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ORIGINAL ARTICLE

The potential protective effects of citrus bergamot extract against amikacin-induced nephrotoxicity in male albino rats

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Abstract

Objective Drug-induced nephrotoxicity is a major cause of kidney injury. Aminoglycosides, like amikacin, are effective gram-negative antibiotics. However, its distinct induction of nephrotoxicity is a problematic clinical issue. This study investigated the preventive benefits of citrus bergamot extract in rats with acute renal injury caused by amikacin.

Methods Five groups of six rats each received treatments for nine days: Group 1, normal control group, was gavaged DW orally. Group 2, negative control group, was gavaged DW orally plus a toxic dose of amikacin (1.2 g/kg) intraperitoneally on day 7 of the research protocol. Group 3, comparison group, was given 100 mg/kg of citrus bergamot extract. Groups (4) and (5), tested groups, gavaged citrus bergamot extract (100 and 200 mg/kg, respectively) orally and a toxic dose of amikacin (1.2 g/kg) intraperitoneally on day 7. Groups (1) and (3) administered intraperitoneal saline on day 7 to mimic the other groups. Serum, urine, tissue indicators of oxidative stress and renal histopathology were used to characterize nephrotoxicity.

Results The results showed that amikacin produced nephrotoxicity by increasing the weight of the rats' kidneys and a substantial increase in the urine beta2-microglobuline as well as serum traditional biomarkers for kidney injury, urea and creatinine. Additionally, amikacin markedly raises serum cytokine levels (IL-6). Amikacin, on the other hand, significantly lowered renal antioxidant activity as measured by changes in serum malondialdehyde levels and reduced glutathione activity in this experiment. This was reinforced by the existence of significant morphological changes in the kidney. Citrus bergamot extract was able to restore renal function, accompanied by significant improvements in the aforementioned parameters of kidney function, inflammation, and oxidative stress indicators, as well as an attenuation of histopathological change.

Conclusion According to biochemical and histological studies, citrus bergamot extract counteracts the detrimental effects of amikacin on renal structure and function.

Keywords Amikacin · Nephrotoxicity · Oxidative stress · Inflammation · Urine β2-micriglubuline · Citrus bergamot

Introduction

Since the kidneys are the key organs that the body requires for maintaining homeostasis, regulating the extracellular environment, and releasing toxic metabolites and medications [1], a large number of exogenous toxicants attack and damage the kidney tissues, affecting renal function in different mechanisms, leading to acute tubular damage, acute interstitial nephritis, or chronic toxicity [2]. The process of kidney toxicity may be thought of as a passage through the cell. Drugs can be metabolized, interact with organelles, change signalling pathways, and eventually induce cell death and inflammation once they enter the cell [3].

Drug-induced nephrotoxicity is the most prevalent disorder that causes worsening kidney function. Renal failure is the most prevalent, constituting 20% of all cases [4]. Antibiotics are still the major cause of drug-induced nephrotoxicity [5]. Antibiotics, especially aminoglycosides (AMG), have been linked directly to the development of nephrotoxicity, which causes acute kidney injury in 10–25% of people who

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take these antibiotics [6]. Since introducing streptomycin in 1944, AMGs have been widely prescribed and considered the most effective antimicrobial agents [7].

Amikacin (AMK) and tobramycin are the most fundamental AMGs, important therapeutically in the clinical management of serious infectious diseases induced by pathogenic gram-negative bacteria. Unfortunately, these antibiotics have been specifically linked to nephrotoxicity, ototoxicity, and neuromuscular blockage [8]. Mechanistically, oxidative stress is the primary underlying cause of nephrotoxicity [9]. Unmetabolized AMGs are recovered in the urine, but 15% of the filtered load is directly bound to megalin, which is expressed at significant levels in the proximal convoluted renal tubules (S1 and S2 segments). Then it will be re-uptake into the renal tubules, resulting in morphological and functional deterioration of the renal plasma membrane, mitochondria, and lysosomal membranes over a longer duration [10]. Furthermore, by inhibiting the synthesis of phospholipase A2 and glutathione, AMGs facilitate the production of massive quantities of hydroxyl radicals by the renal cortex, leading to cellular injury and necrosis [10]. AMGs have also been demonstrated to form a link with mitochondrial Fe + 2 that promotes the production of free radicals [11].

AMK remains the most widely prescribed antibiotic despite its strong renal impact [6]. Substantial work has been directed toward minimizing or preventing such serious nephrotoxicity. Antioxidant supplements, particularly those of natural origin, look like an appealing and promising approach [12]. Citrus fruit is one of the essential fruits in the world because of its health-related features and useful constituents such as vitamins C, carotenoids, flavonoids, pectin, calcium, potassium, taxifolin and so on [13, 14].

Numerous studies have recently proven the nutraceutical advantages of flavonoid-rich citrus fruits. Bidya et al. revealed that the citrus fruit flavonoid naringin possesses anti-apoptosis, antioxidant, and anti-inflammatory effects that protect the kidneys against gentamicin-induced kidney injury [15]. Additional confirmation of its nephroprotective effect, which may be used to alleviate amikacin-induced nephrotoxicity, was provided by Hafsia et al., they found that the essential oil of citrus lemon significantly reversed the nephrotoxicity and hepatotoxicity caused by aspirin in rats [16].

Citrus bergamot, a member of the Rutaceae family that grows predominantly in Calabria, Southern Italy, has gained a lot of interest because of its distinctive composition and particularly high flavonoid content, including rhoifolin, neohesperidin, naringin, rutin, neodesmin, and taxifolin [17–19]. Antioxidant and anti-inflammatory characteristics make bergamot one of the most commonly used traditional medicines in treating a number of medical conditions, such as fever and sore throats in Calabria [20–22].

As far as we know, citrus bergamot extract has not been studied regarding its potential impact on the nephrotoxicity mediated by amikacin in rats. Therefore, we expected that the concomitant use of citrus bergamot extract with amikacin would be a promising strategy for protecting or ameliorating the possible impact of amikacin on the renal system.

Results

In the present study, the body weight changes were determined during the study periods. No significant changes were determined among the animal-studied groups ($P > 0.05$). Increases in body weight appear comparable in all groups, as seen in Fig. 1. On the other hand, significant increases in the RKW were documented in amikacin-treated groups (2) and (4), compared to the remaining groups.

A nephrotoxic rat model was proved by several issues, including urinary ß2-MG before and after AMK administration, issues related to serum biomarkers and clear histopathological changes as seen in Fig. 2. Regarding urinary excretion of biomarkers that reflect kidney injury before and after a highly toxic dose of AMK, urine concentrations of ß2-MG increased significantly following amikacin injection in all AMK-treated groups when compared to the control group. Fortunately, CBE administration in the tested groups showed a marked reduction in the ß2-MG levels with a significant ameliorative and protective role observed in group (5) administered high dose-CBE.

Serum urea and creatinine measurements represent the initial steps in evaluating acute renal injury induced by AMK, as seen in Fig. 3A and 3B. Interestingly, the highest values of both urea and creatinine that were seen in group (2) were reduced by the administration of pharmacological dosages of CBE, with the greatest reduction being documented at a higher dose (200 mg/kg), in which the normalization

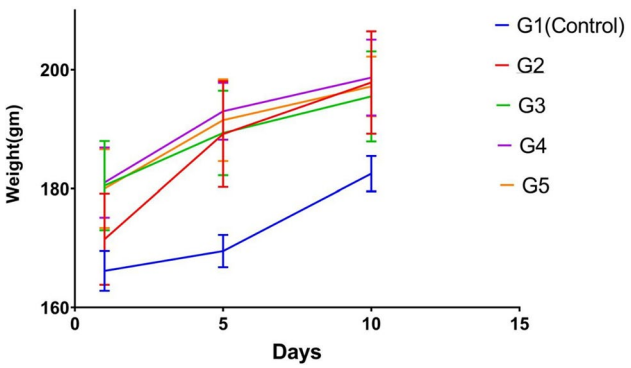

Fig. 1 Changes in rat body weights throughout the experiments and relative kidney weight at the end of the study. *indicate considerable difference $P < 0.05$. The values are expressed as Mean ± SEM. "G1: Group 1, G2: Group 2, G3: Group 3, G4: Group 4, G5: Group 5"

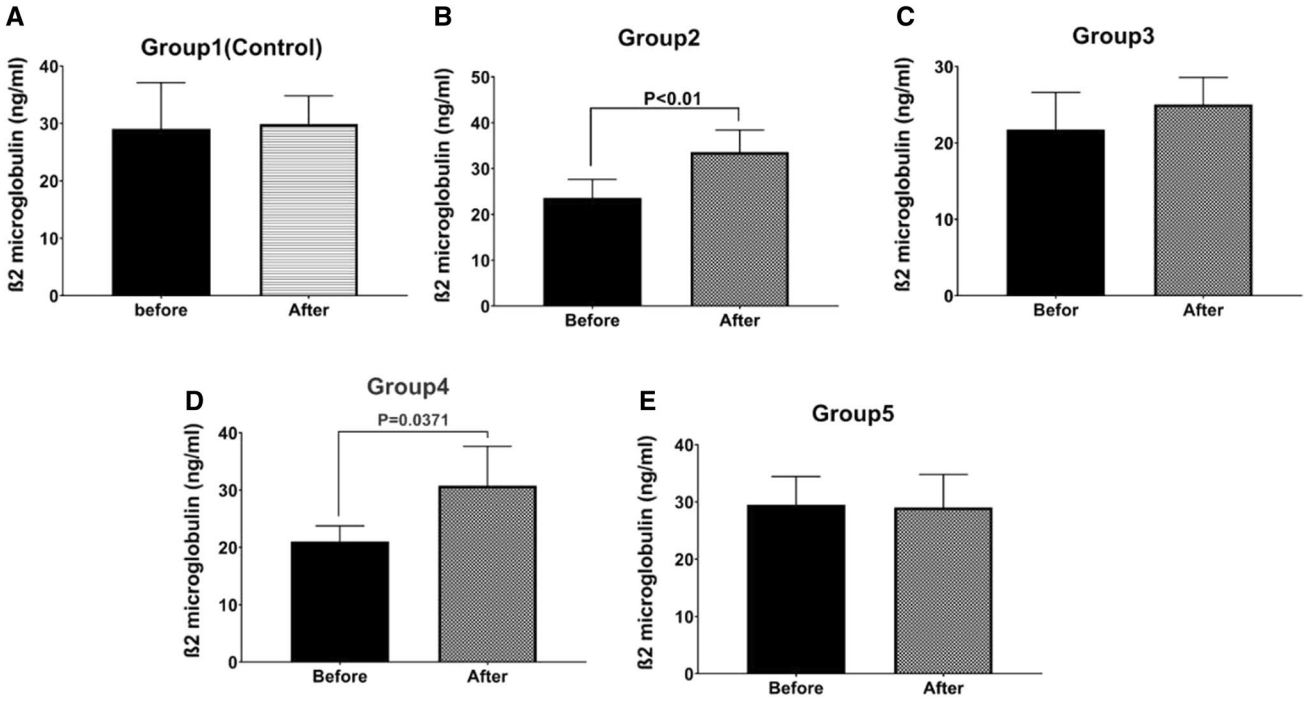

Fig. 2 the effect of Citrus Bergamot Extract (CBE) on urine ß2-microglobuline (ß2-MG) levels before and after high dose intraperitoneal Amikacin administration to rats ($n=30$). The values are represented by Mean ± SEM. "G1: Group 1, G2: Group 2, G3: Group 3, G4: Group 4, G5: Group 5"

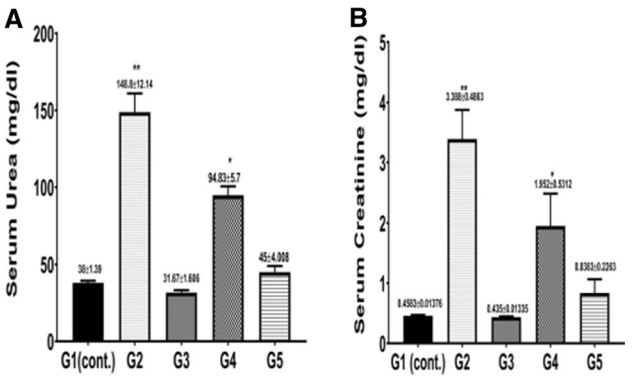

Fig. 3 The effects of Citrus Bergamot Extract (CBE) on renal biomarkers levels after AMK- induced nephrotoxicity ($n=30$). A: represent serum urea concentrations, B: represent serum creatinine concentrations. The values represented by "Mean ± SEM". **represent significant difference $P<0.001$ among groups. * indicate considerable difference $P<0.05$ among groups. "G1:Group(1), G2:Group(2), G3:Group(3), G4:Group(4), G5:Group(5)"

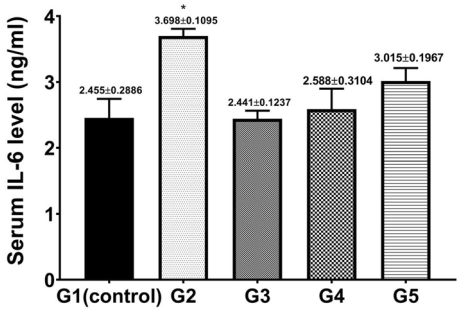

Fig. 4 The effect of Citrus Bergamot Extract (CBE) on serum Interleukin-6 (IL-6) levels after high dose intraperitoneal Amikacin administration to rats ($n=30$). The values represented by Mean ± SEM. *represent significant difference $P<0.05$ among groups. "G1: Group (1), G2: Group (2), G3: Group (3), G4: Group (4), G5: Group (5)"

of renal biomarker values toward normal control group (1). Oral administration of CBE in group (3) did not affect serum urea and creatinine. From this data, it is apparent that serum levels are within normal limits.

The present work provides compelling evidence about immunological involvement in AMK-induced nephrotoxicity, as seen in Fig. 4, measurement of serum IL-6 as an inflammatory marker and its value correlated with tissue injury. There were increases in serum levels of IL-6 in group (2) compared to the control and remaining groups. Pre-administration with CBE significantly dropped the elevation in the levels of IL-6 to values comparable to the normal control group (1). Group (3) had the lowest level, with values that were close to those of the normal control group (1).

To explore the cause and effect of AMK-induced nephrotoxicity, oxidative stress-like tissue (GSH) and serum (MDA) biomarkers were evaluated. AMK administration

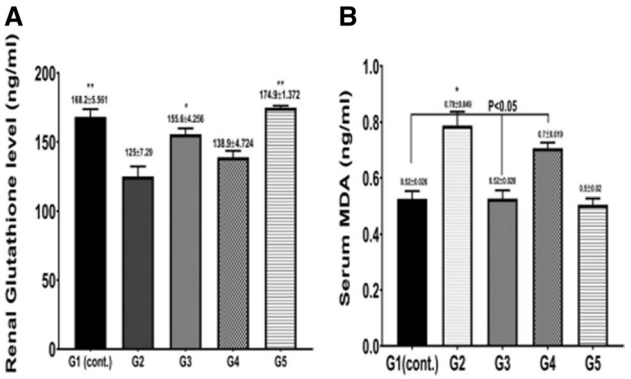

Fig. 5 The effects of Citrus Bergamot Extract (CBE) on oxidative stress markers after high dose intraperitoneal Amikacin administration to rats ($n=30$). **A**: represent renal glutathione levels (GSH), **B**: represent serum Malondialdehyde (MDA). The values represented by Mean ± SEM. * indicate considerable difference $P<0.05$ between groups. "G1: Group 1, G2: Group 2, G3: Group 3, G4: Group 4, G5: Group 5"

negatively impacted these parameters when compared with the normal control group (1). As shown in Fig. 5 A and B, AMK injection resulted in a considerable loss in oxidative buffering ability, as evidenced by a large decrease in renal GSH levels and a significant rise in serum MDA concentrations in group (2) compared to the normal control group (1). Conversely, CBE high dose supplementation (200 mg/kg body weight) significantly attenuated the alteration in renal GSH and serum MDA levels as evidenced by elevated kidney GSH content and lowered serum MDA levels compared with group (2). Rats treated with low doses of CBE group (4) showed non-significant elevation in renal GSH amount and still less than that observed in the normal control group (1).

The damaging effects of AMK on the renal and the preventive effects of CBE on histopathological alterations among experimental groups are depicted in Fig. 6. There were no microscopical changes between the normal control group (1) and the CBE-treated group (3), as H and E sections

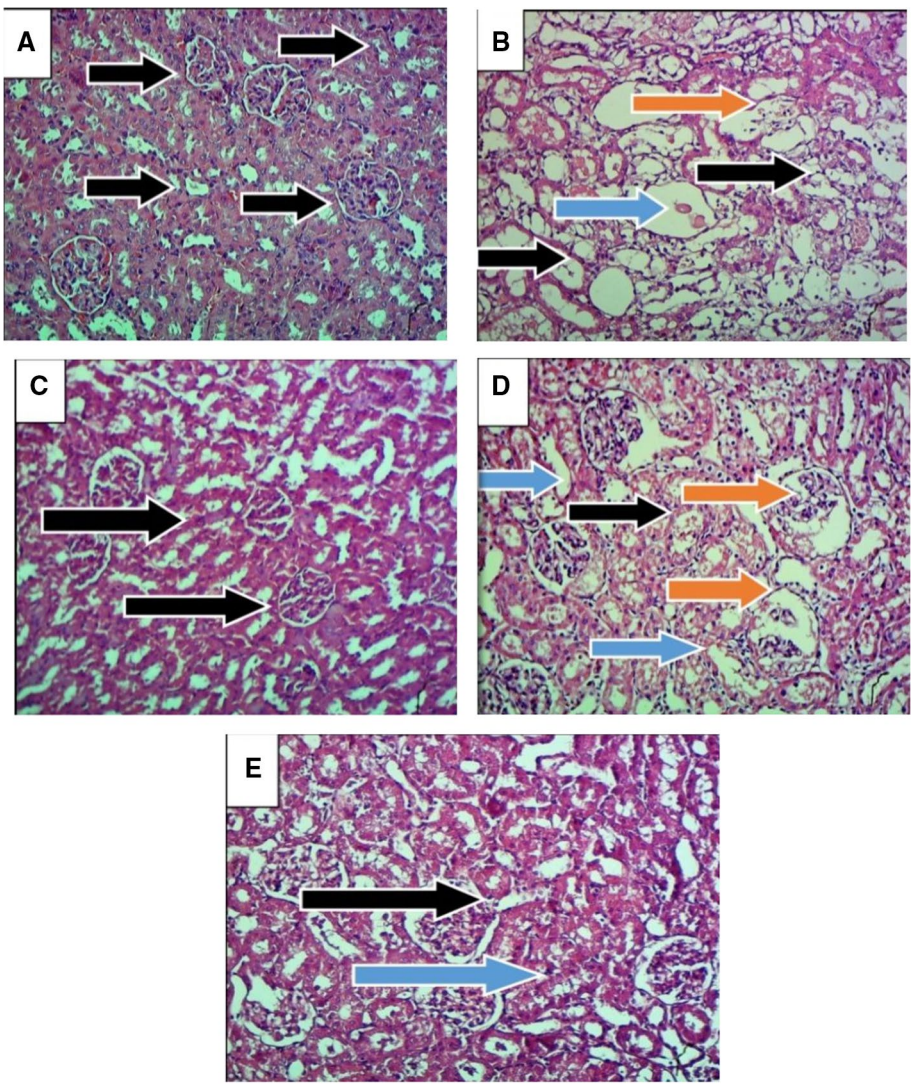

Fig. 6 Light micrographs of the kidneys section stained with H and E × 20. **A** Normal control group, showed normal renal glomeruli, Bowman's capsule and tubules. **B** AMK-treated group showed predominantly massive injury of renal tubules (black arrow), and degeneration of renal glomeruli (orange arrow), also, shrinkage of glomeruli with dilatation of Bowman's space (blue arrow). **C** CBE treated group, showed normal renal glomeruli, Bowman's capsule and tubules. **D** low dose CBE + AMK group, showed epithelium of tubulars is ragged (black arrow), undergoing necrosis, shrinkage of glomeruli with dilatation of Bowman's space (red arrow), and tubulars vacuolization (blue arrow). **E**: high dose CBE + AMK group, showed normal renal tubule (black arrow) and normal glomeruli (blue arrow)

of kidneys from both groups exhibited well-defined cortex and medulla of renal tissues with normal glomeruli and regular organization of tubules (Fig. 6A and C). In contrast, the examination of the kidneys of the AMK-treated groups revealed predominately significant damage to the renal tubules, tubular vacuolization and necrosis, haemorrhage, drug dosage shrinkage and degeneration of the renal glomeruli, and dilation of Bowman's space (Fig. 6B,D and E).

Excitingly, these pathological alterations were improved by the administration of pharmacological doses of CBE, with the greatest improvement reported at a higher dose (200 mg/kg), in which the signals of improvement in the form of normal renal tubules and glomeruli nearly matched the control group (1), associated with a decrease in the intensity of renal lesion triggered by AMK (Fig. 6E).

Discussion

Aminoglycosides are widely prescribed antibiotics in the management of infectious diseases. However, their clinical use is restricted by undesirable consequences, such as nephrotoxicity and ototoxicity[23]. A growing body of studies suggests that oxygen-free radicals may be prime offenders in their pathogenesis [9, 10, 24].

The ten-day duration of the study associated with a single dosage of AMK administered on day 7 of the treatment design did not appear to be sufficient to cause a considerable change in body weight during the period of the trials. Our results align with those of Noori et al., who found that amikacin treatment did not affect the weight of rats[25]. In contrast to our results, Fatima et al., observed that rats treated with aminoglycosides showed significant weight changes compared to the control group [26]. Inversely, AMK caused nephrotoxicity in rats, as seen in a significant increase in kidney weight and relative renal weight in the AMK-treated groups compared to the control group. These outcomes may be attributable to the fact that injection of nephrotoxic substances increased the kidneys' mass and swelled the renal tissue; these findings are supported by many studies documenting the nephrotoxic impact of aminoglycosides [27].

Recently, various indicators for early kidney damage diagnosis have been proposed. In this study, ß2-MG, a novel marker for kidney damage, was investigated in rat urine alongside traditional biomarkers for kidney injury. Because of its small size, the proximal tubular cells normally reabsorb and catabolize ß2-MG, but this process is disturbed in acute renal damage [28]. Urinary excretion of ß2-MG is a biomarker of tubular damage [29]. This supports our findings that AMK injection raised ß2-MG levels in all treatment groups compared to the normal control group. These results align with prior studies that indicate a rise in ß2-MG levels in rats administered AMGs [30]. When CBE was administered pre- and post- AMK injection, it resulted in a dose-dependent decrease in ß2-MG levels compared to the AMK-intoxicated group. This was in line with the research done by Nazari et al., they found that the active flavonoid tangeretin, which is found in citrus peels, significantly decreased tubular injury biomarkers in rats with cisplatin-induced nephrotoxicity [31].

Because blood urea and creatinine are waste products of protein breakdown that the kidney must excrete, a marked increase in serum urea and creatinine, as seen in this research, absolutely proves renal injury [32]. This rise might be explained by the generation of reactive oxygen species (ROS), which are crucially important in the deterioration in glomerular filtration rate and renal tubular necrosis seen in this study. These findings are consistent with prior research that found a considerable increase in urea and creatinine levels in rats given AMGs. These alterations in biochemical parameters were well correlated with the histopathological lesions [33–35]. However, Kaynar et al., reported no correlation in blood creatinine levels after high dosages of AMK therapy compared to the control group [36]. There was a dose-dependent reduction in these parameters when CBE was given orally to the tested groups. Because of its antioxidant and anti-inflammatory properties, bergamot is regarded as the cornerstone and predicate element of CBE mechanistic pharmacology. In line with our results, Cirmi S et al. observed that the nephrotoxicity induced by cadmium in mice was reversed by the administration of a flavonoid-rich extract of bergamot juice [37].

At the molecular level, higher levels of pro-inflammatory cytokines have been linked to renal damage [38]. The serum pro-inflammatory cytokine (IL-6) in this study was significantly elevated, supporting such a strong relationship. The findings indicate that the renal damage induced by AMK has a multidisciplinary aspect. Free radical generation and an inflammatory consequence of an increase in serum pro-inflammatory markers (IL-6) play a pivotal role in renal damage. Numerous studies also conclude that AMGs can cause renal tissue damage via inflammation and excessive oxidative stress [39–41]. The decrease in cytokine levels (IL-6) following oral CBE treatment along with AMK injection confirmed the hypothesis that the extract possesses anti-inflammatory and antioxidant properties. The use of bergamot extract has previously been shown to be effective in improving oxidative stress and inflammatory indicators, which is compatible with the findings of this study [42].

The results of this study, which confirm earlier reports, showed that AMK treatment significantly increased lipid peroxidation in the kidneys, as evidenced by decreased GSH antioxidant activity. In contrast, MDA significantly increased compared to the normal control group. These findings show that the oxidant/antioxidant balance is altered, demonstrating oxidative damage to the kidney,

The renal histopathological changes are well supported, which reveal tubular degeneration and marked necrosis in AMK-treated groups. These data are in harmony with other studies in which a significant consumption of GSH in renal cells results in their damage and elevation of lipid peroxidation [43]. As amikacin-causing nephrotoxicity may be due to oxidative stress, therefore, antioxidant agents could be able to attenuate amikacin-induced nephrotoxicity. Today, researchers are focusing on finding natural supplements that potentially protect the body from the oxidative stress caused by drugs and chemicals.

In this research, CBE administration to rats in a dose-dependent manner significantly increases GSH content in renal tissue and lowers MDA levels in serum as compared to AMK- intoxicated group. These results highlight that Citrus bergamot, a plant rich in antioxidants, protects the kidney from free radical toxicity. A recent study agrees with our findings; researchers have shown that bergamot juice extract decreases CdCl2-induced oxidative toxicity by restoring GSH levels in the kidneys of challenged mice [37]. It has also been reported that the essential oil extract of bergamot reduces MDA levels in rats exposed to AlCl3 and alleviates anxiety-like behavior [44].

These results are compatible with renal histology investigation, demonstrating notable and more severe kidney damage in the AMK-treated groups. Similar alterations were also noted by Doan et al., who showed that AMK administration caused morphological changes in renal tissues with severe renal impairment [45]. These alterations, particularly those caused by concentrated aminoglycoside accumulation in proximal tubular cells, are a major predictor of nephrotoxicity [46]. Aminoglycosides accumulate in the renal cortex due to reabsorption from the tubules' basolateral surfaces [47]. The cortical tubules of the renal cortex are severely harmed by amikacin administration. Morphological alterations include desquamation, tubular epithelial cell vacuolization, tubular epithelial atrophy, and necrosis [48]. According to the literature, it was clear from this study that AMK-treated groups (specifically groups 2 and 4) had severe renal tubular damage, tubular vacuolization, and necrosis in the proximal tubules. Additionally, in these two groups, as compared to the normal control group, there were drug dose shrinking, renal glomeruli degeneration, and Bowman's space enlargement. The interesting facts included the point that administering pharmacological dosages of CBE reversed renal damage, with the greatest improvement being documented at a higher dose (200 mg/kg), in which the signs of improvement in the form of normal renal tubules and glomeruli were almost identical to those in the normal control group and were linked to a decrease in the severity of renal lesion caused by AMK.

Materials and methods

Materials

Amikacin sulphate (Amikozit®, manufactured by Sanofi Co., Istanbul, Turkey) as an aqueous solution for injection; each 1 ml of Amikozit vial contains Amikacin sulfate, equivalent to 250 mg.

Citrus bergamot extract supplement (CBE 500 mg capsules, manufactured by Double Wood Supplements Co., USA).

The other chemicals and instruments we used in our research were of the best quality that could be found.

Animals

After a period of adaptation, thirty sexually mature albino rats "weighing 150–230 g" were purchased from Al-Qadisiyah University, College of Veterinary Medicine and were included in this investigation. This work is part of a master's thesis carried out at the animal house in the College of Pharmacy, University of Basra, from Nov 2021 to Jan 2022. The laboratory conditions are in accordance with internationally agreed-upon standards. The animals were placed in an air-conditioned room with photoperiods of "12 h light/12 h dark", mostly at 25 °C ± 5 °C with appropriate humidity. In big indoor plastic cages, three rats were kept in each cage, unlimited access to fresh water and a standard rodent diet**.**

Experimental design

Randomly and evenly, thirty animals were divided into five experimental groups, including six rats. The animals received the following treatments each morning for nine days. Group (1) represented the normal control group and was administered DW (1 mL) by oral gavage. Group (2) represented the negative control group for AMK-induced nephrotoxicity and received DW (1 ml) gavaged orally, and a single toxic dose of AMK (1.2 g/kg) administered intraperitoneally on day 7 of the study protocol. Group 3 represented the comparison to exclude certain effects of the extract; it was gavaged at 100 mg/kg of CBE. Groups (4) and (5) were the tested groups to investigate the possible nephroprotective effect of the CBE extract against AMK-induced renal damage; these two groups gavaged CBE at (100 mg/kg) and (200 mg/kg) dosages from the first day of the experiment, respectively. On day 7, a single toxic dosage of AMK (1.2 g/kg) was injected intraperitoneally to induce nephrotoxicity. Both groups (1) and (3) were injected intraperitoneally with normal saline on day

7 to resemble the remaining studied groups. The AMK doses were selected in agreement with previous articles to induce a renal toxicity model [49–51].

The weights were measured at three successive points at a five-day interval from the starting time. On day 10, rats fasted overnight, and the live body weight was recorded before they were anaesthetized by inhaling chloroform. Blood samples were collected from the posterior vena cava, centrifuged to isolate clear serum, and then frozen at − 20 °C for biochemical analysis.

According to the following equation, both kidneys were isolated and weighed for relative organ weight measurement (RKW):

RKW = "Absolute kidney weight (g)/Rat body weight on sacrifice day (g)"* 100.

Urine samples from the testing and control groups were collected in sterile Petri dishes before and after AMK i.p. injections. Urine samples were preserved in Eppendorf tubes and frozen until analysis for the selected biomarker.

To determine renal glutathione, the left kidney was submerged in a freshly made phosphate buffer saline solution (PH 7.4) and frozen at -20 °C. For histological examinations, the right kidney was fixed in 10% formaldehyde**.**

Biochemical determination

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 ß2-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). Serum IL-6 was quantified using a rat ELISA kit to determine the immunological profile, and the procedures were performed in accordance with the manufacturer's specifications (Shanghai YL Biotech Company, China). Renal glutathione and serum malondialdehyde (MDA) levels were evaluated by using rat ELISA kits (Shanghai YL Biotech Company, China) as directed by the manufacturer to determine antioxidant enzyme activity.

Histopathological examination

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

For comparison, One-way ANOVA was used among groups. For additional evaluation, Turkey's posthoc analysis test was utilized. The data were presented as Mean ± SEM with $P < 0.05$ significance. GraphPad Prism software was used to analyze the data (Version 8.0).

Conclusion

A final conclusion based on the findings of this study is that AMK effectively produced kidney injury in all of the treated rats. In contrast, CBE alleviated the functional and structural damage caused by AMK by acting as both an anti-inflammatory and an antioxidant. Based on these data, we may infer that CBE supplementation can minimize AMK nephrotoxicity. Future investigations should include a broader range of doses of CBE and explore the effects of CBE on more specific indicators of inflammation and oxidative stress, as well as novel markers of kidney damage, to offer a more accurate explanation of the precise mechanisms of the nephroprotective effects of CBE in this experimental model.

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Declarations

Conflict of interest Fatima. F. Dari, Ausama Ayob Jaccob, and Muhsin S.G. AL-Moziel declare that they have no conflict of interest.

Ethical approval Animal experiments were performed in accordance with the guidelines established by the National Institutes of Health (NIH). Permission for these experiments was obtained by the Animal Ethics Committee of the College of Pharmacy at the University of Basra in October 2021, 3/5/293.

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