



## Original Article



# Titanium-nanostructured and PEGylated Doxorubicin Diminish Chemotherapeutic Resistance in 3-Methylcholanthrene Renal Epithelial Cell Carcinoma via KRAS/FKBP5/P53/JAK2 Signaling

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## Abstract

**Background and Objectives:** Nanoparticle (NP) drug delivery systems have been developed recently to resolve the obstacle of drug resistance, contributing to the effective drug delivery to the target organ. A comparative study was carried out herein between doxorubicin (DOX), doxorubicin-loaded titanium NPs, DOX-loaded lactoferrin NPs, DOX-NPs, and PEGylated-doxorubicin (PEG-DOX) on the reno-carcinogenic impact of 3-methylcholanthrene (CA).

**Methods:** *In-vivo* models were exposed to CA at a dose of 50 mg/kg body weight and left for 3 months till the incidence of chronic kidney disease, followed by one month of treatment with the aforementioned nanomedicines.

**Results:** CA downregulated DOX resistance biomarkers, including the gene expression of *KRAS*, *FKBP5*, *P53*, and *JAK2*, and the kidney tumor marker arginase II. In addition, CA increased the levels of the kidney biomarkers creatinine and urea as well as the minerals chloride and magnesium. Decreased gene expression of *FKBP5*, *KRAS*, *P53*, and *JAK2* was reversed after the treatment with DOX-loaded titanium NPs, DOX-NPs, DOX-loaded lactoferrin NPs, and PEG-DOX. PEG-DOX abolished the detrimental effects of CA via upregulating the gene expression of the immunophilin protein (*FKBP5*), the oncogene (*KRAS*), the tumor suppressor gene (*P53*), and *JAK2*, which indicate DOX drug resistance via regulating cell differentiation, division and apoptosis.

**Conclusion:** PEG-DOX restored renal function and resolved DOX resistance via *KRAS*, *FKBP5*, *P53*, and *JAK2* signaling pathways manipulation; consequently, PEG-DOX may provide a useful adjunct treatment for chronic kidney disease.

## Introduction

A growing body of evidence suggests that about 85% of renal cancers are caused by renal cell carcinoma (RCC), which makes

up about 3% of adult malignancies. Clear cell RCC accounts for nearly 85–90% of RCC.<sup>1</sup> Renal function is gradually lost due to chronic kidney disease (CKD). The kidneys experience this condition when they stop functioning normally, particularly their excretory and regulatory functions, which can be brought on by autoimmune diseases, hypertension, or diabetes. CKD is a major health issue and is spreading like an epidemic throughout the world.<sup>2</sup>

Methylcholanthrene (CA) is a highly carcinogenic polycyclic aromatic hydrocarbon that is created when organic molecules are burned at extremely high temperatures. CA is converted into a highly reactive epoxide through epoxidation, hydrolysis, and subsequent epoxidation. The cytochrome P450 enzyme performs epoxidations and releases metabolites that do not hydrolyze CA right away. The metabolites can move and bind to DNA.<sup>3</sup> Rat liver microsomes can break down CA into an oxygenated form that alkylates DNA, contributing to single-stranded and double-stranded breaks. It has been reported that the liver can inhibit the

**Keywords:** Renal cancer; *P53*; *KRAS*; *JAK2*; *FKBP5*.

**Abbreviations:** AR, androgen receptor; AKT, protein kinase B; ATP, adenosine triphosphate; BW, body weight; CA, 3-methylcholanthrene; CKD, chronic kidney disease; DOX, doxorubicin; EGFR, epidermal growth factor receptor; IP, intraperitoneally; Lac-DOX, doxorubicin-loaded lactoferrin NPs; NP, nanoparticle; PEG-DOX, PEGylated doxorubicin; PHLP, PH domain leucine-rich repeat protein phosphatase; qPCR, quantitative polymerase chain reaction; RCC, renal cell carcinoma; SEM, standard error of the mean; Ti-DOX, doxorubicin-loaded titanium NPs.

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process of CA binding to DNA more than the lung and kidney can. This may explain why CA is more carcinogenic in the lungs and kidneys than liver. CA metabolites must be covalently bonded to DNA and oxygenated to be carcinogenic. In particular, CD44 (a cancer stem cell marker) and vimentin (an epithelial-mesenchymal transition marker) are increased in RCC, and numerous renal tumor cell types (ACHN and Caki-2) are upregulated. In several studies, human renal epithelial cells intoxicated by CA have been utilized as a model of RCC for investigating the mechanisms related to RCC onset.<sup>4</sup>

Genes with significantly altered DNA methylation are biologically relevant in CKD. For example, the genes *KRAS*, *CUX1*, *JAK2*, *ELMO1*, *FKBP5*, and *PRKAG2* are excellent biological candidates for CKD that display statistically significant differential methylation.<sup>5</sup> According to Ni *et al*, FKBP5 promotes the attachment of chaperone p53 to the adenosine triphosphate (ATP)-bound form of Hsp90, creating a superchaperone complex called FKBP5-Hsp90-p53 that promotes androgen receptor (AR)-dependent transcription activation and cell proliferation.<sup>6</sup> As a result, FKBP5 may be a new therapeutic target to assist in stymieing AR-mediated signaling in kidney cancer.

In addition to surgery and radiotherapy, chemotherapy is a key component of kidney cancer therapy. Doxorubicin (DOX), a first-line chemotherapeutic agent and a typical anthracycline drug, is frequently used as a single agent or in combination therapy for advanced kidney cancer as well as postoperative adjuvant chemotherapy. Even though chemotherapy is effective against kidney cancer, drug resistance to DOX has become a significant barrier to contemporary CKD therapy, leaving patients with hazardous side effects and a poor prognosis.<sup>7</sup> Thus, scientists have developed nanoparticles (NPs) for combating drug resistance as well as enhancing pharmacologic properties, bioavailability, and targetability. NPs can differ in charge, size, and compatibility with target ligands. The challenge facing researchers nowadays is to create a side-effect-free renal therapy that precisely targets the medication to the affected area. Renal problems are being resolved via nano-sized delivery systems. The management and treatment of kidney disease today cause a heavy burden worldwide. Many molecular and genetic abnormalities accumulate across multiple steps in the multistep processes of renal disorders, which have been linked to several renal illnesses. Kidney filtration is a growing field in nano-medicine and a crucial pathway for medication elimination. Although it is still in the early stages, the application of nanotechnology in renal disease treatment has great potential.<sup>8</sup>

The current work monitored the impact of doxorubicin-loaded titanium NPs (Ti-DOX), DOX-NPs, doxorubicin-loaded lactoferrin NPs (Lac-DOX), and PEGylated-doxorubicin (PEG-DOX) on the *KRAS*, *P53*, *FKBP5*, and *JAK2* signaling pathways in overcomes DOX-induced drug resistance in a rat model of kidney cancer induced by CA.

## Methods

### Chemicals

DOX, Ti-DOX, Lac-DOX, DOX-NPs, and PEG-DOX were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Quantitative polymerase chain reaction (qPCR) kits for *KRAS*, *P53*, *FKBP5*, and *JAK2* were obtained from Qiagen USA.

### Animals and treatments

Fifty-six male Wistar albino rats from the animal facility of the

National Research Center, weighing 170–190 g (8–10 weeks old), were employed. The animals were housed under controlled circumstances (22.5°C, 55.5% humidity, and a 12-h light-dark cycle). They had unrestricted access to water and a pelleted version of the normal chow diet. The Animal Care and Use Committee of the National Research Center (19302) and the US National Institutes of Health approved the ethical practices and policies that were rigorously followed in all procedures regarding the care and treatment of animals.

### Experimental design

At one-week post acclimatization, the animals were randomly divided into seven groups (eight rats each) as follows: group 1, animals received saline and served as the control group; group 2, kidney cancer was induced experimentally in rats by CA for 3 months and served as the kidney cancer model group.<sup>9</sup> The animals were left for 4 months until the occurrence of kidney injury; group 3, the CA group was treated with DOX at a dose of 18 mg/kg body weight (BW) intraperitoneally (IP) for 30 days; group 4, the CA group was treated with Ti-DOX at a dose of 5 mg/kg BW IP for 30 days; group 5, the CA group was treated with Lac-DOX at a dose of 5 mg/kg BW IP for 30 days; group 6, the CA group was treated with DOX-NPs at a dose of 5 mg/kg BW IP for 30 days; group 7, the CA group was treated with PEG-DOX at a dose of 5 mg/kg BW IP for 30 days.<sup>10</sup>

### Blood sampling and tissue preparation

The animals were monitored for any indication of illness. They were weighed, gently sedated with carbon dioxide, and blood samples were taken from the sublingual vein after the experiment. For subsequent biochemical and molecular results assessment, sera were separated by centrifugation at 4,000 rpm for 10 min.

Then, after thorough kidney tissue separation and portioning, the animals were sacrificed by cervical dislocation. Using a Teflon homogenizer, the first portion was homogenized in phosphate buffer at a pH of 7.4 (Glass-Col homogenizer, Terre Haute, IN, USA). The supernatant from a small portion of this homogenate (20% w/v) was centrifuged at 4,000 rpm for 15 min at 4°C and utilized for biochemical testing. The second portion of the kidney was used for qPCR and mRNA estimation.

### Measured biochemical parameters

#### Serum creatinine

Creatinine was estimated spectrophotometrically via a kit (Randox Laboratories, Crumlin, UK), according to the manufacturer's instructions.<sup>11</sup>

#### Serum urea

Urea was estimated spectrophotometrically via a kit (Randox Laboratories, Crumlin, UK), according to the manufacturer's instructions.<sup>12</sup>

#### Kidney chloride concentration

Chloride was estimated spectrophotometrically via a kit (Randox Laboratories, Crumlin, UK), according to the manufacturer's instructions.<sup>13</sup>

#### Kidney magnesium concentration

Magnesium was estimated spectrophotometry via a kit (Randox Laboratories, Crumlin, UK), according to the manufacturer's instructions.<sup>14</sup>

**Table 1.** Primer sequences designed for quantitative polymerase chain reaction

Gene	Primer sequences	Accession number
FKBP5	Forward: 5'-GAA CCC AAT GCT GAG CTT ATG-3'	NM_010220.4
	Reverse: 5'-ATG TAC TTG CCT CCC TTG AAG-3'	
JAK2	Forward: 5'-GCA GTG ACC TCC AGA GAC AGT CTA TCT TTG AAG CAA TAC GTA TGA-3'	NM_001322199.2
	Reverse: 5'-GCA GTG ACC TCC AGA GAC ACT TAC TTC GTC TCC ACA GAA-3'	
KRAS	Forward: 5'-ATT ATA AGG CCT GCT GAA AAT GAC TGA-3'	AF_465422.1
	Reverse: 5'-ATATGCATATTAAACAAGATTACCTCTA-3'	
P53	Forward: 5'-CAG CGT GAT GAT GGT AAG GA-3'	NM_001263689.1
	Reverse: 5'-GCG TTG CTC TGA TGG TGA-3'	
$\beta$ -actin	Forward: 5'-CTT TGA TGT CAC GCA CGA TTT C-3'	NM_1739979
	Reverse: 5'-GGG CCG CTC TAG GCA CCA A-3'	

### Kidney arginase II level

Arginase was estimated spectrophotometrically via a kit (Randox Laboratories, Crumlin, UK), according to the manufacturer's instructions.<sup>15</sup>

### mRNA gene expression of kidney KRAS, P53, FKBP5, and JAK2

qPCR was used to detect the target gene expression of *KRAS*, *P53*, *FKBP5*, and *JAK2* utilizing particular forward and reverse primers (Table 1).<sup>16–19</sup> Using the SV total RNA isolation system (Promega, Madison, WI, USA), total RNA was first extracted from the kidney tissue samples. The extracted RNA was then reverse-transcribed into cDNA and amplified by PCR using a qPCR kit (Stratagene, La Jolla, CA, USA). A final volume of 50  $\mu$ L was used for the reactions (25  $\mu$ L of SYBR Green Mix (2 $\times$ ), 0.5  $\mu$ L of cDNA, 2  $\mu$ L of primer pair mix (5 pmol/ $\mu$ L each primer), and 22.5  $\mu$ L of water). The following temperatures were used in the PCR: 50°C for 2 min,

95°C for 10 min, 95°C for 45 s, 60°C for 30 s, 72°C for 30 s, and 72°C for 10 min.<sup>20</sup>

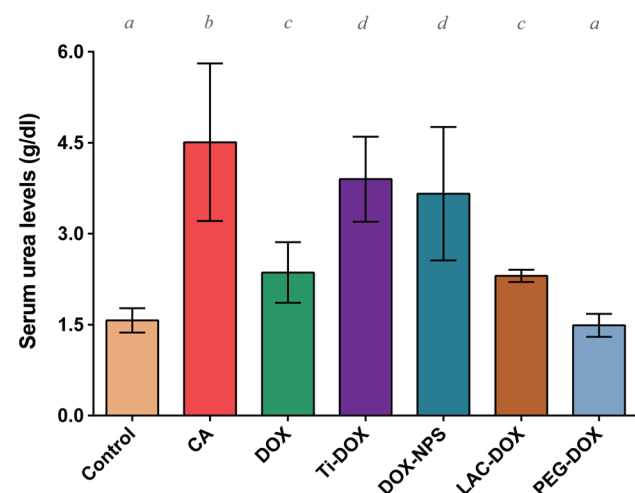
### Statistical analysis

The results were analyzed using a one-way analysis of variance and presented as the mean  $\pm$  standard error of the mean. A value of  $p < 0.05$  indicated a statistically significant difference.

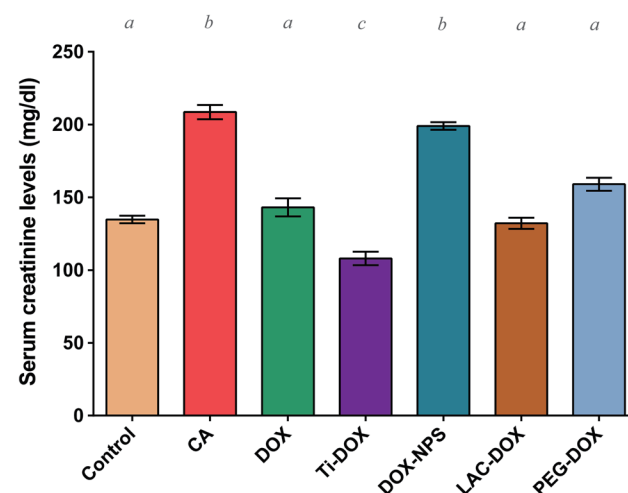
## Results

### Inhibition of CA-induced nephrotoxicity

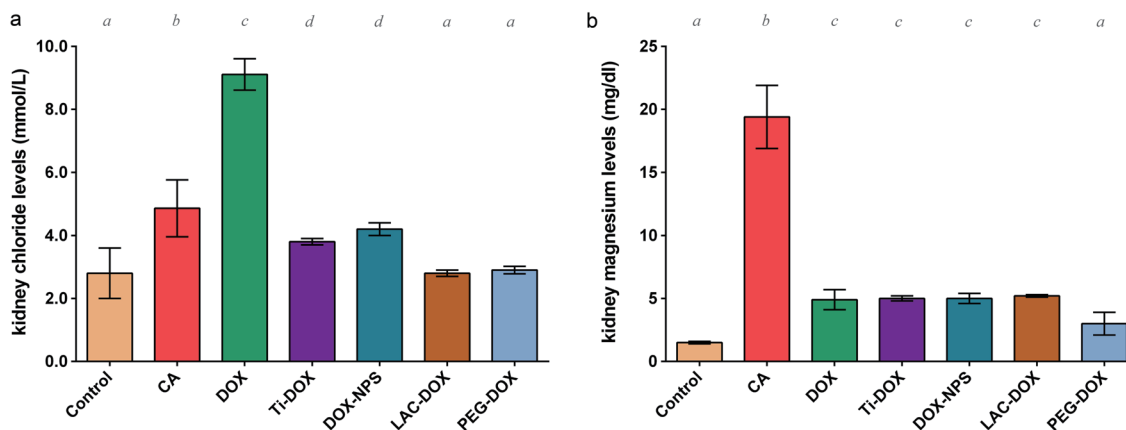
As shown in Figures 1 and 2, CA intoxication significantly increased the serum urea and creatinine levels, compared with the control value. On the other hand, in the groups given DOX, Ti-DOX, Lac-DOX, DOX-NPs, or PEG-DOX, the levels of kidney biomarkers were comparatively less than those of the CA-intoxicated group, with PEG-



**Fig. 1.** Impact of doxorubicin (DOX), DOX-loaded titanium NPs (Ti-DOX), DOX-loaded lactoferrin NPs (Lac-DOX), DOX nanoparticles (DOX-NPs), and PEGylated DOX (PEG-DOX) on the serum urea level after 3-methylcholanthrene (CA) intoxication. Data are expressed as the mean  $\pm$  standard error of the mean (SEM) ( $n = 8$ ),  $p < 0.05$ . Groups having different letters are considered significantly different, while groups having the same letters are not significantly different from each other.



**Fig. 2.** Impact of doxorubicin (DOX), DOX-loaded titanium NPs (Ti-DOX), DOX-loaded lactoferrin NPs (Lac-DOX), DOX nanoparticles (DOX-NPs), and PEGylated DOX (PEG-DOX) on the serum creatinine level after 3-methylcholanthrene (CA) intoxication. Data are expressed as the mean  $\pm$  standard error of the mean (SEM) ( $n = 8$ ),  $p < 0.05$ . Groups having different letters are considered significantly different, while groups having the same letters are not significantly different from each other.



**Fig. 3.** Impact of doxorubicin (DOX), DOX-loaded titanium NPs (Ti-DOX), DOX-loaded lactoferrin NPs (Lac-DOX), DOX nanoparticles (DOX-NPs), and PEGylated DOX (PEG-DOX) on the kidney chloride and magnesium levels after 3-methylcholanthrene (CA) intoxication. Data are expressed as the mean  $\pm$  standard error of the mean (SEM) ( $n = 8$ ),  $p < 0.05$ . Groups having different letters are considered significantly different, while groups having the same letters are not significantly different from each other.

DOX revealing the most significant impact, implying the possible therapeutic impact of DOX-loaded nanocarriers on kidney injury.

#### Modulation of mineral levels

CA intoxication induced a state of mineral imbalance as evidenced by the increase in chloride and magnesium levels compared to the control values (Fig. 3). Meanwhile, the administration of DOX, Ti-DOX, Lac-DOX, DOX-NPs, or PEG-DOX significantly reduced the magnesium and chloride levels compared with those of the CA-intoxicated group. Obviously, the PEG-DOX regimen was superior, thus displaying the most pronounced effect.

#### Modulation of the kidney tumor marker arginase II

CA intoxication caused a significant reduction in kidney tumor marker arginase II compared to the control value (Fig. 4). Meanwhile, the administration of DOX, Ti-DOX, Lac-DOX, DOX-NPs, or PEG-DOX significantly elevated the arginase II level compared with that of the CA-intoxicated group. Obviously, the PEG-DOX regimen was superior, thus displaying the most pronounced effect.

#### Modulation of renal mRNA gene expression of FKBP5, KRAS, P53, and JAK2

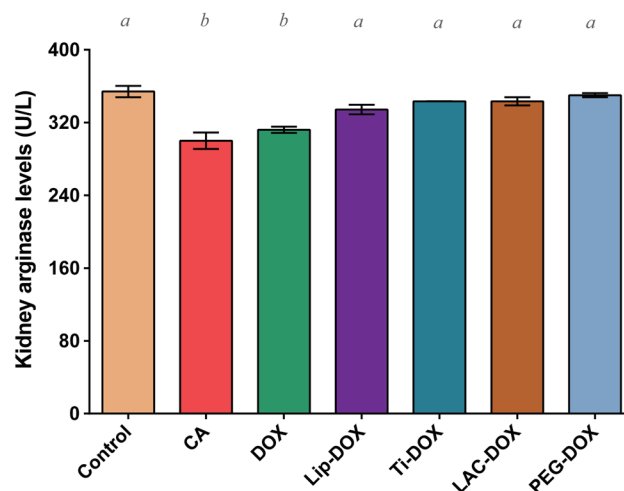
The data shown in Figures 5 and 6 indicate that CA intoxication caused significant downregulation of the gene expression of *FKBP5*, *KRAS*, *P53*, and *JAK2* by almost 4-, 40-, 3-, and 4.5-fold, respectively, compared to the control values. Nevertheless, significant upregulation was apparent in the rats treated with DOX, Ti-DOX, Lac-DOX, DOX-NPs, or PEG-DOX. Besides, the PEG-DOX and Ti-DOX regimens considerably showed the most significant impact regarding *FKBP5* and *KRAS* gene expression compared with the CA group. Meanwhile, the PEG-DOX and Lac-DOX regimens noticeably showed the most significant impact regarding *P53* and *JAK2* gene expression compared with the CA group. In addition, the heatmap representing the expression levels of different genes and their correlation was performed; with red representing a high score and blue representing a low score (Fig. 7).

#### Discussion

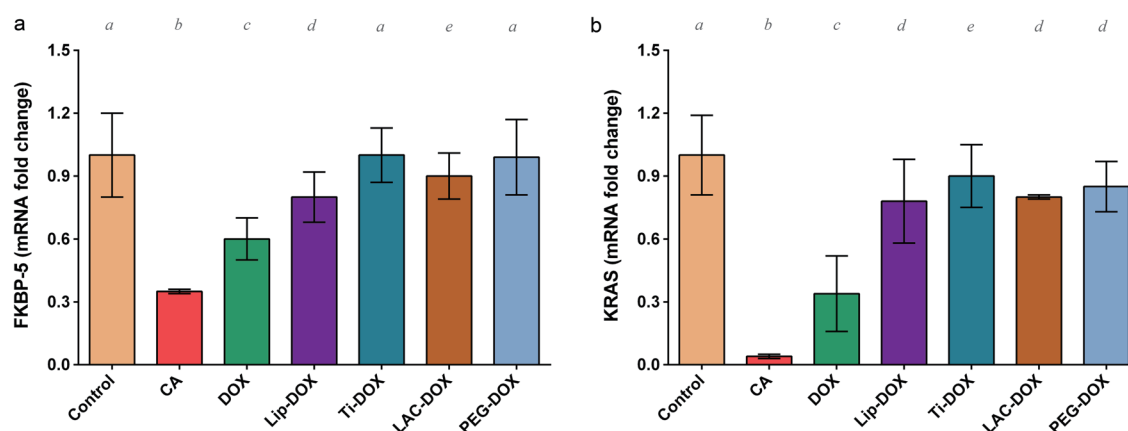
Kidney disorder is a multi-step process that results in a wide range

of molecular and genetic alterations over time. These alterations have an impact on the biological function of the kidney. Many genetic alterations have been related to numerous kidney diseases, including aneuploidy, mutations, insertions, and deletions.<sup>21</sup> In renal proximal tubule, NPs can be effectively delivered and attached to the target tissue. The shape of NPs possesses a significant impact on their functionality and biological distribution. The purpose of the current work is to compare the impact of DOX, Ti-DOX, DOX-NPs, Lac-DOX, and PEG-DOX on renal dysfunction caused by CA by tracking the signaling pathways of *KRAS*, *P53*, *FKBP5*, and *JAK2*.

The present study elucidated that CA intoxication induced a significant elevation in the kidney function biomarkers creatinine and urea. Meanwhile, these parameters were altered by treatment with DOX, Ti-DOX, DOX-NPs, Lac-DOX, or PEG-DOX, with PEG-



**Fig. 4.** Impact of doxorubicin (DOX), DOX-loaded titanium NPs (Ti-DOX), DOX-loaded lactoferrin NPs (Lac-DOX), DOX nanoparticles (DOX-NPs), and PEGylated DOX (PEG-DOX) on the kidney arginase II levels after 3-methylcholanthrene (CA) intoxication. Data are expressed as the mean  $\pm$  standard error of the mean (SEM) ( $n = 8$ ),  $p < 0.05$ . Groups having different letters are considered significantly different, while groups having the same letters are not significantly different from each other.



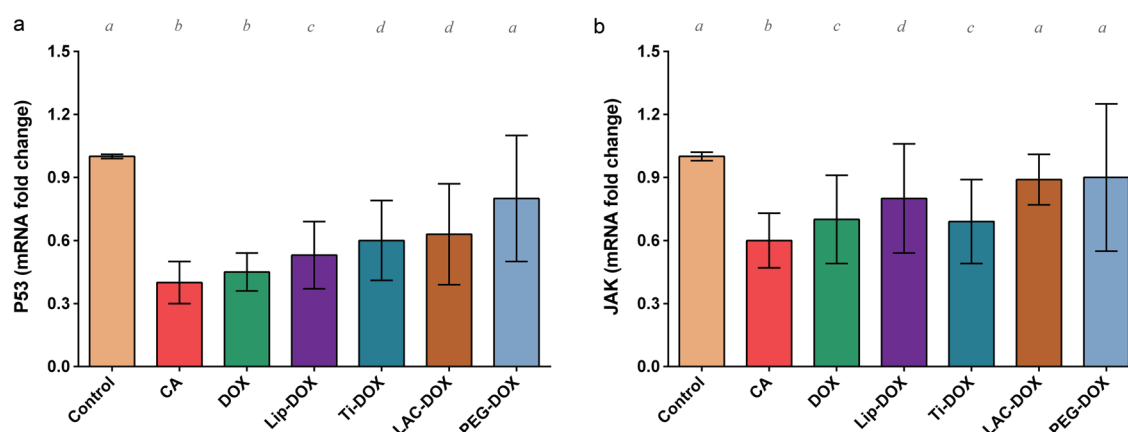
**Fig. 5.** Impact of doxorubicin (DOX), DOX-loaded titanium NPs (Ti-DOX), DOX-loaded lactoferrin NPs (Lac-DOX), DOX nanoparticles (DOX-NPs), and PEGylated DOX (PEG-DOX) on kidney *FKBP5* and *KRAS* gene expression after 3-methylcholanthrene (CA) intoxication.  $\beta$ -Actin was used as a reference gene. Data are expressed as the mean  $\pm$  standard error of the mean ( $n = 8$ ),  $p < 0.05$ . Groups having different letters are considered significantly different, while groups having the same letters are not significantly different from each other.

DOX having the greatest impact. Renal failure is diagnosed when the urea and creatinine levels are increased. Therefore, if the kidneys fail, the blood urea level may increase. Renal failure hinders the elimination of urea by the kidney, which causes an increase in the blood urea concentration. On the other hand, creatinine is formed due to the metabolism of muscle protein. The kidney filters almost all of the creatinine before excreting it in the urine. Renal failure is typically indicated by an increase in the creatinine level.<sup>2</sup>

The current findings revealed a considerable disturbance in the mineral levels after CA intoxication, particularly magnesium and chloride. Meanwhile, these parameters were altered by treatment with DOX, Ti-DOX, DOX-NPs, Lac-DOX, or PEG-DOX, with PEG-DOX having the greatest influence. The physiological functions of chloride ions include the control of extracellular and intracellular volume as well as acid-base equilibrium. The most prevalent extracellular anion, chloride, is responsible for approximately one-third of the tonicity of extracellular fluids. Chloride homeostasis is mostly regulated by the kidney, and chloride tubular reabsorption is essential for preserving the extracellular fluid volume. Many homeostatic systems, such as renin secretion control, tubule-

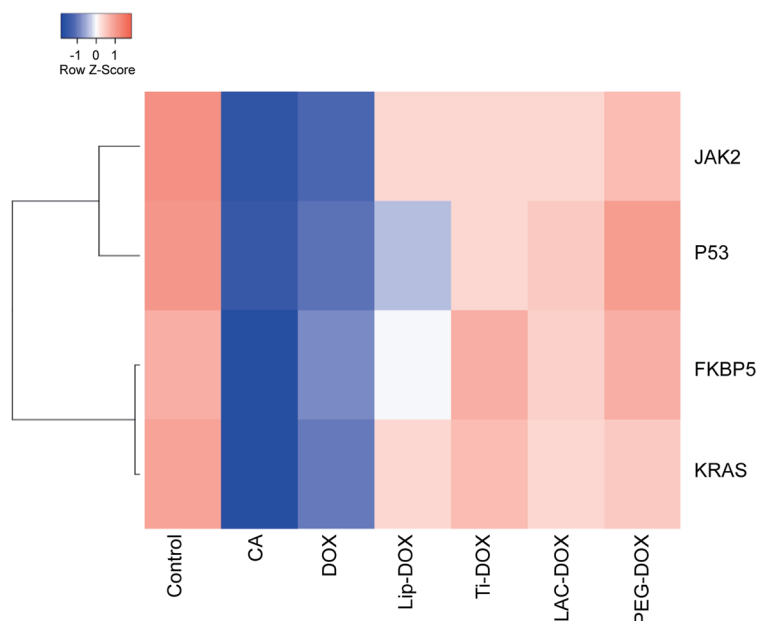
glomerular feedback, blood pressure response, and renal sodium processing, are significantly influenced by serum chloride levels. According to data, heart failure, chronic renal disease, and pulmonary arterial hypertension all increase the risk of death. Moreover, randomized studies have demonstrated that giving crystalloid intravenous fluids with less chloride may improve renal results.<sup>22</sup>

Similar to how the kidney is crucial for maintaining magnesium homeostasis, renal processing of magnesium is extremely flexible, but this ability degrades as the renal function is considerably reduced. In order to maintain a normal serum magnesium level in CKD, magnesium excretion greater than magnesium uptake leads to a decrease in the glomerular filtration rate. This compensatory mechanism, however, becomes insufficient in more advanced CKD, leading to overt hypermagnesemia, which develops in subjects with creatinine clearances less than 10 mL/min. There is evidence that magnesium prevents vascular calcification brought on by phosphate. Magnesium interferes with the maturation of calciprotein particles, which in turn interferes with the crystallization of calcium phosphate. Given that phosphate overload damages the kidneys, magnesium may be able to prevent kidney damage from



**Fig. 6.** Impact of doxorubicin (DOX), DOX-loaded titanium NPs (Ti-DOX), DOX-loaded lactoferrin NPs (Lac-DOX), DOX nanoparticles (DOX-NPs), and PEGylated DOX (PEG-DOX) on kidney *P53* and *JAK2* gene expression after 3-methylcholanthrene (CA) intoxication.  $\beta$ -Actin was used as a reference gene. Data are expressed as the mean  $\pm$  standard error of the mean ( $n = 8$ ),  $p < 0.05$ . Groups having different letters are considered significantly different, while groups having the same letters are not significantly different from each other.





**Fig. 7. Heatmap representing the expression levels of different genes and their correlation.** Red represents a high score, and blue represents a low score. CA, 3-methylcholanthrene; DOX, doxorubicin; LipDOX, doxorubicin nanoparticles; TiDOX, doxorubicin-loaded titanium nanoparticles; LAC-DOX, DOX-loaded lactoferrin nanoparticles; PEG-DOX, PEGylated doxorubicin.

phosphate toxicity, as in the case of vascular calcification.<sup>23</sup> In addition, magnesium inhibits calcium influx into vascular smooth muscle cells by opposing voltage-dependent L-type calcium channels and capacitive calcium entry, which is one of the most fundamental physiological activities of magnesium. As a result, magnesium may lower blood pressure and vascular tone. Magnesium also contributes to the stimulation of vasodilatation by decreasing the expression of endothelin-1 and increasing the generation of prostacyclin and nitric oxide in the endothelium. According to a previous study, eating 300 mg of magnesium daily increases the likelihood of developing incident hypertension, which then progresses to CKD.<sup>24</sup>

Herein, CA intoxication substantially disturbed the level of the kidney tumor marker arginase II. Treatment with DOX, Ti-DOX, DOX-NPs, Lac-DOX, or PEG-DOX resulted in an increase in this parameter, with PEG-DOX having the most significant impact. Arginase II and argininosuccinate synthase-I are two important urea cycle enzymes whose expression is severely suppressed in clear cell RCC. At least two different processes, including preserving the vital biosynthetic cofactor pyridoxal phosphate and preventing hazardous polyamine accumulation, are involved in how reduced arginase II activity encourages clear cell RCC tumor growth.<sup>25</sup>

In the current study, after CA intoxication, the gene expression of *FKBP5*, *KRAS*, *P53*, and *JAK2* was significantly decreased. Meanwhile, these expression levels were altered by treatment with DOX, Ti-DOX, DOX-NPs, Lac-DOX, or PEG-DOX, with PEG-DOX having the highest impact. Human FKBP5 is highly expressed in various organs, including the kidney, liver, heart, and peripheral circulation. Protein folding depends on the peptidyl prolyl isomerase activity of the FKBP domain, which catalyzes the conversion of peptidyl prolyl bonds from cis to trans. Peptidyl prolyl isomerase activity and drug-ligand binding are both carried out by the FKBP domain.<sup>26</sup> By creating a complex with the heat shock proteins Hsp90 and Hsp70, FKBP5 is implicated in the modulation of steroid receptor activity, including progesterone, an-

drogen, and glucocorticoid receptors. After the complex separates, the glucocorticoid receptor can attach to DNA-binding sites in its target genes to control gene transcription.<sup>27</sup> Several studies have suggested that FKBP5 may influence hormone receptors to affect psychiatric conditions like depression in addition to its role in tumorigenesis.<sup>28</sup> The nuclear factor kappa B and AKT-PHLPP signaling pathways, among others, are engaged in FKBP5 function, leading to apoptosis.<sup>29</sup> FKBP5 may be involved in the regulation of inhibitor of nuclear factor kappa B activity in the presence of DOX, which can cause phosphorylated inhibitor of nuclear factor kappa B breakdown, nuclear translocation, and activation of nuclear factor kappa B as well as the production of its target genes, thus inducing cell apoptosis. As a folding protein, FK506 binding protein 5 encourages the connection between protein kinase B (AKT) and PHLPP, enhancing the dephosphorylation of AKT and inactivating AKT, which blocks AKT signaling for cell survival and causes cell death.<sup>5</sup> FKBP5 is overexpressed in prostate cancer, lymphoma, melanoma, and brain cancer. On the other hand, it also has been demonstrated that FKBP5 is downregulated in cancers of the kidney, pancreas, colon, and testicles.

For biological relevance in CKD, genes with significantly altered DNA methylation levels and reduced levels were taken into consideration. The *CUX1*, *FKBP5*, and *PRKAG2* genes are potent biological candidates for CKD that display significant differential methylation.<sup>5</sup> An FKBP5-Hsp90-p23 superchaperone complex is formed when cochaperone p23 is recruited to the ATP-bound form of Hsp90, as demonstrated by Ni *et al*.<sup>6</sup> This complex promotes androgen-dependent transcription activation and cell proliferation. Therefore, FKBP5 could be a new therapeutic target for inhibiting AR-mediated signaling in prostate cancer. It has been hypothesized that screening for *KRAS* and *BRAF* mutations in RCC may be a potential technique to identify patients who would respond to epidermal growth factor receptor (EGFR)-targeted therapy because *KRAS* and *BRAF* mutations are associated with a poor response to anti-EGFR therapy in certain malignancies. A previous

investigation highlighted *EGFR*, *KRAS*, and *BRAF* gene mutations in RCC patients.

Loss of FKBP12 has been associated with a poor prognosis and enhanced resistance to DOX treatment. Overall survival and disease-free survival were both significantly poorer in the low-FKBP12 expression group compared to the high-FKBP12 expression group, according to the Kaplan–Meier survival analysis. Individuals with low FKBP12 expression showed a significantly reduced rate of pathologic complete response while receiving DOX-based preoperative chemotherapy.<sup>30</sup>

The complex issue of multidrug resistance usually results in chemotherapy failure during cancer treatment. The primary mechanism causing multidrug resistance is the overexpression of ATP-binding cassette transporters and the downregulation of *KRAS* gene expression. Clinical trials are being conducted to test the effectiveness of the particular inhibitor Adagrasib (MRTX849) in treating non-small cell lung cancer.<sup>31</sup>

Poor drug penetration into tumor tissues and drug efflux via ATP-driven efflux pumps in tumor cells are two common causes of multidrug resistance. To address DOX resistance in small-cell lung cancer, near-infrared light- and acidity-activated micellar iPUTDN NPs were created. While the PEGylated iPUTDN NPs can remain undetected in the bloodstream, near-infrared radiation at the tumor site can remove the PEG shell of the NPs, and the exposed cyclic peptide iRGD can help the NPs penetrate further into the tumor. The triphenylphosphonium-conjugated DOX can be released from the polyaminoester-based NPs by DOX-resistant H69AR cells and then accumulate in the mitochondria with the help of triphenylphosphonium. As a result, DOX-resistant H69AR cells had lower mitochondrial membrane potential and ATP levels. Moreover, *in-vivo* therapy results have demonstrated that triphenylphosphonium-conjugated DOX-loaded NPs can successfully inhibit DOX-resistant small-cell lung cancer with no side effects.<sup>32</sup>

DOX has been an essential treatment for breast cancer, kidney cancer, lymphoma, and leukemia. However, drug resistance, a significant barrier to its therapeutic utilization, may be a challenge. DOX-induced drug resistance is hypothesized to be caused by a number of processes, including DNA intercalation, free radical production, and cell membrane degradation. The majority of studies concur that oxidative stress is a major contributor to DOX-induced drug resistance. After receiving PEG-DOX therapy, the gene expression profile of the JAK-STAT pathway was upregulated in wild-type mice (MT+/+). The JAK-STAT pathway, which is involved in cancer cell death, is what led to the hypothesis that MT may prevent DOX-induced drug resistance.<sup>33</sup>

The JAK-STAT pathway, which conveys signals from extracellular ligands such as numerous chemokines and cytokines, was studied for its role in the pathogenesis of CKD. *JAK1*, *JAK2*, and *STAT3* are genes whose expression and activity are increased in diabetic nephropathy, and their suppression appears to slow the progression of the condition. Cyst growth in autosomal dominant polycystic CKD may be significantly influenced by JAK-STAT signaling activation. JAK-STAT signaling activation may potentially contribute to the tubular responses to chronic obstructive uropathy and HIV-associated nephropathy. Impairment of JAK and STAT proteins or enhanced expression of the suppressors of cytokine signaling proteins also can be used as a treatment for CKD.<sup>34</sup> In addition, Marrero *et al.* have discovered that angiotensin II directly activated JAK-STAT signaling in mesangial cells, which was the first indication that JAK-STAT activation might be significant in the pathophysiology of CKD.<sup>35,36</sup> JAK2 signaling was also responsible for activating transforming growth factor beta

signaling and fibronectin synthesis.<sup>34</sup>

The well-known tumor suppressor gene *P53* controls several biological processes in cells, including apoptosis, cell cycle arrest, and autophagy, and is essential for CKD and the eventual repair of damaged kidneys. One of the most common side effects of cisplatin as chemotherapy is nephrotoxicity, which may manifest suddenly or develop into CKD. When cisplatin causes acute kidney damage, the protein p53 is crucial in causing p53 activation, apoptosis, and fibrotic alterations.<sup>37</sup> Apoptosis after renal DOX therapy is caused by P53-dependent activation of BAX apoptosis inducing factor.<sup>38</sup> Additionally, death receptors 4 and 5 as well as Fas receptor are induced by P53 to regulate the extrinsic apoptotic pathway.<sup>39</sup> Moreover, tubular cell loss in ischemic and septic CKD has been linked to the tumor necrosis factor alpha and Fas-dependent extrinsic pathway of apoptosis, which raises the possibility that the p53-dependent extrinsic pathway of apoptosis may play a role in CKD.<sup>40,41</sup>

Chemotherapeutic medications like DOX continue to be one of the main RCC treatment options. Regrettably, the use of chemotherapeutic drugs is constrained due to their poor solubility, rapid circulation, nonselective distribution, high risk of treatment resistance, and negative side effects. More significantly, the frequent usage of large dosages of chemotherapy might result in serious side effects like immunosuppression and cardiotoxicity. Thus, it is essential to create a targeted RCC therapy delivery system. Thankfully, numerous inorganic, polymeric, and NP-based drug delivery systems have been developed as a promising means of enhancing the original medicinal and physiological effects of these medications. For instance, Banes *et al.* created a liposomal nanocarrier for the delivery of DOX that showed significant tumor accumulation and anticancer effects in mice, leading to tumor regression *in vivo*.<sup>42</sup> In addition, several NP-based tumor delivery systems are undergoing clinical and preclinical testing, and some of them, including Taxol® and Doxil®, have received US Food and Drug Administration approval for triple-negative breast cancer therapy.<sup>43</sup> In order to address the main issues with ocular drug administration, NP-based drug delivery systems have been developed recently. This has led to the creation of a secure and efficient system that delivers the medication to the desired location.<sup>44</sup> The creation of numerous novel nanomedicines based on nanotechnology, such as nano-structured lipid carriers,<sup>45</sup> liposomes,<sup>46</sup> and nano-emulsions, leads to improved retention time, hydrophobic drug solubility, bioavailability, increased drug penetration, and target-specific sites. Also, the targeted drug is shielded from deterioration by being contained inside a NP.<sup>45–47</sup> Many options for illness perception, prevention, treatment, and preservation are made possible by nanotechnology. Research and development are necessary to help patients with cancer, cardiovascular disease, and chronic renal disease.<sup>48</sup> NPs are important because they provide nucleic acids and different medications with a kidney-targeted delivery pathway.<sup>48</sup> The NPs that have been defined as target ligands can be specifically designed to target particular cells or tissues.<sup>48</sup> The targeting, circulation half-life, and cellular uptake of NPs are significantly influenced by their size. Although particles smaller than 10 nm are more likely to be eliminated via phagocytosis and renal excretion, fewer particles are more likely to pass through the kidneys.<sup>49</sup> Titanium dioxide NPs are utilized extensively in a variety of industries, including pharmacology and medicine. Rats treated with titanium dioxide NPs had considerably less renal impairment as shown by lowered renal indices, increased antioxidant enzyme activity, and suppressed expression of fibrotic genes. Liposomes also have considerable potential for use in cancer treatment. DOXIL®

liposomal NPs are frequently utilized to treat adult malignancies. Compared to their free medication counterparts, these have a better safety profile, are noninvasive, and are utilized for precise targeting of solid tumors. These show wider stratification and can be used as personalized cancer nanomedicine.<sup>50</sup>

Nevertheless, this study does have some limitations that must be addressed. First, the handling of CA may cause asthma, skin cancer, and lung cancer. In addition, the sample size for the described experiments was rather small ( $n = 8$ ). Finally, experiments performed on animals do not exactly mimic the way that the human body acts.

## Conclusion

PEG-DOX restores renal function through KRAS, FKBP5, P53, and JAK2 regulation; therefore, PEG-DOX might serve as an adjunct therapy and is a promising candidate for the treatment of CKD.

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## Conflict of interest

The authors declare no conflict of interests.

## Author contributions

MOK: Conceived and designed the experiments; performed the experiment; analyzed (biochemical parameters and RTPCR gene expression) and interpreted the data; contributed reagents, materials, analysis tools or data; wrote the paper. RMAM: Performed the experiment; Analyzed (biochemical parameters and RTPCR gene expression) and interpreted the data.

## Data sharing statement

No additional data or information is available for this paper.

## Ethics statement

The Animal Care and Use Committee of the National Research Center (19302) and the US National Institutes of Health approved the ethical practices and policies that were rigorously followed in all procedures regarding the care and treatment of animals.

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