



Assessment of Nephroprotective and Antidiabetic Effects of *Epipremnum aureum* from Algeria in Albinos Rats



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Abstract

EPIPREMNUM is a remarkable medicinal herb that has been utilised in various traditional medical systems to cure human illnesses and afflictions. Numerous biochemical substances, including alkaloids, flavonoids, glycosides, terpenoids, and saponins, are said to be present. The objective of this work is to evaluate *in vitro* the antihyperglycaemic activity of the methanolic of *Epipremnum aureum* (EAMeOH) by the inhibition of two enzymes α -amylase and α -glucosidase and to evaluate *in vivo* the anti-nephrotoxic activity against by gentamicin induced nephrotoxicity. Different concentrations of extract were incubated with enzyme substrate solution and enzyme activity was measured. In addition, acarbose was used as a standard inhibitor. The extract was able to inhibit α -amylase and α -glucosidase with a percentage of 28.45 and 57.45% respectively with an IC₅₀ of 222.37 ± 0.12 of α -glucosidase. For anti-nephrotoxic activity, five groups of 6 rats were treated respectively with CMC of sodium, EAMeOH (200 mg/Kg b.w), these treatments were administered orally 1h before the administration of gentamicin except the 1st group did not receive that CMC of sodium). The results showed that the administration of gentamicin decreased significantly ($P \leq 0.05$) the urine volume and the weight of the kidneys in control group, but the oral administration of the extract (200 mg/Kg b.w.) significantly increased ($P \leq 0.05$) the urine volume and the weight of the kidneys compared to the control. Furthermore, the treatment by EAMeOH extract for 7 days at a daily dose of 200 mg / kg b.w; causes the decrease of serum urea with 0.25 g /L with significant manner ($P \leq 0.05$) and creatinine with 7.45 mg /L compared to control group. Histopathological reports showed reduction in the damage of kidneys when treated with the extract. These results suggest that the Methanolic extract of *E. aureum* leaves may be useful in reducing the gentamicin induced nephrotoxicity.

Keywords: *Epipremnum aureum*, gentamicin, methanolic extract, α -glucosidase, α -amylase, nephrotoxicity.

Introduction

Plants are an essential source for the discovery of new medicinal compounds intended for drug development, and secondary metabolites are sources of plant-derived medicines [1]. These secondary metabolites act as natural defenses against pathogens, pests, and environmental stresses like UV radiation, while also contributing to plant development and communication. These compounds, which include alkaloids, terpenoids, and flavonoids, have diverse roles, such as antimicrobial and antioxidant properties, and also serve as signals in plant interactions. Humans benefit from these metabolites,

which have applications in medicine, industry, and agriculture, with playing a role in understanding, enhancing, and potentially engineering these valuable compounds for a sustainable future [2]

The Araceae constitute a large family of plants comprising approximately 105 genera and around 3,000 species of monocots [3].

Epipremnum aureum belongs to the family Araceae, this plant is a common houseplant, also possessing various secondary metabolites, such as alkaloids, flavonoids, and tannins [4]. This plant is used in traditional medicine to treat various

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conditions, particularly as an antimicrobial and anti-inflammatory[5].

In certain traditional medicinal practices, *E. aureum* is used to relieve respiratory symptoms such as cough and bronchial congestion, thanks to its expectorant properties. It is also used to treat gastrointestinal conditions such as indigestion, bloating, and abdominal discomfort [6,7].

E. aureum has both antidiabetic and nephroprotective potential. Leaf extracts have demonstrated significant anti-hyperglycemic (anti-diabetic) effects in alloxan-induced diabetic rats, comparable to the drug metformin, and exhibited protective effects on kidney cells by lowering toxicity and maintaining serum markers. The plant's potential is linked to its rich content of bioactive phytoconstituents, particularly phenolic compounds and flavonoids, which possess antioxidant, anti-inflammatory, and insulin-sensitizing properties [8].

Studying an agent's antidiabetic and nephroprotective activities is significant because uncontrolled diabetes mellitus (DM) frequently leads to diabetic nephropathy, a leading cause of kidney damage and end-stage renal disease. Research in this area aims to discover new, effective, and safer treatments for diabetes and its kidney complications by understanding how agents can lower blood sugar and protect against oxidative stress and inflammation in renal cells, potentially improving overall health and reducing the burden of chronic kidney disease [9].

DM is recognized as a life-threatening disease with numerous dangerous complications. Several medicinal plants have been identified for the treatment of DM [10,11]. The body's incapacity to produce insulin results in type 1 diabetes. Fasting hyperglycemia that happens even when insulin is available is referred to as type 2 DM [12].

The harmful effects of some substances, including hazardous chemicals and medications (nephrotoxins are chemicals that exhibit nephrotoxicity), on the kidneys are known as nephrotoxicity. Numerous antibiotics, such as tetracyclines, cephalosporins, penicillins, aminoglycosides, and sulfonamides, have the potential to be nephrotoxins [13]. Functional manifestations of aminoglycoside nephrotoxicity include moderate glucosuria, tubular proteinuria, lysosomal enzymemuria, decreased ammonium excretion [14].

One of the most frequent causes of drug-induced nephrotoxicity for a long time has been aminoglycosides (AG)[15]. AG continues to be the primary effective treatment choice for bacteria resistant to other antibiotics, despite their unfavourable side effects [16]. Their chemical

stability, instantaneous bactericidal action, low resistance, and low cost are the key reasons for this [17]. Moreover, GM (gentamicin) has been widely used as an appropriate material to induce animal nephrotoxicity in experimental investigations [18]. Numerous studies have demonstrated that oxidative stress brought on by GM metabolism is one of the basic mechanisms underlying the nephrotoxicity caused by this. The production of superoxide and other reactive oxygen species [19].

Many diseases are still incurable with current medications, even though many medications have been created to address the ailments. Finding novel medications with negligible or no adverse effects is also extremely difficult. Because they contain a variety of components that can display surprising biological features, natural products—compounds or chemicals produced from living organisms—may offer solutions to these issues [20].

The current study was aimed to explore the phytoconstituents, and enzyme inhibition of diabetis (Alpha-amylase and Alpha-glucosidase) and nephroprotective activities of *E. aureum* growing in Algeria, specifically in the Aures region.

Material and Methods

Animals and experimental design

The animals used in this study were obtained from the Pasteur institute at Algiers, Algeria. Wistar rats' cages under controlled conditions of humidity (50–60%), temperature (24 °C), with access to food and water ad libitum.

Plant material

In February 2023, the plant under study was gathered from the Ghassira district of Batna, Algeria. For subsequent application, such as extracting the active compounds after grinding, the gathered plant leaves were cleaned and then allowed to dry for 12 days.

This plant was identified by Pr. Oudjhih -University BATNA 1.

Plant extraction

The extraction is done using the Diallo et al. [21] method, which involves macerating 500 g of plant leaf powder with 5L of methanol. We employed filter paper and cotton for the filtration during the 24-hour extraction process, the extraction repeated three times, which was conducted with constant stirring at room temperature. The filtrate was then concentrated by rotational evaporation at 40 °C, yielding methanolic extract (EAMeOH) [22].

Total phenolic content measurement

5 mL of distilled water and 1mL of 20% Na₂CO₃ are used to dilute 1 mL of EAMeOH (0.2 mg/ml). The reaction was allowed to sit at room temperature.

Following the addition of 1 mL of Folin-Ciocateu reagent, the mixture was incubated for 30 min at 40°C in an oven. At 760 nm, the absorbance was measured in comparison to a blank. A gallic acid calibration curve (0-200 µg / ml) is used to express the amount of polyphenols in µg equivalents of gallic acid per mg of extract (µg GAE / mg of extract) [23].

Total Flavonoids content measurement

The trichloroaluminum technique was used to determine the total flavonoid content [24]. To 1ml of EAMeOH extract solution at a concentration of 2 mg/ml, 1ml of AlCl₃ prepared with 2% was added. At 430 nm, the sample's absorbance was measured. The calibration curve for the flavonoid content was established using quercetin as a reference. The results were represented as microgram equivalents of quercetin per milligram of extract (µg QE/mg of extract).

Study of the inhibition of alpha-glucosidase in vitro

Enzyme was obtained from the tissue homogenate made from the rats' small intestine. After being properly cleaned, blotting paper-dried, weighed, and homogenised in glass, a tiny portion of the small intestine was placed in phosphate-buffered saline (PBS) that had been chilled beforehand. The supernatant's ultimate volume was kept at 20% (w/v) after centrifugation at 5,000 G. Yibchok-anun et al. [25] employed the spectrophotometric test method with minor modifications. Here, 80 µl of the standard drug extract EAMeOH (100g/ml) was combined with 40 µl of tissue homogenate, and the mixture was incubated for 15 minutes at 37 °C. Following this, 280 µl of 37 mM maltose was added; after centrifuging the tubes, the glucose oxidase/peroxidase kit based on the peroxidase method (GOD/POD) was used to measure the amount of glucose in the supernatant. The inhibition percentage was expressed as follows: % Inhibition (absorbance control - absorbance test / absorbance control) × 100

Study of alpha-amylase inhibition in vitro (Strategy of treatment of type 2 diabetes)

With a small adjustment, the spectrophotometric analytical method was applied Kumar et al. [26]. 90 µl of the previous homogenate, 180µl of 40mM phosphate buffer (pH 6.9), 100µg/ml of EAMeOH, and a positive control at different concentrations were combined with the supernatant, and the mixture was incubated for 15 minutes at 37 °C. 360µl (0.5 mg/ml) of the substrate 2-chloro-4-nitrophenyl- α -maltotriosidewas added to this reaction. Lastly, the absorbance was taken at 405 nm. There was a control reaction.

Evaluation of nephroprotective activity

This activity was carried out according to Hussain et al. [27]. In this experiment, 24 rats

weighing between (120–150g), which were grouped into four groups, each containing 6 rats.

Group 1 (Control): received 0.5% sodium CMC vehicle (5 ml/kg, b.w., once a day) for 7 days. Group 2 received orally GM (80 mg/kg, b.w., once a day) for 7 days.

Group 3: received GM (80 mg/kg, b.w., once a day) for 7 days and at the same time cysteine (20 mg/kg b.w.) which is considered as a reference.

Group 4: received GM (80 mg/kg, b.w., once a day) for 7 days and at the same time EAMeOH (200 mg/kg b.w.) orally.

Biochemical Analyses

After 7 days of experimentation, the animals are sacrificed, and the kidneys are weighed. Then, blood is collected from the ocular sinus and centrifuged at 3000xg for 10 min. The plasma was analysed for various biochemical parameters such as urea, creatinine, and the urine was collected for the measurement of kidney parameters like using a Metrolab 2300 type Biochemistry analyser.

Histopathological analysis

Samples of kidney were collected for histopathological studies. They were washed in normal saline and fixed immediately in 10% formalin and finally examined under a microscope after staining with hematoxyline and eosine.

Statistical analysis

The results of different evaluations carried out are given in the form of means $\pm$ standard deviations. The statistical analysis by the of One-Way ANOVA followed by Tukey test using Graph pas prism 6. $P \leq 0.05$ = the difference is significant (s).

Results

The extract yield was calculated to be 27.11%. For the results of the dosage of polyphenols and flavonoids, EAMeOH contain 24.12 ± 0.11 mg GAE/g of extract of total phenolic. Regarding flavonoids, the extract is rich in flavonoids 117.70 ± 3.41 µg QE/mg of extract (Table 1).

According to Table 2, we found that the EAMeOH leaves possess the anti α -amylase and anti α -glucosidase activity, and the IC₅₀ of the extract was determined.

The EAMeOH showed an α -amylase inhibitory activity of 28.45% and α -glucosidase inhibitory activity 57.45% and IC₅₀=222.37µg/ml for α -glucosidase. The α -amylase inhibition activity of this extract could not be accurately quantified because, at the maximum tested concentrations, it does not capture more than 28.45% of the enzyme. Using acarbose as a reference medication, it demonstrated α -amylase and α -glucosidase inhibitory activity with IC₅₀ values of 234.65 µg/ml and

520.77 µg/ml, respectively. Acarbose outperformed EAMeOH in terms of enzymatic inhibitory action (α -amylase and α -glucosidase).

Urinary creatinine, serum creatinine, blood urea, urinary urea, and kidney weights were significantly increased in rats that received only GM, whereas treatment with the EAMeOH was found to protect rats against these effects of GM. As shown in (Table 3), it was found that the urine volume was significantly increased in rats treated with the EAMeOH.

In our study, we observed acute renal lesions in the rats of the toxic group after administration of gentamicin/overdose, manifested by abnormal changes in renal function tests (urine creatinine: 298.12 g/L; urine urea: 1.11 g/L; serum urea: 0.79 g/L and serum creatinine: 28.23 mg/L).

Histopathological study

The examination of histological structure and morphology of kidneys which show the presence of a glomerular congestion in kidney of rat treated with gentamicin (Fig.2 B).

Rats treated group with EAMeOH 200mg/Kg b.w (Fig. 2C) also showed the epithelial desquamation. Rats in the cystone treated group (Fig. 2D) had normal architecture. The kidneys of rats in the normal control group (Fig. 2A) showed normal histology.

Discussion

Meshram et Srivastava [28] found that the plant *E. aureum* had total flavonoid levels of 51.156 mg/g of the leaf extract and total phenolic contents of 8.284 mg/g of the leaf extract.

This finding is in line with some research and studies on the presence of active groups in plants, which can occasionally vary depending on several factors. For example, soil composition, texture, depth, humidity, and ventilation can all result in a highly specialised diversity of different chemical compounds even within a single country, and the concentration of secondary metabolites can vary significantly between plants in the same genus and even within the same plant in different parts of it [29].

Postprandial hyperglycemia results from the breakdown of 1,4-glycosidic bonds (starch, glycogen) into disaccharides by α -amylase, which starts the carbohydrate digestion process. α -glucosidase then catalyses the hydrolysis of disaccharides into monosaccharides [30]. More than 800 plants are said to have antidiabetic qualities in ancient Indian literature, and ethnopharmacological studies show that more than 1200 plants have hypoglycemic effects [31]. Therefore, the presence of chemical components both alcoholic and aqueous extracts of *E. aureum*

may be the cause of the anti-diabetic effect seen in the study of Abhinayani et al. [32].

E. aureum with direct anti-diabetic roles are not well-documented, the broader classes of compounds found in plants, such as flavonoids, saponins, and terpenes, have known antidiabetic effects. These compounds can potentially improve diabetes by increasing insulin sensitivity and secretion, enhancing glucose uptake by cells, inhibiting digestive enzymes that break down carbohydrates, reducing oxidative stress, and slowing down gluconeogenesis [33]. Several natural inhibitors of α -glucosidase and α -amylase, including acarbose, voglibose, and miglitol, are clinically used as treatments [34]; a combination of oligosaccharides called acarbose slows down the breakdown of carbs. It prevents pancreatic α -amylase from breaking down artificial starch, but this inhibitor causes side effects such as abdominal pain, diarrhea, and colon problems [34, 35]. Aleixandre et al. [36] reported that phenolic compounds can modulate the enzymatic degradation of carbohydrates by inhibiting α -amylase and preliminary chemical tests of EAMeOH revealed that the plant contains phenolic compounds and flavonoids [37,38].

Extracts from *E. aureum* showed enhanced antioxidant enzyme levels and decreased lipid peroxidation, both of which are indicators of the plant extract's antioxidant efficacy. Reduced glutathione was restored by the extracts, which may aid in avoiding problems from diabetes [39].

In the study of Abhinayani et al. [40], *E. aureum* extracts have demonstrated significant anti-diabetic potential in both normal and alloxan-induced rats. Flavonoids may have a hypoglycemic impact by a mechanism other than insulin secretion, for as by inhibiting intestinal glucose absorption or endogenous glucose synthesis.

When GM was given, serum levels of urea and creatinine increased during GM administration. This supports the idea that GM is harmful to the kidneys, as shown in previous research. This elevation is attributed to GM's capacity to induce oxidative stress and renal inflammation, leading to acute kidney injury [41].

When proteins are directly or indirectly exposed to oxidising chemicals, they can oxidise. Important markers of oxidative stress are oxidised protein products. As a result, variations in urine protein levels serve as indicators of renal injury. Urine shouldn't contain proteins under normal circumstances. However, proteins can be detected in urine if there is nephron malfunction or if the concentration of proteins in the urine surpasses the renal proximal tubules' ability to reabsorb them [42].

It is well known that aminoglycoside antibiotics can cause nephrotoxicity, particularly GN, the most

widely used of these drugs. According to a number of studies, reactive oxygen species are thought to be significant mediators of acute renal failure brought on by GN [43].

Numerous flavonoids have demonstrated nephroprotective properties against a range of nephrotoxic substances that can cause acute or long-term kidney damage. These include baicalin, gossypin and naringin, which lessen the nephrotoxicity that gentamicin causes in rats. The mechanism of action involves several oxidative disruptions and inflammatory cascade pathways, such as the decrease of inflammatory mediators like TNF- α and the elevation of the anti-inflammatory mediator IL-10. Additionally, the findings of this study are consistent with improvements in renal function that have been documented in the past [44].

Conclusion

The antidiabetic activity of methanolic extract of *E. aureum* was screened by the inhibition of α -amylase and anti α -glucosidase assay which shows the important activity. *aureum* possessed an important nephroprotective activity against gentamicin induce nephrotoxicity. The study's findings demonstrated that the chosen plant has nephroprotective properties. The traditional usage of the plants to treat renal issues is supported by these findings. Antioxidant, anti-inflammatory, antibacterial, hepatoprotective, and antidiabetic effects are just a few of the many therapeutic

qualities that make this plant a valuable medicinal herb. Its traditional uses are increasingly supported by scientific evidence, highlighting its potential for developing natural-based therapies. We must explore its pharmacological potential and ensure sustainable utilization.

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Declaration of Conflict of Interest

The authors declare that there is no conflict of interest.

Ethical of approval

All experimental studies were approved by the Committee of the Algerian Association of Sciences in Animal Experimentation (<http://aasea.asso.dz/articles/>) No. 8808/1988, associated with veterinary medical activities and protection of animal health (No. JORA 004/1988).

This study follows the ethics guidelines of the Faculty of Veterinary Medicine, Benha University, Egypt (ethics approval number; 49/11/2023).

TABLE 1. Total phenolic contents and flavonoids of EAMeOH.

Extract	Total phenolic (mg GAE/g)	Total flavonoids (μ g QE/mg)
EAMeOH	24.12 $\pm$ 0.11	117.7 $\pm$ 3.41

EAMeOH: Methanolic extract of *E.aureum*, (mg GAE/g): milligram of gallic acid equivalent per gram of extract, (μ g QE/mg): microgram quercetin equivalent per milligram of extract. Values are expressed as mean + standard deviation of three replicates.

TABLE 2. Enzyme inhibitory properties of EAMeOH

Extract	Inhibition (%) α - amylase	IC 50 α - amylase	Inhibition (%) α - glucosidase	IC 50 α - glucosidase
EAMeOH	28.45	NA	57.45	222.37 $\pm$ 0.12
Acarbose	60.42**	234.65 $\pm$ 1.02	88.42*	520.77 $\pm$ 0.54

IC 50 Values are defined as the concentration required for 50% inhibition. IC 50 was determined by linear regression analysis and presented as the mean $\pm$ SD (n = 3). (*P $\leq$ 0.05, *P $\leq$ 0.001). NA: not active.

TABLE 3. Effect of EAMeOH on urea, creatinine, and uric acid against gentamicin-induced nephrotoxicity.

Groups	Urea (g/L)		Creatinine (mg/L)	
	blood	urinary	blood	urinary
Control	0.12 $\pm$ 0.01	0.41 $\pm$ 0.5	5.9 $\pm$ 0.72	114.12 $\pm$ 3.74
Gentamicin	0.79 $\pm$ 0.07***	1.11 $\pm$ 0.7***	28.23 $\pm$ 0.14***	298.12 $\pm$ 15.12***
Cystone (20mg/Kg)	0.15 $\pm$ 0.04	0.45 $\pm$ 0.12	5.23 $\pm$ 0.24	102.23 $\pm$ 14.23
EAMeOH (200 mg/Kg)	0.23 $\pm$ 0.012*	1.05 $\pm$ 0.15***	8.12 $\pm$ 0.78*	208.78 $\pm$ 17.25***

Values are the mean $\pm$ SEM of 6 animals, Values are the mean $\pm$ SEM of 6 animals; Results are compared to control
*P $\leq$ 0.05, **P $\leq$ 0.001, and ***P $\leq$ 0.0001.

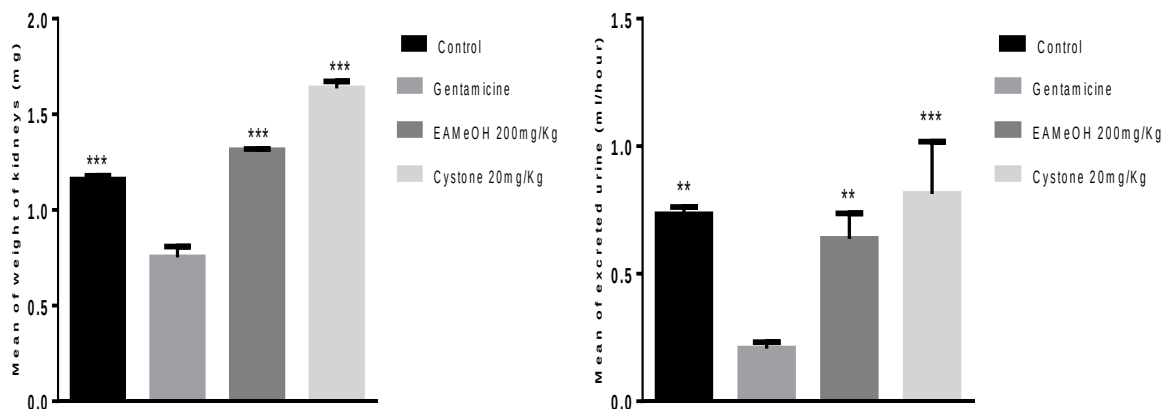


Fig. 1. Kidney Effect of EAMeOH on urine excretion and kidney weight. Values are the mean $\pm$ SEM of 6 animals; Results are compared to group received GM * $P \leq 0.05$, ** $P \leq 0.001$, and *** $P \leq 0.0001$.

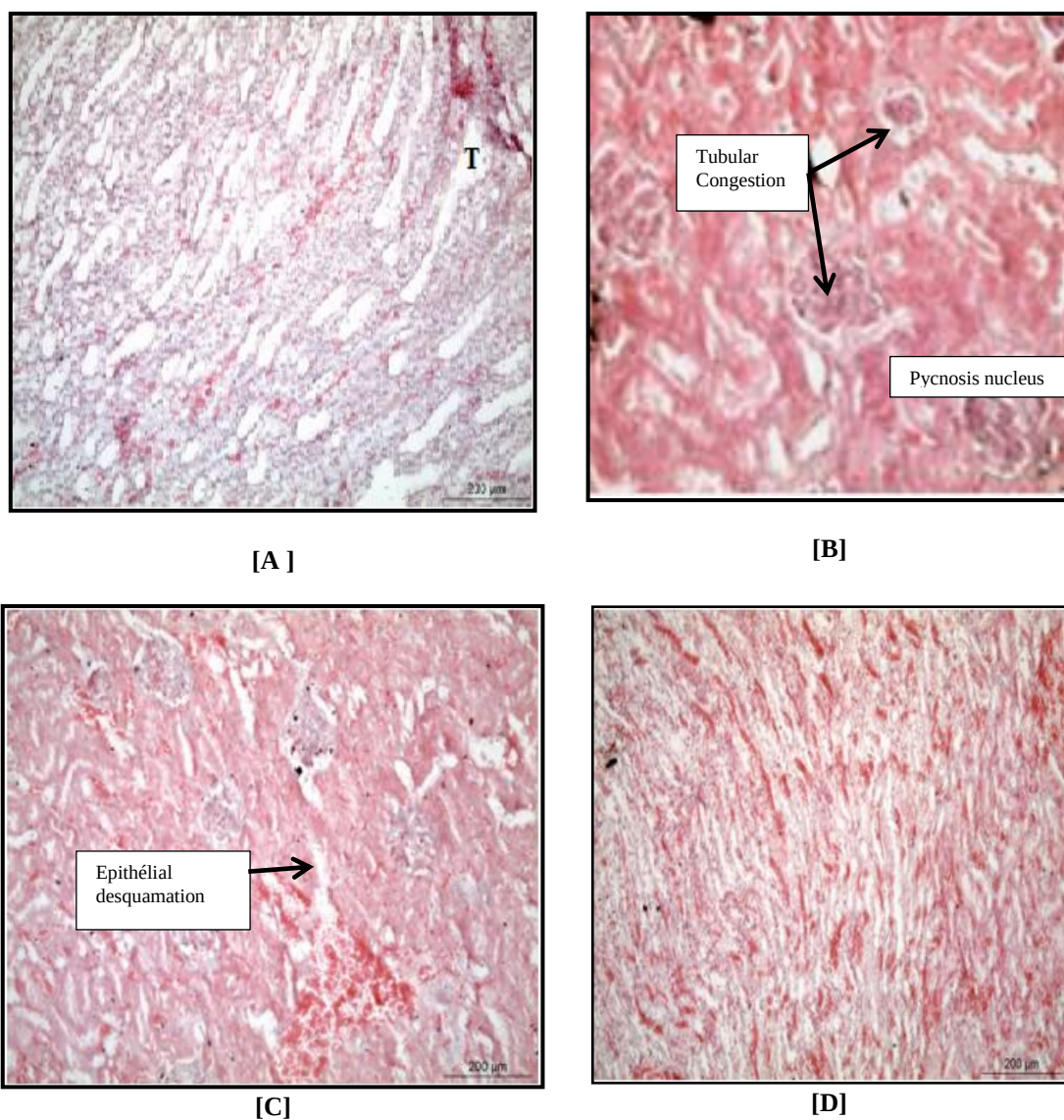


Fig.2. Representation of Kidneys histology (Haematoxylin and eosin staining magnification 40X): (A) Normal kidney histology; (B) (C) (D) Kidney histopathology of rats in treated with Gentamicin, EAMeOH (200mg/kg b.w) and Cystone (20mg/Kg b.w) treated nephrotoxicity rats, respectively.

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تقييم التأثيرات الوقائية الكلوية والمضادة لمرض السكري لنبات *Epipremnum aureum* عند الجرذان البيضاء

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الملخص

الإبيبرمنوم الذهبي (*Epipremnum aureum*) نبات عشبي معمر متسلق، ذو رائحة عطرية خفيفة، وهو عشب طبي قيم يستخدم في مختلف أنظمة الطب التقليدي لعلاج أمراض وعلل الإنسان. وتشير التقارير إلى احتوائه على مركبات كيميائية حيوية متنوعة، مثل القلويدات، والفلافونويدات، والجليكوسيدات، والترينويدات، والسابونينات، وغيرها. يهدف هذا العمل إلى تقييم النشاط المضاد لفرط سكر الدم للميثانول من الإبيبرمنوم الذهبي (EAMeOH) في المختبر، وذلك عن طريق تثبيط إنزيمي ألفا-أميليز وألفا-جلوكوزيداز، وكذلك لتقييم النشاط المضاد للسمية الكلوية في الجسم الحي، ضد السمية الكلوية الناتجة عن الجنتاميسين. حُصنت تراكيز مختلفة من المستخلص مع محلول ركيزة الإنزيم، وقيست فعالية الإنزيم. بالإضافة إلى ذلك، استخدم الأكاربوز كمثبط قياسي. تمكّن المستخلص من تثبيط ألفا-أميليز وألفا-جلوكوزيداز بنسبة 28.45% و 57.45% على التوالي، مع تركيز نصف (IC₅₀) قدره 0.12±222.37 لألفا-جلوكوزيداز. وللحصول على فعالية مضادة للسمية الكلوية، عولجت خمس مجموعات من ستة فئران بـ CMC الصوديوم، وجنتاميسين، 100 و 200 ملغم/كغم من وزن الجسم من MeOHAE على التوالي. أعطيت هذه العلاجات عن طريق الفم قبل ساعة واحدة من إعطاء الجنتاميسين، باستثناء المجموعة الأولى التي لم تتلقَ CMC الصوديوم. أظهرت النتائج أن إعطاء الجنتاميسين أدى إلى انخفاض معنوي ($P \leq 0.05$) في حجم البول ووزن الكلى في المجموعة الضابطة، بينما أدى تناول المستخلص عن طريق الفم (100 و 200 ملغم/كغم من وزن الجسم) إلى زيادة معنوية ($0.05 \leq P$) في حجم البول ووزن الكلى مقارنةً بالمجموعة الضابطة. علاوة على ذلك، أدى العلاج بمستخلص EAMeOH لمدة 7 أيام بجرعة يومية مقدارها 200 ملغم/كغم من وزن الجسم إلى انخفاض معنوي ($P \leq 0.05$) في اليوريا في المصل بمقدار 0.25 غ/لتر، والكرياتينين بمقدار 7.45 ملغم/لتر مقارنةً بالمجموعة الضابطة.

الكلمات الدالة: *Epipremnum aureum* ، جنتاميسين، مستخلص ميثانولي، ألفا جلوكوزيداز، ألفا أميليز، سمية الكلى.