



Amla (*Emblica officinalis*) alleviates doxorubicin-induced cardiotoxicity and nephrotoxicity in rats

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ABSTRACT

Introduction: Doxorubicin (DOX) is a widely used anticancer drug known for its significant cardiotoxic and nephrotoxic effects. Seeking remedies to mitigate these adverse effects is crucial. This study investigates the potential of *Emblica officinalis* (Amla) extract, a prominent component in Chinese and Indian traditional medicine systems, in alleviating DOX-induced cardiotoxicity and nephrotoxicity.

Methods: DOX (20 mg/kg *i.p.*, once) was given to rats to cause acute cardiotoxicity and nephrotoxicity. Rats received 16 similar and cumulative doses of DOX (1.25 mg/kg, *i.p.*) on alternate days for chronic cardiotoxicity and nephrotoxicity. Biochemical and histological evaluations were done to confirm the onset of cardiotoxicity and nephrotoxicity.

Results: The cardioprotective and nephroprotective effects of Amla extract (AE) (150 mg/kg *p.o.* and 300 mg/kg *p.o.*) were evaluated in comparison to Vitamin E (25 mg/kg *p.o.*). The treatment with AE (300 mg/kg/day, *p.o.*) considerably prevented DOX-induced cardiotoxicity, nephrotoxicity, and oxidative stress by positively altering the integrity of glomeruli, restoring the tissue GSH and decreasing serum TBARS. AE (300 mg/kg) was found to be more cardioprotective and nephroprotective than Vitamin E (25 mg/kg *p.o.*).

Discussion: It may be concluded that the induction of cardiotoxicity and nephrotoxicity in rats may be due to DOX-induced oxidative stress, and chronic treatment with AE (300 mg/kg) is an effective way to alleviate the cardiotoxic and nephrotoxic adverse effects of DOX in rats. Moreover, given Amla's historical and contemporary significance in Chinese and Indian traditional medicine systems, its potential therapeutic role merits further exploration in clinical settings.

1. Introduction

Doxorubicin (DOX), an anti-tumor antibiotic, has evolved into a common and widely used medication in treating many cancers [11]. Unfortunately, the drug's significant toxic effects on the body's tissues, such as the liver, heart, kidneys, and nervous system, restrict its use in clinical settings [8,9,18]. The most serious toxic effects associated with DOX use are thought to be cardio- and nephrotoxicity [14]. Conversely, DOX has been rigorously re-evaluated as a chemotherapeutic drug based on its promising therapeutic effects in managing many forms of cancer. In this regard, tremendous efforts have been made to explore the mechanisms involved in the DOX-induced cardiotoxicity and nephrotoxicity, to develop novel therapeutic interventions that may prevent DOX-induced toxicities. DOX has been observed to induce cardiac

muscle oxidative damage and lipid peroxidation. Superoxide is generated when the DOX semiquinone radical lowers oxygen, which is the chemical mechanism for DOX-induced toxicity [13]. In the end, superoxide dismutase, an antioxidant enzyme, reduces the superoxide radical created by DOX's catalysis of the oxygen reduction by NADPH to hydrogen peroxide (H₂O₂) [12]. In the presence of Fe²⁺, H₂O₂ is further reduced to form the highly reactive hydroxyl radical OH⁻. Subsequently, it can react with polyunsaturated fatty acids to produce lipid hydroperoxide [21]. This starts a chain reaction involving lipid radicals that can harm cell membranes by oxidation. As a result, the production of free radicals and damage to the cardiac DNA and membranes are key components of DOX-induced cardiotoxicity. Apart from cardiotoxicity, nephrotoxicity is another major problem associated with DOX [13]. Proteinuria and hypoalbuminemia are features of DOX-induced

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nephrotic syndrome. However, it is still unclear exactly how DOX causes nephrotoxicity. Reactive oxygen species, protein oxidation, and lipid peroxidation are thought to be possible mediators of nephrotoxicity [20]. The harmful side effects of DOX therapy include mechanisms such as NADPH oxidase regulation and apoptosis induction. Additionally, because DOX accumulates preferentially in the kidney, it damages the kidneys. Moreover, it has been discovered that drug-induced nephrotoxicity is linked to altered intraglomerular hemodynamics, tubular cell toxicity, inflammation, thrombotic microangiopathy, necrosis, enlarged glomeruli, and tubulointerstitial tissue sclerosis ([6], Rehman et al., 2014). Taken together, free radical formation is considered a central player in the pathogenesis of DOX-induced cardiac and nephrotoxicity.

With varying degrees of success, several compounds with antioxidant characteristics have been studied *in-vitro*. When conducting *in vivo* experiments, the success rate has been less encouraging. Selenium and other molecules having antioxidant properties *in-vitro* were found to have little use in preventing the cardiotoxicity caused by DOX *in-vivo*. Traditional antioxidants like N-acetylcysteine and tocopherol are also ineffective at preventing the cardiotoxicity caused by DOX.

Emblica officinalis is thought to be the first tree to have been created in the universe, according to ancient Indian mythology. In addition, Amla, recognized as Yuganzi in traditional Chinese medicine (TCM), holds a significant place in the pharmacopeia of both Chinese and Indian traditional medicine systems. *Phyllanthi Fructus*, the fruit of *Emblica officinalis*, has a longstanding history in TCM, with references dating back to ancient texts such as the "Amplification on Materia Medica" by Zongshi Kou during the Song Dynasty, where it was described for its efficacy in treating metal poisoning [10]. Moreover, the "Compendium of Materia Medica" by Shizhen Li notes its use in weight loss and life extension. Amla's utilization in TCM spans various preparations, with its fruit being a primary component in formulations addressing conditions such as diarrhea, jaundice, and inflammation. According to reports, different hazardous states and illnesses related to organs, including the lung, liver, and kidney, can be improved by amla [7,15–17]. Since amla is an antioxidant, it may help prevent free radical damage to important biomolecules such as lipids, low-density lipoproteins (LDL), proteins, and DNA (Schmidt et al., 2015). In contemporary TCM practice, Amla is incorporated into numerous health care functions, with its applications encompassing supplementation of nutrition, enhancement of immunity, alleviation of fatigue, and assistance in reducing blood lipid and blood glucose levels. Amidst this rich historical and contemporary context, our study aims to investigate the efficacy of Amla in mitigating DOX-induced cardiotoxicity and nephrotoxicity in rats by attenuating free radical production in the heart and kidneys.

2. Materials and methods

2.1. Institutional animal ethical committee approval

The Institutional Animal Ethical Committee approved the experimental protocol employed in the current investigation (Proposal No. IAEC/2016/KSOP/E/15/02). In this investigation, Wistar rats were matched for age and weighed between 200 and 250 g were employed. Rats were accommodated in polypropylene cages with a 12-hour light/dark cycle and fed a regular chow diet with unlimited access to water. All animals were exposed to typical day and night cycles while acclimating to institutional animal housing.

2.2. Drugs and chemicals

Doxorubicin was purchased from Fresenius Kabi Oncology Ltd., Baddi, India. All other chemicals and kits were purchased from Hi-Media, India and CDH Fine Chemical, India. Amla extract (AE) was obtained from Sunpure House, Delhi, India, Sunpure Research and Incubation Centre, Sunpure Extracts Pvt. Ltd., India.

2.3. Physical characteristics of Amla powder extract

Test parameters	Specification	Result
Appearance	Brown to dark brown powder	Dark brown powder
Odour	Typical Amla odour	Complies
Taste	Characteristic	Complies
Solubility	Soluble in water	Complies
	IN 50 % Alcohol	
pH	3.0–7.0	3.01
Particle size	100 % passes through 20 mesh	Complies
Density(Tapped)	NLT-0.4 g/ml	0.7200 g/ml
Assay for Tannis	NLT-45.0 %	46.92 %
Heavy metals as per USP	NMT-10ppm	Complies
Microbiological Analysis (Cfu/gm)		
(a) Total plate count	NMT-5000 Cfu/gram	Complies
(a) Yeast and Mould	NMT-100 Cfu/gram	Complies
PATHOGENS		
(a) E.coli	Absent	Complies
(a) Coliforms	Absent	Complies
(a) Salmonella	Absent	Complies
(a) Pseudomonas-aeruginosa	Absent	Complies
(a) Staphylococcus Aureus	Absent	Complies

#Abbreviation -NLT-Not less than, -NMT-Not more than, -Cfu- Colony forming unit /gram.

2.4. Assessment of studied parameters

The biochemical effects of the DOX-induced cardiac damage were evaluated by measuring serum creatinine kinase-MB (CK-MB) and lactate dehydrogenase (LDH). Bio-chemical evaluation of the doxorubicin-induced nephrotoxicity included measuring serum creatinine, blood urea nitrogen, and proteinuria. Proteinuria was assessed using the pyrogallol red method, following the protocol outlined by Watanabe et al. [19]. A commercially available kit sourced from Crescent Biosystems, Goa, India, was utilized for this purpose. This method relies on the reaction between pyrogallol red and protein molecules present in urine samples. Renal and cardiac TBARS and GSH were estimated to determine how much oxidative stress had developed in the kidney and heart. As previously stated, the pathogenic alterations in the glomeruli were evaluated histologically [1–3]. The kidney was removed and placed right away in 10 % formalin. Then the kidney was dehydrated in progressively higher alcohol concentrations, submerged in xylene, and embedded in paraffin. Haematoxylin- and eosin-stained slices of 3–5 µm thickness were cut from the paraffin blocks to examine the pathological alterations in the glomeruli under a light microscope (400X).

2.5. Experimental design and treatment

2.5.1. Acute model

Rats were allocated into six groups (nine animals per group) after acclimation.

Group 1 (Vehicle-treated group): Physiological saline (1 ml/kg, *p.o.*) was given to the normal rats each day for seven days. Group 2 (Disease Control for Pre-Treatment): To serve as a toxic control for pre-treatment, the rats received only a dose of DOX (20 mg/kg, *i.p.*) at 10 a.m. and were sacrificed after 48 h. Group 3 (AE treated for pre-treatment): On day five, two hours after administering the extract, a single dosage of DOX (20 mg/kg, *i.p.*) was injected into the rats. The animals received AE (150 mg/kg, *p.o.*) daily for seven days. Group 4 (AE treated for pre-treatment): On day five, two hours after administering the medication extract, a single dose of DOX (20 mg/kg, *i.p.*) was injected into the rats. The rats were given AE (300 mg/kg, *p.o.*) once a day for seven days. Group 5 (Disease Control for Post-Treatment): As a toxic control for post-treatment, the rats received a single dose of DOX (20 mg/kg, *i.p.*) at 10 a.m. on day one. Group 6 (AE treated for post-treatment): AE (300 mg/kg,

p.o.) was given to the rats daily for seven days, and on the first day, two hours after the medicine was given, a single dose of DOX (20 mg/kg, *i.p.*) was given as post-treatment. All the animals for pre-treatment or post treatment group were euthanized on day 7.

2.5.2. Chronic model

Rats were divided into five groups of nine animals each after acclimation.

Group I (Vehicle control) physiological saline (1 ml/kg/day, *p.o.*) was given to the normal rats for 42 days. Group II (Diseased control): Starting on alternate days, beginning on the tenth day of the vehicle treatment, the rats received 16 similar and cumulative doses of DOX (1.25 mg/kg, *i.p.*). Groups III (Treatment Group I): The rats received 16 similar and cumulative doses of DOX (1.25 mg/kg, *i.p.*) and AE (150 mg/kg/day *p.o.*) for 42 days, the same as group II. Groups IV (Treatment Group II): The rats received 16 similar and cumulative doses of DOX (1.25 mg/kg, *i.p.*) and 300 mg/kg/day of AE (*p.o.*) as group II for 42 days. Group V (Standard Treatment Group): The rats received Vitamin E (25 mg/kg/day *p.o.*) for 42 days in addition to 16 similar and cumulative doses of DOX (1.25 mg/kg, *i.p.*). Blood samples were taken on the 42nd day of treatment to estimate various biochemical evaluations. Animals were sacrificed on 43rd day, and tissues were removed for testing for indicators of oxidative stress and histological analysis.

Cardiotoxicity and nephrotoxicity were induced by giving 16 similar and cumulative doses of DOX (1.25 mg/kg, *i.p.*) on alternate days. AE (150 mg/kg *p.o.*), AE (300 mg/kg *p.o.*), and Vitamin E (25 mg/kg, *p.o.*) were administered to the DOX-treated rats for seven weeks. All the parameters were assessed at the end of seven weeks in both normal and DOX-treated rats, with or without pharmacological treatments. After seven weeks without treatment, a death rate of over 30 % was seen in DOX-treated rats. AE (300 mg/kg *p.o.*)-treated group showed no mortality. However, 1 out of 9 rats in the AE (150 mg/kg *p.o.*) treated group died in the fifth week, and 1 out of the remaining eight rats expired in the seventh week.

2.6. Statistical analysis

The mean $\pm$ SD was used to express all values. One-way ANOVA and Bonferroni multiple comparison tests were followed to statistically analyse the data collected from the diverse groups. $p < 0.05$ was considered statistically significant.

3. Results

3.1. Chronic model

3.1.1. Effect of AE on serum CK-MB

It was observed that rats treated with DOX had a much higher level of CK-MB in their serum than untreated rats. When compared to AE (150 mg/kg *p.o.*, seven weeks) or Vitamin E (25 mg/kg *p.o.*, seven weeks) treatments, AE treatment (300 mg/kg *p.o.*, seven weeks) significantly decreased the CK-MB level in DOX-treated rats (Fig. 1).

3.1.2. Effect of AE on serum LDH

Rats treated with DOX had significantly higher serum LDH concentrations than untreated rats. When compared to AE (150 mg/kg *p.o.*, seven weeks) or Vitamin E (25 mg/kg *p.o.*, seven weeks) in DOX-treated rats, treatment with AE (300 mg/kg *p.o.*, seven weeks) significantly decreased the LDH level (Fig. 2).

3.1.3. Effect of AE on serum creatinine and blood urea nitrogen

DOX-treated rats had considerable greater blood urea nitrogen and serum creatinine levels than untreated animals. Comparing treatment with AE (300 mg/kg *p.o.*, seven weeks) to AE (150 mg/kg *p.o.*, seven weeks) or Vitamin E (25 mg/kg *p.o.*, seven weeks) in DOX-treated rats resulted in a considerable decline in the high levels of blood urea

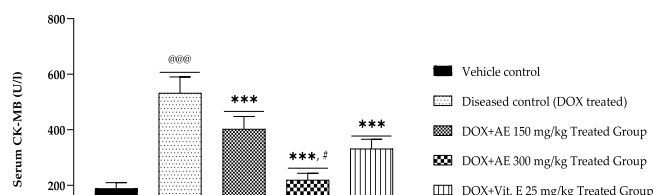


Fig. 1. Effects of AEs (150 mg/kg and 300 mg/kg) and vitamin E (25 mg/kg) on serum CK-MB. All values are shown as mean $\pm$ standard deviation. @@@ on the graph shows comparison of $p < 0.001$ versus the normal control; *** represents $p < 0.001$ versus the disease control comparison; ** represents $p < 0.01$ versus the disease control; ## shows comparison of $p < 0.01$ versus the Vitamin E (25 mg/kg) treated group and # shows comparison of $p < 0.001$ versus the Vitamin E (25 mg/kg) treated group.

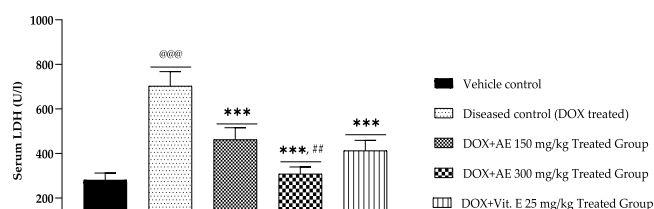


Fig. 2. Effects of AEs at doses of 150 mg/kg, 300 mg/kg, and 25 mg/kg of vitamin E on serum LDH. All values are shown as mean standard deviation. @@@ on the graph shows comparison of $p < 0.001$ versus the normal control; *** represents $p < 0.001$ versus the disease control comparison; ** represents $p < 0.01$ versus the disease control; ## shows comparison of $p < 0.01$ versus the Vitamin E (25 mg/kg) treated group and # shows comparison of $p < 0.001$ versus the Vitamin E (25 mg/kg) treated group.

nitrogen and serum creatinine. (Figs. 3 and 4).

3.1.4. Effect of AE on proteinuria

When compared to untreated rats, proteinuria was seen to occur much more frequently in DOX-treated rats. In comparison to treatments with AE (150 mg/kg *p.o.*, seven weeks) or Vitamin E (25 mg/kg *p.o.*, seven weeks), therapy with AE (300 mg/kg *p.o.*, seven weeks) significantly decreased proteinuria in DOX-treated rats (Fig. 5).

3.1.5. Effect of AE on tissue oxidative stress

Compared to normal rats, administering 16 similar and cumulative doses of DOX (1.25 mg/kg, *i.p.*) every alternate day demonstrated a substantial rise in cardiac and renal TBARS. Additionally, rats treated with DOX were shown to have lower cardiac and renal GSH

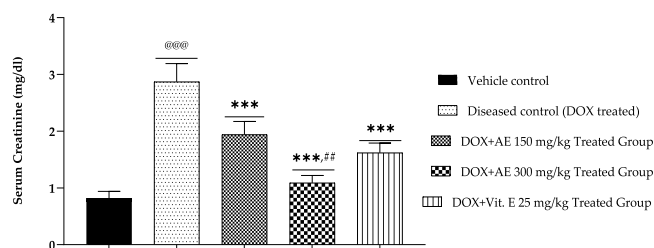


Fig. 3. Effects of AEs at doses of 150 mg/kg, 300 mg/kg, and 25 mg/kg of vitamin E on serum creatinine. All values are shown as mean standard deviation. @@@ on the graph shows comparison of $p < 0.001$ versus the normal control; *** represents $p < 0.001$ versus the disease control comparison; ** represents $p < 0.01$ versus the disease control; ## shows comparison of $p < 0.01$ versus the Vitamin E (25 mg/kg) treated group and # shows comparison of $p < 0.001$ versus the Vitamin E (25 mg/kg) treated group.

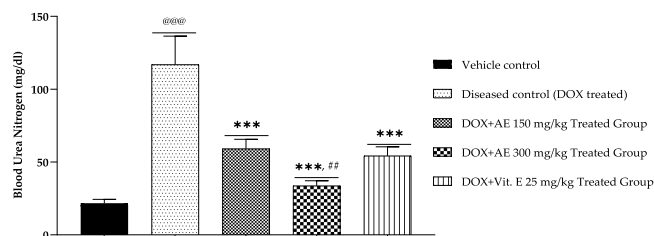


Fig. 4. Effects of AEs at doses of 150 mg/kg, 300 mg/kg, and 25 mg/kg of vitamin E on Blood urea nitrogen. All values are shown as mean standard deviation. @@@ on the graph shows comparison of $p < 0.001$ versus the normal control; *** represents $p < 0.001$ versus the disease control comparison; ** represents $p < 0.01$ versus the disease control; ## shows comparison of $p < 0.01$ versus the Vitamin E (25 mg/kg) treated group and # shows comparison of $p < 0.001$ versus the Vitamin E (25 mg/kg) treated group.

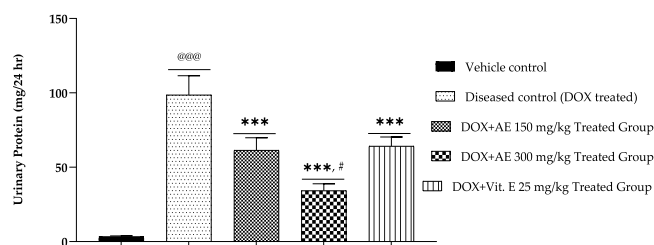


Fig. 5. Effects of AEs at doses of 150 mg/kg, 300 mg/kg, and 25 mg/kg of vitamin E on urinary protein. All values are shown as mean standard deviation. @@@ on the graph shows comparison of $p < 0.001$ versus the normal control; *** represents $p < 0.001$ versus the disease control comparison; ** represents $p < 0.01$ versus the disease control; # shows comparison of $p < 0.01$ versus the Vitamin E (25 mg/kg) treated group and # shows comparison of $p < 0.001$ versus the Vitamin E (25 mg/kg) treated group.

concentrations than untreated rats. When compared to treatments with AE (150 mg/kg *p.o.*, seven weeks) or vitamin E (25 mg/kg *p.o.*, seven weeks), AE (300 mg/kg *p.o.*, seven weeks) significantly restored the DOX-induced rise in cardiac and renal TBARS as well as a decrease in cardiac and renal GSH (Figs. 6 and 7).

3.1.6. Effect of AE on renal histological study

Compared to untreated rats, the DOX-treated animals showed pathological alterations in the glomeruli after seven weeks, including glomerular capillary size reduction and extracellular mesangial enlargement. When compared to treatments with AE (150 mg/kg *p.o.*, seven weeks) or vitamin E (25 mg/kg *p.o.*, seven weeks) in DOX-treated

rats, treatment with AE (300 mg/kg *p.o.*, seven weeks) significantly protected the kidney against pathological alterations in the glomeruli (Fig. 8).

3.2. Acute model

A single dose of DOX (20 mg/kg, *i.p.*) administered elicited cardiotoxicity and nephrotoxicity by increasing tissue TBARS and decreasing tissue GSH levels. Serum levels of CK-MB, LDH, creatinine, and BUN, were found to be higher. After 24 h of receiving a single dose of DOX (20 mg/kg, *i.p.*) without any medication, a death rate of over 30 % was seen. The AE pre-treated group (300 mg/kg *p.o.*) did not experience any deaths during the study. However, 2 out of 9 rats in the post-treatment group with the AE (150 mg/kg *p.o.*) died on the fifth day.

3.2.1. Effect of AE on serum CK-MB

It was observed that rats treated with DOX had a much higher level of CK-MB in their serum than untreated rats. In rats treated with DOX, the increase in CK-MB level was significantly reduced by pre-treatment with AE (300 mg/kg *p.o.*, seven days). However, AE (150 mg/kg *p.o.*, seven days) or AE (300 mg/kg *p.o.*, seven days) pre- or post-treatments partially decreased the CK-MB level in DOX-treated rats (Fig. 9).

3.2.2. Effect of AE on serum LDH

Rats treated with DOX had significantly higher serum LDH concentrations than untreated rats. In rats treated with DOX, the increased LDH level was significantly reduced by pre-treatment with AE (300 mg/kg *p.o.*, seven days). However, AE (150 mg/kg *p.o.*, seven days) or AE (300 mg/kg *p.o.*, seven days) pre- or post-treatments partially decreased the LDH level in DOX-treated rats (Fig. 10).

3.2.3. Effect of AE on serum creatinine and blood urea nitrogen

Rats treated with DOX had significantly higher blood urea nitrogen and serum creatinine levels than untreated rats. When compared to pre-treatment with AE (150 mg/kg *p.o.*, seven days) or post-treatment with AE (300 mg/kg *p.o.*, seven days) in DOX-treated rats, pre-treatment with AE (300 mg/kg *p.o.*, seven days) significantly reduced the high levels of serum creatinine and blood urea nitrogen (Figs. 11 and 12).

3.2.4. Effect of AE on proteinuria

When compared to untreated rats, proteinuria was seen to occur much more frequently in DOX-treated rats. When compared to pre-treatment with AE (150 mg/kg *p.o.*, seven days) or post-treatment with AE (300 mg/kg *p.o.*, seven days) in DOX-treated rats, pre-treatment with AE (300 mg/kg *p.o.*, seven days) significantly reduced the elevated levels of proteinuria (Fig. 13).

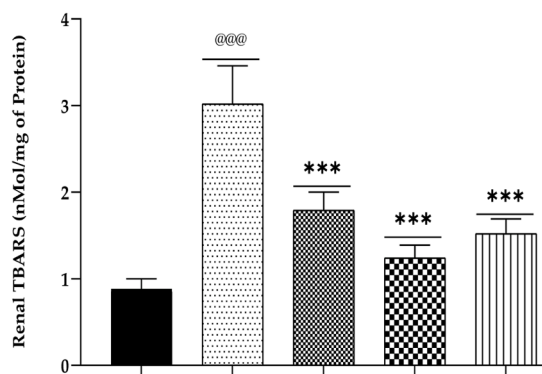
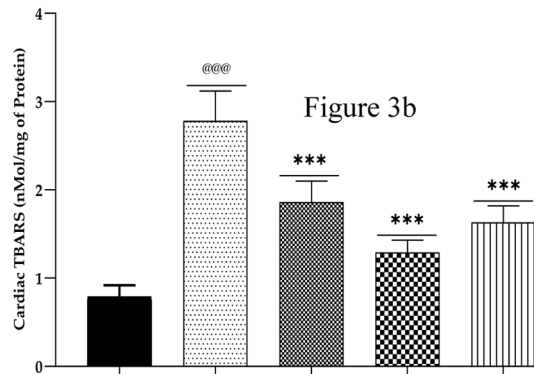


Fig. 6. Effects of AEs at doses of 150 mg/kg, 300 mg/kg, and 25 mg/kg of vitamin E on renal and cardiac TBARS. All values are shown as mean standard deviation. @@@ on the graph shows comparison of $p < 0.001$ versus the normal control; *** represents $p < 0.001$ versus the disease control comparison; ** represents $p < 0.01$ versus the disease control; ## shows comparison of $p < 0.01$ versus the Vitamin E (25 mg/kg) treated group and # shows comparison of $p < 0.001$ versus the Vitamin E (25 mg/kg) treated group.



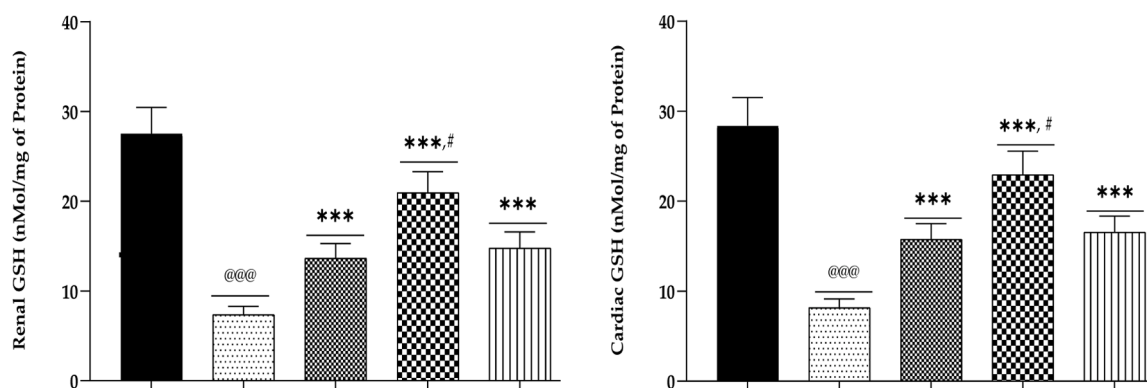


Fig. 7. Effects of AEs at doses of 150 mg/kg, 300 mg/kg, and 25 mg/kg of vitamin E on renal and cardiac GSH. All values are shown as mean standard deviation. @@@ on the graph shows comparison of $p < 0.001$ versus the normal control; *** represents $p < 0.001$ versus the disease control comparison; ** represents $p < 0.01$ versus the disease control; ## shows comparison of $p < 0.01$ versus the Vitamin E (25 mg/kg) treated group and # shows comparison of $p < 0.001$ versus the Vitamin E (25 mg/kg) treated group.

3.2.5. Effect of AE on tissue oxidative stress

Compared to normal rats, administering a single dose of DOX (20 mg/kg, *i.p.*) caused a noticeable rise in cardiac and renal TBARS. Additionally, rats treated with DOX were shown to have lower cardiac and renal GSH concentrations than untreated rats. In comparison to pre-treatment with AE (150 mg/kg *p.o.*, seven days) or post-treatment with AE (300 mg/kg *p.o.*, seven days), pre-treatment with AE (300 mg/kg *p.o.*, seven days) significantly restored the DOX-induced increase in cardiac and renal TBARS along with a decrease in cardiac and renal GSH (Figs. 14 and 15).

3.2.6. Effect of AE on renal histological study

Compared to untreated rats, the DOX-treated animals showed pathological alterations in the glomeruli after seven days, including glomerular capillary size reduction and extracellular mesangial enlargement. As opposed to pre-treatment with AE (150 mg/kg *p.o.*, seven days) or post-treatment with AE (300 mg/kg *p.o.*, seven days) in DOX-treated rats, pre-treatment with AE (300 mg/kg *p.o.*, seven weeks) significantly protected the kidney from pathological alterations in the glomeruli (Fig. 16).

4. Discussion

The therapeutic potential of AE to treat doxorubicin-related cardiotoxicity and nephrotoxicity was examined in the current investigation. For the first time, we showed that doxorubicin-associated cardiac and kidney abrasions were considerably avoided by therapy with AE through regulating oxidative stress. Numerous attempts have been made to ascertain the primary culprit involved in the pathogenesis of this disorder [4,18]. It has been hypothesised that oxidants are the causal factor for the important pathways involved in DOX-associated cardiac and nephrotoxicity based on a substantial body of research indicating that DOX produces a state of "increased oxidative stress" [12,14]. Biomarkers further support the idea of increased oxidative stress in cardiotoxicity and nephrotoxicity for oxidative damage to DNA, lipids, and proteins. The chemical mechanism for DOX-induced toxicity is the superoxide produced when the DOX semi-quinone radical consumes oxygen to produce and regenerate DOX. In the end, superoxide dismutase, an antioxidant enzyme, reduces the superoxide radical to hydrogen peroxide H_2O_2 after DOX has catalysed oxygen reduction by NADPH. H_2O_2 is further reduced to the highly reactive hydroxyl radical ($OH\cdot$) in the presence of Fe^{2+} , after which it can react with polyunsaturated fatty acids to produce lipid hydroperoxide [21]. This starts a chain reaction involving lipid radicals that can harm cell membranes by oxidation. As a result, producing free radicals and causing damage to the cardiac DNA and membranes are two key effects of DOX-induced cardiotoxicity. An

indicator of the onset of oxidative stress is thought to be an increase in cardiac and renal TBARS levels and a subsequent drop in reduced glutathione levels. The development of oxidative stress was indicated by a rise in serum TBARS and aortic superoxide anion production following acute or chronic administration of DOX. Thus, the formation of a significant degree of oxidative stress may explain the observed dramatic induction of cardio- and nephrotoxicity in DOX-treated rats. The fact that DOX-treated rats in the current investigation showed significant oxidative stress and considerable increases in serum LDH, CK-MB, blood urea nitrogen, and serum creatinine concentrations strongly supports this claim.

Phyllanthi Fructus (the ripe fruits of *Phyllanthus emblica* L., Yuganzi in Chinese) also known as Indian gooseberry or Amla is a fruit that is rich in nutrients and is also used as a medicine. *Phyllanthi Fructus* has a long medical history in the system of traditional Chinese medicine and was brought into China with Buddhist scriptures. The Song Dynasty physician Zongshi Kou wrote in Amplification on Materia Medica that *Phyllanthi Fructus* can treat metal poisoning. In Compendium of Materia Medica, Shizhen Li noted that long-term consumption of *Phyllanthi Fructus* can help with weight loss and life extension [7,10]. Additionally, its antioxidant properties suggest a potential role in preventing free radical damage to essential biomolecules. Since amla is an antioxidant, it may help prevent free radical damage to important biomolecules such as lipids, low-density lipoproteins (LDL), proteins, and DNA. As a result, NADPH oxidase inactivation may account for the therapeutic impact of AE in reducing DOX-related cardiotoxicity and nephrotoxicity. In comparison to various herbal remedies and modern adjuvants, AE demonstrates promising cardioprotective and nephroprotective effects. Notably, compounds like ellagic acid, found in nuts and berries, exhibit antioxidative, antiapoptotic, and cardioprotective properties. Similarly, herbs like *Ganoderma lucidum*, *Citrus hystrix*, Pomegranate, Grape seed extract, and others offer diverse pharmacological benefits including antioxidant actions, which mitigate doxorubicin-induced toxicity in cardiac tissue [5]. Amla extract's efficacy aligns with these findings, suggesting its potential as a natural adjuvant in managing doxorubicin-induced toxicity. Blood urea nitrogen and elevated plasma creatinine levels are symptoms of developing nephrotoxicity. CK-MB is an important enzyme normally found in the heart and skeletal muscles. Injury to the myocardial tissue can increase the expression and secretion of CK-MB in the systemic circulation. In this study, increased levels of CK-MB in the diseased control group upon DOX therapy clearly marked the injury to the myocardial tissue, which was subsided upon treatment with both low and high doses of AE along with Vitamin E therapy. The Results of the current study demonstrated that a combination of AE can alleviate the DOX- induced cardiotoxicity.

Previous studies have reported that serum LDH levels can be elevated

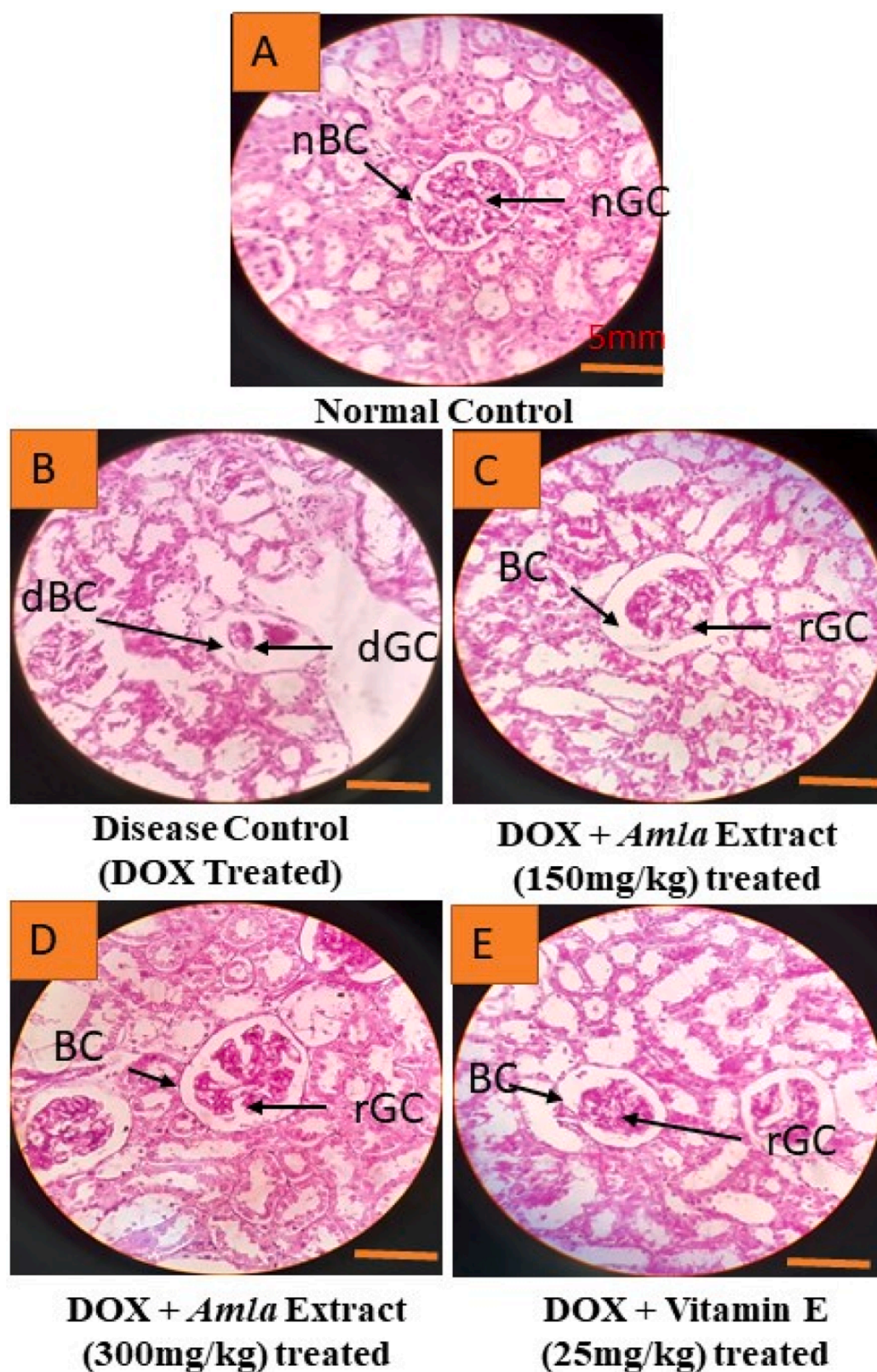


Fig. 8. Chronic model- Microscopical examination of kidney tissue of rats treated with DOX, AE extract and Vitamin E through Haematoxylin & Eosin (H&E) Staining. A.H&E staining of normal rats showing normal shape and structure of Bowman's capsule (nBC) and Glomerular capillaries (nGC). B: H&E staining of rats treated with DOX caused significant damage to the Bowman's capsule (dBC) and Glomerular capillaries (dGC). C-D: H&E staining of kidney tissue of rats treated with low and high dose of AE showing regeneration of BC (rBC) and GC (rGC). E: Kidney histology of rats treated with Vitamin E showing regeneration of BC and GC as Vitamin E is a well-known antioxidant. nBC: normal Bowman's capsule, dBC: damaged Bowman's capsule, dGC: damaged Glomerular capillaries. rBC: regenerated Bowman's capsule nGC: normal Glomerular capillaries, rGC: regenerated glomerular capillaries.

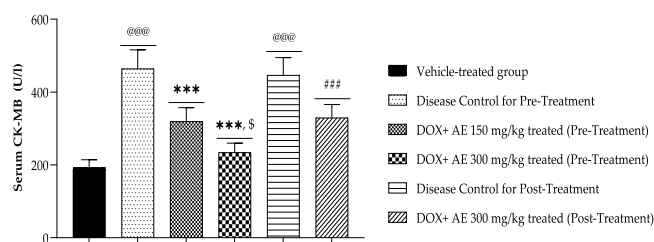


Fig. 9. Effect of pre-treatment with AE at doses of 150 mg/kg and 300 mg/kg, and post treatment with AE at a dose of 300 mg/kg on serum CK-MB. The result outcomes are represented as mean $\pm$ standard deviation. @@@ on the graph shows comparison of $p < 0.001$ versus the normal control; *** represents $p < 0.001$ versus the disease control comparison (for the pre-treated group); ** represents $p < 0.01$ versus the disease control comparison (for the pre-treated group). ### represents comparison of $p < 0.001$ versus the disease control (for the post-treated group); and \$ shows comparison of $p < 0.001$ versus the post-treated group receiving AE (dosage of 300 mg/kg).

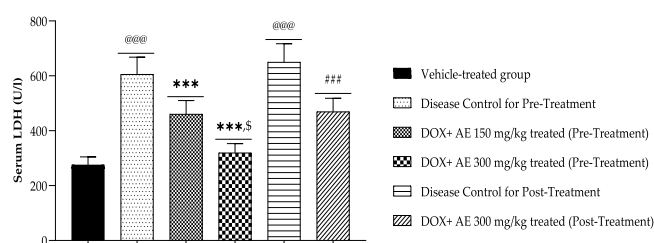


Fig. 10. Effect of pre-treatment with AE at doses of 150 mg/kg and 300 mg/kg, and post treatment with AE at a dose of 300 mg/kg on serum LDH. The result outcomes are represented as mean $\pm$ standard deviation @@@ on the graph shows comparison of $p < 0.001$ versus the normal control; *** represents $p < 0.001$ versus the disease control comparison (for the pre-treated group); ** represents $p < 0.01$ versus the disease control comparison (for the pre-treated group). ### represents comparison of $p < 0.001$ versus the disease control (for the post-treated group); and \$ shows comparison of $p < 0.001$ versus the post-treated group receiving AE (dosage of 300 mg/kg).

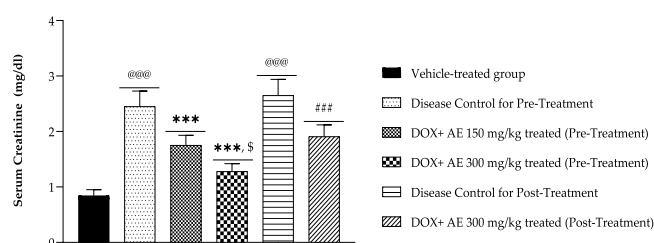


Fig. 11. Effect of pre-treatment with AE at doses of 150 mg/kg and 300 mg/kg, and post treatment with AE at a dose of 300 mg/kg on serum creatinine. The result outcomes are represented as mean $\pm$ standard deviation @@@ on the graph shows comparison of $p < 0.001$ versus the normal control; *** represents $p < 0.001$ versus the disease control comparison (for the pre-treated group); ** represents $p < 0.01$ versus the disease control comparison (for the pre-treated group). ### represents comparison of $p < 0.001$ versus the disease control (for the post-treated group); and \$ shows comparison of $p < 0.001$ versus the post-treated group receiving AE (dosage of 300 mg/kg).

in various conditions, like liver disease, heart disease, kidney disorders, anaemia, and cancer. The results of the current study evidenced an increase in LDH levels upon DOX administration in both acute and chronic models. An increase in LDH level might have caused damage to the cardiac muscle fibres and kidney tissue due to the elevated LDH level observed in toxic control. Interestingly, treatment with low and high doses of AE worked well and prevented further damage to the cardiac and renal tissues. Rats treated with DOX have been found to have greater

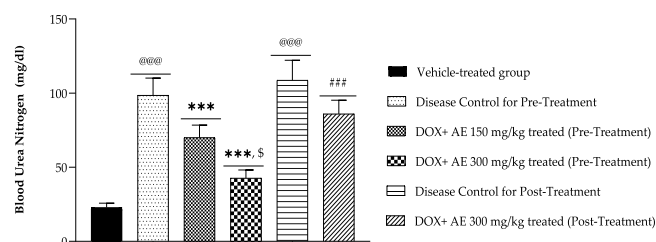


Fig. 12. Effect of pre-treatment with AE at doses of 150 mg/kg and 300 mg/kg, and post treatment with AE at a dose of 300 mg/kg on blood urea nitrogen. The result outcomes are represented as mean $\pm$ standard deviation. @@@ on the graph shows comparison of $p < 0.001$ versus the normal control; *** represents $p < 0.001$ versus the disease control comparison (for the pre-treated group); ** represents $p < 0.01$ versus the disease control comparison (for the pre-treated group). ### represents comparison of $p < 0.001$ versus the disease control (for the post-treated group); and \$ shows comparison of $p < 0.001$ versus the post-treated group receiving AE (dosage of 300 mg/kg).

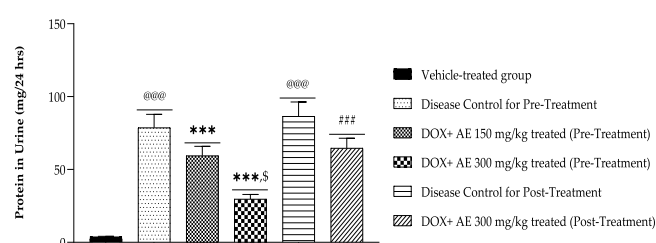


Fig. 13. Effect of pre-treatment with AE at doses of 150 mg/kg and 300 mg/kg, and post treatment with AE at a dose of 300 mg/kg on urinary protein. The result outcomes are represented as mean $\pm$ standard deviation. @@@ on the graph shows comparison of $p < 0.001$ versus the normal control; *** represents $p < 0.001$ versus the disease control comparison (for the pre-treated group); ** represents $p < 0.01$ versus the disease control comparison (for the pre-treated group). ### represents comparison of $p < 0.001$ versus the disease control (for the post-treated group); and \$ shows comparison of $p < 0.001$ versus the post-treated group receiving AE (dosage of 300 mg/kg).

blood urea nitrogen and creatinine levels than untreated rats. In contrast to pre-treatment with AE (150 mg/kg/day, *p.o.*) or post-treatment with AE (300 mg/kg/day, *p.o.*), AE may play a part, either directly or indirectly, in providing protection against DOX-induced nephrotoxicity or delaying its development, as seen by the maintenance of certain biochemical variables closer to those in control rats. Additionally, the progression of glomerulosclerosis and tubulointerstitial fibrosis correlates with the level of proteinuria, and it has been hypothesised that alterations in renal hemodynamics are a significant cause of proteinuria [2,3].

In rats treated with DOX, pre- or post-treatment with AE (300 mg/kg/day, *p.o.*) significantly lowers the rate of severe proteinuria and improves renal histopathologic alterations, including decreased glomerular hypertrophy and tubular atrophy. But compared to pre-treatment with AE (150 mg/kg/day, *p.o.*) or post-treatment with AE (300 mg/kg/day, *p.o.*), pre-treatment with AE (300 mg/kg/day, *p.o.*) was more effective. Preventing DOX-induced cardiotoxicity, nephrotoxicity, and oxidative stress with AE (300 mg/kg/day, *p.o.*) improved glomerular integrity, increased tissue GSH concentrations, and decreased serum TBARS. However, AE (150 mg) pre- and post-treatment had a minor impact on DOX-induced toxicity. Thus, it may be deduced that the production of cardiotoxicity and nephrotoxicity in rats may be due to DOX-induced oxidative stress. Vitamin E is less effective when used frequently and as a pre-treatment than AE (300 mg/kg). Renal and cardiac tissue were further subjected to histological examination in order to validate the above findings. Histology of the tissue is one of the most reliable methods to observe the damage to the tissue architecture at the cellular level. Several studies have reported that chronic and acute

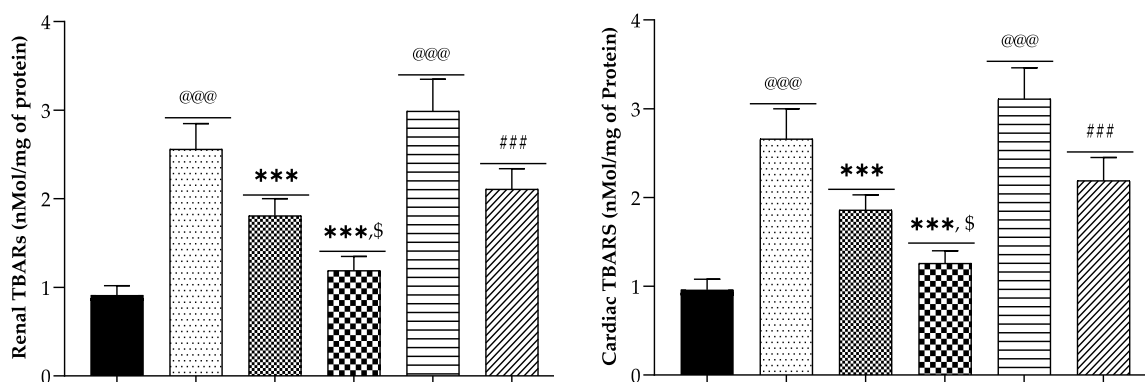


Fig. 14. Effect of pre-treatment with AE at doses of 150 mg/kg and 300 mg/kg, and post treatment with AE at a dose of 300 mg/kg on renal and cardiac TBARs. The result outcomes are represented as mean $\pm$ standard deviation. @@@ on the graph shows comparison of $p < 0.001$ versus the normal control; *** represents $p < 0.001$ versus the disease control comparison (for the pre-treated group); ** represents $p < 0.01$ versus the disease control comparison (for the pre-treated group). \$\$\$ represents comparison of $p < 0.001$ versus the disease control (for the post-treated group); and \$ shows comparison of $p < 0.001$ versus the post-treated group receiving AE (dosage of 300 mg/kg).

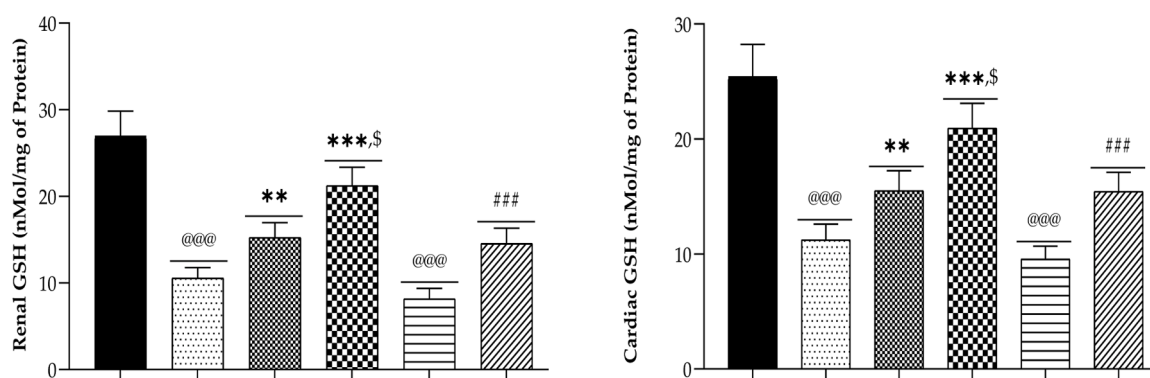


Fig. 15. Effect of pre-treatment with AE at doses of 150 mg/kg and 300 mg/kg, and post treatment with AE at a dose of 300 mg/kg on renal and cardiac GSH. The result outcomes are represented as mean $\pm$ standard deviation. @@@ on the graph shows comparison of $p < 0.001$ versus the normal control; *** represents $p < 0.001$ versus the disease control comparison (for the pre-treated group); ** represents $p < 0.01$ versus the disease control comparison (for the pre-treated group). \$\$\$ represents comparison of $p < 0.001$ versus the disease control (for the post-treated group); and \$ shows comparison of $p < 0.001$ versus the post-treated group receiving AE (dosage of 300 mg/kg).

DOX therapy can damage the Bowman's capsule and cause necrosis of glomerular capillaries. Results of the histological examination of renal tissue did not show any significant damage to the BC and GC, while DOX therapy considerably altered the shape and size of the BC and caused necrosis of GC cells. These findings are consistent with the previous findings. Treatment with low and high doses prevented any further damage to the BC and GC, as evidenced by the histology.

5. Limitations

This study has some limitations-

1. Although we proposed oxidative stress as a potential mechanism underlying the protective effects of Amla extract, our study did not delve deeply into the specific molecular pathways involved. Further mechanistic studies are necessary to elucidate the precise mechanisms of action.
2. The research was conducted solely on animal models, and therefore, the extrapolation of these findings to human subjects should be approached with caution.

6. Strengths

Despite these limitations, our study also possesses several strengths that bolster the validity and significance of our findings.

Novel therapeutic approach: To the best of our knowledge, this is the first study to investigate the potential of Amla extract in mitigating doxorubicin-induced cardiotoxicity and nephrotoxicity. Our findings pave the way for exploring alternative therapeutic strategies for managing chemotherapy-induced adverse effects.

Comprehensive evaluation: We employed biochemical and histological evaluations to comprehensively assess the cardioprotective and nephroprotective effects of Amla extract, providing multiple lines of evidence supporting our conclusions.

Comparative analysis: By comparing the efficacy of Amla extract with that of Vitamin E, a well-known antioxidant, we were able to demonstrate the superiority of Amla extract in attenuating doxorubicin-induced toxicity.

7. Conclusion

Taken together, it may be concluded that amla extract at a preferentially higher dose (300 mg/kg) alleviated DOX-induced cardiotoxicity by lowering cardiac oxidative stress, CK-MB, and LDH levels, as well as demonstrating nephroprotective potential by lowering serum creatinine, blood urea nitrogen, urinary protein, and renal oxidative stress. Both pre-treatment and post-treatment with AE (300 mg/kg) appear to be effective in preventing the DOX-associated toxicity. However, further research is warranted to elucidate the precise mechanisms underlying amla's cardio- and nephro-protective effects. Moreover, considering the

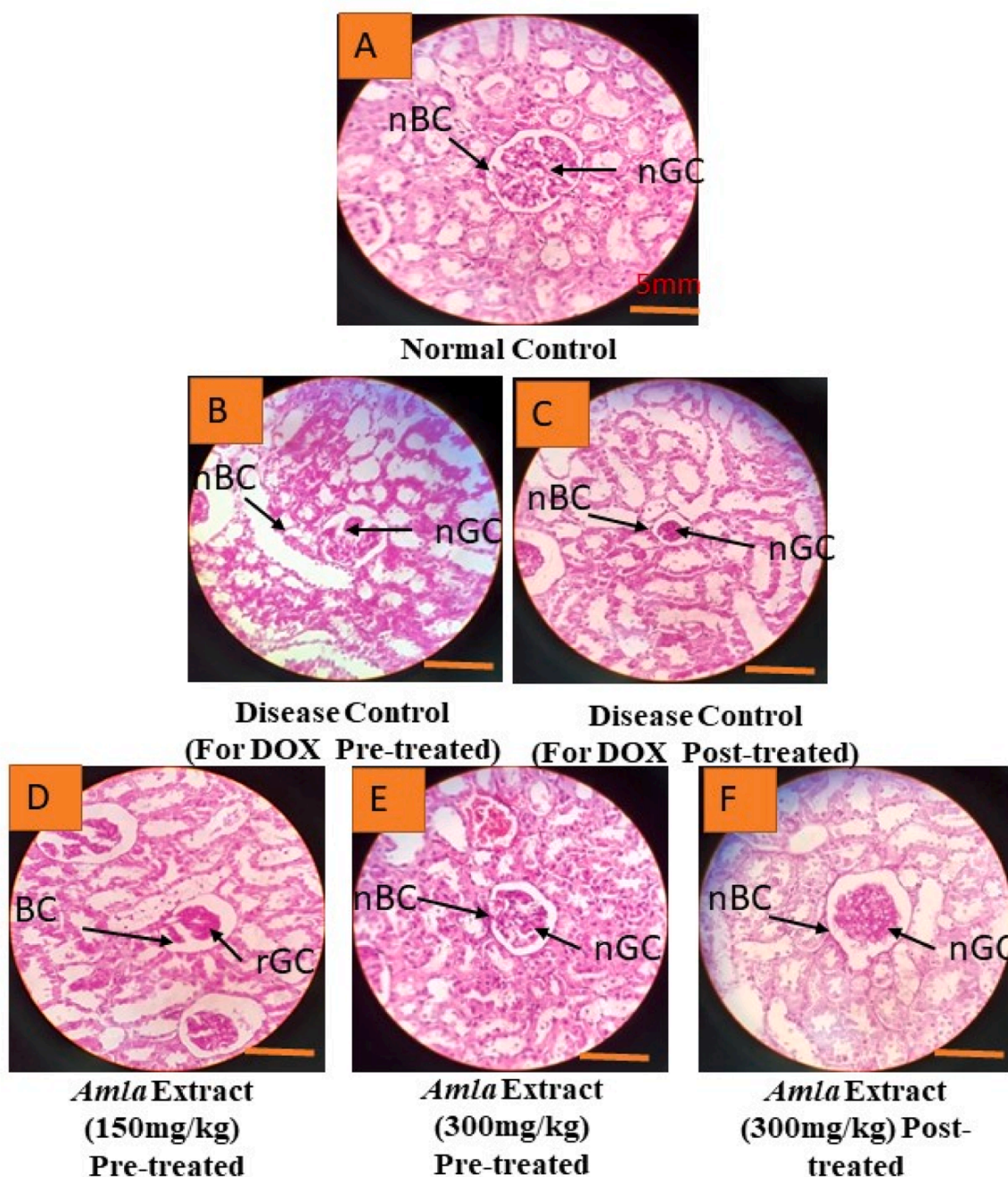


Fig. 16. Acute model- Microscopical examination of kidney tissue of rats pre-treated and post treated with AE (150 mg/kg) and AE (300 mg/kg) through Hae-matoxylin & Eosin(H&E) Staining. A: H&E staining of normal rats showing normal shape and architecture of kidney tissue as only vehicle was administered up to the end of study. B & C: H&E staining of rats' kidney tissue pre-treated with DOX evidenced significant damage tissue architecture as evidenced by damaged Bowman's capsule (dBC) and Glomerular capillaries (dGC). D, E&F: Histology of kidney tissue of rats treated with low and high dose of AE extracts showing regenerated Bowman's capsule (rBC) and Glomerular capillaries (rGC).

historical and contemporary significance of amla in TCM, exploring its therapeutic efficacy in the context of TCM formulations may provide valuable insights into its broader application and potential synergistic effects with other herbal remedies.

CRediT authorship contribution statement

Mandeep Kumar Arora: Writing – review & editing, Software, Resources, Investigation, Data curation, Conceptualization. **Mary Singh:** Validation, Methodology, Formal analysis, Data curation. **Ritu Tomar:** Writing – review & editing, Writing – original draft, Supervision, Project

administration. **Lakhveer Singh:** Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Ashok Jangra:** Writing – original draft, Supervision, Project administration, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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None.

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