International Journal for Multidisciplinary Research (IJFMR)
E-ISSN: 2582-2160 ● Website: www.ijfmr.com ● Email: editor@ijfmr.com

Histomorphological Effects of Vitamin E on Doxorubicin Induced- Skeletal Muscle Toxicity in A Male Wistar Rat Model

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ABSTRACT

Background: Doxorubicin-induced skeletal muscle toxicity leads to muscle atrophy, inflammation, necrosis, and fibrosis, resulting in weakness, fatigue, and impaired quality of life in cancer patients. Vitamin E has antioxidant properties that may offer protective effects, but its role in mitigating doxorubicin-induced muscle toxicity remains unclear.

Aim: This study evaluated the histomorphological protective effects of Vitamin E against doxorubicin-induced skeletal muscle toxicity in male Wistar rats, focusing on anatomical cross-sectional area (ACSA), inflammation, and muscle fascicle integrity.

Methods: A randomized controlled trial was conducted using 42 male Wistar rats. The main treatment group (n = 33) was divided into three subgroups: Negative Control, Doxorubicin-only, and Doxorubicin + Vitamin E. Vitamin E was administered orally from day 1, while doxorubicin was introduced on day 28. ACSA was measured pre- and post-treatment using high-frequency ultrasound, and tibialis anterior muscle samples were analyzed histologically. Non-parametric statistical tests were used, with significance set at $p < 0.05$.

Results: Significant within-group ACSA differences were observed ($p < 0.005$), with high between-group significance ($p < 0.001$). Inflammation scores were significantly elevated in the Doxorubicin group ($p = 0.035$) but reduced in the Doxorubicin + Vitamin E group ($p = 0.001$). Muscle fascicle integrity remained unchanged.

Conclusion: This study provides significant insights into the protective role of Vitamin E against histomorphological changes in skeletal muscle tissue induced by Doxorubicin. The findings suggest that Vitamin E partially mitigates muscle atrophy by preserving the anatomical cross-sectional area of the tibialis anterior muscle. Additionally, it reduces the inflammatory response, thereby improving overall tissue integrity. However, its effect on muscle fascicle distortion remains inconclusive, indicating the need for further investigation. These results highlight the potential of Vitamin E as a therapeutic adjunct in Doxorubicin-induced skeletal muscle toxicity, warranting additional research on its optimal dosage and efficacy in clinical settings.

Keywords: Skeletal Muscle Toxicity, Doxorubicin, Vitamin E, Inflammation, Histoprotective

INTRODUCTION

Doxorubicin is a widely used chemotherapeutic agent for various cancers, including leukemia, breast, lung, and esophageal carcinomas (Zhang et al., 2021; Wang et al., 2022). Despite its efficacy, its clinical utility is hindered by severe side effects, including cardiotoxicity and skeletal muscle toxicity (Amal et al., 2018). Doxorubicin-induced skeletal muscle toxicity manifests as inflammation, fibrosis, necrosis, and muscle atrophy, leading to weakness, fatigue, and impaired mobility, ultimately reducing patients' quality of life (Campelj et al., 2021).

The mechanisms underlying doxorubicin-induced muscle toxicity involve oxidative stress, mitochondrial dysfunction, inflammation, and impaired muscle regeneration. Reactive oxygen species (ROS) accumulation plays a critical role in this process, leading to cellular damage. Given these challenges, research has increasingly focused on antioxidants as potential protective agents against doxorubicin-induced toxicity (Mary et al., 1992; Abullaev F et al., 2000; Shivakumar et al., 2012).

Vitamin E, a lipid-soluble antioxidant, has demonstrated cardioprotective effects against doxorubicin-induced oxidative stress. However, its role in mitigating skeletal muscle toxicity remains underexplored (Van et al., 2009). Preclinical studies suggest that Vitamin E may reduce oxidative damage, modulate inflammation, and support muscle regeneration (Van et al., 2009). The need for effective interventions to mitigate doxorubicin-induced skeletal muscle toxicity underscores the significance of this research.

Cancer patients undergoing doxorubicin-based chemotherapy frequently experience muscle weakness and fatigue, significantly impacting their treatment response and overall well-being. While extensive research has explored doxorubicin-induced toxicity in cardiac and hepatic tissues, limited studies have examined its effects on skeletal muscle. Furthermore, the histomorphological changes in skeletal muscle following doxorubicin exposure, particularly in the presence of Vitamin E, remain inadequately investigated. Addressing this knowledge gap is crucial for optimizing chemotherapy regimens and improving patient outcomes. Investigating the potential protective effects of Vitamin E on skeletal muscle may provide insights into novel therapeutic strategies to counteract chemotherapy-induced muscle toxicity.

Doxorubicin-induced skeletal muscle toxicity is a significant concern in oncology, particularly in low-resource settings where supportive care options are limited (Campelj et al., 2021). Understanding the protective role of Vitamin E in this context could lead to improved management strategies, ultimately enhancing cancer patients' quality of life. This study aimed at bridging the knowledge gap by providing histomorphological evidence of Vitamin E's impact on doxorubicin-induced skeletal muscle toxicity. Vitamin E could serve as an adjunct therapy to reduce muscle-related side effects in chemotherapy patients. The findings may also contribute to future clinical research, informing potential human applications.

This study aimed at answering the question: What are the histomorphological effects of Vitamin E on doxorubicin-induced skeletal muscle toxicity? It is hypothesized that there is a significant difference in skeletal muscle histomorphology among the study groups when Vitamin E is introduced as a potential protective agent.

To investigate this, the study aimed to assess the effect of Vitamin E on the anatomical cross-sectional area of the tibialis anterior muscle, evaluate its impact on the inflammatory response, and determine its role in preserving muscle fascicle integrity following doxorubicin administration. The research was conducted using a male Wistar rat model, focusing exclusively on the histomorphological effects of Vitamin E without exploring other doxorubicin side effects.

Although the study did not encounter significant limitations, findings from animal models may require further validation in human clinical settings. Nonetheless, this research has the potential to provide novel insights into mitigating doxorubicin-induced skeletal muscle toxicity and improving the quality of life for cancer patients undergoing treatment.

MATERIALS AND METHODS

Materials.

Laboratory Materials and Drugs

The study utilized the following materials; male Wistar rats, Doxorubicin Hydrochloride 50mg (Metodox 50, lyophilized and red in color), Manufacturer's license no. 752, batch no. 304105, manufactured date July 2023, expiry date June 2025, manufactured by Neon Laboratories, India; Vitamin E capsules USP 400mg (EDGE 400mg, green in color), Zambia's license number 285/008, batch no. IG-001, manufactured date November 2023, expiry date October 2025, manufactured by Elder Project Limited; Chloroform (clear and volatile liquid), Manufacturer's license no. 1023/567, batch no. CH-89045, manufactured date August 2023, expiry date July 2025, manufactured by Chemo Labs, India; Dental Lignocaine (Lignocaine Hydrochloride Injection USP 2%, clear and colorless solution), Manufacturer's license no. 9801/432, batch no. DL-202310, manufactured date October 2023, expiry date September 2025, manufactured by Prime Pharmaceuticals, India, Sunflower oil, biopsy needles 14G and 16G; ultrasound equipment; histological reagents; and laboratory instruments/equipment, including microscopes.

Methods

Animal selection and group allocation

105 male Wistar rats aged between 14 and 16 weeks and weighing between 90mg and 225mg were purchased from the Biological and Veterinary departments, University of Zambia. All the 105 male Wistar rats were subjected to biopsies, and 72 survived. The time frame for recruitment and healing from biopsies caused wounds was 4 weeks. At the age between 18 to 20 weeks of the male wistar rats, convenience sampling was used to select 42 rats from the large population of the male wistar rats that survived the biopsies. Out of these, 9 rats were assigned to a reservoir group using simple randomization to account for unexpected losses. The remaining 33 rats were divided into three treatment groups through simple randomization.

Housing Conditions

The rats were housed in a clean, well-ventilated room measuring 3 x 3 meters, maintained at an optimal temperature of 22 ± 2°C and a humidity level of 40-60%. The cages, measuring 30 x 20 x 15 cm, provided adequate space for up to 11 rats per cage, minimizing overcrowding and allowing for natural behaviors. Water and commercial food pellets were supplied to meet their nutritional needs. The health and well-being of the rats were closely monitored by a veterinary officer specialized in small animals, with regular checks for signs of distress, injury, or illness.

Baseline Measurements for all the three specific objectives (pre-treatment)

Prior to treatment, the anatomical cross-sectional area (ACSA) of the tibialis anterior muscle was measured by the principle investigator and a specialized personnel for baseline parameters. The measurement served as a reference point for evaluating changes post-treatment. Each rat was weighed and labeled before measurements. The area around the tibialis anterior muscle was shaved for ultrasound measurements. A High-frequency linear (HFL) 38 MHz probe, commonly used for superficial imaging,

including small animal studies and set at 13-6 inch venous for vascular assessments was used. Reference points for pre- and post-treatment measurements were established using anatomical landmarks, specifically the midpoint between the knee joint and the ankle, to ensure consistency. The larger probe provided a broader field of view, enhancing the assessment of muscle morphology while maintaining high-resolution imaging. Baseline biopsies were collected for histological examinations using a 16G and 14G biopsy needle

Table 1: Treatment Groups

Group Description	Treatment Plan
A (Negative Control)	Water for injection (WFI)2ml/kg IP on alternative days in the last days of the 42 days treatment period
B (Doxorubicin)	Doxorubicin 4mg/kg IP, alternative days for 14 days in the last 14 days of the 42 days treatment period
C (Doxorubicin + Vitamin E)	Vitamin E 500mg/kg/day oral gavage for 42 days, Doxorubicin 4mg/kg IP, alternative days for 14 days in the last 14 days of the 42 days treatment

Post-treatment Measurements and examinations for all the specific objectives

After the treatment period (42 days), the following post-treatment assessments were conducted:

1. **ACSA Re-measurement**: The ACSA of the tibialis anterior muscle was re-evaluated using a high frequency linear probe by a specialized personnel.
2. **Histological Examination**: Muscle samples were collected, fixed in 10% formalin, processed, and stained for microscopic evaluation.
3. **Scoring of Inflammation and muscle fascicle Distortion**: This was done by the principle investigator and two independent pathologists using a skeletal muscle multiparametric scoring system developed and validated by Gallet et al.,2011 for the degree of inflammation and by Clara et al.,2023 for degree of muscle fascicle distortion was graded

Data Analysis Plan

Overview of the Statistical Methods Used for the Analysis

The statistical methods applied in this study were strategically chosen to analyze anatomical cross-sectional area (ACSA), inflammation scores and muscle fascicle distortion across different treatment groups. These methods accounted for the non-normal distribution of data for inflammatory scores, small sample size for ACSA which had normally distributed data, and the study's specific objectives. Non-parametric tests, regression analyses, and graphical visualizations were employed to ensure robust and interpretable findings.

Comparisons among the groups

The Kruskal-Wallis test was used to compare the outcomes among the groups. Post hoc pairwise comparisons with Bonferroni correction were conducted to identify specific group-level differences.

Within-group Comparisons

The Wilcoxon signed-rank test was used to assess differences between the baseline and at the sixth week, given the non-parametric distribution of data.

Causal –effect relationship analysis

Ordinal logistic regression analysis was used to explore the causal- effect relationship between exposure (doxorubicin and doxorubicin plus Vitamin E) and outcome (ACSA, degree of inflammation and fibre distortion), while controlling for a number of confounding factors simultaneously. Regression coefficients were used to estimated and determine the strength and direction of the association. All statistical tests were two sided with the significance set at p-value <0.05 and 95% confidence interval. All continuous variables were expressed as medians (range). In terms of visualization, the data was presented through photomicrograph, table format, box plot and bar charts. The SPSS software (version 29.0; IBM, Chicago, IL) was utilized for all statistical analyses.

3.11. Ethical considerations

Permission was obtained from the Department of Paraclinical Sciences, School of Veterinary Medicine, and University of Zambia, to purchase the male Wistar rats and conduct tissue processing/sample analysis. The current study was approved and cleared by the University of Zambia Biomedical Research Ethic Committee (UNZABREC) and the National Health Research Authority (NHRA) respectively.

The rats were housed in a clean, well-ventilated room measuring 3 x 3 meters, maintained at an optimal temperature of 22 ± 2°C and a humidity level of 40-60%. The cages, measuring 30 x 20 x 15 cm, provided adequate space for up to 11 rats per cage, minimizing overcrowding and allowing for natural behaviors. Water and commercial food pellets were supplied to meet their nutritional needs. The health and well-being of the rats were closely monitored by a veterinary officer specializing in small animals, with regular checks for signs of distress, injury, or illness. The experimental procedure involved anaesthetising the rats using established anaesthesia protocols (chloroform) to facilitate immobilization during the weighing process. Dental lignocaine was used to control bleeding and pain during biopsies taking. Research personnel involved in animal handling was hired in compliance with institutional guidelines to ensure humane and ethical treatment of the animals throughout the study. Euthanasia was performed using **Chloroform**, a method recommended by the **Zambian Veterinary Medical Association (ZVMA)** guidelines. The carcasses were then disposed of through incineration at the licensed facility (School of Veterinary Medicine-UNZA), adhering to biosafety and ethical disposal standards of ZVMA. Data collection was systematic and securely stored to prevent any loss or manipulation. The research followed the **ARRIVE (Animal Research: Reporting of in Vivo Experiments)** guidelines to ensure transparent and accurate reporting of all findings, including both positive and negative results. The current study aimed at assessing the histomorphological changes induced by doxorubicin in skeletal muscle tissue mainly, supplemented by kidney and liver histopathological markers, and evaluation of the potential protective effects of Vitamin E.

RESULTS

In the current study, 33 male Wistar rats aged 14 to 16 weeks and weighing between 90 g and 225 g were utilized. These rats were randomly allocated into three groups: the Negative Control Group, the

Doxorubicin Group, and the Doxorubicin plus Vitamin E Group, with each group containing 11 rats. Key outcomes measured included alterations in the area of cross-sectional area (ACSA) of tibialis anterior muscle, the degree of inflammation, and muscle fascicle distortion in the tibialis anterior muscle, assessed both within and between groups. The key problem noted at the end of the study was the fleas' infestation on the rat

Anatomical Cross-Sectional Area (ACSA) Changes

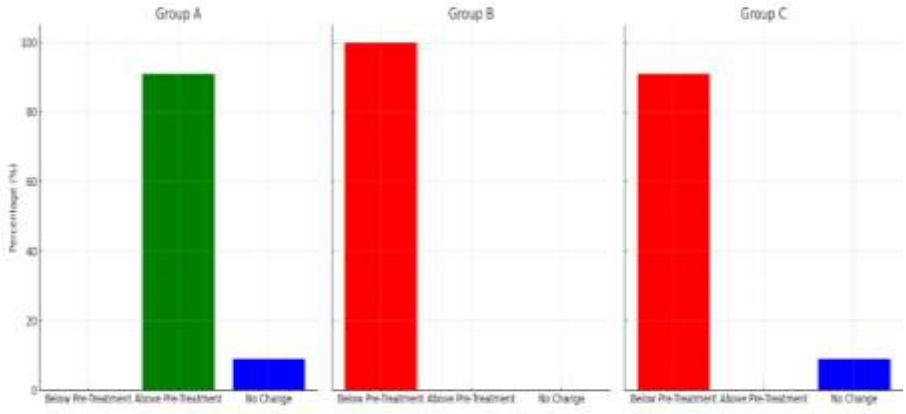
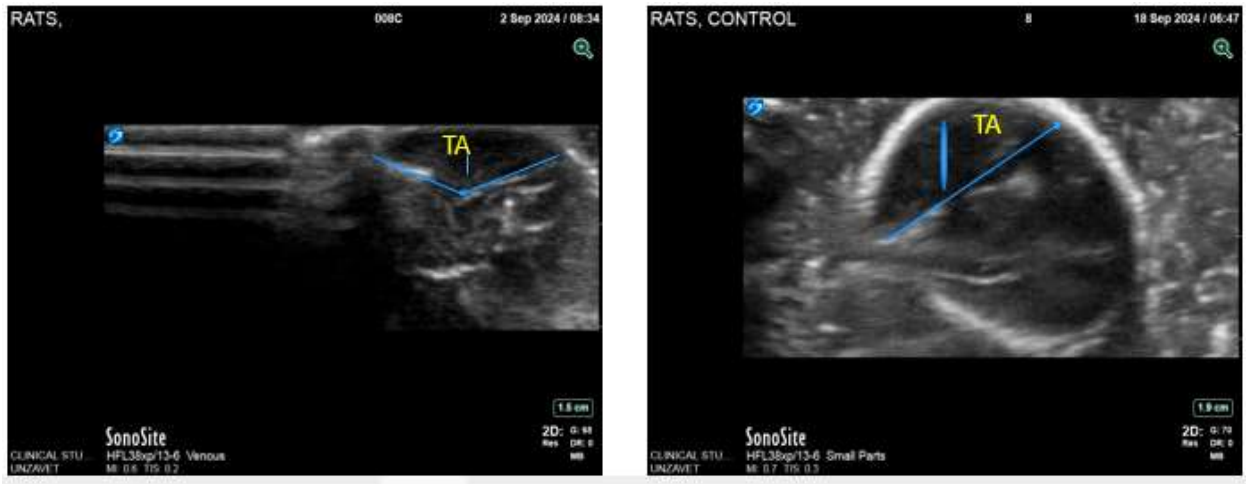


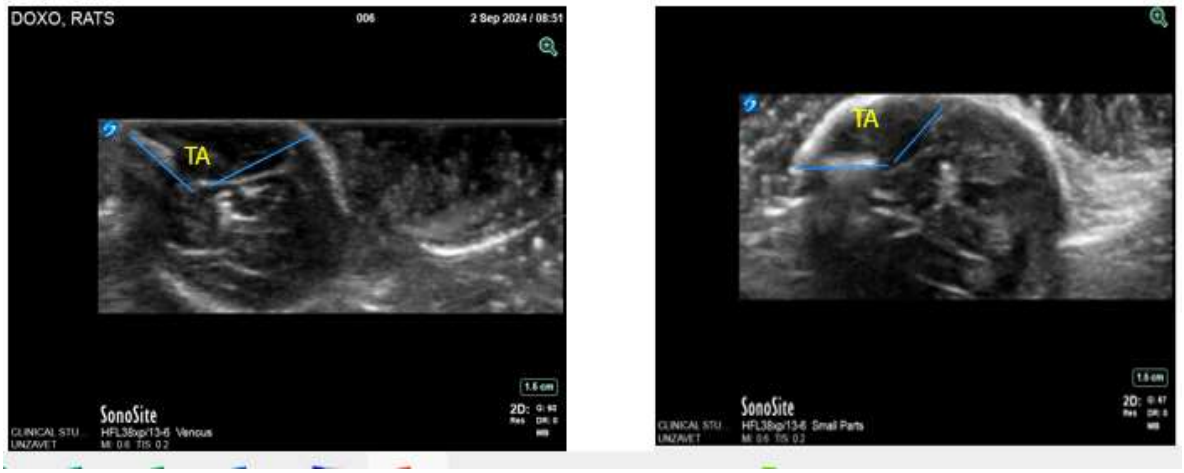
Figure 1. Anatomical Cross-Sectional Area (ACSA) Changes

Red =below pretreatment level**, Green** =above pretreatment level**, blue** = no change,
Showing the bar charts illustrating the percentage of rats in each group whose post-treatment ACSA values fell into one of three categories: below, above, or unchanged compared to their pre-treatment ACSA values. Group A (Control Group): Majority of (91%; n=10) rats showed an increase (above pre-treatment levels) and a small percentage (9%; n=1) exhibited no change. None had values below the pre-treatment levels. Group B (Doxorubicin Group): All rats (100%; n=11) experienced a decrease (below pre-treatment levels). Group C (Doxorubicin + Vitamin E Group): The majority of rats (91%; n=10) showed a decrease (below pre-treatment levels) and a smaller proportion (9%; n=1) showed unchanged values.



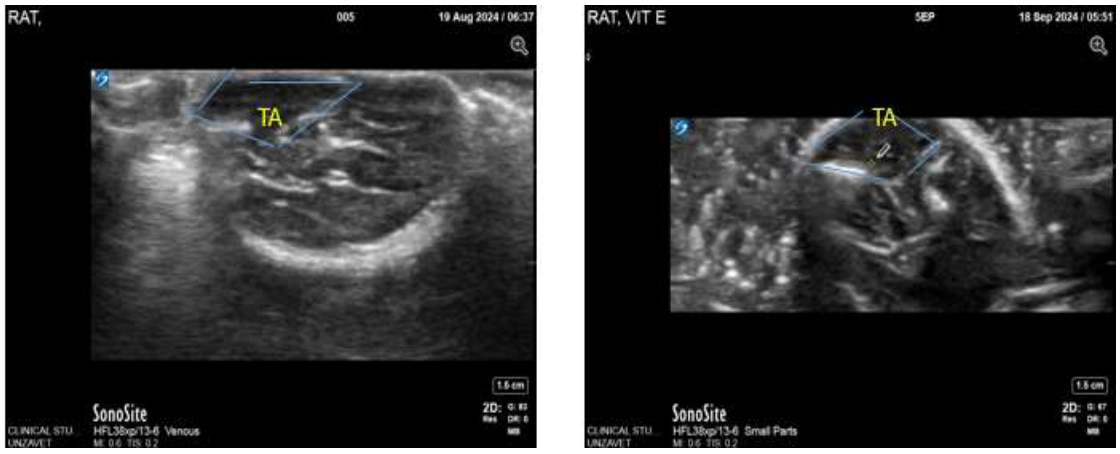
Pre-treatment ACSA 0.20 cm^2 **Post treatment ACSA 0.22 cm^2**

***Figure* 2: Showing ultrasound images of (ACSA) at baseline (pretreatment) and at 6th week (post treatment) for the control group. An increase in ACSA was observed.**



Pre-treatment ACSA 0.22 cm^2 **Post treatment ACSA 0.18 cm^2**

***Figure* 3: Showing ultrasound images of (ACSA) at baseline (pretreatment) and at 6th week (post treatment) for the Doxorubicin group. A reduction in ACSA was observed.**



Pre-treatment ACSA 0.18cm^2 **Post treatment ACSA 0.17 cm^2**

***Figure 4*. Showing ultrasound images of (ACSA) at baseline (pretreatment) and at 6th week (post treatment) for the Doxorubicin plus Vitamin E group. A reduction in ACSA was observed.**

Table 2. Changes in ACSA within and between groups over the six-week study period

Group	Baseline	6 weeks	Absolute ACSA change	% ACSA change	α =0.05. p-value **within each group** between **baseline** and at **6 weeks**	α=0.05. p-value **between groups** at **baseline**	α=0.05. p-value **between groups at 6 weeks** (% change)
A (n=11)	0.19 (0.15-0.22)	0.22 (0.17-0.26)	0.04 (0.00-0.05)	18.18 (0.00-31.25)	0.005	0.816	<0.001

B (n=11)	0.21 (0.20-0.24)	0.13 (0.07-0.19)	-0.09 (-0.41- -0.03)	-40.90 (-63.16- -13.64)	0.003		
C (n=11)	0.19 (0.16-0.21)	0.17 (0.09-0.21)	-0.03 (-0.07- -0.00)	-15.00 (-43.75-0.00)	0.005		

A- Negative Control Group, B-**Doxorubicin Group**, C-**Doxorubicin plus Vitamin E**

Within group ACSA differences were observed in all the groups A (p=0.005), B (p=0.003) and C (p=0.005).

Between group differences, Kruskal-Wallis test was used and showed that at least one group was significantly different from the other (p<0.001) in terms of percentage change in ACSA thickness at 6 weeks of the intervention.

Post hoc analysis was conducted by using Bonferroni correction for multiple tests, and it showed that significant differences were seen between group A (negative control group) and B (Doxorubicin) 27.95 vs. 7.77mm, p<0.001 and between group A (negative control group) and Doxorubicin plus Vitamin E) 27.95 vs. 15.27mm, p= 0.006, and between B (Doxorubicin) and C (Doxorubicin plus Vitamin E) 7.7 vs. 15.27mm, p<0.034.

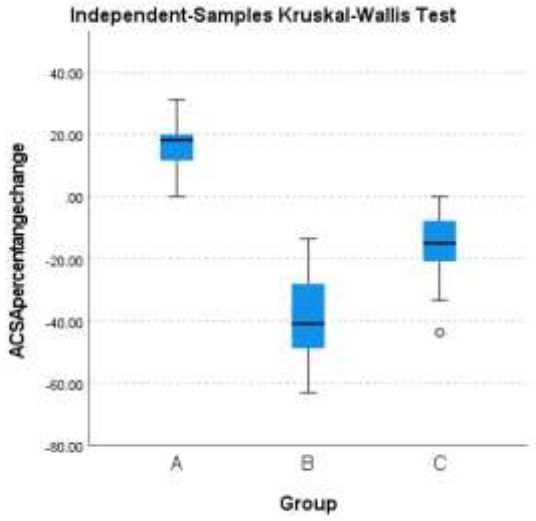


Figure 5: A-Negative Control Group, B-Doxorubicin Group, C-Doxorubicin plus Vitamin E, Showing ACSA percent change

Table 3 showing regression coefficients: - causality due to treatment

Coefficients [a]						
Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	.314	.070		4.511	<.001
	Treatment	-.042	.014	-.651	-3.129	.009

	Baseline weight	.000	.000	-.176	-.846	.414
a. Dependent Variable: ACSA posttreatment						

- Multiple linear regression analysis showed that mode of treatment (p=0.009) and constant (p<0.001) were independent predictors of ACSA thickness as measured by ultrasound after controlling for baseline weight and age (R^2=0.507)
- **Summary of Findings** The results highlight significant within- and between-group differences in ACSA over the six-week period. Group A exhibited a significant increase in ACSA, while Group C showed a significant decrease. Regression analysis confirmed that treatment type was a strong predictor of ACSA thickness after controlling for baseline weight and age. These findings underscore the potential impact of treatment on muscle structure, as measured by ultrasound.

0.1. Inflammation scores

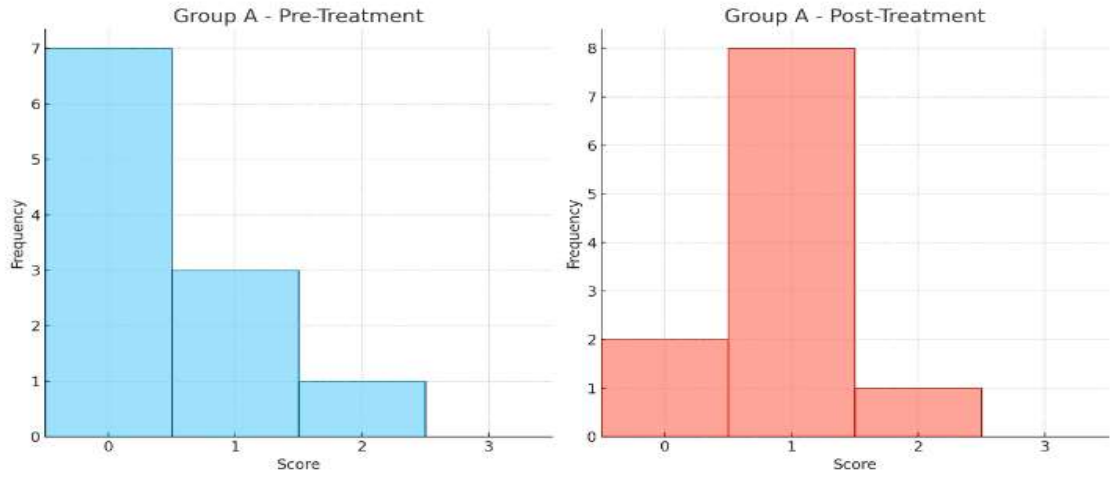


***Figure* 6. Frequencies of the inflammatory scores for pre and post treatment for A-Negative control group.**

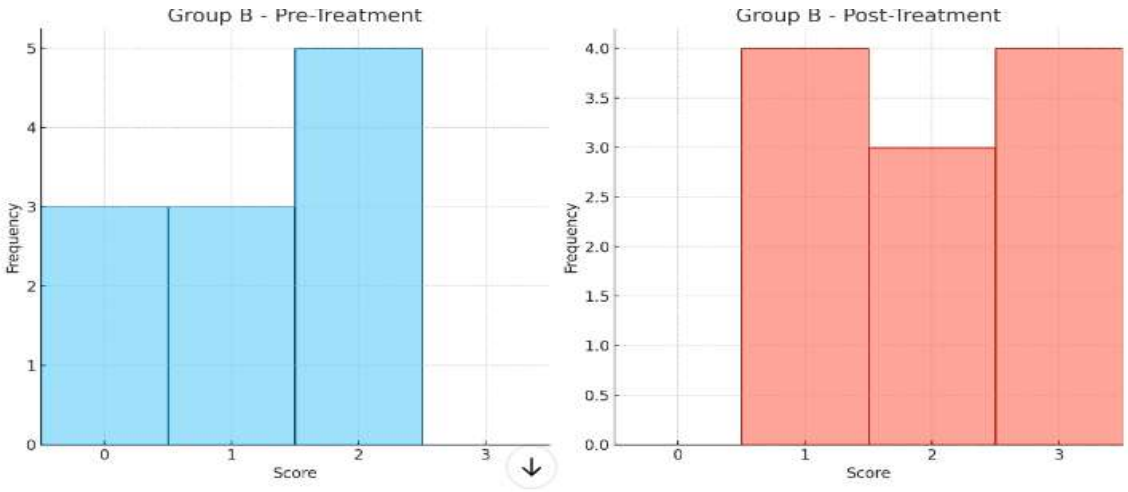


Figure 7: Showing frequencies of the inflammatory scores for pre and post treatment for B-Doxorubicin group.

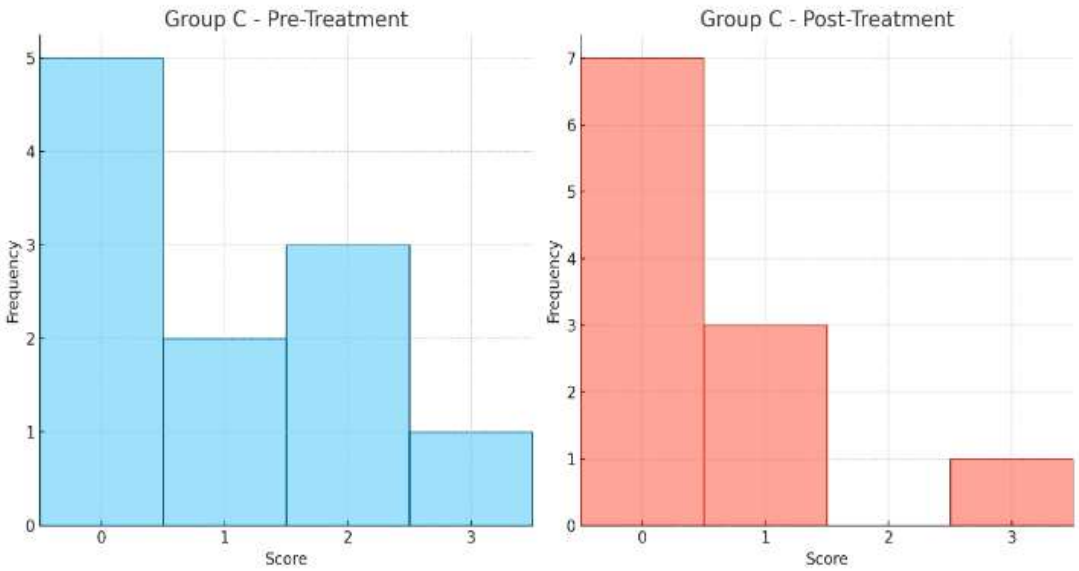
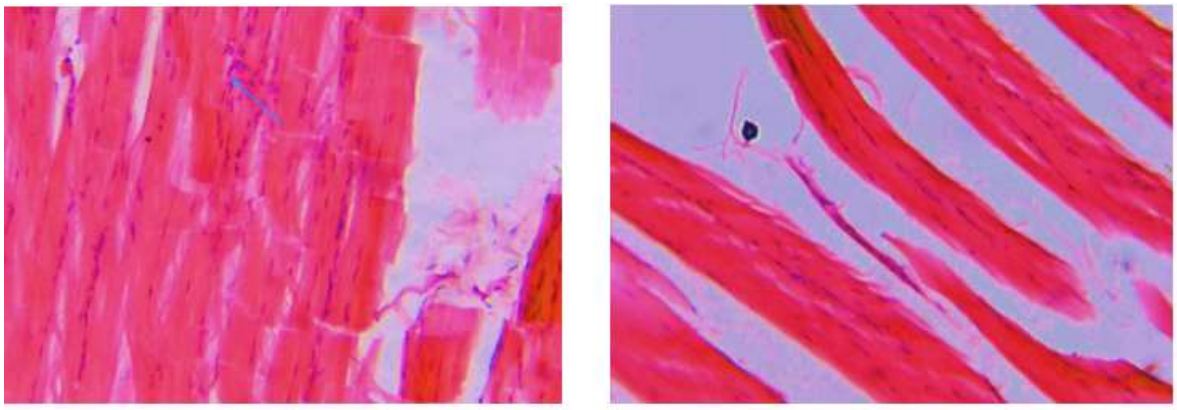
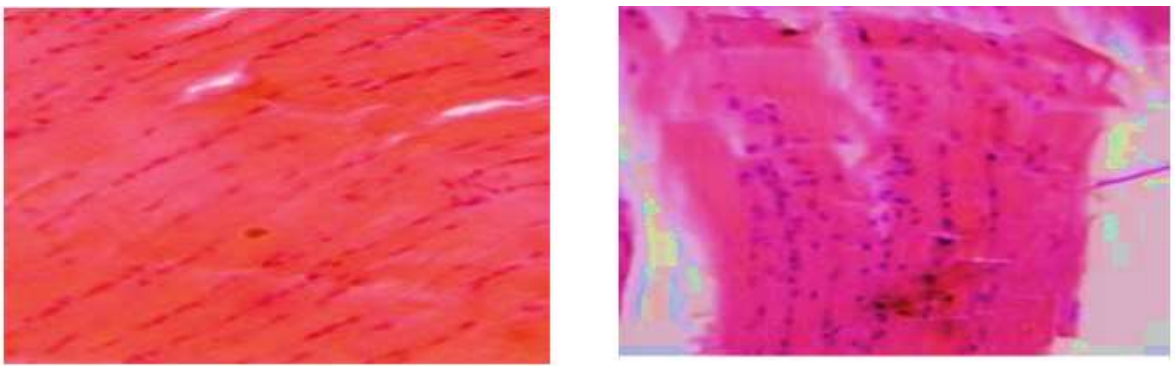


Figure 8: Showing frequencies of the inflammatory scores for pre and post treatment for C-Doxorubicin plus Vitamin E group.



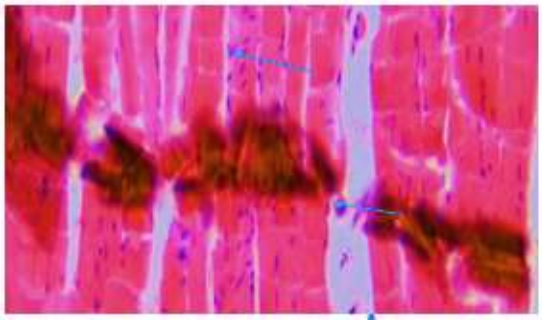
Pre-treatment inflammation: - score 2 **post treatment inflammation: - score 0**

Figure 9: H&E Photomicrographs at Mag 10x showing degree of inflammation sores in tibialis anterior muscle pre (baseline) and post (at week 6) treatment inflammation in the control group.

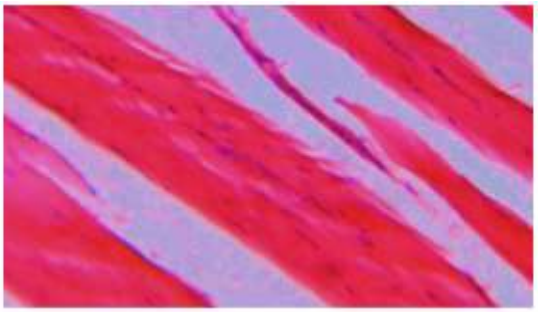


Pre-treatment inflammation: - score 0 **post treatment inflammation: - score 1**

***Figure* 4. 10: H&E Photomicrographs at Mag 10x showing degree of inflammation sores in tibialis anterior muscle pre (baseline) and post (at week 6) treatment inflammation in the doxorubicin group.**

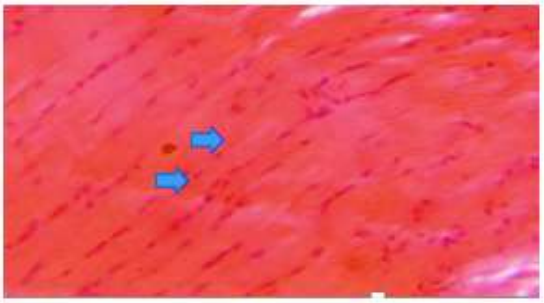


Pre-treatment inflammation: - score 2

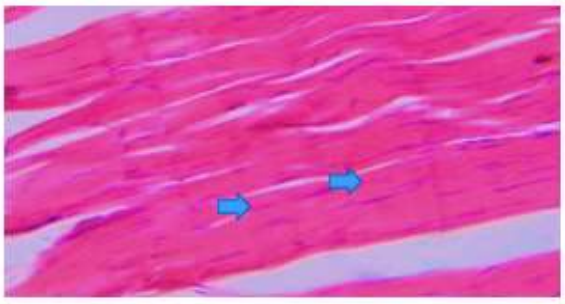


post treatment inflammation: - score 0

***Figure* 4. 11: H&E Photomicrographs at Mag 10x showing degree of inflammation sores in tibialis anterior muscle pre (baseline) and post (at week 6) treatment inflammation in the Doxorubicin plus Vitamin E group.**



Pre-treatment inflammation: - score 0



post treatment inflammation: - score 0

***Figure* 4.12: H&E Photomicrographs at Mag 10x showing degree of inflammation sores in tibialis anterior muscle pre (baseline) and post (at week 6) treatment inflammation in the Doxorubicin plus Vitamin E group.**

Table 4.3: showing details of inflammation scores before and after treatment

Group	Pre-treatment inflammation (n=11)			Post-treatment inflammation (n=11)			α=0.05.p values for **Within group difference** in inflammation scores between pre and post treatment
	Score	Frequency	Percent	Score	Frequency	Percent	
A, n (%)	0	7	63.6	0	2	18.2	0.096
	1	3	27.3	1	8	72.7	
	2	1	9.1	2	1	9.1	
B, n (%)	0	2	18.2	0	4	36.4	0.035
	1	3	27.3	1	3	27.3	
	2	5	45.5	2	4	36.4	
	0	5	45.5	0	7	63.6	0.096

C, n (%)	1	2	18.2	1	3	27.3	
	2	3	27.3	2	0	0.0	
	3	1	9.1	3	1	9.1	

A- Negative Control Group, B-**Doxorubicin Group**, C-**Doxorubicin plus Vitamin E**

- Within group pre and post treatment comparisons only showed a significant difference in group B (p=0.035) in inflammation score.

Table 4.4. Shows Kruskal-Wallis test for inflammation prior to treatment

	Inflammation pre-treatment
Kruskal-Wallis H	4.703
df	2
Asymp. Sig.	**.095**
a. Kruskal Wallis Test	
b. Grouping Variable: Group	

No significant difference in inflammation scores pre-treatment p= 0.095

Table 4.5 shows pairwise group comparisons of inflammation post treatment.

Group	Test Statistic	Std. Error	Std. Test Statistic	Sig.[a]
C vs. A	5.045	3.871	1.303	.577
C vs. B	14.045	3.871	3.629	.001
A vs. B	-9.000	3.871	-2.325	.060

Kruskal-Wallis test showed that at least one group was significantly different from the other (p=0.001). Post hoc analysis was then conducted by using Bonferroni correction for multiple tests, and it showed that a significant difference was between group C and B (p=0.001).

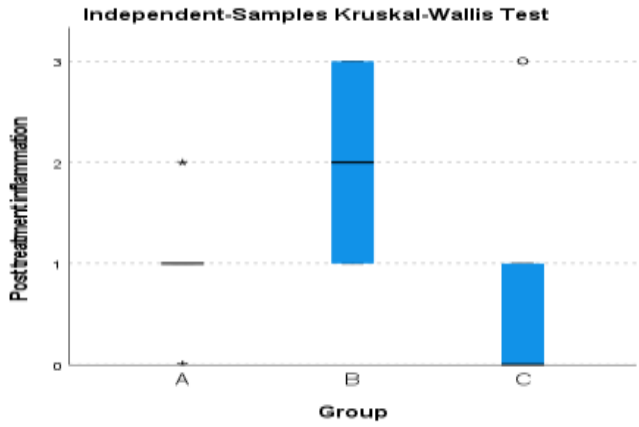


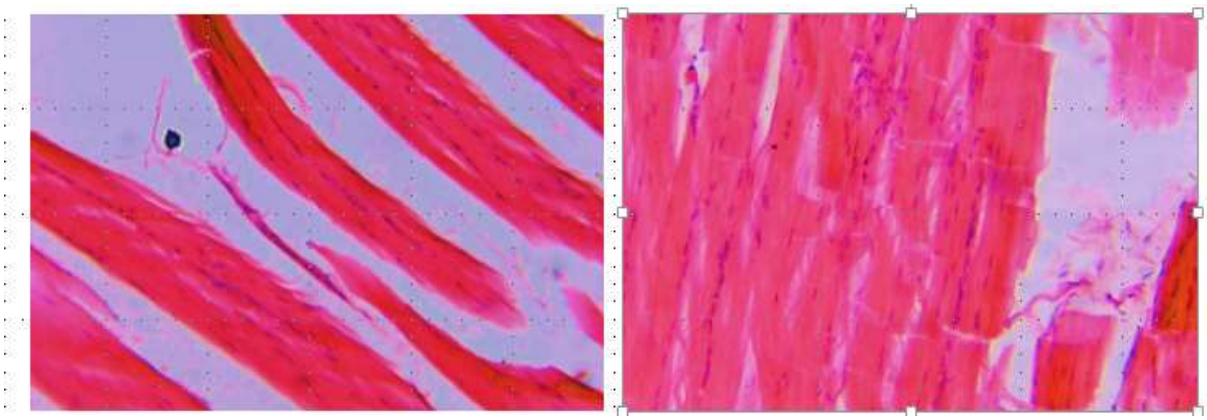
Figure 4.14: A-Negative Control Group, B-Doxorubicin Group, C-Doxorubicin plus Vitamin E, showing post treatment inflammation.

Ordinal logistic Regression analysis showed that mode of treatment (p=0.009) was an independent predictor of inflammation after controlling for base weight, follow up weight and age (R^2=0.310).

Summary of the findings

- Group C vs. Group B: Significant differences were observed (p = 0.001), suggesting that the Doxorubicin + Vitamin E combination was more effective in reducing inflammation compared to Doxorubicin alone.
- Group A vs. Group B: Marginal differences were noted (p = 0.060).
- Group C vs. Group A: No significant differences were observed (p = 0.577)

4.3. Muscle fascicles distortion scores



***Pre-treatment score for fascicle distortion= 0. Post treatment score for fascicle Distortion=0 Figure* 4.14:** H&E Photomicrographs at Mag 10x showing no distortion of muscle fascicles in tibialis anterior muscle pre (baseline) and post (at week 6) treatment in the Doxorubicin group.
In terms of distortion of muscle fascicles, no significant difference was observed (p=1.000).

DISCUSSION

This study provides a thorough investigation into the toxic effects of Doxorubicin and the potential mitigating effects of Vitamin E in skeletal muscle tissue. Conducted within a robust experimental framework, the randomized controlled trial (RCT) design ensures strong evidence for causality by minimizing selection bias. The use of 33 male Wistar rats aged 14–16 weeks further supports the reliability of the findings, as this age range represents a stable phase of skeletal muscle growth, reducing variability due to developmental changes. The rats were divided into three groups: Negative Control, Doxorubicin, and Doxorubicin + Vitamin E, with 11 rats in each group. Over six weeks, the study measured critical outcomes, including tibialis anterior muscle cross-sectional area (ACSA), inflammation scores, and muscle fascicle distortion.

Anatomical Cross-Sectional Area (ACSA)

ACSA measurements revealed significant muscle atrophy in the Doxorubicin group ($p < 0.001$), while the Negative Control group showed increased ACSA, indicative of normal muscle growth. The Doxorubicin + Vitamin E group exhibited partial preservation of ACSA, suggesting that Vitamin E mitigates muscle loss to some extent. Post hoc analyses confirmed significant differences between the groups, particularly between the Negative Control and Doxorubicin groups ($p < 0.001$) and negative control and Doxorubicin plus Vitamin E ($P<0.006$). Least but statistically significant differences were seen between the Doxorubicin and Doxorubicin + Vitamin E groups ($p = 0.034$).

Figure 1 visually presents the percentage of rats in each group with post-treatment ACSA changes. Notably, 91% of the Negative Control group showed increased ACSA, while all rats in the Doxorubicin group exhibited decreases. The Doxorubicin + Vitamin E group predominantly showed decreases (91%), though one rat (9%) exhibited no change. Figures 2, 3, and .4 provide ultrasound images showcasing ACSA differences pre- and post-treatment.

The Control group (Figure .2) displayed increased ACSA from 0.20 cm^2 to 0.22 cm^2, whereas the Doxorubicin group (Figure .3) showed reduced ACSA from 0.22 cm^2 to 0.18 cm^2. The Doxorubicin + Vitamin E group (Figure .4) also showed a slight reduction from 0.18 cm^2 to 0.17 cm^2. Table .1 highlights the detailed statistical changes in ACSA within and between groups over the six-week period. The Doxorubicin group showed a significant decline in ACSA (−40.90%), while the Control group exhibited an increase (18.18%). The Doxorubicin + Vitamin E group experienced a modest decline (−15.00%), supporting the protective effect of Vitamin E. Between-group comparisons confirmed significant differences, particularly between the Control and Doxorubicin groups ($p < 0.001$). Figure .5 illustrates ACSA percent changes across groups, emphasizing the notable difference between the Control and Doxorubicin groups and the partial preservation in the Doxorubicin + Vitamin E group. Multiple linear regression analysis showed that mode of treatment ($p=0.009$) and constant ($p<0.001$) were independent predictors of ACSA thickness as measured by ultrasound after controlling for baseline weight and age ($R^2=0.507$).

These findings align with existing literature, such as Gilliam & St. Clair (2011) and Choi et al. (2016), which attribute muscle atrophy to oxidative damage and mitochondrial dysfunction induced by Doxorubicin, and demonstrate the protective effects of antioxidants like Vitamin E.

Inflammation

Inflammation scores were highest in the Doxorubicin group, consistent with its known pro-inflammatory effects due to reactive oxygen species (ROS) generation and tissue damage (Octavia et al., 2012). In contrast, the Doxorubicin + Vitamin E group exhibited reduced inflammation scores, highlighting Vitamin E's antioxidant properties in attenuating inflammatory responses. Between-group differences were significant ($p = 0.001$), particularly between the Doxorubicin and Doxorubicin + Vitamin E groups ($p = 0.001$). Figures .6, .7, and .8 show the frequency distributions of inflammatory scores pre- and post-treatment for the three groups. The Control group (Figure .6) exhibited minimal inflammation changes, while the Doxorubicin group (Figure .7) demonstrated pronounced increases. The Doxorubicin + Vitamin E group (Figure 8) showed marked reductions in post-treatment inflammation scores. Figures .9 to 12 provide H&E photomicrographs illustrating inflammation levels. The Control group's photomicrographs (Figures 9 and 10) showed minimal changes, while the Doxorubicin group (Figure .10) exhibited heightened inflammation. The Doxorubicin + Vitamin E group (Figures 11 and .12) demonstrated reduced post-treatment inflammation. Table .2 details the pre- and post-treatment inflammation scores. A significant within-group difference in inflammation was observed only in the Doxorubicin group ($p = 0.035$). Post hoc analysis revealed significant post-treatment differences between the Doxorubicin and Doxorubicin + Vitamin E groups ($p = 0.001$). Figure 13 further highlights the post-treatment inflammation differences, showcasing the superior anti-inflammatory effect of the Vitamin E combination compared to Doxorubicin alone. Binary regression analysis confirmed treatment mode as an independent predictor of inflammation ($p = 0.009$).

Muscle Fascicle Distortion

There was no significant differences in skeletal muscle fascicle distortion observed p=1.000. No accumulation of connective tissue in the perimysium between fascicles was observed suggesting that treatment effects were primarily localized to anatomical cross-sectional area (ACSA) and inflammation parameters rather than structural alterations in muscle fascicles. This finding aligns with prior research by Aughsteen, Khaair, and Suleiman (2006), who observed that acute metabolic disturbances impact muscle fiber morphology without significantly altering fascicle structure. Similarly, Choi et al. (2016) reported that while oxidative stress leads to muscle atrophy, fascicle organization remains relatively preserved unless subjected to prolonged degenerative conditions. Conversely, Campelj et al. (2021) highlighted chemotherapy-induced myopathy as a potential cause of muscle fascicle distortion, particularly in cases of severe cachexia.

Conclusion:

This study provides significant insights into the protective role of Vitamin E against histomorphological changes in skeletal muscle tissue induced by Doxorubicin. The findings suggest that Vitamin E partially mitigates muscle atrophy by preserving the anatomical cross-sectional area of the tibialis anterior muscle. Additionally, it reduces the inflammatory response, thereby limiting muscle degeneration and improving overall tissue integrity. However, its effect on muscle fascicle distortion remains inconclusive, indicating the need for further investigation. The results align with existing literature, reinforcing the potential of Vitamin E as a therapeutic adjunct to mitigate Doxorubicin-induced skeletal muscle toxicity, warranting additional research on its optimal dosage and efficacy in clinical settings.

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