Effect of Ellagic Acid, Cilostazol and Their Combination on Amikacin Induced Nephrotoxicity in Rats

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Abstract

Amikacin(AK) has the largest spectrum of aminoglycosides. However, its use is limited due to nephrotoxicity and ototoxicity. Ellagic acid (EA) is a plant phenolic structure. it has antioxidant, anticarcinogenic and antimutagenic properities. Cilostazol (CTZ) is a PDE Ш inhibitor, it is a potent vasodilator and antiplatelet drug. This study aimed to determine if EA and cilostazol have a protective effect against nephrotoxicity caused AK. Forty nine rats were divided into seven equal groups: control normal; AK 400mg/kg; EA 10 mg/kg; CTZ 10mg/kg; AK 400mg/kg plus EA 10mg/kg; AK 400mg/kg plus CTZ 10mg/kg; AK 400mg/kg plus EA 10mg/kg and CTZ 10mg/kg. For seven days, Drugs were given orally one hour before intramuscular injection of AK. After twenty-four hours from the last dosage, samples of blood were obtained to determine blood urea nitrogen (BUN) and creatinine levels in serum, kidneys were extracted and longitudinally divided into two parts, part for measuring the following parameters: malondialdehyde (MDA), catalase (CAT), reduced glutathione (GSH), interleukin 6 (IL6), superoxide dismutase (SOD), tumor necrosis factor-alpha (TNFα), nuclear factor kappa B (NFκB) and Bcl-2 associated x protein (Bax), the other part was placed in formaldehyde solution and examined under light microscopy for routine histopathologic examination. The results of the present study proved that EA, CTZ and their combination protected rats against AK - induced nephrotoxicity; This effect might be a result of the antioxidant, anti-inflammatory and anti-apoptotic properties of these compounds.

Introduction

Amikacin (AK) has the broadest spectrum and the least resistance of all aminoglycosides. AK is preferred due to its advantageous characteristics, which include rapid and robust bactericidal activity, synergy with β-lactam antibiotics, low cost, chemical stability and low resistance; however, its use is limited due to the risk of nephrotoxicity and ototoxicity (Abdel-Daim et al. 2019).

Because AK is not metabolized in the body and is eliminated in large amounts in the urine, it builds up in the proximal convoluted tubules, causing free radical production. Nephrotoxicity is caused by these free radicals (Ozer et al. 2020).

Multiple mechanisms, such as inflammation, blockage of particular transporters, production of oxidative stress and decreased renal blood flow are involved in amikacin-induced renal damage (Prajapati and Singha 2010).

Ellagic acid (EA) is a polyphenolic compound found in plants naturally. Several studies have shown that EA has antioxidant, anti-apoptotic and anticarcinogenic properties. This antioxidant action of this compound is determined by its chemical structure, specifically the number of hydroxyl groups and their ability to boost the stability of the phenoxyl radical (Firdaus et al. 2018).

EA decreases the expression of proinflammatory and profibrogenic cytokines such as tumor necrosis factor-alpha (TNFα), tumor growth factor-alpha (TGFα), and many interleukins which are involved in alcohol-induced inflammation and fibrosis (Ciuclan et al. 2010).

Cilostazol (CTZ) is a strong antiplatelet and vasodilator that is a specific PDE III inhibitor. It raises intracellular cyclic adenosine monophosphate(cAMP) levels (Ragab et al. 2014). It also raises cyclic guanosine monophosphate (cGMP),it has many effects in different tissues (Rondina and Weyrich 2012).

Materials And Methods

Drugs and reagents:

Amikacin (Amikacin®) 500mg/2ml vial, Amoun Pharmaceutical Co. (EL-obour city, Cairo, Egypt). All other drugs were purchased from Sigma Aldrich (St. Louis, MO).

Animals and experimental design:

Rats weighing 150–180 g were obtained from Zagazig University Faculty of Veterinary Medicine. The animals were placed in hygienic and standard environmental conditions (25 ±2˚ C) and 12 hours light/dark cycle. They were given access to water and food on an ad-libitum basis. The Zagazig University Ethics Committee approved all experimental protocol. Forty nine albino male rats were randomly divided into seven groups, each group seven rats as follows: Group 1: non treated (control normal); Group 2: received AK 400mg/kg, intramuscular injection once daily for 7days for induction of experimental nephrotoxicity as described by Parlakpinar et al. (2003); Groups 3: received EA 10 mg/kg, p.o. dissolved in distilled water and given by oral tube according to Sepand et al. (2016); Groups 4: received CTZ 10 mg/kg, p.o. dissolved in distilled water and given by oral tube according to Abdelsameea et al. (2016) ; Group 5: received EA 10mg/kg, one hour before intramuscular injection of AK 400mg/kg. Group 6: received CTZ 10mg/kg, one hour before intramuscular injection of AK 400mg/kg. Group 7: received EA 10mg/kg plus CTZ 10mg/kg, one hour before intramuscular injection of AK 400mg/kg. Blood samples from the retro-orbital plexus of veins were collected using a microcapillary tubes and centrifugation at 3000 × g for 10 minutes was performed to separate serum for determining serum BUN and creatinine concentrations on the 8th day. For histopathological examination, the left kidney longitudinal section was designated. Additionally, for biochemical estimation of malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), nuclear factor kappa B (NFκB), interleukin 6 (IL6), tumor necrosis factor-alpha (TNFα), and Bcl-2 associated x protein (Bax), the remaining kidneys were frozen at - 80°C and ice-cold 0.05 M phosphate buffer pH 7.4 was used.

Determination of BUN and creatinine:

Using kits purchased from Spinreact (Gerona, Spain).

Estimation of lipid peroxidation:

MDA levels in kidney homogenate were measured by spectrophotometry, in which kidney samples were homogenised in ice-cold 50 mM potassium phosphate buffer (pH 7.5), centrifuged for 15 minute at 4 C 12,000 g, then supernatant was collected. MDA in the supernatant has the ability to generate a colourful complex with thiobarbituric acid (TBA) which was absorbed maximally at 535nm (Ohkawaet al. 1979).

Estimation of GSH:

The level of GSH in the kidney was evaluated using the colorimetric method proposed by Ellman (1959).

Catalase determination (CAT):

Colorimetric kits obtained from Dokki Biodiagnostic Company in Giza, Egypt, were used to determine it. The chromophore formed is inversely proportional to the catalase present in the initial sample (Aebi 1984).

Superoxide dismutase activity determination (SOD):

Colorimetric method was used determine SOD activity (Sun et al. 1988).

Quantitative estimation of tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL6) concentration in renal tissue:

It was assessed using USCN life Science Inc. ELISA kits. The competitive inhibition enzyme immunoassay technique is used in this assay.

Estimation Bcl-2 associated x protein (Bax) and nuclear factor kappa B (NFκB) in renal tissue:

It was determined using quantitative real-time PCR after total RNA was isolated according to the manufacturer's instructions using the Qiagen tissue extraction kit (Qiagen, USA). Using a high-capacity cDNA reverse transcription kit (Fermentas, USA), total RNA was converted to cDNA. Using Applied Biosystems with Step One TM software version 3.1 (USA), amplification and analysis of the real-time qPCR product were performed. The primer sequence of the studied genes; BAX: Forward primer:5'-CCCTGTGCACTAAAGTGCCC-3. Reverse primer: 5'-CTTCTTCACGATGGTGAGCG-3 NFκB: Forward primer:5'-CATTGAGGTGTATTTCACGG -3

Reverse primer: 5'-GGCAAGTGGCCATTGTGTTC -3

Histopathological studies:

Prior to sacrifice, the animals were anesthetized with a 50 mg/kg intraperitoneal injection of sodium pentobarbital. Both kidneys were quickly removed and opened. The specimens were fixed in 10% formalin, sectioned into 5mm thick paraffin blocks and hematoxylin and eosin stains (H&E) were used for light microscopy (Bancroft and Gamble 2002)

Analysis of data:. To compare all groups, one-way analysis of variance (ANOVA) was performed, while to compare between every two groups the least significant difference (LSD) was utilized. All data are expressed as mean ± SEM. A P-value less than 0.05 is considered significant. Computer analysis of the collected data was performed using the Statistical Package for Social Services version 25 (SPSS).

Results

Effect on BUN and Creatinine:

AK 400mg/kg significantly increased BUN and creatinine levels in comparison to the control normal group. EA 10mg/kg or CTZ 10mg/kg alone produced a non significant reduction in both parameters in relation to the control normal group. AK plus EA and AK plus CTZ produced a significant reduction of BUN and creatinine compared with AK group. AK plus EA and CTZ produced more significant reduction of both parameters than each drug alone (Table 1).

Effect on MDA, GSH, SOD and CAT:

AK 400mg/kg significantly increased MDA levels in renal tissue and caused a significant reduction of CAT, SOD, and GSH in renal tissue compared to the control group. EA10mg/kg or CTZ 10mg/kg alone produced non significant results in relation to the control normal group. AK plus EA and AK plus CTZ significantly reduced MDA

and significantly increased GSH, SOD and CAT in renal tissue in relation to the AK group. AK plus EA and CTZ produced more significant reduction of MDA and more significant rise of GSH, SOD and CAT than each drug alone (Table 2).

Effect on TNFα and IL6

AK 400mg/kg produced a significant increase in renal tissue TNFα and IL6 as compared to the control normal group. EA 10mg/kg or CTZ 10mg/kg alone produced non significant results in relation to the control group. AK plus EA and AK plus CTZ produced a significant reduction of TNFα and IL6 in renal tissue as compared to the AK group. AK plus EA and CTZ produced more significant reduction of TNFα and IL6 than each drug alone (Table 3).

Effect on expression of NFκB and Bax

AK 400mg/kg resulted in a significant increase of NFκB and BAX expression in renal tissue compared with the control normal group. EA 10mg/kg or CTZ 10mg/kg alone produced a non significant results in relation to the control normal group, while AK plus EA and AK plus CTZ showed a significant decrease of NFκB and BAX expression in relation to AK group. AK plus EA and CTZ produced more significant reduction of NFκB and BAX expression in renal tissue than each drug alone (Table 4).

Table (1): EA, CTZ and their combined effect on serum BUN and creatinine.

Groups	Control normal	AK 400mg/kg	EA 10mg/kg	CTZ 10mg/kg	AK+ EA	AK+ CTZ	AK+ EA+CTZ
BUN (mg/dl)	28.59±1.7 a	85.8 ± 2.8 b	27.5 ± 1.5 a	29.1±1.2 a	38.54 ± 2.56 c	40.56±1.9 C	28.6 ±2.3 a
Creatinine (mg/dl)	0.22±0.03 a	1.8±0.26 b	0.21 ±.02 a	0.23±0.5 a	0.56±0.02 c	0.61±0.02 C	0.3±0.04 a

Data represent mean ± SE

BUN, blood urea nitrogen;AK, amikacin; EA, ellagic acid;CTZ,cilostazol

Values without common small letters are significantly different

Table (2): EA, CTZ and their combined effect on MDA, GSH, SOD and CAT in renal tissue.

Groups	Control normal	AK 400mg/kg	EA 10mg/kg	CTZ 10mg/kg	AK+ EA	AK+ CTZ	AK+ EA+CTZ
MDA (nmol/gm)	25.13±2.74 a	107.9±4.58 b	24.11±2.5 a	25.5±3.2 a	61.83 ±4.6 c	68.61±3.32 c	40.61±2.71 d
GSH (nmol/gm)	64.54±2.18 a	24.51 ±2.5 b	62.5 ± 3.2 a	63.2±2.1 a	44.74 ±4.5 c	43.04±2.1 c	50.83±3.14 d
SOD (u/gm)	10.04±0.54 a	2.29 ±0.21 b	9.50 ± 0.5 a	10.4±1.1 a	6.72 ±0.24 c	5.91 ±0.16 c	8.24±0.27 a
CAT (u/gm)	120 ± 1.72 a	63.95 ±4.8 b	118 ± 2.5 a	121.3±3.4 a	90.59±2.7 c	84.3±2.28 c	110.34±6.6 a

Data represent mean ± SE

MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; AK, amikacin; EA, ellagic acid;CTZ,cilostazol

Values without common small letters are significantly different

Table (3): EA, CTZ and their combined effect on TNFα and IL6 in renal tissue.

Groups	Control normal	AK 400mg/kg	EA 10mg/kg	CTZ 10mg/kg	AK+ EA	AK+ CTZ	AK+ EA+CTZ
TNFα (Pg/mg,pt)	15.79±1.6 a	104.4±5 b	15.33±1.2 a	16.2±2.1 a	49.2±1.13 c	51.2±2.02 c	34.56±1.77 d
IL6 (Pg/mg,pt)	33.6±2.06 a	126.2±2.5 b	32 ± 1.2 a	33.8±1.5 a	65.53±3.44 c	73.44±2.17 c	36.7±3.84 a

Data represent mean ± SE

TNFα, tumor necrosis factor-alpha; IL6, interleukin 6; AK, amikacin; EA, ellagic acid; CTZ, cilostazol

Values without common small letters are significantly different

Table (4): EA, CTZ and their combined effect on NFκB and BAX expression in renal tissue.

Groups	Control normal	AK 400mg/kg	EA 10mg/kg	CTZ 10mg/kg	AK+ EA	AK+ CTZ	AK+ EA+CTZ
NFκB	1.02±0.005 a	6.24 ±0.11 b	1 ± 0.003 a	1.02±0.003 a	2.66 ±0.13 c	2.76±0.08 c	1.56±0.07 a
BAX	1.02 ±0.004 a	7.7 ± 0.37 b	1.01±002 a	1.02±0.002 a	3.69 ±0.17 c	4.12±0.07 c	1.77± 0.16 d

Data represent mean ± SE

NFκB, nuclear factor kappa B; BAX, bcl-2 associated x protein; AK, amikacin; EA, ellagic acid;CTZ,cilostazol

Values without common small letters are significantly different

Histopathological result

Histopathological findings showed normal structure of renal cortex, tubules, and glomeruli [A]. After nephrotoxicity was induced by amikacin, the kidney showed karyolysis, loss of the outer basement membrane of tubules and accumulation of necrotic material in the lumen [B].

EA 10mg/kg produced no changes in normal kidney structure[C].Also,CTZ 10mg/kg produced no changes in normal kidney structure[D]. Administration of EA 10 mg/kg or CTZ 10mg/kg one hour before AK as a prophylactic agents showed improvement of AK-induced nephrotoxicity in the form of reduction of the percentage of the area of inflammation, eosinophilia and necrosis [E]&[F] respectively. Administration EA plus CTZ one hour before AK as a prophylactic agents showed more reduction of percentage of the area of inflammation, eosinophilia, and necrosis[G] than each drug alone.

Discussion

Drugs such as aminoglycosides, chemotherapeutic agents, non-steroidal anti-inflammatory drugs, vancomycin, amphotericin B, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers as well as chemicals and radiocontrast produce about 20% of cases of nephrotoxicity (Hlail et al. 2020). Acute kidney injury (AKI) occurs in 20–33% of children exposed to aminoglycosides (McWilliam et al. 2017).

Despite the aminoglycosides toxicity, the growth of bacterial resistance to frequently used antibiotics has necessitated their continued usage as a viable therapeutic option. Chemical stability, rapid bactericidal activity, synergy with β-lactam antibiotics, limited resistance and reliability make aminoglycosides an appealing therapy option in these cases (Wargo and Edwards 2014).

EA is an antioxidant, antimutagenic and anticarcinogenic compound found naturally in plant phenol structures. The antioxidant action is determined by their molecular structure, specifically the number of hydroxyl groups and their ability to increase the stability of phenoxyl radicals (Firdaus et al. 2018).

According to Ding et al. (2014), EA activates the antioxidant response via the nuclear erythroid 2-related factor 2 (Nrf2) protein. EA has an effect on a variety of growth factors, including transforming growth factor-beta (TGF-β),

hepatic growth factor (HGF), and platelet-derived growth factor (PDGF) (Kesavan et al. 2013).

CTZ is a drug that inhibits the enzyme phosphodiesterase-3 (PDE-3) and is used to treat vascular diseases (Ragab et al. 2014). It raises cAMP, which has antithrombotic and vasodilator properties (Hafez et al. 2019). It also raises cGMP, which has a role against inflammation in central nervous system (Raposo et al. 2014).

CTZ has been demonstrated to have numerous pharmacological effects in different animal models, including effects against oxidation, inflammation, and apoptosis (Mohamed et al. 2018).

The classic specific indicators of nephrotoxicity and renal failure are BUN and creatinine (Campos et al., 2018). Increased serum creatinine level is a simple and widely available measure of glomerular filtration rate in clinical practice (McWilliam et al. 2017). The level of creatinine is particularly important in the early stages of renal injury (sepand et al. 2016).

The results of this work showed that AK 400mg/kg, in relation to the control group, significantly increase serum BUN and creatinine levels. These results are in agreament with Hlail et al. (2020), who demonstrated that intraperitoneal injection of AK 120 mg/kg for 14 days produced a significant increase in serum creatinine and urea level. Also, Abdel-Daim et al. (2019) found that i.m injection of AK 100mg/kg for 7days produced a significant rise in creatinine, uric acid and urea.

Multiple pathophysiological effects of AK-induced kidney damage include the creation of reactive oxygen and nitrogen species, as well as the stimulation of apoptosis, as AK forms a complex with mitochondrial Fe2+, resulting in the development of free radicals. These free radicals and reactive species are important in drug-induced renal impairment and BUN and creatinine increase (Prajapati and Singha 2010).

In this study, oral administration of EA 10mg/kg one hour before AK significantly reduced BUN and creatinine. These findings support those of Sepand et al. (2016), who reported that EA 10mg/kg produced a significant reduction of urea and creatinine in gentamycin 100mg/kg induced nephrotoxicity. Also, Ateşşahín et al. (2007) reported that EA 10mg/kg produced a significant reduction of urea and creatinine in nephrotoxicity induced by intraperitoneal injection of cisplatin 7mg/kg.

The improvement in RBF and GFR could explain ellagic acid's favorable effect in improving kidney function tests and lowering creatinine and BUN levels (Nejad et al. 2017).

Also, this study showed that oral administration of CTZ 10mg/kg one hour before AK significantly reduced BUN and creatinine. These findings support those of Abdelsameea et al. (2016) who demonstrated that administration of CTZ 10mg/kg once daily for 8 days reduced creatinine, urea and uric acid level in nephrotoxicity induced by gentammycn. Also, Gokce et al. (2012) reported that concomitant use of CTZ 10 mg/kg.rat /d orally with cyclosporine reduced urea and creatinine level.

CTZ's renoprotective impact could be due to its antioxidant properties as it increases GSH, CAT, SOD and reduces oxidation parameters as MDA and it has antiapoptotic effect by increasing BCL2/Bax(Bcl2 associated-x protein) ratio (Abdelsameea et al. 2016).

In this study, oral adminsteration of EA 10mg/kg and CTZ 10mg/kg before AK 400mg/kg produced more reduction of BUN and creatinine than each drug alone due to the additive effect of both drugs.

Antioxidant enzymes like SOD and CAT are important for cellular antioxidative defense. SOD catalyzes the formation of hydrogen peroxide (H_2O_2) by superoxide radicals dismutation (Ateşşahn et al. 2007).MDA is a lipid peroxidation end product that can be utilized as a biological biomarker to describe the degree of oxidative stress (Rahardjani 2016).

GSH act as a potent electron donor acting against free radicals. With the help of glutathione peroxidase enzymes, GSH can degrade H_2O_2 to H_2O (Lushchak 2012).

The results of this work showed that AK 400mg/kg produced a significant reduction of the antioxidant parameters; GSH, SOD and CAT, and a significant increase of oxidation parameter; MDA in renal tissue. These results are in accordance with Abdel-Daim et al. (2019), who reported a significant elevation of MDA and a significant reduction of SOD, CAT, and GSH caused by AK 100mg/kg.

AK is not metabolised in the body and is largely eleminated in the urine. As a result, it accumulate in proximal tubules and glomeruli leading to activation of renin-angiotensin-aldosterone system, lowering glomerular filtration rate, and increasing the production of platelet-activating factor (PAF), reactive oxygen species (ROS) and vasoconstrictors (Wargo and Edwards 2014). Excessive reactive oxygen species production results in oxidative stress, which causes major interconnected disturbances in cellular metabolism such as protein and nucleic acid structure changes, DNA damage, induction of apoptosis, the elevation of intracellular free calcium, damage to membrane ion transport and cell damage from lipid peroxidation (Hlail et al. 2020).

In the present study, EA 10mg/kg orally one hour before AK produced a significant reduction of oxidation parameter; MDA and a significant increase of the antioxidant parameters; GSH, CAT, and SOD in renal tissue in relation to the amikacin group. Sepand et al. (2016) agreed with these results as they found that EA 10mg/kg produced a preventive effect against nephrotoxicity caused by gentamycin as it increased SOD, CAT and GSH levels. Also, Bhattacharjee et al. (2021) reported that oral administration of EA 25,50 mg/kg, p.o for two months exhibited a preventive effect against nephrotoxicity caused by lead by increasing CAT, SOD, GSH, and reducing MDA compared to the control nephrotoxic group.

The ability of EA to scavenge free radicals has been related to its intrinsic antioxidant activity. This is due to the fact that it can transfer the phenolic H-atom to a free radical. Lactone systems and EA hydroxyl groups can create Hydrogen bonds, and they can act as hydrogen donors and electron acceptors. As a result, EA possesses ability to participate in antioxidant redox reactions, resulting in a highly efficient free radical scavenger (Ríos et al. 2018).

Oxidative stress has been demonstrated to be reduced by EA through modulation of multiple mechanisms. These involve antioxidant response activation via Nrf2, suppression of cytokines, such as IL1, IL6, and TNF, and cyclooxygenase 2 (COX-2) via NF-kB, and cell survival or apoptosis control via NF-kB (ALTamimi et al. 2021). EA is classed as a multiple-function antioxidant since it exerts its beneficial antioxidant properties in both primary and secondary modes (Alfei et al. 2020).

The results of this work showed that oral administration of CTZ 10mg/kg one hour before AK produced significant reduction of oxidation parameter; MDA and significant increase of the antioxidant parameters; GSH, CAT and SOD in renal tissue in relation to the AK group.These results are in accordance with Gokce etal. (2012) who reported that administration of CTZ 10mg/kg for 7 days ameliorates cyclosporine induced nephrotoxicity by reducing MDA and increasing SOD and CAT activity. CTZ prevents oxidative stress by activating redox defence systems via increased expression of PI3K/Akt and Nrf2/HO-1 mRNAs, resulting in oxidative stress reduction and restoration of

mitochondrial dysfunction (Hafez etal. 2019). In this study,the combined effect of EA 10mg/kg plus CTZ10mg/kg as a prophylactic, produced more significant results than each drug alone may be due to the additive anti oxidant effect of both drugs.

TNF is a proinflammatory cytokine formed by macrophages and monocytes and is capable of activating neutrophils and lymphocytes, enhancing vascular endothelial cell permeability and triggering the production and release of other cytokines. It acts on tumor necrosis factor receptor 1 (TNFR1) and 2 (TNFR2). TNFR1 is involved in mediating inflammation and increasing fibroblast proliferation by activating nuclear factor (NF). TNFR2 has a role in cell migration, regeneration, proliferation and TNF1-mediated apoptosis regulation. TNFα may stimulate the NF-B pathway, which regulates the transcription and production of inflammatory mediators. This is a vicious cycle that exacerbates inflammatory reactions (Zhou et al. 2019).

The results of this work proved that AK 400mg/kg produced a significant increase of NFκB, TNFα and IL6 in relation to control normal group. Ozbek et al. (2009) agreed with these results and stated that intraperitoneal injection of gentamycin 100 mg/kg significantly increased NFκB expression in renal tissue.

AK - induced nephrotoxicity could be due to upregulation of TNF-expression or due to AK induced oxidative stress, which induces oxygen-containing derivatives and cytokines production, which function as a second messenger for activating NF-B, resulting in the transcription of cytokines, growth factors, and extracellular matrix proteins (Abdel-Daim et al. 2019).

In this present work, EA 10mg/kg orally one hour before AK significantly reduced TNFα, IL6 and NFκB expression. These findings are consistent with Marn et al. (2013), who suggested that EA reduced NF-B, IL-6 and TNF levels in comparison to the control group in mice with ulcerative colitis. EA inhibits inflammation via modulating the NF-B signaling pathway (Ghasemi-Niri et al. 2016).

These findings are consistent with Cornélio Favarin et al. (2013), who found that EA 10 mg/kg increased the anti-inflammatory cytokine IL-10 and decreased the proinflammatory cytokine IL-6 in bronchoalveolar lavage fluid.

EA reduces toll-like receptor 4 (TLR4) and high mobility group protein 1 (HMGB1) in the kidney tissue by cutting down TLR4 downstream protein leading to reduction of inflammatory factors (Zhou et al. 2019).

In this study oral administration of CTZ 10mg/kg reduced TNFα, IL6 and NFκB expression in renal tissue. These results are in accordance with Hermes et al. (2016), who reported that oral administration of CTZ 100mg/kg for14 days reduced TNFα and NFκB in dystrophic diaphragm muscle. Also, Sakamoto et al. (2018) demonstrated that CTZ 50mg/kg for 7 day reduced interleukin-6 and TNFα.

CTZ prevents nitric oxide (NO), prostaglandin E2 (PGE2), cytokines such as IL1, TNF α and monocyte chemoattractant protein-1 (MCP-1) production through inhibits extracellular signal-regulated kinases 1 and 2 (ERK1/2) and c-Jun N-terminal kinase (JNK) (Jung et al.2010). In this study,the combined effect of EA 10mg/kg plus CTZ10mg/kg as a prophylactic, produced more significant reduction of TNFα, IL6 and NFκB expression than each drug alone may be due to the additive anti-inflammatory effect of both drugs.

BAX (Bcl2 associated –x protein) is an apoptotic marker. BAX is thought to interact with the voltage-dependent anion channel in mitochondria. This causes cytochrome c and other pro-apoptotic substances release from the mitochondria, resulting in caspase activation and apoptosis induction (Westphal et al. 2011).

The results of this work showed that AK 400mg/kg significantly increase BAX expression in renal tissue. Helmy et al. (2020) agreed with this result and revealed that AK 1.2 g/kg single intraperitoneal injection increased expression of BAX in renal tissue.

Aminoglycosides can cause apoptosis in the kidney by increasing the content of cytosolic Bax protein, which leads to activating the mitochondrial pathway of apoptosis, it includes caspase 9 activation as an initiator, caspase 3 activation as an effector and DNase activation, resulting in DNA fragmentation and apoptosis (Servais et al. 2006).

In this study, EA 10mg/kg orally one hour before AK reduced the expression of BAX in renal tissue. Sepand et al. (2016) agreed with this finding and reported that EA 10mg/kg decreased gentamycin-induced nephrotoxicity in rats by increasing Bcl2/BAX ratio and decreasing Caspase-3. It is thought to be one of the main executors of apoptosis.

EA's antioxidant and anti-apoptotic properties may be attributed to increased SIRT1 expression in renal tissues (Mohammed et al. 2020). SIRT1 (sirtuin1) is the mammalian homolog of the yeast Sir2 (silent information regulator 2). It protects against oxidative stress by deacetylating forkhead box O (FOXO) and tumor suppressor protein (p53). SIRT1 deacetylates p53 and FOXO, resulting in transcriptional activities suppression and loss of stress-induced apoptosis (Yun et al., 2012). FOXOs also contribute to the viability of cells through the transactivation of enzymes that detoxify ROS, such as SOD2/MnSOD and CAT (Mohammed et al. 2020).

In the present study CTZ 10mg/kg orally as a prophylactic, significantly reduced expression of BAX in relation to control diseased. This results are in accordance with Abdelsameea et al. (2016) who reported that CTZ 10mg/kg for 8days produced a significant reduction in Bax expression in gentamycin induced nephrotoxicity model. CTZ suppresses signals of mitochondria-dependent apoptosis. In addition it decreases cytochrome c release from mitochondria and down-regulates Bax expression (Park et al. 2011).

This study proved that the combined effect of EA 10mg/kg plus CTZ 10mg/kg before AK produced more significant reduction of BAX expression in renal tissue than each drug alone which may occur may be due to the additive effect of both drugs.

The current histopathology findings revealed that AK 400mg/kg was associated with disturbances in the kidney histopathological picture, including inflammatory cell infiltration, tubular epithelial lining degeneration and tubular necrosis. These results are in accordance with Abdel Fattah and Gaballah (2020), who demonstrated that marked degenerative changes in the kidney and marked tubular necrosis occurred with AK. EA administration before AK showed improvement of the histopathological changes as it reduced inflammation and necrosis. These results are in consistent with Bhattacharjee et al. (2021), who stated that EA 25,50 mg/kg reduced histopathological changes and renal tubular necrosis in lead induced nephrotoxicity. Also CTZ administration one hour before AK show reduction in inflammation and tubular necrosis. This results are in agreement with Abdelsameea et al. (2016) who reported that administration of CTZ 10mg/kg rat ameliorates degenerative changes in renal cortex in gentamycin induced nephrotoxicity model. In this wok,administration of EA and CTZ before AK showed more reduction of areas of inflammation and necrosis than each drug alone .

Conclusion

EA and CTZ have a renoprotective effect partially due to their antioxidant, anti-inflammatory and anti-apoptotic effects.

Declarations

Author contributions

MK and NK conceived and designed the research. ZS and AS conducted experiments. ZS and AS analyzed the data. ZS wrote the manuscript. All authors read and approved the manuscript and all data were generated in-house and that no paper mill was used.

Compliance with ethical standards

The study was approved by Zagazig University's local animal Ethical Committee in Egypt. Approval number is ZU-IACUC/3/F/57/2019. National Institutes of Health's guidelines (USA) were followed all over the experiment.

Conflict of interest

The author declares no conflict of interest.

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Data availability

Link of raw data: https://docs.google.com/spreadsheets/d/1IlJkbziTziZN4gRzpg6q2RHkvp3ND8o4/edit?usp=sharing&ouid=103429316171603633606&rtpof=true&sd=true

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Figures

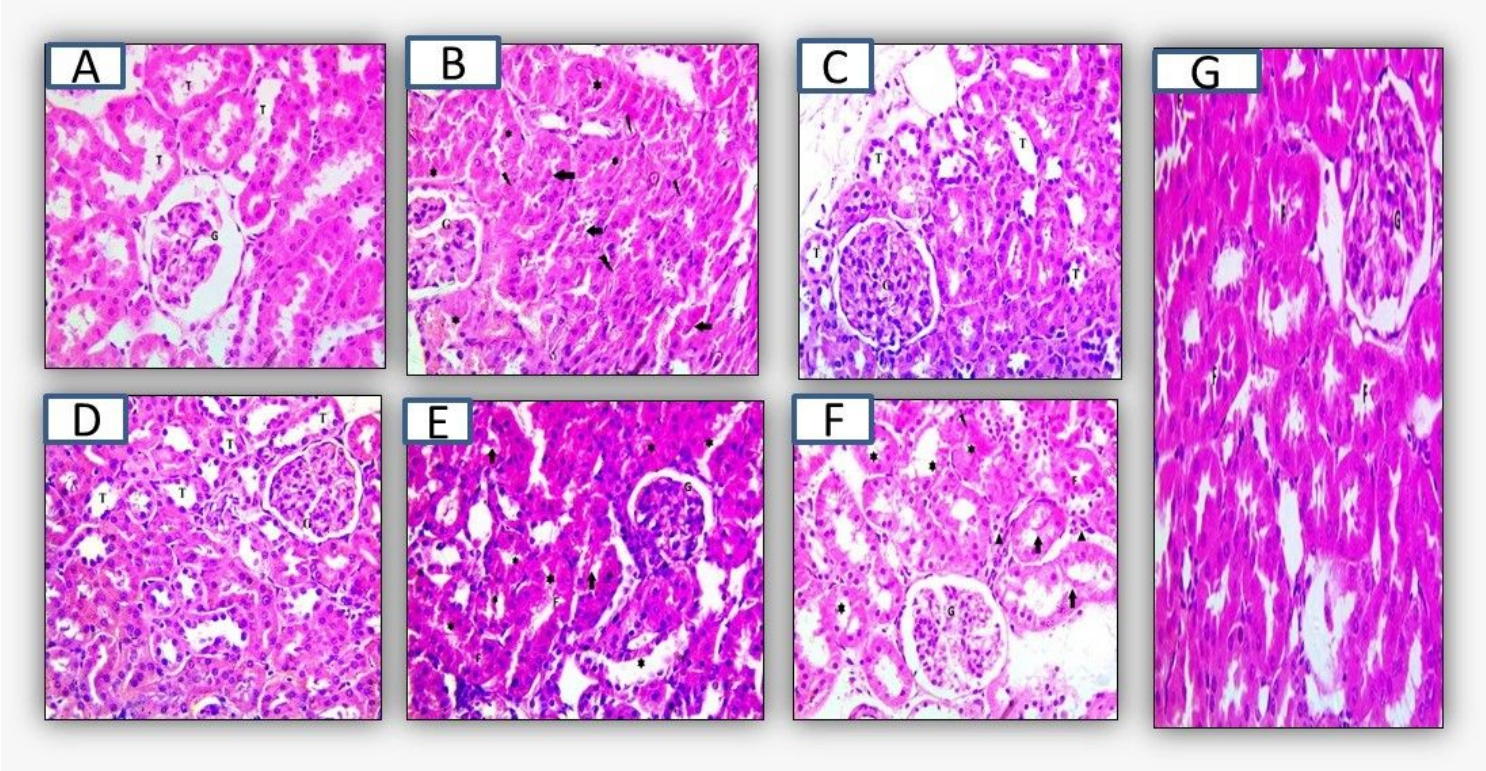

Figure 1

A photomicrograph of renal tissue showing a normal structure of renal cortex, tubules (T), and glomeruli(G); control normal. (H&E x 400). B photomicrograph showing marked tubular necrosis (>75%) in almost all the field as seen karyolysis (fading or lost nuclei [asterisks]), loss of outer basement membrane of tubules [broken arrows], and accumulation of necrotic material in the lumen [arrows], glomerulus; AK group (H & E x 400). C photomicrograph of renal tissue showing normal structure of renal cortex, tubules, and glomeruli ; EA 10mg/kg (H&E x 400). D photomicrograph of renal tissue showing normal structure of renal cortex, tubules, and glomeruli ; CTZ 10mg/kg (H & E x 400) E&F Photomicrographs of renal tissue showing epithelial lining degeneration of the tubules shown by cellular swelling, eosinophilia, fragmentation (F) of the cytoplasm in many tubules with few areas (<25%) of necrosis (loss of nuclei of some tubular cells (asterisks), flattening of the epithelial lining and accumulation of necrotic cells in the lumen [arrows]), glomerulus(G); AK + EA & AK + CTZ respectively (H & E x 400) G photomicrograph of renal tissue showing epithelial lining degeneration of the tubules shown by cellular swelling and eosinophilia with no significant areas of necrosis/apoptosis, glomerulus(G); AK+EA+CTZ (H&E stain x400).