration remains at present the first choice in preventing CIN (Mamoulakis et al., 2017; Pattharanitima and Tasanarong, 2014). Intravenous volume expansion using isotonic fluids prior to CM administration is the intervention proven most effective. The value of using compounds with antioxidant properties other than sodium bicarbonate remains controversial, warranting further clinical investigation (Dutta et al., 2018; Mamoulakis et al., 2017).

Phosphodiesterase 5 (PDE5) inhibitors (PDE5Is) are currently recommended as first-line therapy of erectile dysfunction (ED) in humans by enhancing the vasodilatory effects of nitric oxide (NO) (Hatzimouratidis et al., 2019). Acting via the selective inhibition of cyclic guanosine monophosphate (cGMP)-specific PDE5 that metabolizes cGMP, the principal mediator of NO-induced smooth muscle relaxation, PDE5Is cause vasodilatation in the
corpora cavernosa promoting erection. Recently, research has been focused on the preventive and therapeutic use of these agents that are supposed to have a favorable effect on renal hemodynamics, given that the endogenous vasodilator NO is crucial for medullary oxygenation as well by enhancing regional blood flow (Morcos, 2014). Sildenafil's first official use was in 1998 as a pioneer medication for the treatment of ED at 25-100 mg on-demand use. Its onset of action is estimated after 30-60 min, with a duration of up to 12 h and pharmacokinetics affected by fatty food (Goldstein et al., 1998; Moncada et al., 2004). Apart from ED (Hatzimouratidis et al., 2019), sildenafil has been used for treating pulmonary hypertension (Ramani and Park, 2010) and as an off-label medication with unclear role for vasospasm related to other pathologies such as recurrent priapism (Tzortzis et al., 2009), and Raynaud’s syndrome (Roustit et al., 2018). Tadalafil was launched in 2003 with a similar onset of action but the prolonged duration of effects lasting up to 36 h. The dose is 10-20 mg for ED (on-demand use); a dose of 5 mg/day has also been approved for treating ED (Hatzimouratidis et al., 2019), and lower urinary tract symptoms secondary to benign prostatic obstruction (Gravas et al., 2019; Oelke et al., 2014; Sakalis et al., 2017). Tadalafil has been approved at a dose of 40 mg for pulmonary arterial hypertension as it promotes vasodilation/inhibits vascular remodeling, resulting in lower pulmonary pressures (Curran (Curran and Keating, 2003; Gacci et al., 2016; Roehrborn et al., 2016; Ventimiglia et al., 2016). Additionally, it has been used as an off-label medication with an unclear role for the vasospasm related to other pathologies such as recurrent priapism (Tzortzis et al., 2009). The protective effect of sildenafil in CIN has been evaluated in some animal studies (Altintop et al., 2018; Lauver et al., 2014), only one study investigated till now the efficacy of tadalafil in the prevention of experimentally induced CIN (Özbek et al., 2015), but no study evaluated the difference and the efficacy of these two agents in the prevention of CIN. Tadalafil can have some advantages over sildenafil regarding the longer duration of action determined by a
half-life of 17.5 hours compared to only 4-5 hours for sildenafil (Forgue et al., 2006; Nichols et al., 2002). The aim of the present study was to evaluate the protective role of sildenafil and tadalafil on a CIN rat model and to evaluate whether this potential effect is associated with oxidative stress modulation.

2. Material and Methods

2.1. Reagents

All oxidative stress markers reagents, as well as sildenafil, tadalafil, \( \text{N}\)-nitro-L-arginine methyl ester (L-NAME), indomethacin, and N-acetyl cysteine (NAC) were purchased from Sigma-Aldrich (USA). The CM used was iopromide (Ultravist 300 mg iodine per mL, Bayer Pharma AG, Germany). The stock solution of sildenafil, tadalafil, and N-acetyl cysteine were made in dimethyl sulfoxide (DMSO) in a final dose of 0.5% DMSO (v/v) and dissolved in phosphate-buffered saline (PBS). DMSO is a good solvent for lipophilic compounds and could be used in-vivo in low doses without any toxic effect (Mus et al., 2018).

2.2. Animals

Thirty Wistar male rats, 6 months old, were obtained from the University of Medicine and Pharmacy Craiova Animal House. The animals were acclimatized for 1 week to the new laboratory conditions (constant temperature 21±2°C, 12 h/12 h dark/light cycle) before starting the experiment. Food and water were provided ad libitum. All the requirements for the animal experiments from EU Commission Directive 2010/63/EU were respected. The animal experiment protocol was approved by the Ethical Committee of the University of Medicine and Pharmacy of Craiova, Romania.
2.3. Experimental design

The rats were assigned into 5 groups as follows:

- Sham group (n=6; PBS once per day by gavage for 7 days before CIN induction)
- CIN group (n=6; PBS once per day by gavage for 7 days before CIN induction)
- CIN+SIL (sildenafil) group (n=6; sildenafil 10 mg/kg bw/day by gavage for 7 days before CIN induction). The dose of sildenafil was chosen based on previously reported doses used in rats (Cadirci et al., 2011).
- CIN+TAD (tadalafil) group (n=6; tadalafil 5 mg/kg bw/day by gavage for 7 days before CIN induction). The dose of tadalafil was chosen based on previously reported doses used in rats (Benli et al., 2017).
- CIN+NAC group (n=6; NAC 100 mg/kg bw/day by gavage for 7 days before CIN induction: positive control for the protection against CIN prevention (Gong et al., 2016).

CIN was induced based on previous protocols (Agmon et al., 1994; Goldfarb et al., 2006) as follows: on day 6 the rats were kept for 12 h without water for dehydration. On day 7, after they received the daily treatment by gavage, CIN, CIN+SIL, CIN+TAD, and CIN+NAC rats received an inhibitor of the synthesis of prostaglandin (indomethacin; 10 mg/kg bw) dissolved in PBS intraperitoneally. The sham group received the same quantity of PBS. After 15 minutes the experimental groups received an inhibitor of NO synthesis (L-NAME; 10 mg/kg bw) dissolved in PBS intraperitoneally. The sham group received the same quantity of PBS. After another 15 minutes, the experimental groups received iopromide (3 g/kg bw iodine) administered intravenously in the tail vein within 10 minutes under light sevoflurane anesthesia. The sham group received a similar dose of PBS.
2.4. Serum creatinine level evaluation

Whole blood was drawn from the tail vein prior to and 24 h after CIN induction, using a 23G needle after immobilizing the rats with a restrainer. A 250 µl centrifuge tube for serum separation (Arkay, Japan) was used to collect the blood. CRE was determined using an automated biochemistry analyzer with veterinary software, SPOTCHEM EZ SP-4430 Automated Analyser (Arkay Japan). The diagnosis of CIN was defined as an increase in CRE level > 25% at 24-72 h after CM exposure compared to baseline values (Mehran and Nikolsky, 2006; Stacul et al., 2011).

2.5. Oxidative stress markers evaluation

For the evaluation of oxidative stress markers, blood samples were collected 24 h after CIN induction in ethylenediaminetetraacetic acid (EDTA) vacutainers following the same procedure described for CRE level evaluation. Samples were centrifuged at 1,370 x g for 10 min at 4°C, the plasma and erythrocytes were separated and stored at – 80°C till the analysis.

Plasma was used to determine the total antioxidant capacity (TAC), protein carbonyl (PROTC) levels and thiobarbituric reactive species (TBARS). Packed erythrocytes were pre-treated as follows: they were lysed with distilled water (1:1 v/v) and centrifuged at 4,020 g for 15 min at 4°C and then the erythrocyte lysate was collected and used for reduced glutathione (GSH) levels and catalase (CAT) activity evaluation.

The oxidative stress biomarkers GSH, CAT, PROTC, TBARS and TAC were determined based on the methods previously described (Docea et al., 2018; Fountoucidou et al., 2019; Gerasopoulos et al., 2015; Padureanu et al., 2019). Briefly, for GSH the erythrocyte lysate was treated with trichloroacetic acid (TCA) and mixed with 67 nM phosphate buffer (pH=7.95) and 1 nM 5,5dithiobis (2 nitro benzoic acid) (DTNB) and incubated for 45 min in
the dark at room temperature. After incubation, the absorbance of the samples was evaluated at 412 nm and the GSH concentration was calculated based on the extinction coefficient of DTNB.

For CAT activity, the erythrocyte lysate (diluted 1/10) was mixed with 67 nM sodium-potassium phosphate (pH=7.4) and incubated for 10 min at 37°C. After incubation, the samples were mixed with 30% hydrogen peroxide (H₂O₂), and the absorbance was measured at 240 nm for 130 s. CAT activity was calculated based on the extinction coefficient of H₂O₂.

For PROTC evaluation, the plasma was mixed with 20% TCA and incubated for 15 min in an ice bath. After incubation, the samples were centrifuged at 15,000 g for 5 min at 4°C, the supernatant was discarded, and 2,4 –dinitrophenylhydrazine (DNPH) in 2.5 N HCl was added and incubated for 1 h in the dark at room temperature. After incubation, the samples were centrifuged in the same conditions as previous, the supernatant was discarded, and the samples were mixed and centrifuged with ethanol-ethyl acetate (1:1 v/v) in the same conditions twice, and the supernatant was discarded. In the final step, urea (pH=2.3) was added and incubated with the samples at 37°C for 15 min, centrifuged in the same conditions and the absorbance was monitored at 375 nm. The PROTC level was calculated based on the extinction coefficient of DNPH.

The TBARS levels were determined as follows: the plasma was mixed with 35% TCA and Tris-HCl (200 nM, pH=7.4) and incubate at room temperature for 10 min. After incubation, Na₂SO₄ and thiobarbituric acid (TBA) was mixed with the samples and incubated at 95°C for 45 min. After cooling for 5 min on ice and adding 70% TCA and centrifuged at 15,000 g for 3 min at 20°C, the supernatant was separated, and the absorbance was measured at 530 nm. TBARS level was calculated based on the extinction coefficient of malonyl dialdehyde.
The evaluation of TAC levels was determined as follows: the plasma was mixed with 10 mM sodium phosphate buffer (pH = 7.4) and 0.1 mM 2,2-diphenyl-1-picrylhydrazyl radical (DPPH\(^*\)) solution and incubated in the dark at room temperature for 60 min. Then the samples were centrifuged at 20,000 g for 3 min at 4°C, and the absorbance was determined at 520 nm. The TAC is expressed as mmol of DPPH\(^*\) reduced to 2,2-diphenyl-1-picrylhydrazine (DPPH:H) by the antioxidants that are found in plasma.

A Bradford assay was used to determine the concentration of total protein in the plasma (Bradford, 1976). The hemoglobin concentration was determined using a BC-5000 Vet auto hematology analyzer (Mindray, North America).

### 2.6. Statistical analysis

The data were collected in Microsoft Excel (Microsoft Corporation, USA), which was also used for constructing some graphs. We used STATA 13 (StataCorp. 2013. Stata Statistical Software: Release 13. College Station, TX: StataCorp LP) for all statistical analyses and to generate some of the charts. All continuous parameters were expressed as mean and standard deviation. The values were also expressed as percentages of increase or decrease compared with all the other groups.

A comparison between the groups was performed by analysis of variance (one-way ANOVA), after assessing for normality using the Shapiro-Walk test. The post-hoc two by two comparisons were performed with Sidak adjustment for multiple comparisons. A statistical significance was considered when the P-value was less than 0.05.

### 3. Results

#### 3.1. Increase of CRE levels at 24 h after CIN compared to baseline
The validity of our CIN model was documented by the fact that all animals in the CIN group presented an increase of CRE levels > 25% compared to baseline at 24 h after CM administration. The percentage of CRE increase at 24 h after CIN induction compared to baseline in all groups is presented in Figure 1.

**Figure 1. Percentage of mean CRE level increase at 24 h after CIN induction compared to baseline.** *P< 0.05 compared to sham group, # P<0.05 compared to CIN group

### 3.2. TAC levels

TAC levels decreased in all groups, compared to sham group reaching the statistical significance only for CIN and CIN+SIL groups (Table I). TAC levels increased in CIN+SIL, CIN+TAD and CIN+NAC groups compared to CIN group from (0.3±0.02) to (0.43±0.09), (0.51±0.11) and (0.49±0.13), respectively; the increase was statistically significant only in CIN+TAD and CIN+NAC groups (Table I and Figure 2).

**Table I. Redox status parameters at 24 h after CM administration**
<table>
<thead>
<tr>
<th>Parameter</th>
<th>Statistic</th>
<th>GROUPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TAC</td>
<td>Average</td>
<td>Sham</td>
</tr>
<tr>
<td>(mM DPPH/L)</td>
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<tr>
<td>SD</td>
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<tr>
<td>% to Sham</td>
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<tr>
<td>% to CIN</td>
<td></td>
<td>113.13%</td>
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<tr>
<td>% to CIN + SIL</td>
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<td>48.84%</td>
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<tr>
<td>% to CIN + TAD</td>
<td></td>
<td>25.49%</td>
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<tr>
<td>% to CIN + NAC</td>
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<td>30.61%</td>
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<tr>
<td>TBARS</td>
<td>Average</td>
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<tr>
<td>(µM TBARS/L)</td>
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<tr>
<td>SD</td>
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<tr>
<td>% to Sham</td>
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<tr>
<td>% to CIN</td>
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<tr>
<td>% to CIN + SIL</td>
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<tr>
<td>% to CIN + TAD</td>
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<td>-5.26%</td>
</tr>
<tr>
<td>% to CIN + NAC</td>
<td></td>
<td>-14.29%</td>
</tr>
<tr>
<td>PROTC</td>
<td>Average</td>
<td>Sham</td>
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<tr>
<td>(nmol/mg)</td>
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<td>SD</td>
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<tr>
<td>% to CIN</td>
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<tr>
<td>% to CIN + SIL</td>
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<tr>
<td>% to CIN + TAD</td>
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<td>-1.49%</td>
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<tr>
<td>% to CIN + NAC</td>
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<td>4.35%</td>
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<td>GSH</td>
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<td>% to CIN + NAC</td>
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<td>+46.43%</td>
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<tr>
<td>CAT activity</td>
<td>Average</td>
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<tr>
<td>(U/gHb)</td>
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<tr>
<td>SD</td>
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<td>0.97</td>
</tr>
<tr>
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</tr>
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<tr>
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<td>% to CIN + TAD</td>
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<td>+14.29%</td>
</tr>
<tr>
<td>% to CIN + NAC</td>
<td></td>
<td>+10.34%</td>
</tr>
</tbody>
</table>

Note: * P< 0.05 compared with sham group, † P< 0.05 compared to CIN group, ‡ P< 0.05 compared to CIN + SIL group, ° P< 0.05 compared to CIN + TAD group, † P< 0.05 compared to CIN + NAC group.
Figure 2. TAC levels at 24 h after CM administration. *P < 0.05 compared to sham group, #P < 0.05 compared to CIN group

3.3. TBARS levels

In the CIN group, TBARS levels increased significantly compared to sham group (Table I). TBARS levels significantly decreased in CIN+SIL, CIN+TAD and CIN+NAC groups compared to CIN group from (0.63±0.11) to (0.4±0.06), (0.38±0.06) and (0.42±0.03), respectively; the levels in the treatment group being similar to those in the sham group (Table I and Figure 3).
Figure 3. TBARS levels at 24 h after CM administration. * P< 0.05 compared to sham group, # P<0.05 compared to CIN group

3.4. PROTC levels

In the CIN group, PROTC levels increased significantly compared to sham group (Table I). TBARS levels significantly decreased in CIN+SIL, CIN+TAD and CIN+NAC groups compared to CIN group from (1.06±0.08) to (0.68±0.07), (0.67±0.02) and (0.69±0.05), respectively; the levels in the treatment group being similar with those in the sham group (Table I and Figure 4).
Figure 4. Protein Carbonyl (PROTC) levels at 24 h after CM administration. * p< 0.05 compared to sham group, # p<0.05 compared to CIN group

3.5. Reduced glutathione (GSH) levels

In the CIN group, GSH levels decreased significantly compared to sham group (Table I). GSH levels significantly increased in CIN+SIL, CIN+TAD and CIN+NAC groups compared to CIN group from (1.41±0.12) to (2.42±0.28), (2.34±0.16) and (1.96±0.24), respectively (Table I and Figure 5).
Figure 5. GSH levels at 24 h after CM administration. * P< 0.05 compared to sham group, # P<0.05 compared to CIN group, $ P<0.05$ compared to CIN + SIL group, & P<0.05 compared to CIN + TAD group, † P<0.05 compared to CIN + NAC group

3.6. CAT activity levels

In the CIN group, CAT levels decreased significantly compared to sham group (Table I). CAT levels increased dramatically in CIN+SIL, CIN+TAD and CIN+NAC groups compared to CIN group from (7.64±1.31) to (13.78±1.05), (12.6±0.78) and (13.05±0.18), respectively (Table I and Figure 6).
Figure 6. CAT activity levels at 24 h after injection of contrast agent. * P< 0.05 compared to sham group, # P<0.05 compared to CIN group

4. Discussion

CIN represents a severe complication of CM use, especially in patients with risk for renal injury (Mamoulakis et al., 2017). A patient who develops CIN is at higher risk for worse clinical outcomes and complications, presents more extended hospital stay, and higher mortality. CM administration results in vasoconstriction with decreased local prostaglandin synthesis and vasodilatation mediated by NO and also a direct cytotoxic effect on tubular cells determined by increasing oxidative stress. All these changes lead to ischemia in the renal medulla and acute kidney injury (Persson et al., 2005). In vitro studies showed that descending vasa recta (DVR) are directly constricted by CM and this effect is mainly determined by the ability of CM to disturb the balance between vasodilatory factors, such as NO and vasoconstrictive factors, such as ROS. The impairment of endothelial cell viability determines the decrease in NO production, and this may be aggravated by oxidative stress (Sendeski et al., 2009). Along with NO reduction, oxidative stress has been postulated as one
of the main mechanisms related to CIN (Goldenberg and Matetzky, 2005). Modulating the levels of ROS can be one of the main targets for preventing CIN.

In this study, we induced CIN in a rat model based on previous protocols, using indomethacin as an inhibitor of prostaglandin synthesis and L-NAME as an inhibitor of NO synthesis (Agmon et al., 1994; Goldfarb et al., 2006). Iopromide, a nonionic, water-soluble, tri-iodinated CM was used. In the CIN group, all animals developed CIN defined as increased CRE levels by 25% compared to baseline values, showing that the model was valid. Sildenafil and tadalafil pre-treatment significantly limited the increase of CRE levels compared to the CIN group and were comparable to those of the NAC group.

The oxidative stress was evaluated using GSH, CAT, TBARS, TAC and PROTC. Reduced GSH is the main non-enzymatic antioxidant involved in scavenging of free radicals and in normal conditions it is found in equilibrium with its oxidized form (GSSG) in a ratio of 100:1, while in oxidative stress models the level of GSH decreased compared with an increase in GSSG reaching a ratio of 10:1 and even 1:1 (Chai et al., 1994; Lu, 2009). CAT is an antioxidant enzyme implicated in the protection of cells from peroxide damage (Iwase et al., 2013). TBARS are markers of lipid peroxidation, and their levels are increased in oxidative stress (Pryor, 1991). PROTC is a marker of oxidative damage of the proteins, and its levels increase in oxidative stress. The determination of PROTC levels is preferred compared to other makers of oxidative products due to the early formation and the stability of carbonylated proteins formed (Berlett and Stadtman, 1997). TAC is a marker of the antioxidant capacity of plasma (Weydert and Cullen, 2010).

We showed that in the CIN group, the TAC, GSH, and CAT levels were significantly decreased while TBARS and PROTC levels were significantly increased compared to the sham group, in accordance with previous studies in (Chu et al., 2016; de Souza Santos et al.,
We hereby verify therefore that oxidative stress is one of the molecular mechanisms involved in the pathogenesis of CIN and the determination of blood levels of oxidative stress markers can contribute to early diagnosis.

PDE5Is, such as sildenafil and tadalafil act by inhibiting the cGMP-binding PDE5 and inhibit the hydrolysis of cGMP, an effector molecule of NO. By inhibiting cGMP degradation, the action of NO is prolonged, stimulating vasodilatation. They have been first approved for facilitating penile erection, by stimulation of the vasodilatation within the corpora cavernosa (Hatzimouratidis et al., 2019). Recently more and more studies are focused on the effect of PDE5Is on kidney vasoconstriction as PDE5 is highly expressed in this tissue, and the increase of NO by modulating the NO-cGMP pathway can solve the problems related to NO deficit that appear in chronic or acute kidney diseases (Dousa, 1999). PDE5Is also showed the capacity to reduce inflammation and oxidative stress in different conditions (Duranti and Ceci, 2017; Garcia et al., 2014).

In our study, TAC was increased in sildenafil, tadalafil, and NAC pre-treatment groups compared to the CIN group, getting the statistical significance only in the last two groups. The effect of 5 mg/kg bw/day tadalafil was superior to that of the 100 mg/kg bw/day NAC used as the positive control, (70% vs 63.33% increase compared to CIN group, respectively) but without reaching statistical significance. Interesting is that the tadalafil and NAC succeeded in keeping the TAC in the levels compared with the sham, with no statistically significant differences detected between the TAC levels in the sham group, CIN+TAD and CIN+NAC groups. The other two protective markers of oxidative stress, GSH and CAT activity, were significantly increased by sildenafil, tadalafil and NAC pre-treatment compared to the CIN group. Sildenafil tadalafil showed a significantly superior effect on increasing GSH levels compared with NAC. Sildenafil, tadalafil, and NAC pre-treatment
protect against lipid peroxidation and protein oxidation secondary to CM administration, significantly decreasing the level of TBARS with 36.51%, 39.68%, and 33.33%, respectively compared to CIN group and the level of PROTC with 35.85%, 36.79%, and 34.91%, respectively. Both sildenafil and tadalafil showed superior effects compared with NAC but without reaching statistical significance. Our results are in accordance with other studies that investigated the protective effects of sildenafil in the prevention of CIN. Almeida et al. (Almeida et al., 2016) showed that pre-treatment with 50 mg/kg/day sildenafil decreased CIN-induced overproduction of ROS like peroxynitrite and hydroxyl. Tadalafil has been shown to decrease lipid peroxidation by modulating serum and tissue malondialdehyde (MDA) levels in a CIN murine model (Özbek et al., 2015). 15 days of treatment with sildenafil (3 mg/kg/day) showed to have protective effects on renal function and oxidant/antioxidant parameters in a rat model of streptozocin-induced diabetes. Sildenafil treatment reversed till almost the normal values the increase of CRE, urea, MDA levels and the decrease of GSH, superoxide dismutase (SOD), CAT, and TAC observed in diabetic rats, supporting the potential role of PDE5Is in preventing the progression of diabetic kidney injury (Mehanna et al., 2018).

It is known that oxidative stress involvement in CIN development has a significant role. ROS are increased by CM cytotoxicity by two mechanisms: direct endothelial and tubular damage. In both ways, the antioxidant/pro-oxidant balance is impaired and determines the reduction of NO and vasoconstriction (Yang et al., 2018). Till now, several strategies for modulation acute kidney injury using antioxidant compounds have been tested in humans and animal models, showing beneficial effects (Dennis and Witting, 2017). In this study, we showed that pre-treatment with 10 mg/kg bw/day sildenafil and 5 mg/kg bw/day tadalafil for 7 days before the administration of CM protects against CIN also by modulating
the oxidative stress associated with the primary mechanism of PDE5I to increase the NO and produce vasodilatation (Figure 7). The effects were comparable with NAC and are also supported by the histopathological changes observed in the kidney of the animals (data under preparation in another article). No significant differences were observed between sildenafil and tadalafil regarding the modulation of oxidant/antioxidant balance.

**Figure 7. The protective mechanism of PDE5Is in CIN**

5. Conclusions

Induction of CIN in a murine model leads to disturbances in redox status translated by a decrease of TAC, GSH, and CAT levels and an increase of TBARS and PROTC levels. Pre-treatment with sildenafil and tadalafil reduce the incidence of CIN by modulating the oxidant/antioxidant balance in a rat model of CIN. Their effects are superior compared to NAC, even if they did not reach statistical significance, making them good candidates for future studies intending to demonstrate their protective effects in CIN. The determination of oxidative stress markers as TAC, GSH, CAT, TBARS and PROTC can be used as biomarkers for CIN monitoring.
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Conflicts of Interest

All authors declare that there is no conflict of interest.

References


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Highlights:

- Contrast-induced nephropathy is determined by disturbances in oxidant/antioxidant balance

- TAC, GSH and CAT levels decrease while TABRS and PROTC levels increase in CIN

- Sildenafil and tadalafil reduce CIN risk

- Sildenafil and tadalafil increase TAC, GSH and CAT levels

- Sildenafil and tadalafil decrease TBARS and PROTC levels
Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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