HSI Health Sciences Investigations Journal

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ISSN Online 2704-4890 | ISSN Print 2720-7609

Online first publication

Original Research Article

HSI Journal (2025) Volume 7 (Issue 2):1271-1279. https://doi.org/10.46829/hsijournal.2025.12.7.2.1271-1279

Open Access

Comparative renoprotective and haematological effects of gallocathechin and Anona muricata in rats treated with 7,12-dimethylbenz[a]anthracene (DMBA)

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Received June, 2025; Revised October 2025; Accepted Noverber, 2025

Abstract

Background: Chemically induced carcinogenesis often leads to renal dysfunction and haematological abnormalities.

Objective: This study compared the effects of doxorubicin, gallocatechin, and *Annona muricata* leaf extract (AMLE) on renal function, oxidative stress, and haematological markers in rats treated with cancer-inducing DMBA.

Methods: Fifty female Sprague-Dawley rats were randomised into five groups (n = 10). Group A served as control; Group B received DMBA (80 mg/kg); Groups C – E received DMBA followed by doxorubicin (4 mg/kg weekly), gallocatechin (40 mg/kg daily), or AMLE (40 mg/kg daily) for 21 days, respectively. Renal function markers (urea, creatinine, electrolytes), oxidative stress indices (SOD, CAT, MDA, total protein), and haematological parameters (PCV, RBC, Hb, WBC, platelets) were measured. Kidney histology was also evaluated.

Results: DMBA-treated rats (Group B) showed increased kidney weight, elevated serum urea and creatinine, oxidative stress (↑MDA, ↓SOD, ↓CAT), electrolyte imbalance,↓PCV, ↓RBC, ↓Hb, and ↑WBC. Doxorubicin (Group C) reduced kidney weight and partially reversed electrolyte imbalance but exacerbated renal and haematological toxicity. In contrast, gallocatechin (Group D) and AMLE (Group E) significantly improved renal markers, restored antioxidant enzyme levels, corrected electrolyte and hematologic imbalances, and ameliorated kidney histopathology. AMLE showed the most pronounced histological recovery.

Conclusion: While doxorubicin retained antitumor effects, it contributed to renal and haematological damage. Gallocatechin and AMLE demonstrated superior nephroprotective and hematopoietic benefits, primarily via antioxidant and anti-inflammatory mechanisms, making them promising adjuncts in cancer therapy.

Keywords: Doxorubicin, gallocatechin, *Annona muricata*, DMBA, renal function, haematological parameters, phytotherapy

Cite the publication as Oyesola OA, Iquot IS, Soetan OA, Olayemi OE, Ojo-Adebayo EO (2025) Comparative renoprotective and haematological effects of gallocathechin and Anona muricata in rats treated with 7,12-dimethylbenz[a]anthracene (DMBA). HSI Journal 7 (2):1271-1279 . https://doi.org/10.46829/hsijournal.2025.12.7.2.1271-1279

INTRODUCTION

Renal impairment and haematological alterations are frequent consequences of exposure to environmental and chemical carcinogens such as 7,12-dimethylbenz[a]anthracene (DMBA), a polycyclic aromatic hydrocarbon extensively used to induce experimental models of carcinogenesis [1]. DMBA induces oxidative stress, lipid peroxidation, and systemic inflammation, with kidneys being particularly susceptible due to their role in detoxification and excretion [2]. Assessing renal function markers such as urea, creatinine, total protein, blood electrolytes, and oxidative stress indicators—superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA)—alongside histological and haematological parameters, provides an extensive analysis of systemic toxicity and organ function.

Doxorubicin, a widely used anthracycline antibiotic in cancer chemotherapy, is effective against a broad spectrum of malignancies but is associated with dose-limiting nephrotoxicity and haematological suppression [3]. These

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adverse effects are largely mediated by the generation of reactive oxygen species (ROS), leading to oxidative stress and mitochondrial damage in renal tissue [4]. Doxorubicin was selected as the chemotherapeutic comparator in this study because it is one of the most extensively used anthracycline agents in oncology, and its nephrotoxic and haematological adverse effects are well documented. Importantly, it shares mechanistic pathways with DMBA in generating ROS, inducing oxidative stress, and promoting mitochondrial dysfunction, thereby serving as a clinically relevant and mechanistically appropriate reference point for evaluating potential protective interventions. In contrast, natural antioxidants such as gallocatechin, a bioactive flavonoid found in green tea and other plant sources, exhibit free radical scavenging, anti-inflammatory, and cytoprotective properties that may counteract these toxic effects[5]. Emerging studies suggest that gallocatechin may counteract oxidative damage induced by xenobiotics, but its potential renoprotective and hematomodulatory effects in carcinogen-challenged models remain underexplored.

Similarly, *Annona muricata* (soursop) leaf extract has garnered attention for its high levels of acetogenins, flavonoids, and phenolic compounds, which contribute to its antioxidative, nephroprotective, and haematological restorative properties [6,7]. Evidence suggests that *A. muricata* may ameliorate chemical-induced renal dysfunction and oxidative imbalance in experimental models. Despite these advances, no study to date has directly compared the renoprotective and haematological effects of gallocatechin and *Annona muricata* leaf extract (AMLE) within the same carcinogen-induced experimental framework. Such a comparative analysis is essential not only for clarifying whether these natural agents offer differential or complementary benefits, but also for evaluating the translational relevance of using an isolated bioactive compound versus a crude extract. Therefore, this study comparatively evaluated the effects of doxorubicin, gallocatechin, and AMLE on renal function and haematological parameters in DMBA-treated rats, integrating biochemical, histopathological, and haematological analyses to assess their relative therapeutic or toxicological effects and to provide new insights into plant-based alternatives for mitigating drug-induced organ damage.

MATERIALS AND METHODS

Plant collection, authentication, and preparation of extract

Fresh *A.muricata* leaves were collected from Sagamu, Ogun State, Nigeria. The plant material was authenticated by a taxonomist at the Federal Herbarium, Ibadan, and a voucher specimen was deposited with the herbarium number FHI. 113982 for future reference. The collected leaves were washed thoroughly, air-dried at room temperature, and then pulverised into coarse powder using a mechanical grinder. The powdered material was macerated in 99% ethanol for 72 hours with intermittent shaking. After maceration, the extract was filtered and concentrated using a rotary evaporator under reduced pressure to obtain a crude ethanol extract. After preparation of the crude extract, it was divided into two portions: one portion was used directly as AMLE without further manipulation, while the other portion was subjected to gallocatechin isolation.

Isolation and characterisation of gallocatechin

Gallocatechin was isolated from the ethanolic extract of *A. muricata leaves by* solvent partitioning followed by chromatographic separation. Column chromatography was performed using silica gel as the stationary phase and hexane as the mobile phase. Fractions exhibiting antioxidant activity, as determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, were pooled for further analysis. Preliminary identification was carried out using Thin Layer Chromatography (TLC). High-Performance Liquid Chromatography (HPLC) was employed to assess the purity of the isolated compound. Structural elucidation and confirmation of gallocatechin were achieved through spectroscopic techniques, including UV-Visible (UV-Vis) spectroscopy, Fourier-Transform Infrared (FTIR) spectroscopy, and Nuclear Magnetic Resonance (NMR) spectroscopy.

Chemicals and reagents

DMBA (Union City, CA 94587, USA) and Doxorubicin (Zuvius Lifescience Pvt Ltd, India) were purchased. All reagents and assay kits for biochemical and haematological tests were of analytical grade.

Experimental animals, induction of carcinogenesis, and experimental design

Fifty healthy adult female Sprague-Dawley rats weighing between 150 and 200 g were used for the study. The animals were housed under standard laboratory conditions, including a 12-hour light/dark cycle, and were provided with a standard pellet diet and water ad libitum. Ethical approval for the study was obtained from the OlabisiOnabanjo University Teaching Hospital Research Ethics Committee (OOUTHREC) with approval number OOUTH/HREC/698/2023AP. Carcinogenesis was induced by a single intraperitoneal injection of DMBA at a dose of 80 mg/kg body weight, dissolved in sesame oil, as described by Wang & Zhang [2]. After induction of carcinoma, treatment was carried out with gallocatechin (40 mg/kg) and *Annona muricata* leaf extract (AMLE, 100 mg/kg). The selected doses were based on prior experimental studies that demonstrated efficacy without overt toxicity.

Gallocatechin has been shown to exert significant antioxidant and protective effects in rodents at doses between 20 – 50 mg/kg, with 40 mg/kg representing a commonly adopted midpoint that produces consistent biochemical and histological benefits [9]. In contrast, crude plant extracts, such as AMLE, generally require higher doses due to their lower concentrations of active phytoconstituents. Previous studies have reported the

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protective effects of A. muricata leaf extract at doses of 100 – 250 mg/kg in chemically induced toxicity models without signs of toxicity [10]. Accordingly, this study employed 40 mg/kg for gallocatechin and 100 mg/kg for AMLE to reflect biologically relevant, literature-supported doses that allow meaningful comparison between a purified phytoconstituent and a crude extract in terms of renoprotective and haematological outcomes. The animals were randomly divided into five experimental groups (A-E), with ten rats in each group (n = 10). The treatment given to each group of rats is summarised in Table 1. Randomisation was performed using a computer-generated random number sequence to assign animals to treatment groups, ensuring that allocation was unbiased. Investigators responsible for treatment administration and outcome assessment were blinded to group assignments. A research investigator in our team prepared and coded the treatment solutions, while another conducted biochemical, haematological, and histopathological analyses without knowledge of the treatment codes. Blinding was maintained until all data were collected and analysed to minimise experimental bias.

Animal sacrifice and sample collection

At the end of the treatment period, the rats were fasted overnight and then euthanised using an overdose of an approved barbiturate anaesthetic solution, administered in accordance with the AVMA Guidelines for the Euthanasia of Animals (2020) and the protocol approved by the Institutional Animal Care and Use Committee. Blood samples were collected via cardiac puncture for haematological and biochemical analyses. The kidneys were carefully excised, weighed, and processed for both biochemical and histopathological examinations.

Biochemical analysis of renal function

Urea and Creatinine

Serum urea and creatinine concentrations were determined using standard enzymatic colourimetric methods. Commercial diagnostic kits (Randox Laboratories Ltd., UK) were used for these assays following the manufacturer's instructions. The urea assay was based on the urease-Berthelot enzymatic method, while the creatinine assay employed the Jaffe reaction modified for enzymatic colourimetric measurement. Absorbance was read using a UV-Visible spectrophotometer at appropriate wavelengths (580 nm for urea and 510 nm for creatinine), and concentrations were calculated according to the manufacturer's protocol.

Blood Electrolytes

Serum electrolytes, including sodium (Na^+), potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-), were measured using an automated electrolyte analyser (Roche 9180 Electrolyte Analyser). Blood samples were centrifuged to separate serum, and the analyses were conducted immediately to ensure accuracy. The ion-selective electrode (ISE) method was used for quantification of sodium, potassium, and chloride, while bicarbonate was assessed using a CO2 electrode method. Calibration of the analyser and quality control checks were performed regularly in accordance with the manufacturer's instructions to ensure precision and reliability of the results.

Assessment of oxidative stress markers

To evaluate oxidative stress in renal tissue, kidney samples were excised immediately after sacrifice and rinsed with ice-cold saline. Each kidney was weighed and homogenised in cold phosphate buffer saline (PBS, pH 7.2) using a Teflon-glass homogeniser (Ace Glass, USA). The homogenates were centrifuged at 3,000 rpm for 15 minutes, and the resulting supernatant was used for biochemical assays of oxidative stress markers, including Superoxide Dismutase (SOD), Catalase (CAT), Malondialdehyde (MDA), and Total Protein (TP).

Superoxide dismutase (SOD):

SOD activity was determined using the method based on the inhibition of autoxidation of epinephrine at alkaline pH or the reduction of nitroblue tetrazolium (NBT) by superoxide radicals, as described by Misra and Fridovich [11]. The inhibition rate was monitored spectrophotometrically, and one unit of SOD activity was defined as the amount of enzyme causing 50% inhibition of NBT reduction per minute.

Catalase (CAT)

Catalase activity was assessed according to the method of Aebi [12], which measures the rate of decomposition of hydrogen peroxide (H_2O_2) at 240 nm. One unit of catalase activity was defined as the amount of enzyme required to

Table 1. Animal grouping and experimental design

Group		Treatment	References
Group A	Negative Control	None	
Group B	DMBA only	DMBA (80 mg/kg)	[2]
Group C	Doxorubicin-treated	DMBA (80 mg/kg) + doxorubicin (4 mg/kg) i.p weekly	[8]
Group D	Gallocatechin-treated	DMBA (80 mg/kg) + gallocatechin (40 mg/kg) p.o daily	[9]
Group E	*Annona muricata* Leaf Extract-treated (AMLE)	DMBA (80 mg/kg) (AMLE) (100 mg/kg) p.o daily	[10]

All treatments were administered for 21 consecutive days. Abbreviation: p.o stands for per os (by mouth)

decompose 1 µmol of H_2O_2 per minute under the assay conditions.

Total protein (TP)
The total protein content in the kidney homogenates was determined using the Bradford assay, with bovine serum albumin as the standard. Absorbance was read at 595 nm, and protein concentration was expressed in mg/mL. This was used to normalise the enzyme activities and oxidative marker levels.

Malondialdehyde (MDA)
Lipid peroxidation was estimated by measuring MDA levels, a major end-product of lipid peroxidation. The thiobarbituric acid reactive substances (TBARS) assay was used, based on the reaction of MDA with thiobarbituric acid (TBA) under acidic and high-temperature conditions to form a pink chromogen, which was measured spectrophotometrically at 532 nm. The MDA concentration was expressed in nmol/mg protein.

Assessment of haematological parameters
Blood samples were collected into EDTA-containing tubes at the time of sacrifice via cardiac puncture for haematological analysis. The haematological indices were analysed using an automated haematology analyser (Mindray BC-2800Vet), which provided precise and standardised results. The following parameters were evaluated: Packed Cell Volume (PCV), Haemoglobin Concentration (Hb), Red Blood Cell (RBC) Count, Total White Blood Cell (WBC) Count, Differential White Blood Cell Count (neutrophils, lymphocytes), and Platelet Count. All values were automatically calculated by the analyser and cross-checked with peripheral blood smear examination, where necessary for accuracy and confirmation of differential counts.

Histological procedure
Kidney tissues were carefully excised and immediately fixed in 10% neutral buffered formalin for at least 24 hours to preserve tissue architecture. Following fixation, the tissues were subjected to standard histological processing, including dehydration in a graded series of ethanol, clearing in xylene, and embedding in paraffin wax. Paraffin-embedded tissues were sectioned at a thickness of 5 µm using a rotary microtome (Leica, Leica Biosystems, USA). The sections were mounted on glass slides, deparaffinised, and stained with Hematoxylin and Eosin (H&E) for general histopathological evaluation. The stained slides were examined under a light microscope for structural alterations and pathological changes in renal tissue architecture. Representative photomicrographs were captured using a digital microscope camera (Celestrone Microscope Imager, Model #44420, China) for documentation and comparison among experimental groups.

Statistical analysis
Data were analysed using GraphPad Prism statistical software (version 10; GraphPad Software, San Diego, CA, USA). All results were expressed as mean ± standard error of the mean (SEM). Comparisons between experimental groups were performed using one-way analysis of variance (ANOVA) followed by a Bonferroni post hoc test to determine significant differences between means. A p-value less than 0.05 was considered statistically significant.

RESULTS

Effect of doxorubicin, gallocatechin and *A. muricata* on kidney weight in DMBA-treated rats
As shown in Figure 1, kidney weight was significantly higher in group B than in group A. In contrast, Group C rats had a statistically lower kidney weight compared to Groups A and B. Rats in Groups D and E had significantly lower kidney weights compared to those in Group B. There were significantly lower urea and creatinine levels in groups B and C rats compared with group A rats (Figure 2). Rats in Group C had a non-significant difference in urea and creatinine levels compared with animals in Group B. Groups D and E rats had significantly lower urea and creatinine levels compared with Group B rats.

There were significantly lower CAT, SOD, and TP levels and a significantly higher MDA in groups B and C compared with group A (Figure 3). Group C showed no significant differences in CAT, SOD, TP, and MDA levels compared with group B. As further shown in Figure 3, groups D and E had significantly higher CAT, SOD, and TP levels and a significantly lower MDA levels when compared with group B. Data presented in Table 2 show a

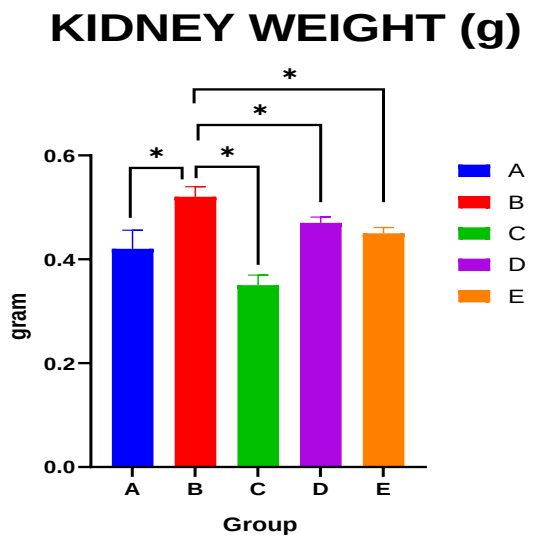

Figure 1. A bar chart showing the effect of doxorubicin (C), gallocatechin (D), and A. muricata (E) on kidney weight in DMBA-treated rats. Group A animals were untreated controls, and group B Animals were DMBA-treated without intervention. The error bars represent standard error of means (SEM). Asterisks indicate significant differences at P > 0.05.

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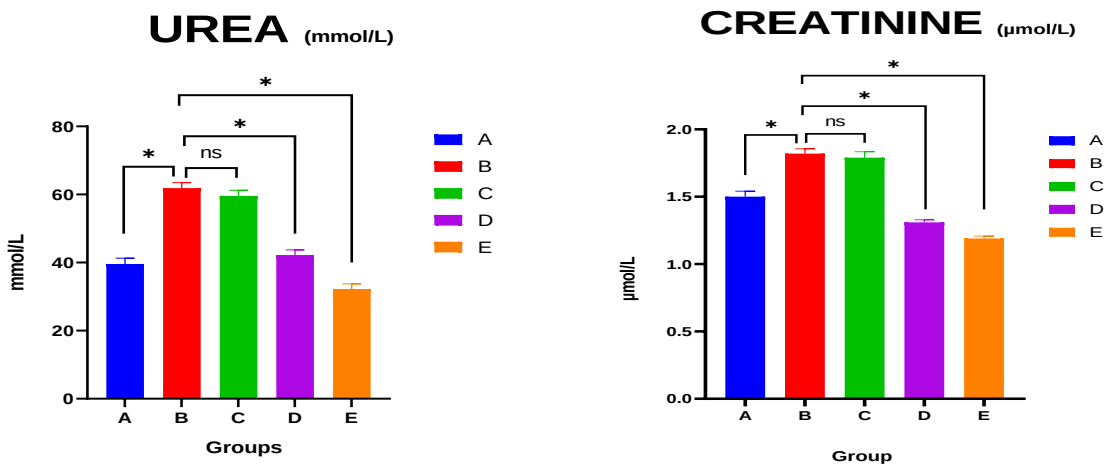

Figure 2. Bar charts showing serum levels of urea and creatinine in DMBA-treated rats with subsequent administration of doxorubicin (C), gallocatechin (D), and A. muricata (E) on kidney weight in DMBA-treated rats. Group A animals were untreated controls, and group B Animals were DMBA-treated without intervention. The error bars represent the standard error of means (SEM). Asterisks indicate significant differences at $p > 0.05$.

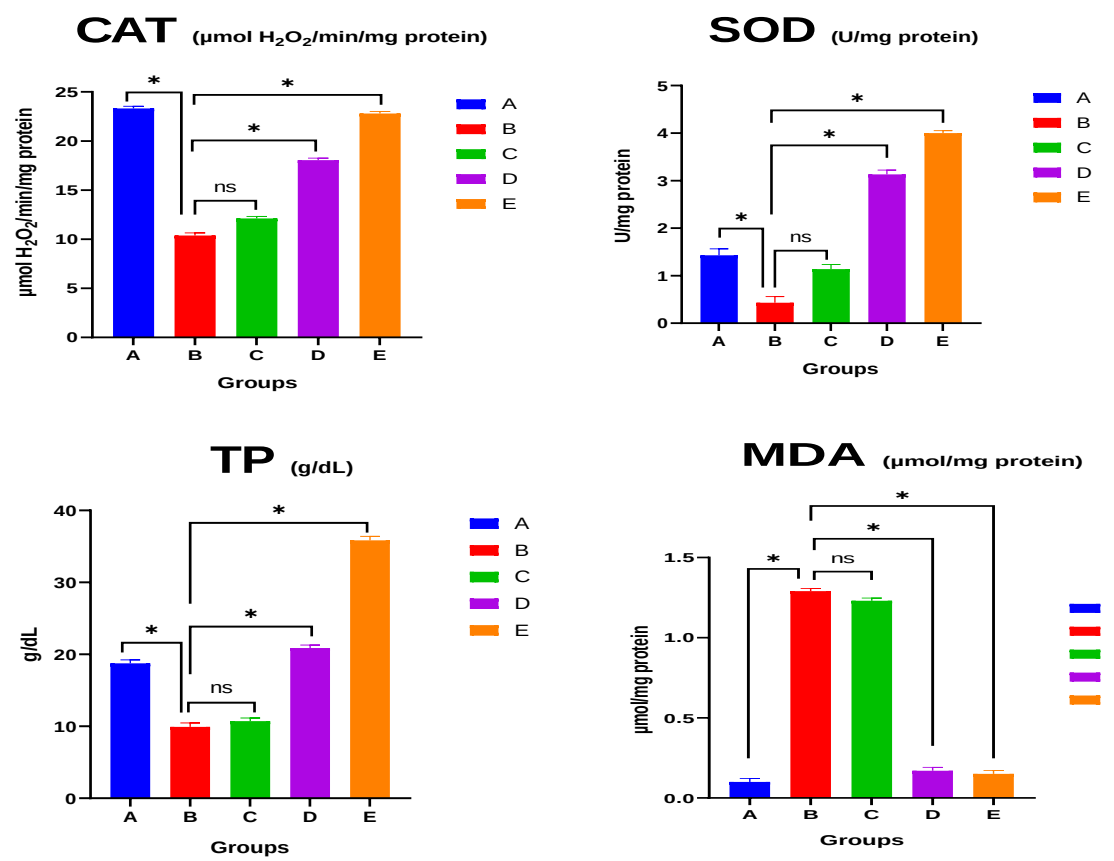

Figure 3. Bar charts showing the renal levels of catalase (CAT), superoxide dismutase (SOD), total protein (TP), and malondialdehyde (MDA) in DMBA-treated rats subsequently given doxorubicin (C), gallocatechin (D), and A. muricata leaf extract (E). Group A animals were untreated controls, and group B Animals were DMBA-treated without subsequent intervention. The error bars represent the standard error of means (SEM). Asterisks indicate significant differences at $p > 0.05$.

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Table 2. Effect of doxorubicin (C), gallocatechin (D), and A. muricata leaf extract (E) on blood electrolytes in DMBA-treated Sprague-Dawley rats. Group A animals were untreated controls, and group B Animals were DMBA-treated without subsequent intervention.

Groups	Na+	K+	Cl^-	HCO_3^-
A	133.5±0.69	3.19±0.40	65.51±0.57	24.75±0.62
B	141.5±0.74a	5.16±0.29a	42.48±0.61a	18.52±0.58a
C	112.4±0.69ab	1.45±0.43ab	85.43±0.50ab	31.70±0.41ab
D	134.8±0.36b	3.39±0.40b	70.10±0.54b	25.33±0.51b
E	134.2±0.13b	3.27±0.51b	67.42±0.47b	25.12±0.39b

Each value is an expression of mean± SEM
a = $p \leq 0.05$ when compared with group A, b = $p \leq 0.05$ when compared with group B.

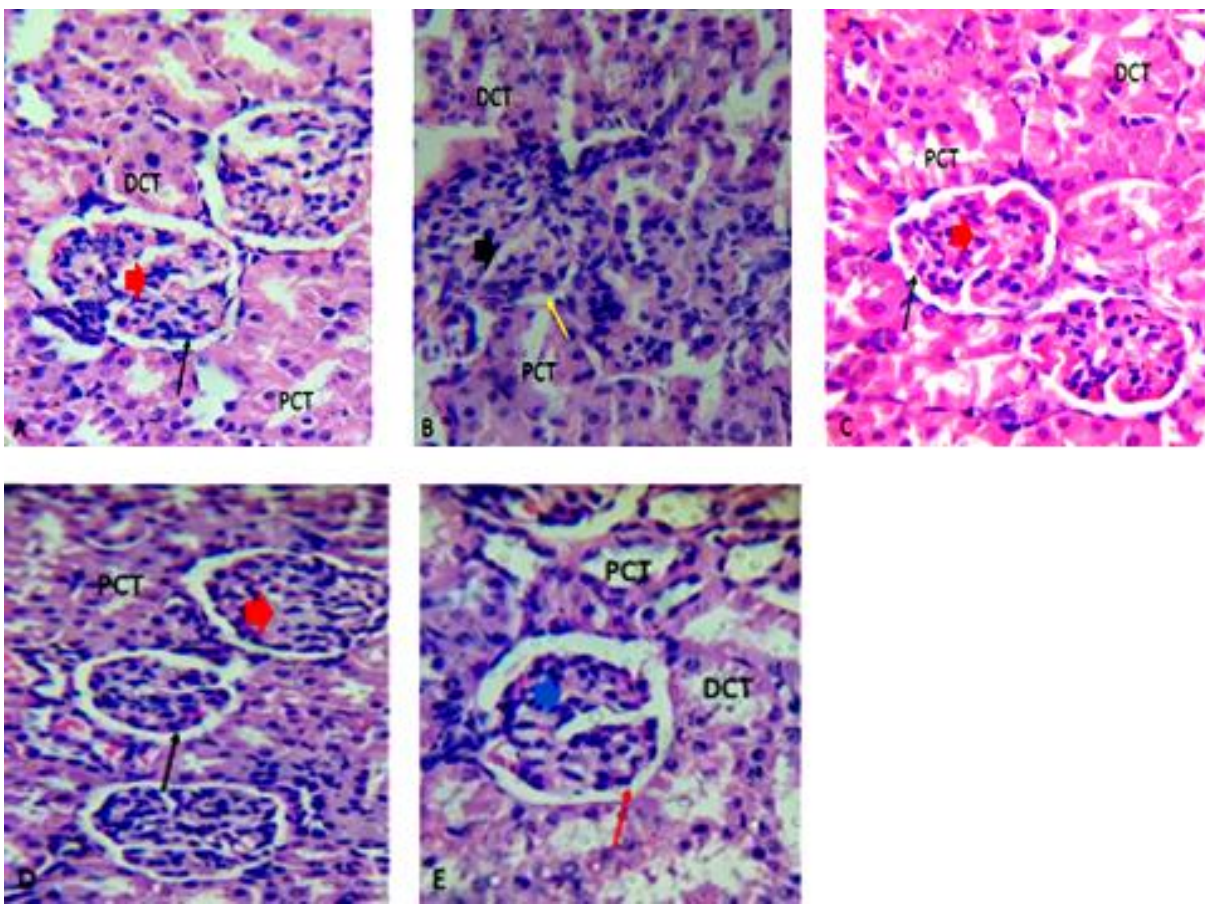

Figure 4. Photomicrograph of kidney tissue shows (magnification 100x)

a. Untreated Control group (A) had well-differentiated and organized glomerulus with podocytes (thick red arrow), capsular space (thin black arrow), proximal and distal convoluted tubules (PCT and DCT)
b. DMBA only group (B) had severely degenerated and distorted architecture of the glomerulus (thick black arrow), constricted capsular space (yellow thin arrow), and proximal and distal convoluted tubules (PCT and DCT)
c. Post DMBA-treatment with Doxorubicin treatment group (C) had normal glomerulus (thick red arrow), capsular spaces (thin black arrow), and slight constriction of the proximal and distal convoluted tubules (PCT and DCT)
d. Post-DMBA-treatment with Gallocatechin treatment group (D) had slight changes of the glomerulus with increased podocytes (thick red arrow), capsular space (thin black arrow), and constricted proximal and distal convoluted tubules (PCT and DCT)
e. Post-DMBA treatment with AMLE treatment group (E) had well-preserved glomerulus (thick blue arrow), capsular spaces (thin red arrow), and proximal and distal convoluted tubules (PCT and DCT)

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Table 3. Effect of doxorubicin (C), gallocatechin (D), and *A. muricata* leaf extract (E) on haematological variables in DMBA-treated Sprague-Dawley rats. Group A animals were untreated controls, and group B Animals were DMBA-treated without subsequent intervention

GROUP	RBC	Hb	PCV	Platelets	WBC	NEU	LYMPH
A	6.31±0.36	18.05±0.41	54.05±0.41	196.1±0.42	6001±1.37	41.90±0.38	29.90±0.38
B	4.72±0.35a	12.05±0.21a	42.05±0.41a	154.1±0.14a	14501±3.91a	19.90±0.38a	53.90±0.13a
C	4.92±0.53a	13.05±0.23a	45.05±0.41a	159.1±0.34a	4901±3.91ab	18.90±0.38ab	22.90±0.41b
D	5.62±0.22bc	16.85±0.41b	49.75±0.41bc	186.1±0.12abc	7009±13.70abc	36.90±0.38bc	34.90±0.33bc
E	6.12±0.35bc	17.65±0.41b	51.05±0.41bc	192.1±0.29abc	6639±13.70abc	39.90±0.38bc	31.90±0.38bc

Each value is an expression of mean± SEM
A = $p \leq 0.05$ when compared with group A, b = $p \leq 0.05$ when compared with group B, c = $p \leq 0.05$ when compared with group C.

significantly higher Na^+, K^+, and a significantly lower Cl^-, HCO_3^- level in group B when compared with group A. Group C showed a significantly lower Na^+, K^+, and a significantly lower Cl^-, HCO_3^- level when compared with groups A and B, respectively. Groups D and E rats showed significantly lower blood Na^+, K^+, and a significantly lower Cl^-, and $HCO3^-$ levels when compared with group B rats. Data summarised in Table 3 show significantly lower RBC, Hb, PCV, Platelet count, and Neutrophil levels, and significantly higher total WBC and Lymphocyte counts in groups B and C when compared with group A. Groups D and E showed a significantly higher RBC, Hb, PCV, Platelet count, Neutrophil count, and a significantly lower total WBC and Lymphocyte count when compared with group B. Comparative histological sections of the kidney from the five treatment groups of rats are presented in Figure 4.

DISCUSSION

This study evaluated the effects of physiological doses of doxorubicin, gallocatechin, and *A. muricata* leaf extract (AMLE) on renal and haematological variables after DMBA-induced carcinoma in Sprague-Dawley rats. Significantly higher kidney weight of group B (DMBA-only) rats may be associated with renal inflammation, oedema, and tumour infiltration due to neoplastic transformation, in line with prior findings by Kwok et al. [13], who reported renal hypertrophy following chemical carcinogen exposure. Lower kidney weight in doxorubicin-treated animals (Group C) relative to rats in Groups A and B, without correspondingly higher markers of kidney function (urea and creatinine) or better markers of anti/oxidative stress (CAT, SOD, MDA, and TP), suggests reduced tumour burden but not improved renal health. However, lower kidney weight and higher levels of kidney function markers and anti/oxidative stress in rats treated with gallocatechin (Group D) and AMLE (Group E) are ascribable to anti-inflammatory and anti-proliferative effects. This finding is comparable with that of Khan and Mukhtar [14] and Gavamukulya et al. [6], who reported tissue-protective and tumour-suppressive roles of polyphenols and A. muricata, respectively.

Groups B and C showed significant elevations in serum urea and creatinine, indicating compromised glomerular filtration and renal dysfunction. Doxorubicin is known to induce nephrotoxicity through oxidative injury to tubular cells and glomeruli [15]. The non-significant difference between groups B and C reinforces doxorubicin's known renal toxicity. In contrast, Groups D and E showed significantly lower levels of these markers, suggesting nephroprotection. Gallocatechin likely enhanced renal function not only by directly scavenging reactive oxygen species (ROS), but also by activating the nuclear factor erythroid 2–2-related factor 2 (Nrf2) pathway, which induces antioxidant enzymes such as SOD, CAT, and glutathione peroxidase, thereby strengthening endogenous redox defence [16,17]. It also preserves mitochondrial integrity by limiting permeability transition pore opening, maintaining ATP production, and reducing fibrosis and apoptosis in renal tissue [18]. Similarly, AMLE contains acetogenins, flavonoids, and phenolic compounds that exert pleiotropic effects, including Nrf2 activation, NF-κB suppression, and mitochondrial preservation [6,19,20]. These actions converge to limit lipid peroxidation, preserve renal architecture, and support haematopoietic restoration.

An important mechanistic distinction lies between bioavailability and compound synergy. Gallocatechin, as a purified compound, may achieve higher, more predictable systemic concentrations and directly engage molecular targets such as Nrf2 and mitochondrial proteins. In contrast, AMLE represents a complex phytochemical mixture, where synergistic interactions among flavonoids, phenolics, and acetogenins may broaden its protective spectrum but also necessitate higher doses to achieve efficacy. Such compound synergy could explain why AMLE demonstrated stronger histological preservation despite gallocatechin's more direct molecular activity. This highlights the trade-off between the potency of isolated compounds and the multi-targeted resilience conferred by crude extracts.

Groups B (DMBA-only) and C (doxorubicin) showed elevated MDA levels (a marker of lipid peroxidation) and reduced antioxidant enzyme activity (CAT, SOD, and TP), indicating oxidative renal damage. Doxorubicin's redox cycling and ROS generation may have further exacerbated

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oxidative injury [21]. Conversely, gallocatechin and AMLE significantly enhanced antioxidant enzyme levels and reduced MDA, underscoring their potent free radical scavenging activities. Green tea catechins are well-documented for upregulating endogenous antioxidant defences and downregulating NADPH oxidase [16]. *A. muricata* also enhances antioxidant enzyme expression and prevents lipid peroxidation, which supports renal integrity [19]. DMBA-only-treated rats (Group B) exhibited significant dysregulation of blood electrolytes marked by higher Na^+ and K^+, and lower Cl^- and HCO_3^- - a typical signature of renal tubular dysfunction and acidosis. Doxorubicin, gallocatechin, and AMLE (Groups C–E) all prevented this pattern, likely by preserving renal tubular reabsorption and acid-base balance. While doxorubicin's reversal may be due to anti-tumour effects rather than renal healing, both gallocatechin and AMLE appear to have modulated ion channels and transporters involved in electrolyte balance [22,23], supporting their renoprotective potential. DMBA-only treated rats (Group B) and doxorubicin-treated rats (Group C) showed significant anaemia (↓RBC, Hb, PCV), thrombocytopenia (↓platelets), and leukocytosis with lymphocytosis, likely reflecting bone marrow suppression and paraneoplastic immune responses. These findings are consistent with those of Sonneveld et al. [24] and Stone et al. [25], who reported haematopoietic disruption and immune dysregulation in cancer and chemotherapy models. Groups D and E displayed notable recovery in haematopoietic function. Gallocatechin may support erythropoiesis and thrombopoiesis not only by limiting oxidative bone marrow damage [10], but also through Nrf2-mediated protection of hematopoietic progenitor cells [17]. AMLE has been shown to stimulate haematopoietic growth factors and suppress oxidative and inflammatory insults in the marrow, promoting recovery of RBC and platelet counts [26].

Renal histology corroborated biochemical findings in the present study; DMBA caused severe glomerular degeneration and tubular damage, confirming its nephrotoxic and carcinogenic nature. Doxorubicin showed mild tubular constriction, suggesting limited morphological recovery. Gallocatechin displayed moderate glomerular improvement, while AMLE exhibited the most notable histo-architectural preservation, in terms of glomeruli and normal tubules. These observations support the protective and regenerative roles of polyphenols and phytochemicals in kidney physiology. The findings with doxorubicin highlight its dual role as both a potent anticancer agent and a source of systemic toxicity. While its DNA intercalation and topoisomerase II inhibition explain its tumour-suppressive effects, these mechanisms are coupled to ROS generation, mitochondrial dysfunction, and apoptosis in non-tumour tissues, leading to nephrotoxicity and haematotoxicity [15,21,24]. This therapeutic–toxic paradox has been widely documented in preclinical and clinical studies, reinforcing the clinical challenge of balancing anticancer efficacy against organ safety.

Conclusion

This study demonstrated that while doxorubicin retained anticancer efficacy, it aggravated renal toxicity and haematological suppression in DMBA-treated rats. In contrast, gallocatechin and *Annona muricata* leaf extract showed superior renoprotective and haematoprotective properties, effectively mitigating oxidative stress, restoring renal and haematologic parameters, and improving histological integrity. The comparative analysis underscores the potential of gallocatechin and AMLE as renoprotective and haematoprotective adjuncts in carcinogen-induced pathology. While gallocatechin offers potency through targeted molecular actions, AMLE provides broader multi-compound synergy. The translational implication is that plant-derived compounds, whether isolated or used in standardised extracts, may serve as adjuvant therapies to reduce chemotherapy-associated organ toxicities. Future studies should evaluate pharmacokinetics, bioavailability, and combination strategies with conventional drugs to determine their relevance in clinical oncology.

This study did not evaluate tumour regression or direct anticancer efficacy, which restricts the interpretation of the therapeutic thresholds between efficacy and toxicity. Without tumour outcome data, it remains unclear whether the renoprotective and haematological benefits observed with gallocatechin and *A. muricata* leaf extract (AMLE) would translate into preserved or enhanced anticancer activity when compared to doxorubicin. Furthermore, the absence of long-term follow-up or a recovery phase prevents assessment of whether the protective effects were transient or could be sustained beyond the treatment period. This limitation is particularly relevant given that chemotherapy-associated toxicities often accumulate over prolonged treatment cycles, and natural compounds may exert delayed or adaptive effects on organ systems. Additionally, the study employed only a single dosing regimen, which precludes evaluation of potential dose–response relationships. These limitations highlight the need for future investigations that integrate tumour regression analysis, extended follow-up, and multiple dose levels to better inform translational applications.

DECLARATIONS

Ethical consideration

Ethical approval for the study was obtained from the Olabisi Onabanjo University Teaching Hospital Research Ethics Committee (OOUTHREC) with approval number OOUTH/HREC/698/2023AP.

Consent to publish

All authors agreed on the content of the final paper.

Funding

None

Competing Interest

The authors declare no conflict of interest

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Author contribution

OOA and IIS contributed to conceptualisation and methodology. IIS, OOA, OOE, and OEO collected and analysed the data. IIS, OOA, OOE, and OEO interpreted the results. OOA supervised the study. All authors contributed to the writing and revision of the manuscript, and all approved the final version.

Acknowledgement

The authors acknowledge the support of the Department of Physiology, Olabisi Onabanjo University, which facilitated the successful completion of this study by providing access to core laboratory equipment, dedicated laboratory space, and essential administrative assistance.

Availability of data

Data is available upon request from the corresponding author

REFERENCES

1. Al-Asady AM, Ghaleb NK, Jasim Alnasrawi AM, ALhamed TA (2020) Effect of carcinogenic substance (7,12 dimethylbenz[a]anthracene (DMBA)) on tissue, haematology character and enzyme activity in rat. Indian J Forensic Med Toxicol. 14:1
2. Situmorang PC, Wibowo S, Sari RM, Nugraha AP, Ibrahim A, Fitrianita A, et al. (2025) Nephroprotective effects of Rhodomyrtus tomentosa in 7,12-dimethylbenz[a]anthracene (DMBA)-induced nephrotoxicity: Computational and physiological analysis of cytokine expression. J Agric Food Res. 102155
3. Kciuk M, Gielecińska A, Mujwar S, Kołat D, Kałuzińska-Kołat Ż, Celik I, Kontek R (2023) Doxorubicin—an agent with multiple mechanisms of anticancer activity. Cells. 12:659
4. Afsar T, Razak S, Almajwal A, Al-Disi D (2020) Doxorubicin-induced alterations in kidney functioning, oxidative stress, DNA damage, and renal tissue morphology; improvement by Acacia hydaspica tannin-rich ethyl acetate fraction. Saudi J Biol Sci. 27:2251–2260
5. Yan Z, Zhong Y, Duan Y, Chen Q, Li F (2020) Antioxidant mechanism of tea polyphenols and its impact on health benefits. Anim Nutr. 6:115–123
6. Ilango S, Sahoo DK, Paital B, Kathirvel K, Gabriel JI, Subramaniam K, et al. (2022) A review on Annona muricata and its anticancer activity. Cancers. 14:4539
7. Omiyale BO, Ekundayo BE, Mathenjwa-Goqo MS, Ajiboye BO, Oyinloye BE (2025) Protective effect of phenolic-rich extract of Anona muricata Linn leaf on renal oxidative stress and inflammation in streptozotocin-induced diabetes in diabetic rats. Sci Afr. 27:e02515
8. Kang Y, Wang W, Zhao H, Qiao Z, Shen X, He B (2017) Assessment of subclinical doxorubicin-induced cardiotoxicity in a rat model by speckle-tracking imaging. Arq Bras Cardiol. 109
9. Nazir N, Zahoor M, Ullah R, Ezzeldin E, Mostafa GA (2020) Curative effect of catechin isolated from Elaeagnus umbellata Thunb. berries for diabetes and related complications in streptozotocin-induced diabetic rats model. Molecules. 26:137
10. Chen B, Liu G, Zou P, Li X, Hao Q, Jiang B, et al. (2015) Epigallocatechin-3-gallate protects against cisplatin-induced nephrotoxicity by inhibiting endoplasmic reticulum stress-induced apoptosis. Exp Biol Med. 240:1513–1519
11. Misra HP, Fridovich I (1972) The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 247:3170–3175
12. Aebi H (1984) Catalase in vitro. Methods Enzymol. 105:121–126
13. Kwok SH, Liu Y, Bilder D, Kim J (2024) Paraneoplastic renal dysfunction in fly cancer models driven by inflammatory activation of stem cells. bioRxiv. 2024.03.21.586173
14. Khan N, Mukhtar H (2008) Multi-targeted therapy of cancer by green tea polyphenols. Cancer Lett. 269:269–280
15. Tacar O, Sriamornsak P, Dass CR (2013) Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. J Pharm Pharmacol. 65:157–170
16. Kanlaya R, Thongboonkerd V (2019) Protective effects of epigallocatechin-3-gallate from green tea in various kidney diseases. Adv Nutr. 10:112–121
17. Khan IM, Gul H, Khan S, Nassar N, Khalid A, Swelum AA, Wang Z (2025) Green tea polyphenol epigallocatechin-3-gallate mediates an antioxidant response via Nrf2 pathway in heat-stressed poultry: a review. Poult Sci. 104:105071
18. Oliveira MR, Nabavi SF, Daglia M, Rastrelli L, Nabavi SM (2016) Epigallocatechin gallate and mitochondria—a story of life and death. Pharmacol Res. 104:70–85
19. Moghadamtousi SZ, Fadaeinasab M, Nikzad S, Mohan G, Ali HM, Kadir HA (2015) Annona muricata (Annonaceae): a review of its traditional uses, isolated acetogenins and biological activities. Int J Mol Sci. 16:15625–15658
20. Naik AV, Dessai SN, Sellappan K (2021) Anti-tumour activity of Annona muricata L. leaf methanol extracts against Ehrlich Ascites Carcinoma and Dalton's Lymphoma Ascites mediated tumours in Swiss albino mice. Libyan J Med. 16:1846862
21. Thorn CF, Oshiro C, Marsh S, Hernandez-Boussard T, McLeod H, Klein TE, Altman RB (2011) Doxorubicin pathways: pharmacodynamics and adverse effects. Pharmacogenet Genomics. 21:440–446
22. Sheng Y, Sun Y, Tang Y, Yu Y, Wang J, Zheng F, Li Y, Sun Y (2023) Catechins: protective mechanism of antioxidant stress in atherosclerosis. Front Pharmacol. 14:1144878
23. Oladele J, Oyewole O, Oyeleke M, Adewale O, Adeloju O (2019) Annona muricata attenuates cadmium-induced oxidative stress and renal toxicity in Wistar rats. J Biosci Appl Res. 5:543–550
24. Sonneveld P, Mulder JA, van Bekkum DW (1981) Cytotoxicity of doxorubicin for normal hematopoietic and acute myeloid leukemia cells of the rat. Cancer Chemother Pharmacol. 5:167–173
25. Stone JB, DeAngelis LM (2016) Cancer-treatment-induced neurotoxicity—focus on newer treatments. Nat Rev Clin Oncol. 13:92–105
26. Usunobun U, Okolie NP (2015) Phytochemical analysis and mineral composition of Annona muricata leaves. Int J Res Curr Dev. 1:38–42