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Prophylactic and Curative Effects of *Citrus aurantifolia* Peels Extract on Doxorubicin-Induced Ovarian Oxidative Stress and Histopathological Alterations in Female Wistar Rats

E. O. OYINLOYE[1*A-F], A. A. MURTALA[2 A-E], F. A. OLADOJA[1 A-E], O. L. OKUNYE[3 A-D]

[1*]*Department of Pharmacology and Toxicology, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu Campus, Ogun State. Nigeria.*
[2]*Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, Obafemi Awolowo College of Health Sciences, Olabisi Onabanjo University, Sagamu Campus, Ogun State. Nigeria.*
[3]*Department of Pharmaceutical Microbiology, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu Campus, Ogun State. Nigeria.*

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: Doxorubicin (DOX), a potent chemotherapeutic agent, is widely used in treating various malignancies but is limited by its dose-dependent toxicity, particularly in reproductive organs. Ovarian toxicity resulting from DOX administration leads to oxidative stress, follicular atresia, and impaired fertility. Natural antioxidants from medicinal plants have been explored as protective agents against chemotherapy-induced organ damage. *Citrus aurantifolia* (lime) peel contains flavonoids, phenolic acids, and vitamin C, which possess strong antioxidant and anti-inflammatory activities. This study investigated the prophylactic and curative effects of *Citrus aurantifolia* peels extract (CAPE) on DOX-induced ovarian toxicity in female Wistar rats.
Methods: Sixty female Wistar rats were divided into prophylactic and curative experimental models, each with six subgroups (n=5). CAPE (100, 200, and 400 mg/kg) and a reference antioxidant, alpha lipoic acid {ALA}(150 mg/kg), were administered orally for seven days either before or after a single intraperitoneal dose of DOX (15 mg/kg). Ovarian tissues were evaluated for oxidative stress biomarkers, malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), catalase, and vitamin C and examined histologically. Statistical analysis was conducted using ANOVA with significance set at $p < 0.05$.
Results: DOX administration significantly increased MDA levels while decreasing GSH, SOD, catalase, and vitamin C concentrations. CAPE markedly reversed these alterations in a dose-dependent manner, with the 400 mg/kg dose demonstrating comparable effects to alpha lipoic acid. Histopathological analysis revealed restoration of normal follicular structure, reduced edema, and improved ovarian morphology, especially in prophylactic groups.
Conclusion: CAPE exhibited significant prophylactic and curative antioxidant and histoprotective effects against DOX-induced ovarian damage, suggesting its potential as a natural adjunct for preserving female fertility during chemotherapy.
Keywords: *Citrus aurantifolia*, Doxorubicin, Ovarian toxicity, Antioxidant, Prophylactic, Curative, Oxidative stress.

INTRODUCTION

The advent of modern chemotherapy has significantly improved survival outcomes in many cancers; however, the clinical benefits of agents such as doxorubicin (DOX) are accompanied by notable off target toxicities, including adverse effects on the female reproductive system. Ovarian injury resulting from chemotherapy is now widely recognised as a major cause of infertility and premature ovarian insufficiency among female cancer survivors (Spears *et al*., 2019). Doxorubicin, an anthracycline commonly used in the treatment of breast cancer, lymphoma, and other solid tumours, has been reported to reduce ovarian size and weight, deplete follicular reserves, and impair ovulation in experimental models (Ben-Aharon *et al*., 2010; Nishi *et al*., 2018). For instance, Zhang *et al*. (2017) demonstrated that a single dose of DOX in mice induced follicular apoptosis, reduced ovulation rates, and caused persistent reductions in ovarian size one month after exposure. Although these studies clearly establish DOX as a potent gonadotoxic agent, much of the literature remains largely descriptive, with limited emphasis on evaluating interventions capable of preventing or reversing this damage.

Mechanistically, DOX-induced ovarian toxicity involves multiple pathways, with oxidative stress playing a central role. Excessive generation of reactive oxygen species (ROS) leads to lipid peroxidation, DNA damage, and activation of apoptotic pathways within ovarian tissue (dos Santos Silva *et al*., 2023; Spears *et al*., 2019). Granulosa cells and early-stage follicles appear particularly susceptible to these effects, which may accelerate follicular depletion and compromise ovarian reserve (Spears *et al*., 2019). In addition, chemotherapy has been shown to disrupt ovarian blood supply, induce stromal injury, and promote fibrotic changes, all of which further impair ovarian function (Zhang *et al*., 2017). These mechanistic insights provide a strong biological basis for exploring antioxidant-based strategies as potential protective approaches against chemotherapy-induced ovarian damage.

In recent years, increasing attention has been given to the use of natural antioxidants as adjuncts for mitigating chemotherapy related toxicities. Citrus peel-derived extracts have attracted particular interest due to their reported antioxidant, anti-inflammatory, and cytoprotective properties across different biological systems (Li *et al*., 2024; Oyinloye *et al*., 2024). The peels of *Citrus aurantifolia* are rich in flavonoids, phenolic compounds, vitamin C, and other bioactive constituents known to scavenge free radicals, enhance endogenous antioxidant defenses, and modulate inflammatory pathways (Li *et al.,* 2024; Oyinloye *et al*., 2024). Supporting this, Sandhiutami *et al*. (2024) reported that C. aurantifolia peel extract attenuated DOX-induced renal toxicity by reducing malondialdehyde (MDA) levels and increasing the activities of superoxide dismutase (SOD) and catalase. However, most investigations of citrus-derived products have focused predominantly on hepatic, renal, and cardiovascular models, with relatively little attention given to the female reproductive system, despite its high vulnerability to chemotherapeutic injury.

Evidence for the ovarian-protective potential of *C. aurantifolia* remains limited. Oboma *et al*. (2020) demonstrated that lime juice extract ameliorated cadmium-induced ovarian toxicity in rats, suggesting that the plant possesses bioactivity relevant to ovarian protection. Nonetheless, this study was conducted in a heavy metal toxicity model rather than a chemotherapeutic context, and it utilised juice rather than peel, which is generally considered to contain higher concentrations of polyphenolic compounds. Consequently, a clear gap remains regarding whether citrus peel extracts can effectively protect ovarian tissue against DOX-induced oxidative and structural damage.

In addition to this knowledge gap, the timing of intervention represents an important yet underexplored dimension in studies of chemotherapy induced gonadotoxicity. From a clinical perspective, two distinct scenarios commonly arise in oncology practice. In some cases, fertility preservation strategies or protective agents may be administered before or alongside chemotherapy, with the goal of preventing or minimising ovarian injury. In other situations, patients seek intervention only after chemotherapy exposure, when ovarian damage may already be established and therapeutic options are aimed at mitigating ongoing dysfunction or promoting recovery. Despite their differing clinical implications, few experimental studies have systematically evaluated both preventive and post-injury treatment strategies within the same model.

In light of these considerations, the present study was designed to investigate both the prophylactic (pre-treatment) and curative (post-treatment) effects of a methanolic extract of *Citrus aurantifolia* peels extract (CAPE) in female Wistar rats exposed to doxorubicin. The study evaluated key ovarian oxidative stress markers, including MDA, GSH, SOD, catalase, and vitamin C, alongside histopathological assessment of ovarian architecture, such as follicular development, corpora lutea, and stromal integrity. Alpha-lipoic acid (ALA) was included as a reference antioxidant to enable comparative evaluation of efficacy. By focusing specifically on ovarian outcomes and by comparing the effects of treatment timing, this study seeks to provide a

more comprehensive preclinical assessment of the potential relevance of citrus peel-derived antioxidants in the context of chemotherapy associated ovarian injury.

METHODOLOGY

Materials
Drugs, Chemicals, and Reagents

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, USA); and methanol (Sigma Chemical Co., 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 grade.

Preparation of Plant Material

The peels of *Citrus aurantifolia* were removed, shade dried at room temperature (27–30°C) for seven days to constant weight, and coarsely powdered using a mortar and pestle, followed by pulverization with 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 filtered and concentrated on a water bath at 60°C. Fresh solutions of the extract were prepared for each experimental use.

Experimental Animals

Female Wistar rats (120–140 g) were obtained from the Animal House Facility, College of Medicine, University of Lagos. The animals were housed under standard laboratory conditions with free access to feed and water and were allowed to acclimatize for two weeks. The ethical approval was obtained from the College of Medicine's Animal Care and Use Research Ethics Committee (CMUL/ACUREC)., University of Lagos (Approval No.: (CMUL/ACUREC/06/22/1116).

Experimental Design

Evaluation of Prophylactic Effects of CAPE in Doxorubicin-Induced Ovary toxicity

Thirty female rats were divided into six (6) groups (n=5). Distilled water only, Distilled water, CAPE (100, 200, 400mg/kg) and alpha lipoic acid {ALA} (150 mg/kg) were administered orally respectively to all the groups for seven (7) days. On day 8, group II, III, IV V, and VI received Doxorubicin (mixed with normal saline, 15 mg/kg i.p.)

Group I = Distilled water D/Water) (only)
Group II = Distilled water (D/Water) + DOX 15 mg/kg)
Group III = CAPE 100 mg + DOX 15 mg/kg
Group IV = CAPE 200 mg + DOX 15 mg/kg
Group V = CAPE 400 mg + DOX 15 mg/kg
Group VI = Alpha lipoic acid (150 mg/kg) + DOX 15 mg/kg

Evaluation of Curative Effects of CAPE in Doxorubicin-Induced Ovary toxicity

Thirty female rats were divided into six (6) groups (n=5). Distilled water, Doxorubicin (mixed with normal saline, 15 mg/kg i.p.) were administered to rats in groups II, III, IV and V, group VI. Twenty-four (24) hours after, group I to VI received Distilled water, Distilled water, CAPE (100, 200, 400 mg/kg) and alpha lipoic acid (150 mg/kg) for a period of seven (7) days, respectively.

Group I = Distilled water (D/Water) (only)
Group II = DOX 15 mg/kg + Distilled water (D/Water)
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)

Treatments were administered for seven days. Twenty-four (24) hours after Doxorubicin and CAPE administration in the prophylaxis and curative studies, respectively, animals were anesthetized, and ovarian tissues were collected for antioxidant and histological analyses. The ovaries were blotted on filter paper, rinsed with normal saline, and preserved in 10 % formalin for histological examination.

In Vivo Antioxidant Assays

The harvested ovaries were rinsed in ice-cold 1.15 % KCl, dried and weighed. The tissues were homogenised in four volumes of 50 mM phosphate buffer (pH 7.4) using a Potter Elvehjem homogeniser and centrifuged at 10,000 x g for 15 minutes to obtain post-mitochondrial supernatant (PMS), which was used for the determination of Malondialdehyde (MDA), reduced glutathione (GSH), catalase, Superoxide dismutase (SOD) and Vitamin C levels using established methods.

The ovarian samples for histology were excised, rinsed in normal saline, blotted on filter paper and fixed in 10 % formalin. All procedures were done at a temperature of 4^0C.

Histopathological Examination

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

Statistical Analysis

The experimental data were presented as Mean ± standard error of the 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

Prophylactic effects of *Citrus aurantifolia* peels on Antioxidant Parameters in doxorubicin-induced ovarian toxicity in rats.

Doxorubicin administration led to a marked ($p<0.001$) suppression of all antioxidant defense markers GSH, catalase, SOD, and vitamin C, between the groups administered with control (D/Water) and (DW+ DOX) groups. CAPE (200 and 400 mg/kg) and alpha lipoic acid significantly ($p<0.001$) reduced the MDA levels, respectively, relative to distilled water+ DOX. *Citrus aurantifolia* peels extract effectively counteracted DOX-induced oxidative stress in (DW+ DOX) group, restoring ($p<0.001$) SOD, catalase, GSH, vitamin C, in CAPE and α-lipoic acid relative to distilled water plus DOX (Table 1).

Table 1: Prophylactic effects of *Citrus aurantifolia* peels on Antioxidant Parameters in doxorubicin induced ovarian toxicity in rats.

Treatments	MDA (nmol/ mg protein)	GSH (µg/mg protein)	Catalase (U/mg protein)	SOD (U/mg protein	Vitamin C (µmol/mg protein)
D/Water	0.19±0.22	18.70±0.03	18.23±1.20	22.45±0.13	8.43±0.43
D/Water+ DOX	8.11±0.13$^{\#}$	1.58±0.05$^{\#}$	1.93±0.06$^{\#}$	2.28±0.07$^{\#}$	0.70±0.02$^{\#}$
CAPE 100 mg/kg+ DOX	5.01±0.10***	2.15±0.06	4.24±0.03*	3.55±0.16	1.07±0.32
CAPE 200 mg/kg+ DOX	3.75±0.03***	5.98±0.2***	7.31±0.03***	8.64±0.11***	
2.66±0.01***					
CAPE 400 mg/kg+ DOX	2.66±0.01***	8.43±0.07***	10.30±0.09***	12.18±0.10***	
3.75±0.03***					
ALA 150 mg/kg+ DOX	0.70±0.02***	10.89±0.06***	13.78±0.03***	16.29±0.33***	
5.01±0.10***					

Legend: D/Water = Distilled water; DOX=Doxorubicin; ALA= Alpha lipoic Acid
Results were expressed as the mean ± SEM of 5 rats; $^{\#}P<0.001$ vs D/Water; $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ vs DOX+ D/Water

Curative effects of *Citrus aurantifolia* peels on Antioxidant Parameters in doxorubicin induced ovarian toxicity in rats

Doxorubicin caused a significant ($p<0.001$), increase in MDA, relative to distilled water administered group. In contrary, CAPE significantly and dose-dependently reduced MDA levels, comparable to alpha lipoic acid at 400 mg/kg ($p < 0.001$). GSH, Catalase, SOD, Vitamin C

levels were markedly (p< 0.001) increased by CAPE, with significant (p< 0.001), increase with dose. CAPE 400 mg/kg nearly matched α-lipoic acid in restoring antioxidant balance (Table 2).

Table 2: Curative effects of *Citrus aurantifolia* peels on Antioxidant Parameters in doxorubicin-induced ovarian toxicity in rats

Treatments	MDA (nmol/ mg protein)	GSH (µg/mg protein)	Catalase (U/mg protein)	SOD (U/mg protein	Vitamin C (µmol/mg protein)
D/Water	0.88±0.02	22.79±0.23	27.85±0.28	32.92±0.33	10.13±0.10
DOX+D/Water	10.13±0.10$^{\#}$	1.98±0.05$^{\#}$	2.42±0.05$^{\#}$	2.86±0.06$^{\#}$	0.88±0.02$^{\#}$
DOX+CAPE 100 mg/kg	6.30±0.10***	7.77±0.09***	9.50±0.10***	11.23±0.12***	3.45±0.04***
DOX+CAPE 200 mg/kg	4.6±0.07***	10.48±0.15***	12.81±0.18***	15.14±0.2***	4.88±0.07***
DOX+CAPE 400 mg/kg	3.45±0.04***	14.1±0.24***	17.34±0.29***	20.49±0.35***	6.30±0.11***
DOX+ALA 150 mg/kg	3.32±0.19***	15.12±0.43***	18.48±0.52***	21.83±0.62***	6.72±0.19***

Legend: D/Water = Distilled water; DOX=Doxorubicin; ALA= Alpha lipoic Acid
Results were expressed as the mean ± SEM of 5 rats; $^{\#}P$<0.001 vs D/Water; $^{***}P$<0.001 vs DOX+ D/Water

Prophylactic effects of C*itrus aurantifolia* peels on Histological examination in doxorubicin induced Ovarian toxicity in rats.

Histological examination revealed normal ovarian architecture with well-developed follicles and corpora lutea in the DW group. The D/Water + DOX group showed marked follicular degeneration and moderate inflammatory infiltration. Treatment with CAPE (100 mg/kg) moderately restored follicular growth and corpora lutea integrity, while CAPE+DOX (200–400 mg/kg) groups exhibited near-normal ovarian structure with evident fluid-filled corpora lutea. The ALA + DOX group demonstrated well-preserved follicles and corpora lutea, comparable to the control, indicating strong ovarian protection (Figure 1-A, B, C, D, E and F).

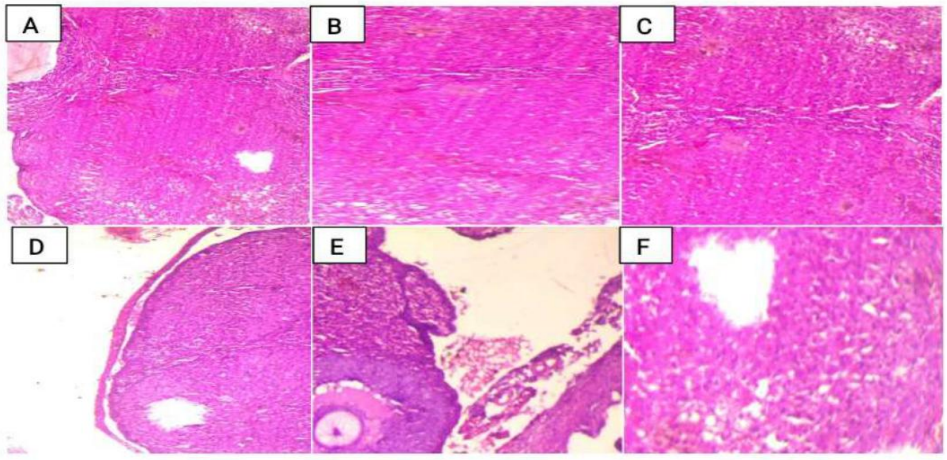

Figure 1: Prophylactic effects of C*itrus aurantifolia* peels on the histological examination in Doxorubicin-induced ovarian toxicity in rats

Representative photomicrographs of ovarian sections (H&E, ×400). A: D/Water group showing normal ovarian architecture with growing primary follicles and corpora lutea containing fluid-filled cavities. B: D/Water + DOX group showing severe follicular degeneration and moderate inflammatory infiltration. C: CAPE + DOX (100 mg/kg)

group showing moderate follicular growth and partially preserved corpora lutea. D–E: CAPE + DOX (200–400 mg/kg) groups showing progressive restoration of ovarian structure with near-normal follicles and corpora lutea. F: ALA + DOX group showing well-developed follicles and corpora lutea comparable to the control, indicating strong ovarian protection (Figure 1-A, B, C, D, E and F).

Curative effects of C*itrus aurantifolia* peels on Histological examination in doxorubicin induced ovarian toxicity in rats.

Histological analysis showed normal ovarian architecture in the DW group, with numerous growing follicles and corpora lutea containing fluid-filled cavities. The DOX + D/Water group exhibited distorted follicles and moderate inflammatory infiltration, indicating severe cytotoxic damage. Treatment with DOX+CAPE (100–400 mg/kg) showed dose-dependent restoration, with improved follicular development and presence of healthy corpora lutea. The DOX+200 mg/kg group showed mild recovery, while 400 mg/kg revealed better structural preservation. The DOX + ALA group displayed nearly normal ovarian histology, comparable to control, confirming strong antioxidant protection (Figure 2-G, H, I, J, K and L).

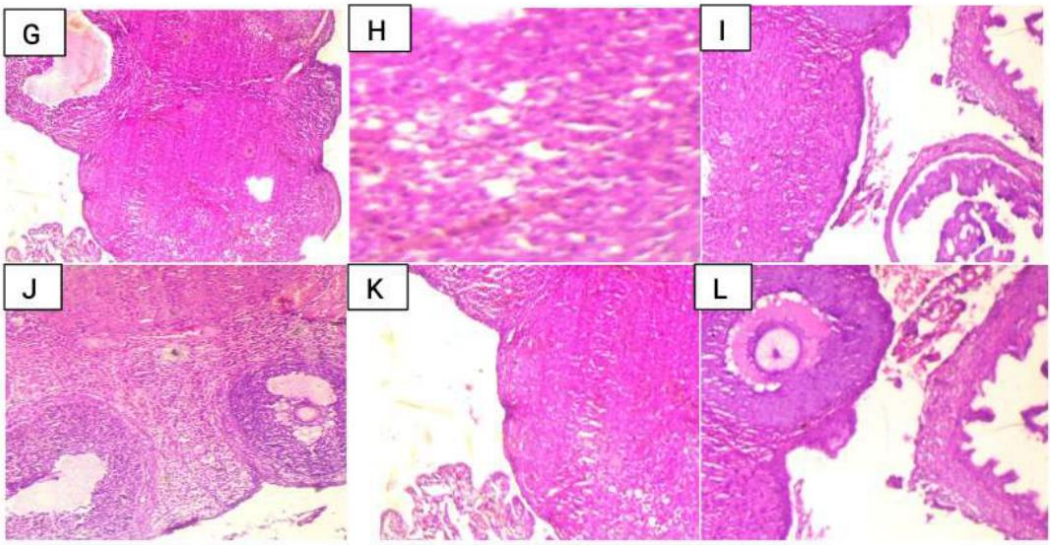

Figure 2: Curative effects of C*itrus aurantifolia* peels on Histological examination in Doxorubicin-induced ovarian toxicity in rats

Representative photomicrographs of ovarian sections (H&E, ×400). G: D/Water group showing normal follicles and corpora lutea with fluid-filled cavities. H: DOX + D/Water group showing follicular degeneration and inflammatory infiltration. I: DOX + CAPE (100 mg/kg) showing moderate follicular recovery. J: DOX + CAPE (200 mg/kg) showing mild restoration of ovarian structure. K: DOX + CAPE (400 mg/kg) showing near-normal follicles and corpora lutea. L: DOX + ALA showing well-preserved ovarian architecture similar to control (Figure 2-G, H, I, J, K and L).

DISCUSSION

The present study evaluated the prophylactic and curative effects of *Citrus aurantifolia* peels extract (CAPE) against doxorubicin (DOX) induced ovarian toxicity in female Wistar rats. The findings showed that DOX administration produced marked oxidative stress and histological alterations in ovarian tissue, consistent with earlier reports on DOX associated gonadotoxicity (Spears *et al*., 2019; Zhang *et al*., 2017). Treatment with CAPE was associated with improvement in antioxidant status and ovarian histoarchitecture, with effects comparable to those observed with α-lipoic acid, a standard reference antioxidant.

The untreated DOX group demonstrated increased malondialdehyde (MDA) levels alongside reductions in glutathione (GSH), catalase, superoxide dismutase (SOD), and vitamin C, reflecting increased lipid peroxidation and weakened antioxidant defenses. These

findings are consistent with existing evidence that oxidative stress plays a central role in DOX-induced tissue injury (dos Santos Silva *et al*., 2023). The mechanism is thought to involve redox cycling of the quinone moiety of DOX, leading to the generation of reactive oxygen species (ROS) that can induce mitochondrial dysfunction, DNA damage, and apoptosis in granulosa and theca cells (Spears *et al*., 2019; Nishi *et al.,* 2018).

Histopathological observations supported the biochemical findings, as ovaries from DOX + D/water exhibited follicular degeneration, interstitial edema, and inflammatory cell infiltration. Similar pathological changes have been reported by Ben-Aharon *et al.* (2010) and Zhang *et al*. (2017), who showed that DOX preferentially affects actively growing follicles, contributing to follicular loss and impaired ovarian function. Histological examination further showed that ovaries from CAPE-treated rats exhibited relatively preserved follicular structures, with reduced inflammatory changes compared with the DOX + D/water group. The higher dose of CAPE (400 mg/kg) produced effects comparable to α-lipoic acid. Similar ovarian-related protective observations have been reported in a cadmium-induced toxicity model using *C. aurantifolia* preparations (Oboma *et al*., 2020), although the experimental context differed from the present study.

Administration of *Citrus aurantifolia* peels extract attenuated DOX-induced oxidative alterations in a dose dependent manner. Reductions in MDA levels and improvements in GSH, catalase, SOD, and vitamin C suggest that the extract may support endogenous antioxidant defense mechanisms. The antioxidant potential of CAPE is plausibly linked to its phytochemical composition, as *C. aurantifolia* peels are known to contain flavonoids (e.g., hesperidin and naringenin), phenolic compounds, and ascorbic acid, which possess established antioxidant properties (Li *et al*., 2024; Oyinloye *et al*., 2024).

Flavonoids have been reported to limit lipid peroxidation, chelate transition metals, and modulate antioxidant enzyme activity, potentially through pathways such as Nrf2 signaling (Sandhiutami *et al*., 2024). The elevated GSH levels observed in CAPE treated groups may indicate improved redox balance within ovarian tissue. These findings are consistent with previous reports describing antioxidant related protective effects of citrus extracts in models of DOX-induced hepatic and renal toxicity (Oyinloye *et al.*, 2024; Li *et al*., 2024).

Comparison of the prophylactic and curative protocols indicated that both treatment schedules were associated with measurable improvements in biochemical and histological parameters. The slightly better outcomes observed with prophylactic administration may suggest that prior enhancement of antioxidant defenses could reduce susceptibility to DOX induced oxidative damage. However, the improvements observed in the curative model also indicate that CAPE may influence recovery processes following established injury. These interpretations should be considered cautiously and warrant further mechanistic investigation.

The protective effects observed in this study may be linked to multiple complementary mechanisms, including free radical scavenging, support of endogenous antioxidant systems, and modulation of inflammatory pathways. Previous studies have suggested that citrus-derived phytochemicals can influence cytokine signaling and pathways such as NF-κB (Li *et al*., 2024; Sandhiutami *et al*., 2024), which could contribute to reduced tissue damage. However, these mechanisms were not directly examined in the present work and therefore remain speculative.

From a translational perspective, these findings suggest that *Citrus aurantifolia* peels extract may have potential for further investigation as a candidate for reducing chemotherapy-associated ovarian damage in preclinical settings. However, the present results are limited to an animal model, and no pharmacokinetic, toxicological, or drug–plant interaction assessments were performed. In addition, ovarian oxidative stress markers and histological features are known to vary across the estrous cycle; however, estrous cycle monitoring or synchronization was not performed in this study, which represent a potential limitation and should be considered when interpreting the findings. Therefore, conclusions regarding clinical applicability or fertility preservation in humans should be drawn with caution.

CONCLUSION

The findings of this study demonstrate that methanolic extract of *Citrus aurantifolia* peels provides substantial protection against doxorubicin-induced ovarian oxidative stress and histological damage in female Wistar rats. CAPE effectively reduced lipid peroxidation, enhanced antioxidant enzyme activities, and preserved ovarian follicular integrity. Both prophylactic and curative treatments were beneficial, though the prophylactic regimen showed slightly greater efficacy. The antioxidant and cytoprotective properties of *C. aurantifolia* are attributed to its bioactive constituents particularly flavonoids and ascorbic acid that modulate oxidative and inflammatory pathways.

Thus, CAPE represents a promising natural therapeutic candidate for mitigating chemotherapy-induced gonadotoxicity. Future studies should focus on isolating specific active molecules, exploring molecular pathways, and conducting clinical trials to validate these preclinical observations.

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**Address for correspondence: Elijah Oladapo Oyinloye*

Department of Pharmacology and Toxicology, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu Campus, Ogun State, Nigeria,

Telephone: +2348039196240 & +2349020531914,
E-mail: oyinloye.oladapo@oouagoiwoye.edu.ng
OCID ID: 0000-0003-2094-1855.

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