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Renal Protective Effects of Soy Isoflavones Compared to Carvedilol in Rat Models of Glycerol Induced Acute Renal Injury

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Ethics statement. All the animal experiments were carried out in accordance with guidelines evaluated and approved by the National Institute of Health (86/609/EEC) Guidelines for using Laboratory Animals. Furthermore, College of Pharmacy / University of Basrah / Ethical Committee approval number 3/5/414, October 2022 were obtained.
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Abstract

Introduction. Acute kidney injury (AKI) is a thoughtful clinical problem with a high degree of morbidity and mortality. Therefore, searching for medicinal products with pronounced therapeutic and prophylactic effects in acute renal pathology appears to be relevant.
Purpose. To investigate possible protective effect of soy isoflavones genistein (GN) and daidzein (DZ) compared to carvedilol (CV) against glycerol induced AKI in rats.
Materials and methods. A total of 30 adult female Wister rats were divided into five groups. Group 1 (6 rats) represented the control. Group 2 (6 rats) represented the glycerol-induced ARI model, Groups 2 (6 rats), 3 (6 rats), 4 (6 rats) and 5 (6 rats) rats were administered 8 ml/kg water, 2.5 mg/kg CV, 21.7 mg/kg GN and 17.4 mg/kg DZ respectively, orally daily for 14 days and after that animals of the last four groups were deprived from water for 24h, and then administered hypertonic solution (8 ml/kg body weight/day) IM for 3 days to induce ARI. Fourth day, the animals sacrificed, blood were collected for biochemical tests and kidneys for histopathological analysis.
Results. No deaths or changes in animals appearance were reported throughout the study. Rises in body weight was significant in all groups, except AKI induced group. Urinary β_2-microglobulin, urine albumin, BUN and serum creatinine were increased in the induced group compared to control and preserved normal in pretreatment groups with CV, GN and DZ. Histopathology results of rat kidneys are consistent with the biochemical results.
Conclusion. Glycerol induces AKI and treatment with CV, GN and DZ reversed the adverse effects. Therefore, CV drug and soy isoflavones (GN and DZ) have a renal protective effect; however, CV and GN have more pronounced protective effect than DZ. It was shown that soya proteins might be of health values in recovering patients with acute kidney injury induced by rhabdomyolysis.
Keywords: carvedilol, genistein, daidzein, kidney histopathology, soy isoflavones

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Протекторное действие изофлавонов сои в сравнении с аналогичным влиянием карведилола при остром глицерол-индуцированном повреждении почек у крыс

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Этическое заявление. Все эксперименты на животных проводились в соответствии с рекомендациями, представленными в Руководстве по использованию лабораторных животных Национального института здравоохранения (86/609/EEC). Кроме того, было получено одобрение этического комитета Фармацевтического колледжа Университета Басры (№ 3/5/414, октябрь 2022 г.).
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Резюме

Введение. Острое повреждение почек (ОПП) – опасная клиническая патология с высокой степенью заболеваемости и смертности. В связи с этим представляется актуальным поиск лекарственных средств, оказывающих выраженный лечебно-профилактический эффект при острой ренальной патологии.
Цель. Исследовать возможный протективный эффект изофлавонов сои (GN и DZ) в сравнении с таковым карведилола (CV) на модели глицерол-индуцированного ОПП у крыс.
Материалы и методы. Объектом исследования явились взрослые крысы линии Вистар (30 самок), контингент которых включал 5 групп. Группа 1 (6 самок) была контрольной. Каждая же из последующих четырех групп включала в себя лабораторных животных, которым накануне моделирования ОПП производилось введение ежесуточно на протяжении 14 суток: воды из расчета 8 мл/кг массы тела (группа № 2 – 6 самок), карведилола (CV) – 2,5 мг/кг массы тела (группа № 3 – 6 самок), изофлавона GN (5,7-dihydroxy-8-[4-hydroxyphenyl]chromen-4-one) – 21,7 мг/кг (группа № 4 – 6 самок), изофлавона DZ (4,7 Dihydroxyisoflavone) – 17,4 мг/кг (группа № 5 – 6 самок). После этого животных последних четырех групп лишали воды на 24 часа, затем им вводили гипертонический раствор глицерола (8 мл/кг массы тела в день) в течение 3 сут. внутримышечно (или ВМ) для индуцирования ОПП. На четвертые сутки животных умерщвляли, кровь отбирали на биохимический анализ, а почки подвергали гистопатологическому анализу.
Результаты. В продолжение всего эксперимента не было зарегистрировано ни одного случая гибели или изменения внешнего вида животных. Увеличение массы тела было достоверно значимым во всех группах, кроме группы индуцированного ОПП. Уровень β_2-миоглобина в моче, альбумина в моче, остаточного азота и креатинина в сыворотке крови был повышен в группе индуцированного ОПП по сравнению с

контрольной группой и оставался в пределах нормы в группах предварительного лечения CV, GN и DZ. Результаты гистопатологического исследования почек крыс совпали с результатами биохимических исследований.
Заключение. Глицерин индуцирует ОПП, а профилактическая терапия препаратами CV, GN и DZ устраняет связанные с повреждением почек неблагоприятные явления. Таким образом, и карведилол (CV), и изофлавоны сои (GN и DZ) оказывают нефропротекторное действие, однако CV и GN обладают более выраженным защитным эффектом, чем DZ. Показано, что протеины сои могут быть полезны для выздоровления пациентов с острым повреждением почек, вызванным распадом мышечной ткани (рабдомиолизом).
Ключевые слова: карведилол, генистеин, дайдзеин, гистопатология почек, изофлавоны сои

■ INTRODUCTION

A clinical case called rhabdomyolysis produced from an acute skeletal muscle disturbance that caused by chemical or physical damaging due to comprehensive exercise, injury, extreme temperature or due to toxins [1]. Skeletal muscle damage causes the myoglobin and another cellular proteins to leak from the cells to the circulation resulting in myoglobinuria, that can lead to acute kidney injury (AKI) if it is severe. About 10% of the AKI developed from rhabdomyolysis [2].

Treatment with glycerol causes skeletal muscle disturbance that can be used as an investigational model mimics the case of rhabdomyolysis that induces AKI in the human [3]. Pathophysiology of AKI induced by glycerol revealed variant mechanisms, specifically oxidative stress, apoptosis and inflammatory factors. Skeletal muscle damage causes the myoglobin and another cellular proteins to leak from the cells to the circulation causing myoglobin concentrated in the urinary tubules and subsequent leak of the ROS that lead to tubules injury [4]. Furthermore, increase of macrophage numbers [5] and the production of cytokines for instance tumour necrosis-factor-α (TNF-α) [6] and subsequently, TNF-α causes the release of IL-6 in abundant amounts by the endothelium cells [7].

Genestein (GN) and Daidzein (Dz) are the principal isoflavone phytoestrogens in the soy products, their structures are similar to 17-β-oestradiol structure and produces oestrogenic actions [8–10]. Soybean, is a legume species, which is a natural isoflavonoid type and the soy benefits have been studied clinically, with some indication related to decreased occurrences of atherosclerosis, coronary – heart disease and type II DM [11]. Furthermore, soy isoflavones can decrease the risk of numerous chronic diseases, including some cancers, especially BC, and prostate adenocarcinoma [12, 13].

Studies revealed that soy isoflavones show antioxidant effect in both of in vivo and in vitro. They have a direct effect on scavenging free radicals, mainly effective are GN and DZ, which are exposed to stop the oxidative damage of DNA and reduces the lipid peroxidation [14]. Furthermore, they can result in reduction of the oxidative cell damage by an indirect effects like the induction of ant oxidation enzymes [14].

Carvedilol (CV) is a non-selective, third-generation β-blocker having α1-adrenergic blockade action [15]. It is prescribed for the management of the essential hypertension, heart failure, and also for myocardial infarction [16]. It was indicated that CV causes

vasodilation effect mediated by both of α1-adrenergic blocking and endothelial release of NO [17]. It was established that CV possess antioxidant, anti-ischemic, anti-inflammatory and anti-proliferative effects, in addition to Ca2+ antagonistic effect [18]. It was founded that CV improves hemodynamics and decreases vascular resistance of the renal system [19]. Previous study in rats indicated that administration of CV (2.5 mg/kg/day, orally) has a renal protective effect against the rhabdomyolysis induced by glycerol, mimicking to AKI [20].

■ PURPOSE OF THE STUDY

To investigate possible protective effect of soy isoflavones genistein (GN) and daidzein (DZ) compared to carvedilol (CV) against glycerol induced AKI in rats.

■ MATERIALS AND METHODS

Materials and animals

Hypertonic Glycerol solution (50% v/v in normal saline), CV obtained from Denk pharma (Germany) that dissolved in distilled water. GN (5,7-dihydroxy-8-[4-hydroxyphenyl] chromen-4-one) and DZ (4,7 Dihydroxyisoflavone) powders both from axenic research and formulation materials, China. GN and DZ were dissolved in distilled water. Thirty adults female Wister rats weighing 150–250 g were utilized in the present study. Rats obtained from college of veterinary medicine/university of basrah and acclimated for a period of one week before starting experiment. Each three animals caged in a plastic cage with standard bedding. Standard food pellets and water were supplied ad libitum unless otherwise stated.

Experiment

Thirty adult female rats equally divided into five groups of 6 rats each. Animal's weights measured at the beginning and the end of experiment. Group 1 rats represent the control group, administered distilled water (8 ml/kg body weight) / day orally by gavage for 20 days. Group 2 rats represent the glycerol-induced ARI model, Groups 2, 3, 4 and 5 rats administered 8 ml/kg body weight water, 2.5 mg/kg body weight CV, 21.7 mg/kg body weight GN and 17.4 mg/kg body weight DZ respectively, daily for 14 days orally by gavage and after that animals of the last four groups deprived from water for 24 h then administered hypertonic solution (8 ml/kg body weight/day) IM for 3 days to induce ARI. The injected volume was divided equally between the two hind limbs.

Urine samples from the control and treatments groups were collected in sterile Petri dishes, preserved in Eppendorf tubes and frozen until analysis for the selected biomarker. After that, the animals were sacrificed on day 20 (at the end of experiment and 24h after last dose) and the blood samples were taken by intracardiac puncture and the sera collected by centrifugation at a speed of 1000 rpm for 10 min. Sera were kept for biochemical tests at – 20 °C. Kidneys from rats in all groups removed and washed with saline solution (0.9%), then separated longitudinally into two parts and fixed in 10% formalin for histopathological examination.

Biochemical analysis

Urine samples were centrifuged for 20 min at 2000 rev/min. The supernatant was carefully collected according to the manufacturer's instructions in order to test for

β2-microglobulin using a rat ELISA kit (Shanghai YL Biotech Company, China). The serum urea and creatinine concentrations were determined using a diagnostic automated laboratory analyzer per the manufacturer's instructions (Abbott Architect 4000c, USA).

Spectrophotometric analysis was used for kidney function tests (serum urea and creatinine) Renal function tests, includes urinary β_2-microglobulin, urine albumin, blood urea nitrogen (BUN) and serum creatinine, were determined by colorimetric methods using kits supplied by Spinreact (Girona, Spain) and spectrum (Cairo, Egypt), respectively, according to the manufacturer's instructions.

Histopathological investigation

Histopathological investigation was prepared by fixing the tissues in 10% solution of formalin for about 4 days, after that the tissues prepared as sections, and covered with paraffin. The size of cut of histological pieces is 4 to 5 µm, stained by routine hematoxylin (H) and eosin (E) stains. After staining, the sections were examined under a light dissection microscope.

Following extraction, the right kidneys were fixed in a 10% formalin solution, dehydrated, and then embedded in paraffin for histological analysis. For structural characterization of renal impairment, 4–5 µm histological slices were cut using a microtome, followed by dewaxing and hematoxylin and eosin staining (H and E). An electronic light microscope with a 20 × magnification was utilized for this purpose.

Statistical analysis

Statistics achieved using Graph Pad Prism software (version 7.0, Inc., San Diego, CA). Descriptive data presented statistically as mean ± SEM for wholly estimated parameters. One-way analysis of variance (ANOVA) and Tuckey's Multiple Comparison tests used for comparison between groups, p-values less than 0.05 considered as significantly different.

■ RESULTS

This study exposed no deaths in all experiment groups during the study period and no alteration in the general appearance of animals was reported. The animal's weights measured at the beginning and the end of experiment. Rises in body weight appear significant in all groups, except the AKI induced group as illustrated in fig. 1.

Renal function

As illustrated in Fig. 2, induction of AKI in female rats using a single dose of glycerol distinctly elevate the normal urinary β_2-microglobulin level on the 2nd day of glycerol administration and increased urine albumin, BUN and serum creatinine compared to control. Pretreatment of rats with 2.5 mg/kg/day CV, 21.7 mg/kg/day GN and 17.4 mg/kg/day DZ preserved the normal levels of urinary β_2-microglobulin, urine albumin, BUN and serum creatinine.

Histopathological investigation of kidney

Control or normal rats had normal glomeruli and tubules (Fig. 3a), whereas rats of AKI induced group showed enlargement, bleeding and necrosis of the epithelial cells lining glomeruli and renal tubules, atrophy in the glomerular capillary tuff and dilation of renal tubules. In addition to sloughing and bleeding in cortical tubules (Fig. 3b). Rats

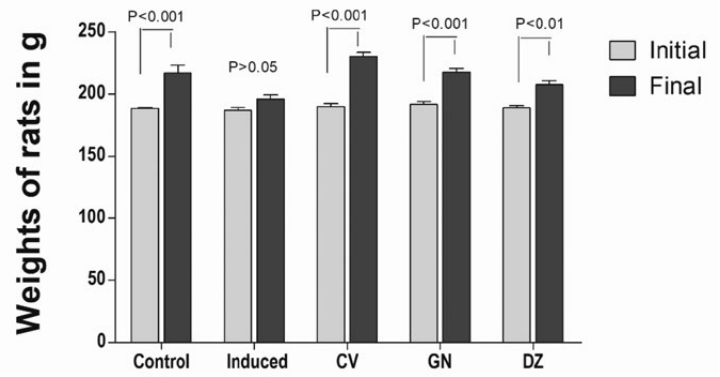

Fig. 1. Changes in animal's body weight in different experiment groups. All data showed as mean ± SEM. Grey columns signify weights of rats at the start point of experiment while black columns represent weights of rats at the end after treatment with 2.5 mg/kg/day Carvedilol (CV), 21.7 mg/kg/day Genestein (GN) and 17.4 mg/kg/day Daidzein (DZ). P<0.05 is significant difference

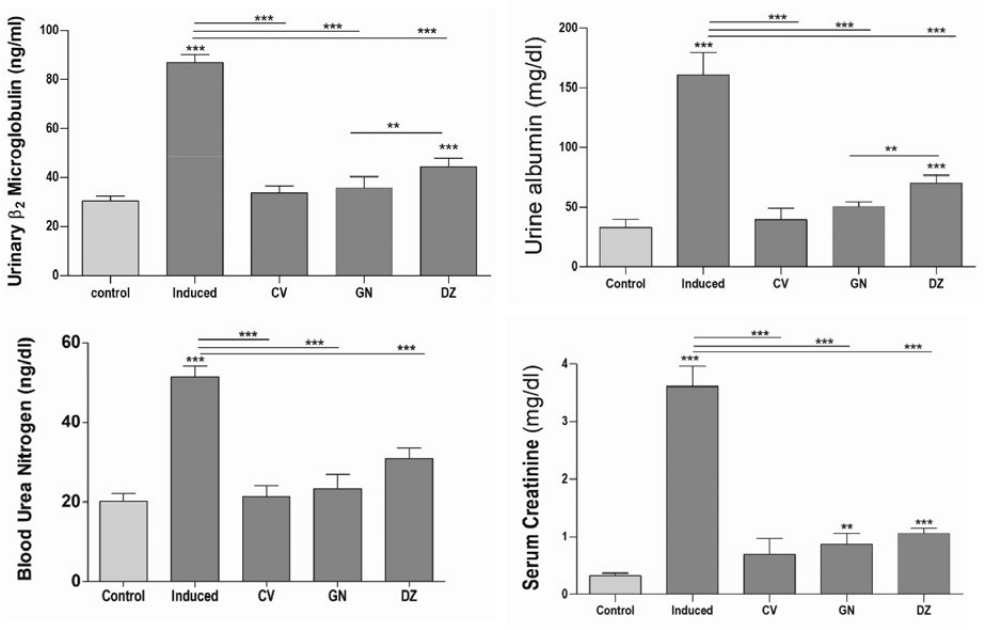

Fig. 2. Influence of treatment with 2.5 mg/kg/day Carvedilol (CV), 21.7 mg/kg/day Genestein (GN) and 17.4 mg/kg/day Daidzein (DZ) on urinary β2-microglobulin, urine albumin, BUN and serum creatinine

Notes: * represents significant difference P<0.05 among groups; ** represent significant difference P<0.001 among groups.

treated with 2.5 mg/kg/day CV displayed health and normal structure of glomeruli and renal tubules (Fig. 3c), while rat treated with 21.7 mg/kg/day GN displayed normal structure of glomeruli and dilated renal tubules with vaculation (Fig. 3d). Rats treated with 17.4 mg/kg/day DZ displayed the epithelium of tubules ragged due to the degeneration

of the cells lining atrophied renal tubules, in addition to shrinkage of glomeruli with dilatation of Bowman's space (Fig. 3e).

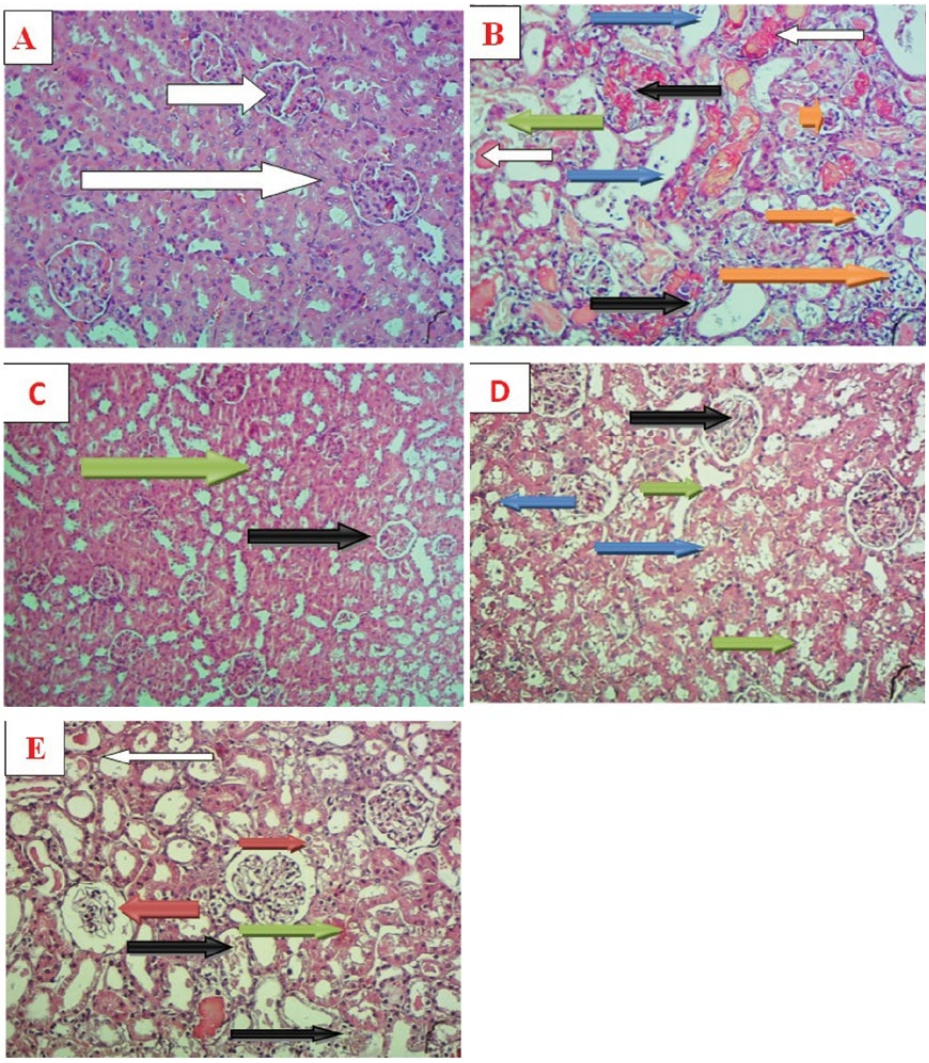

Fig. 3. Light micrographic section of female rat kidney (stained by the H and the E) 20X. A – Control: displays normal glomeruli and tubules structures (thick arrow). B – AKI induced group showed enlargement, bleeding in glomeruli (black arrow), necrosis (orange arrows) of the epithelial cells lining glomeruli and renal tubules, atrophy in the glomerular capillary tuff (green arrows) and dilation of renal tubules (blue arrow), also, sloughing and bleeding in cortical tubules was noted (white arrow). C – Carvedilol (CV) 2.5 mg/kg/day treatment group displayed health and normal structure of glomeruli (black arrow) and renal tubules (green arrow). D – Genestein (GN) 21.7 mg/kg/day treatment group displayed normal structure of glomeruli (black arrow) and dilated renal tubules with vaculation (blue arrow). E – Daidzein (DZ) 17.4 mg/kg/day treatment shows the epithelium of tubules ragged due to the degeneration of the cells lining atrophied renal tubules (black arrow), hemorrhage (green arrow) dilated in tubules (white arrow) and shrinkage of glomeruli with dilatation of Bowman's space (red arrow)

■ DISCUSSION

Kidneys are the important organ in the human body, it is required to maintain homeostasis, regulate extracellular fluid and to release the medications and toxic substances [21], a huge number of external toxicants can injured the kidneys, affecting their function causes acute damage of tubules, acute nephritis or chronic intoxication [22]. This study aimed to evaluate the effects of soy isoflavones (GN and DZ) compared to CV on renal biochemical functions and histopathology on the rhabdomyolysis induced by glycerol, mimicking to AKI, in female rats.

Rises in body weight appear significant in all groups, except the AKI induced group and this may be caused by oxidative stress in AKI induced group [23]. The significant increase in body weights of rats treated with CV are in line with a study revealed that CV attenuated cachexia in patients of chronic cases of severe heart failure [24]. Furthermore, rises in body weights of rats treated with soy isoflavones (GN and DZ), which are phytoestrogens with neuroprotective and antioxidant effects [8–11] observed reliable with the study on fat Zucker rats [25].

Induction of AKI in female rats using a single dose of glycerol distinctly increases urinary β_2-microglobulin level and this increase may be related to either renal vessels constriction, obstruction of renal tubules or oxidative stress [23]. Furthermore, urine albumin, BUN and serum creatinine were elevated compared to control and this elevation because they are protein breakdown waste excreted by kidneys, indicates renal injury by glycerol. Serum creatinine level depends on the amount of muscle mass, gender and age [26]. These issues were constant, as rats were the same weight, sex and all rats had limited movement. Pretreatment of rats with CV, preserved the normal levels of urinary β_2-microglobulin, urine albumin, BUN and serum creatinine compared to glycerol group. These results are in line with previous study indicated renal protective effect of CV against rhabdomyolysis induced by glycerol [27] and they explained this protection effect related to the antioxidant activity of CV, numerous studies reported the antioxidant activity of CV [28, 29]. Moreover, pretreatment with soy isoflavones, also preserved the normal levels of urinary β_2-microglobulin, urine albumin, BUN and serum creatinine compared to glycerol group and these results are in line with previous studies stated that soy milk has Reno protective in animal models of renal disease [30, 31]. These protective effects may be related to the effects of soy phytochemicals on enzymes gene expression, which can increase the antioxidant activity [32].

Kidney histopathological findings revealed that rats treated with CV, GN and DZ displayed health and normal structure of glomeruli and renal tubules and restored the kidney disrupted by glycerol, confirmed that all of these treatments have a renal protective effect, however, CV and GN have more renal protective effect than DZ. These results are consistent with the results of biochemical analysis.

■ CONCLUSION

This study indicated that glycerol induces AKI and that CV, GN and DZ reversed all the adverse effects. Therefore, our findings illustrates that CV drug and soy isoflavones (GN and DZ) have a renal protective effect, however, CV and GN have more renal protective effect than DZ. Soya proteins might be of health values to rhabdomyolysis induced AKI patients.

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