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Dose-Dependent Protective Effects of *Persea americana* Mill. Seed Extract Against Doxorubicin Induced Multi-Organ Toxicity in Rats

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ABSTRACT

The most popular chemotherapeutic agent, Doxorubicin (DOX), is restricted by the dose-related multi-organ toxicity which is mediated mainly by oxidative stress and damage of cells. The paper assessed the dose-effective protective action of *Persea americana* ethanolic seed extract on the toxicity of DOX on Wistar rats. Forty two rats were randomly selected into six groups, namely, normal control, DOX control (5 mg/kg), DOX + Vitamin C (100 mg/kg), and DOX + *P. americana* extract at 500, 1000 and 1500 mg/kg. The treatments were given after four weeks. Standard methods were used to measure hematological parameters, indices of liver and renal functions, lipid profile, antioxidants enzyme activities, and cardiac biomarkers. The use of DOX led to a serious decrease in the number of RBC, hemoglobin, PCV, and platelets, and the simultaneous increase in the level of WBC ($P < 0.05$). Hepatic dysfunction was supported by high levels of AST, ALT, ALP, and bilirubin and low indices of protein levels whereas renal dysfunction was supported by high levels of urea, creatinine, and uric acid. DOX also caused dyslipidemia, lipid peroxidation (MDA), drop in antioxidant enzyme (GSH, SOD, CAT, GPx) and cardiac biomarker increase (LDH, CK-MB, troponin). Dose-dependently acute effects of *P. americana* extract had a significant effect in reducing these changes with the highest dose (1500 mg/kg) showing the highest inhibitory effect, which is similar to Vitamin C. The acute toxicity analysis showed

no mortality up to 5000 mg/kg. Conclusively, *Persea americana* seed extract provides serious multi-organ coverage against DOX-induced toxicity, mainly due to the antioxidant and cytoprotective effects, which demonstrates its potential in the safe use as an adjunct in chemotherapy.

Keywords: *Persea americana*, Doxorubicin, multi-organ toxicity, damage of cells, rats

1. INTRODUCTION

Doxorubicin (DOX), an anthracycline antibiotic, has continued to be a cornerstone chemotherapeutic agent that is extensively used in the therapy of solid tumors and in the treatment of the hematological malignancies such as breast cancer, lymphomas and leukemias. It acts as an antitumor by mainly intercalating DNA, inhibiting topoisomerase II, and inducing double-strand DNA breaks, which culminate into the apoptosis of rapidly dividing cancer cells (Bhutani et al., 2025; Radeva and Yoncheva, 2025). Although effective clinically, due to cumulative and dose-dependent toxicity, especially of the cardiovascular, hepatic, renal, and hematopoietic systems, the therapeutic use of doxorubicin has been severely limited (Arrigoni et al., 2025). These toxicities do not only undermine the outcomes of treatment but also lead to serious morbidity and mortality in survivors of cancer in the long run.

The pathophysiology of multi-organ toxicity caused by doxorubicin is complicated and comprises of a meeting of multiple pathways which are interrelated and one of which is identified as oxidative stress that is the central and unifying pathway. Doxorubicin is an enzymatic and non-enzymatic redox cycling drug through its quinone moiety, and this causes overproduction of reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl radicals (Radeva & Yoncheva, 2025). The consequences of this excessive production of ROS are the disruption of cellular redox homeostasis leading to oxidative damage of lipids, proteins, and nucleic acids that eventually leads to the deterioration of cellular integrity and cellular functioning (Pigg et al., 2025). Moreover, doxorubicin disrupts mitochondrial respiration, which further increases the production of ROS and leads to the development of mitochondrial dysfunction, which is one of the events in organ injury (Bhadra et al., 2025; Qing et al., 2025).

In addition to oxidative stress, new evidence shows that iron dysregulation and ferroptosis are important factors in toxicity induced by doxorubicin. Doxorubicin alters the intracellular iron homeostasis by increasing the accumulation of iron and Fenton reactions, which increases the production of highly reactive hydroxyl radicals (Bhadra et al., 2025). The process also leads to lipid peroxidation and the ferroptotic cell death, a recently defined type of regulated cell death mediated by iron-dependent oxidative damage (Wu et al., 2024; Wang et al., 2025).

Moreover, doxorubicin triggers various types of programmed cell death, such as apoptosis, necroptosis, and autophagy and increases tissue damage in organ systems (Li et al., 2024). Interactions among oxidative stress, mitochondrial dysfunction, inflammation, and dysregulated cell death pathways all end up in progressive organ damage (Vitale et al., 2024; Linders et al., 2024).

The other important pathway of the doxorubicin toxicity is the prevention of topoisomerase IIb (TOP2b) in healthy tissues especially cardiomyocytes. It causes DNA damage, transcriptional dysregulation, and mitochondrial impairment due to this inhibition, which causes cardiomyopathy and heart failure (Xie et al., 2024).

Also, the inflammatory signaling pathways, such as NF- κ B and NLRP3 inflammasome cascades, are activated by doxorubicin and contribute to tissue damage further by enhancing cytokine release and immune cells infiltration (Shi et al., 2023). Taken together, the mentioned mechanisms indicate the multifactorial and systemic character of the toxicity caused by doxorubicin, which validates the necessity of effective preventive measures.

Considering the key contribution of oxidative stress and inflammation to doxorubicin toxicity, natural antioxidants have become the potential treatment of interest. Compounds with high polyphenols and flavonoid and other bioactive components derived from plants have been shown to be able to scavenge free radicals, stimulate native antioxidant responses, and regulate inflammatory responses. Notably, natural products were also found to suppress ferroptosis and replenish redox balance, which also provides multi-target protection against doxorubicin-induced damage (Wang et al., 2025).

Due to its variety of pharmacological properties, *Persea americana* (avocado) has been receiving growing scientific interest (Dreher and Davenport, 2013). Although the pulp of the fruit is also commonly used, the seeds that are commonly discarded as wastes contain bioactive phytochemicals, such as flavonoids, phenolic acids, tannins and saponins, that have potent antioxidant and anti-inflammatory properties. These phytoconstituents have been identified to decrease lipid peroxidation, stabilize cellular membrane and also increase the performance of the endogenous antioxidant enzymes like superoxide dismutase, catalase and glutathione peroxidase (Dabas et al., 2013). These mechanisms could help *P. americana* seed extract to reverse oxidative stress-induced tissue damage and maintain organ functionality.

It has been shown by preclinical research that plant-derived antioxidants have the potential to reduce the doxorubicin-induced cardiotoxicity, hepatotoxicity, and nephrotoxicity by regulating the oxidative stress, mitochondrial dysfunction, and inflammatory signaling pathways (Vitale et al., 2024). Nonetheless, although *Persea americana* seed extract has demonstrated the prospective protective effects in experimental models, the current literature is still narrow, as it tends to concentrate on the outcomes of single organs and has not provided the overall analysis of dose-dependent effects on the multiple organ systems. In addition, the integrative evaluation of the hematological, biochemical, and histopathological changes within the single experimental design is not well-researched.

Thus, the current research was aimed at exploring the dose-sensitive protective implications of *Persea americana* seed extract against the doxorubicin-induced multi-organ toxicity in rats with special consideration to the mechanisms of oxidative stress and the systemic impact of the organ. This study would fill knowledge gaps in the literature by offering the ultimate assessment of its protective potential and can help in the creation of safer and more plant-based adjunct therapies that can reduce the toxicity caused by chemotherapy without reducing the anticancer effects.

2. METHODOLOGY

2.1. Study Area

The research was conducted in the Medical Biochemistry Laboratory in Imo State University in Owerri, Nigeria, although some biochemical tests were performed at the Biochemistry Laboratory of Michael Okpara University of Agriculture in Umudike.

2. 2. Chemicals and Reagents

All the reagents utilized in this study were of an analytical grade. The toxicant was Doxorubicin hydrochloride and Vitamin C was taken as the reference antioxidant. Biological analyses were performed according to the instructions of the manufacturers with the help of commercial assay kits (Randox Laboratories Ltd., UK; TECO Diagnostics, USA).

2. 3. Collection and Extraction of the Plant Material

Persea americana fruits were fresh and were purchased in Eke Onunwa Market in Owerri, Nigeria and were verified by a taxonomist. The seeds were isolated, washed, dried in the air, and ground. Cold maceration was used to extract the products as explained by Egbonu et al. (2018). The powdered sample was impregnated with 90% ethanol (1:2 w/v), filtered, concentrated with water bath at 60 °C, and dried in an oven at 50 °C to get the crude extract.

2. 4. Experimental Animals

The Animal House, College of medicine, Imo state University, Owerri was used and 42 adult Wistar rats (male and female) were purchased. The acclimatization of animals took two weeks in the normal laboratory conditions, and animals had free access to food and water.

2. 5. Experimental Design

Animals were randomly grouped into six (n = 7 animals in each group) groups:

- Group I: Control of normalcy.
- Group II: Doxorubicin (5 mg/kg, i.p.)
- Group III: Vitamin C (100 mg / kg) + DOX.
- Group IV: DOX + *P. americana* extract (500 mg/kg).
- Group V: DOX + extract (1000 mg/kg)
- Group VI: DOX + extract (1500 mg/kg)

Therapy was done on a weekly basis after four weeks. In the end of the experiment animals were starved overnight, killed and blood samples were collected through cardiac puncture. Organs were picked to be analyzed.

2. 6. Acute Toxicity Study

Acute toxicity was evaluated using Lorke's method (Lorke, 1983) as modified by Orieke et al. (2019). Animals received graded doses (10–5000 mg/kg), and observations for toxicity and mortality were made over 24 hours and up to 7 days. LD₅₀ was calculated as:

$$LD_{50} = \sqrt{A \times B}$$

where: A = Maximum dose that produced no mortality, B = Minimum dose that killed all animals in a group

2. 7. Hematological Analysis

An automated hematology analyzer (Mindray BC-2300, China) was used to determine hematological parameters such as RBC, Hb, PCV, WBC, platelet count, MCV, MCH, and MCHC based on the instructions given by the company.

2. 8. Biochemical Analysis

2. 8. 1. Liver Function Tests

Serum ALT, AST, ALP, total protein, albumin, and bilirubin were determined using standard colorimetric methods with commercial kits, as described by Ugbogo et al. (2017).

2. 8. 2. Renal Function Tests

Serum urea, creatinine, uric acid, and electrolytes (Na^+ , K^+ , Cl^- , HCO_3^-) were analyzed using established enzymatic methods with commercial kits following Ugbogo et al. (2017).

2. 8. 3. Lipid Profile

Serum total cholesterol, triglycerides, and HDL-cholesterol were determined enzymatically, while LDL and VLDL levels were calculated using standard equations.

2. 8. 4. Oxidative Stress and Antioxidant Assays

Antioxidant enzyme activities and lipid peroxidation were evaluated in tissue homogenates:

- Superoxide dismutase (SOD) – Rotruck et al. (1993)
- Catalase (CAT) – Hadwan and Abed (2016)
- Glutathione peroxidase (GPx) – Rotruck et al. (1993)
- Reduced glutathione (GSH) – Rotruck et al. (1993)
- Malondialdehyde (MDA) – Alam and Fareed (2016)

All assays were conducted using established spectrophotometric methods.

2. 8. 5. Cardiac Biomarker Assay

The serum LDH activity was identified by the procedure of Beutler et al. (1993). The CK-MB was determined through immunoinhibition assay as described by Neumeier (1981) where the troponin levels in the heart were determined through ELISA kits according to the manufacturer protocols.

2. 9. Statistical Analysis

Data were analyzed using SPSS version 20.0 (IBM, USA). Results were expressed as mean $\pm$ standard deviation (SD). Statistical differences among groups were evaluated using one-way ANOVA followed by Duncan multiple range test and Student's t-test for post hoc comparisons. A value of $P < 0.05$ was considered statistically significant.

3. RESULTS

3. 1. Acute Toxicity (LD_{50}) of *Persea americana* Seed Extract

No mortality was observed in either phase of the acute toxicity study following oral administration of *Persea americana* ethanolic seed extract at doses up to 5000 mg/kg body weight. Animals remained physically stable with no observable signs of toxicity, including

changes in posture, locomotion, or behavior. Continued observation over 7 days revealed no delayed mortality. The LD₅₀ of the extract was therefore estimated to be greater than 5000 mg/kg body weight.

Table 1a. Phase 1 LD₅₀ results.

Group	Dose (mg/kg)	No. of death	Observation
1	10	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
2	100	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
3	1000	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.

Table 1b. Phase 2 LD₅₀ results.

Group	Dose (mg/kg)	No. of death	Observation
1	1600	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
2	2900	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
3	5000	0/3	Animals were calm for about 30 hours, but regained physical activity thereafter. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.

LD₅₀ > 5000 mg/kg body weight.

3. 2. Acute Toxicity (LD₅₀) of Doxorubicin

Intraperitoneal administration of doxorubicin produced dose-dependent toxicity. No mortality was observed at doses of 1–5 mg/kg; however, behavioral alterations such as reduced

activity and agitation were noted. At higher doses, mortality was observed, with 33.3% mortality at 20 mg/kg and 100% mortality at 50 mg/kg. The LD₅₀ was calculated to be 22.36 mg/kg body weight.

Table 2a. Phase 1 LD₅₀ results.

Group	Dose (mg/kg)	No. of death	Observation
1	1	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
2	2	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.
3	5	0/3	Animals were active and physically stable. Signs of toxicity like agitations, roughness of hairs, depression, writhing reflexes and death were absent.

Table 2b. Phase 2 LD₅₀ results.

Group	Dose (mg/kg)	No. of death	Observation
1	10	0/3	Signs of toxicity like agitations, depression, and writhing reflexes were observed, but no mortality was recorded.
2	20	1/3	33.33 percent mortality observed. Other toxicity signs observed include reduced movement/calmness, agitations and display of writhing reflexes.
3	50	3/3	100 percent mortality observed. Before mortality, animals looked calm and agitated.

$$\begin{aligned}
 LD_{50} &= \sqrt{10 \times 50} \\
 &= \sqrt{500} \\
 &= 22.36 \text{ mg/kg body weight}
 \end{aligned}$$

3. 3. Hematological Parameters

Doxorubicin significantly reduced RBC, PCV, hemoglobin, and platelet counts, while increasing WBC levels (P < 0.05). Treatment with *Persea americana* extract attenuated these

changes in a dose-dependent manner, with the highest dose (1500 mg/kg) showing the greatest restoration toward control values.

Table 3. Effects of *Persea americana* (PA) ethanolic seed extract on haematological parameters in DOX treated rats.

Treatments	Normal control	DOX, 5 mg/kg body weight.	Vitamin C (100 mg/kg BW) + DOX, 5 mg/kg BW	P.A seed extract (500 mg/kg BW) + DOX, 5 mg/kg BW	P.A seed extract (1000 mg/kg BW) + DOX, 5 mg/kg BW	P. A seed extract (1500 mg/kg BW) + DOX, 5 mg/kg BW
RBC ($\times 10^6/\text{mm}^3$)	7.72 $\pm$ 0.47	6.38 $\pm$ 0.07	7.33 $\pm$ 0.38	6.96 $\pm$ 0.26	6.57 $\pm$ 0.14	7.32 $\pm$ 0.28
PCV (%)	48.33 $\pm$ 2.89	40.67 $\pm$ 1.16	45.67 $\pm$ 3.31	43.67 $\pm$ 1.53	41.33 $\pm$ 1.16	45.00 $\pm$ 1.00
Hb (g/dl)	18.13 $\pm$ 1.33	15.60 $\pm$ 0.20	17.47 $\pm$ 1.10	16.13 $\pm$ 0.31	15.60 $\pm$ 0.20	17.00 $\pm$ 0.87
WBC ($\times 10^3/\text{mm}^3$)	9.82 $\pm$ 0.18	14.02 $\pm$ 0.18	10.40 $\pm$ 0.60	11.10 $\pm$ 0.89	13.55 $\pm$ 0.13	10.92 $\pm$ 0.28
PLT ($\times 10^3/\text{mm}^3$)	400.67 $\pm$ 19.66	350.67 $\pm$ 6.11	373.33 $\pm$ 14.01	379.33 $\pm$ 20.55	394.67 $\pm$ 42.71	376.67 $\pm$ 19.60
MCV (fl)	62.64 $\pm$ 0.51	63.77 $\pm$ 1.21	62.28 $\pm$ 0.41	62.71 $\pm$ 0.19	62.91 $\pm$ 0.45	61.53 $\pm$ 1.01
MCH (pg)	23.49 $\pm$ 0.32	24.46 $\pm$ 0.27	23.81 $\pm$ 0.41	23.18 $\pm$ 0.42	23.75 $\pm$ 0.27	23.23 $\pm$ 0.37
MCHC (g/dl)	37.50 $\pm$ 0.56	38.37 $\pm$ 0.70	38.23 $\pm$ 0.53	36.96 $\pm$ 0.60	37.75 $\pm$ 0.69	37.76 $\pm$ 1.20

Values are presented as mean $\pm$ standard deviation (n = 3); and values different letter superscripts are significantly (P < 0.05) different from paired mean across the row.

3. 4. Serum Hepatic Biomarkers

Doxorubicin significantly increased serum AST, ALT, ALP, and total bilirubin levels while reducing total protein, albumin, and globulin (P < 0.05). These alterations were significantly reversed following treatment with *Persea americana* extract in a dose-dependent manner.

Table 4. Effects of *Persea americana* (PA) seed extract on hepatic function parameters in DOX treated rats.

Treatments	Normal control	DOX, 5 mg/kg body weight.	Vitamin C (100 mg/kg BW) + DOX, 5 mg/kg BW	P.A seed extract (500 mg/kg BW) + DOX, 5 mg/kg BW	P.A seed extract (1000 mg/kg BW) + DOX, 5 mg/kg BW	P. A seed extract (1500 mg/kg BW) + DOX, 5 mg/kg BW
Total protein (g/dl)	7.43 $\pm$ 0.09	5.33 $\pm$ 0.09	6.84 $\pm$ 0.59	6.58 $\pm$ 0.39	6.51 $\pm$ 0.21	7.12 $\pm$ 0.73
Albumin (g/dl)	4.02 $\pm$ 0.11	2.93 $\pm$ 0.14	3.30 $\pm$ 0.11	3.49 $\pm$ 0.37	3.53 $\pm$ 0.04	3.86 $\pm$ 0.43
Globulin (g/dl)	3.41 $\pm$ 0.05	2.40 $\pm$ 0.16	3.54 $\pm$ 0.49	3.10 $\pm$ 0.03	2.98 $\pm$ 0.22	3.25 $\pm$ 0.30

ALT (u/l)	37.00±2.65	51.33±3.51	42.67±2.08	43.33±1.16	43.67±1.53	41.33±0.58
AST (u/l)	43.67±1.53	98.00±3.61	68.67±5.77	81.00±7.00	70.33±1.53	72.67±3.06
ALP (u/l)	75.00±3.00	108.00±9.85	89.67±11.59	86.67±6.66	85.00±5.29	77.67±4.04
Total Bilirubin (mg/dl)	0.45±0.04	0.88±0.13	0.65±0.06	0.60±0.06	0.63±0.06	0.58±0.05

Values are presented as mean ± standard deviation (n = 3); and values different letter superscripts are significantly (P < 0.05) different from paired mean across the row

3. 5. Renal Function Parameters

Doxorubicin significantly elevated serum urea, creatinine, uric acid, and electrolyte levels (P< 0.05). Administration of *Persea americana* extract significantly reduced these parameters in a dose-dependent manner, with no significant changes observed in bicarbonate levels.

Table 5. Effect of *Persea americana* (PA) seed extract on renal function parameters in DOX treated rats.

Treatments	Normal control	DOX, 5 mg/kg body weight.	Vitamin C (100 mg/kg BW) + DOX, 5 mg/kg BW	P.A seed extract (500 mg/kg BW) + DOX, 5 mg/kg BW	P.A seed extract (1000 mg/kg BW) + DOX, 5 mg/kg BW	P. A seed extract (1500 mg/kg BW) + DOX, 5 mg/kg BW
Urea (mg/dl)	20.20±1.30	26.92±1.03	21.69±0.83	23.02±1.23	23.70±0.30	21.60±0.67
Creatinine (mg/dl)	0.80±0.03	1.90±0.14	1.22±0.03	1.28±0.09	1.24±0.06	1.19±0.04
Na ⁺ (mEq/L)	128.70±1.95	136.30±1.06	132.00±1.28	132.83±2.41	133.37±1.60	132.23±1.08
K ⁺ (mEq/L)	4.79±0.10	4.20±0.17	4.43±0.07	4.32±0.13	4.30±0.09	4.25±0.08
Cl. (mEq/L)	87.23±1.50	91.77±0.97	91.27±2.20	92.90±0.62	90.50±0.36	91.30±1.31
HCO ₃ ⁻ (mmol/L)	19.83±0.15	20.37±0.15	20.13±0.21	20.27±0.12	20.07±0.15	20.07±0.23
Uric acid (mg/dl)	4.41±0.07	7.10±0.21	5.31±0.32	5.80±0.21	5.45±0.26	5.12±0.22

Values are presented as mean ± standard deviation (n = 3); and values different letter superscripts are significantly (P < 0.05) different from paired mean across the row

3. 6. Lipid Profile

Doxorubicin significantly increased total cholesterol, triglycerides, LDL, and VLDL levels, while reducing HDL (P < 0.05). Treatment with *Persea americana* extract significantly reversed these alterations, demonstrating dose-dependent effects.

Table 6. Effect of *Persea americana* (PA) seed extract on lipid profile parameters in DOX treated rats.

Treatments	Total cholesterol (mg/dl)	HDL-C (mg/dl)	TAG (mg/dl)	LDL-C (mg/dl)	VLDL-C (mg/dl)
Normal control	95.28±4.18	63.83±1.34	81.03±2.72	15.24±3.49	16.21±0.54
DOX, 5 mg/kg body weight.	124.14±6.64	61.23±1.33	123.00±2.60	38.30±8.24	24.60±0.52
Vitamin C (100 mg/kg BW) + DOX, 5 mg/kg BW	100.33±2.06	62.63±3.61	101.87±2.63	17.32±3.99	20.37±0.53
P.A seed extract (500 mg/kg BW) + DOX, 5 mg/kg BW	99.76±0.47	62.77±1.47	91.33±2.74	18.73±1.44	18.27±0.55
P.A seed extract (1000 mg/kg BW) + DOX, 5 mg/kg BW	98.54±1.86	65.37±1.34	94.63±3.52	14.25±2.91	18.93±0.70
P. A seed extract (1500 mg/kg BW) + DOX, 5 mg/kg BW	93.66±1.98	64.93±0.87	94.07±1.22	9.91±2.90	18.81±0.24

Values are presented as mean ± standard deviation (n = 3); and values different letter superscripts are significantly (P < 0.05) different from paired within the column.

3. 7. Oxidative Stress Markers

Doxorubicin significantly increased MDA levels and reduced antioxidant enzyme activities (GSH, SOD, CAT, GPx) (P < 0.05). These changes were significantly attenuated following treatment with *Persea americana* extract in a dose-dependent manner.

Table 7. Effect of *Persea americana* (PA) seed extract on oxidative stress and lipid peroxidation parameters in DOX treated rats.

Treatments	GSH (mg/dl)	GPx (u/l)	SOD (u/l)	CAT (u/l)	MDA (mg/dl)
Normal control	13.93±0.45	47.30±1.35	31.83±0.86	24.10±0.96	4.11±0.13
DOX, 5 mg/kg body weight.	10.37±0.5	38.70±1.71	25.03±0.42	21.27±0.65	7.01±0.15
Vitamin C (100 mg/kg BW) + DOX, 5 mg/kg BW	12.67±0.72	43.90±0.56	27.20±1.40	22.37±0.59	5.05±0.48
P.A seed extract (500 mg/kg BW) + DOX, 5 mg/kg BW	12.73±0.31	41.20±0.98	28.87±0.71	24.03±0.87	4.93±0.23
P.A seed extract (1000 mg/kg BW) + DOX, 5 mg/kg BW	13.00±0.44	45.47±1.02	30.17±0.78	23.13±0.85	4.96±0.25
P. A seed extract (1500 mg/kg BW) + DOX, 5 mg/kg BW	13.30±0.56	44.00±0.36	30.70±0.98	22.67±1.55 ^a	4.93±0.12

Values are presented as mean $\pm$ standard deviation (n = 3); and values different letter superscripts are significantly ($P < 0.05$) different from paired within the column.

3. 8 .Cardiac Biomarkers

Doxorubicin significantly elevated LDH, CK-MB, and cardiac troponin levels ($P < 0.05$). Treatment with *Persea americana* extract significantly reduced these biomarkers, with the highest dose showing the greatest effect.

Table 9. Effect of *Persea americana* (PA) seed extract on some cardiac parameters in DOX treated rats.

Treatments	LDH (pg/dl)	CK-MB (iu/l)	Cardiac troponin level (ng/ml)
Normal control	125.67 $\pm$ 9.29	653.77 $\pm$ 39.13	0.53 $\pm$ 0.02
DOX, 5 mg/kg body weight.	401.33 $\pm$ 7.57	955.98 $\pm$ 30.95	0.90 $\pm$ 0.04
Vitamin C (100 mg/kg BW) + DOX, 5 mg/kg BW	251.33 $\pm$ 19.73	773.36 $\pm$ 9.77	0.65 $\pm$ 0.02
P.A seed extract (500 mg/kg BW) + DOX, 5 mg/kg BW	292.67 $\pm$ 20.23	747.89 $\pm$ 67.05	0.63 $\pm$ 0.04
P.A seed extract (1000 mg/kg BW) + DOX, 5 mg/kg BW	252.00 $\pm$ 9.64	694.38 $\pm$ 39.09	0.64 $\pm$ 0.06
P. A seed extract (1500 mg/kg BW) + DOX, 5 mg/kg BW	244.00 $\pm$ 12.17	686.12 $\pm$ 12.99	0.68 $\pm$ 0.03

Values are presented as mean $\pm$ standard deviation (n = 3); and values different letter superscripts are significantly ($P < 0.05$) different from paired within the column.

4. DISCUSSION

The current experiment showed that doxorubicin caused serious multilateral organ toxicity and *Persea americana* seed extract treated experiment showed a dose-dependent alleviation of these effects. These results align with the recent data that oxidative stress, mitochondrial dysfunction, inflammatory signaling, and regulated cell death pathways such as apoptosis and ferroptosis mediate doxorubicin toxicity (Wallace, 2020; Cappetta et al., 2017; Rawat et al., 2021).

The decrease in the RBC, hemoglobin, PCV, and platelet count after the administration of doxorubicin are indicative of hematological toxicity that is related to bone marrow suppression and oxidative injury of the circulating erythrocytes. Doxorubicin causes the production of reactive oxygen species (ROS) through redox cycling and iron interaction with the effect of membrane lipid peroxidation and decreased erythrocyte survival (Octavia et al., 2012; Cappetta et al., 2017). The increase in WBC is an indication of inflammatory response due to tissue damage. These parameters have been restored by *Persea americana* extract, and these results indicate attenuation of oxidative stress and potential hematopoiesis stimulation, which have been reported in the case of plant-derived antioxidants (Valko et al., 2007; Sies, 2017).

The hepatotoxic changes described, such as the increase in AST, ALT, ALP, and bilirubin and the decrease in protein synthesis indicators, are in line with the ROS-mediated hepatocellular damage and mitochondrial dysfunction (Carvalho et al., 2009; Wallace, 2020; Rawat et al., 2021). Doxorubicin interferes with the integrity of the hepatocyte membrane that results in the release of intracellular enzymes. The fact that *Persea americana* extract reverses these changes implies hepatoprotective activity, which is probably mediated by the action of antioxidant and membrane-stabilizing activity. Equivalent results have been achieved with plant extracts rich in polyphenols in experimental hepatotoxicity models (Dreher and Davenport, 2013).

Dysfunction of the kidneys that is observed by the increase of urea, creatinine, and uric acid is consistent with the literature indicating that doxorubicin causes nephrotoxicity through oxidative stress, inflammation, and mitochondrial damage (Octavia et al., 2012; Wallace, 2020). The increase in the renal parameters after the use of *Persea americana* extract indicates that there is preservation of renal functions, which is probably due to the alleviation of the oxidative damage and preservation of nephron integrity.

The dyslipidemia of the current research is in line with the findings that doxorubicin disturbs lipid metabolism by exerting an oxidative stress and mitochondrial dysfunction causing lipid peroxidation increase and lipid transport alterations (Tacar et al., 2013). The lipid profiles after the treatment with *Persea americana* extract normalized, which is an indication of the hypolipidemic effect, probably by the mechanism of antioxidant activity and enhanced lipid metabolism.

One of the key results of the research is the increase in malondialdehyde (MDA) and the decrease in the activity of antioxidant enzymes (GSH, SOD, CAT, GPx) which proves the fact of the contribution of the oxidative stress to the toxicity caused by doxorubicin. The generation of ROS by Doxorubicin results in the loss of endogenous antioxidant defenses and oxidative stress of cellular components (Sies, 2017; Wallace, 2020). The recovery of antioxidant enzyme activity and lipid peroxidation decrease after treatment with *Persea americana* extract demonstrate that this extract has a high antioxidant potential, which can probably be explained by a high phytochemical content.

Doxorubicin-induced injury is characterized by cardiac toxicity that is manifested by high levels of LDH, CK-MB, and cardiac troponin. Mechanically, doxorubicin is stored in the cardiac mitochondria, which results in a lack of oxidative phosphorylation, excessive production of ROS, and the death of cardiomyocytes (Zhang et al., 2012; Wallace et al., 2020). The fact that these biomarkers were reduced after treatment with *Persea americana* extract is an indication of cardioprotective properties, which are probably mediated by antioxidant and mitochondrial protective processes.

The protective effects on all the parameters were dose-dependent, which confirms the biological plausibility of the results and the therapeutic potential of *Persea americana* extract. The similar effects of Vitamin C also support the importance of the antioxidant action in the mediation of the protection against the toxicity of doxorubicin.

The results of the acute toxicity showed that *Persea americana* extract did not show any mortality at doses up to 5000 mg/kg, and thus has a large safety margin, and doxorubicin had a high toxicity at a relatively low dose. This underscores the possibilities of *Persea americana* as a safe adjunctive agent in the minimization of chemotherapy induced toxicity.

Altogether, the results are good evidence that *Persea americana* seed extract can protect multiple organs against toxicity caused by doxorubicin by acting as an antioxidant, anti-

inflammatory, and cytoprotective agent. These findings justify the increasing attention to the use of bioactive compounds of plant origin as complementary treatment of cancer.

5. CONCLUSIONS

This experiment has shown that doxorubicin caused severe multi-organ toxicity, which was reflected in the changes of hematological indices, parameters of hepatic and renal functions, lipid profile, antioxidant status, and cardiac biomarkers. These alterations indicate the systemic effects of doxorubicin, which is mostly achieved by oxidative stress and related cellular damage. Toxicity of *Persea americana* ethanolic seed extract was considerably reduced by administration of the extract in a dose-dependent manner. The extract replenished hematological, promoted liver and kidney, normalized lipid profile, antioxidant defense systems, and minimized cardiac injury biomarkers. The best effects were always recorded at the maximum dose (1500 mg/kg), which was similar to Vitamin C.

The fact that *Persea americana* seed extract does not cause mortality even at high doses is further evidence of a high safety margin of this extract that can be used in the treatment process. The results indicate that the antioxidant and cytoprotective processes play the major role in mediating the protective effects of the extract.

In general, *Persea americana* seed extract has a good potential to be used as a natural supplement in the prevention of toxicity caused by doxorubicin. The use of molecular characterization and clinical assessment should be further investigated to confirm its effectiveness and translational usefulness in cancer therapy.

References

- [1] Alam, Z., and Fareed, U. C. (2016). A simple spectrophotometric method for the determination of thiobarbituric acid reactive substances in fried fast foods. *Journal of Analytical Methods in Chemistry*, 2016, 1–5
- [2] Arrigoni, R., Jirillo, E., & Caiati, C. (2025). Pathophysiology of doxorubicin-mediated cardiotoxicity. *Toxics*, 13(4), 277
- [3] Beutler, E. (1993). Enzyme replacement therapy for Gaucher's disease. *Baillière's Clinical Haematology*, 10(4), 751–763
- [4] Bhadra, P., Yadav, P., Kaur, S., Hanumantharayudu, P. T., & Arunachalam, S. (2025). The role of ferroptosis in doxorubicin-induced cardiotoxicity—An update. *Life Sciences*, 123945
- [5] Bhutani, V., Varzideh, F., Wilson, S., Kansakar, U., Jankauskas, S. S., & Santulli, G. (2025). Doxorubicin-induced cardiotoxicity: a comprehensive update. *Journal of Cardiovascular Development and Disease*, 12(6), 207
- [6] Cappetta, D., et al. (2017). Oxidative stress and cellular response to doxorubicin: a common factor in the complex milieu of anthracycline cardiotoxicity. *Oxidative Medicine and Cellular Longevity*, 2017(1), 1521020

- [7] Carvalho, C., Santos, R. X., Cardoso, S., Correia, S., Oliveira, P. J., Santos, M. S., & Moreira, P. I. (2009). Doxorubicin: the good, the bad and the ugly effect. *Current Medicinal Chemistry*, 16(25), 3267-3285
- [8] Dabas, D., Elias, R. J., Lambert, J. D., & Ziegler, G. R. (2011). A colored avocado seed extract as a potential natural colorant. *Journal of Food Science*, 76(9), C1335-C1341
- [9] Dreher, M. L., & Davenport, A. J. (2013). Hass avocado composition and potential health effects. *Critical Reviews in Food Science and Nutrition*, 53(7), 738-750
- [10] Egbuonu, A. C., Opara, I. C., Onyeabo, C., and Uchenna, N. O. (2018). Proximate, functional, antinutrient and antimicrobial properties of avocado pear (*Persea americana*) seeds. *Journal of Nutritional Health and Food Engineering*, 8(2), 00260
- [11] Li, Y., Yan, J., & Yang, P. (2024). The mechanism and therapeutic strategies in doxorubicin-induced cardiotoxicity: Role of programmed cell death. *Cell Stress and Chaperones*, 29(5), 666-680
- [12] Linders, A. N., Dias, I. B., LópezFernández, T., Tocchetti, C. G., Bomer, N., & Van der Meer, P. (2024). A review of the pathophysiological mechanisms of doxorubicin-induced cardiotoxicity and aging. *NPJ Aging*, 10(1), 9
- [13] Neumeir, D. (1981). Tissue-specific distribution of Creatine kinase isoenzyme. In H. Lang (Ed.), Springer-Verlag (pp. 85–109).
- [14] Octavia, Y., Tocchetti, C. G., Gabrielson, K. L., Janssens, S., Crijns, H. J., & Moens, A. L. (2012). Doxorubicin-induced cardiomyopathy: from molecular mechanisms to therapeutic strategies. *Journal of Molecular and Cellular Cardiology*, 52(6), 1213-122
- [15] Orieke, D., Ohaeri, O. C., Ijeh, I. I., and Ijioma, S. N. (2019). Gastrointestinal and uterine smooth muscle relaxant and anti-inflammatory effects of *Corchorusolitorius* leaf extract in laboratory animal models. *Journal of Ethno Pharmacology*, 247, 112224
- [16] Pigg, Q. W., Harris, D. R., Inoue, D. S., & Janini Gomes, M. (2025). Mitigating doxorubicin-induced skeletal muscle toxicity: a review of oxidative stress mechanisms and the therapeutic role of exercise. *Antioxidants*, 14(7), 870
- [17] Qing, G., Huang, C., Pei, J., & Peng, B. (2025). Alteration of cardiac energetics and mitochondrial function in doxorubicin-induced cardiotoxicity: Molecular mechanism and prospective implications. *International Journal of Molecular Medicine*, 56(5), 183
- [18] Radeva, L., & Yoncheva, K. (2025). Doxorubicin Toxicity and Recent Approaches to Alleviating Its Adverse Effects with Focus on Oxidative Stress. *Molecules*, 30(15), 3311
- [19] Rawat, P. S., Jaiswal, A., Khurana, A., Bhatti, J. S., & Navik, U. (2021). Doxorubicin-induced cardiotoxicity: An update on the molecular mechanism and novel therapeutic strategies for effective management. *Biomedicine & Pharmacotherapy*, 139, 111708
- [20] Rotruck, J. T., Pope, A. L., Ganther, H. E., Swanson, A. B., and Hafeman, D. G. (1993). Selenium biochemical role as a component of glutathione peroxidase. *Science*, 179(4073), 588–590

- [21] Shi, S., Chen, Y., Luo, Z., Nie, G., & Dai, Y. (2023). Role of oxidative stress and inflammation-related signaling pathways in doxorubicin-induced cardiomyopathy. *Cell Communication and Signaling*, 21(1), 61
- [22] Sies, H. (2017). Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biology*, 11, 613-619
- [23] Tacar, O., Sriamornsak, P., & Dass, C. R. (2013). Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. *Journal of Pharmacy and Pharmacology*, 65(2), 157-170
- [24] Ugbogo, A. E., Okezie, E., and Ijioma, S. N. (2017). Introductory biochemistry practical. Justman Publishers.
- [25] Valko, M., Leibfritz, D., Moncol, J., Cronin, M. T., Mazur, M., Telser, J. (2007). Free radicals and antioxidants in normal physiological functions and human disease. *The international Journal of Biochemistry & Cell Biology*, 39(1), 44-84
- [26] Vitale, R., Marzocco, S., & Popolo, A. (2024). Role of oxidative stress and inflammation in doxorubicin-induced cardiotoxicity: a brief account. *International Journal of Molecular Sciences*, 25(13), 7477
- [27] Wallace, K. B., Sardão, V. A., & Oliveira, P. J. (2020). Mitochondrial determinants of doxorubicin-induced cardiomyopathy. *Circulation Research*, 126(7), 926-941
- [28] Wang, B., et al., (2025). Ferroptosis: Latest evidence and perspectives on plant-derived natural active compounds mitigating doxorubicin-induced cardiotoxicity. *Journal of Applied Toxicology*, 45(1), 135-158
- [29] Wu, L., Zhang, Y., Wang, G., & Ren, J. (2024). Molecular mechanisms and therapeutic targeting of ferroptosis in doxorubicin-induced cardiotoxicity. *Basic to Translational Science*, 9(6), 811-826
- [30] Xie, S., et al., (2024). An update of the molecular mechanisms underlying anthracycline induced cardiotoxicity. *Frontiers in Pharmacology*, 15, 1406247