



Contrasting renal effects of metformin and allicin in doxorubicin-induced nephrotoxicity in experimental animals

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ABSTRACT

Doxorubicin (DOX) remains a cornerstone in cancer chemotherapy; however, its therapeutic benefit is often compromised by progressive organ toxicity, with renal impairment representing a major clinical concern. Although metformin at conventional doses has been associated with renoprotective actions mediated by redox and inflammatory modulation, the renal safety profile of supratherapeutic dosing is still debated. Allicin, a garlic-derived organosulfur molecule, has attracted attention due to its strong antioxidant and tissue-protective capabilities. In this experimental investigation, the renal responses to high-dose metformin and allicin, either alone or combined, were examined in a DOX-induced nephrotoxicity model. Adult male Wistar rats ($n = 70$) were distributed across seven study arms comprising untreated controls, DOX-treated animals, metformin-treated animals, allicin-treated animals, and three combination regimens involving DOX with metformin, allicin, or both agents. Doxorubicin was administered intraperitoneally at spaced intervals to achieve a cumulative exposure of 18 mg/kg, while metformin (300 mg/kg/day) and allicin (40 mg/kg/day) were delivered orally throughout a 21-day period. At the end of the experimental protocol, comprehensive biochemical, oxidative, inflammatory, and histological assessments were performed. Administration of high-dose metformin resulted in marked deterioration of renal status, reflected by worsened functional indices, impaired antioxidant capacity, intensified lipid peroxidation, suppression of nitric oxide availability, and extensive structural damage, surpassing alterations induced by DOX alone. In contrast, allicin markedly mitigated renal injury associated with both DOX and metformin, leading to biochemical improvement and preservation of renal tissue integrity. Collectively, these results indicate that excessive metformin dosing may potentiate renal toxicity, whereas allicin demonstrates a robust protective effect against drug-related renal injury.

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1. INTRODUCTION

Doxorubicin (DOX) is an anthracycline chemotherapeutic agent extensively used to manage a wide range of hematological malignancies and solid tumors. Despite its potent antineoplastic activity, the clinical utility of DOX is substantially constrained by cumulative dose-related toxicities that affect several organs, most notably the heart and kidneys [1, 2]. Renal tissue is particularly susceptible owing to its central role in xenobiotic clearance

and systemic drug elimination. Experimental evidence indicates that doxorubicin preferentially accumulates in renal structures, provoking marked glomerular and tubular damage accompanied by inflammation and oxidative stress [3, 4].

The mechanisms underlying DOX-induced nephrotoxicity are multifaceted and involve excessive production of reactive oxygen species (ROS), impairment of mitochondrial integrity, and persistent stimulation of proinflammatory signaling pathways [5]. These inter-

connected processes drive lipid peroxidation, protein modification, and DNA injury, culminating in the apoptotic loss of renal tubular epithelial cells and progressive deterioration of kidney function. In parallel, elevated levels of inflammatory mediators, particularly tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6), further intensify renal damage by amplifying inflammatory cascades and disrupting intrarenal vascular homeostasis [5].

Metformin, a first-line antidiabetic medication, has attracted considerable interest because of its pleiotropic biological actions that extend beyond glucose regulation. A growing body of experimental research suggests that metformin exerts antioxidant and anti-inflammatory effects and may protect against drug-induced organ injury. At modest doses, metformin has been shown to mitigate DOX-associated cardiac and renal toxicity, largely through the activation of AMP-activated protein kinase (AMPK) and preservation of mitochondrial function [6]. Nevertheless, the renal consequences of metformin administered at higher doses remain inadequately characterized, and emerging evidence indicates that its effects may diverge from the traditionally recognized protective profiles.

However, the renal impact of metformin at higher doses remains incompletely understood, and emerging observations indicate that its pharmacological profile may shift away from protection under suprathreshold conditions. Allicin, a major organosulfur compound derived from garlic (*Allium sativum*), exhibits pronounced antioxidant, anti-inflammatory, and cytoprotective activities in various experimental settings [7]. Previous studies have demonstrated that allicin effectively neutralizes free radicals, augments endogenous antioxidant defenses, and attenuates chemically induced tissue injury, including renal damage [8]. These properties highlight allicin as a potential therapeutic candidate for counteracting doxorubicin-mediated nephrotoxicity.

Given the clinical imperative to limit chemotherapy-related organ damage, exploring the interactions between doxorubicin and adjunctive agents with putative protective effects is of substantial scientific and therapeutic importance. While the cardioprotective potential of both metformin and allicin has been extensively documented, their renal actions, particularly in the context of high-dose metformin exposure, remain insufficiently characterized. Accordingly, the present study was undertaken to assess the renal consequences of high-dose metformin and evaluate the nephroprotective capacity of allicin in a rat model of DOX-induced nephrotoxicity, with a specific focus on renal functional indices, oxidative stress parameters, inflammatory biomarkers, and histopathological changes.

The importance of this investigation stems from addressing a potential therapeutic paradox, whereby metformin,

widely recognized for mitigating doxorubicin-induced cardiotoxicity, may exert detrimental renal effects when administered at elevated doses. Clarifying the renal safety profile of high-dose metformin may assist clinicians and researchers in optimizing treatment strategies that preserve cardiac benefits while minimizing renal liability, thereby supporting rational dose selection and balanced risk–benefit evaluation in patients undergoing doxorubicin-based chemotherapy.

2. MATERIALS AND METHODS

2.1. DRUGS AND CHEMICALS

Doxorubicin was procured from GLS Pharma Ltd. (India), metformin from Denk Pharma GmbH & Co. KG (Germany), and allicin from Now Foods (USA). Diagnostic kits used for the measurement of serum creatinine, urea, blood urea nitrogen (BUN), and total protein were obtained from Biomed Diagnostics (USA). Reagents for evaluating antioxidant capacity and oxidative stress indices—including reduced glutathione (GSH), glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and nitric oxide (NO)—were supplied by Bio-Diagnostic (Egypt). Enzyme-linked immunosorbent assay (ELISA) kits for interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) quantification were purchased from BT LAB (Bioassay Technology Laboratory, Shanghai, China). All remaining chemicals and reagents were of analytical grade.

2.2. ANIMALS

Seventy adult male Wistar albino rats (150 ± 30 g) were obtained from the Animal House of the Department of Biology, Sana'a University. Animals were maintained in polypropylene cages under standardized environmental conditions (25 ± 2 °C; 12 h light/dark cycle) with unrestricted access to standard chow and tap water ad libitum. Prior to the experimental procedures, the rats were allowed a one-week acclimatization period.

2.3. EXPERIMENTAL DESIGN AND SAMPLE COLLECTION

Animals were randomly assigned to seven groups ($n = 10$ each): Control, DOX, Metformin, Allicin, DOX + Metformin, DOX + Allicin, and DOX + Metformin + Allicin. Doxorubicin (6 mg/kg) was administered intraperitoneally on days 7, 14, and 21 to achieve a cumulative dose of 18 mg/kg [9]. Metformin (300 mg/kg/day, p.o.) and allicin (40 mg/kg/day, p.o.) were administered based on previously reported experimental protocols employing comparable doses in rodent models to investigate cardioprotective and antioxidant effects [10, 11]. The drugs were administered orally once daily for 21 consecutive days. Oral treatments were freshly prepared in normal saline and



Table[1]: Experimental Design Summary

Group	Treatment	Dose	Route	Duration
Control	Normal saline	2 ml/kg	Oral	21 days
DOX	Doxorubicin	6 mg/kg	i.p.	Days 7, 14, 21
MET	Metformin	300 mg/kg/day	Oral	21 days
Allicin	Allicin	40 mg/kg/day	Oral	21 days
DOX + MET	DOX + Metformin	same doses	i.p + oral	21 days
DOX + Allicin	DOX + Allicin	same doses	i.p + oral	21 days
DOX + MET + Allicin	Combination	same doses	i.p + oral	21 days

delivered by gavage, with doses adjusted daily according to the individual body weight.

On day 22, the animals were fasted overnight and anesthetized using diethyl ether inhalation in a closed chamber pre-saturated with approximately 2% vapor. This concentration (1.9–3%) reliably produces surgical anesthesia in rodents [11, 12]. Adequate anesthesia was verified by the complete absence of the pedal withdrawal reflex. Euthanasia was subsequently performed by cardiac exsanguination in compliance with the American Veterinary Medical Association (AVMA) Guidelines for the Euthanasia of Animals (2020), which recognize exsanguination as an acceptable secondary method following deep anesthesia. This approach has been widely applied to validated rat experimental models [13, 14].

Blood was collected via cardiac puncture, allowed to clot at room temperature, and centrifuged at 3000 rpm for 15 min (Centurion Scientific, model K2002, United Kingdom). The separated serum was stored at -20 °C until analysis [12]. The kidneys were immediately excised, washed in ice-cold phosphate-buffered saline (PBS, pH 7.4), gently blotted, and weighed. One kidney from each animal was preserved in 10% neutral-buffered formalin for histopathological evaluation [15], whereas the contralateral kidney was stored at -80 °C for biochemical assays [13].

2.4. BODY WEIGHT AND RELATIVE KIDNEY WEIGHT

Body weight was recorded at baseline, throughout the experimental period, and at the end of the study. At the end of the experiment, kidney weights were obtained, and the relative kidney weight (RKW) was calculated using the following formula:

$$\text{RKW (\%)} = (\text{Kidney weight} / \text{Body weight}) \times 100$$

2.5. BIOCHEMICAL ANALYSES

2.5.1. Serum Renal Biomarkers

Serum urea, creatinine, BUN, and total protein concentrations were quantified using commercial colorimetric kits (Biomed Diagnostics, USA). Absorbance readings were obtained using a semi-automated spectrophotometer

(Rayto RT-9200, India). Total protein was determined using the Biuret method described by Gornall et al. (1949), based on the formation of a violet complex between copper ions and peptide bonds under alkaline conditions [14].

2.5.2. Oxidative Stress and Antioxidant Parameters

Approximately 0.1 g of renal tissue was homogenized in 1 mL of PBS (pH 7.7) using an Omni homogenizer (USA). Homogenates were centrifuged as specified in each assay protocol, and the resulting supernatants were used for further analysis. Oxidative and antioxidant markers were assessed using standard colorimetric kits (Bio-Diagnostic, Egypt), including malondialdehyde (MDA) [16], nitric oxide (NO) [15], reduced glutathione (GSH) [17], glutathione peroxidase (GPx) [18], superoxide dismutase (SOD) [19], and catalase (CAT) [20].

2.5.3. Anti-inflammatory Parameters

Serum levels of TNF- α and IL-6 were measured using sandwich ELISA kits (BT LAB, Bioassay Technology Laboratory, Shanghai, China; TNF- α Cat. No. E0082Hu; IL-6 Cat. No. E0090Hu) according to the manufacturer's instructions. Absorbance was recorded at 450 nm using a Rayto RT-9200 spectrophotometer (Rayto Life and Analytical Science Co., Ltd., Shenzhen, China), and cytokine concentrations were derived from standard curves and expressed in pg/mL [15].

2.6. HISTOPATHOLOGICAL EXAMINATION

Renal tissues fixed in formalin were processed using routine paraffin embedding techniques [17, 18]. The samples were dehydrated in graded ethanol, cleared with xylene, and embedded in paraffin. Sections of 4–5 μ m thickness were prepared and stained with Periodic Acid–Schiff (PAS) following Bancroft and Gamble (2008) [19]. Microscopic examination was performed at 400 $\times$ magnification (Olympus BX51, Japan) to assess glomerular integrity, tubular alterations, and interstitial inflammatory changes in the kidney.

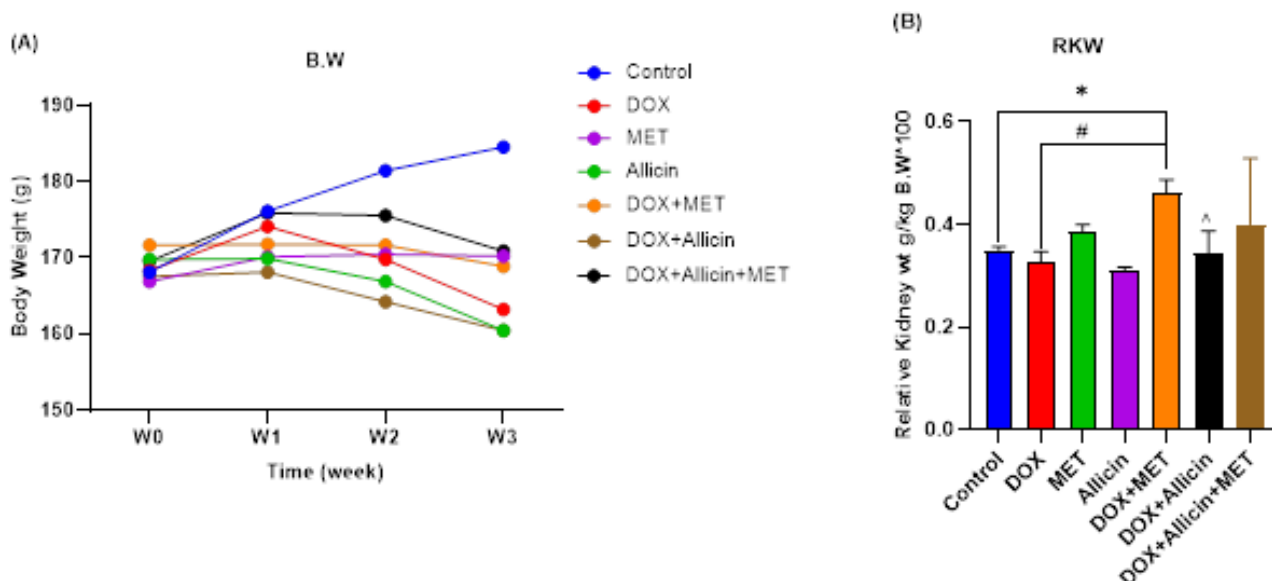


Figure 1. Effects of treatments on (A) body weight and (B) relative kidney weight of experimental rats. Metformin (MET, 300 mg/kg) and its combination with doxorubicin (DOX + MET) significantly increased relative kidney weight compared with control and DOX groups, whereas Allicin treatment alone maintained normal values and partially normalized the increase when combined with DOX + MET. Values are expressed as mean $\pm$ SD ($n = 10$). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$ vs control; # $p < 0.05$ vs DOX; @ $p < 0.05$ vs MET; \$ $p < 0.05$ vs Allicin; ^ $p < 0.05$ vs DOX + MET.

2.7. STATISTICAL ANALYSIS

Data are presented as mean $\pm$ standard deviation (SD). Statistical evaluation was performed using GraphPad Prism version 8.4 (GraphPad Software, San Diego, CA, USA). Normality was verified using the Shapiro–Wilk test. Group differences were analyzed using one-way ANOVA followed by Tukey's post hoc multiple-comparison test, with $p < 0.05$ considered statistically significant.

3. RESULTS

3.1. EFFECT ON BODY WEIGHT AND RELATIVE KIDNEY WEIGHT

Body weights did not differ significantly among the experimental groups throughout the 3-week period (Figure 1A). In contrast, the relative kidney weight was significantly increased in the DOX + MET group compared to that in the control and DOX groups ($p < 0.05$), indicating renal hypertrophy and possible fluid retention. Allicin alone maintained normal kidney weight, whereas the DOX + MET + Allicin group showed partial improvement in kidney weight compared to the control values (Figure 1B).

3.2. EFFECT ON SERUM RENAL-FUNCTION MARKERS

Serum renal function markers, including creatinine, urea, BUN, and total protein, are illustrated in Figures (2A–D). DOX administration caused a mild, non-significant eleva-

tion in serum creatinine, urea, and BUN levels compared with the control group. In contrast, high-dose metformin (300 mg/kg) and DOX + MET treatments significantly increased serum creatinine levels and notably reduced total protein levels ($p < 0.05$). The DOX + MET group exhibited the highest levels of urea and BUN compared with the other groups ($p < 0.05$). Allicin, either alone or in combination with DOX + MET, markedly improved these parameters and restored near-normal renal function indices, indicating its nephroprotective potential.

3.3. EFFECT ON RENAL OXIDATIVE-STRESS AND ANTIOXIDANT BIOMARKERS

Renal oxidative stress and antioxidant biomarkers are shown in Figures (3A–F). DOX and high-dose metformin induced marked oxidative stress, as reflected by increased lipid peroxidation and depletion of enzymatic and non-enzymatic antioxidants.

GSH, GPx, SOD, and CAT were significantly lower in the DOX + MET group than in the control group ($p < 0.05$). Allicin treatment restored most antioxidant parameters to near-normal values. Similarly, nitric oxide levels were significantly reduced by DOX + MET treatment and improved after Allicin co-treatment. MDA, an index of lipid peroxidation, increased markedly in the DOX + MET group ($p < 0.05$) and was markedly reduced by Allicin, confirming its antioxidant capacity.

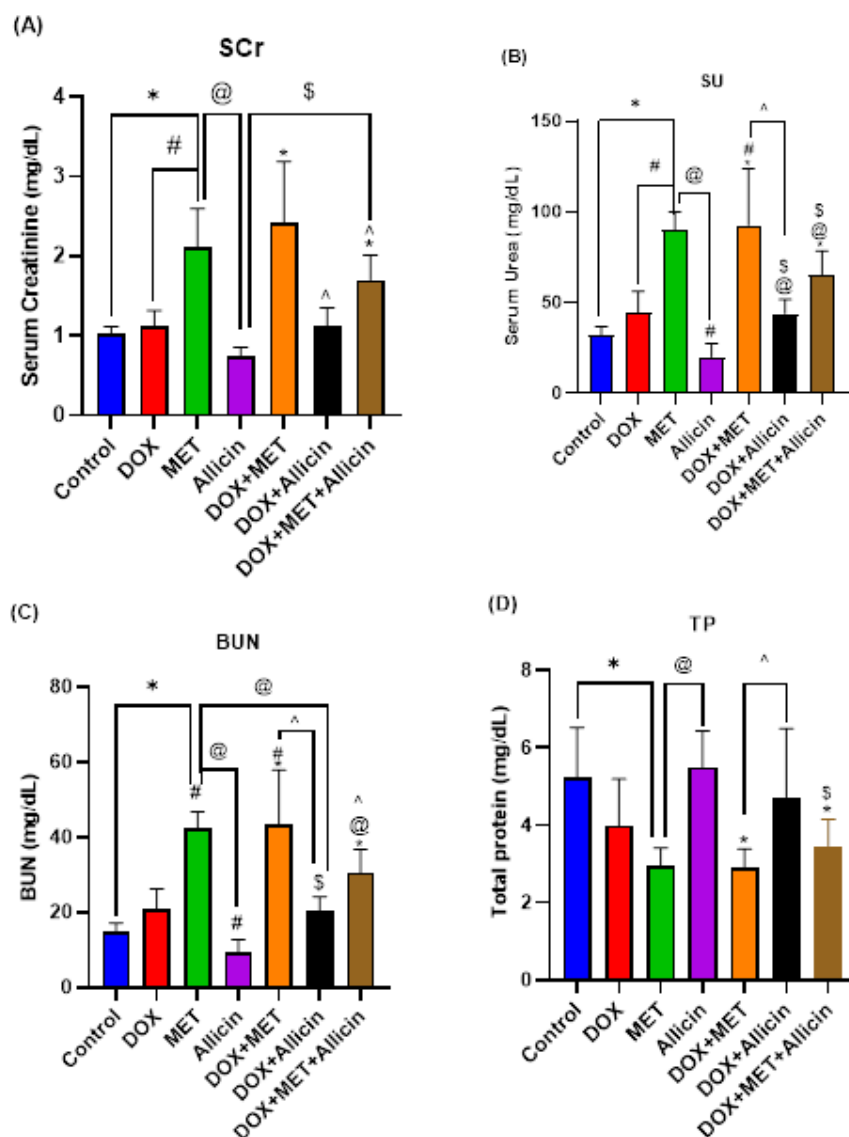


Figure 2. Serum renal-function markers in experimental groups: (A) creatinine, (B) urea, (C) BUN, and (D) total protein. DOX + MET markedly elevated creatinine, urea, and BUN while reducing total protein compared with control; Allicin significantly ameliorated these alterations either alone or when combined with DOX + MET. Values are expressed as mean $\pm$ SD (n = 10). One-way ANOVA followed by Tukey's post hoc test was used. *p < 0.05 vs control; #p < 0.05 vs DOX; @p < 0.05 vs MET; \$p < 0.05 vs Allicin; ^p < 0.05 vs DOX + MET.

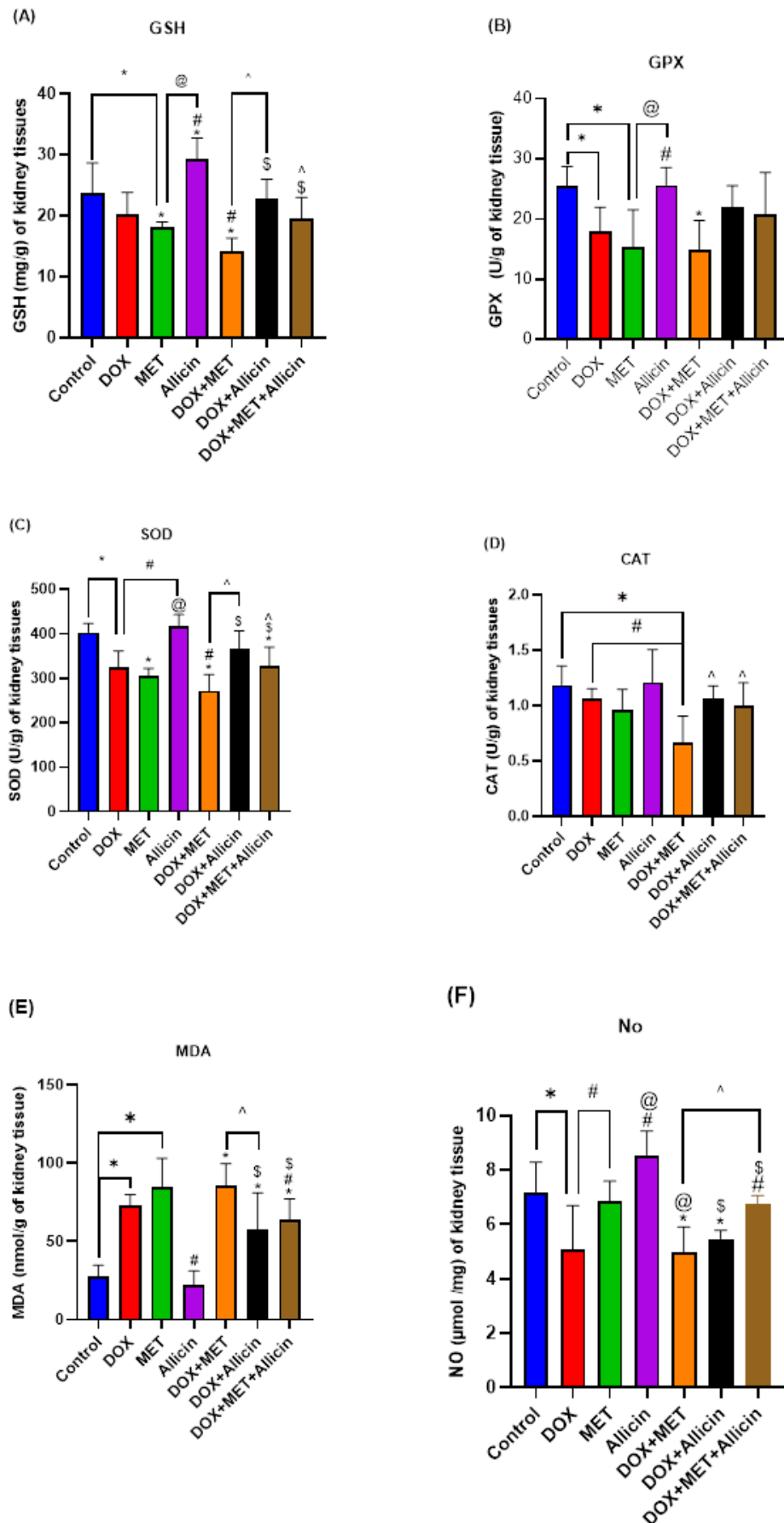


Figure 3. Renal oxidative-stress and antioxidant biomarkers: (A) GSH, (B) GPx, (C) SOD, (D) CAT, (E) MDA, and (F) NO. DOX and MET treatments significantly decreased antioxidant enzyme activities and NO levels while increasing MDA compared with control, indicating oxidative stress. Allicin markedly enhanced antioxidant activities and reduced lipid peroxidation in both single and combined regimens. Values are expressed as mean $\pm$ SD (n = 10). One-way ANOVA with Tukey's post hoc test. *p < 0.05 vs control; #p < 0.05 vs DOX; @p < 0.05 vs MET; \$p < 0.05 vs Allicin; ^p < 0.05 vs DOX + MET.

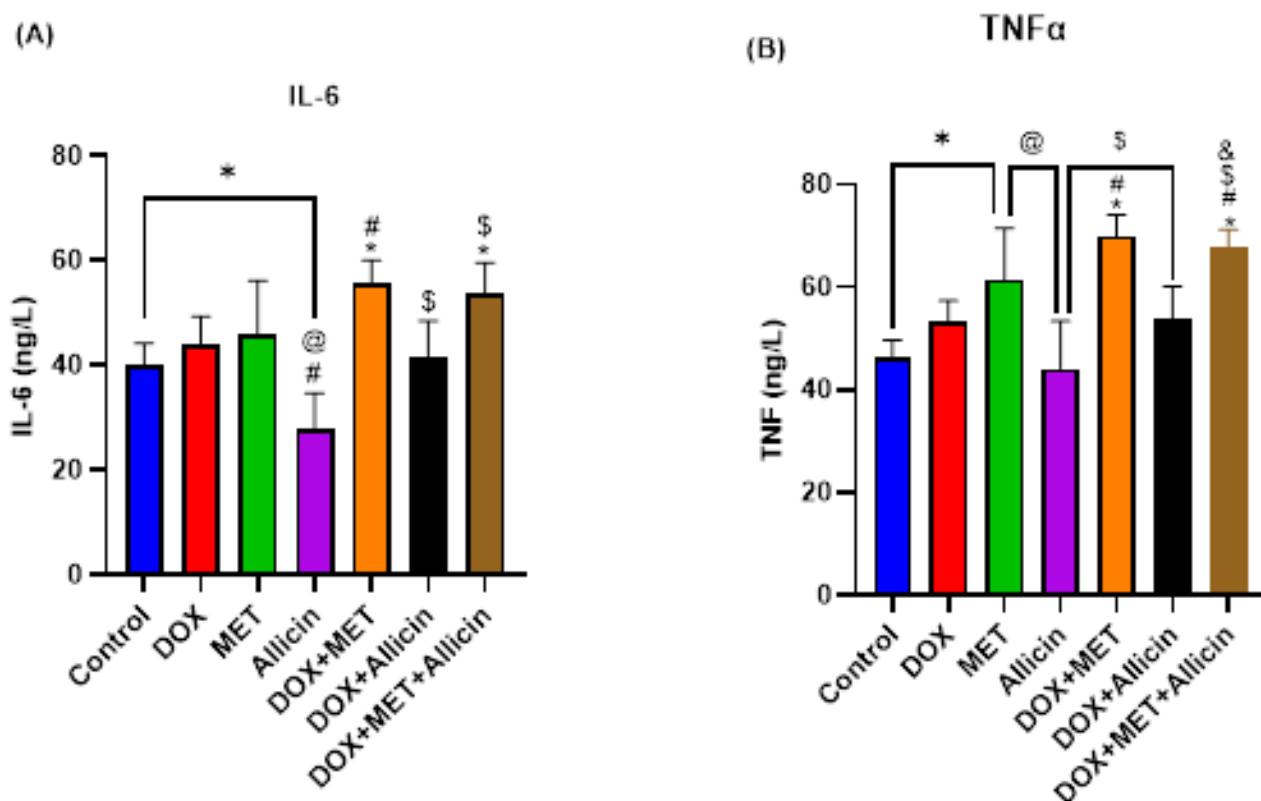


Figure 4. Serum inflammatory cytokines in experimental rats: (A) IL-6 and (B) TNF- α . DOX + MET significantly increased both cytokines compared with control and DOX groups, confirming activation of inflammatory pathways. Allicin alone or combined with DOX + MET significantly reduced these elevations and normalized cytokine profiles. Values are expressed as mean $\pm$ SD (n = 10). One-way ANOVA with Tukey's post hoc test. *p < 0.05 vs control; #p < 0.05 vs DOX; @p < 0.05 vs MET; \$p < 0.05 vs Allicin; ^P < 0.05 vs DOX + MET

3.4. EFFECT ON INFLAMMATORY CYTOKINES

The levels of inflammatory cytokines IL-6 and TNF- α are shown in Figures (4A–B). DOX + MET markedly elevated IL-6 and TNF- α compared with the control and DOX groups (p < 0.05), confirming the pro-inflammatory effect of high-dose metformin in the presence of DOX. Allicin administration significantly reduced the levels of both cytokines, either alone or in combination with DOX + MET, demonstrating its anti-inflammatory and nephro-protective effects.

3.5. HISTOPATHOLOGICAL EVALUATION OF RENAL TISSUE

Table (2). Semi-quantitative histopathological scoring of renal injury in experimental groups. Renal lesions including tubular degeneration, tubular necrosis, glomerular shrinkage, widening of Bowman's space, and interstitial edema were graded according to severity using a scoring scale (0 = normal; 1 = mild; 2 = moderate; 3 = severe). The total score represents the sum of individual lesion scores for each group.

4. DISCUSSION AND CONCLUSION

The present investigation offers new mechanistic insights into the renal impact of high-dose metformin (300 mg/kg) and the protective role of allicin in a validated rat model of doxorubicin (DOX)-induced nephrotoxicity. Administration of DOX alone produced pronounced biochemical, oxidative, inflammatory, and histopathological disturbances characteristic of anthracycline-induced renal injury.

Elevations in serum creatinine, urea, and BUN levels, together with increased lipid peroxidation and cytokine release, indicate compromised glomerular filtration and tubular dysfunction. These observations are consistent with earlier studies demonstrating that DOX provokes oxidative stress, mitochondrial impairment, and inflammatory activation through excessive ROS generation, NF- κ B signaling, and endothelial damage [1, 3, 4]. Consistently, histological evaluation revealed tubular degeneration, glomerular contraction, and widening of Bowman's space, reflecting the classical features of oxidative-inflammatory renal injury secondary to DOX accumulation.

One of the central mechanistic findings of this study

Table[2]: Semi-quantitative histopathological scoring of renal tissue injury

Group	Tubular Degeneration	Tubular Necrosis	Glomerular Shrinkage	Bowman's Space Widening	Interstitial Edema / Hemorrhage	Total Score
Control	0	0	0	0	0	0
DOX	2	1	1	1	1	6
MET	1	1	1	1	1	5
Allicin	0	0	0	0	0	0
DOX + MET	3	3	2	2	2	12
DOX + Allicin	1	1	1	1	1	5
DOX + MET + Allicin	1	1	1	1	1	5

is the marked elevation of IL-6 and TNF- α following DOX exposure, signifying the strong activation of innate inflammatory pathways. These cytokines are key regulators of renal inflammation, driving endothelial dysfunction, tubular apoptosis, and interstitial fibrosis via the NF- κ B and MAPK signaling cascades [20, 21]. Their increase closely mirrored the biochemical and structural abnormalities observed, reinforcing the concept that inflammation is a primary driver of DOX-induced nephrotoxicity rather than a secondary consequence. This interpretation aligns with previous reports documenting increased renal IL-6 and TNF- α expression after DOX administration [1, 4, 22].

Notably, high-dose metformin (300 mg/kg) exacerbated oxidative and inflammatory disturbances rather than attenuating them. This was evidenced by further elevation of renal biomarkers, increased MDA levels, depletion of antioxidant defenses (GSH, SOD, GPx, CAT), and pronounced increases in IL-6 and TNF- α , accompanied by aggravated histological injury compared with DOX alone. These findings are consistent with reports indicating that supratherapeutic metformin exposure induces mitochondrial Complex I inhibition, metabolic acidosis, and redox imbalance, thereby heightening renal susceptibility to injury [23, 24]. Given the kidney's reliance on oxidative phosphorylation, such metabolic perturbations render renal tissue particularly vulnerable, which explains the observed potentiation of DOX-induced nephrotoxicity.

This apparent biphasic response to metformin reflects its dose-dependent pharmacodynamics, particularly its interaction with mitochondrial energetics and oxidative stress pathways. At lower doses (≤ 250 mg/kg), metformin partially and reversibly inhibits mitochondrial Complex I, increasing the AMP/ATP ratio and activating AMPK [25]. This adaptive response improves mitochondrial efficiency, limits ROS generation, promotes autophagy, and suppresses NF- κ B-mediated inflammatory signaling, including reductions in IL-6 and TNF- α [26, 27].

Several investigations have demonstrated that metformin (200–250 mg/kg) attenuates DOX-induced nephrotoxicity by enhancing antioxidant defenses and preserving renal architecture [26–28]. In contrast, higher doses (≥ 300 mg/kg) cause excessive Complex I inhibition, leading to ATP depletion, NADH accumulation, enhanced anaerobic glycolysis, and lactic acidosis [23, 24, 29]. These metabolic disturbances foster ROS overproduction and weaken endogenous antioxidant systems, thereby amplifying oxidative stress and NF- κ B/MAPK-driven inflammation [4, 24].

Proximal tubular cells, which depend heavily on oxidative phosphorylation and possess limited glycolytic reserves, are particularly prone to energy failure under such conditions.

In contrast, cardiomyocytes exhibit greater metabolic flexibility, which may explain why equivalent or higher metformin doses can confer cardioprotection while inducing nephrotoxicity [30, 31]. Moreover, lactate accumulation at supratherapeutic doses further destabilizes mitochondrial homeostasis, reduces intracellular pH, impairs enzymatic activity, and aggravates oxidative-inflammatory injury [24, 29]. Collectively, these mechanisms clarify why metformin displays cytoprotective properties at moderate doses but becomes deleterious at higher doses, especially within renal tissues.

Although metformin has been widely studied as a nephroprotective agent, no evidence supports renal protection at a dose of 300 mg/kg in DOX-induced nephrotoxicity models. Protective outcomes have largely been reported at lower doses (200–250 mg/kg), which improve antioxidant capacity, preserve glomerular and tubular integrity, and attenuate oxidative-inflammatory damage [26–28].

In contrast, the renoprotective effects of 300 mg/kg metformin have been observed mainly in alternative contexts, such as chronic diabetic nephropathy or

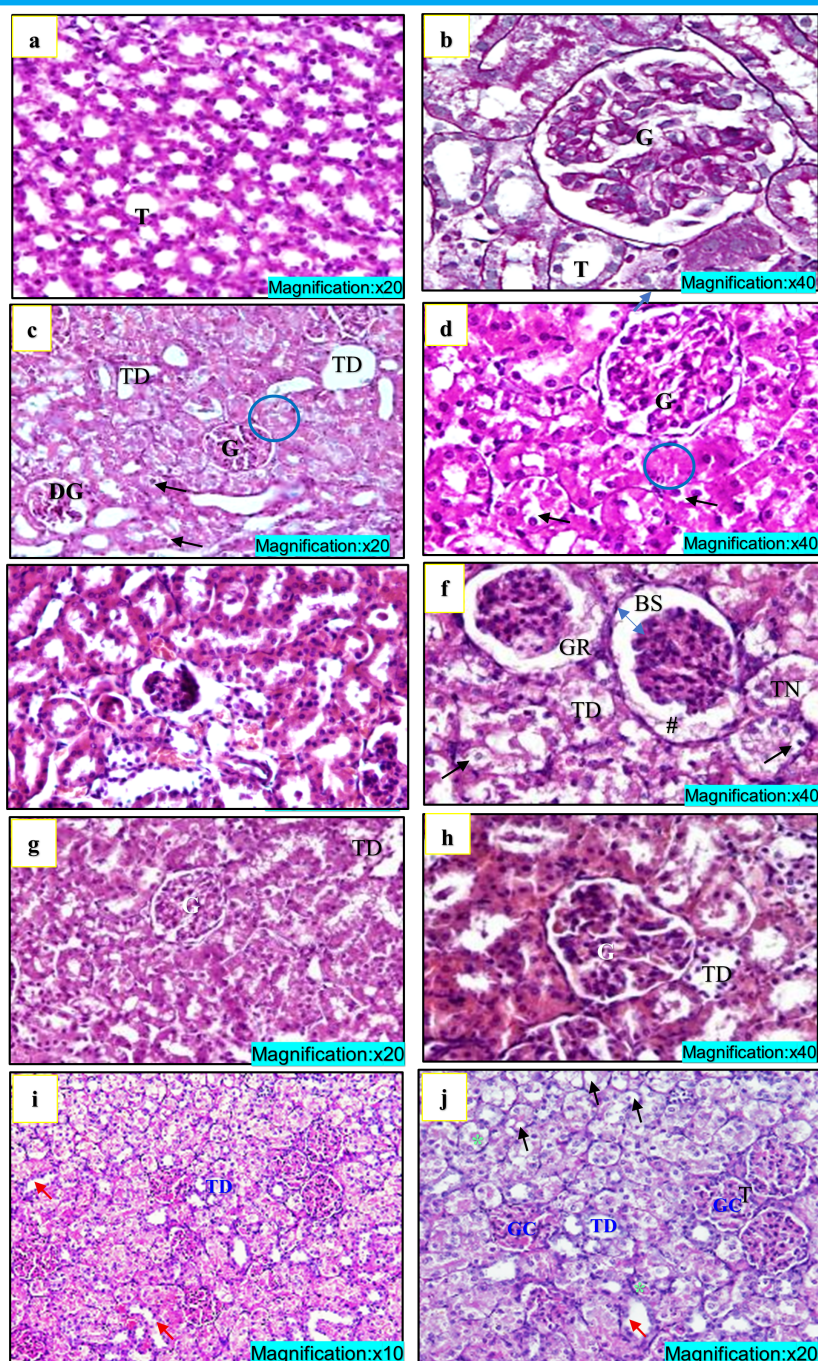


Figure 5. Histological photomicrographs at kidney sections tissue from experimental Wistar albino rats were stained with Hematoxylin and Eosin (H&E) showing: (a & b): Kidney sections from the normal control group exhibited preserved renal architecture, characterized by normal glomeruli (G) and well-organized renal tubules (T) without signs of degeneration or inflammation. (c & d): kidney sections administration of doxorubicin (DOX), significant histopathological alterations were observed. These included tubular degeneration (TD), cytoplasmic vacuolization (black arrow), distorted glomerular structures (DG), and tubular necrosis (blue circle), indicating pronounced nephrotoxicity. (e & f): The DOX + Metformin (300 mg/kg) group, the kidneys showed more extensive structural damage, including widespread tubular necrosis (TN), Increased Bowman's space (BS) lead to glomerular retraction (GR) cytoplasmic vacuolization (black arrow), tubular deletion (TD), hydronephrosis and atrophy (#), and disorganization of renal architecture. This suggests that the administration of a high dose of metformin may exacerbate DOX-induced renal injury. (g & h): The DOX + allicin-treated group showed histological improvement, with reduced tubular damage (TD), and partial restoration of glomerular morphology (G), indicating a potential nephroprotective effect of allicin after doxorubicin administration. (i & j): The DOX + metformin + allicin group shows; Improved glomerular preservation was evident compared to the DOX + MET group, though occasional glomerular contraction was noted (GC); moderate vacuolar and hydropic degeneration, especially in proximal tubular epithelial cells (black arrows); Some tubular dilation (TD); with relatively intact brush border in some areas (red arrows) and eosinophilic intraluminal proteinaceous casts consistent with residual tubular stress were observed (an asterisk).

ischemia–reperfusion preconditioning, where prolonged or anticipatory treatment enhances AMPK activation, strengthens mitochondrial resilience, and suppresses inflammatory signaling [32–34].

Such models permit adaptive metabolic remodeling and regulate redox signaling, potentially accounting for their favorable outcomes. Acute toxic insults, including DOX nephrotoxicity, represent a far less permissive metabolic environment in which suprathreshold metformin may exceed the mitochondrial adaptive capacity, shifting from protection toward metabolic stress and toxicity. Supporting this interpretation, multiple studies have shown that high-dose metformin promotes lactate accumulation, inhibits oxidative phosphorylation, and increases renal vulnerability during acute injury [23, 24, 35]. The present findings align with this framework, demonstrating that 300 mg/kg metformin not only fails to confer protection but also aggravates DOX-induced renal damage, underscoring the narrow therapeutic window and context-dependent nature of metformin's renal effects.

In contrast, allicin markedly reduced IL-6 and TNF- α concentrations, highlighting its strong anti-inflammatory activity, in addition to its antioxidant effects. Allicin significantly improved renal function markers, restored antioxidant status, lowered MDA levels, and enhanced both enzymatic and non-enzymatic defenses. Histological evaluation further revealed the preservation of glomerular and tubular architecture with intact basement membranes and brush borders.

These outcomes are consistent with prior evidence indicating that allicin, a bioactive organosulfur compound, exerts potent antioxidant and anti-inflammatory actions by scavenging ROS and modulating redox-sensitive transcription factors, such as Nrf2 and NF- κ B [8, 36, 37]. The suppression of IL-6 and TNF- α likely plays a central role in disrupting the oxidative–inflammatory loop initiated by DOX, thereby maintaining renal structural and functional integrity. The present data support the concept that allicin effectively restores redox homeostasis.

Collectively, these results confirm that DOX-induced nephrotoxicity is primarily mediated by oxidative stress and inflammatory responses. High-dose metformin intensified these pathogenic pathways, whereas allicin interrupted this cycle and provided substantial nephroprotection against AKI. These findings emphasize the importance of dose selection when considering metformin as an adjunct to chemotherapy. While lower doses (~200–250 mg/kg) may offer renal benefits, higher doses ($\geq$ 300 mg/kg) can exacerbate kidney injury despite reported cardioprotective effects, highlighting the

need for organ-specific safety assessments and careful dose optimization.

In conclusion, the present study demonstrates that metformin (300 mg/kg) aggravates renal injury through excessive mitochondrial inhibition, lactic acidosis, and intensified oxidative inflammatory stress. Conversely, allicin confers significant nephroprotection by preserving the renal architecture, strengthening antioxidant defenses, and downregulating IL-6 and TNF- α signaling. These findings underscore the necessity of exercising caution when administering high-dose metformin. Although metformin has established cardioprotective benefits, excessive dosing may compromise renal safety. Accordingly, routine monitoring of renal function and judicious dose adjustment are recommended to balance therapeutic efficacy and minimize nephrotoxic risk, particularly during combination therapy with doxorubicin.

5. ETHICS STATEMENT

All experimental procedures were conducted in accordance with the institutional guidelines for animal care and were approved by the Ethics Committee of Sana'a University, Yemen (Code No. (25) 7-10).

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