



The Cardioprotective Effect of Olive leaf extract against Cardiotoxicity Induced by Carfilzomib. Modulation of cardiac biomarkers, oxidative stress, inflammation and histopathological damage

Nabaa Ali Abd Alradha Al_Saeedi

Wasit University , College of Science, Department of pathological Analysis

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Abstract

Background: Carfilzomib is a powerful proteasome inhibitor that is a broad-spectrum therapy used for multiple myeloma but may be clinically accompanied by cardiotoxicity. There was a need to investigate the protective effect of olive leaf extract against the cardiac toxicity caused by carfilzomib in male albino rats and to compare its effect with carvedilol in this study. **Methodology:** Thirty-six rats were randomly assigned to six groups: control group, group treated with olive leaf extract (400 mg/kg), group treated with carfilzomib, two groups where rats were treated with olive leaf extract (200 or 400 mg/kg) before they were treated with carfilzomib, and a group treated with carvedilol before carfilzomib. b. Cardiac damage markers, inflammatory markers, oxidative stress markers, and histopathological changes were then evaluated at the end of the experiment. **Results:** Administration of carfilzomib caused a marked increase ($p < 0.05$) in serum levels of troponin-I, CK-MB, LDH, TNF- α , and IL-6 as compared to the control group. In addition, the content of malondialdehyde (MDA) was found to be elevated in cardiac tissue with a significant decrease in reduced glutathione (GSH) level and superoxide dismutase (SOD) activity. The histopathological analysis showed severe myocardial damage with the presence of edema, vascular congestion, inflammatory cell infiltration, hemorrhage, myofibrillar fragmentation, and cardiomyocyte degeneration. The administration of olive leaf extract at a preventive level was very effective in reducing these biochemical and histological changes, depending on the dose. The higher dose (400 mg/kg) showed better protection than the lower dose (200 mg/kg), with the results showing an improvement in cardiac biomarkers, a reduction in inflammatory responses, restoration of antioxidant defense, and preservation of myocardial architecture. The cardioprotective effect was most significant in the group that was treated with carvedilol before the treatment. Finally, olive leaf extract protected against cardiotoxicity associated with carfilzomib administration, which was probably due to antioxidant and anti-inflammatory effects. **Conclusions:** While carvedilol proved to be more effective, OLE clearly exhibited significant cardioprotective properties and could be a potent natural preventive approach against cardio-toxic effects of carfilzomib.

Keywords: Carfilzomib, Olive leaf extract, Cardiotoxicity, Oxidative stress, Inflammation, Carvedilol.

Introduction

Multiple myeloma is a uniformly fatal condition of the plasma cell system and represents around 10% of all hematological malignancies in the world(Rajkumar, 2020). Over the last few years, there has been great progress in the treatment, and patient survival is now better, especially due to the availability of proteasome inhibitors(Sutanto *et al.*, 2025). Of these agents, Carfilzomib is a second generation irreversible Proteasome Inhibitor that has shown great promise in the treatment of Relapsed/Refractory Multiple Myeloma(Jayaweera *et al.*, 2021). Carfilzomib selectively inhibits the proteasomal activity, thereby disrupting the intracellular protein degradation pathways leading to accumulation of damaged proteins and induction of apoptosis in malignant cells(Yee, 2021). Although therapeutic, evidence is emerging that there is a higher risk of cardiac side effects with Carfilzomib therapy, potentially restricting the clinical application and impacting patients' outcomes(Efentakis *et al.*, 2024).

Cardiotoxicity is one of the most severe side effects of a number of chemotherapy drugs. A number of clinical and experimental studies have shown that administration of Carfilzomib may cause hypertension, arrhythmias, myocardial ischemia, left ventricular dysfunction and heart failure(Buck *et al.*, 2022; Kelly *et al.*, 2025). Although the exact mechanisms responsible for Carfilzomib-induced cardiotoxicity have not been fully elucidated, oxidative stress has been recognized as a major contributor to cardiac injury(Efentakis *et al.*, 2019). However, overproduction of ROS may lead to lipid peroxidation, protein oxidation, mitochondrial dysfunction and eventually damage to the cardiomyocytes as a result of the failure of endogenous antioxidant defense systems to combat the excessive ROS formed(Ali and Alkarbolii, 2024). Moreover, oxidative stress may activate inflammatory response by activating different signaling pathways and through the production of pro-inflammatory cytokines, thus enhancing myocardial injury(George *et al.*, 2024).

Due to the energy needs and large number of mitochondria, the heart is one of the most susceptible organs to oxidative damage. Thus, anti-oxidative and anti-inflammatory therapy could offer an effective protection from chemotherapy-induced cardiac injury(Gao *et al.*, 2024; Liu *et al.*, 2025). In this context, natural products have been a great deal of interest due to their wide range of biological properties and good safety profile(Najmi *et al.*, 2022). One of these natural compounds, olive leaf extract (OLE), has garnered significant interest for its strong antioxidant and anti-inflammatory benefits(Bucciantini *et al.*, 2021).

Olive leaf extract is derived from the leaves of *Olea europaea* and is rich in several bioactive phenolic compounds, such as oleuropein, hydroxytyrosol, tyrosol and flavonoids(de Oliveira *et al.*, 2024; Panou and Karabagias, 2025). Oleuropein has been described as the major component and it is mainly responsible for the pharmacological properties of olive leaves(Elhrech *et al.*, 2024). Many studies have shown that olive leaf extract possesses a high free-radical scavenging activity, increases endogenous antioxidant system, reduces inflammatory mediators and has protective effect against tissue damage from free radicals(Silvestrini *et al.*, 2023). Furthermore, olive leaf polyphenols have been demonstrated to protect mitochondrial function, decrease apoptotic cell death and promote cardiovascular health in several experimental models(Lanfranchi *et al.*, 2026). There have been a number of studies which have demonstrated the positive impact of olive leaf extract on cardiovascular diseases, such as hypertension, ischemic damage and heart damage caused by oxidative stress(Razmpoosh *et al.*, 2022). They have been attributed to its antioxidant properties, ability to inhibit lipid peroxidation, augmenting the activity of antioxidant enzymes and reducing inflammatory responses(van Stratum *et al.*, 2026). The interest in the therapeutic use of olive leaf extract is growing, but little information is available as to its protective effect against the cardiotoxicity of Carfilzomib. In view of this, the present study was conducted to assess the protective action of OLE against cardiotoxic effects of Carfilzomib in male albino rats by determining the cardiac injury markers, oxidative stress markers and histopathological changes in the cardiac tissue.

Material and Methods:

2.1 Experimental Animals

Adult male Sprague–Dawley albino rats (*Rattus norvegicus*) weighing 290–325 g were used in the present study. These animals were captured and kept in the animal house of the Department of biology at college of education for pure sciences, university of Anbar. During the experiments, they received standard laboratory conditions (a 12 h light/12 h dark cycle and free access to standard pellet food and drinking water) and were kept at controlled temperature ($22 \pm 2^\circ\text{C}$). Before the start of the experiment, animals were introduced to the laboratory for two weeks to adapt their physiology to the new environment and to reduce environmental stress.

2.2 Ethical Approval

The Research Ethics Committee of University of Anbar approved the experimental protocol (No. EAC155; 3rd May, 2026). All animal use and procedures were carried out in accordance with the guidelines for the care and use of laboratory animals.

2.3 Preparation of Alcoholic Olive Leaf Extract

Fresh olive (*Olea europaea L.*) leaves were harvested, washed with distilled water and air dried under shade at room temperature. The dried leaves were powdered with electric grinding machine. Maceration was carried out for 72 hours with the powdered material in 70% ethanol with occasional shaking. This extract was then filtered through Whatman No.1 filter paper and further concentrated by rotary evaporator at 40°C . Crude alcoholic extract was dried, weighed and kept in air tight containers at 4°C until use (Wainstein *et al.*, 2012).

2.4 Preparation of Carfilzomib and Carvedilol

Carfilzomib was acquired as a commercial lyophilized pharmaceutical and was reconstituted with the use of sterile water for injection as per the manufacturer's guidelines. The prepared solution was shaken gently until complete dissolution and then freshly prepared before administering. The tablets of carvedilol were procured from a licensed pharmacy and finely powdered for suspension in 0.5 % CMC for providing the requisite dose. All the doses of Carfilzomib and Carvedilol were based on rat body weight. Carfilzomib was given by i.p. injection and Carvedilol was orally by gavage.

2.5 Experimental Design

The rats were randomly allocated into six experimental groups, with six rats in each group. The Group-I was kept as control group which was given orally 0.5% of carboxymethyl cellulose (CMC) and intraperitoneally the vehicle. The group II was given olive leaf extract at the dose of 400 mg/kg body weight, orally once daily. Group III was the Carfilzomib induced cardiotoxicity group to which Carfilzomib was administered at the dose of 4 mg/kg body weight twice a week by IP injection. The olive leaf extract group was treated with 200 mg/kg of olive leaf extract for 30 days, followed by Carfilzomib treatment. Group V was given with olive leaf extract at 400 mg/kg body weight, orally every day and then was administered Carfilzomib. Group VI were treated with Carvedilol (10 mg/kg body weight, orally once a day) and then received Carfilzomib treatment and were used as a reference group for cardioprotection. The olive leaf extract and Carvedilol were dissolved in 0.5% of CMC and the same oral vehicle was used in the groups without these treatments in order to get uniformity in the oral vehicle. Olive leaf extract or Carvedilol was given 1 hour prior to Carfilzomib injection on the days of Carfilzomib injection. The experiment was conducted for four weeks.

2.6 Biochemical and Molecular Assessments

2.6.1 Cardiac Function Biomarkers

Cardiac Troponin-I (cTnI): The levels of serum cardiac troponin-I were measured by commercial rat-specific ELISA kit, which was supplied by the manufacturer. Since its introduction by (Cummins *et al.*, 1987), cardiac troponin-I (cTn-I) is a highly sensitive and specific biomarker of myocardial injury that has been widely used for the diagnosis of cardiac damage.

Creatine Kinase-MB (CK-MB): The serum CK-MB activity was determined by a commercial diagnostic kit by immunoinhibition method. Well established as a marker of myocardial cell injury, CK-MB has been widely used for the evaluation of cardiac damage, as detailed by (Rosalki, 1967).

Lactate Dehydrogenase (LDH): Serum LDH activity was measured spectrophotometrically by the method of (Wróblewski and Ladue, 1955) with a commercial assay kit. High levels of LDH are thought to be signs of tissue damage, including myocardial damage.

2.6.2 Preparation of Cardiac Tissue Homogenates

The freshly excised cardiac tissue was then homogenized in ice cold phosphate buffered saline (PBS pH 7.4) at 10% (w/v) homogenate with a tissue homogenizer. The homogenates were centrifuged at 10,000 rpm for 15 min at 4°C and the supernatant was collected and stored at -20°C for oxidative stress measurements such as malondialdehyde (MDA), reduced glutathione (GSH), and superoxide dismutase (SOD).

2.6.3 Oxidative Stress Markers

Malondialdehyde (MDA): The levels of MDA in cardiac tissue were detected as an index of lipid peroxidation based on the method for the detection of thiobarbituric acid reactive substances (TBARS) of (Ohkawa *et al.*, 1979). The assay is done by a colorimetric reaction between MDA and thiobarbituric acid.

Reduced Glutathione (GSH): The content of GSH in cardiac tissue was measured by the method of (Ellman, 1959). It is based on the reaction of sulfhydryl groups with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) that forms a yellow colored chromogen which can be measured spectrophotometrically

Superoxide Dismutase (SOD): The SOD activity of cardiac tissue homogenates was determined by the method developed by (Misra and Fridovich, 1972). This assay is based on the competitive inhibition of autoxidation of epinephrine by SOD under alkaline conditions.

2.6.4 Inflammatory Biomarkers

Tumor Necrosis Factor-Alpha (TNF- α):

Sandwich enzyme-linked immunosorbent assay (ELISA) kit was used to measure the level of TNF- α in the serum, following the manufacturer's instructions (Elabscience, China).

Interleukin-6 (IL-6):

The concentration of serum IL-6 was measured by the commercially available rat ELISA kit (Elabscience, China) following the manufacturer's directions.

2.6.5 Histological Examination of Cardiac Tissue

The rats were then killed at the end of the experimental period and their hearts were removed, washed in cold normal saline to exclude the blood residues and gently blotted. Representative samples were taken from the left ventricle and fixed in 10% neutral buffered formalin for 48 h. Fixed tissues were then routinely processed in graded ethanol dehydration, xylene and embedded in paraffin wax. For histopathological examination, 5 μ m thick sections were cut with a rotary microtome and stained with hematoxylin and eosin (H&E) and examined under a light microscope (Suvana *et al.*, 2018).

2.7 Statistical Analysis

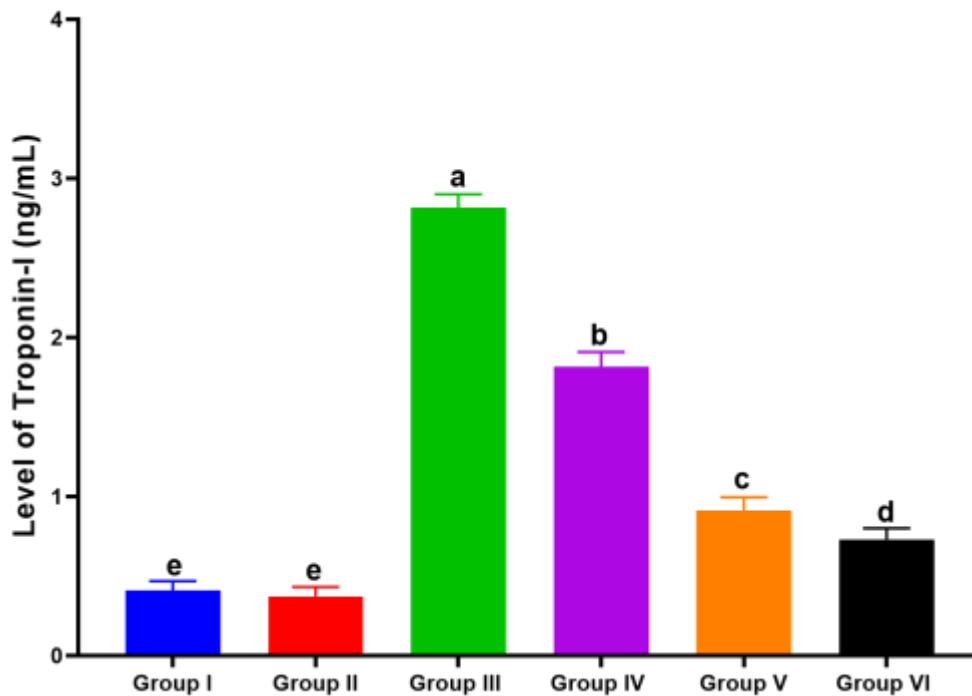
All data are presented as mean $\pm$ standard deviation (Mean $\pm$ SD). GraphPad Prism software (GraphPad Software, San Diego, CA, USA) was used for statistical analysis. One-way analysis of variance (One-Way ANOVA) was used to analyze the differences among experimental groups and multiple comparisons were determined by Tukey's test. $P < 0.05$ was regarded as being statistically significant.

Results and Discussion:

Cardiac Injury Biomarkers

Figures 1–3 show the effect of olive leaf extract and carvedilol on the cardiac injury biomarkers measured in serum. The results indicated that the serum level of troponin-I, CK-MB, and LDH were significantly high ($P < 0.05$) in Carfilzomib-treated rats (Group III) compared to the control rats (Group I). There were no significant differences between the olive leaf extract-alone group (Group II) and the control group. All biomarkers were significantly reduced and there was a significant difference between Group V (400 mg/kg) and Group IV (200 mg/kg) when compared to the rest of the groups. Pretreatment with olive leaf extract significantly reduced the Carfilzomib-induced elevation in all biomarkers with Group V (400 mg/kg) having significantly lower values than Group IV (200mg/kg). Furthermore, the carvedilol-treated group (Group VI) had the lowest levels of troponin-I, CK-MB and LDH, but the figures were still statistically significantly different from the control group. These results suggest that the olive leaf extract had a dose-dependent cardioprotective activity, and carvedilol was the most cardioprotective agent against the myocardial injury induced by Carfilzomib.

Figure 1. Carvedilol and OLE effects on the serum troponin-I level in Carfilzomib-treated rats. Data represent mean $\pm$ SD ($n = 6$). Significant differences (at $P < 0.05$) are shown by different letters.



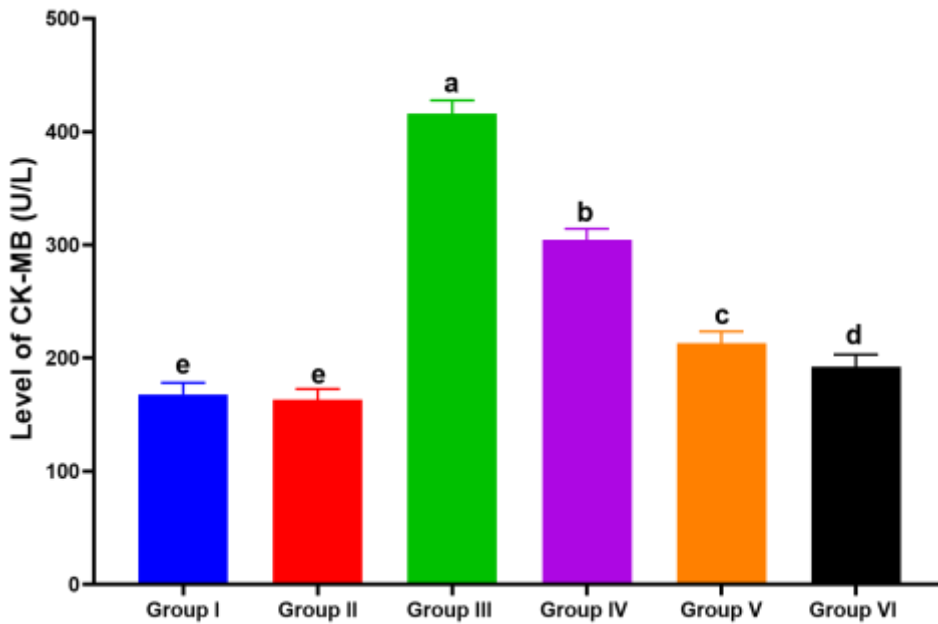


Figure 2. Carvedilol and OLE effects on the serum CK-MB activity in Carfilzomib-treated rats. Data represent mean $\pm$ SD (n = 6). Significant differences (at $P < 0.05$) are shown by different letters.

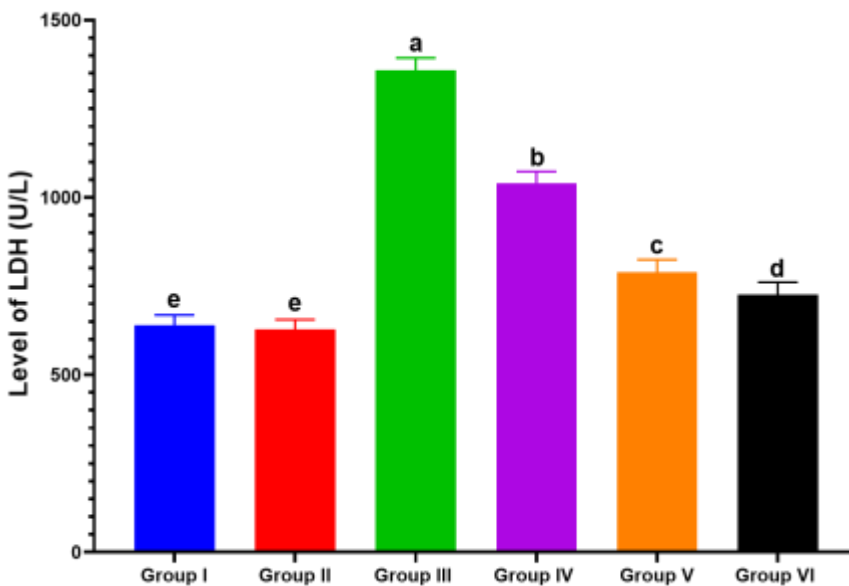


Figure 3. Carvedilol and OLE effects on the serum LDH activity in Carfilzomib-treated rats. Data represent mean $\pm$ SD (n = 6). Significant differences (at $P < 0.05$) are shown by different letters.

These high values of serum troponin-I, CK-MB and LDH levels reported after the use of Carfilzomib suggest serious myocardial damage and loss of the integrity of the membrane of cardiomyocytes, as Attanasio et al.,2024 pointed out in their study . This study confirms previous findings that the cardiotoxicity from Carfilzomib is attributed to the inhibition of the proteasome leading to the accumulation of damaged proteins in the cell, mitochondrial dysfunction and generation of reactive oxygen species (ROS) (George *et al.*, 2024). The cardiac tissue is very sensitive to oxidative damage, as it depends on the production of energy through the mitochondria, leading to the leakage of enzymes into

the blood(Verma *et al.*, 2025). There was no significant difference in the measured biomarkers as a result of taking olive leaf extract alone, suggesting that olive leaf extract was tolerated and was not detrimental to cardiac function. However, olive leaf extract pretreatment completely prevented the increases in troponin-I, CK-MB and LDH levels induced by Carfilzomib. These findings are consistent with several recent studies investigating natural cardioprotective agents against chemotherapy-induced cardiotoxicity. George *et al.* (2024) reported that empagliflozin significantly attenuated carfilzomib-induced myocardial injury by reducing serum troponin-I and oxidative stress. Likewise, recent experimental studies using naturally derived antioxidants, including resveratrol, curcumin, and quercetin, demonstrated significant reductions in cardiac biomarkers such as CK-MB, LDH, and troponin following doxorubicin-induced cardiotoxicity. These protective effects were mainly attributed to attenuation of oxidative stress, suppression of inflammatory cytokines, preservation of mitochondrial function, and inhibition of cardiomyocyte apoptosis.

The observed difference between the two dose groups indicated a dose-dependent cardioprotective effect with the high dose group showing greater improvement. It may be due to the presence of bioactive phenolic compounds, such as oleuropein and hydroxytyrosol, in olive leaf extract that possesses beneficial action(Menezes *et al.*, 2022). These compounds have strong antioxidant activity, which may mitigate oxidative stress, maintain the functions of mitochondria and minimize the damage of peroxidation of membrane lipids(Berköz *et al.*, 2023). They also have anti-inflammatory effects that could help prevent damage to cardiomyocytes and retain cellular integrity, reducing the levels of cardiac injury biochemicals in the blood(Romeh *et al.*, 2022). Within the treated groups, carvedilol resulted in the largest decrease in troponin-I, CK-MB and LDH levels. This is a better effect that could be due to its combined β -adrenergic blocking, antioxidant and anti-inflammatory effects. It is reported that carvedilol can protect the myocardial resistance to oxidative injury, protect the mitochondrial function and prevent the damage to the cells during the cardiac load(Wang *et al.*, 2021). The significant reduction in all the biomarkers measured suggests that both olive leaf extract and carvedilol were able to mitigate the myocardial damage caused by Carfilzomib. But, the higher response seen with carvedilol indicates that it has a wider cardioprotective activity. However, the fact that olive leaf extract proved to be effective in reducing the cardiotoxicity associated with Carfilzomib, even at high doses, suggests that it could be used as a natural adjunctive therapy.

Oxidative Stress Parameters

In Figures 4–6 the effects of olive leaf extract and carvedilol on cardiac oxidative stress markers are shown. The MDA level was significantly elevated ($P < 0.05$) in Carfilzomib-treated rats (Group III) when compared to the control rats (Group I) and there was a corresponding decline in GSH content ($P < 0.05$) and SOD activity ($P < 0.05$) in the same group. (Figure 4 & 5). The group that was given olive leaf extract alone did not differ significantly from the control group (Group II). Phytochemicals found in Olive Leaf extract (OLE) were highly effective in reducing the oxidative changes caused by Carfilzomib (CFZ). MDA value was significantly lower and GSH and SOD were significantly higher in Group V (400 mg/kg) than in Group IV (200 mg/kg). Furthermore, the lowest MDA levels and highest GSH and SOD values were observed in the group which was treated with carvedilol (Group VI) among all treated groups. However, none of the treated groups completely returned to the control values. The results showed a dose-dependent antioxidant activity of OLE and the highest antioxidant activity was in the group treated with carvedilol against the antioxidant activity of the Carfilzomib induced oxidative stress.

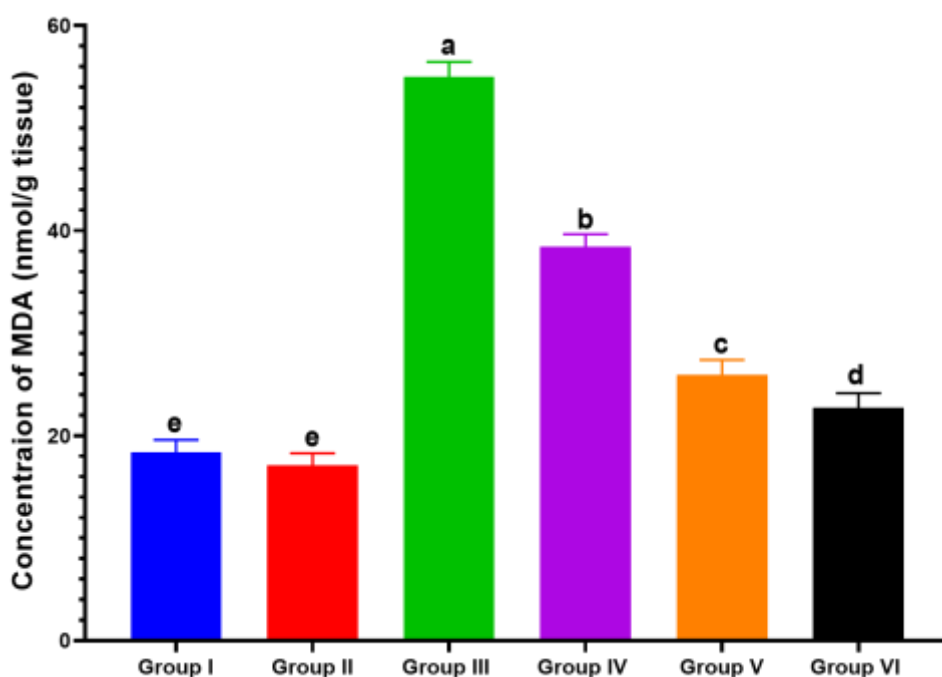


Figure 4. Cardiac lipid peroxidation (MDA) in Carfilzomib treated rats after olive leaf extract and carvedilol treatment. Data is presented as the mean $\pm$ SD (n = 6). Significant differences at $P < 0.05$ indicated by different letters.

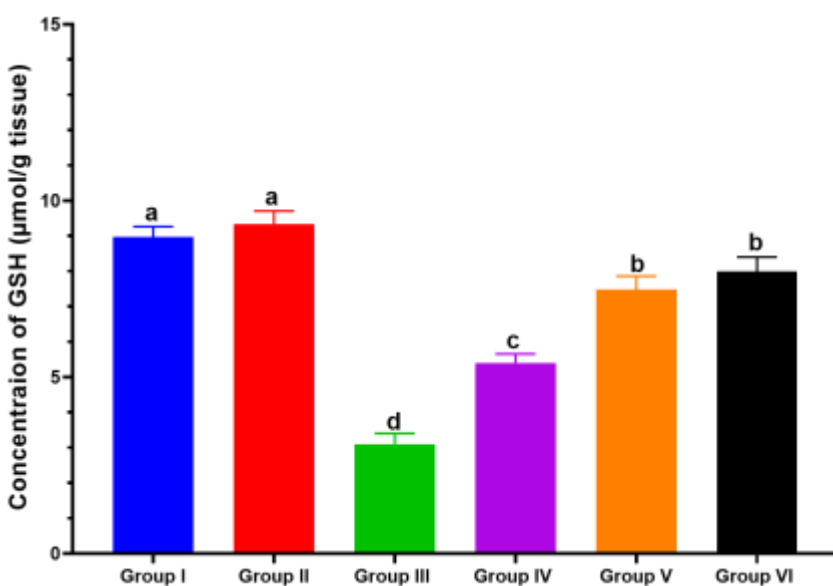


Figure 5. Cardiac reduced glutathione (GSH) level in Carfilzomib treated rats after olive leaf extract and carvedilol treatment. Data is given as mean $\pm$ SD (n = 6). Significant differences ($P < 0.05$) are indicated by different letters.

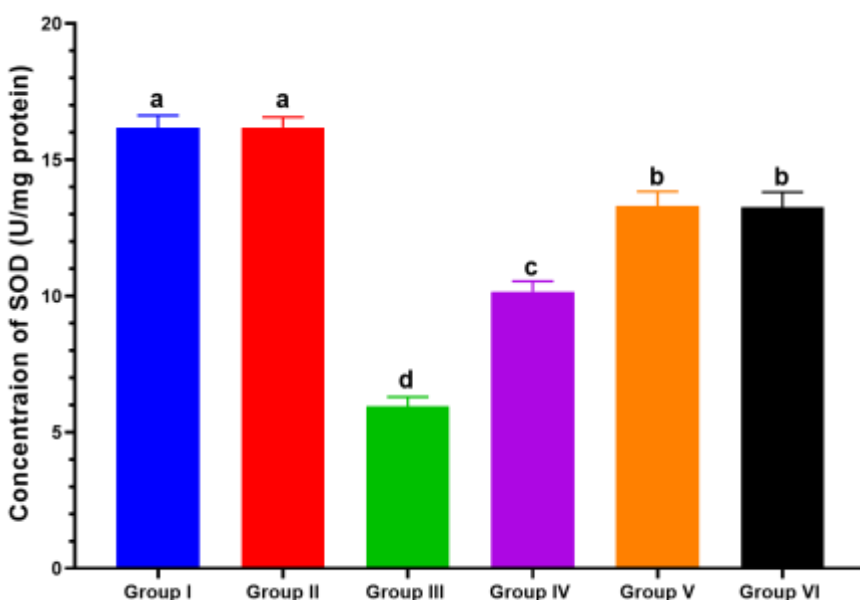


Figure 6. Cardiac superoxide dismutase (SOD) activity in Carfilzomib treated rats after olive leaf extract and carvedilol treatment. Data is given as mean $\pm$ SD (n = 6). Significant differences ($P < 0.05$) are indicated by different letters.

From the alterations observed in the levels of MDA, GSH and SOD, it can be concluded that oxidative stress is a major mechanism of cardiotoxicity induced by Carfilzomib. This marked rise in cardiac MDA levels is indicative of increased lipid peroxidation of cellular membranes, probably due to the excessive production of ROS and thus oxidative injury (Al-Harbi *et al.*, 2019). The simultaneous significant decrease in GSH content and SOD activity suggest that the endogenous antioxidant defense system was exhausted and the ability of cardiac tissue to protect itself against oxidative damage was impaired (Efentakis *et al.*, 2021).

The results are consistent with previous reports of Carfilzomib-induced mitochondrial dysfunction and disruption of cellular redox balance that make the cardiomyocytes more susceptible to oxidative injury (Buck *et al.*, 2022). The levels of MDA were significantly decreased, while all other oxidative stress parameters, such as GSH and SOD were restored by the olive leaf extract administration. The enhancement could be due to the high level of polyphenolic compounds, mainly oleuropein and hydroxytyrosol, with high antioxidant activity (van Stratum *et al.*, 2026).

These bioactive components have the ability to neutralize the free radicals, inhibit lipid peroxidation, and protect the antioxidant capacities of cardiac tissue (de Oliveira *et al.*, 2024). Results showed that the high dose group had significantly better results than the low dose group, which indicates the antioxidant effect of olive leaf extract is dose dependent. A key point of the restoration of GSH and SOD levels is the importance of both molecules in protecting the cardiomyocytes from oxidative stress.

GSH is an important anti-oxidant in the cell, while SOD is the enzyme that helps to convert the superoxide radical to a less toxic product. Thus, the rise in their concentrations after olive leaf extract therapy indicates that the antioxidant defense system of the myocardium was improved and not simply an abatement of the oxidative damage (Kaeidi and Hassanshahi, 2025). Carvedilol had the most significant effect on improving oxidative stress markers in all treated groups. This effect could be attributed to its β -adrenergic blocking action along with its ability to inhibit free radical induction and reduce lipid peroxidation and preserve mitochondrial function (Hafez *et al.*, 2024).

This improvement in the responses seen in this group further underscores the role of oxidative stress in the pathophysiology of cardiac injury induced by Carfilzomib. The observed cardioprotective effects were strongly correlated with the observed decrease in MDA and increase in GSH and SOD levels, suggesting that the antioxidant effect of the compounds was closely related to the cardioprotective activity. As such, the enhancement induced by olive leaf extract could be mainly explained by its ability to preserve redox homeostasis and to reinforce the antioxidant activities of tissues in the heart (Amraoui *et al.*, 2025). Our findings are in agreement with the recent review by Frontiers in Pharmacology by Gao *et al.*, 2025, which emphasized that oxidative stress and excessive ROS generation are central mechanisms underlying carfilzomib-induced cardiotoxicity. The review described mitochondrial dysfunction and lipid peroxidation as key events leading to cardiomyocyte injury, supporting the observed increase in MDA and depletion of endogenous antioxidants (GSH and SOD) in the present study.

Also, Alam *et al.*, 2022, who demonstrated that carfilzomib markedly increased cardiac MDA while reducing GSH and SOD activities. Pretreatment with the antioxidant compound zingerone significantly restored these parameters, indicating that attenuation of oxidative stress represents a common cardioprotective mechanism.

The study by Hamza *et al.*, 2021, demonstrates that olive leaf extract markedly reduced lipid peroxidation and improved endogenous antioxidant defenses in experimental cardiotoxicity models. These beneficial effects were mainly attributed to its high polyphenol content, particularly oleuropein and hydroxytyrosol.

Inflammatory Biomarkers

The effects of olive leaf extract and carvedilol on inflammatory biomarkers in serum are shown in Figures 7 and 8. The levels of serum TNF- α and IL-6 were significantly higher in Carfilzomib-treated rats (Group III) than in the control rats (Group I) ($P < 0.05$). There were no significant differences between the group which received the olive leaf extract alone (Group II) and the control group. Pre-treatment with olive leaf extract significantly reduced the increase of both inflammatory markers induced by Carfilzomib, with the 400 mg/kg group (Group V) have significantly lower values compared to the 200 mg/kg group (Group IV). In addition, the carvedilol-treated group (Group VI) had the lowest level of the TNF- α and IL-6 levels of the treated groups, but these were still significantly higher than the levels observed in the control group. These results suggest that olive leaf extract has a dose-dependent anti-inflammatory activity and carvedilol is able to induce the highest suppression of the inflammatory response caused by Carfilzomib.

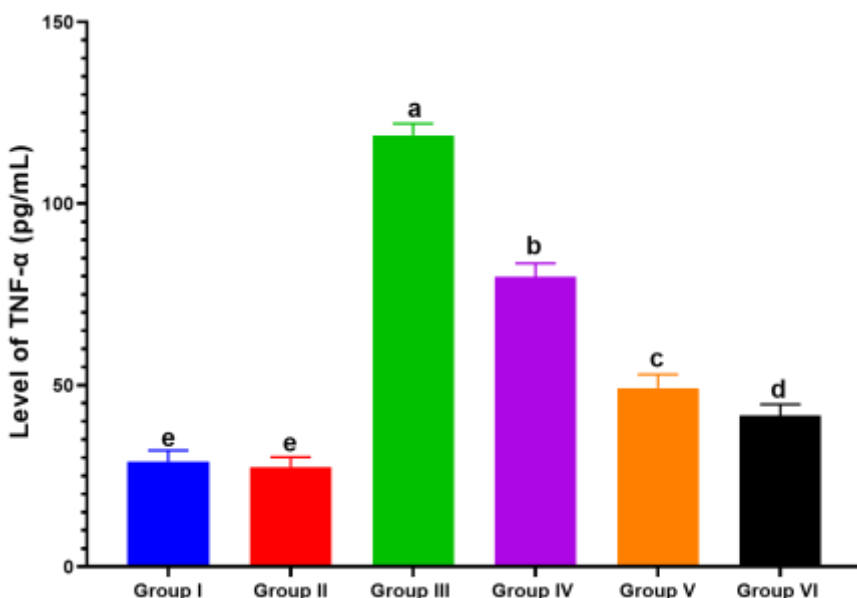


Figure 7. Effect of Olive Leaf Extract and carvedilol on the level of serum Tumor Necrosis Factor (TNF- α) in carvedilomib treated rats. Data is given as mean $\pm$ SD (n = 6). Significant differences (P < 0.05) are indicated by different letters.

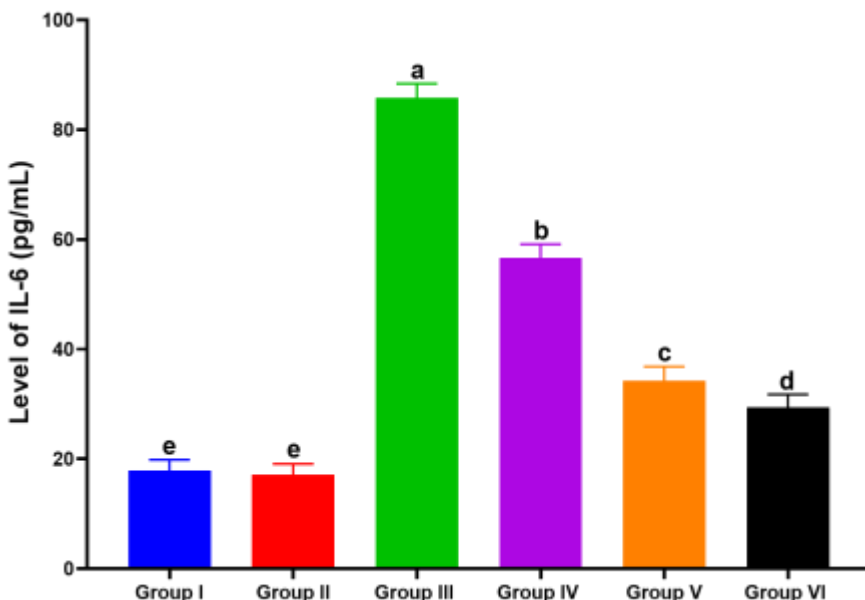


Figure 8. Effect of Olive Leaf Extract and carvedilol on the level of serum IL-6 levels in carvedilomib treated rats. Data is given as mean $\pm$ SD (n = 6). Significant differences (P < 0.05) are indicated by different letters.

A significant increase in the levels of TNF- α and IL-6 after Carfilzomib administration suggests the onset of a strong inflammatory response, which is associated with cardiac damage(Gao *et al.*, 2025). Increased levels of these cytokines are associated with myocardial stress and are markers of activation of signaling pathways associated with myocardial damage and cell dysfunction(Georgiopoulos *et al.*, 2023). TNF- α has been shown to be associated with cardiomyocyte apoptosis and inflammatory cell infiltration, while IL-6 is said to help maintain and exacerbate inflammatory responses in damaged tissues(Hanna and Frangogiannis, 2020).

The inflammatory changes seen in the present study could be a result of the oxidative imbalance created by Carfilzomib. An increase of ROS can trigger the production of pro-inflammatory mediators and increase cytokine production. This interpretation is supported by the simultaneous increase in MDA and decrease in GSH and SOD that was observed in the present study, indicating a close relationship between oxidative stress and inflammation as a stage of the development of cardiotoxicity(Kastritis *et al.*, 2021). The treatment with olive leaf extract was effective in decreasing the levels of the inflammatory cytokines (TNF- α and IL-6), with the higher dose being more effective than the lower dose. This effect could be due to the high levels of phenolic compounds, including oleuropein and hydroxytyrosol, which have been claimed to control inflammatory pathways and inhibit the synthesis of cytokines(Fayez *et al.*, 2023). The enhancing trend in the concentration of these bioactive constituents as per the dose dependent improvement in the present study indicate that with the increase in concentration these bioactive constituents increase their ability to suppress inflammatory activity. Only carvedilol caused the largest decrease in inflammatory markers(Banaei *et al.*, 2025).

In addition to its β -blocking activity, carvedilol has antioxidant and anti-inflammatory properties that could help to decrease cytokine production and minimize tissue damage. The greater response seen in this group further demonstrates the role of inflammation in damage to the heart in the presence of Carfilzomib(Hassanein *et al.*, 2023). The reduction in inflammatory and oxidative stress markers may

have been mediated by a decrease in oxidative injury, which may have helped to moderate cytokine production. The present findings agree with Gao et al. (2025), who reported that carfilzomib significantly increased circulating TNF- α and IL-6 through activation of inflammatory signaling pathways, particularly NF- κ B. Similar to our findings, elevated cytokine levels were closely associated with oxidative stress and myocardial injury.

Histopathological Findings

The microscopic examination of the myocardial architecture of the control group (G1) and the OLE 400 mg/kg (G2) group showed the presence of well-preserved myocardial architecture, normally arranged cardiac muscle fibers, and normal pattern of cardiomyocyte nuclei, indicating the safety of the extract. The carfilzomib-treated group (G3), however, showed thickened walls, severe myocardial injury, diffuse interstitial edema and vascular congestion, and significant inflammatory cell infiltration and myocardial hemorrhage, as well as myofibrillar fragmentation and widespread cardiomyocyte degeneration (Figures 3 and 4). In comparison, the OLE 200 mg/kg + carfilzomib group (G4) exhibited moderate histopathological improvements, with decreased edema, congestion and minimal inflammatory infiltration without pathological changes (Figure 5). Of particular interest, minimal inflammation and well-organized cardiac fibers were seen in the high-dose group (OLE 400 mg/kg + carfilzomib; G5) with significant protection when compared to G4 (Figure 6). Likewise in the carvedilol + carfilzomib group (G6), myocardial structure was almost normal and inflammatory and degenerative lesions were reduced almost to normal (Figure 7), thus displaying an excellent cardioprotection efficacy similar to the high dose OLE group (G5).

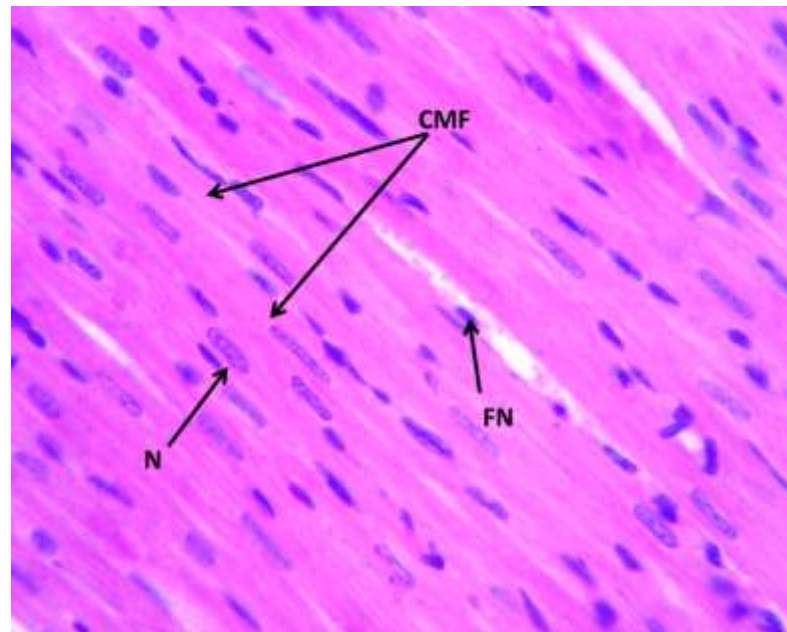


Figure 1. Normal architecture of myocardium (Control group – G1). The branching cardiac muscle fibers (CMF) are well organized with central oval nuclei (N), and a normal fibroblast nucleus (FN) in the narrow interstitial space. (H&E, 400 $\times$).

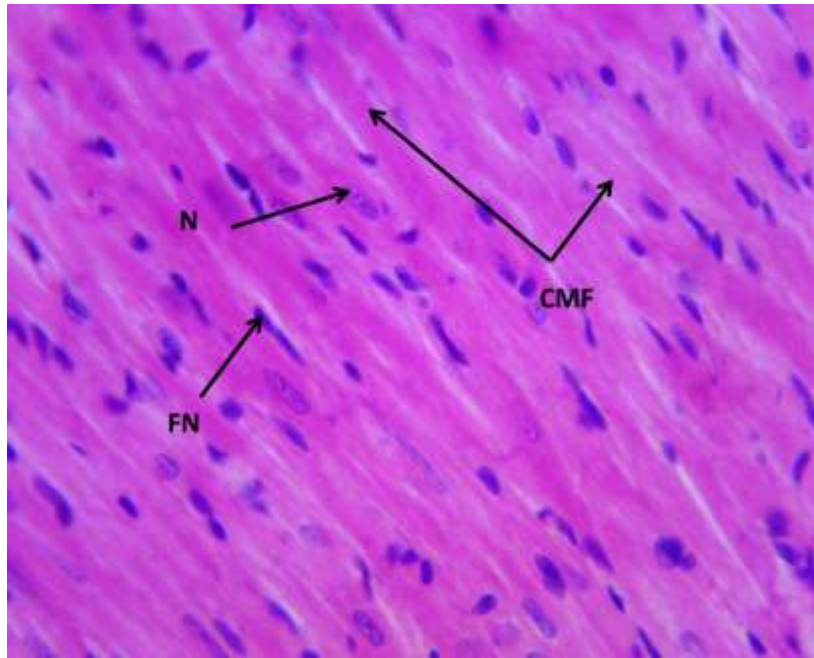


Figure 2. Normal architecture in the myocardium of olive leaf extract group. Organized branching cardiac muscle fibres (CMF) and central oval nuclei (N), a normal fibroblast nucleus (FN) in the narrow interstitial space. (H&E, 400×).

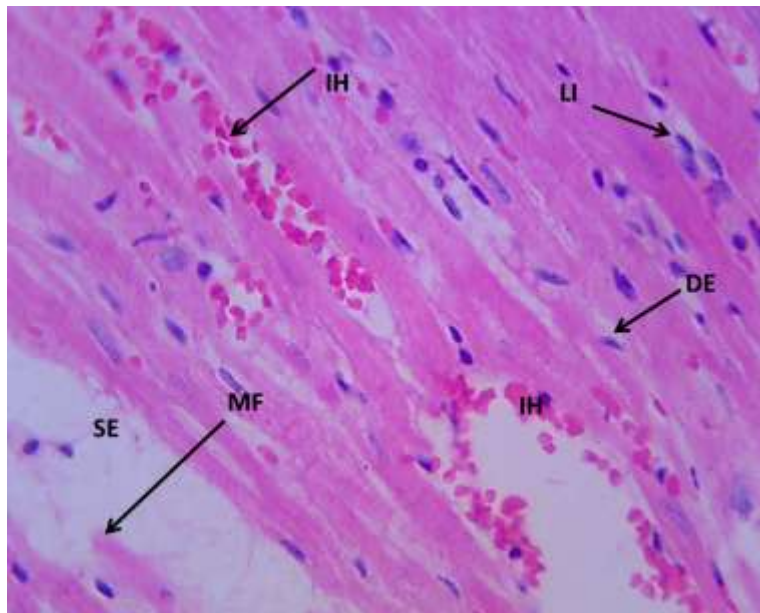


Figure 3. Severe cardiotoxicity in Myocardium of Carfilzomib group (G2). Interstitial hemorrhage (IH) and dense leukocyte infiltration (LI) are present. There is significant severe edema (SE), extensive myofibrillar fragmentation (MF) and advanced cardiomyocyte degeneration (DE). (H&E, 400×).

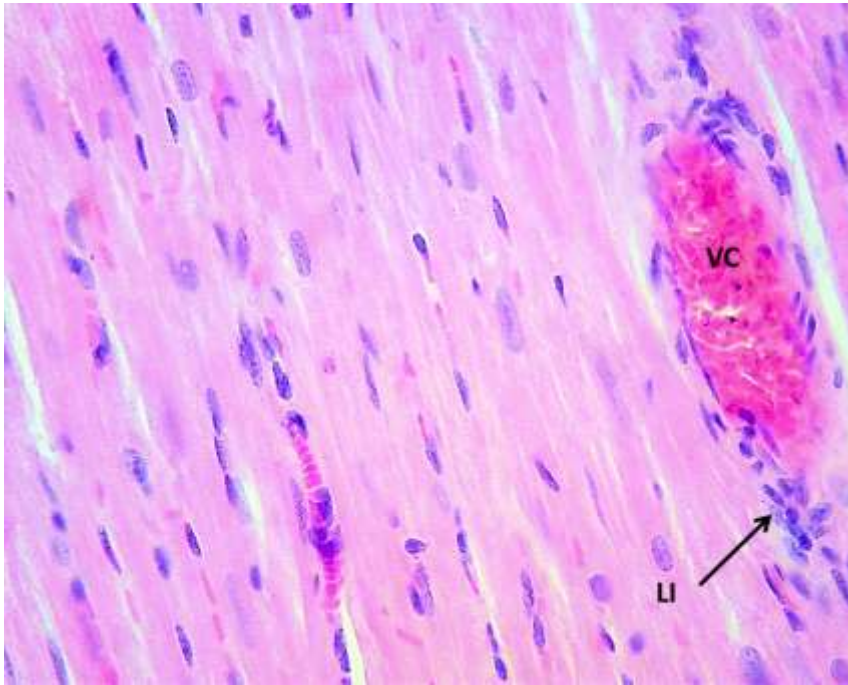


Figure 4. section of the Carfilzomib group (G2) myocardium with severe vascular changes. In a large blood vessel, vascular congestion (VC) is prominent and there is focal perivascular leukocyte infiltration (LI) (H&E, 400×).

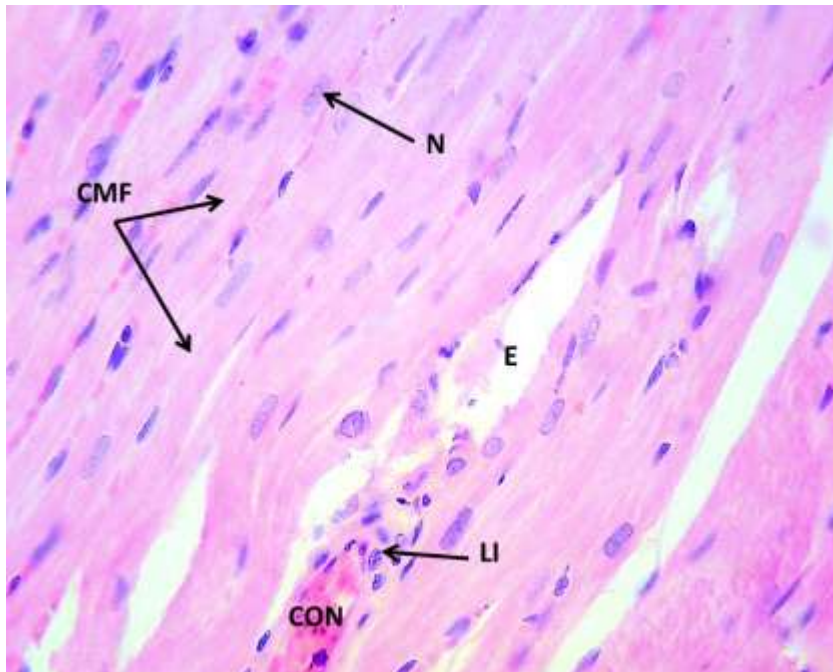


Figure 5. Myocardium of OLE 200 mg/kg + Carfilzomib group with significant improvement. Cardiac muscle fibres (CMF) and central nuclei (N) demonstrate good morphology. The interstitial edema (E), leukocyte infiltration (LI) and mild congestion (CON) are still present, but mild. (H&E, 400×).

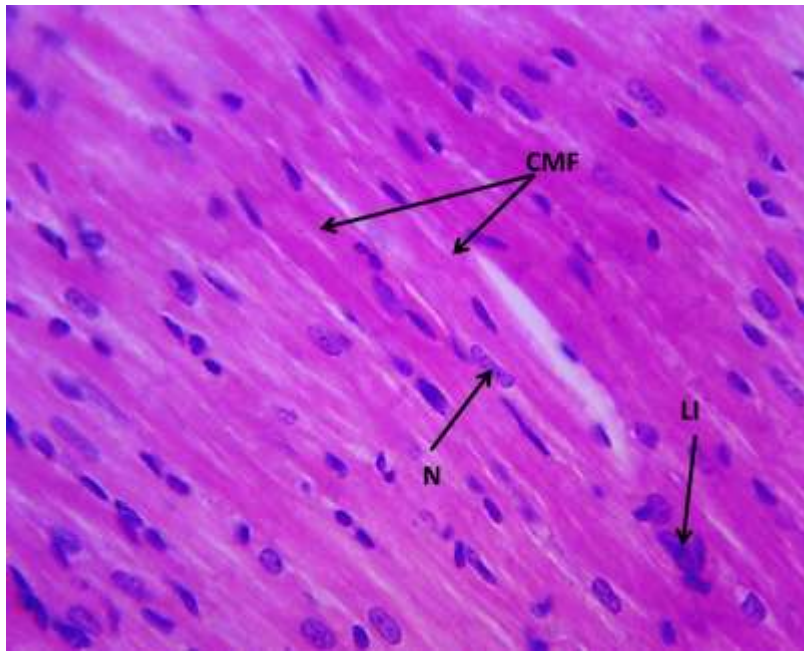


Figure 6. Normal architecture of the myocardium of the OLE 400 mg/kg + Carfilzomib group. Only a minimal amount of interstitial leukocyte infiltration (LI) is seen, and well-preserved cardiac muscle fibers (CMF) and central oval nuclei (N) can be seen. (H&E, 400×).

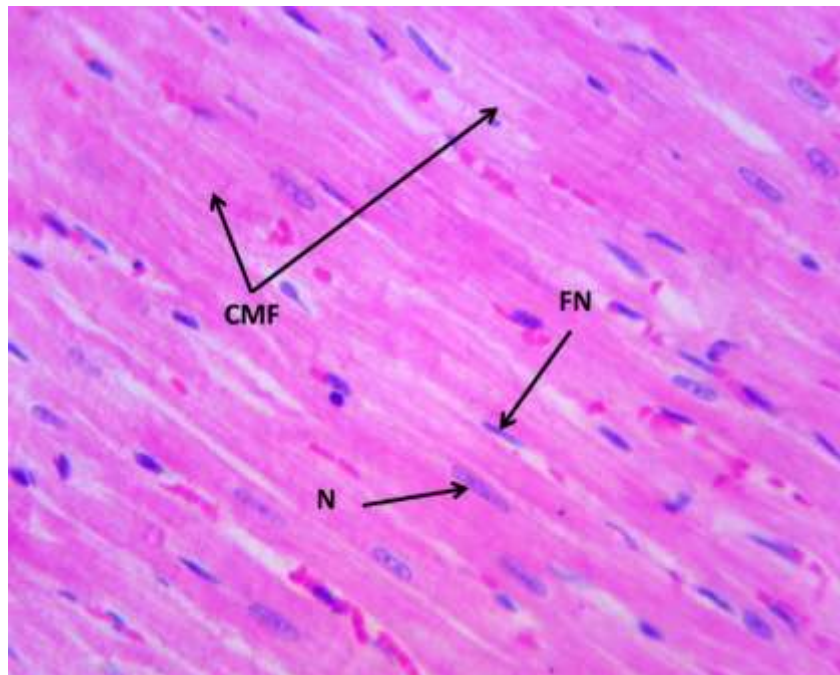


Figure 7. Carvedilol + Carfilzomib (Positive control) excellent cardioprotection and Normal architecture in myocardium. Note the well-organized branching cardiac muscle fibers (CMF) with central oval nuclei (N), and a normal interstitial fibroblast nucleus (FN). (H&E, 400×).

The histopathological changes in the carfilzomib group (G3) are a direct result of the changes in the biochemical biomarkers. In particular, rupture of the cell membrane leads to severe cardiomyocyte degeneration and fragmentation of the myofibrillar structure, which is biochemically confirmed by

massive leakage of cTnI, CK-MB and LDH into the circulation (Yang *et al.*, 2026). The intense lipid peroxidation, reflected by an increase in MDA level and depletion of endogenous antioxidants (GSH and SOD) is the major driving factor for this membrane damage (Hajinezhad *et al.*, 2025). In addition, increased expression of pro-inflammatory cytokines, such as TNF- α and IL-6, directly causes inflammation with a dense inflammatory cell infiltrate and interstitial oedema, leading to increased vascular permeability and recruitment of inflammatory cells to the injured myocardium (Alhareth *et al.*, 2023). On the other hand, the protective action of OLE (G4 and G5) is due to its dose-dependent preservation of myocardial architecture. OLE scavenged free radicals and replenished the levels of GSH and SOD, which kept the cell membranes intact to prevent leakage of enzymes and result in well-organized cardiac fibers with minimal fragmentation (Buzdar *et al.*, 2025; Shalan, 2025). Not only that, but OLE's direct suppression of expression of these two cytokines, TNF- α and IL-6, explains the significant decrease in inflammatory infiltration and edema noted histologically, particularly in the high dose group (G5). This tissue-protective effect was similar to carvedilol (G6), which also has antioxidant and anti-inflammatory effects and was able to modulate these same molecular pathways, leading to an almost normal myocardial structure (Amirshahrokhi and Abzirakan, 2022). The histopathological findings observed in the present study are consistent with the recent review published in *Frontiers in Pharmacology* by Gao *et al.*, 2025, which described cardiomyocyte degeneration, myofibrillar disruption, inflammatory infiltration, and myocardial edema as characteristic pathological features of carfilzomib-induced cardiotoxicity. Similar histological protection has been reported by de Oliveira *et al.* (2024), who demonstrated that olive leaf extract preserved myocardial architecture, reduced inflammatory cell infiltration, and attenuated myocardial degeneration through its potent antioxidant and anti-inflammatory activities.

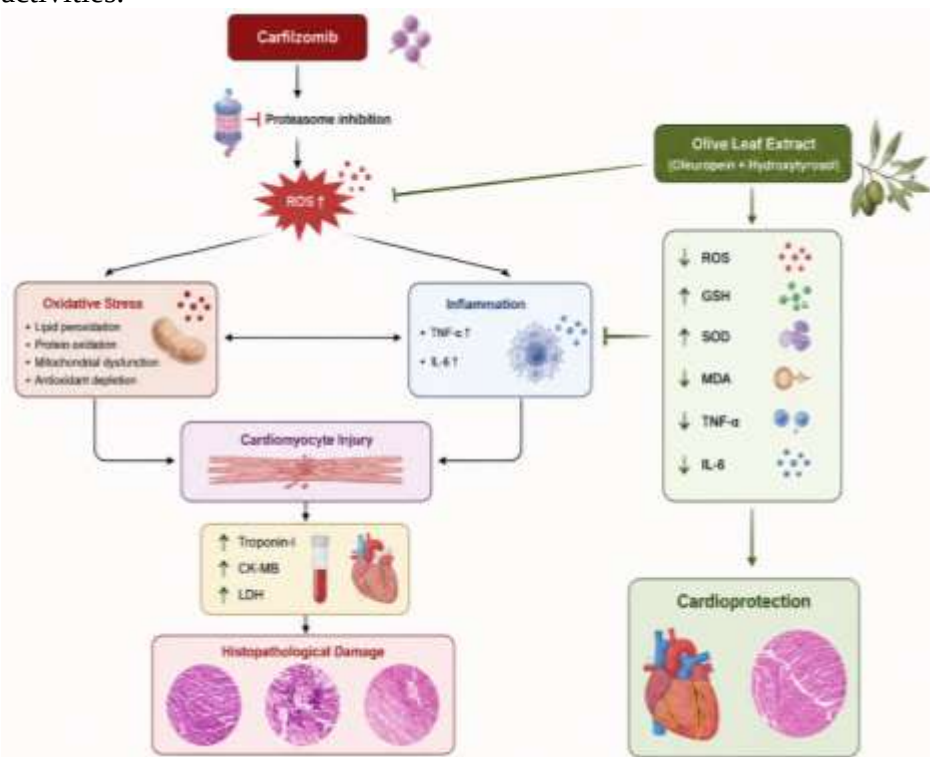


Figure 8: Proposed mechanism by which olive leaf extract, attenuates carfilzomib induced cardiotoxicity.

Limitation of Study

This study has several limitations included, first, the sample size was relatively small. Second, this study included only male rats, therefore sex-related differences could not be evaluated.

Conclusion

The administration of carfilzomib caused a substantial cardiac insult with elevated serum cardiac markers, increased levels of oxidative stress, elevated inflammatory response markers and marked histopathological changes in myocardial tissue. These detrimental effects were greatly reduced by pretreatment with olive leaf extract in a dose-dependent fashion and the high dose of 400 mg/kg afforded better protection than the low dose of 200 mg/kg. The cardioprotective activity of olive leaf extract seems to be due to its antioxidant and anti-inflammatory action. Carvedilol showed the highest protection, but olive leaf extract had significant efficacy and could be a natural source to reduce cardiotoxicity induced by Carfilzomib.

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