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RESEARCH ARTICLE

Treatment with human umbilical cord blood serum in a gentamicin-induced nephrotoxicity model in rats

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ABSTRACT

Sold under the brand name of Garamycin, gentamicin (GM) is an antibiotic in the category of aminoglycoside, that although does have many antibacterial properties, owing to several side effects, its consumption is confined. The current study is aimed at gauging the protective influences of human umbilical cord blood serum (hUCBS) on nephrotoxicity which is induced by GM. In this regard, in the present experimental design, twenty-eight male Wistar rats with the weights of 220 ± 20 g were categorized randomly into 4 groups of seven. The groups included GM (100 mg/kg), control as well as hUCBS at doses of one and two percent together with GM (100 mg/kg) for ten days in an intraperitoneal manner. Blood sampling was collected from the heart directly 24 h after the final injection for obtaining blood serum; the parameters of C-reactive protein (CRP), total oxidant status (TOS), interleukin (IL)-6, lactate dehydrogenase (LDH), total antioxidant capacity (TAC), creatinine (Cr), blood urea nitrogen (BUN), blood serum glutathione (GSH) were gauged in blood serum samples to evaluate renal function. Moreover, for histology, an examination of kidney tissue was performed. In comparison to those of the GM group, in the treatment group, hUCBS significantly decreased the levels of BUN, Cr, LDH, TOS, IL-6, and the CRP levels, and significantly increased the TAC and GSH levels. It was revealed that the treatment of the animals with hUCBS culminates in the reduction of GM' toxic impacts on the kidney.

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Gentamicin; nephrotoxicity; umbilical cord blood serum; rat; inflammation

Introduction

The kidneys are quintessential organs for regulating homeostasis and the extracellular environment as well as detoxifying and excreting the drugs and toxic metabolites (Ferguson *et al.* 2008). Therefore, kidney is a target organ for the detoxification of foreign substances. As Finn *et al.* (2003) contended, kidney damage or nephrotoxicity, which is generally caused by various toxins in the kidneys, is one of the most principal factors in which the kidneys do not excrete toxic chemicals and medications. About twenty percent of nephrotoxicity cases are caused by drugs; long-term drug use raises the nephrotoxicity incidence by up to 66% Acute kidney injury (AKI) is a common disease with a high mortality rate. The epidemiologic data indicate that a significant proportion of cases of this disease are associated with exposure to nephrotoxins due to chemotherapy and the use of anticancer drugs (Kohli *et al.* 2000).

Gentamicin (GM), an antibiotic from the aminoglycoside family, is widely used to treat infections caused by gram-negative bacteria. The GM administration generally results in a dose-dependent nephrotoxicity in 10–20% of treatment cases (Tavafi 2012). Gentamicin causes tubular necrosis and subsequently increases blood urea and serum creatinine (Cr), and decreases the filtration of glomerular. This antibiotic causes the production of hydrogen peroxide in the mitochondria of rat kidney cortex and elevates the production of ROS or reactive oxygen species (Li *et al.* 2013). GM also induces nephrotoxicity through mechanisms such as inducting oxidative stress, necrosis, apoptosis, increased permeability of macrophages as well as monocytes to the cortex and medulla of the kidney and reduced energy of renal epithelial cells (Lopez-Novoa *et al.* 2011).

Kidney transplantation and dialysis are the therapeutic strategies for renal patients. However, the lack of donors and the high cost limit the use of these therapies (Nash *et al.* 2002). That said, mesenchymal stem cell (MSC) transplantation-based therapies to stimulate renal reconstruction due to secretion of paracrine or biologically active factors including anti-apoptotic, angiogenic, antioxidant, and immune mediators have been reported to be auspicious (Tögel *et al.* 2005). It should be mentioned, nonetheless, that poor transplantation and uncontrolled growth are two major concerns for the use of these cells (Rota *et al.* 2019). Therefore, it is more clinically desirable to choose cell-free therapeutic options, provided more significant positive effects are observed. Following the cell-free therapeutic approaches, the use of stem cell culture media has been previously studied, in which the preparation and cultivation of cells have incurred some costs (Moghadasali *et al.* 2013).

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The human umbilical cord blood serum (hUCBS), which is obtained during the separation of cells from the umbilical cord blood, is largely a waste product. The umbilical cord serum has been used as a standard alternative for *in vitro* stem cell growth and differentiation as well as proliferation of MSCs in culture medium containing hUCBS has increased *vs.* culture medium containing fetal bovine serum (FBS). hUCBS is responsible for these beneficial effects (Shetty *et al.* 2007, Huang *et al.* 2011, Tekkatte *et al.* 2012). Moreover, it has recently been documented that it has beneficial effects in experimental animal models of GM-induced hepatotoxicity (Mirazi *et al.* 2021). The proteomics of the umbilical cord blood indicates the presence of cytokines, immune mediators and growth factors in hUCBS which influence the proliferation and function of immune cells and stem cells (Fan *et al.* 2011, Doorn *et al.* 2012). On the other hand, it has been shown that growth factors can be effective in repairing and reconstructing the tissues of kidney (Humes *et al.* 1989, Coimbra *et al.* 1990). Cytokines also are a regulator of host responses to the inflammation, immune responses, trauma and infection. Some of them also act as anti-inflammatory and inflammation reducers (Dinarello 2000).

Due to the beneficial compounds present in hUCBS, these substances may partially prevent the adverse effects of GM on the kidney and reduce the limitation of the use of this antibiotic in patients prone to acute renal failure and exert a protective influence. To recap, using male Wistar rats, the present study is aimed at examining the influence of hUCBS on the nephrotoxicity induced by GM.

Material and methods

Animals and their maintenance

Twenty-eight male rats (Wistar) with the weights of 220 ± 20 g were bought from the University of Medical Sciences, Hamadan, Iran. These rodents were then sent to the University of Bu-Ali Sina and were kept in an animal room. They were kept in a 12-hour dark-light cycle with a humidity of fifty to sixty percent at a temperature of 22 ± 3 °C. They also had unconstrained access to food and water. For their acclimatization to the new situation, they received treatments 2 weeks after their accommodation. All the experiments in the present study were performed based upon the guidelines for the standard ethics stipulated by European Directive Committee (2010/63/EU). The experiments had also the approval of Bu-Ali Sina's Local Ethics Committee (permit No.: IR.BASU.REC.1398.035).

hUCBS preparation

Royan Institute, which is under the auspices of Iran's Academic Center for Education, Culture and Research, provided us with the required hUCBS. Having obtained the consents of the cesarean mothers (those who had gone through the screening process for infectious maladies including, hepatitis B, hepatitis C, and HIV), we took the cord blood from the newborn males. Next, having received the placenta along with the umbilical cord, we injected the latter in a falcon tube which was sterile. Then, the tube which contained the pumped umbilical cord blood was moved to a lab with ice. For 2 hours at a room temperature, it was incubated and centrifuged at 20 °C for twenty minutes after its coagulation at 3000 g. A sampler was used to separate the serum of blood out of the upper section of the tube. The serum was then kept in a tube that was sterile. For the minimization of the differences in the batches, gleaned serums of various donors were blended. The serums were stored at a −20 °C for later usage. The preparations of hUCBS at doses of 1 and 2% were followed based on a previously published study (Caseiro *et al.* 2018).

Study groups design

Alborz Darou Corporation, Tehran, Iran, provided the 80 mg/ml ampoules of GM. For inducing nephrotoxicity, all rats received 100 mg/kg body weight (Ademiluyi *et al.* 2013). We divided the rats randomly into four groups of seven: the group receiving GM 100 mg/kg, control group, and the groups receiving one and two percent of hUCBS along with GM (100 mg/kg) for ten days. An insulin syringe was used for all the intraperitoneal injections. A minimum interval of 1 h was observed between the injection of GM and that of hUCBS. Twenty-four hours after the final injection, a xylazine-ketamine (10 mg/kg, and 80 mg/kg, respectively) combination was used for the anesthetization of the rats. Approximately 5 mL of blood samples was taken directly from their hearts and put into tubes specified for test. A record of each group's names was done. The ten-minute centrifuging through blood centrifugation took place after 1 h, separating the rats' serums. After making the incision in all animals' abdomens and removing the tissue in their right kidneys, the tissue was decapsulated; saline also was used for washing. The kidney was divided equally into two longitudinal slices. Then, a container with ten percent of formalin (with ten times of the volume of the sample) was used to keep it. After dehydration and blocking, the tissues of the kidneys were retained for histological inspections.

Biochemical and inflammatory cytokine measurements

The amount of LDH or lactate dehydrogenase, blood urea nitrogen (BUN) and Cr were determined by kinetic colorimetric methods with the use of commercial kits (Pars Azmun, Iran), using the BT-3000 auto-analyzer machine (Biotechnical, Rome, Italy). The assay range, intra- inter-assay coefficients of variation (CV) of the kit for BUN were 0.9–60 mg/dL, 3.27%, and 4.17%, respectively and read at 340 nm wavelength based on the manufacturer instruction. The kit's assay range and intra-inter-assay CV for Cr were 0.2–15 mg/dL, 3.22%, and 1.78% respectively and read at 500 nm wavelength based on the manufacturer instruction. The intra-inter-assay CV as well as the sensitivity of the kit for LDH were 2.52%, 1.73% and 5 U/L, respectively and read at 340 nm wavelength based on the manufacturer instruction.

To measure the total antioxidant capacity (TAC) and oxidant status (TOS) (Novin Navand Salamat Pishtaz Co., Iran),

glutathione (GSH), C-reactive protein (CRP) and interleukin (IL)-6 (Invitrogen, California, USA), the serums were transferred to ELISA reader (BioteTek ELx808, USA). The sensitivity, intra-assay, and inter-assay CV of the kit for TOS were 0.023 U/mL, 4.5%, and 3.6% respectively and read at 530 nm wavelength based on the manufacturer instruction. Respectively, the kit's intra-inter assay and sensitivity for TAC were 3.7%, 2.5% and 2 mmol/L, and read at 593 nm wavelength based on the manufacturer instruction. Respectively also, the kit's intra-inter assay and sensitivity for GSH were 3.4%, 9.73% and 0.634 μM, and read at 405 nm wavelength based on the manufacturer instruction. The kit's intra-inter assay and sensitivity for the rats' IL-6 were <5%, <10% and 12 pg/mL, respectively and read at 450 nm wavelength based upon the manufacturer instruction. The kit's intra-inter assay and sensitivity for CRP of the rats were respectively <10%, <12% and 200 pg/mL and read at 450 nm wavelength according to the instruction of the manufacturer.

Histopathological staining

Prior to the implementation of eosin and hematoxylin staining, from the kidney tissues, 5 μ-size sections were deparaffinized and rehydrated. The reagents and stains were bought from Sigma (St. Louis, MO, USA). An Olympus BX-50 microscope, with the integration of a color digital camera (Olympus, Japan, DP-72), was utilized for observing the histological sections. Protective effects of hUCBS on kidney were quantitatively assessed by measuring the glomerular surface area (GA), glomerular volume (GV) and number of proximal tubular cell nuclei (PTN). For each group, 20 superficial cortical glomeruli were selected according to the following criteria: 1) presence of afferent or efferent arterioles at the glomerular vascular pole; 2) presence of the beginning of proximal convoluted tubule at the glomerular urinary pole. GA was measured by outlining a perimeter along the glomerular periphery (Gilbert *et al.* 1991) using image analysis software (Image J v1.53). Assuming glomeruli as spheres, we calculated GV using the following formula: $GV = 1.2545 \times GA$ (Rangan and Tesch 2007). PTN was counted in 10 proximal convoluted tubular sections close to the renal corpuscles on which the glomerular measurements were performed. The numbers of epithelial cells were calculated as number of nuclei per 100 μm of perimeter of proximal convoluted tubules using Olympus DP2-BSW application software (v2.2). The images were all taken with a magnification of ×400 from H&E-stained renal tissue sections.

Statistical analyses

The presentation of the data was done as mean ± standard deviation (SD). Shapiro-Wilk test was adopted for checking the normality of data. Version eight of GraphPad Prism software was used for the data analysis and one-way ANOVA was utilized for the determination of significance level. The Tukey *post hoc* test was adopted in case a significant level was found. 0.05 was deemed the level of significance for the interpretation of the results. For the microscopic scrutiny, the extracted slides with the magnification at 400 were analyzed.

Table 1. Comparison of the serum BUN, Creatinine (Cr), and LDH level in the control, GM 100, hUCBS 1%, and hUCBS 2% groups of male Wistar rats.

Experimental groups	BUN (mg/dL)	Cr (mg/dL)	LDH (U/L)
Control	23.29 ± 4.89	0.90 ± 0.43	109.1 ± 10.42
GM 100	57.00 ± 10.20[b]	3.86 ± 0.82[b]	483.9 ± 198.3[b]
GM 100 + hUCBS 1%	43.00 ± 8.10[a]	2.73 ± 0.75[a]	444.6 ± 48.25[b]
GM 100 + hUCBS 2%	31.00 ± 3.37[d]	2.16 ± 0.46[c]	191.4 ± 82.96[d,e]

Data are shown as mean ± SD of seven male Wistar rats per group. [a]$p < 0.01$; [b]$p < 0.001$; vs. control; [c]$p < 0.05$; [d]$p < 0.001$; vs. GM 100; [e]$p < 0.05$ vs. GM 100 + hUCBS 1% group. BUN: blood urea nitrogen, LDH: lactate dehydrogenase, GM: gentamicin, hUCBS: human umbilical cord blood serum.

Result

The effects of hUCBS on the biochemical parameters

Table 1 displays the alterations in the levels of serum in the biochemical parameters after being treated with either hUCBS or GM. A significant increase ($p < 0.001$) was identified in degree of BUN in the group receiving gentamicin. Compared with the GM group, the hUCBS (2%) treatment group demonstrated a significant decline ($p < 0.001$). The amount of Cr in the group receiving gentamicin showed a significant increase ($p < 0.001$). In the experimental group receiving 1% of hUCBS, Cr did not demonstrate a significant decrease compared with that of the gentamicin-receiving group. However, compared with the GM group, a significant decline was shown in the experimental group receiving 2% of hUCBS ($p < 0.05$). A significant elevation in the LDH enzyme level of the gentamicin receiving group was witnessed ($p < 0.001$). Compared with that of the control group, in the experimental group receiving 1% of hUCBS, the enzyme level did not show significant changes. Moreover, hUCBS at higher a dosage (i.e., 2%) resulted in a significant decline in comparison to the gentamicin group ($p < 0.001$).

The effect of hUCBS on the oxidative damage

The serum levels evaluation of TOS showed that gentamicin elevated the rats' enzyme levels in a significant manner. Compared with the GM group, treatment with hUCBS 1% significantly decreased TOS. As displayed in Figure 1(A), TOS in the group receiving a higher dose of hUCBS (e.g., 2%) experienced a significant decrease in comparison to the group receiving gentamicin ($p < 0.001$). In the tested rats, the levels of TAC serum were shown to indicate a significant decrease due to gentamicin ($p < 0.001$). As regards by Figure 1(B), the group receiving hUCBS at a dose of 2% also experienced significant increase in these levels in comparison to the GM receiving group ($p < 0.05$) The amount of GSH in the group receiving GM showed a significant decline ($p < 0.001$). Compared with the group receiving GM, the treatment with the doses of 1 and 2% of hUCBS revealed a significant increase ($p < 0.05$ and $p < 0.01$, respectively).

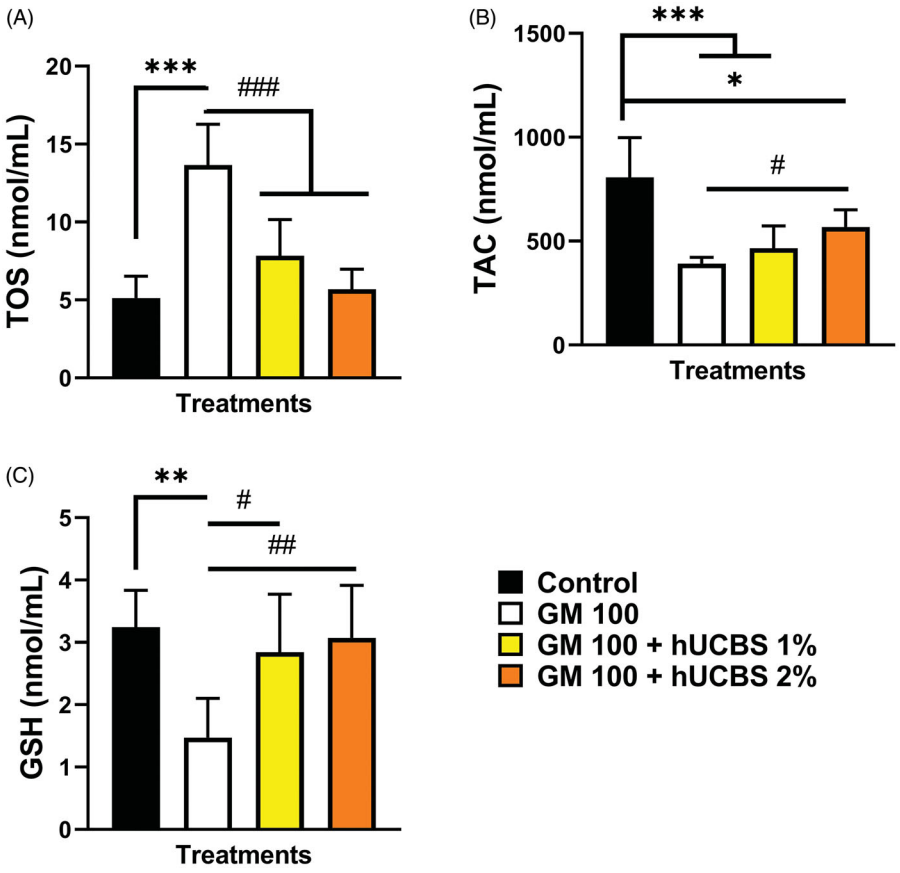

Figure 1. Comparison of serum levels of total oxidant status (TOS) (A), total antioxidant capacity (TAC) (B), and glutathione (GSH) (C) in all groups. Each bar presents the mean ± SD pertaining to seven male Wistar rats. Signs give us the significant meaningfulness in changing the levels of TOS, TAC, and GSH after the treatments. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$; *vs.* control; #$p < 0.05$; ##$p < 0.01$; ###$p < 0.001$; *vs.* GM 100 group.

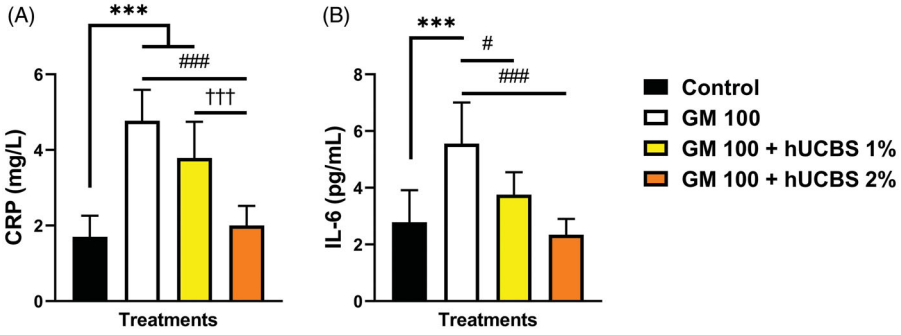

Figure 2. Comparison of serum levels of C-reactive protein (CRP) (A) and IL-6 (B) in different experimental groups. Each bar represents the mean ± SD levels pertaining to 7 male Wistar rats. Signs signal the significant meaningfulness in changing the levels of CRP and IL-6 after the treatments. $^{***}p < 0.001$; *vs.* control; #$p < 0.05$; ###$p < 0.001$; *vs.* GM 100; †††$p < 0.001$; *vs.* GM 100 + hUCBS 1% group.

hUCBS effects on the inflammatory cytokines

The results on CRP serum levels of the rats unveiled that gentamicin elevated the levels of CRP in a significant manner. In comparison with the GM group, 1% of hUCBS failed to decrease CRP levels. Nonetheless, as exhibited in Figure 2(A), two percent dose of hUCBS decreased the levels in a significant manner in comparison with the gentamicin group. The findings on the levels of IL-6 serum in the tested rats demonstrated that whereas gentamicin in the rats' treatment significantly raises the IL-6 serum levels, hUCBS reduces their IL-6 serum levels significantly. It was also shown that the rats' levels of IL-6 serum were decreased significantly in the group receiving hUCBS at a higher dosage compared with the ones that took the lower dosage (see Figure 2(B)).

Histopathological results

As displayed in Figure 3(A), the histopathological inspections on the slides of the tissue in the group of control tell us that different sections of the tissue in the kidney include normal glomeruli, renal tubules and blood vessels, without any signs of necrosis, inflammation or the presence of lymphocyte in the renal tissue. As exhibited in Figure 3(B), examining the kidney tissue in the gentamicin group microscopically, we

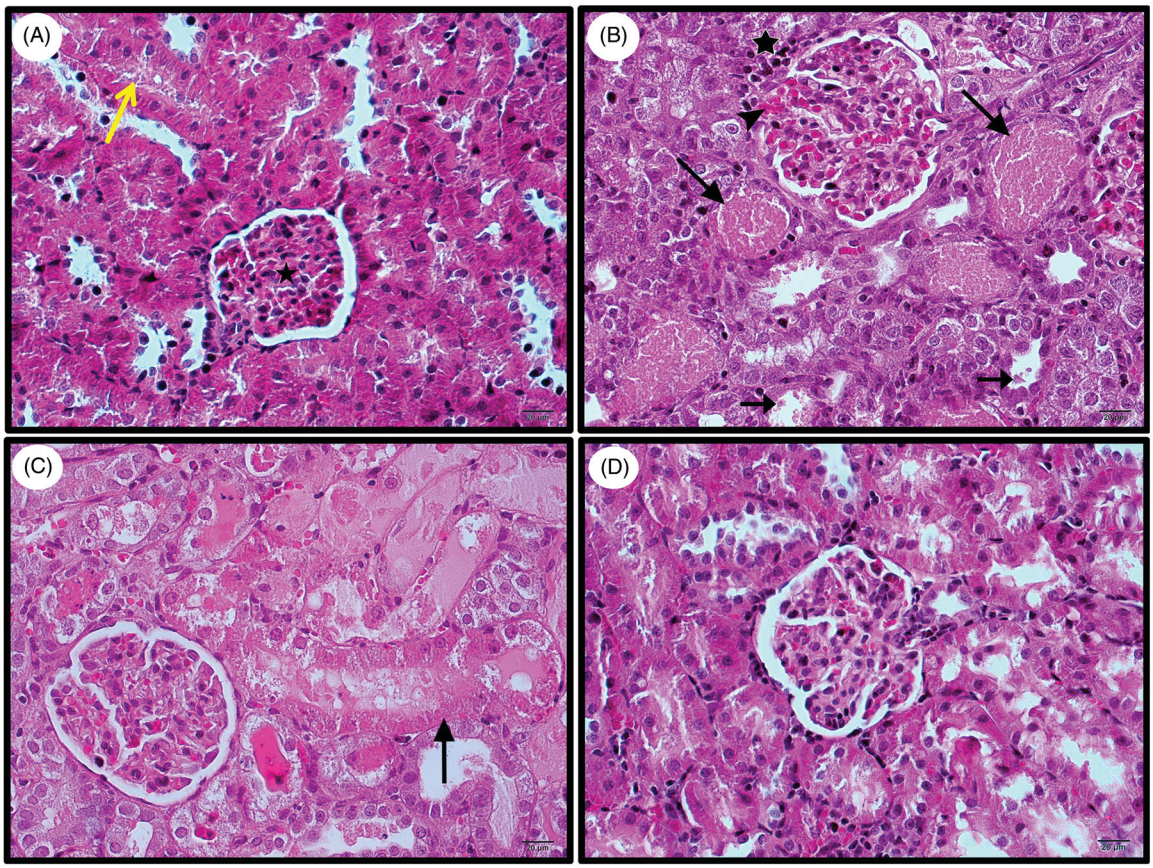

Figure 3. Micrographs of renal sections both treated and normal rats. (A) Tissue section of kidney in the group of control. Glomeruli (asterisk), renal tubes (yellow arrow) of the kidney cortex are normal. Proximal and distal convoluted tubules are without any disruption; (B) Tissue section of GM group. Diffuse coagulative necrosis of proximal convoluted tubular epithelium (long arrows), intra-tubular eosinophilic hyaline cast in distal convoluted tubules (short arrows), glomerular capillary congestion (arrowhead), and peritubular infiltration of lymphocyte (asterisk) seen; (C) Tissue section of the experimental hUCBS 1% group. Tissue necrosis observed in the proximal convoluted tubules (arrow) and lymphocyte infiltration in the interstitial space; (D) Tissue section prepared from the kidney of group receiving 2% of hUCBS. The cellular scaffold is reaching normal. The necrosis rate has declined. Infiltration of lymphocytic has disappeared and inflammation has decreased. Hematoxylin-eosin staining with 400× magnification.

Table 2. Glomerular surface area (GA), glomerular volume (GV) and number of proximal tubular cell nuclei (PTN) per 100 μm of tubular perimeter in different treatment groups of male Wistar rats.

Experimental groups	GA (μm^2)	GV (μm^3)	PTN (per 100 μm)
Control	7463 ± 315.2	9362 ± 395.3	6.31 ± 0.522
GM 100	8175 ± 489.0[a]	10255 ± 613.4[a]	4.24 ± 0.558[a]
GM 100 + hUCBS 1%	7819 ± 388.5	9808 ± 487.3	4.78 ± 0.397[a]
GM 100 + hUCBS 2%	7637 ± 559.4[b]	9580 ± 701.8[b]	5.86 ± 0.409[c,d]

Data are shown as mean ± SD. [a]$p < 0.001$; vs. control; [b]$p < 0.01$, [c]$p < 0.001$; vs. GM 100; [d]$p < 0.001$ vs. GM 100 + hUCBS 1% group. Abbreviations: GM: gentamicin, hUCBS: human umbilical cord blood serum.

observed that gentamicin shrank glomeruli and caused necrosis in the proximal convoluted tubules; GM also resulted in an extensive influx of inflammatory cells (lymphocyte) amongst the renal tubes. Renal histology inspections in the group receiving 1% of hUCBS showed the convoluted tubular cells' necrosis and leukocytes' infiltration in the interstitial space; that said, compared to the gentamicin group, tissue damage was observed to a lesser degree (Figure 3(C)). The histology on the kidneys of the experimental group receiving 2% of hUCBS exhibited that the protection of tissue was performed satisfactorily against the damage inflicted by GM; moreover, no shrinkages of the glomeruli and tubular necrosis or inflammatory cell aggregations were witnessed (Figure 3(D)). As shown in Table 2, GA and GV were highest in GM-treated group due to the hypertrophy of glomerular tuft compared with those of the control group ($p < 0.001$). These scores reached a normal level after treatment with hUCBS. On the other hand, PTN per 100 μm of the tubular perimeter was lowest in the GM-treated group in comparison with the group of control ($p < 0.001$). Compared with the gentamicin group, treatment with hUCBS at a dose of 2% resulted in a significant increase ($p < 0.001$).

Discussion

The findings of the present study exhibited that GM increased the levels of serum in BUN and Cr. These results are in harmony with those obtained in previous studies on GM-induced nephrotoxicity (Rafieian-Kopaei *et al.* 2013). Moreover, the serum levels of renal injury-determining enzymes such as Cr and BUN diminished compared to those of the gentamicin group. In comparison to that of the GM group, LDH – an index of tissue necrosis (Kumar *et al.* 2018)—also was decreased significantly. This specific result is in line with that of El-Kashef *et al.* (2015) that showed that the consecutive administration of GM for seven days would produce a significant rise in the activity of Cr, BUN, and LDH. Treatment with hUCBS leads to an improvement in renal

biochemical BUN, Cr and LDH. This result is consistent with that of Li P *et al.* (2015). They reported that the administration of multipotent human umbilical cord mesenchymal stem cells (HUC-MSCs) significantly decreased the damage indexes of serum creatine kinase, and LDH in comparison to those of the GM-treated group.

The current study assessed the reno-protective influences of hUCBS by evaluating the oxidative stress markers such as TOS, TAC, and GSH in the serum of GM-treated rats. It appeared that the kidneys in the rats treated with GM are more vulnerable to ROS injury due to the antioxidant enzymes being insufficient (Vysakh *et al.* 2018). GM induces the production of hydrogen peroxide in the mitochondria of the rat kidney cortex and increases the production of ROS (Li PKT *et al.* 2013). GM also induces nephrotoxicity through mechanisms such as the induction of oxidative stress, apoptosis, and necrosis (Lopez-Novoa *et al.* 2011). The decreased levels of antioxidant and increased levels of oxidant enzymes in the kidney of gentamicin-induced rats (TOS, TAC, and GSH) were restored by hUCBS treatment. These impacts might boil down to higher hUCBS antioxidant activities. Thus, along with the production of ROS, hUCB can be used as the cogent free radical scavenger for the treatment of a variety of maladies (Ebrahimi *et al.* 2018, Yao *et al.* 2019).

The role of inflammatory factors such as CRP and IL-6 in the pathology of GM-induced nephrotoxicity was also investigated in the present study. The results from this study show that CRP and IL-6 serum levels increased in the rats treated with GM. Treatment with hUCBS restores the increased CRP and IL-6 levels to a normal state. Studies have shown that the ROS production enhances the nuclear factor kappa B's (NF-κB) activation (González-Ramos *et al.* 2012). The NF-κB mediated regulation of genes involved in the proliferation of cells, production of cytokines and inflammation is also well-established. Oxidative stress has a crucial role in the elevated NF-κB expression of the rats treated by GM. The increased NF-κB expression could consequently result in the induction of the synthesis in other inflammatory-associated molecules that boost renal injures (Karimi *et al.* 2020). Therefore, at least in part, hUCBS's anti-inflammatory action might be helpful in protecting the kidney from pernicious side effects induced by gentamicin.

GM increases kidney tissue damages including the shrinkage in glomeruli and necrosis in the renal tubules. Damage to glomeruli and renal tubules during GM-induced acute tubular necrosis process causes ductal obstruction by necrotic cells due to the retention of the nitrogenous substances such as urea and Cr (Rafieian-Kopaei *et al.* 2013, Karimi *et al.* 2020). The findings in the current paper demonstrate that the renal tissue damage was decreased in the hUCBS group *vis-à-vis* the GM group. These effects are probably due to anti-inflammatory and antioxidant features of hUCBS. Based on the results, it seems that hUCBS improves renal function by inhibiting renal and endothelial cell death and increasing angiogenesis thanks to its antioxidant and anti-inflammatory properties. Furthermore, hUCBS lacks any cells and its use does not pose a risk for tumor or cell mass formation. In addition, due to the absence of cells lacking any antigens, whether membrane or cellular antigens, it may be a new therapeutic option in kidney disease. Another advantage of hUCBS is that it is easy to prepare, store and maintain for clinical applications.

Conclusion

The present research demonstrated that hUCBS plays a protective role against the GM-induced kidney damage that might be pertaining to its antioxidant and anti-inflammatory properties; hUCBS could also ameliorate the complications, and might be able to regenerate the renal biochemical and morphology to a normal state. These results may pave the way for randomized clinical trials on hUCBS, which will help researchers to usher in new treatments for patients with renal dysfunction in the future.

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Ethical approval

All experiments in this study regarding the animal rights and conservation were performed according to the standard ethical guidelines (European Communities Directive 2010/63/EU) and were endorsed by the University of Bu-Ali Sina's Local Ethics Committee (permit No.: IR.BASU.REC.1398.035).

Disclosure statement

The authors wish to declare that they have no conflict of interest.

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Data availability statement

The data that support the findings of the research are available and can be attained from the corresponding author, upon reasonable request.

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