Research Article

Erlotinib and Garcinia Cambogia Prevent Adenine Induced Nephrotoxicity in Adult Male Albino Rats Through Modulating EKR1/2, STAT3, and p53 Apoptotic Pathways

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Abstract | **Backgrounds:** A major global public health concern is chronic renal disease (CRD). Thus, it is necessary to search for beneficial and helpful medications that can stop CRD from progressing to end-stage renal failure. **Aims:** The purpose of this study was to evaluate how erlotinib and garcinia cambogia alleviate the harmful effects of adenine on the rat's kidneys. **Methods:** Forty animals were divided into four groups: Control group: received normal vehicle, adenine group: received orally (50 mg/kg/day) that is subdivided into 3 groups: adenine group, garcinia cambogia (GC) group: received orally GC (200 mg/kg/day)+adenine, and erlotinib (E) group: received 80 mg/kg/day + adenine. Antioxidant biomarkers and renal function tests were evaluated. Histopathological scoring of tubular damage and renal fibrosis as well as immunohistochemistry evaluation of Bcl2 and p53 expression in the kidneys were performed. In renal tissue, the concentrations of transforming growth factor-1, pERK1/2, and pSTAT3 were assessed. **Results:** The group that received adenine treatment had considerably higher blood urea nitrogen and serum creatinine levels as well as significant pathological alterations as tubular injury and infiltration of infammatory cells. Elevated TGF1, pERK1/2, and pSTAT3 levels were also present. Bcl-2 levels were down while p53 levels were up. All of the detected adenine-induced biochemical and histological alterations were reduced by erlotinib and Garcinia cambogia. **Conclusions:** we concluded that oral administration of erlotinib and garcinia cambogia may be able to reduce the destructive effects of CRD that may be brought on by their anti-fibrotic, antioxidant, and antiapoptotic pathway capabilities.

Keywords | Adenine, Apoptosis, Biochemical indices, Chronic renal disease, Erlotinib, Garcinia cambogia, and herbal medicine.

Received | May 07, 2023; **Accepted** | May 20, 2023; **Published** | June 05, 2023
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Citation | Amin DM, Halloull NM, Omar WE, Ibrahim BA, Ibrahim NM (2023). Erlotinib and garcinia cambogia prevent adenine induced nephrotoxicity in adult male albino rats through modulating ekr1/2, stat3, and p53 apoptotic pathways. Adv. Anim. Vet. Sci. 11(7): 1165-1175.
DOI | https://doi.org/10.17582/journal.aavs/2023/11.7.1165.1175
ISSN (Online) | 2307-8316

INTRODUCTION

Chronic renal disease (CRD) has a significant negative effect on public health and exihibited a significant cause of a disease that affects more than 200 million people globally (Manoj and Hartmann, 2019). CRD forced patients to undergo terrible medical procedures, which drastically reduced their quality of life. The morbidity and mortality problem is getting worse every year (Ali et al., 2017). Up till now, dialysis or kidney transplantation are the only available treatment options as, and there hasn't been at least one medicine discovered that can be utilized to sustain kidney function in CRD patients (Cai et al., 2018).

To delay the reduce in kidney function, it is necessary to discover beneficial therapies or nutritional supplements. It is undeniable that oxidative stress, inflammation, and ap-

optosis play roles in the progression of CRD and its complications. Renal fibrosis is the most common structural pathogenesis in CRD (Shara et al., 2020).

Renal fibrosis is characterized by high mononuclear inflammatory cell infiltration, tubulointerstitial fibrosis, and glomerular degeneration in both humans and animals. Adenine is metabolized to form 2,8 dihydroxyadenine, which accumulates in renal tissues. This metabolite causes tubulointerstitial tissue injury and damage, resulting in tubular fibrosis (Chen et al., 2018).

Garcinia cambogia, also known as Malabar tamarind. According to La et al. (2018) it is high in oxy-guttiferone-K, M, and I, as well as irbenzophenone analogues, which are topoisomerase II inhibitors and bioflavonoids (Espirito Santo et al., 2020) Numerous studies have found that they have vasodilatory and antihistaminic properties (AL-Askalany, 2018).

A putative therapeutic target and mediator in the development of CRD is transforming growth factor (TGF) (Corden et al., 2020). miRNA has recently been discovered to play a role in the development of CRD. Tyrosine kinases can hasten the development of fibrotic disorders and are crucial in cell signaling. The epidermal growth factor receptor (EGFR), a tyrosine kinase receptor, which is largely located in the epithelial cells of all tubules, arteries, and glomeruli, has been discovered with increased expression in diseased renal tissue (Zhao et al., 2019). Recently, it has been demonstrated that EGFR can halt renal fibrosis by blocking or reducing its signaling pathway which is a therapeutic approach for the management of renal illness (Yoshioka, 2020).

Erlotinib is a tyrosine kinase receptor competitive inhibitor that competes with ATP for the intracellular part of the receptor. As a result, it inhibits receptor phosphorylation and interferes with downstream signals. Erlotinib is used to treat lung cancer (Skibba, 2016). Several studies have shown that it can help with various kidney injuries (Yang, 2019).

The purpose of this study was to determine the effect of erlotinib and garcinia cambogia in an experimental model of chemically induced nephrotoxicity and fibrosis.

Ethics approval

This study was approved by the local ethical committee of the Faculty of Medicine, Zagazig, Egypt. (approval number :ZU-IACUC/3/F/62/2023).

MATERIAL AND METHODS

Chemicals

Erlotinib: Erlotinib free base as white powder (LC laboratories, MA, USA; PubChem CID: 176870).
Garcinia cambogia: It was purchased from Eva pharm.
Adenine: Adenine (99%) as fine white powder (Sigma Chemicals, St. Louis, MO, USA).
Distilled Water: used as diluent for all chemicals.

Kits

Kits for creatinine, urea, and uric acid were purchased from (Diamond Diagnostics, USA).
Oxidative Stress Markers: Kits for malondialdehyde (MDA), SOD, and catalase were obtained from (Biodiagnostic Company, Dokki, Giza, Egypt).
The transforming growth factor-1 kit was obtained from (Cloud Clone Corp., USA), as were the phospho-STAT3 and p-ERK1/2 kits obtained from (Assay Biotechnology Co., CA, USA)

Animals

A total of 40 adult male albino rats weighing 150 grams were used in this study. The study was created at the Zagazig University School of Medicine. All animals were cared for in accordance with "The Handbook for the Care and Use of Laboratory Animals" (Institute of Laboratory Animal Resources' Animal Care Guidelines and Ethical Standards). The following environmental factors were standardized in accordance with Cuschier and Backer (1997) in order to eliminate misconceptions: 1) The climate conditions in the animal house and in the cage free from any source of chemical contamination under room temperature (22°C ± 2°C), relative humidity 50% ± 5% and a 12 h light cycle with free access to tap water with proper ventilation. 2) Strength, character, regularity, and length of light were those of natural light. 3) To keep the animal clean, bedding and wood shavings were routinely replaced in galvanized iron-mesh cages with solid bottoms. 4) Ten rats were placed in each cage to avoid both isolation and overcrowding. 5) A low noise level was maintained because it might change how the animals behave. Before beginning the experiment, the rats were housed in this setting for one week to allow them to acclimate to any potential stress brought on by the transit process from the animal supplier or by a rapid environmental change, as well as to eliminate any sick animals. The rats were fed a balanced diet before and after the administration of the medication that was rich in every thing they needed to stay healthy. It is composed of ad libtium. In separate, spotless containers, distiller water was provided (Semler, 1992).

Induction of renal fibrosis

According to a previous study by Nemmar (2016), rats

were given adenine with food (0.2% w/w) every day for 4 weeks in order to cause renal fibrosis.

The 40 rats were weighed and divided into an adenine group (30 rats) and a control group at random (10 rats).

Experimental design

All medications are administered once /day, six days a week, and are diluted in 1 cc of purified water. The adult male rats were divided into four groups of ten at random and studied for four weeks as the followings:

Group I (control group) (n=10 rats): Each rat received only regular diet and distilled water to determine the basic values of performance. These rats were left without intervention to measure the basic parameters.

Group II (Adenine group) (n=30 rats): Each rat was administered orally 50 mg/kg adenine (suspended in distilled water). Further, adenine group was divided into three subgroups:

Group IIa (Disease control group, A) (n=10): Each rat was gavaged orally with adenine (0.2% w/w) daily with food (Diwan et al., 2018).

Group IIb (Garcinia cambogia group, GC) (n=10 rats): Each rat was gavaged orally with 50 mg/day garcinia cambogia according to Espirito Santo et al. (2020). It was prepared by dissolving one capsule in 10 ml D.W.

Group IIc (Erlotinib group, E) (n=10 rats): Each rat was gavaged orally with 80 mg/kg/day erlotinib according to Zhang et al. (2014).

Garcinia cambogia and erlotinib groups were suspended in distilled water and administered with adenine for 4 weeks. It was administered one hour prior to adenine administered in the previous doses and ways.

An appropriate-sized metallic tube designed for rat stomach intubation was used for oral delivery. In order to prevent gastric distention, overflow, or regurgitation—which could result in tracheal aspiration—excessive vehicle use was avoided. One month injection in rats is equivalent to 24 months in human being (Kari et al., 2019).

The experimental design is presented schematically in Table (1).

Methods

The rats were intraperitoneally injected with sodium pentobarbital (50 mg/kg body weight) 24 hours after the last administration to make them unconscious. Retro-orbital plexuses were used to collect blood samples, which were then centrifuged to separate the serum (3000–4000 rpm, 4 °C, 15 min) and stored at 20 °C for subsequent analysis of several serum biochemical parameters such creatinine, blood urea nitrogen (BUN), and uric acid. Kidneys were removed, placed in saline, and then cut longitudinally. Kidney pieces were manually immersed in PBS (10% w/v) using a handheld homogenizer on ice to create kidney homogenate. The kidney pieces were then centrifuged at 4000 rpm for 20 minutes at 4 °C to separate and collect the supernatant. For histological examination, the kidneys were fixed in 10% neutral-buffered formalin. According to Jennifer et al. (2020), they were removed and studied under a light microscope for histological examination after being preserved in Bouin's fixative, processed to create 5-mm-thick paraffin sections, and stained with hematoxylin and eosin. Using a manual tissue arrayer (Pathology Device, Westminster, MD), four 1.5-mm-diameter tissue cores were taken from variously fixed specimens of each tissue and combined into a tissue array in a single paraffin block. In the Leica Auto Stainer XL, hematoxylin and eosin (H&E) staining was carried out in accordance with protocol.

All groups of rats were utilized to measure:

1) Biomarkers of kidney function (serum creatinine, uric acid, and Blood urea nitrogen)
2) Biomarkers for antioxidants status (Malondialdehyde, catalase, and superoxide dismutase)
3) Measuring the expression of the genetic markers for renal profibrogenesis (phospho-STAT3, TGF-1, and p-ERK1/2).
4)Histopathology study of the kidney (hematoxyline and eosin).
5)Immunohistochemical study of the kidney(p-53, Bcl-2)
6)Electron Microscope Examination.

Table 1: Schematic presentation of experimental design. n=10/group.

Group	Week				
	1	2	3	4	
Control	○	○	○	○	Sacrifice
Adenine (A)	○ ■	○ ■	○ ■	○ ■	
Garcinia cambogia (GC)	○ ■ ●	○ ■ ●	○ ■ ●	○ ■ ●	
Erlotinibe (E)	▲ ○ ■	▲ ○ ■	▲ ○ ■	▲ ○ ■	

○ Rats received water and food. ■ Rats received 50mg/kg adenine (orally/day with food). ● Rats received garcinia cambogia 50mg/kg (orally/day with food). ▲ Rats received 80 mg/kg erlotinib (orally /day with food).

Biochemical Assays

Kidney function tests: Blood urea nitrogen, uric acid, and serum creatinine levels (measured in mg/dl) were determined spectrophotometrically as directed by the manufac-

turer (Bauer et al., 1982).

Levels of antioxidant biomarkers: Malonyldialdehyde (MDA) Level in Kidney was identified in kidney homogenates using the Rangel et al. (2019) method. Following Ratliff et al. (2016), catalase activity (CAT) was found by homogenizing hydrogen superoxide (H2O2) with phosphate buffer. According to Ahmed et al. (2021), superoxide dismutase activity (SOD) was measured by adding potassium phosphate buffer, EDTA, L-methionine, riboflavin, and nitro blue tetrazolium (NBT). ErbaChem 7 was used to measure the reaction's color and absorbance spectrophotometrically.

Measurement of renal phosphoSTAT3, TGFβ1 and pERK1/2: Signal transducer and activator of transcription 3 (STAT3), transforming growth factor (TGF), and pERK1/2 were quantified in kidney homogenate using the sandwich ELISA technique in accordance with the manufacturer's instructions (Kinomura, 2008).

Histopathological and Immunohistochemical Studies

Histopathological evaluations: Tissues were dehydrated using ascending grades of ethyl alcohol (50-100%) , then cleared using xyelene (2/change), then embedded in melted paraffin wax (59c) , then blocked and cutted into five micrometer slices, stained with hematoxylin and eosin, and then inspected under a light microscope. Using a light microscope (total magnification power 400), sections were evaluated for tubular damage and renal fibrosis in accordance with Kinomura's (2008) modified pathologic scoring system. Using a score from 0 to 5, the randomly chosen fields were quantified for swelling, desquamation from the tubular basement membrane, necrosis, and vacuolar degeneration of the tubules affected; 0: normal; 1: <20% of the tubules are involved; 2: 20–40%; 3: 40–60%; 4: 60–80%; and 5: 80–100%).

Immunohistochemical evaluations: Serial sections on slides that were cut to a thickness of 4 m were immunostained. The tissue sections were rehydrated in graded ethanol after being deparaffinized in xylene. To stop a general peroxidase response, deparaffinized portions were submerged in hydrogen peroxide for 10 minutes. Sections were combined with EDTA for microwave antigen retrieval, and then rabbit serum was added for pre-incubation to prevent antibody non-specific binding. The kidney sections were then treated overnight at 4 °C with rabbit primary antibodies (dilution 1:50) against p53 (ab131442, Abcam) and Bcl-2 (PA5-20068, Invitrogen, Thermo Fisher Scientific, CA). According to Kane and Greenhalgh's description, secondary antibodies were added. The horseradish peroxidase-diaminobenzidine detection technique was then used to visualize antibody binding on stained (H&E) slides (GBI Labs, WA, USA). Expression of p53 (cytoplasmic and nuclear) and Bcl-2 (cytoplasmic) under a light microscope (X100) (Percicote, 2013).

Method of evaluation of IHC results

According to Zimmermann et al's (2014) method of evaluation, qualitative interpretation of IHC data was applied. The classifications on a continuum that describe the various intensities of IHC expression in the groups under investigation are negative (-), mild (+), moderate (++), and strong (+++).

Electron microscope examination

The specimens were fixed in 2% buffered glutaraldehyde, cleaned in PBS, fixed in 1% osmium tetroxide, dehydrated in alcohol, and embedded in epoxy resins for transmission electron microscopy. Semi-thin Sections were seen under a light microscope after being stained with 1% toluidine blue. With a Leica Ultra microtome (Leica Microsystems, Vienna, Austria), ultrathin sections (50–60 nm) were cut, placed on copper grids, and stained with uranyl acetate and lead citrate. The Faculty of Agriculture Mansoura University's Electron Microscope Research Laboratory (EMRL) examined the grids using a Jeol JEM-2100 transmission electron microscope (Egypt). The current study's biochemical and histological findings have been recorded and statistically analyzed (Bozzola and Russell, 1999).

Statistical analysis

Data (n=10) were analyzed using SPSS Software's statistical Package for the Social Sciences, version 17, and were presented as mean standard deviation (SD). Analysis of variance (One Way ANOVA) was used in the statistical analysis of data with Gaussian distribution, and was followed by the Bonferroni multiple comparisons test. Dunnett's test for multiple comparisons was used after the Kruskal-Wallis test for parameters with non-Gaussian distributions. At P 0.05, differences were deemed significant (Prabhakernet al., 2019).

RESULTS

The death rate was 0% in the different groups.
No characteristic clinical signs observed during running our investigation, the animal were apparently doing well.

Biochemical Results

Kidney Function tests: When compared to the control, (GC+A), and (E+ A) groups, there was a highly significant increase in the levels of (blood urea nitrogen, creatinine, and uric acid) in the rats treated with (A) (Table (2) & Fig. 1a,b,c). Nevertheless, when combined with adenine, the effects of garcinia cambogia and erlotinib greatly reverted

Table 2: Serum kidney tests in all rats of studied groups

Parameters	**Control** mean±SD *n*=10	**Adenine (A)** mean±SD *n*=10	**Garcinia cambogia (GC)** mean±SD *n*=10	**Erlotinibe (E)** mean±SD *n*=10
Creatinine (mg/dL)	0.63±0.08	1.05±0.107***	0.69±0.11$$$	0.67±0.18$$$
Blood urea nitrogen(mg/dL)	12.42±1.04	52.93±5.95***	15.88±2.19$$$	14.78±2.19$$$
Uric acid(mg/dL)	2.14±0.40	4.17±0.46***	2.16±0.34$$$	2.09±0.34$$$

Data are expressed as mean±SD. *n*=10 rats in each group. ***P<0.001 versus normal control group. $$$$P$<0.001 versus adenine group.

Table 3: Malondialdehyde, superoxide dismutase and catalase levels in kidney of rats in all studied groups

Parameters	**Control**	**Adenine (A)**	**Garcinia cambogia (GC)**	**Erlotinibe (E)**
MDA (nmol/g tissue)	215.34±54.72	366.86±31.03***	230.07±19.4*,$$$	234.05±19.4*,$$$
SOD (U/g tissue)	1468.40±390.19	952.25±181.71***	1831±288*,$$$	1722±279*,$$$
Catalase (K unit/g tissue)	2.73±0.11	1.595±0.19***	2.41±0.15***,$$$	2.29±0.16***,$$$

Data are expressed as mean±SD. *n*=10 rats in each group. *,***P<0.05, P<0.001 respectively versus normal control group. $$$$P$<0.001 versus adenine group. MDA: Malondialdehyde; SOD: Superoxide dismutase; SD: Standard deviation

Table 4: Efect of garcinia cambogia and erlotinib on renal tubular injury and fbrosis score in adenine induced renal injury in rats of all studied groups.

Groups	**Tubular injury^**	**Fibrosis score^**
Control	0.00±0.000	0.00±0.000
Adenine (A)	2.51±0.149***	3.32±0.084***
Garcinia cambogia (GC)	1.45±0.141$$	1.16±0.043$$$
Erlotinibe (E)	1.60±0.112$$	1.36±0.050$$$

^ Scores were calculated /20 felds. ***P<0.001 compared to control group. **$$, $$$** P<0.01, P<0.001 respectively compared to adenine group

all of these alterations back to normal (Table (2) & Fig. 1a,b,c).

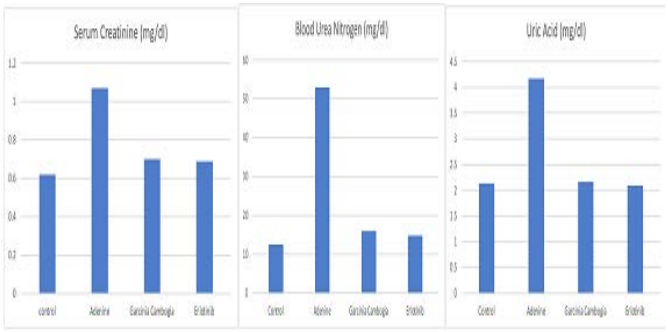

Figure 1: Efect of garcinia cambogia and erlotinib administration on serum levels of (**a**) creatinine (**b**) blood urea nitrogen and, (**c**) uric acid in adenine induced nephrotoxicity in rats.

Malonyldialdehyde (MDA) Level in Renal tissues: When compared to the control, (GC+A), and (E+ A) groups, renal tissue (MDA) concentration increased in a highly significant manner (Table (3) & Fig. 2a). Nevertheless, when combined with adenine, the effects of garcinia cambogia and erlotinib greatly reverted all of these alterations back to normal (Table (3) & Fig. 2a). Whereas simultaneous injection of garcinia cambogia, erlotinib, and adenine significantly reduced the amount of MDA in kidney tissue (Table (3) & Fig. 2).

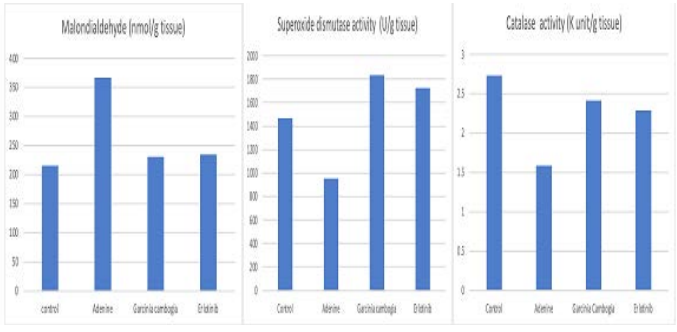

Figure 2: Efect of garcinia cambogia and erlotinib administration on renal levels of (**a**) Malondialdehyde (**b**) superoxide dismutase activity and, (**c**) catalase activity in adenine induced nephrotoxicity in rats.

Antioxidant Enzyme Activities: Rat kidney homogenates were used to measure the antioxidant enzyme activities (CAT and SOD) of the renal tissues. These activities were considerably lower in the (A) treated group compared to the control, (GC+A) and (E+ A) groups (Table (3) & Fig. 2 b,c) in both cases. Yet, when combined with adenine, garcinia cambogia and erlotinib greatly raised the enzymes' activity toward normal levels (Table (3) & Fig. 2 b,c).

Profibrogenic markers (TGFb1, pERK1/2 and phosphoSTAT3).

When TGF-1 levels in renal tissue were evaluated, it was found that the adenine group had significantly higher levels than the control group. TGF-1 level significantly lowered when garcinia cambogia and erlotinib were administered together compared to the adenine group (Fig. 3a).When compared to the control group, the renal level of phosphorylated signal transducer and activator of transcription-3 (phospho-STAT3) significantly increased in the adenine group. When garcinia cambogia and erlotinib were combined with adenine, the level of phospho-STAT3 was much lower than that of the adenine group (Fig. 3b). Similar results were observed when extracellular signal-regulated kinases-1/2 (p-ERK1/2) levels were assessed in renal tissue (Fig. 3c).

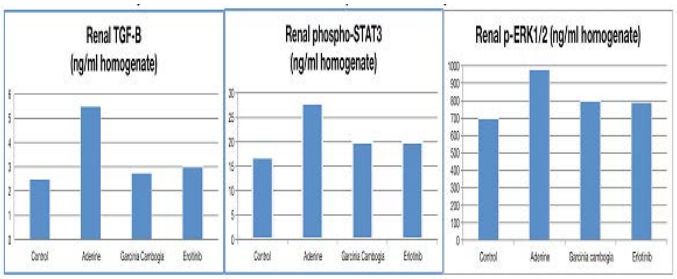

Figure 3: Efect of garcinia cambogia and erlotinib administration on renal levels of (**a**) transforming growth factor (TGF)-β1, (**b**) phospho-signal transducer and activator of transcription (STAT)-3, (**c**) p-extracellular signalregulated kinase (ERK)-1/2 in adenine induced nephrotoxicity in rats

Histopathological Changes of the Kidneys

Macroscopic examination: It demonstrated that the kidneys in both the control and treatment groups showed aberrant masses or cystic abnormalities. Cut sections were gritty sensation.

Light microscopic examination: Tubular damage and fibrosis score: When garcinia cambogia and erlotinib were combined with adenine, the tubular injury and fibrosis scores were significantly reduced by 1.5 and 2.4 folds, respectively, compared to the adenine group score Table (4). The renal glomeruli and tubules' normal histological structure was visible in hematoxylin and eosin-stained sections (Fig. 4). Congested and shrunken renal glomeruli, as well as degenerative characteristics and necrosis of epithelium lining renal tubules. When garcinia cambogia and erlotinib were administered together with adenine, renal glomeruli displayed mesangial cell growth and few congested interstitial capillaries (Fig. 4).

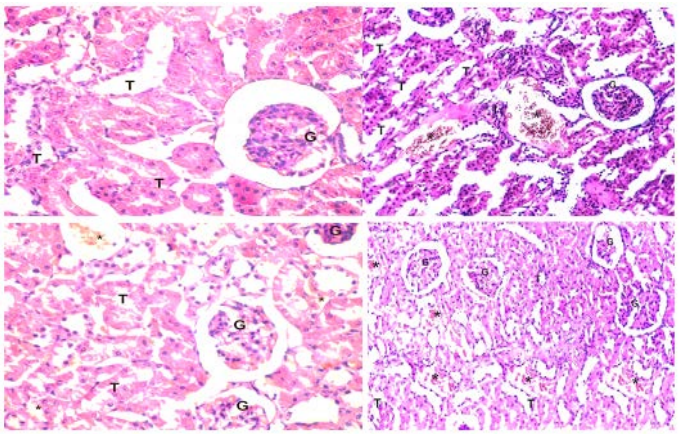

Figure 4: Kidney sections stained with Hematoxylin–eosin stain (×400) showing glomerulai (G), tubules (T) and tubular injury and infltration of infammatory cells (astriks). A: control group B: adenine group C: garcinia cambogia D: erlotinib group.

Immunohistochemical examination (Expression of Bcl2 and p53).

There was significant Bcl-2 immunostaining in the renal tissues. High Bcl-2 expression and no morphologic alterations were seen in the control group (Fig. 5). Bcl-2 was only mildly expressed in the adenine group compared to the control group (Fig. 5). Significantly enhanced Bcl-2 expression was seen when garcinia cambogia and erlotinib were administered along with adenine (Fig. 5). The distribution of Bcl-2 immunoreactivity within renal tissues were mildly, moderately and strongly correlated with histopathologic changes affecting tissues appeared scattered, patchy, and diffuse.

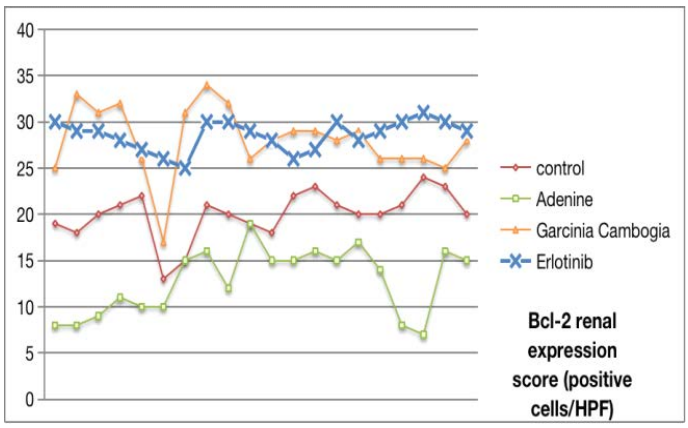

Figure 5: Efect of garcinia cambogia and erlotinib administration on kidney expression scores of B-cell lymphoma (Bcl)-2 in adenine induced nephrotoxicity in rats.

When compared to the control group, the tumor protein p-53 immunostaining expression was significantly higher in the adenine group (Fig. 6) Garcinia cambogia and erlotinib given with adenine significantly reduced the

expression of the p-53 tumor suppressor gene and renal tissue's p-53 immunoreactivity's distribution (Fig. 6). The distribution of p-53 immunoreactivity within renal tissues were mildly, moderately and strongly correlated with histopathologic changes affecting tissues appeared scattered, patchy, and diffuse.

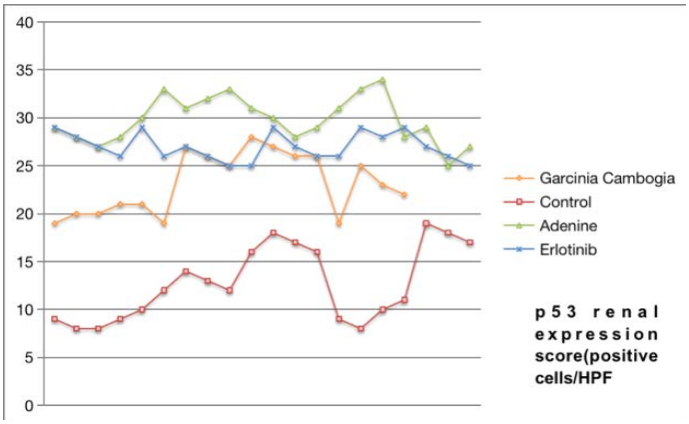

Figure 6: Efect of garcinia cambogia and erlotinib administration on kidney expression scores of tumor protein p-53 in adenine induced nephrotoxicity in rats.

Electron microscopic results

Control group cells displayed a single oval nucleus with expanded chromatin, as seen by ultrathin sections. Sarcoplasm was seen and showed normal cytosomes, mitochondria and rough endoplasmic reticulum. Many granules of glycogen were seen (Fig. 7).

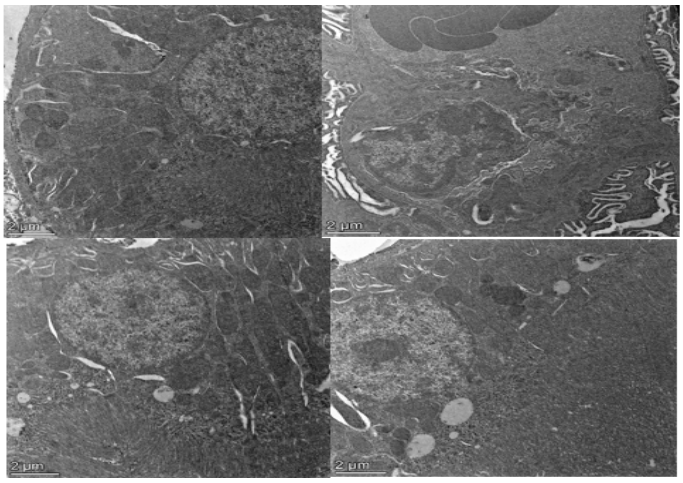

Figure 7: Electron micrograph of a proximal tubule for kidney sections from A: control group (normal ultrastructure of cells) B: adenine group (Large irregular cytosomes contain large numerous myeloid bodies, mitochondria and cisternae of rough edoplasmic reticulum are swollen. Large vacuoles are present near the base of the cell) C: garcinia cambogia D: erlotinib group (both groups show the cell ultra structure is combarable to that of the control).

The nephrocytes in the adenine-treated group saw significant modifications in ultrastructure. Several nuclei appeared to have thickets of heterochromatin and uneven serrations (Fig. 7). Furthermore, sarcoplasmic vacuolation could be seen, the structure and size of the mitochondria and cisternae of rough endoplasmic reticulum looked to be distorted and enlarged. Large irregular cytosomes contain large numerous myeloid bodies are scattered. Large vacuoles are present near the base of the cell. Cell ultrastructure of garcinia cambogia and erlotinib groups is comparable to that of the control (Fig. 7).

DISCUSSION

Our investigation showed that adenine causes elevations in serum uric acid, creatinine, and BUN because renal excretion of urea and creatinine is impeded, indicating a decreased glomerular filtration rate and renal failure. These findings are consistent with those of prior research (Törmänen et al., 2017; Cai et al., 2018; Wang et al., 2018) In our investigation, BUN, creatinine, and uric acid levels in rats who received erlotinib and garcinia cambogia together with adenine decreased to levels that were close to normal. These findings suggest that it has a positive impact on how severe renal problems are.

Tyrosine kinase inhibitors have an impact on the urea cycle enzyme N-acetylglutamate synthase (Li, 2019). By inhibiting glutamate receptors, erlotinib may prevent glutamate from reacting with ERK1/2 stimulatory signals. Many biological processes, including survival, differentiation, and proliferation, are controlled by the EGF/EGFR system. Tubular cell growth in an autocrine/paracrine pattern is detectable. Excessive EGFR expression can be seen in the glomerulus and the tubulointerstitial compartment, and both human and experimental chronic renal disorders have been linked to EGFR overexpression and activation. Glomerulonephritis, glomerulosclerosis, diabetic nephropathy, and chronic renal disease all progress in part due to the EGFR pathway (Pham-Danis, 2019).

In the nephrectomy model, erlotinib targets EGF/EGFR, protects against glomerular injury, and enhances glomerular fltration.

The beneficial effects of garcinia cambogia may be mediated by a number of mechanisms, such as: (a) chelating Ca2+ ion, which reduces the nucleation and aggregation of calcium crystals in the renal tubules; and (b) disintegrating and dissolving calcium stones that have already formed in the renal tubules by forming water-soluble calcium complexes, which prevents renal function impairment. Also, the group that had been given garcinia cambogia demonstrated restored SOD and CAT activities in the renal tissues, providing protection for the kidneys from ROS-induced

oxidative stress (Mao and Wang, 2016).

It's interesting to note that the nephroprotective effects of garcinia cambogia may be attributed to its flavonoids and gallic acid, which are phenolic antioxidants (Kaul et al., 2021). Citrate and hydroxycitrate have been found to suppress the nucleation of calcium oxalate monohydrate (COM) in a previous study. By adhering to crystal surfaces, hydroxycitrate inhibits crystal formation (Russo et al., 2020).

According to our study, garcinia cambogia can guard against histopathological changes. Kidney slice H&E microscopic analysis indicated nearly normal histological structure. Due to its phenolic components, it demonstrated notable protection against oxidative stress-related cellular damage in the kidneys.

As a highly active organ, the kidney is vulnerable to the harm caused by oxidative stress. Hence, renal disease progression is promoted by oxidative stress (Daenen, 2019).

In our investigation, renal tissue homogenates from the adenine group showed a significant rise in MDA levels and a decline in the antioxidant enzyme activity of SOD and catalase, indicating an oxidative stress state. It is well known that all cellular activities are decreased when free radicals cause damage. According to Ali et al. (2018) and Chang et al. (2017), adenine dramatically elevated oxidative stress state indicators and lowered antioxidant enzyme activities in renal tissue. These findings are consistent with their findings.

In our investigation, delivery of adenine led to elevated MDA levels and a decline in endogenous antioxidant indicators. Increased lipopolysaccharide-induced renal fbrosis and ureteral obstruction were two tests that showed elevated renal MDA levels. MDA denotes the release of ROS that causes tissue damage and fibrosis. Data gained suggests that erlotinib has renoprotective effects via an antioxidant potential, which is consistent with other studies. It lowers MDA levels and encourages a variety of antioxidant mechanisms, including GSH synthesis, catalase activation, and an overall increase in antioxidant capacity (Ren et al., 2017).

In our study, MDA levels in tissue decreased after erlotinib and garcinia cambogia administration, and SOD and catalase activity increased as a result. Hydroxycitrate-SX (HCA-SX, Super CitriMax), a component of garcinia cambogia, weakens the elevated oxidative stress biomarker by lowering (MDA) and increasing the activities of antioxidant enzymes (Bargi et al., 2017).
Reactive oxygen species (ROS) release and EGF-EGFR have been linked in numerous studies. The relationship between the EGF-EGFR axis and the NADPH oxidase enzyme is a key component of the ROS signaling pathway. In many different cell types, this enzyme is regarded as a significant ROS generator (Cai et al., 2018).

Furthermore, it has been demonstrated that EGFR/ROS stress signaling plays a significant role in the advancement of CRD (Weng et al., 2018).

Several signaling pathways, particularly those that contain mitogen-activated protein kinases (MAPKs), are activated in renal damage, and growth factors and hormones are integrated into and activate ERK1/2 (mitogenic stimuli). ERK1/2 inhibition plays a significant role in preventing renal apoptosis and tubular damage caused by cisplatin. In many induced models of fibrosis, activating the EGFR/ERK1/2 pathway via its inhibitors also retarded the progression of renal fbrosis (Qian, 2016).

In many nephropathies, STAT3 is also substantially expressed. By contributing to the synthesis of collagen type 1 and the consequent excessive deposition of collagen, it causes fibrosis. Renal fibrosis models attribute the rise in TGF-1 and the impending synthesis of collagen I to STAT3. Pharmacological suppression of STAT3 has been shown to slow the course of several nephropathies (Pang, 2020).

While kidney damage and fibrosis are slowed in our investigation, erlotinib has the capacity to block STAT3 and ERK1/2 phosphorylation brought on by adenine. An essential fibrogenic cytokine that plays a role in the various pathogenic processes of CKD is TGF-1. TGF-1 production in renal fibrosis required activated EGFR signaling (Chen, 2011). According to Chen (2011), in cultured renal interstitial fibroblasts, EGFR inhibition can delay TGF-1-induced cell mitogenesis via the EGFR-ERK1/2 kinase pathway. These data show that erlotinib lowered the profibrogenic marker TGF-1, which may have been a result of blocking the ERK1/2 pathway that was activated.

Apoptosis is a type of controlled cell death that can happen either the intrinsic mitochondrial system or the extrinsic receptor-mediated mechanism (Gajate et al., 2009). The Bcl-2 family of proteins comes from the mitochondrial pathway. The balance between Bax, a pro-apoptotic regulator, and Bcl-2, an anti-apoptotic component, can control when caspases are activated. Recent research has shown that chronic renal failure is accompanied with caspase pathway activation and Bcl-2 downregulation in renal tissue (Tang, 2019).

According to the findings of our study, erlotinib treatment

boosted Bcl-2 expression in the kidneys, counteracting the anti-apoptotic effect of the drug that protects against renal tissue damage. Bcl-2 was little expressed in animals given adenine.

Moreover, the tumor suppressor p53 has a role in the onset and healing of acute kidney damage in tubular cells. A possible modality for treating acute injuries and their progression into CRD is p53 inhibition. According to Samarakoon (2013), p53 is a profibrotic initiator necessary for profibrotic genes to activate in the near future in response to TGF-1 (Samarakoon, 2013). They discovered a strong connection between the expression of profibrotic genes and the p53 and EGFR signaling pathways. According to these findings, erlotinib's ability to reduce p-53 expression in renal tissue allowed us to observe a regression of renal fibrosis in our investigation. The chemical mechanisms underpinning G. cambogia's effects on cellular functions are still not fully known. Here, we found that G. cambogia reduced STAT3 expression, which was important for G. cambogia's ability to control autophagic flow (Han et al., 2022).

CONCLUSION

Renal fibrosis and damage can progress more slowly with the use of erlotinib and garcinia cambogia. As they were given alongside adenine and demonstrated the ability to slow the progression of fibrosis, more study into their capacity to reverse fibrosis is advised. Further studies are recommended on larger population, longer duration and other markers to assess the toxicity.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the support and help provided by all the staff of animal House, Zagazig University Hospitals.

CONFLICT OF INTEREST

The authors declare no conflict of interests in relation to this article.

NOVELTY STATEMENT

The achieved results highlightened the value of erlotinib and garcinia cambogia as a novel promising protective strategy to halt drug nephrotoxicity.

AUTHOR CONTRIBUTIONS

Experiments were created and designed by Dalia M. Amin. They were performed by Dalia M. Amin, Nahla M. Ibrahim, and Noha M. Halloull. Biochemical analyses, statistics, and discussion were performed by Dalia M. Amin and Walaa E. Omar and Basma A. Ibrahim.All authors contributed to writing and revising the manuscript.

DATA AVAILABILITY

This published article contains all of the data created or analyzed during this investigation.

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