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Protective effect of aqueous extract of Adiantum capillus leaves against renal toxicity induced by gentamicin

Measer Abdullah Ahmed, Jasim Mohammad Jasim

Dep. Of biology, college of education for pure science, Tikrit university, Iraq

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***Corresponding author:**

Email : measer80@gmail.com

Mobile : 009647701827668

Contact To Journal

E-mail: tjops@tu.edu.iq

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Abstract

The study aimed to investigate the therapeutic effect of Aqueous Extract of Adiantum capillus-veneris leaves (AEA) against nephrotoxicity induced by Gentamicin. Twenty four adult male rats housed in animal house unit / college of veterinary, underwent to the necessary laboratory conditions. Study period was 8 days. Rats divided into four groups: first was normal control, second treated gentamicin(80mg/kg/day.im), third group treated with gentamicin(80mg/kg/day.im) and AEA(250mg/kg/day. oral) and fourth group treated with gentamicin (80mg/kg/day.im) and metformin (100mg/kg/day.oral). Blood were taken by cutting jugular vein serum were used to estimate levels of albumin, urea, creatinine, malondialdehyde(MDA), glutathione (GSH) and superoxide dismutase (SOD). Also Kidney were weighted and homogenate to estimate oxidant-antioxidant parameters. Results showed a significant increase in albumin, urea, creatinine, MDA and a significant decrease in GSH and SOD in rats treated by gentamicin compared to control in $P<0.01$, while treating by AEA and metformin showed a significant decrease in Albumine, Urea, creatinine, MDA and a significant increase in GSH and SOD and CAT in AEA group compared to gentamicin group. The significance was higher in AEA than metformin. We concluded that AEA has a therapeutic activity against nephrotoxicity induced by gentamicin in male rats.

التأثير الوقائي للمستخلص المائي لأوراق كزبرة البئر ضد سمية الكلى المستحث بوساطة عقار الجنتامايسين

ميسر عبدالله احمد جاسم محمد جاسم

الخلاصة

هدفت الدراسة لمعرفة التأثير العلاجي للمستخلص المائي لأوراق كزبرة البير ضد سمية النفرونات المستحث بوساطة الجنتامايسين. اربع وعشرون ذكراً من الجرذان البالغة وضعت في وحدة البيت الحيواني وخضعت للظروف المختبرية الضرورية لمدة ثمانية ايام. قسمت الجرذان الى اربعة مجاميع: الاولى السيطرة الطبيعية، الثانية مجموعة الجنتامايسين (80ملغم/كغم/يوم/ في العضلة)، الثالثة المعاملة بالجنتامايسين (80ملغم/كغم/يوم/ في العضلة) مع المستخلص المائي (250ملغم/كغم/يوم. فموياً) و المجموعة الرابعة المعاملة بالجنتامايسين(80ملغم/كغم/يوم/ في العضلة) مع عقار الميتفورمين (100ملغم/كغم/ يوم. فموياً). اخذ الدم من خلال قطع الوريد الوداجي واستخدم المصل لتقدير مستويات الالبومين، اليوريا، الكرياتينين والمالون ثنائي الالديهايد، الكلوتاثيون، وانزيم السوبر اوكسيد دسميوتيز. كذلك وزنت الكلى و سحقت لتقدير مستويات الاكسدة – مضادات الاكسدة. اظهرت النتائج زيادة معنوية في مستويات الالبومين ،اليوريا، الكرياتينين و المالون ثنائي الالديهايد وانخفاضاً معنوياً في مستويات الكلوتاثيون وانزيم السوبر اوكسيد دسميوتيز في المجموعة المعاملة بالجنتامايسين بالمقارنة مع مجموعة السيطرة عند مستوى معنوية $P<0.01$. بينما اظهرت المعاملة بالمستخلص المائي والميتفورمين انخفاضاً معنويا في مستويات البومين، اليوريا، الكرياتينين و المالون الثنائي الالديهايد وارتفاعاً معنوياً في مستويات الكلوتاثيون، الكاتاليز وانزيم الدسميوتيز بالمقارنة مع مجموعة الجنتامايسين فقط. وكان التأثير المعنوي في مجموعة المستخلص المائي اعلى من مجموعة الميتفورمين. نستنتج من ذلك الفعالية العلاجية للمستخلص المائي ضد السمية النفرونية المستحثة بوساطة الجنتامايسين في ذكور الجرذان.

Introduction

Kidney diseases has been newly d3eveloped and increased, especially in patients who already have some kidney problems[1]. The incidence of kidney disease varies among those taking antibiotics from one drug to another, but it is especially high among those who are treated with aminoglycosides especially if kidney function has deteriorated since Start, or excretion of urine slightly every 24 hours or in patients with dehydration, and older people are more vulnerable to these drugs than children[2]. The most important types of aminoglycosides are: streptomycin, kanamycin, gentamycin, tobramycin, amicassine, naltymycin, all of which have toxic effects on the kidneys and on the ear nerves responsible for hearing and balance [3]. Aminoglycosides are one of the most powerful types of antibiotics and kill bacteria quickly, causing serious diseases (eg, peritoneal inflammation and septicemia). Antibiotics (especially gentamicin) should be taken in the serious diseases caused by bacteria, and note should not be used for a period of one week at most increases until the patient does not exceed the ratio of toxicity [4]. Several chemotherapy drugs are used in the treatment of kidney diseases such as metformin, which is commonly used to treat type 2 diabetes chronic kidney disease, etc., but there is damage associated with the use of this drug, such as increased lactic acid, which leads to negative side effects such as stomach pain Muscle, tiredness, frequent sleep, general weakness, etc.[5]. Therefore, scientific thinking tends to return to the treatment of various diseases with plant drugs because of its high efficiency in treating many diseases and the absence of most of the side effects when used, even for a long time, unlike chemically manufactured pharmaceutical compounds laboratory [6,7]. Plants and herbs contain chemicals from secondary metabolisms such as phenols and flavonoids that are effective against many pathogens[8]. High liquid performance chromatography (HPLC) analysis studies recorded a lot of chemical materials in

Adiantum capillus-veneris. The leaves are the most useful part contain alkaloids compounds like (kaemmpfertin, Adiantone, Quanic acid and Isoquinoline) and phenolic compound such as (Kaempferol, caffic acid, ferulic acid, Naringin and Coumaric acid) in addition to minerals like zinc and copper. These compound have a bioactivity against various diseases and inflammations. [9].

Aim of the study

The study aimed to investigate the biological effect of aqueous extract of *Adiantum capillus-veneris* leaves (AEA) to protect kidney against toxicity induced by Gentamicin, and evaluate its effect in compare to metformin.

Materials and methods

Collection and preparation of plant

The *Adiantum capillus-veneris* plant was collected in August to October 2018 from the banks of the Tigris River in Alalam district. The plant were washed with distilled water before the drying phase to obtain a pure sample free of impurities. Plant dried at room temperature (27C) then leaves were taken and placed in a non-transparent containers, at room temperature until extraction.

Extraction of plant

The *Adiantum capillus-veneris* leaves were extracted in cold water in accordance with the study of AL-shaikhani, 2015.[10]. Leaves crushed with the electric mixer, 40g of powder were mixed with 160 ml of distilled water, placed in a glass flask. After mixing, the mixture was kept in the refrigerator at 4 ° C for at least 24 hours to ensure the solubility was well completed. The mixture was filtered using filtration papers to remove the plant fiber and obtain a raw extract, then the filter mixture was transfer to the rotary evaporator at 40 C degree in order to avoid any change in the chemical composition of the mixture. This process is performed for 30 minutes until it reaches a semi-solid strength and then transferred to a device vibrator incubator at 35C degree until drying, and kept in plastic packaging until use [10].

Animals and Experimental Design

Twenty four animals of white male rats *Sparque Dawley* were enrolled in this study weighed between 210 - 220 g and ages between 10-12 weeks. The animals were placed in plastic cages in the animal house at the Faculty of Veterinary Medicine / University of Tikrit and underwent the necessary laboratory conditions during the 8 days. The animals were assigned to four groups. First group is a control group, given a standard diet and water orally. Second group treated with Gentamicin (80mg/kg/day. intramuscular). Third group treated with aqueous extract of *Adiantum capillus-veneris* leaves (250mg / kg/day. oral) and gentamicin (80mg/kg/day. Intramuscular); forth group treated with gentamicin (80mg/kg/day. Intramuscular) and metformin (100mg/kg/day.oral) and considered as a positive control to compare with AEA.

Preparation of blood sample

At the end of the study period (8 days), animals were starved for 24 hours, then blood samples were taken by cutting the jugular vein, placed in the test tubes, kept in the incubator at 37 ° C for 30 minutes, Using the centrifuged at 3000 rpm for 15 minutes, separated serum from the other ingredients using the micropipettes. Serum kept at -80 ° C until the biochemical tests were done.

Preparation of kidney homogenization

After animals were sacrificed kidney tissue were removed immediately, weighed and placed in cooling homogenization medium (0.25M sucrose, 1mM EDTA, 20mM tris-HCl buffer, pH 7.5) 10ml/gram of tissue. A gram of Kidney tissue was homogenated using homogenizer type (SHM1)16000 rpm for 30 second then placed in cooling centrifuge (-4C) 10.000 rpm for 15 minutes. The supernatant was used to

estimate the concentration of antioxidant parameters.[11]

Parameters Analyze

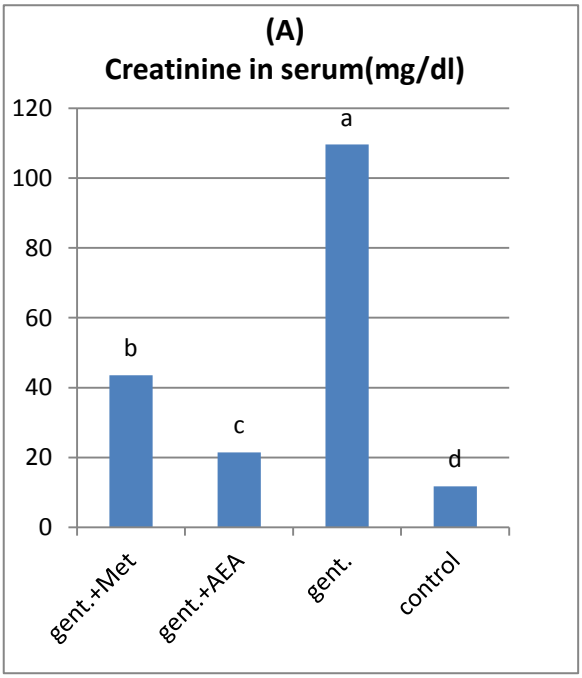
Serum was used to determine the concentration of Albumine, Urea, Creatinine, Glutathione (GSH), Super oxide dismutase (SOD) and, Malondialdehyde (MDA) using animal analysis kit processed by Spanish Biosystems and instruction of manufacture.

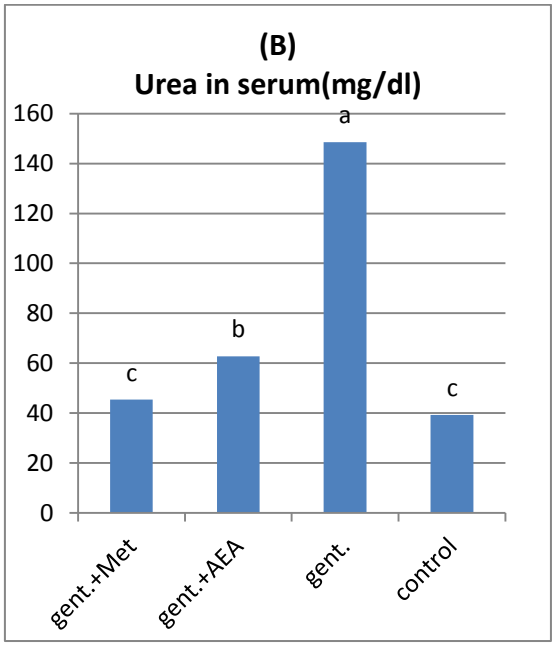
Statistical analysis

Significance differences were determined using the ANOVA-one way test. These differences were confirmed by the standard error. The differences were determined by Duncan's multiple ranges and at a significant level ($p<0.01$) [12].

Results

The results showed that the treatment with gentamicin resulted in a significant increase in the levels of Creatinine, Urea and Albumin in serum and in kidney weight compared with the control group, The treatment with the aqueous extract of *Adiantum capillus* leaves was result in significantly lower levels in Creatinine, Urea, albumin in compare to the gentamicin treatment group. The treatment with metformin was also showed a significant decrease in Creatinine, Urea, and Albumin levels in compared with the gentamicin treatment group. Figure(1).





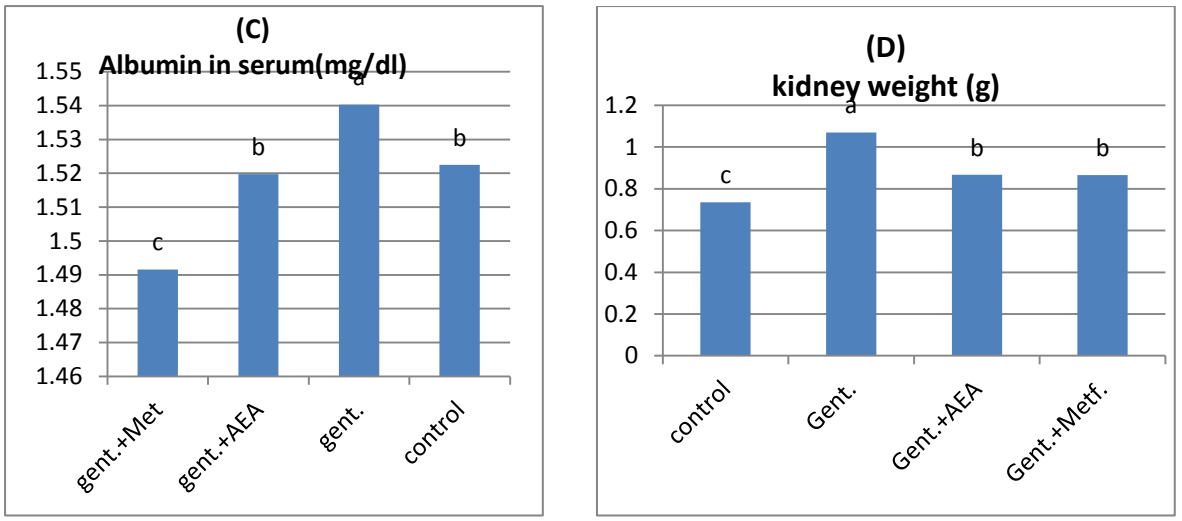


Fig. (1):- Effect of aqueous extract of adiantum leaves and metformin in concentration of (A) Urea, (B) creatinine, (C) albumin and (D) kidney weight, in rats exposed to nephropathy induced by gentamicin. Gentamicin group was compared to control group P≤0.01. Treatment groups were compared to gentamicin group. P≤0.01. Gent.: Gentamicin (80mg/kg/day. i.m). Met.: metformin.(100mg/kg/day. Oral). AEA.: Aqueous extract of *Adiantum capillus-veneris* leaves. (250mg/kg/day. Oral). Different letters mean a significant differences in p≤0.01.

The results of the study showed that the treatment with gentamicin resulted a significant increase in the levels of MDA in serum and kidney tissue compared with the control group, while a decrease in the levels of GSH, CAT and SOD was observed in serum and kidney tissue. The treatment with the aqueous extract of *Adiantum capillus* leaves was resulted in a significantly higher levels of GSH,CAT and SOD(in serum and kidney tissue) and significantly lower in MDA in serum and kidney tissue compared to the gentamicin treatment group. The treatment with metformin also showed a significant increase in GSH, and SOD levels in serum and kidney tissue and a significant decrease in MDA in compared with the gentamicin treatment group, while No significant difference found in CAT level Table(1).

Table (1):- Effect of AEA and metformin on oxidant-antioxidant parameters in serum and kidney tissue in gentamicin-induced nephropathy (Mean ± SEM).

	Control	Gent.	Gent.+AEA	Gent.+Met
GSH(µmol/L)	4.051 ± 0.214^{b}	3.637 ± 0.122^{c}	4.598 ± 0.512^{b}	8.154 ± 433^{a}
SOD(µmol/L)	10.089 ± 0.132^{a}	8.916 ± 0.217^{b}	10.711 ± 0.342^{a}	10.444 ± 0.165^{a}
MDA(µmol/L)	21.623 ± 0.402^{b}	23.046 ± 0.163^{a}	18.123 ± 0.213^{c}	19.124 ± 0.435^{c}
GSH-Kidney (µmol/g)	4.828 ± 0.735^{b}	1.165 ± 0.101^{d}	5.571 ± 0.472^{a}	3.643 ± 0.232^{c}
SOD- Kidney (µmol/g)	9.358 ± 0.264^{a}	7.590 ± 0.293^{b}	10.732 ± 0.488^{a}	9.832 ± 0.219^{a}
CAT-kidney (µmol/g)	969.145 ± 8.203^{a}	657.32 ± 5.815^{c}	743.695 ± 4.089^{b}	606.19 ± 4.879^{c}
MDA- Kidney (µmol/g)	6.790 ± 0.119^{c}	10.455 ± 0.184^{a}	5.160 ± 0.624^{c}	7.987 ± 0.144^{b}

Gentamicin group compared to control group, $p<0.01$.
Treatment groups compared to gentamicin group, $p<0.01$.
SEM: standard error of mean.

Gent.: Gentamicin (80mg/kg/day. i.m)
Met.: metformin.(100mg/kg/day. Oral)
AEA.: Aqueous extract of *Adiantum capillus-veneris* leaves.(250mg/kg/day. Oral).
Different letters mean a significant differences in $p \leq 0.01$.

The treatment using aqueous extract was significantly higher in GSH, CAT and SOD, lower in levels of MDA and Creatinine than in the metformin group, while the decrease in Albumin and Urea levels was significantly higher than the aqueous extract treatment group.

Discussion

Gentamicin is an antibiotic that used for the treatment of a bacterial infection**,** evidence indicated the nephrotoxic effect of gentamicin that causes a renal failure by inducing oxidative stress that cause renal damages[13]. Previous studies indicated the role of gentamicin in increased levels of reactive oxygen species ROS like superoxide anion and hydroxyl radicals that cause cellular damage and contribute in proximal tubular necrosis through several mechanisms including lipid peroxidation, membrane destabilization and DNA damage. [14]. Gentamicin accumulation in kidney cortex make a morphological changes which led to pathological effect [4]. References have documented the role of metformin in raises the antioxidant system and reduces cell death induced by oxidative stress [15]. Due the protective role to metformin we used it in this study as a positive control to compare the protective potential with AEA. Kidney is over sensitive to damage due reactive oxygen species[16], an increasing in kidney weight marked in gentamicin group (fig.1.D) which might refer to inflammation, oxidative stress and edema caused by gentamicin, while a decrease in kidney weight noticed in treatment groups in compare to gentamicin which indicate to the protective role of treatments against oxidative stress and inflammation (fig1.D). gentamicin attenuates to renal function is reflected to the increase in creatinine, albumin and urea levels in serum in compare to control (fig. 1.A.B.C). The treatment using AEA enhanced the nephroprotective due decreasing creatinine, albumin and urea levels in serum which mean the renal efficiency in compare to gentamicin group and that could be attributed to the antioxidant effect of biochemical materials that exist in AEA. Nephropathy induced by gentamicin is related to the decrease in antioxidant system activity in renal cortex [17] such as GSH, SOD and CAT also increase level of MDA which made oxidative damage in rats kidney (table.1). The protective role of metformin and AEA is associated to attenuate the lipid peroxidation and increase the activity of antioxidant enzyme SOD and CAT in addition to increase GSH as a scavenger of free radicals to prevent oxidative damage in rats exposed to oxidative stress by gentamicin. (table 1).

Conclusions

We concluded from data of this study the bioactivity of vital compounds in the aqueous extract of *Adiantum capillus-veneris* in renal protective against oxidative damage induced by gentamicin by elevating antioxidants levels and prevent lipid peroxidation. In general the significance in AEA treatment was higher than metformin.

Recommendations

According to the study result, we recommend to avoid using gentamicin for a period over than a week. Also we suggest to use medicinal herbs that approved their ability to protect renal from toxicity.

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