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Antioxidant-Mediated Hepatoprotective Effects of *Citrus aurantifolia* Peels Extract in Doxorubicin-Induced Liver Injury: Acute and Sub-Acute Toxicity Assessment in Rats

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of the article.

Abstract

Background: The liver plays a crucial role in metabolism and detoxification but remains highly susceptible to oxidative stress induced by chemotherapeutic agents such as doxorubicin (DOX). DOX promotes the generation of reactive oxygen species (ROS), resulting in lipid peroxidation, inflammation, and hepatocellular degeneration. Medicinal plants have attracted growing interest for their potential to alleviate oxidative damage.
Objective: This study aimed to evaluate the antioxidant-mediated hepatoprotective effects of *Citrus aurantifolia* peels extract against doxorubicin-induced liver injury in rats, alongside assessing its acute and sub-acute toxicity profile.
Method: Acute and sub-acute toxicity studies were carried out in accordance with OECD guidelines. Hepatotoxicity was induced by intraperitoneal administration of DOX (15 mg/kg), followed by oral treatment with CAPE (100, 200, and 400 mg/kg) or alpha-lipoic acid (150 mg/kg) for seven days. Changes in body weight, hematological indices, and antioxidant markers (superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA) and Vitamins C) were assessed. Liver tissues were also examined histologically.
Results: CAPE was well tolerated up to 2000 mg/kg, indicating a broad safety margin. Repeated administration at 200–400 mg/kg caused no mortality or clinical signs of toxicity, though mild lethargy was observed at 800 mg/kg. CAPE significantly ($p<0.05$) decreased MDA levels while enhancing superoxide dismutase activities in a dose-dependent manner. Histopathological findings showed remarkable improvement in hepatic architecture, comparable to alpha-lipoic acid treatment.
Conclusion: *Citrus aurantifolia* peels extract exhibits strong antioxidant and hepatoprotective effects against DOX-induced oxidative liver injury, demonstrating safety and therapeutic potential for managing drug-related hepatotoxicity.

Keywords: *Citrus aurantifolia*, hepatoprotection, doxorubicin, antioxidants, liver injury.

INTRODUCTION

The liver is a vital organ responsible for numerous metabolic, synthetic, and detoxification processes, including the biotransformation of endogenous and exogenous substances. However, because of its central metabolic role, the liver is particularly vulnerable to damage caused by xenobiotics, drugs, and environmental toxins (Jaeschke and McGill, 2019). Drug-induced liver injury (DILI) is a leading cause of acute liver failure worldwide and remains one of the most common reasons for post-marketing drug withdrawal and termination of clinical trials. Mechanistically, DILI may occur through intrinsic (dose-dependent and predictable) or idiosyncratic (dose-independent and unpredictable) pathways, involving oxidative stress, mitochondrial dysfunction, immune-mediated reactions, and disruption of bile acid homeostasis (Chalasani *et al*., 2021).

Hepatotoxicity remains a major clinical problem, especially with the use of chemotherapeutic agents such as Doxorubicin, which generates reactive oxygen species (ROS), leading to oxidative stress, lipid peroxidation, and ultimately hepatic tissue damage (Zhao et al., 2021). Doxorubicin is an anthracycline antibiotic widely used in the treatment of various malignancies, including breast cancer, lymphomas, leukemias, and sarcomas. Its antineoplastic activity is primarily mediated through intercalation into DNA, inhibition of topoisomerase II, and generation of free radicals that induce apoptosis in rapidly dividing tumor cells (Thorn *et al*., 2021).

Despite its effectiveness, doxorubicin is associated with dose-limiting toxicities affecting the heart, kidneys, and liver. In the liver, its metabolism by hepatic cytochrome P450 enzymes and redox cycling promotes excessive ROS production, mitochondrial injury, inflammatory cytokine release, and activation of apoptotic pathways, thereby contributing to DILI (Octavia *et al*., 2019; Zhang et al., 2022). These toxic effects underscore the need for adjunct therapies capable of mitigating oxidative and inflammatory hepatic damage during chemotherapy. Consequently, there is a growing interest in identifying natural hepatoprotective agents capable of reducing drug-induced oxidative injury.

Medicinal plants have long been recognized as valuable sources of bioactive compounds with diverse therapeutic potentials. Many modern pharmaceuticals are derived directly or indirectly from plant metabolites (Newman and Cragg, 2020). Among such plants, *Citrus aurantifolia* (Christm.) Swingle, commonly known as key lime, belongs to the family Rutaceae. *Citrus aurantifolia* is believed to have originated in Southeast Asia and is now widely distributed across tropical and subtropical regions, including West Africa, East Africa, South Asia, Central and South America, and parts of the Middle East. In sub-Saharan Africa, it is extensively cultivated in countries such as Nigeria, Ghana, Kenya, and Tanzania for both nutritional and ethnomedicinal purposes (Oboh *et al*., 2023). The plant has been traditionally used in the management of fever, cough, sore throat, jaundice, and digestive disturbances (Yunusa *et al.,* 2022).

Phytochemical analyses of *C. aurantifolia* have revealed a rich composition of flavonoids, alkaloids, coumarins, limonoids, and essential oils containing compounds such as limonene, citral, and naringenin (Abdurahman et al., 2022). These constituents have been reported to exhibit antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Liñán-Atero *et al*., 2024). The antioxidant activity of *C. aurantifolia* peels extract, in particular, has been attributed to its high levels of phenolic and flavonoid compounds, which can scavenge free radicals and enhance the body's endogenous antioxidant defense mechanisms (Pandey *et al*., 2023). Flavonoids such as hesperidin and naringenin have also been shown to modulate signaling pathways involved in oxidative stress and inflammation, including the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway, thereby strengthening cellular antioxidant responses (Zhou et al., 2022).

Oxidative stress plays a key role in the mechanism of doxorubicin-induced liver injury. Natural antioxidants have been shown to protect the liver by enhancing antioxidant parameters such as superoxide dismutase (SOD) and glutathione (GSH), and by reducing lipid peroxidation products such as malondialdehyde (MDA) (Chen *et al*., 2022). Given the high reliance on plant-based remedies in sub-Saharan Africa and the increasing exposure to hepatotoxic drugs, exploring locally

available medicinal plants with potential hepatoprotective effects is of both scientific and public health importance (WHO, 2023). Despite the wide traditional use of *C. aurantifolia*, there is limited scientific evidence on the safety profile and hepatoprotective efficacy of its peel extract in experimental models.

Therefore, this study was designed to investigate the antioxidant-mediated hepatoprotective effects of Citrus aurantifolia peels extract (CAPE) against doxorubicin-induced liver injury in Wistar rats, while also evaluating its in vivo antioxidant potential and assessing its acute and sub-acute oral toxicity profile. The findings from this study are expected to contribute to the growing body of evidence supporting the pharmacological relevance of C. aurantifolia and its potential role as a natural agent in mitigating oxidative liver damage.

MATERIAL AND METHODS

Plant Collection and Identification

Swingle fruits of *Citrus aurantifolia* (Chrism.) were gathered in Forest Research Institute of Nigeria (FRIN) Ibadan, which is located in southwest Oyo State, Nigeria. A Taxonomist from the University of Ibadan's Department of Botany, Ibadan, Oyo State, Nigeria, D.P.O. Esimekhuai, identified the plant. The plant sample was given Herbarium voucher number, UIH-22957, and deposited in the Botany Department

Drugs, Chemicals, and Reagents

Methanol (Sigma Chemical Co., Burlington, MA, USA) were used. Other chemicals, including formalin, ethylenediaminetetraacetic acid (EDTA), and sodium dihydrogen phosphate, were obtained from Sigma (St. Louis, MO, USA). All reagents used were of analytical grad; and North West Life Science Specialties (NWLSSTM) antioxidant kits; Enzopak biochemical kits; alpha-lipoic acid capsules (Uni Pharma, Cairo, Egypt); doxorubicin; Folin Ciocalteu reagent (BDH Chemicals, Radnor, Pennsylvania, USA).

Preparation of Plant Material

The peels of *Citrus aurantifolia* were removed and shade-dried under adequate ventilation, protected from direct sunlight to preserve heat-sensitive phytochemicals at controlled ambient conditions (25–30 °C), slightly above standard room temperature (~25 °C) due to tropical climate, for seven days until constant weight. The dried peels were then coarsely powdered with a mortar and pestle and further pulverized using an electric blender.

Extraction of *Citrus aurantifolia* Peels

The powdered plant material was extracted with aqueous methanol (methanol: water, 80:20 v/v) for 72 hours at room temperature by maceration. The extract was first filtered through sterile muslin cloth to remove coarse plant debris, followed by further clarification using Whatman No. 1 filter paper. The muslin cloth (Surgical Gauze/Muslin Cloth; Johnson & Johnson Medical Ltd., Lagos, Nigeria) was used for the initial filtration step to obtain a clear filtrate prior to concentration.The results of extraction of *Citrus aurantifolia* with methanol/water gave Percentage yield of 8.04% w/w.

Experimental Animals

Wistar rats (120–140 g) were obtained from the Animal House Facility, College of Medicine, University of Lagos, Lagos, Nigeria. The animals were housed under standard laboratory conditions with free access to feed and water and were allowed to acclimatize for two weeks before the commencement of experimental procedures. Ethical approval was obtained from the College of Medicine, University of Lagos Animal Care and Use Research Ethics Committee (CMUL ACUREC), approval no. CMUL/ACUREC/06/22/1116.

Experimental Design

Acute Toxicity Study

The acute oral toxicity study followed OECD guideline 423. Two groups of Wistar rats (n=6; three males and three females each) were fasted overnight. One group received *Citrus aurantifolia* peels extract (CAPE) at 2000 mg/kg (p.o.), while the control group received distilled water (10 mL/kg, p.o.). The animals were monitored for 14 days for signs of toxicity and mortality, including salivation, lacrimation, piloerection, diarrhea, and behavioral changes.

Sub-Acute Toxicity Study

Forty Wistar rats (20 males, 20 females) were randomly divided into four groups (n=10/group; 5 males and 5 females), using OECD guideline 407.The extract was administered orally for 28 consecutive days. The treatment schedule is outlined below:
Group 1: Control (distilled water, 10 mL/kg)
Group 2: CAPE 200 mg/kg
Group 3: CAPE 400 mg/kg
Group 4: CAPE 800 mg/kg

The animals were observed for clinical signs of toxicity. On day 29, rats were sacrificed under chloroform anesthesia, and blood was collected via cardiac puncture, and liver samples were collected for antioxidant, and histopathological analyses.

Hematological Parameters

Blood collected via cardiac puncture into EDTA tubes was analyzed for red blood cells (RBC), hemoglobin (Hb), white blood cells (WBC), packed cell volume (PCV), and platelet counts using
Automated Hematology Analyzer (XT-1800i, Japan) on the EDTA collected blood.

***In-Vivo* Antioxidant Assays**

Liver tissues were homogenized in phosphate buffer (0.05 M, pH 7.0) and centrifuged at 4000 rpm for 20 minutes to obtain post-mitochondrial supernatant (PMS), which was used for the analyses of Malondialdehyde (MDA), reduced glutathione (GSH), catalase, Superoxide dismutase (SOD) and Vitamin C levels using established methods.

Histopathological Examination

Liver tissues were fixed in 10% buffered formalin, dehydrated in graded ethanol, embedded in paraffin, sectioned at 2 μm thickness, and stained with hematoxylin and eosin (H&E, ×400). Slides were examined under an Olympus BX-51 microscope, and images were captured using a CCD camera.

Evaluation of Curative Effects of CAPE in Doxorubicin-Induced Hepatotoxicity

Thirty wistar rats were divided into six (6) groups (n=5). Doxorubicin (dissolved with normal saline, 15 mg/kg i.p.) was injected to rats in Groups II, III, IV, V, and VI. Twenty-four (24) hours after, Group I to VI rats received Distilled water, Distilled water, CAPE (100, 200, 400 mg/kg) and Alpha-lipoic acid {ALA}(150 mg/kg) for a period of seven (7) days, orally respectively. The treatment schedule is outlined below:

Group I = Distilled water {10mL} (only)
Group II = DOX 15 mg/kg + Distilled water {10mL}
S
Group III = DOX 15 mg/kg + CAPE 100mg
Group IV = DOX 15 mg/kg + CAPE 200mg
Group V = DOX 15 mg/kg + CAPE 400mg
Group VI = DOX 15 mg/kg + Alpha lipoic acid (150 mg/kg)

At the end of the treatments period for sub-acute and curative studies, rats were anesthetized using chloroform, and liver tissues were collected for antioxidant and histological analyses as described above.

Statistical Analysis

All experimental results are expressed as Mean ± SEM. Data were analyzed using One-way analysis of variance (ANOVA) and followed by Dunnett's/Tukey's post-hoc tests using Graph Prism 6 Software (Graph Pad Soft Inc., CA, USA). The results were considered significant at $p<0.05$.

RESULTS

Acute Oral Toxicity of CAPE in Rats

No mortality or signs of toxicity were observed in rats administered *Citrus aurantifolia* peels extract (CAPE) at a single oral dose of 2000 mg/kg during the 14-day observation period. Behavioral and physical parameters, including posture, grooming, feeding, and activity, remained normal throughout the study. Therefore, the median lethal dose (LD_{50}) of CAPE is estimated to be greater than 2000 mg/kg.

Sub-Acute (28-Day) Oral Toxicity of CAPE in Rats

Rats administered CAPE at doses of 200 and 400 mg/kg body weight exhibited no visible signs of toxicity or behavioral abnormalities throughout the 28-day study. However, animals treated with 800 mg/kg showed mild lethargy, generalized weakness, and reduced motor and reflex responses. No mortality, respiratory distress, or changes in fur texture were observed in any group during the experimental period.

Effects of 28 days administration of *Citrus aurantifolia* peels extract on body weight

A progressive increase in body weight was observed in the control and CAPE-treated rats at 200 and 400 mg/kg doses throughout the experimental period. Rats treated with 800 mg/kg CAPE showed a higher increase in body weight gain compared to the control group (Figure 1).

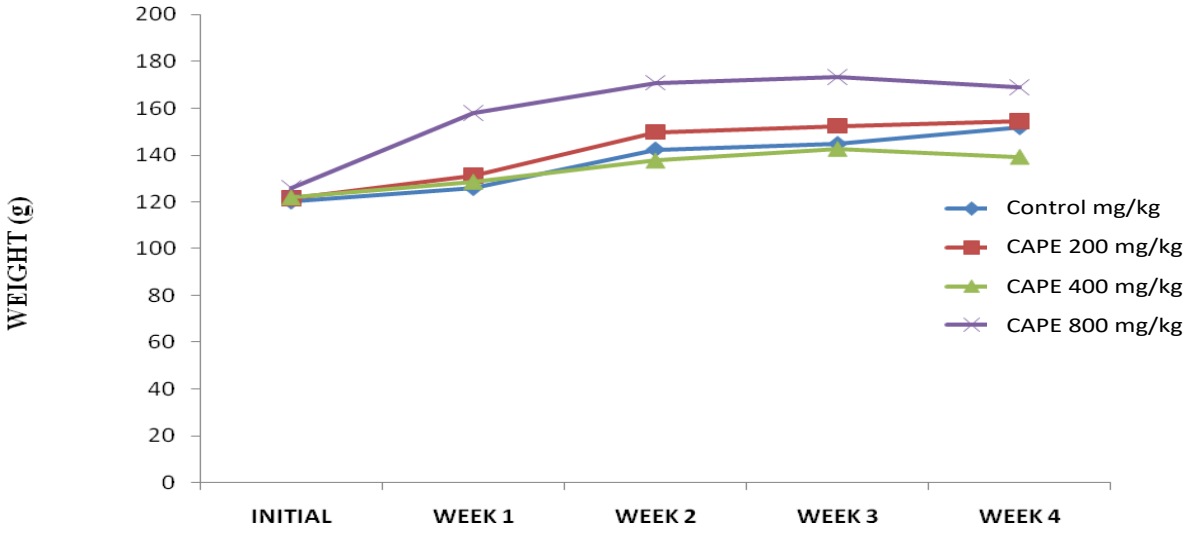

Figure 1: Body weight (g) of rats in the sub-acute toxicity studies of CAPE

Effects of 28 days administration of *Citrus aurantifolia* peels extract on hematological parameters in rats

Hemoglobin, PCV, and RBC levels showed a dose-dependent decline, with significant reductions at 400 and 800 mg/kg ($p<0.05$) relative to distilled water. The 200 mg/kg group showed no significant change ($p>0.05$). WBC counts increased significantly ($p<0.05$) at 200 mg/kg, but decreased significantly ($p<0.05$) at higher doses (CAPE 400 and 800 mg/kg) in comparison with distilled water. Platelet counts were significantly elevated ($p<0.05$) in all treated groups, most notably at 200 mg/kg. Overall, CAPE induced mild hematological alterations in a dose-dependent manner (Table 1).

Table 1: Effects of 28 days administration of *Citrus aurantifolia* peels extract on hematological parameters in rats

Parameters	Distilled water (10 mL/kg)	CAPE 200 mg/kg	CAPE 400 mg/kg	CAPE 800 mg/kg
Haemoglobin (g/dl)	14.7±1.2	13.4±1.0	10.8±1.3*	9.1±1.4*
PCV (%)	45±1.1	40.7±1.3	32±1.4*	27±2.2*
RBC (10^{12}/L)	9.2±2.1	9.0±2.2	7.2±1.0*	6.1±2.1*
WBC (10^{9}/L)	10.1±1.4	14.6±1.6*	7.7±1.1*	2.9±1.1*
Platelets (10^{9}/L)	2.7±2.0	5.5±1.1*	4.1±2.1*	5.0±2.3*

Results are expressed as mean ± SEM of 6 rats; *=Significantly different ($p<0.05$) from the control.

Effects of 28 days administration of *Citrus aurantifolia* peels extract on organ weight

Liver weight decreased significantly with increasing doses of the extract. At 200 mg/kg, the reduction was mild and not statistically significant compared to distilled water. At 400 mg/kg and 800 mg/kg, the decreases were significant, $p<0.05$ and $p<0.01$ respectively (Table 2).

Table 2: Weights (g) of the liver in the sub-acute toxicity studies of CAPE

		Citrus aurantifolia peels extract (CAPE) {mg/kg}		
	Distilled water (10 mL/kg)	**CAPE 200**	**CAPE 400**	**CAPE 800**
Liver	1.05± 0.12	0.97±0.3	0.80±0.11*	0.70±0.3**

Results are expressed as the mean ± SEM of 6 rats; *=Significantly different (p<0.05) from the control; **= Significantly different (p<0.01) from the control.

Effects of 28 days administration of *Citrus aurantifolia* peels extract on *in-vivo* antioxidants of the liver in rats

The result revealed that CAPE treatment significantly ($p<0.05$) increases hepatic MDA levels while a dose-dependent significant increase ($p<0.05$) in GSH concentration and SOD activity were observed in CAPE (200 400 and 800 mg/kg) treated groups relative to distilled water. Only CAPE 200 mg/kg exhibited a significant ($p<0.05$) increase compared with distilled water. Furthermore, a reduction in Vitamin C concentration were observed in CAPE 400 and 800 mg/kg, in contrary a significant increase ($p<0.05$) in Vitamin C concentration was exhibited in CAPE 200 mg/kg (Table 3).

Table 3: Effects of 28 days administration of *Citrus aurantifolia* peels extract i*n vivo* antioxidants of the liver in rats

Treatments	**MDA (nmol/ mg protein)**	**GSH (µg/mg /protein)**	**SOD U/mg protein)**	**Vitamin C (µmol/mg/ protein)**
Distilled water	0.15±0.10	115.79±3.23	23.94±0.69	4.94±0.14
CAPE 200 mg/kg	0.24 ±0.001*	150.53±4.21*	31.10±0.86*	6.43±0.17*
CAPE 400 mg/kg	0.73±0.02*	312.64±8.74*	64.59±1.81*	3.35±0.37
CAPE 800 mg/kg	1.34±0.03*	925.65±2.67*	193.79±5.41*	4.04±1.12

Results are expressed as mean ± SEM of 6 rats; *=Significantly different (p<0.05) from the control

Effects of 28 days administration of *Citrus aurantifolia* peels extract on histological studies of the liver in rats

Histological evaluation of liver sections revealed that Groups A and C showed normal hepatic architecture with closely packed hepatic plates and no visible lesions. Group B exhibited mild hepatocellular changes

characterized by foci of hepatocytes with finely reticulated cytoplasmic appearance. In contrast, Group D displayed multiple foci of moderate thinning of hepatic cords with resultant dilation of the sinusoids (black arrows × 400), indicating mild hepatocellular alterations associated with higher exposure (Table 4 and Figure 2)

Table 4: Effects of 28 days administration of *Citrus aurantifolia* peels extract on histological studies of the liver in rats

Treatments	Liver
Distilled water	No visible lesion
CAPE 200 mg/kg	No visible lesion
CAPE 400 mg/kg	Mild lesion
CAPE 800 mg/kg	Moderate thinning of hepatic cords with resultant sinusoids dilation

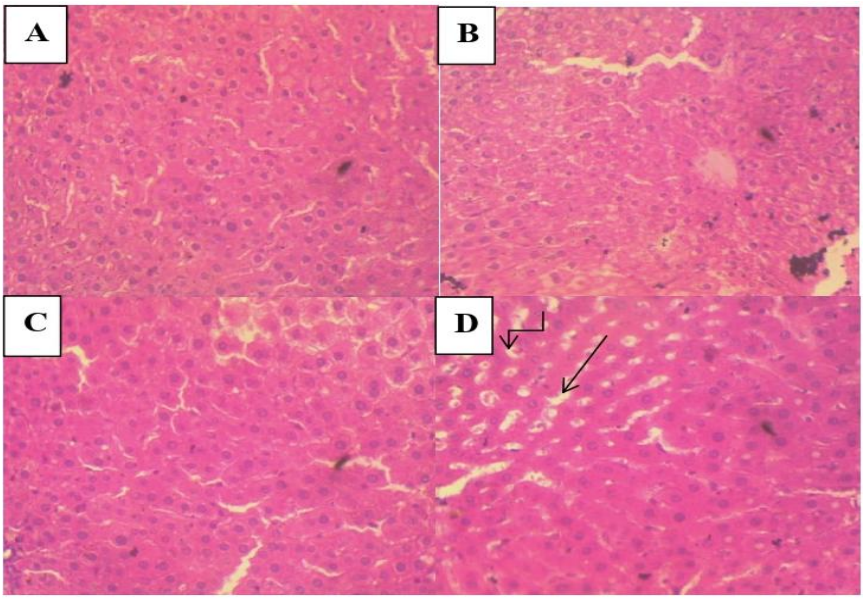

Figure 2: Histopathological examination of the liver in the sub-acute toxicity studies of CAPE

(A) Distilled water liver showing normal hepatic architecture with closely packed hepatic plates and no visible lesions. (B) 200 mg/kg CAPE liver showing no visible lesion with moderately packed hepatic plates (C) 400 mg/kg CAPE liver showing mild reticulated cytoplasmic appearance in some hepatocytes. (D) 800 mg/kg CAPE liver showing moderate thinning of hepatic cords with dilated sinusoids (black arrows), indicating mild hepatocellular alteration.

Curative effects of *Citrus aurantifolia* peels extract in Doxorubicin-induced hepatotoxicity in rats

Doxorubicin plus distilled water significantly ($p<0.05$) increased oxidative stress marker (MDA) and reduced GSH, SOD and Vitamin C) in the liver, indicating hepatotoxicity ($p<0.05$) relative to distilled water. CAPE 200, 400 and 800 mg/kg, and α-Lipoic acid produced significant ($p<0.05$) reduction in MDA and increases in GSH, SOD and Vitamin C compared with the DOX + distilled water group. Overall, CAPE showed significant ($p<0.05$) hepatoprotective and antioxidant effects against DOX-induced liver damage (Table 5).

Table 5*: Curative effects of *Citrus aurantifolia* peels extract on *in-vivo* antioxidant parameters of the liver in Doxorubicin-induced hepatotoxicity rats

Treatments	MDA (nmol/ mg protein)	GSH (µg/mg /protein)	SOD (U/mg protein)	Vitamin C (µmol/mg/ protein)
Distilled water	0.71±0.05	19.97±0.6	28.85±0.87	8.80±0.20
DOX+ Distilled water	8.88±0.27#	1.73±0.11#	2.50±0.15#	0.77±0.05#
DOX+CAPE 100 mg/kg	3.70±1.62*	3.25± 1.12*	9.83±0.32*	3.03±0.09*
DOX+CAPE 200 mg/kg	3.12±0.18*	6.81±0.22*	13.27±0.57*	4.08±0.18*
DOX+CAPE 400 mg/kg	2.27±0.09*	9.18±0.39*	17.96±0.91*	5.23±0.28*
DOX+ α-Lipoic acid 150 mg/kg	1.57±1.68*	12.43±0.63*	19.1±1.62*	7.38±0.5*

Results are expressed as mean ± SEM of 10 rats; #=Significantly different ($p<0.05$) from the Distilled water group; *=Significantly different ($p<0.05$) from the DOX + Distilled water

Histological evaluation of the curative effects of *Citrus aurantifolia* peels extract in Doxorubicin-induced liver toxicity

Histological examination (Figure 3) revealed normal hepatic architecture with closely packed hepatocytes and mild Kupffer cell hyperplasia (KCH) in the distilled water group. The DOX + distilled water group showed mild vacuolar degeneration, random single-cell necrosis, and mild KCH, indicating hepatocellular damage. CAPE-treated groups exhibited dose-dependent improvement: mild to moderate hepatocellular changes at 100–200 mg/kg, and restoration of normal architecture with reduced necrosis and pigmentation at 400 mg/kg. The DOX + Alpha-lipoic acid group showed near-normal hepatic structure with mild KCH.

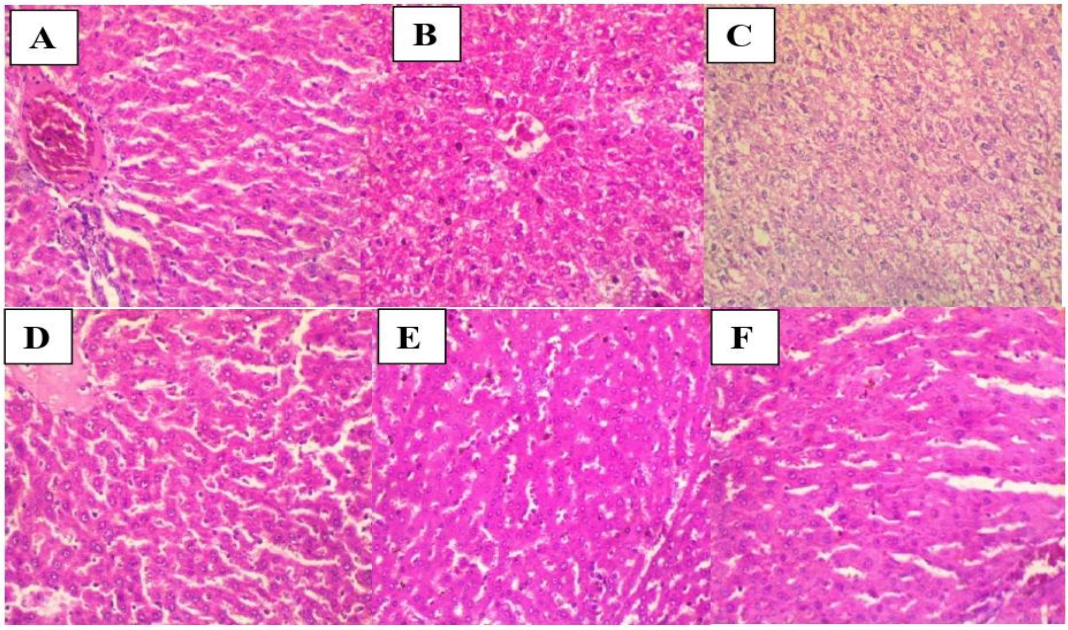

Figure 3: Histological evaluation of the curative effects of *Citrus aurantifolia* peels extract in Doxorubicin-induced liver toxicity

Photomicrographs of liver sections (H&E, ×400). (A) Distilled water shows normal hepatic architecture with mild Kupffer cell hyperplasia (KCH). (B) DOX + Distilled water exhibits vacuolar degeneration and single-cell necrosis. (C–E) CAPE (100–400 mg/kg) shows dose-dependent improvement with reduced necrosis and restoration of hepatic cords. (F) Alpha-lipoic acid shows near-normal hepatic structure with mild KCH.

DISCUSSION

The present study revealed that *Citrus aurantifolia* peels extract (CAPE) exhibits strong hepatoprotective and antioxidant effects against doxorubicin (DOX)-induced liver injury in rats. Acute and sub-acute toxicity assessments indicated that CAPE was safe up to 2000 mg/kg, with no mortality or behavioral abnormalities observed. These findings agree with earlier reports showing that methanolic extracts of *C. aurantifolia* possess low toxicity in rodents (Adegoke *et al.,* 2019; Ibrahim *et al*., 2021). However, mild behavioral changes at 800 mg/kg suggest possible dose-dependent effects. In addition, body weight increased progressively in control and CAPE-treated rats at 200 and 400 mg/kg, indicating normal growth. A higher gain in body weight at 800 mg/kg suggests enhanced anabolism and reduced oxidative stress, consistent with the findings of Chen *et al*. (2022).

Doxorubicin-induced liver injury is primarily mediated through oxidative stress, characterized by excessive reactive oxygen species (ROS) formation and lipid peroxidation (Zhao *et al*., 2021). In this study, DOX treatment significantly increased hepatic malondialdehyde (MDA) level and reduced the activities of antioxidant parameters such as superoxide dismutase (SOD) and glutathione (GSH). Administration of CAPE restored these parameters toward normal values, indicating potent antioxidant activity. Similar antioxidant responses have been reported for citrus flavonoids such as naringenin and hesperidin, which enhance endogenous antioxidant defenses and suppress lipid peroxidation (Ghasemi *et al*., 2018; Sharma *et al*., 2023). Meanwhile, in the sub-acute toxicity study, *Citrus aurantifolia* peels extract treatment groups caused a dose-dependent increase in MDA levels with corresponding increases in GSH, SOD, and vitamin C, indicating enhanced antioxidant defense and reduced oxidative stress. This observation agrees with previous reports that CAPE mitigates oxidative damage and lipid peroxidation (Zhao *et al.,* 2021; Chen *et al*., 2022).

The hepatoprotective effect of CAPE at 400 mg/kg was comparable to that of alpha-lipoic acid, a well-known antioxidant. Comparable restoration of antioxidant balance has been documented for *Moringa oleifera* and green tea extracts in DOX-treated animals, both of which reduced hepatic oxidative stress and preserved tissue integrity (Laftah *et al.,* 2025). Likewise, flavonoids such as quercetin and rutin protect against DOX-induced liver injury by enhancing antioxidant enzyme activities (Sahlan *et al.,* 2021). These findings support the role of *Citrus aurantifolia* peels extract as a natural antioxidant capable of mitigating DOX-induced oxidative damage.

The antioxidant potential of CAPE may be attributed to its rich content of flavonoids, phenolics, and essential oils, particularly limonene, citral, and naringenin, as reported by our previous study (Oyinloye *et al*., 2024), *Citrus aurantifolia* scavenges free radicals and prevent oxidative cell damage (Abdurahman *et al.*, 2020; Ehiowemwenguan *et al*., 2020). The synergistic interaction among these phytochemicals likely contributes to the overall protective effect. AlAsmari *et al*. (2021). similarly reported that diosmin, a citrus flavonoid, attenuated DOX-induced hepatotoxicity by maintaining redox balance and preserving hepatocyte morphology. Furthermore, tannic acid, another phenolic compound, has been shown to protect the liver from DOX toxicity by suppressing oxidative stress and apoptosis (AlAsmari *et al*., 2021).

Histopathological findings supported the biochemical results. The distilled water only and CAPE-treated groups maintained normal hepatic architecture with intact hepatocytes, while the DOX+ distilled water group displayed necrosis, vacuolar degeneration, and sinusoidal dilation, hallmarks of oxidative liver injury (Chen *et al*., 2022). CAPE at 400 mg/kg markedly reduced these lesions, offering protection comparable to alpha-lipoic acid. These observations align with previous studies showing that citrus flavonoids preserve hepatocellular structure by stabilizing mitochondria and reducing ROS generation (Sahlan *et al*., 2021; AlAsmari *et al*., 2021).

Hematological results in sub-acute toxicity study revealed that moderate CAPE doses (200–400 mg/kg) maintained normal blood parameters, whereas the high dose (800 mg/kg) caused mild anemia, possibly due to interference with erythropoiesis or iron metabolism. Similar dose-dependent hematological changes have been observed in animals treated with concentrated citrus extracts (Ghasemi *et al*., 2018). Interestingly, *Citrus aurantifolia* peels extract increased platelet counts across all treatment groups, suggesting possible stimulation of thrombopoiesis, which may enhance recovery under oxidative stress (Emmanuel *et al*., 2023).

Overall, this study demonstrates that *Citrus aurantifolia* peels extract provides significant protection against DOX-induced liver injury through antioxidant-mediated mechanisms. These effects are comparable to those reported for other natural antioxidants such as diosmin and tannic acid (AlAsmari *et al*., 2021). The results further support previous findings that phenolic-rich plant extracts enhance hepatic resistance to xenobiotic-induced damage by modulating redox balance (Newman and Cragg, 2020; Pandey *et al*., 2023). Future research should explore the molecular mechanisms involved, particularly pathways such as Nrf2/Keap1, NF-κB, and MAPK, which regulate oxidative and inflammatory responses (AlAsmari *et al*., 2021).

CONCLUSION

Citrus aurantifolia peels extract exhibited notable hepatoprotective activity against DOX-induced oxidative stress. The findings highlight the therapeutic promise of *Citrus aurantifolia* peels as a natural source of antioxidants for preventing drug-induced hepatotoxicity, and further pharmacological and clinical evaluations are recommended.

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