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Anti-inflammatory and antioxidant activity of essential amino acid α-ketoacid analogues against renal ischemia–reperfusion damage in Wistar rats

α-ketoacid analogues in renal ischemia reperfusion

Actividad anti inflamatoria y antioxidante de α-cetoanálogos de aminoácidos esenciales en un modelo de daño por isquemia reperfusión en ratas Wistar

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Introduction: Essential amino acid α-keto acid analogues (EAAs) are used in the treatment of chronic kidney disease (CKD) to delay the symptoms of uremia. However, it is unknown whether EAAs affect oxidative stress and inflammation present in acute renal injury, such as that produced by ischemia-reperfusion (I/R).

Objective: The purpose of this study was to evaluate the effect of EAAs on renal I/R injury in Wistar rats.

Materials and methods: Rats were divided into 11 groups (*n* = 6/group): two groups received physiological saline with or without I/R injury (45 min/24 h), six groups received EAAs (400, 800, or 1200 mg/kg/24 h/7d) with or without I/R injury (EAAs+I/R), and two groups received allopurinol (ALO) (50 mg/kg/24 h/7d) with or without I/R injury. Biochemical markers included creatinine and blood urea nitrogen (BUN), proinflammatory cytokines (IL-1β, IL-6, and TNF-α), renal damage markers (cystatin C, KIM-1, and NGAL), and markers of oxidative stress such as malondialdehyde (MDA) and total antioxidant activity (AOxT).

Results: The EAAs- and ALO-treated groups had lower levels of creatinine, BUN, renal damage markers, proinflammatory cytokines, and MDA than their corresponding I/R groups; these changes related to the EAAs dosage. AOxT was lower in EAAs- and ALO-treated groups than in corresponding I/R groups.

Conclusions: This is the first report of the nephroprotective effects of EAAs against I/R injury. EAAs decreased the levels of biochemical markers, kidney injury markers, proinflammatory cytokines, and MDA while minimizing total antioxidant consumption.

Keywords: Ischemia; reperfusion injury; renal insufficiency, chronic; inflammation; oxidative stress; models, theoretical.

Introducción. Los α-cetoanálogos de aminoácidos esenciales (EAAs) se utilizan en el tratamiento de la enfermedad renal crónica (ERC) para retrasar los síntomas de la uremia. Sin embargo, se desconoce si los EAAs afectan el estrés oxidativo y la inflamación presentes en la lesión renal aguda como en la producida por isquemia-reperfusión (I/R).

Objetivo. Evaluar el efecto de las EAAs sobre la lesión renal por I/R en ratas Wistar.

Materiales y métodos. Se emplearon 11 grupos de ratas (n= 6): dos grupos recibieron solución salina fisiológica con o sin lesión I/R (45 min/24 h), seis grupos recibieron EAAs (400, 800 o 1200 mg/kg/24). h/7d) con o sin lesión I/R (EAAs + I/R), y dos grupos recibieron alopurinol (ALO) (50 mg/kg/24 h/7d) con o sin lesión I/R. Los marcadores bioquímicos incluyeron creatinina y nitrógeno ureico en sangre (BUN), citoquinas proinflamatorias (IL-1β, IL-6 y TNF-α), marcadores de daño renal (cistatina C, KIM-1 y NGAL) y marcadores de estrés oxidativo como malondialdehído (MDA) y la actividad antioxidante total (AOxT).

Resultados. Los grupos tratados con EAAs y ALO tuvieron niveles inferiores de creatinina, BUN, marcadores de daño renal, citocinas proinflamatorias, AOxT y MDA que los grupos I/R correspondientes fue menor en los grupos con EAAs y ALO que en los grupos I/R correspondientes.

Conclusiones. Este es el primer informe de los efectos nefroprotectores de las EAAs contra la lesión I/R. Los EAAs disminuyeron los niveles de marcadores bioquímicos, marcadores de lesión renal, citoquinas proinflamatorias y MDA, a la vez que minimizan el consumo total de antioxidantes.

Palabras clave: isquemia; daño por reperfusión; insuficiencia renal crónica; inflamación; estrés oxidativo; modelos teóricos.

Essential amino acid ⟨-keto acid analogues (EAAs) are used as conservative treatment of chronic kidney disease (CKD) with the intention of reducing the production of metabolites of nitrogen degradation and thereby improving the glomerular filtration rate and delaying the need for substitution treatment (1). The beneficial effect of EAAs along with a low-protein diet in treating CKD was first described in the 1970s (2), and this treatment is still used. A recent meta-analysis reported that EAAs can delay the progression of CKD by limiting hyperphosphatemia, preventing hyperparathyroidism, and improving the control of arterial blood pressure and malnutrition. EAAs are recommended in the treatment of stages 3 to 5 CKD (3).

However, it is not clear whether EAAs have other beneficial effects, especially in the early stages of CKD or in acute kidney injury (AKI). AKI can be caused by acute tubular necrosis as a result of various types of ischemia. Renal ischemia is observed in a variety of clinical situations, such as recovery after cardiac arrest, liver and kidney transplantation, and partial nephrectomy. AKI observed after ischemia is characterized by decreased glomerular filtration rate, tubular necrosis, and increased renal vascular resistance. These changes can cause damage to tubular cells through the release of reactive oxygen species (ROS) and nitric oxide, and increased intracellular calcium concentration, which can in turn trigger mitochondrial damage through the depletion of antioxidants, cell-mediated cytotoxicity, and an inflammatory response (4). These processes are similar to those occurring in CKD, in which a series of events triggers the process of fibrosis or scarring through a cytokine-mediated inflammatory response, which can activate extracellular matrix-producing cells in the glomerulus and tubules, and lead to the repair or scarring of various renal components (5).

Ischemia–reperfusion (I/R) injury occurs when the blood supply is cut off for a prolonged period of time and is then suddenly perfused with oxygenated blood (6). This process is characterized by the release of ROS and an intense inflammatory response provoked by the cellular response to the damaged tissue. I/R injury can be studied in animal models involving bilateral ischemia or heminephrectomy followed by ischemia of the remaining kidney. Bilateral ischemia is the preferred model because of its similarity to the pathological process in humans (7,8). Heminephrectomy has been used to evaluate the nephroprotective activity of drugs such as allopurinol (ALO), rosuvastatin, aliskiren and rutin that are considered as nephroprotective (8-11).

EAAs may provide new agents for nephroprotection strategies, mainly for treating AKI and the early stages of CKD. Understanding whether EAAs have nephroprotective activity is the first step in developing new therapeutic strategies for the use of EAAs as more than a nutritional supplement. The principal objective of this study was to evaluate the effects of EAAs on the inflammatory response and oxidative stress in a model of renal damage caused by I/R injury in Wistar rats. As secondary objectives, it was initially evaluated to establish the dose to be used in order not to generate hepatotoxicity or nephrotoxicity through biochemical markers and renal damage, based on this established dose, the nephroprotective effect was evaluated through biochemical markers, proinflammatory mediators and oxidative stress markers. Finally, the effect of EAAs on various typical markers of renal damage and histological changes in renal architecture post I/R with and without administration of the selected dose of EAAs was evaluated.

Materials and methods

Animals

Experiments were performed using Wistar rats weighing 250–300 g in the Pharmacology and Toxicology Department of the Medicine Faculty at the Universidad Autónoma de Nuevo León, Mexico. All animals were maintained in polypropylene cages at a standard temperature (23-27°C) with 12 h light/dark periods and *ad libitum* access to water and standard food for rodents (Prolab diet 2500). Every experiment complied with the Mexican Official Norm NOM-062-ZOO-1999 specifications and was approved by the committee for the institutional care and use of laboratory animals with the registration number (HI17-00001).

Experimental design

The animals were randomly divided into 11 groups with six rats in each group. The first five groups were used for evaluation of toxicity and groups 6 to 11 were used to evaluate nephroprotection. All doses evaluated were adjusted to a volume of 0.5 mL (figure 1).

Chemicals

Saline solution (CS PISA® solution, GDL México, sodium chloride 0.9%), alopurinol (Zyloprim® tablets, Aspen Port Elizabeth Pty, Ltd, Reg. No. 86270 SSA IV), EAAs (Cetolán Laboratorios Columbia® Reg. No. 122M2016 SSA IV), xylazine (Sedaject, Vedilab S.A. de C.V. Reg. SAGARPA Q-0088-122), ketamine (Anesket, PiSA Agropecuaria, S.A. de C.V. Reg. SAGARPA Q7833-028)

The rats were anesthetized with xylazine (10 mg/kg, intraperitoneally (ip) and ketamine (100 mg/kg, ip). A small incision was made at the midline of the abdomen, through which the renal pedicle was occluded with microvascular clamps, and adequate occlusion was verified by cyanotic changes in both kidneys. The microvascular clamps were removed

after 45 min to induce reperfusion, which was confirmed by observation of blood flow restoration. The abdominal incision was closed with a 4-0 suture. All experiments were performed under aseptic conditions. The animals were monitored for 24 h and were allowed *ad libitum* access to water and food. Rats of sham group received the same surgical procedure but without I/R. Every animal was euthanized, and blood and kidney tissue samples were obtained for evaluation.

Blood and tissue samples

Blood was collected from each animal, allowed to clot, and centrifuged at 3,500 rpm for 15 min, and the serum was separated for later measurement of the concentrations of proinflammatory cytokines, biochemical markers, and biomarkers of kidney injury. The kidneys were removed and divided longitudinally. One side was placed in a cryotube and immediately frozen in dry ice for later measurement of the concentrations of oxidative stress markers. The other side was fixed with formaldehyde in phosphate buffer (pH 7.4) for 24 h, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) to evaluate the histological alterations.

Measurement of proinflammatory cytokine concentrations

Serum concentrations of the proinflammatory cytokines interleukin 6 (IL-6), interleukin 1-beta (IL-1β), and tumor necrosis factor-alpha (TNF-α) were measured using commercial sandwich ELISA kits (Rat IL-6 ELISA Development Kit, Rat IL-1β ELISA Development Kit, and Rat TNF-α ELISA Development Kit, respectively, PeproTech, Mexico City, México). Avidin–peroxidase and 2,2-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid were used as the chromogen, and the absorbance was measured by spectrophotometry at 405 nm.

Biochemical markers

The serum concentrations of total proteins (TPs), albumin (Alb), urea nitrogen (BUN), creatinine (Creat), uric acid (UA), aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP) were measured using an ILAB-Aries self-analyzer spectrophotometer using diagnostic kits (Instrumentation Laboratory, Milan, Italy) according to the supplier's specifications.

Kidney injury biomarkers

The serum levels of cystatin C (CysC), neutrophil gelatinase associated lipocalin (NGAL), and kidney injury molecule-1 (KIM-1) were measured using commercial ELISA-sandwich kits (Quantikine ELISA, Mouse/Rat Cystatin C Immunoassay, R&D Systems, (Minneapolis, MN, USA), Lipocalin-2 (NGAL) Rat ELISA Kit, Abcam 119602, Cambridge, MA, USA, KIM-1 (TIM-1) Rat ELISA Kit, Abcam 119597, respectively). These were analyzed by spectrophotometry at 450 nm.

Oxidative stress markers

Malondialdehyde (MDA) concentration was measured using a thiobarbituric acid-reactive substance (TBARS) assay using a TBARS Assay Kit (Cayman Chemical Co., Ann Arbor, MI, USA). The reaction product, which is proportional to the concentration of MDA in the sample, was measured spectrophotometrically at 540 nm. The total antioxidant activity (AOxT) was measured in homogenized kidney tissue using a commercial kit (Antioxidant Assay Kit, Cayman Chemical Co.). AOxT activity included both enzymatic and nonenzymatic activities'and was measured by spectrophotometry at 405 nm.

Statistical analysis

The results are expressed as mean ± standard deviation (SD). The homogeneity of variance was first established, and variables with a normal distribution were analyzed

using analysis of variance with multiple group comparisons using Dunnett's test and Prism software (v. 7.0; GraphPad, San Diego, CA, USA). A *p*-value of <0.05 was considered to be significant.

Results

Evaluation of toxicity

EAAs did not cause liver or renal toxicity when given at the different doses. The only markers that differed significantly between some of the study groups and the sham group were BUN, Creat, NGAL, IL-1□, and MDA levels (table 1, figure 2).

Effects of EAAs on damage induced by I/R injury

Biochemical markers

Compared with the levels in the saline group, the levels of some biomarkers were significantly elevated in the saline +I/R group: Creat, BUN, and ALT (figure 3). The levels of other biomarkers were significantly lower in the saline+ I/R group than in the salinegroup: TPs, Alb, and UA. Compared with the saline+I/R group, groups ALO50+I/R, EAAs-800+I/R, and EAAs-1200+I/R had significantly lower Creat and BUN levels. Group EAAs-400+I/R also had lower Creat and BUN levels, but the difference was significant only for Creat level. Of the other biochemical parameters, only ALT concentration was significantly lower in the EAAs-1200+IR group than in the saline+I/R group (figure 3).

Kidney injury biomarkers

The levels of renal damage indicators (CysC, KIM-1, and NGAL) were significantly higher in the saline+I/R group than in the salinegroup. These levels were significantly lower in the groups treated with ALO50 and EAAs at various doses than in the saline+I/R group (figure 4).

Proinflammatory cytokines

Compared with the saline group, the saline+I/R group has significantly higher serum levels of the proinflammatory cytokines IL-1β and TNF-α. Control group showed a tendency to increase IL-6 levels compared to sham group. Cytokine levels were significantly lower in the ALO50+IR group than in the saline+I/R group. A similar pattern was seen in groups treated with EAAs at various doses (figure 5).

Oxidative stress markers

AOxT was significantly higher in the saline+I/R group than in the salinegroup. AOxT was significantly lower in the ALO50+I/R and EAAs+I/R groups than in the saline+I/R group, and this effect increased with the dose of EAAs. The MDA level was significantly higher in the saline+I/R group than in the saline-only group. The increment in MDA level was significantly smaller in the ALO50+I/R and EAAs+I/R groups than in the saline+I/R group, and this effect increased with the dose of EAAs (fFigure 6).

Histological analysis

The saline-treated group showed a normal renal parenchyma and preserved tubules and glomeruli, whereas the I/R group showed necrosis of the tubular epithelium in 90% of the medulla and 80% of the cortex. The ALO50+I/R group showed ischemic necrosis in 50% of the cortex and acute necrosis of the distal convoluted tubule The other EAAs+IR groups showed similar effects as those observed in the ALO50+I/R group (figure 7).

Discussion

CKD is currently considered to be a threat to global health as the number of patients increases rapidly (12). CKD is characterized by the development of glomerulosclerosis and interstitial fibrosis, and involves the interactions of various factors such as angiotensin II, growth factors, cytokines, and oxygen metabolites (13).

The restriction of proteins in the diet is an important therapy for people with CKD. Keto acid (KA) effects have been evaluated in patients with CKD on a protein-restricted diet (14) but not in AKI patients. One study compared the efficacy of a low-supplemented protein diet (LPD) with keto acids (LPD+KA) and an LPD alone in stopping the development of kidney injury associated with CKD in a 5/6 nephrectomy model in Sprague Dawley rats (15). This animal model of CKD is characterized by proteinuria, decreased renal function, glomerular sclerosis, and tubulointerstitial fibrosis. Protein restriction decreased the extent of these changes. The effect was greater in the LPD+KA group than in the LPD group, and the LPD+KA group had lower serum levels of BUN and Creat compared with the LPD group (15).In our current study, animals treated with various doses of EAAs also had lower BUN and creatinine levels, and this effect was dose dependent, in addition to presenting normal liver enzyme levels so it was shown that at these doses the EAAs were neither nephrotoxic or hepatotoxic. However, the levels of Alb, TPs, and UA did not decrease. These findings suggest that EAAs may have protected the kidneys from progressive injury by correcting the protein malnutrition in the remnant kidney tissue.

On the other hand , in the renal I/R model, the levels of mediators of kidney damage, such as MCP-1, KIM-1, and CysC increase with reperfusion time (16). We found that the levels of some of these damage mediators, such as KIM-1, CysC, and NGAL, decreased considerably in the EAAs-treated groups that received the I/R injury. These data support the nephroprotective effect of EAAs.

CKD can be exacerbated by oxidative stress, which promotes the production of reactive carbonyl compounds and lipoperoxides that lead to advanced glycosylation accumulation and lipoxidation. ROS react with nitric oxide to produce reactive cytotoxic nitrogen species that are capable of producing nitrate proteins that can damage other molecules (17).

Oxidative stress plays an important role in the development and progression of sclerosis and fibrosis in the remnant tissue in models of chronic renal failure (17,18). A low-protein diet, with or without KAs, has an antioxidant effect in humans with CKD and animal models of CKD (18,19). Previous studies in animals have found that EAAs combined with a low-protein diet prevents weight loss, normalizes Alb levels, maintains nutritional status, and improves the protein malnutrition and injury caused by oxidative stress in the remnant kidney tissue (18). In addition, in rats submitted to 5/6 nephrectomy, the application of advanced oxidation protein products, which are associated with deterioration of renal function, imposes greater oxidative stress during the fibrotic process (15). Recently the nephroprotective effect of EEAs in early stages of diabetic nephropathy type 2 was described, mediated by the inhibition of oxidative stress through the decrease of MDA and increase of superoxido dismutase (20). In our study, we induced kidney damage in Wistar rats by I/R and found that EAAs modified the levels of oxidative stress markers, as shown by the attenuated damage in the renal cortex and decreased AOxT and production of MDA in EAAs/I/R rats compared with I/R rats. Again, these effects were EAAs dose dependent. The factors that initiate the cascade of cell damage and the inflammatory response after I/R leading to kidney damage are not completely understood. Increases in protein concentration have been described, such as the high-mobility group box-1 protein, which is released by kidney cells (particularly vascular and tubular cells) into the venous circulation after renal I/R damage. This protein induces a rapid release of cytokines (TNF-α, G-CSF, IFN-Y, IL-10, IL-1β, IL-6) into the systemic circulation (21,22). One study has reported decreases in the levels of IL-1β, IL-6, and TNF-α in patients receiving peritoneal dialysis and treated with KAs (18). In a rat experimental model with 5/6 nephrectomy treated with KAs, IL-1β and IL-6 levels increased but TNF-α and IL-18 levels did not

change significantly (15). We also observed decreases in the levels of IL-1β, IL-6, and TNF-α and the kidney damage markers CysC, KIM, and NGAL in the EAAs-treated groups; this effect was dependent on the dose of EAAs. These findings suggest that EAAs decreased the inflammatory response to I/R injury.

The inflammatory response is extremely important in the development of kidney damage; I/R models are relevant when wanting to demonstrate an anti-inflammatory effect resulting in delayed kidney damage. This has been demonstrated in several studies, such as the one published by Mori da Cunha, where in their I/R model they demonstrate a nephroprotective effect of the application of amniotic fluid stem cells with increased regulation of vascular endothelial growth factor, which is dose-dependent (23). Other studies have also demonstrated the relationship of other inflammatory markers in the use of EAAs, for example those involved in mineral and bone metabolism disorders mediated by FGF-23 and Klotho involved in inflammation, oxidative stress and energetic protein malnutrition in stages 3b and 4 of chronic renal disease, associated with low-protein diet. The nephroprotective effect observed in our study, in acute damage, has not been previously reported. This study opens up the possibility of EAAs efficacy in both acute and chronic CKD damage (24).

In conclusion, we show for the first time that EAAs have a nephroprotective effect against renal I/R damage. EAAs decreased the levels of biochemical markers, markers of kidney damage, proinflammatory cytokines, and MDA, and this effect was related to the dose of EAAs. EAAs exerted these protective effects while avoiding the consumption of total antioxidants. There is the possibility of a continuous protective effect, even without a restriction of proteins, which would strengthen its use in early stages of chronic kidney disease as well as its potential application in acute kidney damage and transplantation of

solid organs to reduce injury by I/R or improve the response in non-optimal organs, so studies are required to evidence such applications.

Conflict of interest

The authors declare no conflict of interest

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Table 1. Changes in the seric levels of biochemical markers of kidney damage and proinflammatory cytokines in the toxicity evaluation groups.

Variable	Sham	ALO 50 mg/kg	EAAs 400 mg/Kg	EAAs 800 mg/Kg	EAAs 1200 mg/Kg
CysC (ng/mL)	381,30±37,85	350,80±24,38	287,00±19,07	346,80±23,04	395,50±26,54
KIM–1 (pg/mL)	24,96±9,85	38,98±2,91	26,28±6,40	53,53±21,07	29,23±8,06
NGAL (pg/mL)	113,3±15,4	169,5±13,1*	159,0±11,9	104,0±5,5	170,0±14,1*
IL-1β (ng/mL)	0,35±0,19	0,41±0,09	0,82±0,06*	0,46±0,06	0,93±0,13*
IL-6 (ng/mL)	1,25±0,25	1,36±0,31	1,33±0,15	1,92±0,38	0,92±0,29
FNTα (ng/mL)	1,05±0,28	0,79±0,14	0,93±0,25	1,14±0,23	0,82±0,20
MDA (uM)	0,75±0,30	3,50±0,34***	1,46±0,25	1,96±0,39*	1,97±0,27*
AOxT (mM)	2,59±0,10	2,63±0,13	2,78±0,06	2,72±0,08	2,58±0,11

ALO: allopurinol; EAAs: Essential amino acid α ketoacid analogues; CysC: Cystatin C; KIM-1: kidney injury molecule-1; NGAL: neutrophil gelatinase associated lipocalin; IL-1β: interleukin 1 beta; IL-6: interleukin 6; FNT-α: tumor necrosis factor alpha; MDA: malondialdehyde; AOxT: activity of the total antioxidants. Values are expressed as mean+SD, Sham group vs. study groups. * $p < 0,05$, ** $p < 0,01$, *** $p < 0,001$.

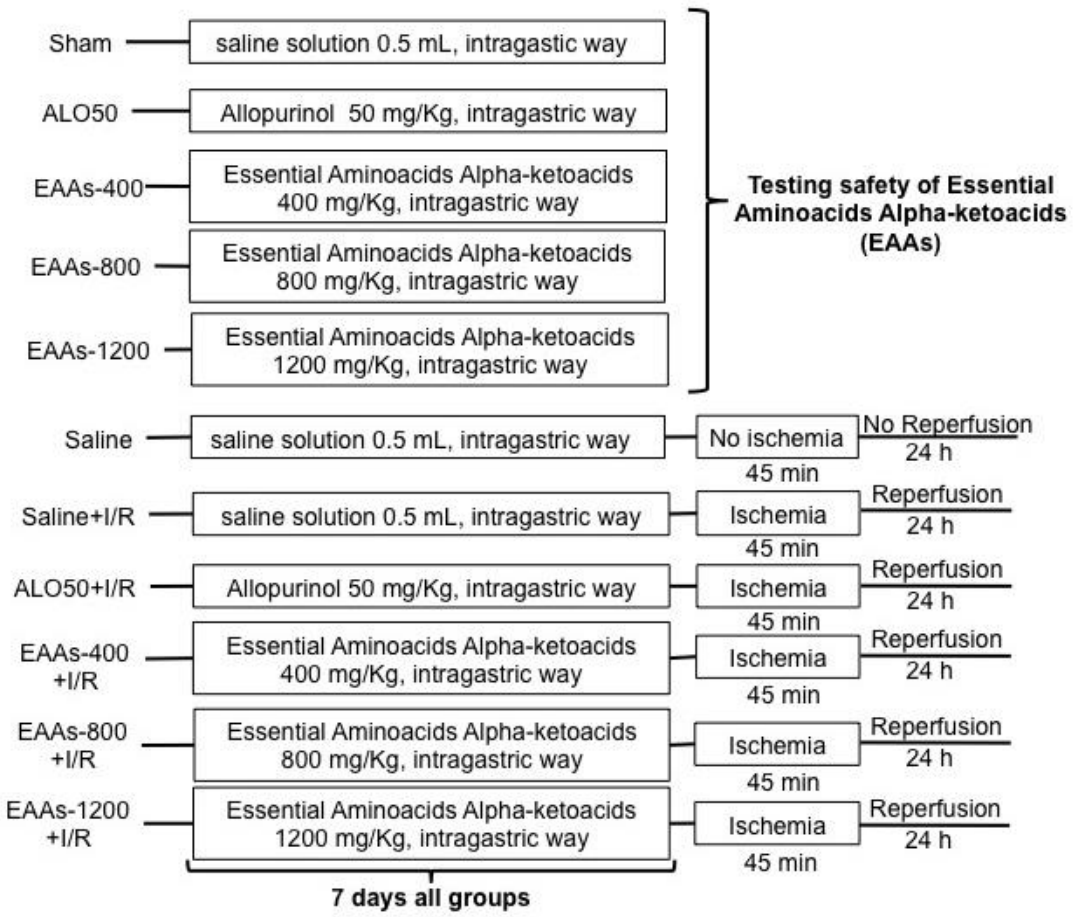

Figure 1. Experimental design of the study groups in the timeline.

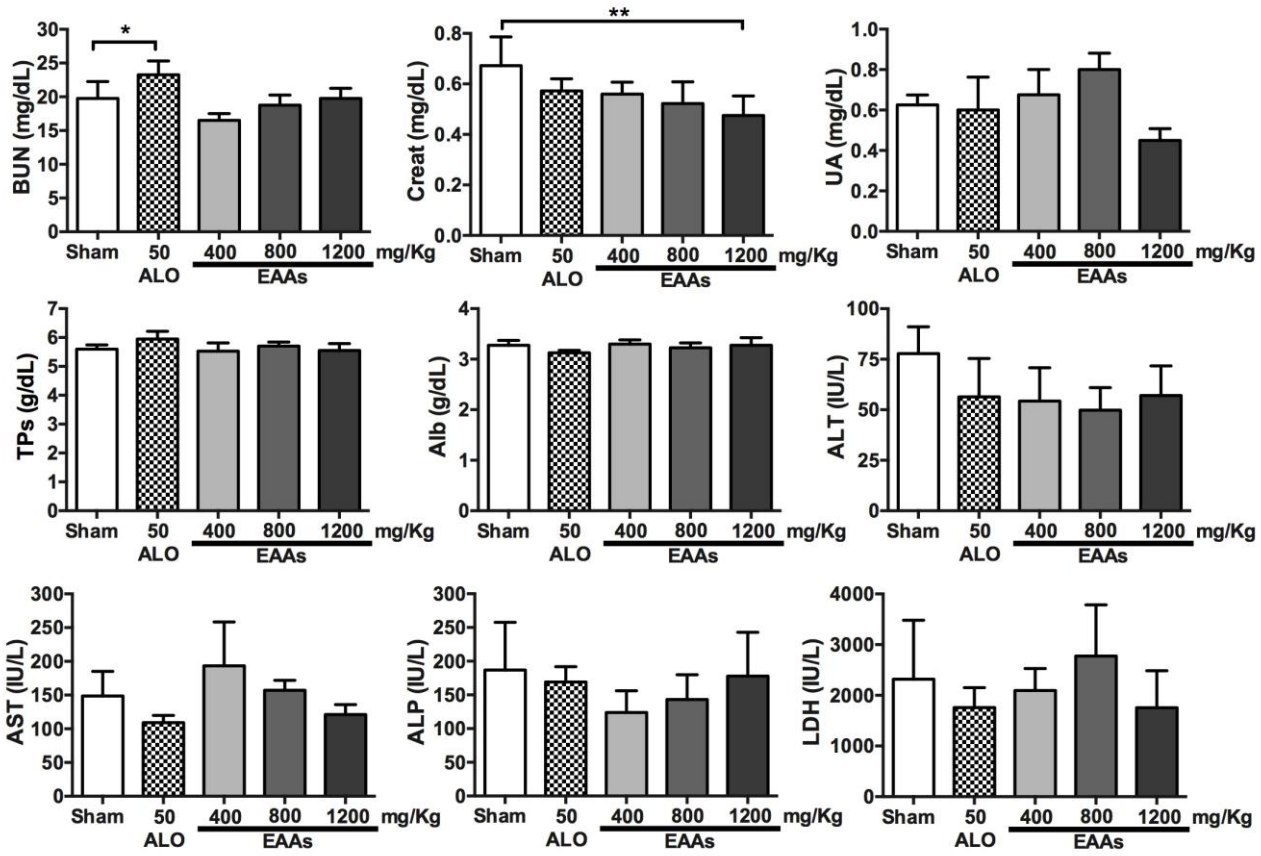

Figure 2. Changes in the seric levels of biochemical markers in toxicity study depending of treated groups (2-5) and sham control (Group 1). BUN: blood urea nitrogen; Creat: creatinine; UA: uric acid; TPs: total proteins; Alb: albumin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; LDH: lactate dehydrogenase.; I/R: Ischemia-reperfusion; ALO: Allopurinol; EAAs: Essential amino acid α-ketoacid analogues. Values are expressed as mean+SD. * p <0,05, ** p <0,01.

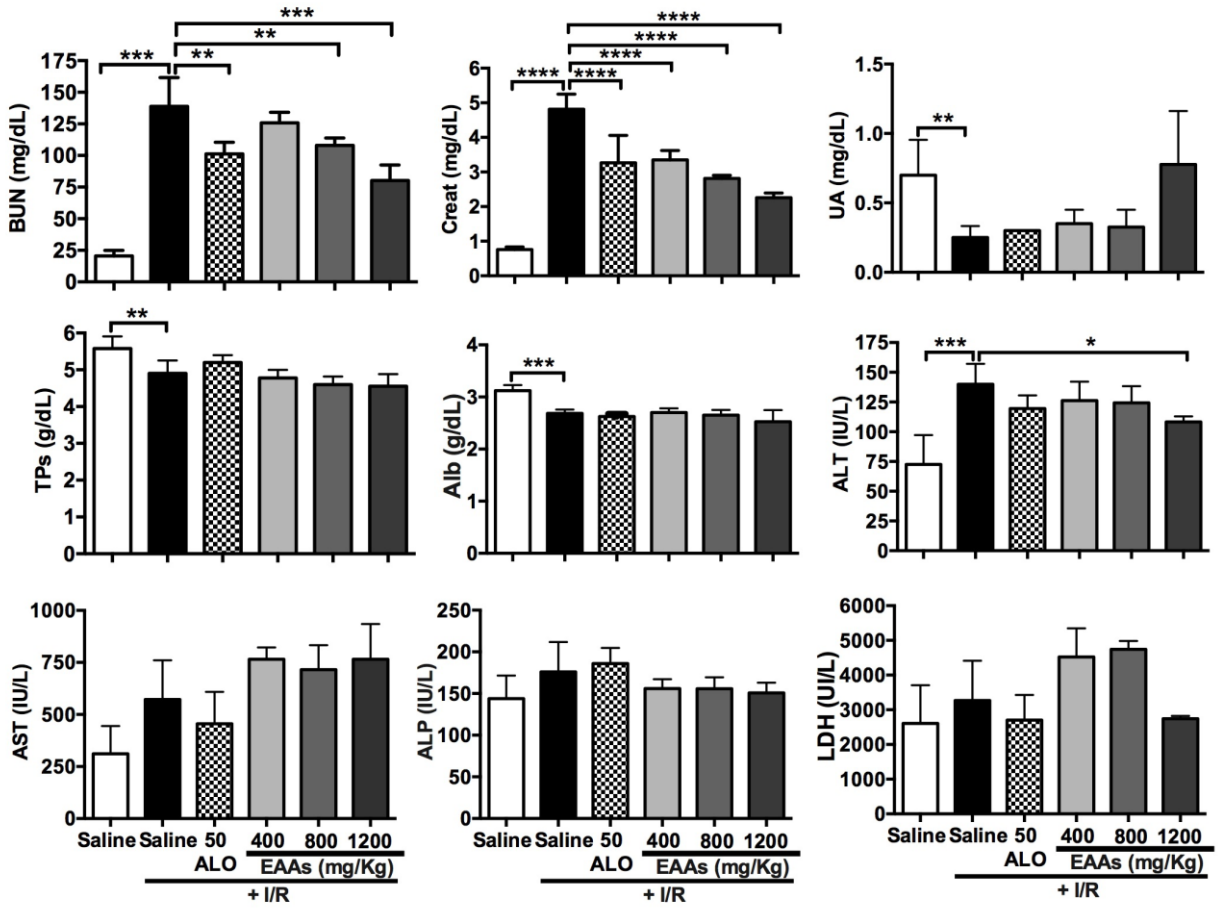

Figure3. Changes in serum levels of biomarkers in the study of nephroprotection. BUN: blood urea nitrogen; Creat: creatinine; UA: uric acid; TPs: total proteins; Alb: albumin; ALT: alanine aminotransferase; AST: aspartate aminotransferase; ALP: alkaline phosphatase; LDH: lactate dehydrogenase.; I/R: Ischemia-reperfusion; ALO: Allopurinol; EAAs: Essential amino acid α-ketoacid analogues. Saline group +I/R (7) vs. sham group (6) and treated groups (8-11)

Values are expressed as mean+SD. * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

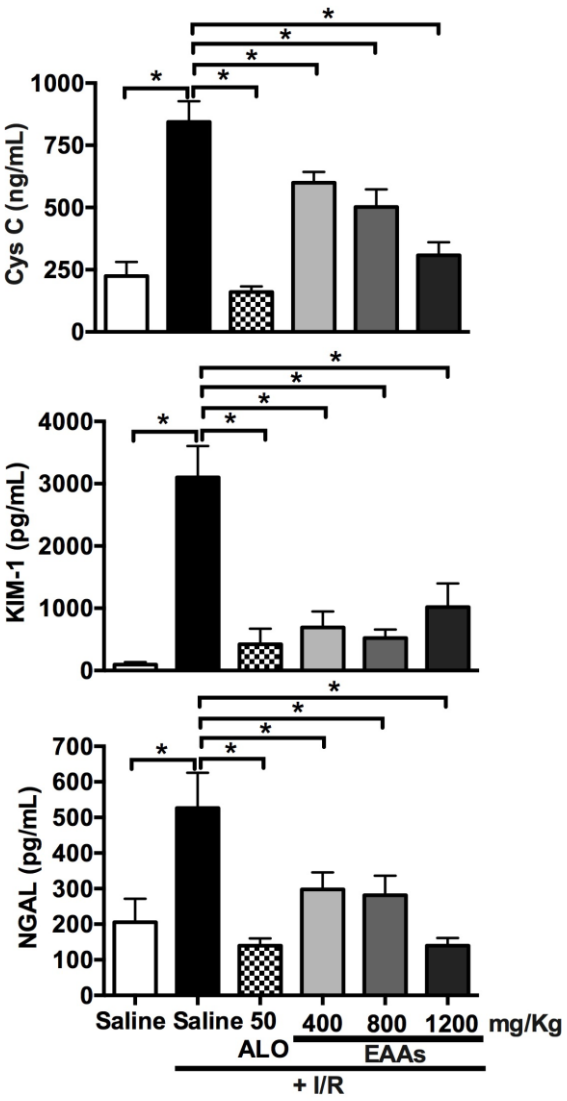

Figure 4. Changes in kidney injury biomarkers in the nephroprotection study . CysC: Cystatin C; KIM-1: kidney injury molecule-1; NGAL: neutrophil gelatinase associated lipocalin.; I/R: Ischemia-reperfusion; ALO: Allopurinol; EAAs: Essential amino acid α-ketoacid analogues. Saline group +I/R (7) vs. sham group (6) and treated groups (8-11). Values are expressed as mean+SD. .* $p<0.0001$.

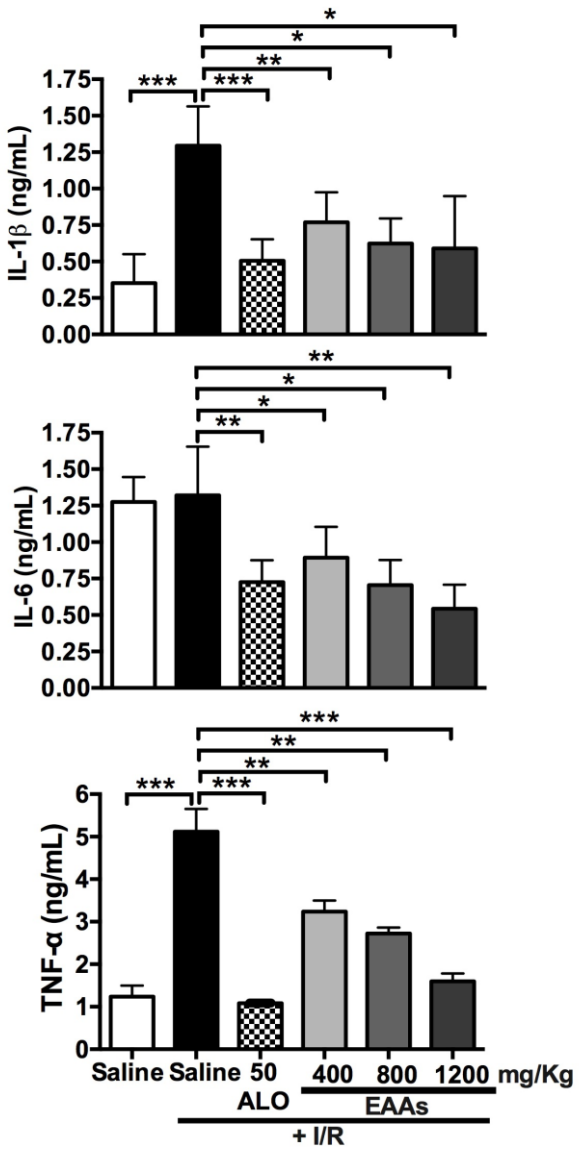

Figure 5. Changes in proinflammatory cytokines in the nephroprotection study . IL-1β: interleukin 1beta; IL-6: interleukin 6; TNF-α: tumor necrosis factor alpha; I/R: Ischemia-reperfusion; ALO: Allopurinol; EAAs: Essential amino acid α-ketoacid analogues. Saline group +I/R (7) vs. sham group (6) and treated groups (8-11). Values are expressed as mean+SD. , * $p<0.01$, ** $p<0.001$, *** $p<0.0001$.

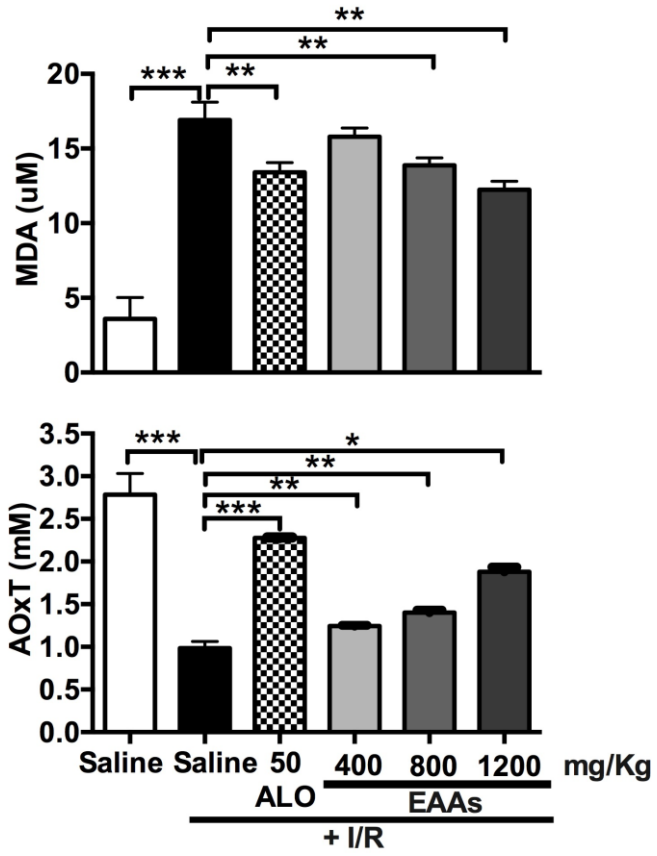

Figure 6. Changes in oxidative stress markers in the nephroprotection study.. MDA: Malondialdehyde; AOxT: activity of the total antioxidants; I/R: Ischemia-reperfusion; ALO: Allopurinol; EAAs: Essential amino acid α-ketoacid analogues. Saline group +I/R (7) vs. sham group (6) and treated groups (8-11). Values are expressed as mean+SD. $p<0.05$, ** $p<0.001$, *** $p<0.0001$.

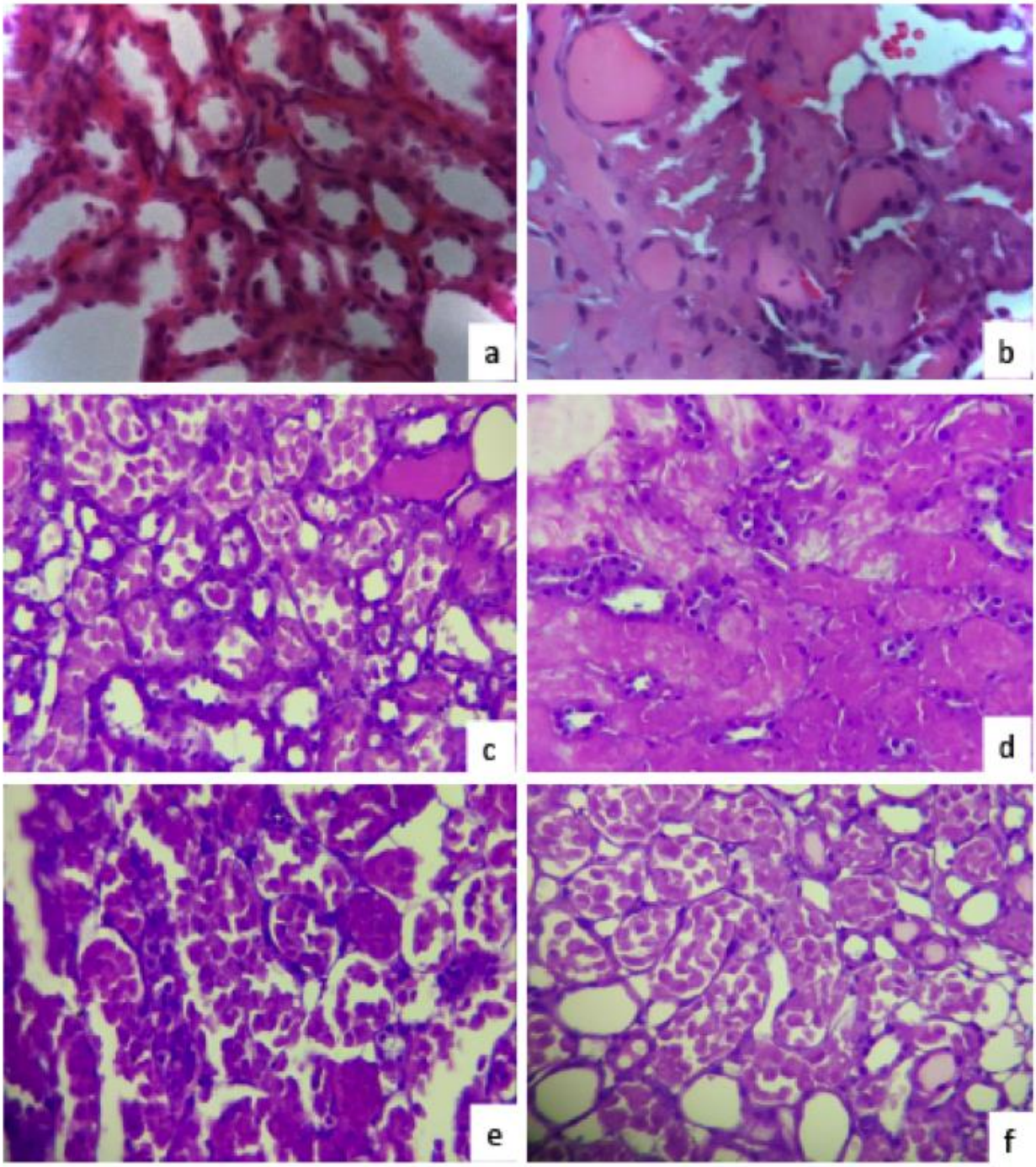

Figure 7. Histological findings in the study groups with and without damage by ischemia-reperfusion (H&E, x40). a: S.S: Preserved tubules b: I/R: Acute tubular necrosis and intratubular cylinders. c: ALO + I/R: medulla with acute tubular necrosis of convoluted tubules. d: EAAs 400 + I/R: diffuse ischemic necrosis. e: EAAs 800 + I/R: acute necrosis of

juxtamedullary convoluted tubule. f: EAAs 1200 + I/R: Acute tubular necrosis of proximal tubules in medulla, preserved cortex.; I/R: Ischemia-reperfusion injury; ALO: Allopurinol; EAA: Essential amino acid α-ketoacid analogues.