

The Impact of Citrus Bergamot Extract on Hemato- Biochemical, Inflammatory and Oxidative Stress Parameters Induced by Acute Amikacin Toxicity in Male Albino Rats

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Abstract

One of the most efficient aminoglycosides is amikacin. Yet, it has been linked to unwanted renal toxicity, which has resulted in negative alterations in various biochemical indicators, particularly those related to oxidative stress, kidney function, and inflammation. The goal of this research was to see how citrus bergamot extract affected hemato-biochemical, inflammatory, and oxidative stress parameters in male rats triggered by acute amikacin toxicity. A total of 30 male rats were divided into five equal groups, each with six rats. The control group received 1 ml DW orally for ten days. Group 1 was given 1 ml of DW orally for ten days, and I.P. AK (1.2 g/kg) on day seven. Group 2 was given 100 mg/kg citrus bergamot extract orally for ten days. Groups (3) and (4) were given 100 mg/kg and 200 mg/kg of citrus bergamot extract, respectively, orally for ten days. On the seventh day of the experiments, I.P. AK (1.2 g/kg) was given to the test groups. At the end of the trial, blood samples were used to assess oxidative stress, renal function, inflammatory markers, and some hemato-biochemical parameters. Significantly higher levels of serum MDA, IL-6, urea, and creatinine, and adversely affected lipid profile, are indications of AK-induced nephrotoxicity. Supplementation of CBE attenuated AK-induced change in these biomarkers. It was concluded that CBE supplementation protects against the nephrotoxic effects of AK because of its antioxidant, anti-inflammatory, and hypolipemic properties.

Keywords: Amikacin, Nephrotoxicity, Oxidative stress, Inflammation, Citrus bergamot.

تأثير مستخلص برغموت الحمضيات على عوامل الإجهاد البيوكيميائية والالتهابية والأكسدة التي تسببها السمية الحادة لأميكاسين في ذكور الجرذان.

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الخلاصة

الاميكاسين هو أحد أكثر المضادات الحيوية للأمينوكلينوسيد كفاءة. ومع ذلك، فقد تم ربطه بالتسمم الكلوي غير المرغوب فيه، مما أدى إلى تغييرات سلبية في مؤشرات كيميائية حيوية مختلفة، لا سيما تلك المتعلقة بالإجهاد التأكسدي، ووظائف الكلى، والالتهابات. كان الهدف من هذا البحث هو معرفة كيفية تأثير مستخلص البرغموت الحمضي على عوامل الإجهاد الكيميائي الحيوي والالتهابات والأكسدة في ذكور الجرذان الناتجة عن السمية الحادة للاميكاسين. تم تقسيم إجمالي 30 من ذكور الجرذان إلى خمس مجموعات متساوية، كل منها ستة جرذان. تلقت مجموعة السيطرة 1 مل من الماء المقطر عن طريق الفم لمدة عشرة أيام. اما المجموعة (1) تلقت 1 مل من الماء المقطر عن طريق الفم لمدة عشرة أيام و (2، 3) (1.2 / كغ) من الاميكاسين في التجويف البيرييتوني في اليوم السابع. اما المجموعة (2) تلقت 100 / كغ / مغ / كغ من المستخلص شفويا لمدة عشرة أيام. تم إعطاء المجموعات (3، 4) 100 / كغ / مغ / كغ و 200 / كغ / مغ / كغ من المستخلص شفويا لمدة عشرة أيام. في اليوم السابع من التجارب، تم إعطاء (1.2 / كغ) من الاميكاسين في التجويف البيرييتوني لمجموعات الاختبار. في نهاية التجربة، تم استخدام عينات الدم لتقييم الإجهاد التأكسدي، ووظيفة الكلى، وعلامات الالتهاب، وبعض المتغيرات الكيميائية الحيوية للدم. اظهرت هذه الدراسة بارتفاع مستويات كل من المالونديالدهايد (MDA)، الانترلوكين-6 (IL-6)، اليوريا، والكرياتينين، بالإضافة إلى تأثير سلبي على الدهون، التي تعتبر مؤشرات على السمية الكلوية التي يسببها الاميكاسين. في حين سببت المعالجة بالمستخلص بتقليل التغيير الناتج عن الاميكاسين في هذه المؤشرات الحيوية مع بعض التحسن في ملف تعريف الدهون. استنتجت هذه الدراسة انه مستخلص البرغموت الحمضي يحمي من التأثيرات السامة للكلى بسبب الاميكاسين بسبب خصائصها المضادة للأكسدة والمضادة للالتهابات وتقليل نسبة دهون الدم.

الكلمات المفتاحية: أميكاسين، السمية الكلوية، الإجهاد التأكسدي، الالتهاب، البرغموت الحمضي.

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Introduction

Aminoglycosides (AMGs) are one of the earliest antibiotics. They were first made in the 1940s and used to treat various infectious diseases caused by gram-negative aerobic bacteria and gram-positive *Staphylococcus aureus*. AMGs inhibit the synthesis of proteins and induce changes in the integrity of the bacterial cell membrane, which leads to bactericidal cell death⁽¹⁾. The semi-synthetic aminoglycoside derivative amikacin (AK) has the broadest spectrum among semi-synthetic aminoglycoside derivatives. It is produced by the acetylation of kanamycin, a natural medication⁽²⁾. Amikacin is the most preferred and frequently used to treat severe hospital-acquired diseases caused by multidrug-resistant bacterial strains for its beneficial features like intense bactericidal activity, limited resistance to many bacterial enzymes, synergistic effects with lactam antibiotics, and low cost⁽³⁾. Dose-dependent and reversible nephrotoxicity is the most significant and life-threatening side effect of AK, which limits its usage⁽⁴⁾. Many pathophysiological pathways are involved in AK-induced nephrotoxicity, involving inflammation, transporter blockade, oxidative stress, and vascular alterations⁽⁵⁾. It also forms a connection with mitochondrial Fe²⁺, which stimulates the formation of free radicals⁽⁶⁾. AK accumulates most prominently in the renal cortex, where it produces free radicals that destroy unsaturated fatty acids in the cell membrane, leading to an increase in lipid peroxidation, an increase in MDA levels, and a decrease in glutathione levels in renal tissue⁽⁷⁾. AMGs-induced nephropathy is linked to biochemical and structural changes in the renal cortex, which include phospholipase C inhibition associated with increased phospholipid content in the cortex and the aggregation of myeloid bodies in the tissue⁽⁸⁾. According to Salem et al., 2011, gentamicin (GM) therapy raised blood TC, TG, and LDL levels while decreasing serum HDL values⁽⁹⁾. Rashid et al., 2005, discovered that after gentamicin exposure, blood cholesterol raised significantly in rats and caused significant damage to renal tissue⁽¹⁰⁾. It was also demonstrated that an increase in blood cholesterol levels had a major effect on the progression of GM-induced nephrotoxicity⁽¹¹⁾. Natural remedies have gained increasing popularity for treating various illnesses, and they may be the primary ingredients employed by the pharmaceutical industry. Plants contain a wide spectrum of compounds that are beneficial to one's health. Various phenolic compounds found in fruits and vegetables have antioxidant, anti-inflammatory, and antibacterial activities⁽¹²⁻¹⁴⁾. Citrus fruits have been shown to have health-promoting characteristics, owing to their high flavonoid content, which has a wide range of biological characteristics, including anti-oxidant, metal ion

chelating, vasoprotective, hepatoprotective, anti-inflammatory, anti-cancer, and anti-infective activity^(15,16). Among these, bergamot has recently received a lot of attention due to its unique characteristics. Bergamot (*Citrus bergamia* RissoetPoiteau) is a Rutaceae plant that grows mostly in Southern Italy, especially in Calabria⁽¹⁷⁾. Around 1660, Bergamot was initially employed as a pain reliever, and later as a perfume. Bergamot has been a vital component of the Calabrian economy for a long time, especially because it is the main source of essential oil used in the cosmetics sector⁽¹⁸⁾. Bergamot has a unique set of flavonoids and glycosides, like neohesperidin, neoeriocitrin, poncirin, naringin, and, rutin that make it different from other citrus fruits. not only for the composition of its flavonoids but also for its high content^(12,13). Recent scientific data have established the powerful anti-oxidative, anti-inflammatory⁽¹⁹⁾ and beneficial effect on the lipid profile of *Citrus bergamia* in animals and human studies⁽²⁰⁾, shining new light on its usefulness as a nutraceutical

Accordingly, this study attempts to look at how AK affects some Hemato-biochemical parameters and the possible ameliorative role of CBE in male albino rats who were exposed to acute amikacin toxicity.

Materials and Methods

Chemicals and Drugs

The drug Amikacin (Amikozit®, 500 mg/2 ml vial) was bought from Sanofi (Istanbul, Turkey). A citrus bergamot extract supplement (CBE 500 mg capsule) was bought from Double Wood Supplements (USA). Almost all of the biochemical analysis kits were bought from Abbott Company (USA). IL-6 and MDA ELISA kits came from Shanghai YL Biotech Company (China). The other chemicals and instruments we used in our research were of the best quality that could be found.

Experimental animals

In this study, 30 adult healthy albino male rats weighing 150 to 230 grams (aged 2-3 months) have been used. The rats were purchased from the university of Al-Qadisiyah, College of Veterinary Medicine, Iraq. After getting permission from the ethics committee at the University of Basra to do this experimental research. This study was conducted in the College of Pharmacy on October 2021, 3/5/293. The animals were kept in the animal house of the College of Pharmacy at the University of Basra under clean and standard conditions (25°C± 2°C, moderate humidity, and a 12-hour light/dark cycle). As part of the study, the rats were kept in large indoor plastic cages with wood chips as flooring. They also had ad libitum access to essential rat food and fresh water.

Experimental design

After 14 days of acclimatization, thirty rats were divided into five experimental groups, each with six rats. The normal control group received 1 ml of DW gavaged orally for 10 days. Group (1) was given 1 ml of DW gavaged orally for ten days, and I.P. AK (1.2 g/kg) on day seven. For group (2), 100 mg/kg citrus bergamot extract was gavaged orally for ten days. Groups 3 and 4 represent the test groups were given 100 mg/kg and 200 mg/kg of citrus bergamot extract, respectively, and were gavaged it orally for ten days and they represent the test groups. On the seventh day of the experiments, I.P. AK (1.2 g/kg) was given to the test groups. On day 7, the control group and group (2) were given DW intraperitoneally to mimic a rat model of AK intraperitoneal injection. The doses were chosen in agreement with prior studies(21–23). For nine days, all administered doses were provided once a day in the morning. The weights of the rats were measured three times during the experiment: at the start, five days later, and before the animals were sacrificed. [Relative kidney weight (RKW) = (kidney weight/ rat weight)* 100] calculated for comparison.(24).

Hemato-biochemical assessment

Twenty-four hours after the final dosage was administered, blood samples from the posterior vena cava were obtained for examination of hemato-biochemical parameters after the rats entered profound unconsciousness produced by breathing chloroform. Two tubes were used to collect blood samples. The blood in the first tube, was kept at room temperature for a brief period to enable clotting, and serum samples were obtained following centrifugation at 10,000 rpm for 20 minutes. Samples of clear serum were stored frozen at -20 °C until they were utilized for biochemical

analysis. While the other tube contained an anticoagulant (Na₂ EDTA) for hematological parameter testing. For both the experimental as well as control groups, ELISA kits were used to check the levels of both MDA and IL-6 markers in serum according to kit instructions. The spectrophotometric method was used to analyze kidney function tests (serum urea and creatinine) and lipid profiles (LDL, VLDL, HDL, cholesterol, and triglycerides). A diagnostic automated laboratory analyzer (Abbott Architect 4000c, USA) was used to test all of these parameters, except Non-HDL-C, which was calculated by subtracting HDL-C from the total cholesterol and adding it back. The hemoglobin levels were estimated using animals' whole blood by a Semi-Auto Chemistry Analyzer following the manufacturer's instructions (mindray BA-88A, China).

Statistical analysis

For comparison, One-way ANOVA was utilized among groups. Turkey's posthoc analysis test for further assessment was used. Data were expressed as Mean± SEM with p<0.05 significance. Data was analyzed using GraphPad Prism software (Version 6.0).

Results

The effects of amikacin on the body weights and relative kidney weight
In the current research, no significant changes were documented P>0.05 when compared and correlate the changes in body weights in the control and treated groups with respect to the number of days of the experiments (table1). Increase in body weights appear to be comparable in all groups. In contrast, the calculation of relative kidney weights suggests significant increase in RKW in both group1 and group3 compared to control.

Table 1. Changes in rat body weights with respect to days of the experiments.

Days	Rat weight (g)			P-Value	Relative Kidney weight
	1	5	10		
Control	166.16±3.35	169.5±2.7	182.5±2.97	0.1688	0.732± 0.023
Group1	171.5±7.68	189.16±8.9	197.8±8.62	0.1648	0.89±0.028*
Group2	180.5±7.48	189.3±7.12	195.5±7.58	0.1059	0.649±0.021
Group3	181±5.89	193±4.788	198.6±6.38	0.1707	0.871±0.045*
Group4	180±6.61	191.5±6.87	197.16±5.02	0.1641	0.861±0.033

*Indicate significant difference if we compare it to control P<0.05.

The influence of citrus bergamot extract (CBE) on serum malon dialdehyde (MDA) levels after high dose intraperitoneal Amikacin administration to rats

Experimental rats exposed to toxic dose of Amikacin had significant rise in MDA levels compared to control and remaining groups (figure1). This value reflects oxidative stress associated with AK toxicity. High pharmacological dose (200 mg/kg of CBE) ameliorates and counteracts oxidative damage rendering AK less toxic. Compared to group1, the levels of MDA dropped significantly in all of the groups that were treated with CBE and the normal control group (intoxicated groups).

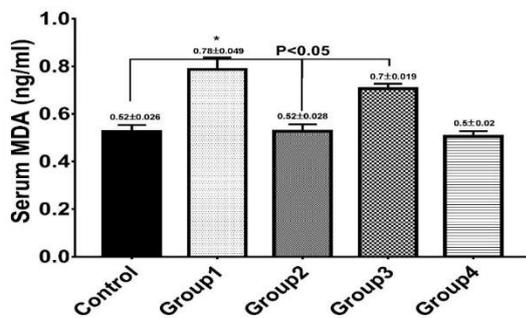


Figure 1. Influence of Citrus Bergamot Extract (CBE) on serum Malon dialdehyde (MDA) levels after high dose intraperitoneal Amikacin administration to rats. *represent significant difference P<0.05 between groups.

The influence of citrus bergamot extract (CBE) on serum Interleukin-6 (IL-6) levels after high dose intraperitoneal Amikacin administration to rats

Figure (2) clearly shows a significant increase in IL-6 level in group1 treated with AK alone compared to control and remaining groups. In both doses, CBE lowers the level to similar levels of the control group. The lowest level was found in Group 2.

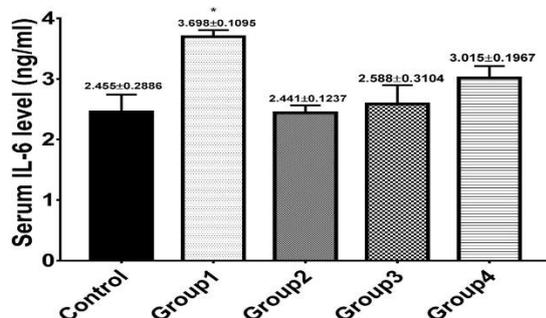


Figure 2. Influence of Citrus Bergamot Extract (CBE) on serum Interleukin-6 (IL-6) levels after high dose intraperitoneal Amikacin administration to rats. *indicate significant difference P<0.05 among groups.

The influence of Citrus Bergamot Extract (CBE) on renal biomarkers levels after high dose intraperitoneal Amikacin administration to rats

Serum concentrations of urea and creatinine were significantly elevated in group1 as seen in figures 3 (A and B). Rats in group1 injected with AK associated with clear increment in renal biomarkers mostly occur in renal injury. Administration of pharmacological doses of CBE decrease both creatinine and urea values with greater reduction associated with dose 200mg/kg of CBE were normalization of renal biomarkers values resemble to control group. Group2 administered CBE alone show normal values of both urea and creatinine.

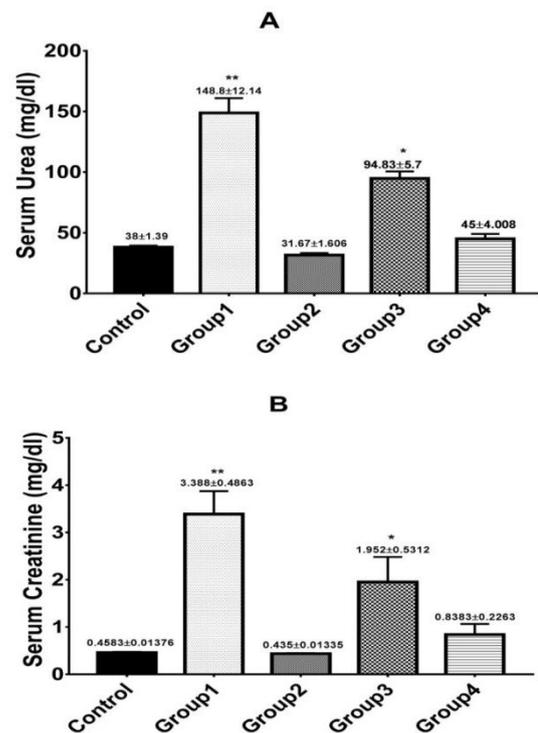


Figure 3. Influence of Citrus Bergamot Extract (CBE) on renal biomarkers levels after high dose intraperitoneal Amikacin administration to rats. A: represent serum urea concentrations, B: represent serum creatinine concentrations. **represent significant difference P<0.001 among groups. *represents significant difference P<0.05 among groups.

The influence of citrus Bergamot Extract (CBE) on lipid profile after high dose intraperitoneal Amikacin administration to rats

Fig. 4 showed serum concentrations of LDL, VLDL, cholesterol, TG, UHDL and Non-HDL in the control and treated groups of rats after CBE oral gavage and intraperitoneal Amikacin administration. Serum LDL significantly increased in all groups administered AK, serum VLDL significantly increased in group1 associated with a bad effect on lipid profile. Oral treatment with CBE

in both doses reduced serum VLDL elevation and led to normalization towards serum VLDL level in the control and group3. It was found that serum levels of cholesterol and TG in the studied groups expressed in resemble manner to LDL and VLDL respectively. Serum cholesterol levels still elevated in spite of CBE oral administration for 10 days. On the other hand, serum TG levels in CBE treated

groups decrease significantly as documented in figure4. Regarding serum UHDL the highest value observed in group2 this level indicates the good effect of CBE on lipid profile. Actually, the non-HDL levels in all groups administered AK increased significantly indicated it is bad effect on lipid profile.

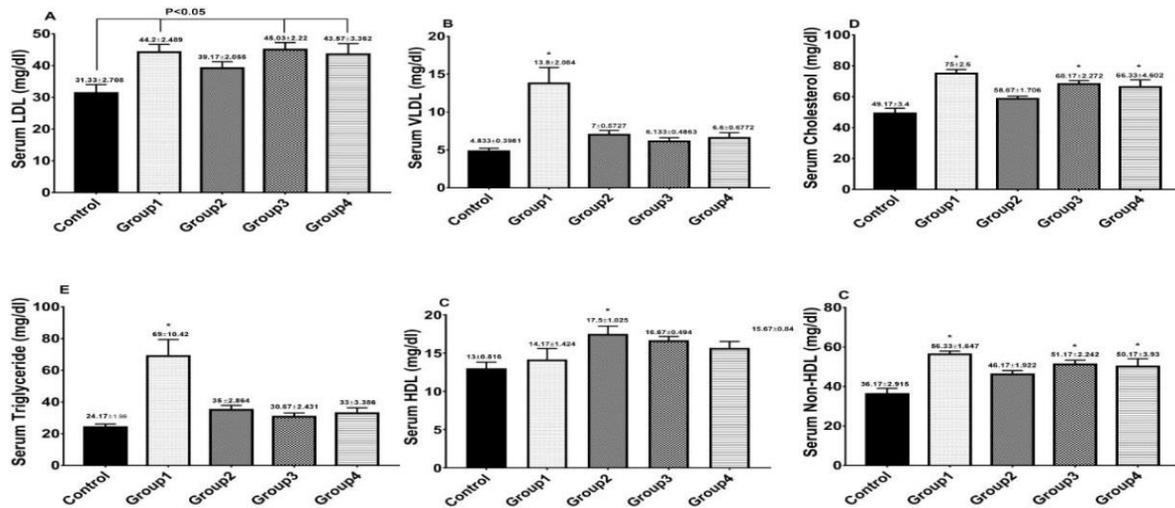


Figure 4. Influence of citrus Bergamot Extract (CBE) on lipid profile after high dose intraperitoneal Amikacin administration to rats. * indicate significant difference P<0.05 among groups in each lipid profile panel.

Influence of Citrus Bergamot Extract (CBE) on hemoglobin levels (Hb) after high dose intraperitoneal Amikacin administration to rats

Hemoglobin levels were evaluated in order to obtain complete idea about the effect of CBE and AK on HB serum concentration. There was no significant difference between the tested and control groups in this study. All groups well matched as seen in figure 5.

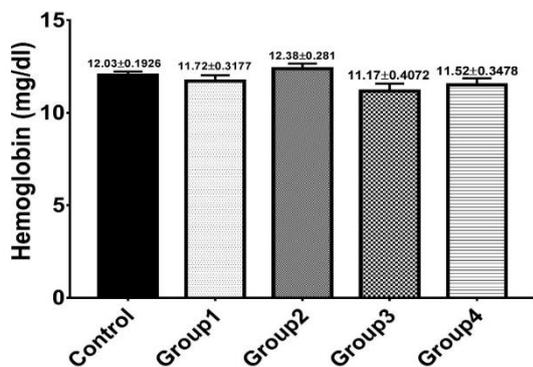


Figure 5. Influence of Citrus Bergamot Extract (CBE) on hemoglobin levels (Hb) after high dose intraperitoneal Amikacin administration to rats.

Discussion

Aminoglycoside antibiotics, , specifically AK, have long been recommended as a first-line antimicrobial treatment option for severe gram-negative infectious diseases (25). Despite their benefits, aminoglycosides have considerable oto- and nephro-toxicity, which are limiting considerations for their clinical usage, in which the oxygen free radicles have been involved in mechanistic toxicity (4).

The results of the existing work showed anon-significant correlations regarding the effect of times on changes in body weight of the tested and control groups. This finding can be explained by single dose administration of AK on day7 of the treatment schedule appear not enough to induce a significant change in body weight during 10 days' period of the study. The present findings are in line with those of Noori et al., 2019, who showed that the body weight of rats was not affected after amikacin administration (26). Opposite to our findings, Fatima et al., 2021, discovered that when rats that given aminoglycosides, their body weights change when compared to control groups, However, AK induced nephrotoxicity in rats evidenced by significant elevation in relative kidney weight in the AK-treated groups compared to the control group. These findings may be attributed to the fact that injection

of nephrotoxic substances caused an increase in kidney weight and swelling of renal tissue, these findings are supported and came in line with a large number of articles explained the nephrotoxic effect of aminoglycoside⁽²⁶⁾.

In this study, rats that had been given AK had higher levels of MDA in their serum than rats in normal control group. This elevation can be explained by excess ROS production by the accumulation of AMGs in renal proximal tubular cells, which causes an increase in lipid peroxidation (MDA) and the destruction of unsaturated fatty acids in the cell membrane⁽⁷⁾.

This is supported by other investigations which revealed the involvement of oxidative stress in nephrotoxicity as indicated by elevation in MDA levels in animals⁽²⁷⁻²⁹⁾. Today, researchers are focus 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 significantly lowers MDA levels as compared to AK-treated rats (group 1). These finding highlights that bergamot extract possesses a robust antioxidant activity.

In tune of our results, Zhang et al., 2021, demonstrated the antioxidant activity of bergamot products in rats with primary dysmenorrhea by marked decreased in MDA content, a marker of lipid peroxidation⁽³⁰⁾.

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