Synergistic renoprotective effect of a compiled branched-chain amino acids and *Cymbopogon schoenanthus* extract against experimentally induced oxido-nitrosative renal insult

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**Objective:** To better investigate the protective role of branched-chain amino acids (BCAAs) and *Cymbopogon schoenanthus* (CS) extract against the potassium dichromate (PDC)-induced oxido-nitrosative nephrotoxic insult in the experimental rat model.

**Methods:** Thirty male rats were randomly divided into five equal groups: The 1st group served as control; the 2nd was injected with a single dose of PDC (15 mg/kg b.w. i.p.); the 3rd, 4th, and 5th groups were respectively treated with BCAAs, CS, and their combination for 15 d prior to induction of renal insult via PDC single dose (15 mg/kg b.w. s.c.). The experimental period was terminated in all groups 2 d after induction of renal insult. The harvested kidney samples were divided for biochemical assays and histological examination.

**Results:** The PDC-induced nephrotoxic effect caused a depletion of renal oxidative scavengers glutathione, superoxide dismutase with consequent lipo-oxidative cellular membrane deterioration manifested by a rise in malonaldehyde, oxidized glutathione, myeloperoxidase and the concomitant increase in inflammatory response elements tumor necrosis factor α, nitric oxide, and interleukin 1 β. Moreover, the comet assay and increased 8-hydroxy-2-deoxyguanosine proved an accelerated apoptotic DNA fragmentation. These local renal changes were met with global altered blood biochemistry. The BCAAs and CS or their compiled administration showed an ameliorative effect against PDC-induced nephrotoxic in a synergistic pattern.

**Conclusions:** Both BCAAs and CS or their combined administration afford potential competitors against renal insult induced by polyvalent anion pollutants in experimentally studied animals model. As a route for novel drug discovery, further investigation should be attempted to optimize their augmenting reno-protecting potential.

1. Introduction

Environmental pollution is a global problem with increasing concern due to blasting growth of industrialization. For decades, the hexavalent chromium (chromium VI) is a common air born and groundwater pollutant with over hundreds of tons annual production[1]. Chromium salt is used in different industrial applications (U.S. Department of the Interior and U.S. Geological Survey; Mineral Commodity Summaries 2004). Its air pollution effect is a previously unrecognized risk for kidney disease; however, it is recently accused of kidney disease

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progression. The increased concentration of the fine particulate matter with an aerodynamic diameter (less than 2.5 µm) potentiates the risk of incident chronic kidney diseases by mechanical hindrance of glomerular filtration rate with consequent end-stage renal disease[2]. Former investigation on animal models showed that prolonged exposure for these particles provokes oxidative stress and inflammation with altered renal hemodynamics[3]. Moreover, the soluble chromium found in leather tanning, groundwater, and industrial waste is recognized as an environmental contaminant with teratogenic mutagen and potent nephrotoxic pollutant[4].

On molecular basis, the chromium VI has a structural similarity to other circulating sulfate and phosphate anions. This facilitates its cellular entry via competition for their non-specific anion channels[5,6]. On entry, extra damage would occur during its reduction via depleting cellular anti-oxidant reserve, e.g., active thiol-containing glutathione. After reduction, chromium seeks the formation of a thermodynamically stable adduct with cellular nucleophiles like DNA and other proteins. These events result in increased oxidative stress with disrupted cellular redox potential, double DNA breakage manifested by elevated 8-hydroxy-2-deoxyguanosine (8OHdG) level and marked denaturation of vulnerable cellular proteins and perturbed cellular membranes[7]. Although the chromate is recently proved to induce nephotoxic cellular damage, the study on amelioration of its pro-oxidant renal insult is still in its infancy[8].

The branched-chain amino acids (BCAAs), such as valine, leucine, and isoleucine, are indispensable amino acids. Their altered plasma profile is the common finding of de-compensated renal failure with blood acidosis[9]. BCAAs metabolic disturbance inflicts cellular performance especially in organs with high protein and energy turnover. The kidney plays a key role in acid-base balance with major contribution in metabolic homeostasis[10]. The renal regenerative capacity is consequently improved with enhanced protein synthetic machinery. BCAAs cope with local oxidative stress[11] and participate in protein synthesis by activating a specific mammalian kinase that stimulates eukaryotic initiation factor 4E and enhancement of polysomes enrichment[12]. Moreover, renal parenchyma is highly dependent on BCAAs because of its high BCAA-transferase and low branched-chain keto-acid-dehydrogenase activities[11]. Despite the large body of evidence concerning the regenerative capacity of BCAAs[13], their actual role on ameliorating the heavy metal-nephritic insult in lab model is not fully recovered.

The herbal extract, as an alternative medicine, exerts favorable neutralizing effect against toxin-including drugs, pollutants, and chemicals. Many herbal extracts have an antioxidant protective effect[14]. Their observed mild action could explain their current blasting use in parallel with their synthetic pharmaceutical counterparts. Cymbopogon schoenanthus (CS) has antioxidant, anti-inflammatory, detoxification, and chemoprotective properties[15]. The fresh leaves of CS are widely used as a traditional medicine in Arab peninsula. While inducing hepatoprotective effect, oral intake of their water extract is believed as a potent remedy against kidney stones in North African arid Sahara[16]. Their actual guardian effect against the deleterious influence of PDC as a potent nephrotoxic pollutant in animal lab model is still underestimated. Therefore, this study was adopted to evaluate the localized regenerative capacity of BCAAs as well as the possible defensive role of CS extract during renal PDC-induced nephrotoxicity in lab animal model. Furthermore, their compiled possible synergistic action was investigated against this kind of renal insult for the first time.

2. Materials and methods

2.1. Rats

Adult male Sprague–Dawley rats weighting (220–250 g) from NODCAR animal house (Egypt) were maintained at (27±1) °C with equal daily dark/light cycle. They had free access to water and food (standard pellet chow) during the whole experimental period. The experiments were carried out between 12:00 and 15:00 with a previously approved protocol[17] and in accordance with the guideline standards of the experimental animals’ treatment at the Faculty of Veterinary Medicine, Cairo University, Egypt.

2.2. Chemicals

Methanol (HPLC grade) were purchased from Loba, India. Perchloric acid were purchased from Loba, India. Sulphosalicylic acid and P-aminobenzyl glutamate and pyrogallol were purchased from TMMEDIA Co, India. The 1,1,3,3-tetraethoxypropane, glutathione (GSH) and oxidized glutathione (GSSG) from Sigma Aldrich (USA). Potassium dichromate (PDC), monobasic potassium phosphate, nitrites and nitrate were from Al Nasr chemicals, Abozabal (Qualybia, Egypt). All used chemicals were either HPLC or analytical grade.

2.3. Preparation of extract

Approximately 50 g of CS leaves were soaked with 10 volumes of 70% ethanol for 3 d. Extraction was performed by 40-minute water bath sonication. The content was then filtrated via Whatman filter paper (Bibby RE200, Sterilin Ltd., UK) prior to hot drying at 45 °C to obtain the gummy extract[18].

2.4. Grouping of animals

Animals were divided into five equal groups (n=6) with the following treatments: Group 1 served as control. Group 2 was the treated modeling group with PDC intra-peritoneal injection (15 mg/kg b.w) according to Biber[19]. Group 3 was with an oral administration of BCAAs for 15 d prior to PDC injection (116, 76, 76 mg/kg b.w. for leucine, isoleucine, and valine respectively), according to Smriga[20]. Group 4 was with an oral administration of CS ethanolic leaves extract (200 mg/kg b.w.) for 15 d prior to PDC injection[15]. Group 5 was with mixed oral administration of BCAA and CS for 15 d before PDC injection.

The experiment lasted for 17 d designed for prophylactic treatment for 15 d and then 2 d after induction of nephrotoxicity by PDC. At the end of the experiments (day 18) animals were slaughtered to get tissue samples.

2.5. Blood collection

At the end of the experiment, blood samples were collected and serum samples were obtained after centrifugation at 3 000 r/min for 20 min and kept at −20 °C until further analysis. Other blood samples were collected in heparinized tubes for determination of hemoglobin
and centrifuged (3 000 r/min for 30 min) to obtain plasma for total plasma protein determination.

2.6. Preparation of kidney homogenates

Rats were sacrificed and kidneys were collected and then washed with cooled saline (0.9%). The harvested kidney samples were divided for biochemical assays [malonaldehyde (MDA), GSH, GSSG, superoxide dismutase (SOD), 8OHdG, tumor necrosis factor α (TNF-α), myeloperoxidase (MPO), nitric oxide (NO), and interleukin-1β (IL-1β)] and histological examinations. The kidney tissues under investigation were homogenized with ice-cold phosphate buffer saline (pH 7.4) using Potter–Elvehjem glass homogenizers. The kidney homogenates were used for different enzyme assays.

2.7. Determination of biochemical parameters

Urea, creatinine, total protein, hemoglobin, ferritin, iron, sodium, potassium, calcium, and phosphorus were determined with colorimetric method by spectrophotometer according to kit procedure (Spectrum, Egyptian company of biotechnology-Egypt). The biochemical parameters were determined by spectrophotometer (UV–Visible spectrophotometer, Shimadzu 2450 Kiyamachi Nijo Minami, Nakagyo-kU, Japan).

2.7.1. Determination of MDA by HPLC

HPLC standardization condition for MDA detection utilized an Agilent instrument equipped with Supelcosil C18 column and the flow rate of 1.5 mL/min. The 250 nm wave length detection was performed using standard 1,1,3,3 tetraethoxypropane solution and phosphate buffer solution (3.6 pH): methanol (82.5:17.5) as previously stated[21]. Kidney tissue preparation for MDA determination by HPLC followed our previous work[22].

2.7.2. Determination of nitrites and nitrates by HPLC

Nitrites and nitrate were determined according to the method of Papadoyannis et al[23] by HPLC. A standard mixture of nitrite and nitrate was used to determine the retention times and separation of the peaks. The samples were resolved by anion exchange (PRP-X100 Hamilton, 150 mm × 4.1 mm, 10 µm) analytical column using 0.1 mol/L NaCl:methanol (45:55, v/v) mobile phase with 2 mL/min flow rate and 230 nm wavelength detection limit.

2.7.3. Determination of GSH and GSSG by HPLC

The concentration of reduced or oxidized glutathione was measured by HPLC-tracing of their thiol groups after the method of Jayatilleke and Shaw[24] using reduced and oxidized glutathione reference standards (1 mg/mL in 70% methanol).

2.7.4. Determination of kidney 8-OhdG

Determination of 8-OHdG was determined in kidney tissue homogenates by HPLC according to our previous study of Abd-Elarzeak and Ahmed-Farid[25] against standard solution of 8OHdG using Supelcosil C18 column and phosphate buffer mobile phase (50 mm KH2PO4, in water:methanol (85:15, v/v) at 5.5 pH) with 0.6 mL/min flow rate and 245 nm detection limit.

2.7.5. Determination of SOD

SOD activity was assayed in the kidney homogenate by the method of Marklund and Marklund[26]. The enzyme activity was expressed as 1 U/g tissue, and enzyme activity is the activity capable for 50% inhibition of pyrogallol auto-oxidation in 1 min.

2.7.6. Determination of inflammatory mediators

TNF-α, MPO, and IL-1β were determined with ELISA technique according to RayBio® Rat, kits procedure (RayBio, Spain).

2.8. Histopathological examination

Kidney tissue samples were collected. The specimens were prepared and stained with hematoxylin and eosin stains for histopathological examination according to Banchroft et al[27] and visualized using electric light microscope (Optic, CF160 Infinity, USA).

2.9. Immunohistochemical staining of proliferating cell nuclear antigen

TNF-α polyclonal antibody (Thermo Scientific Co., USA) was used to evaluate renal inflammation parallel with hematoxylin counterstaining. The detail immunohistochemical procedure followed our previous work[28].

2.10. DNA comet assay

The comet assay of DNA in kidney homogenate was estimated according to the classic alkaline single-cell electrophoresis protocol[29]. Samples were stained with ethidium bromide and analyzed by Comet Score 1.5 software. Percent of DNA in comet tails was considered as the marker of genotoxic effect.

2.11. Statistical analysis

Data are expressed as the mean ± S.E.M for the six rats in each group. Statistical differences between groups were evaluated by one-way analysis of variance (ANOVA) using SAS. Statistical analysis of the obtained data was performed using the general linear model (GLM). Significant differences among means were evaluated using Duncan’s Multiple Range Test.

3. Results

3.1. Effects of BCAA, CS, and their combination on electrolytes balance and biochemical parameters in rats treated with PDC

The PDC injected group (Table 1) showed a generalized disturbance in all measured serum electrolytes comparable to control (P<0.05) in the form of hypernatremia and hyperphosphatemia with recorded hypokalemia and hypocalcemia. A partial restoration of plasma electrolytes homeostasis was noticed in BCAAs and CS treated groups. The compiled administration of BCAAs and CS, however, restored the plasma electrolytes to its normal control values (Table 1). The observed serum electrolytes disturbance was tightly coherent with other organ dysfunction. It is clear from the result
that the kidney insult induced by PDC (15 mg/kg b.w) altered blood chemistry in the form of elevated levels of serum creatinine and blood urea nitrogen (Table 1) with an evidence of renal insufficiency rather than liver dysfunction. Maximal amelioration of this alteration was observed in the group with compiled BCAAs and CS administration where the level of both urea and creatinine decreased to near normal values. The separate administration of either BCAAs or CS extraction induced a non-significant decrease in their blood values than that in PDC group but the blood values were still higher than control group. The data in Table 1 showed a significant decrease in total protein and oxygen carrying capacity in PDC group when compared with the control. This decrease was restored to normal values after compiled BCAAs and CS extraction treatment.

### 3.3. Effects of BCAAs, CS, and their combination on oxidative stress markers in rats treated with PDC

The result of this study showed that in PDC treated rats a recognized increase (P<0.05) in inflammatory mediators levels (Table 2). While the BCAAs treatment significantly restored the level of MPO (P<0.05) to its original normal value in control kidney tissue; however, other treatments partially restored this value. This effect was potent with BCAAs administration and their combination with CS extract.

The obtained biochemical data were extra-confirmed by microscopic evidences. As seen from Figure 1, the PDC induced congestion in glomerular capillaries with edema and necrosis in renal tubular epithelium comparable with control. The BCAAs treated group showed dilatation tubules and mild congestion. The CS group showed a slight increase in glomerular capillaries with negligible necrosis in renal tubular epithelium. Renal tissue of compiled BCAAs and CS treatment group showed much restoration in nephron morphology. To prove the microscopic finding of increased inflammatory response, the anti-TNF-α immune-staining in nephrons after different treatments (Figure 2) showed the strong positivity against TNF-α expression in PDC-insult was markedly attenuated after BCAAs treatment and completely abolished after

### Table 1
Effects of branched chain amino acids (BCAAs), Cymbopogon schoenanthus (CS), and their combination on kidney function in rats treated with potassium dichromate (PDC).

<table>
<thead>
<tr>
<th>Group</th>
<th>Sodium (mEq/L)</th>
<th>Potassium (mEq/L)</th>
<th>Calcium (mg/dL)</th>
<th>Phosphorus (mg/dL)</th>
<th>Urea (mg/dL)</th>
<th>Creatinine (mg/dL)</th>
<th>Total protein (g/dL)</th>
<th>Hemoglobin (g/dL)</th>
<th>Ferritin (µg/dL)</th>
<th>Iron (mg/dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>35.21±1.02</td>
<td>12.51±0.41</td>
<td>0.445±0.013</td>
<td>55.23±1.66</td>
<td>0.32±0.010</td>
<td>9.58±0.88</td>
<td>26.52±0.39</td>
<td>11.45±0.33</td>
<td>29.73±0.33</td>
<td></td>
</tr>
<tr>
<td>PDC</td>
<td>50.19±1.80</td>
<td>8.43±0.26</td>
<td>0.852±0.023</td>
<td>27.24±0.42</td>
<td>0.37±0.012</td>
<td>17.62±0.33</td>
<td>10.17±0.46</td>
<td>13.26±0.92</td>
<td>31.23±0.99</td>
<td></td>
</tr>
<tr>
<td>PDC+BCAAs</td>
<td>41.20±1.36</td>
<td>9.16±0.26</td>
<td>0.524±0.016</td>
<td>36.41±1.16</td>
<td>0.37±0.012</td>
<td>13.26±0.92</td>
<td>13.26±0.92</td>
<td>31.23±0.99</td>
<td>33.41±0.41</td>
<td></td>
</tr>
<tr>
<td>PDC+CS</td>
<td>46.91±1.40</td>
<td>10.82±0.24</td>
<td>0.597±0.024</td>
<td>40.12±1.35</td>
<td>0.39±0.015</td>
<td>12.61±1.14</td>
<td>33.41±0.41</td>
<td>33.41±0.41</td>
<td>33.41±0.41</td>
<td></td>
</tr>
<tr>
<td>PDC+BCAAs+CS</td>
<td>41.73±1.32</td>
<td>10.77±0.34</td>
<td>0.531±0.017</td>
<td>47.52±1.37</td>
<td>0.38±0.013</td>
<td>11.45±0.33</td>
<td>29.73±0.33</td>
<td>29.73±0.33</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2
Effects of branched chain amino acids (BCAAs), Cymbopogon schoenanthus (CS), and their combination on kidney function in rats treated with potassium dichromate (PDC).

<table>
<thead>
<tr>
<th>Group</th>
<th>MDA (nmol/g tissue)</th>
<th>GSH (µmol/g tissue)</th>
<th>GSSG (µmol/g tissue)</th>
<th>SOD (U/g tissue)</th>
<th>8OHdG (µg/dL)</th>
<th>MPO (µg/g tissue)</th>
<th>NO (µmol/g tissue)</th>
<th>TNF-α (pg/mg tissue)</th>
<th>IL-1β (pg/mg tissue)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>137.4±1.33</td>
<td>25.2±0.63</td>
<td>1.37±0.04</td>
<td>65.1±1.45</td>
<td>0.37±0.012</td>
<td>13.26±0.92</td>
<td>13.26±0.92</td>
<td>31.23±0.99</td>
<td>33.41±0.41</td>
</tr>
<tr>
<td>PDC</td>
<td>198.0±5.37</td>
<td>2.54±0.016</td>
<td>36.41±1.16</td>
<td>0.37±0.012</td>
<td>13.26±0.92</td>
<td>31.23±0.99</td>
<td>33.41±0.41</td>
<td>33.41±0.41</td>
<td>33.41±0.41</td>
</tr>
<tr>
<td>PDC+BCAAs</td>
<td>234.26±2.86</td>
<td>0.862±0.031</td>
<td>40.12±1.35</td>
<td>0.39±0.015</td>
<td>12.61±1.14</td>
<td>33.41±0.41</td>
<td>33.41±0.41</td>
<td>33.41±0.41</td>
<td>33.41±0.41</td>
</tr>
<tr>
<td>PDC+CS</td>
<td>167.19±5.12</td>
<td>0.705±0.026</td>
<td>47.52±1.37</td>
<td>0.38±0.013</td>
<td>11.45±0.33</td>
<td>29.73±0.33</td>
<td>29.73±0.33</td>
<td>29.73±0.33</td>
<td>29.73±0.33</td>
</tr>
<tr>
<td>PDC+BCAAs+CS</td>
<td>167.19±5.12</td>
<td>0.705±0.026</td>
<td>47.52±1.37</td>
<td>0.38±0.013</td>
<td>11.45±0.33</td>
<td>29.73±0.33</td>
<td>29.73±0.33</td>
<td>29.73±0.33</td>
<td>29.73±0.33</td>
</tr>
</tbody>
</table>
compiled BCAAs and CS treatment. The accelerated apoptosis was confirmed by the comet assay (Figure 3 & Table 3), where the higher comet score presented after PDC-renal insult disappeared after the compiled treatment with BCAAs and CS.

4. Discussion

The exposure to hexavalent chromium (chromium \( \text{Cr}^{6+} \)) compounds has an increasingly global concern. For decades, they are defined

<table>
<thead>
<tr>
<th>Group</th>
<th>Head diameter (px)</th>
<th>%DNA in head</th>
<th>Tail length (px)</th>
<th>%DNA in tail</th>
<th>Tail moment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>22.70±0.61</td>
<td>86.26±2.28</td>
<td>4.01±0.11</td>
<td>12.13±0.33</td>
<td>0.652±0.018</td>
</tr>
<tr>
<td>PDC</td>
<td>13.81±0.38 (^a)</td>
<td>66.8±1.86</td>
<td>12.07±0.33 (^b)</td>
<td>32.61±0.88 (^b)</td>
<td>1.223±0.033 (^b)</td>
</tr>
<tr>
<td>PDC+BCAAs</td>
<td>16.84±0.45 (^ab)</td>
<td>76.34±2.16 (^a)</td>
<td>7.91±0.21 (^a)</td>
<td>22.62±0.62 (^a)</td>
<td>0.835±0.018 (^b)</td>
</tr>
<tr>
<td>PDC+CS</td>
<td>18.88±0.51 (^ac)</td>
<td>72.60±1.91 (^b)</td>
<td>9.83±0.26 (^b)</td>
<td>26.75±0.70 (^a)</td>
<td>1.075±0.031 (^b)</td>
</tr>
<tr>
<td>PDC+BCAAs+CS</td>
<td>20.83±0.55 (^bcd)</td>
<td>80.86±2.17 (^bcd)</td>
<td>5.20±0.14 (^bcd)</td>
<td>18.22±0.48 (^abc)</td>
<td>0.639±0.025 (^bcd)</td>
</tr>
</tbody>
</table>

Data are expressed as Mean±S.E.M for six rats/group. * significant difference from control group at the same column with one way ANOVA at \( P<0.05 \). 
\(^a\) significant difference from PDC at the same column with one way ANOVA at \( P<0.05 \). 
\(^b\) significant difference from PDC+BCAAs group at the same column with one way ANOVA at \( P<0.05 \). 
\(^c\) significant difference from PDC+CS group at the same column with one way ANOVA at \( P<0.05 \).
as potent multivalent toxins and carcinogens. They could induce nephrotoxicity, since these pro-oxidants are easily absorbed and diffusible across cell membrane with strong oxidative potential. The non-specific sulfate anion channels are the main route of entry where chromium ions compete with their similar charged sulfates[5,6].

This followed by a concomitant massive reactive oxygen species production during their intracellular reduction, which is the main characteristic of chromium \[\text{III}\] metabolism[5]. The reactive oxygen species gush could induce renal tubular damage[30] with MDA flooding[31] and exhaustion of antioxidant markers, e.g., GSH and SOD. Meanwhile, chromium \[\text{III}\] ion induces high oxidative damage to proteins and other biomolecules. Previous studies proved that chromium \[\text{III}\] activates several mitogens-like protein kinases extracellular regulated protein kinases-1, extracellular regulated protein kinases-2, c-Jun N-terminal kinase and p38[32] with enhanced phosphorylation of nuclear factor \(\kappa\) B transcription factor. Indeed the activation of nuclear factor \(\kappa\) B triggers various inflammatory mediators[33]. An increasing body of evidence suggests the common link between disrupted metabolism with cellular immune response and inflammation[34]. Our obtained data explain, in part, the observed global disrupted homeostasis in our study after PDC-intoxication in the form of altered blood biochemical parameters. The decreased hemoglobin concentration together with lowering of serum iron, total protein and serum ferritin in PDC logically implies a decreased oxygen saturation capacity with a state of global hypoxia. An earlier related study demonstrated that chromium may compete with iron for apo-transferrin binding sites. This kind of competition decreases serum iron and badly influences iron metabolic role[35]. The elevated blood urea nitrogen and serum creatinine levels, on the other hands, clearly indicate the renal insult rather than liver damage. This renal insult was proved microscopically on the nephron level by defected filtration power evidenced by shrunken glomerular tuft epithelium with necrosis and disturbed reabsorption capacity manifested by the congested peritubular capillaries with tubular dialition and loss of brush borders. The increased level of inflammatory mediators, e.g., myeloperoxidase activity and IL-1 \(\beta\) level with TNF- \(\alpha\) over-expression in glomerular capillaries and proximal tubules, announces a complicated inflammatory infiltration. Nevertheless, the nitro-oxidative nature of this damage was clearly deduced from the increased MDA and NO levels with collapsed renal antioxidant activities signified by GSH depletion and mitigated SOD activity. Like the previous study on acute intoxication[36], the long-term chromium renal intoxication during this investigation demonstrated a lowering in GSH/GSSG ratio, which impacts a concomitant decrease in its reductase/peroxidase ratio. Together with the decreased SOD activity, our obtained data confirm the collapsed redox potential with prioritized renal oxidative damage. The cellular oxidative stress culminated further lipo-oxidative membrane damage with elevated renal MDA level. As a final outcome, an accelerated apoptosis could be expected from the increased renal level of 8OHdG together with distorted nuclei after PDC insult. Interestingly, the observed increase in inflammatory mediators is proved to be in positively correlation with lipoxative stress parameters and both of them correlate positively with the increase in blood urea nitrogen. Moreover, serum electrolytes, the real narrators of the renal intracellular story, are also affected. Sodium is the most extracellular cation that plays an important role in maintaining fluid balance. The observed PDC-induced hypernatremia could imply the impaired renal functions with the decreased excretory ability[37]. Other serum ions like calcium and phosphate are indispensable multivalent ions modulating the vast majority of cellular activities. The kidneys play a central role in their homeostasis via complicated ion gated pathways. Their deviated values in the serum of PDC treated group could also support the nation of disrupted renal function. It is worth noting that the observed hypocalcemia might reflect the membrane re-absorption defective function at the proximal convoluted tubules where 80% of the filtered calcium is passively reabsorbed by the ambient electrochemical gradient for calcium[38]. Since calcium ions are potent anti-inflammatory cations, it is logically to find a negative correlation between the decreased serum calcium level and the reported elevation in inflammatory response mediators. Similarly, the decreased level of serum phosphate could largely attribute to a malformation in apical brush border membrane renal proximal tubule where most of the phosphate re-absorption is dependent on its integrity[39].

Yet so far the ability of BCAAs or herbal extract of CS as natural products with therapeutic potential against the pro-oxidative PDC-induced renal-insult is not fully resolved. The benefit of the use of BCAAs in improving cellular regeneration and limiting oxidative stress is a matter of controversy[39,40]. A recent study favored the supplementation with BCAAs as a safe intervention with positive impact in patients with cirrhosis[41]. In addition, BCAA-containing peptides modulated oxidative stress and reduced the severity of fatty liver in an experimental animal model[42]. The decreased blood levels of total protein and ferritin after PDC-insult might be compensated by BCAAs. The increased level of BCAAs, on the other hand, provokes an inflammatory response guarded by pro-oxidative mediators in cultured leukocytes[43]. This type-dependent discrepancy in cellular behavior acknowledges a peculiarity of response as a function of cellular location. During renal insult, kidney cells have special strategy to compete their energy and regenerative demands from the available BCAAs, where they are extra-hepatically metabolized in tricarboxylic acid cycle to anapleurotic or energy-producing intermediates[44,45].

The value of BCAAs appears to go further in contribution of protein synthesis[46]. \(L\)-leucine, a member of BCAAs, activates mammalian protein synthesis via rapamycin (mammalian target of rapamycin) signaling pathway[47]. The up-regulation of albumin synthesis induced by BCAAs administration may be attributed to the accelerated phosphorylation of p70 S6 kinase and 4E-BP1 in livers with promotion of hepatic mammalian target of rapamycin signaling[48]. Therefore, it is logically to notice an improvement in plasma total protein level after BCAAs addition.

These data can explain the role of BCAAs supplementation with or without CS in restoring homeostasis as well as attenuation of PDC-induced nephrotoxicity and renal oxidant stress. Leakage and depletion of filtration function of kidney may be compensated by BCAAs which enhance protein syntheses. As demonstrated from our finding the BCAAs might act as cell membrane protective antioxidant that lead to normalized kidney function and prevent minerals leakage through urine excretion. Antioxidant features of BCAAs come from the enhancement of endogenous antioxidant glutathione synthetase (EC 6.3.2.3) via glutamine amino acid in the presence of cysteine to produce \(\gamma\) glutamyl cysteine prior to conversion of active glutathione when coupled with glycine[49]. The present finding is inconsistent with previous data[50] who reported that the BCAAs-rich protein increases the GSH level and decreases MDA in rats under stress. Zemel reported that leucine
has an anti-inflammatory action by reduction of oxidative stress and inflammatory mediators[51].

Moreover, cellular increased level of BCAAs in duodenal mucosal side is recently proved to be potent stimulators of the duodenal calcium absorption[52]. This fact adds an advantage to the use of BCAAs to restore normocalcimia and to cope against the decreased serum calcium level after PDC-intoxication.

We screened the use of CS extract as traditional alternative medicine with a potential protective effect against the PDC-induced renal insult. Previous studies reported that the leaves of CS contain saponins, flavonoids and serve as good scavenger against superoxide anions. Interestingly, the saponins as terpene glycosides are recognized recuperative power stimulants with natural resistance improvement[51,53]. Worthwhile, the decreased in oxidative stress markers in our results may attribute to the indirect tannins chelation or direct antioxidant properties of flavonoid. This finding is in agreement with that of Singh et al. who stated that flavonoid has a beneficial effect for removal of urea and creatinine from plasma of normal mice treated with its alcoholic extract and considered as a therapeutic herb to manage renal function[54]. The normalization of total protein, hemoglobin, as well as iron and ferritin level in the CS group may be due to saponin, which was successfully used to promote blood circulation while iron is important in the production of hemoglobin and it plays an important role in flavor protein cytochrome system activities[55]. The correction of serum electrolytes in the CS group could be induced by the steroidal like saponin action that preserves the level of serum cations and enhances their intestinal absorption. Further renoprotective effect of saponin is attributed by inhibition of the intra-renal renin-angiotensin-aldosterone system[56]. Normalized of inflammatory mediators may be due to suppression of the production of lipoprotein-induced NO and chemotactic cytokines by down-regulation of reactive oxygen species generation, inducible nitric oxide synthase and cyclooxygenase-2 expression[57]. A combined BCAAs and CS treatment significantly attenuated renal dysfunction and the observed morphological alterations. The combined treatment also decreased the MDA level and attenuated the reduction of SOD activity in the kidney after PDC-induced renal insult. These data, however, met with the general recovery of blood parameter to normal values. The restored homeostasis was confirmed at the histological level together with a normal return of the level inflammatory mediators and decreased rate of apoptosis. The BCAAs administration markedly reduced the PDC-induced renal DNA damage. Moreover, a recognized restoration of the renal antioxidant capacity with reduced lipid peroxidation was observed after a combined CS and BCAA treatment.

In conclusion, the PDC treatment in rat animal model induced an oxidative renal damage with accelerated local inflammatory response and global alteration in blood parameters. The PDC-induced renal insult was corrected by treatment of either BCAAs or CS. The combined BCAAs and CS treatment has a better renoprotective effect from being separately used. The study warrants against the PDC renal intoxication and affords novel natural product candidates for recovery.

Conflict of interest statement

The authors declare that they have no conflict of interest.

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