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Restoration of the PINK1/Parkin/PGC-1α axis by Liraglutide and Pravastatin co-therapy mitigates Doxorubicin-induced cardiotoxicity

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ABSTRACT

Purpose: Doxorubicin (Dox) is a widely used anthracycline antibiotic with dose-dependent cardiotoxicity. Cardiac myocytes are highly energy-demanding cells that harbour a dense and well-structured mitochondrial network, which facilitates the accumulation of reactive Dox metabolites, causing disturbance of the mitophagy/biogenesis balance. We investigated the potential cardioprotective properties of liraglutide, a GLP-1 receptor agonist, either alone or in combination with pravastatin, in Dox-induced cardiotoxicity (DIC). **Methods:** Thirty-five male Sprague-Dawley rats were allocated into five groups (n= 7/group): normal control, doxorubicin, pravastatin & doxorubicin, liraglutide & doxorubicin, and pravastatin-liraglutide & doxorubicin groups. Cardiac histopathological assessment, as well as, echocardiography was performed at the end of the experiment to asses cardiac function along with measurement of CK-MB and troponin-T levels using ELISA. PINK1, Parkin, and PGC-1α gene expression levels were quantified using qRT-PCR while oxidative stress markers were analyzed biochemically. **Results:** Our findings demonstrated significant improvement in cardiac function, evidenced by the reduction in serum levels of cardiac injury markers, CK-MB and troponin-T, and restoration of mitophagy/biogenesis balance. This was supported by increased expression of PINK1, Parkin, and PGC-1α. Furthermore, co-administration of both drugs enhanced myocardial antioxidant capacity and reduced mitochondrial lipid peroxidation levels. Echocardiographic imaging and histopathological evaluations revealed preserved cardiac architecture and improved ventricular performance and fractional shortening, particularly in the combination therapy group. **Conclusion:** The enhanced effects highlight the therapeutic promise of combining pravastatin with liraglutide to counteract DIC, restoring mitochondrial dynamics, and strengthening antioxidant activity. Such modification of chemotherapeutic regimens may help safeguard cardiac health, improve quality of life, and reduce treatment-related complications.

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1. Introduction

Mitochondria are the main metabolic hub in every nucleated cell that maintains cellular bioenergetics. These dynamic organelles undergo synchronized cycles of biogenesis, fission, fusion, and degradation processes that fine-tune cellular metabolism and mitochondrial homeostasis.[1] The interplay between mitophagy (mitochondrial-specific autophagy) and biogenesis is essential for mitochondrial content and ensures proper metabolic homeostasis.[2] Any disruption of this delicate equilibrium impinges on numerous pathological disorders, particularly in highly demanding organs.

Mitophagy, first described by Lemasters[3], is a selective autophagic pathway that removes damaged or long-lived mitochondria via autophagolysosomal degradation. The canonical mitophagy pathway relies on mitochondrial inner and outer membrane proteins, PINK1 (PTEN-induced putative kinase-1) & E3 ubiquitin ligase Parkin, which tag damaged mitochondria for autophagic removal. Under physiological conditions, PINK1 levels are kept low via proteolysis mediated by protease presenilin-associated rhomboid-like (PARL), thereby preventing unnecessary mitophagy.[4] However, under stress conditions, loss of outer membrane potential creates conditions that allow the inner membrane protein PINK1 to stabilize on the outer surface, where it recruits and activates "Parkin".[5] The PINK1-Parkin complex traffics damaged mitochondria for sequestration, which subsequently fuse with lysosomes for degradation.[6]

Mitophagy is balanced by mitochondrial biogenesis to prevent an energetic crisis.[7] This complex process is regulated via different transcription factors, including nuclear respiratory factors NRF-1 and NRF-2, and peroxisome proliferator-activated receptor gamma co-activator-1 alpha (PGC-1α).[8,9] PGC-1α is a master regulator that maintains mitochondrial density, structure, integrity, and mt-DNA transcription. The orchestrated PGC-1α expression following stressors is important for regulating mitochondrial oxidative phosphorylation and biogenesis, as observed in neurodegenerative disorders.[10] Yao et al. demonstrated that PGC-1α upregulation and its downstream factors enhance mitochondrial mass, respiration, and oxygen consumption.[11]

The interplay between mitophagy and biogenesis was illustrated by Shin et al. in the rodent Parkinson's model, where the Parkin knockout was associated with downregulation of biogenesis transcription factors.[12] Conversely, the stimulation of mitobiogenesis following the Parkin overexpression enhances muscular integrity and strength.[13] Those seemingly opposing findings highlight their coordinated role in preserving mitochondrial dynamics and protecting cellular function from detrimental consequences.

Doxorubicin (Dox) is a broad-spectrum chemotherapeutic antibiotic that profoundly disturbs mitochondrial dynamics.[14] Its clinical application is constrained by multi-organ toxicity.[15] The chemical structure of Dox, combined with the fact that mitochondria occupy more than one-third of cardiomyocyte volumes, facilitates its accumulation in cardiac mitochondria, where it binds to the inner membrane phospholipid cardiolipin.[16,17] Oxidized cardiolipin disrupts the mitochondrial membrane potential and promotes excessive reactive oxygen species (ROS) production, leading to mitochondrial DNA damage and contributing to the cardio-selective toxicity of the drug.[6,18] Preclinical studies have demonstrated that mitochondrial dynamic imbalance, oxidative perturbation, and mitochondrial fragmentation is the main pillar of Dox-linked cardiotoxicity.[19–21] Dox-associated cardiotoxicity draws scientists' attention to the fact that orchestrated mitophagy/biogenesis in myocytes is the fundamental machinery for preventing myocyte toxicity.

Additional therapeutic strategies are required alongside chemotherapy to restore the balance between mitophagy/biogenesis, thereby maintaining organ function. Statins and Glucagon-like peptide-1 (GLP-1) receptor agonists are promising pharmacological interventions with pleotropic properties and an emerging role in mitochondrial medicine. Statins remain first-line therapy for managing dyslipidemia and cardiovascular events.[22] Although statins can exert deleterious effects on skeletal muscle mitochondria[23], Studies have highlighted the off-target effects of pravastatin, a hydrophilic statin, in balancing cardiac mitochondrial redox status and reducing reactive radical production.[24] Pravastatin has been shown to preserve mitochondrial structure and hinder the organelle swelling and DNA fragmentation in different rodent models.[25–27]

Huang et al. investigated the clinical application of statins in patients with breast cancer, demonstrating that statin therapy may help mitigate cardiovascular complications in those undergoing breast-conserving surgery or breast radiotherapy, particularly with hydrophilic statins. Notably, the five-year incidence of cardiovascular events was lower in the pravastatin group (12.2%) compared with the placebo group (31.7%).[28] The hydrophilic nature of pravastatin limited its placental transfer, which contributes to a more favorable safety profile and has supported its evaluation in clinical studies for the management of preeclampsia in women.[29] In addition, pravastatin has demonstrated cardioprotective effects in human myocardium when administered during the reoxygenation phase, where it reduces the expression of apoptotic markers associated with ischemia-reperfusion injury.[30]

GLP-1 is an incretin that regulates insulin secretion in a glucose-dependent manner. GLP-1R agonists exhibit pleiotropic properties, beyond glycemic control effects, owing to their widespread distribution in different organs.[31,32] Liraglutide (LIR) is a GLP-1receptor (GLP-1R) licensed for glycemic regulation in diabetic patients. Beyond glycemic regulation, LIR maintains neuronal mitochondrial integrity by upregulating mitophagy proteins, PINK1, and Parkin expression in the hippocampus.[33] In addition to mitophagy activation, LIR enhances the nuclear expression of mitochondrial biogenesis proteins, PGC-1α, for renewing mitochondrial dynamics. Conversely, downregulation of PGC-1α in the substantia nigra abolished the liraglutide's neurotropic effect in the Parkinson's rat model.[34] The conspicuous ability of liraglutide to attenuate oxidative perturbation and mitochondrial dysfunction ascertained its role as a mitochondrial regulator. The subcutaneous administration of liraglutide in patient experienced non-alcoholic steatohepatitis was evaluated over extendend treatment duration. Clinical findings indicate that liraglutide is associated with a reduction in hepatic steatosis, liver enzyme concentrations, and incidence of fibrosis. Regarding safety, liraglutide is generally well tolerated, with most adverse events being mild to moderate in severity. The most commonly reported side effects are gastrointestinal in nature, including diarrhea, constipation, and loss of appetite.[35,36] Pharmacokinetic analysis from phase III studies conducted in the united states and Asia suggest that body weight and sex can influence liraglutide kinetics.[37,38] Importantly, no dose adjustment is required in patients with hepatic or renal impairments.[39,40] Liraglutide also has a low potential for clinically significant drug-drug interactions due to its minimal involvement with cytochrome P450 mediated metabolism. Accordingly, no meaningful interactions have been observed when liraglutide is co-administered with commonly used medications as atorvastatin, paracetamol, oral contraceptives, and digoxin.[41,42]

Collectively, the intricate interplay between mitophagy and biogenesis has attracted scientific interest as a therapeutic target to manage disorders linked to mitochondrial dysfunction. We hypothesized that liraglutide and pravastatin co-administration would mitigate Dox-induced cardiotoxicity by restoring the balance between mitophagy and mitochondrial biogenesis via modulation of the PINK1/Parkin/PGC-1α axis.

2. Materials & Methods

2.1 Animals

Thirty-five male Sprague-Dawley mature rats (150-170 g), approximately 8-9 weeks old, were acclimatized in the Cairo University animal house under homeothermic conditions with a fixed diurnal period (12-hour light/dark). Animals had unrestricted access to standard rodent chows and free access to fresh drinking water. The body weight of all rats was recorded at the beginning of the experiment and monitored weekly till the day of sacrifice.

All procedures were conducted in precise accordance with the Guide for Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85–23, revised 2011). Ethical approval of the experiment was obtained by the Institutional Animal Care and Use Committee at Cairo University (Permit number: PT3459).

2.2 Drugs & Chemicals

Doxorubicin hydrochloride was purchased as a concentrated solution (2 mg/ml) from EBEWE Pharma (Unterach, Austria). Liraglutide (LIR) was supplied as an injectable pen (6 mg/ml) from Novo Nordisk (Denmark). Pravastatin powder was purchased from Uni Pharma (Egypt). Pravastatin solution was freshly prepared by dissolving it in distilled water before administration.

2.3 Experimental Design

Rats were acclimatized to the laboratory conditions and then randomly allocated into five groups (n= 7/group). The selected doses of pravastatin (20 mg/kg/day) and liraglutide (0.2 mg/kg/day) were based on prior preclinical studies demonstrating cardioprotective efficacy. Using body surface area normalization (Km factor), these doses correspond to human equivalent doses within or near clinically relevant therapeutic ranges.[43]

Group 1; (**Normal control)**, Rats received no pharmacological interventions, only rodent chow, water, along with intraperitoneal (i.p.) saline injection.

Group 2; (**Doxorubicin)**, Rats were administered a single 20mg/kg doxorubicin (i.p.), on the 20th day of the experiment to establish a model of cardiotoxicity.[44]

Group 3; (**Pravastatin + Doxorubicin)**, Rats were pretreated with 20 mg/kg pravastatin solution daily by oral gavage from day 1 to the 21st day of the experiment.[45,46] The cardiotoxic Dox regimen was concurrently applied as described in Group 2.

Group 4; (**Liraglutide + Doxorubicin)**, Rats were pretreated with daily 0.2 mg/kg liraglutide by (i.p.) from day 1 to the 21st day of the experiment.[47,48] The cardiotoxic Dox regimen was concurrently applied as described in Group 2.

Group 5; (**Pravastatin + Liraglutide + Doxorubicin)**, Rats were pretreated daily with a combination of (20 mg/kg pravastatin solution) + (0.2 mg/kg liraglutide) by oral gavage from day 1 to the 21st day of the experiment. The cardiotoxic Dox regimen was concurrently applied as described in Group 2.

2.4 Echocardiography

At the end of pharmacological interventions, rats underwent LV transthoracic echocardiographic examination. Rats were lightly anaesthetized with an i.p injection of ketamine (50 mg/Kg) and xylazine (10 mg/kg) combination[49] and were then positioned in a left lateral recumbent position on the heated monitoring platform. The anterior chest hair was removed for proper probe coupling. ECHO images were performed using the commercially available echocardiogram (Honda HS-2200 V, Tokyo, Japan), with a 12.5 MHz transducer, and the proper parasternal long-axis view at the level of papillary muscles was selected for displaying the maximum LV area. The M-mode tracing automatically measured the left ventricular internal-diastolic and systolic diameter

(LVIDd, LVIDs), ejection fraction (EF%), and fractional shortening (FS%). The cardiac tracings were followed for at least 3 consecutive cardiac cycles to ensure reproducibility.

Following ECHO assessment, blood samples were collected via the retroorbital sinus puncture, centrifuged, and then serum was separated in a new Eppendorf tube for subsequent biochemical examination. Animals were humanely sacrificed, and cardiac tissues were harvested for histopathological examination and protein quantification.

2.5 Biochemical analysis

Commercial colorimetric assay kits (Bio-diagnostic company, Cairo, Egypt) were used to quantify malondialdehyde (MDA) (Cat. No. MD 25 29) and superoxide dismutase (SOD) (Cat. No. SD 2521) levels. In erythrocyte lysates, SOD activity was assayed at 560 nm by measuring its ability to inhibit the reduction of nitro-blue tetrazolium dye, whereas the MDA levels were assessed based on measuring the absorbance of the pink Thio-barbituric acid product at 534 nm.

2.6 Enzyme-linked immunosorbent assays (ELISA)

Creatinine kinase-MB isoenzyme (CK-MB) and Troponin-T were assayed using SUNLONG Sandwich-ELISA kits (Hangzhou, China), catalogue numbers SL0203Ra and SL0713Ra, respectively. Micro-ELISA strip plates, precoated with specific antibody against each biomarker, were used. Samples were incubated with horseradish peroxidase-conjugated secondary antibody in the precoated wells. After the antigen-antibody complex formation, the stop reaction was added, which produced a colour change from blue to yellow, which was quantified spectrophotometrically at a wavelength of 450 nm. The analyte concentrations were calculated from the standard calibration curve.

2.7 Quantitative real-time polymerase chain reaction (Q-PCR)

The Q-PCR technique was used to measure the expression of PINK1, Parkin, and PGC-1α proteins in cardiac tissues. The freshly isolated heart tissues were snap-frozen in liquid nitrogen to preserve RNA from degradation. Up to 30 mg of cardiac tissues were ground in liquid nitrogen, then the resulting fine powder was dissolved in lysis buffer for centrifugation and incubation. The lysate was centrifuged at 14,000 x g for 1 min using an RT column to facilitate RNA binding. Contaminants and residues were removed.

Total RNA was eluted with buffer according to the manual (Cat. No. NA021-0004). Complementary DNA (cDNA) was synthesized from the purified RNA template via RevertAid First Strand cDNA Synthesis Kit (Cat. No. K1621). The cDNA template was mixed with a combination of an optimized PCR buffer and Thermo Scientific Maxima SYBR Green qPCR Master Mix in PCR tubes. PCR tubes filled with cDNA template and q-PCR master mix were placed in a thermal cycler based on Thermo Scientific (Cat. No. K0251).

2.8 Histopathological examination

Cardiac tissues (n= 3/group) were harvested immediately after euthanasia and fixed in 10% buffered formalin. Tissues were prepared for tissue fixation via washing with different ethanol concentrations. Cardiac sections were deparaffinized and stained with hematoxylin & eosin dye following a procedure for morphology inspection. The microscopical examination was performed by a pathology expert following Bancroft's technique.[50]

2.9 Statistical analysis

For normally distributed data, the one-way ANOVA test was used to assess the difference between multiple groups, followed by the Tukey post hoc test. If the P value is less than 0.05, the differences are considered statistically significant. Data sets of the results were expressed as mean +/- SEM, after being processed using GraphPad Prism version 9.

3. Results

3.1 Pravastatin with liraglutide suppressed cardiac injury provoked by Dox

Firstly, we assessed the toxic effects of doxorubicin on cardiac myocytes following a single 20 mg/kg doxorubicin. The cardiotoxicity was confirmed by a marked elevation in creatine kinase (CK-MB) and troponin-T (cardiac injury parameters) by ~6- and 5-fold, respectively, as shown in Fig. 1 (A & B). Liraglutide (0.2 mg/kg/day) treatment suppressed cardiac injury as indicated by a significant decrease in (CK-MB) ($p=0.0001$) and Troponin-T ($p < 0.0001$) levels by 50% and 95%, respectively. Pravastatin (20 mg/kg/day) showed a comparable reduction in injury markers by 44% and 88% respectively, relative to the doxorubicin group. In the combined group, both treatments showed significant cardioprotective effects via restoring Troponin-T and (CK-MB) levels to approximately normal values ($p < 0.0001$).

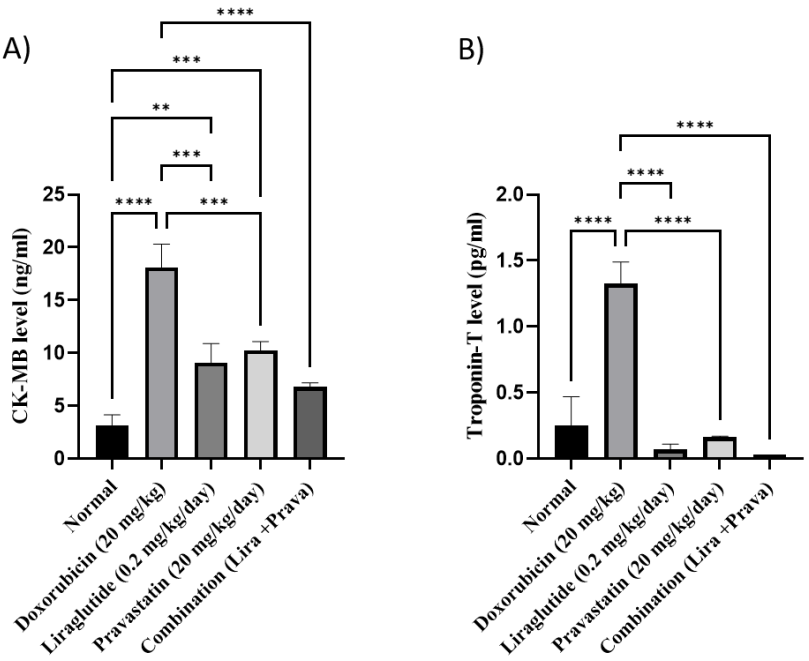

Fig. 1: Levels of A) CK-MB and B) Troponin-T after administration of doxorubicin (20mg/kg), liraglutide (0.2 mg/kg/day), pravastatin (20 mg/kg/day), and a combination of liraglutide and pravastatin (Lira + Prava). One-way ANOVA followed by Tukey post-hoc analysis was performed for the interpretation of data. The results were presented as a mean +/- SE with the significance represented as follows: **** p value < 0.0001, *** p value < 0.001, ** p value < 0.01.

3.2 Treatment regimens stimulate the dampened mitophagy pathway associated with Dox administration

As shown in Fig. 2 (A & B), a single administration of 20mg/kg doxorubicin inhibited the cardiac mitochondrial autophagic pathway (mitophagy). Dox reduced the mitophagy canonical protein expression (PINK1 & Parkin) in cardiac tissues by 56% and 36%, respectively. Daily administration of 0.2 mg/kg liraglutide significantly increased PINK1 and Parkin expression in myocytes by ~4-fold each ($p < 0.0001$), whereas 20mg/kg/day pravastatin elevated their expression by ~ 5 and 6-fold, respectively, compared to the Dox group. The combined treatment, as shown in Fig. 2 (A & B), restored PINK1 & Parkin expression to 6.5-fold of Dox group ($P < 0.001$), exceeding liraglutide or pravastatin monotherapy, thereby enhancing dysfunctional mitochondrial disposal via mitophagy.

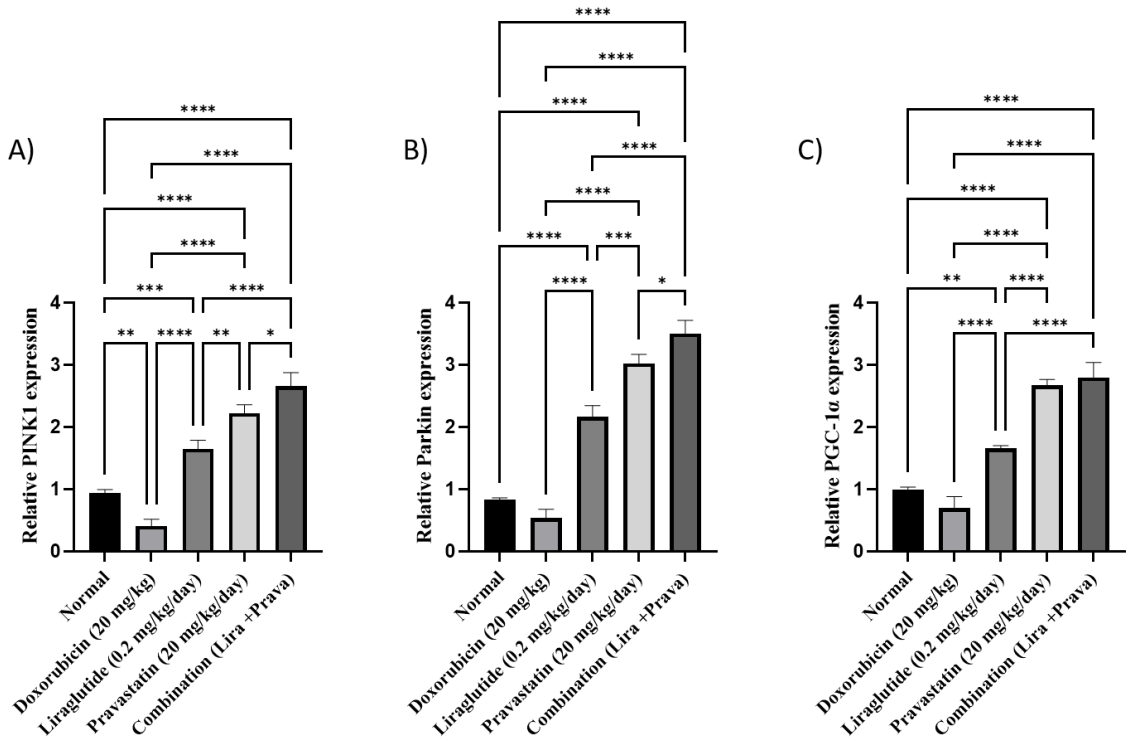

Fig. 2: Levels of A) PINK1, B) Parkin, and C) PGC-1α after administration of doxorubicin (20mg/kg), liraglutide (0.2 mg/kg/day), pravastatin (20 mg/kg/day), and a combination of liraglutide and pravastatin (Lira + Prava). One-way ANOVA followed by Tukey post-hoc analysis was performed for the interpretation of data. The results were presented as a mean +/- SE with the significance represented as follows: **** p value < 0.0001, *** p value < 0.001, ** p value < 0.01, * p value < 0.05.

3.3 Liraglutide alongside Pravastatin boosts mitochondrial biogenesis

Mitochondrial biogenesis, together with mitophagy, sustains mitochondrial homeostasis, which was disturbed by a single 20 mg/kg doxorubicin administration, as shown in (Fig. 2). Dox administration dampened mitochondrial biogenesis and reduced PGC-1α expression by 30% compared to normal controls. The 0.2 mg/kg liraglutide daily administration showed cardioprotective effects via stimulating mitochondrial biogenesis, which was confirmed by a 2.4-fold enhancement in PGC-1α expression in myocytes relative to the Dox group. A combined liraglutide and pravastatin treatment as well as pravastatin alone renewed the mitochondrial pool and produced a significant upsurge in protein expression by 4-fold and 3.8-fold, respectively, compared to the normal control group ($p < 0.0001$), as illustrated in (Fig. 2C).

3.4 Treatment protocols mitigate oxidative stress and reestablish the mitochondrial antioxidant system in cardiac tissues

A single 20mg/kg Dox disrupted mitochondrial oxidative status and significantly reduced the superoxide dismutase (SOD) level by 45%, and increased malondialdehyde (MDA) by ~ 5-fold ($p < 0.0001$), as shown in Fig. 3, compared with the normal control group. Rats in the liraglutide and pravastatin treatment showed a significant increment in SOD levels by 1.2 ($p = 0.006$) and 1.4-fold ($p = 0.0001$), respectively, relative to the Dox group. A combined treatment of both tested drugs significantly restored antioxidant properties and increased SOD levels in a manner equivalent to the normal control group ($p < 0.0001$). Co-treatment with liraglutide and pravastatin significantly decreased the dox-induced elevation in MDA levels by 40% ($p < 0.0001$), while

liraglutide and pravastatin monotherapies reduced MDA levels by 23% and 14% respectively, when compared to the Dox group. Treatment regimens restored the orchestration between mitochondrial antioxidant/oxidative stress in cardiac tissues and enhanced cardioprotective effects.

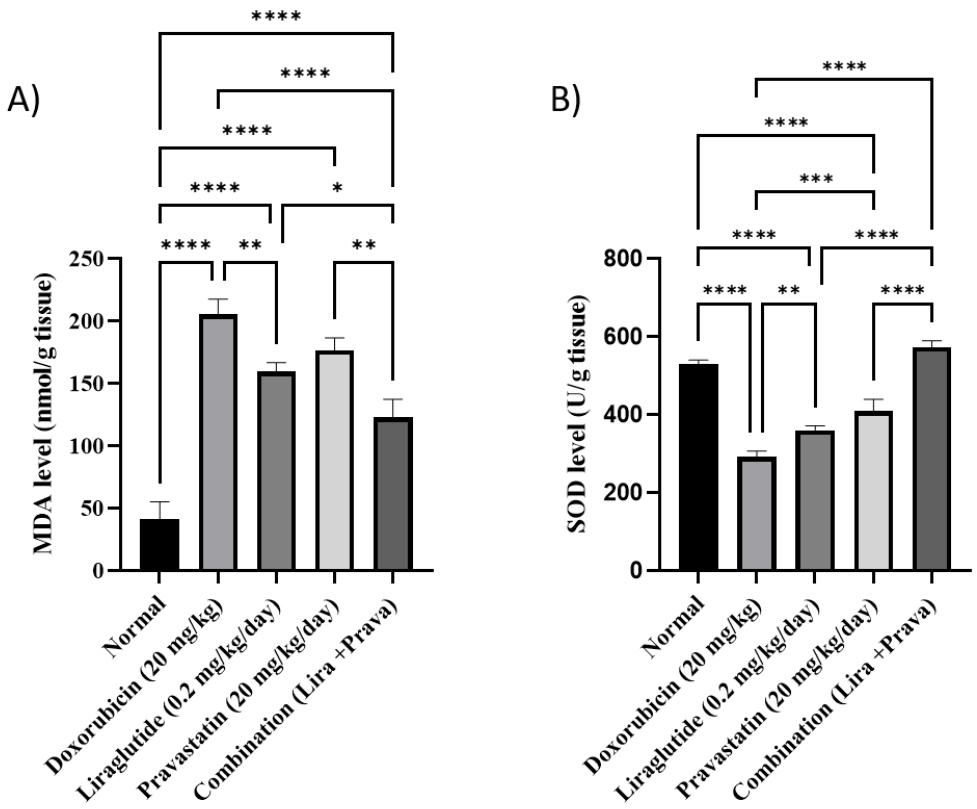

Fig. 3: Levels of A) SOD and B) MDA after administration of doxorubicin (20mg/kg), liraglutide (0.2 mg/kg/day), pravastatin (20 mg/kg/day), and a combination of liraglutide and pravastatin (Lira + Prava). One-way ANOVA followed by Tukey post-hoc analysis was performed for the interpretation of data. The results were presented as a mean +/- SE with the significance represented as follows: **** p value < 0.0001, *** p value < 0.001, ** p value < 0.01, * p value < 0.05.

3.5 Echocardiographic examination results

Echocardiographic images in (Fig. 4) demonstrate the effects of doxorubicin and the various treatments on cardiac function. Doxorubicin showed a significant reduction in ejection fraction (EF) and fractional shortening (FS) by 54% and 69% respectively ($p < 0.0001$), compared with normal control (Fig. 5). Liraglutide improved EF & FS by 1.3- and 1.4-fold, respectively, while pravastatin elevated both parameters by 1.5 and 1.7-fold, compared with the Dox group. Combined liraglutide and pravastatin thereby produced a notable increase in EF and FS by ~ 2- and 2-fold, respectively, significantly surpassing the effects of either drug alone ($p < 0.0001$).

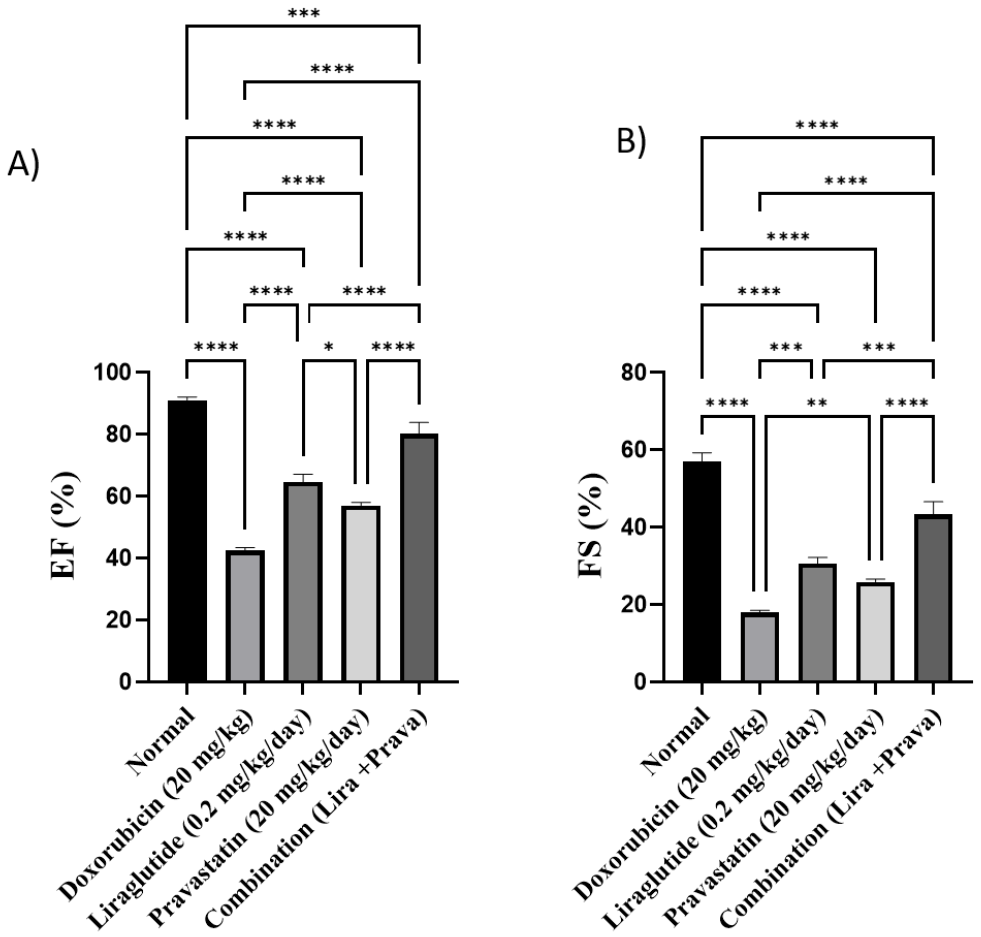

Fig. 4: Levels of A) EF and B) FS after administration of doxorubicin (20mg/kg), liraglutide (0.2 mg/kg/day), pravastatin (20 mg/kg/day), and a combination of liraglutide and pravastatin (Lira + Prava). One-way ANOVA followed by Tukey post-hoc analysis was performed for the interpretation of data. The results were presented as a mean +/- SE with the significance represented as follows: **** p value < 0.0001, *** p value < 0.001, ** p value < 0.01, * p value < 0.05.

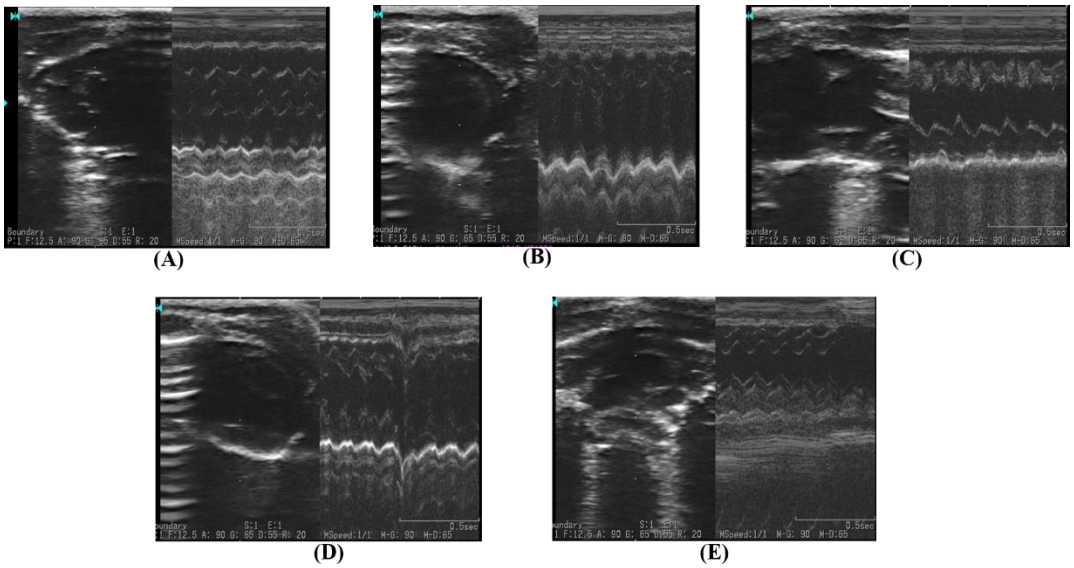

Fig. 5: Echocardiographic images in M mode for (A) normal control, (B) doxorubicin (20mg/kg), (C) liraglutide (0.2 mg/kg/day), (D) pravastatin (20 mg/kg/day), and (E) combination of liraglutide and pravastatin. One representative image for each group is shown.

3.6 Pharmacological interventions optimize myocyte abnormalities induced by Dox

Cardiomyocytes in the normal control group exhibited normal histological features, including striated, branched muscle fibres with oval vesicular nuclei. Intercalated discs between cells are clearly visible and enable cellular organization and synchronized contraction. Cardiac sections from doxorubicin-treated groups, as illustrated in Fig. 6, revealed marked loss of muscular integrity and striation, along with nuclear loss. Degenerated myocytes displayed swollen fibres with a high degree of vascular congestion and inflammatory cell infiltration. Pravastatin and liraglutide mono treatment exert cardioprotective effects as evidenced by moderate inflammatory infiltrates and fewer recorded congested blood vessels. Additionally, both treatments preserved well-organised cardiac architecture with oval-shaped nuclei and mild congestion and cellular inflammatory infiltrates.

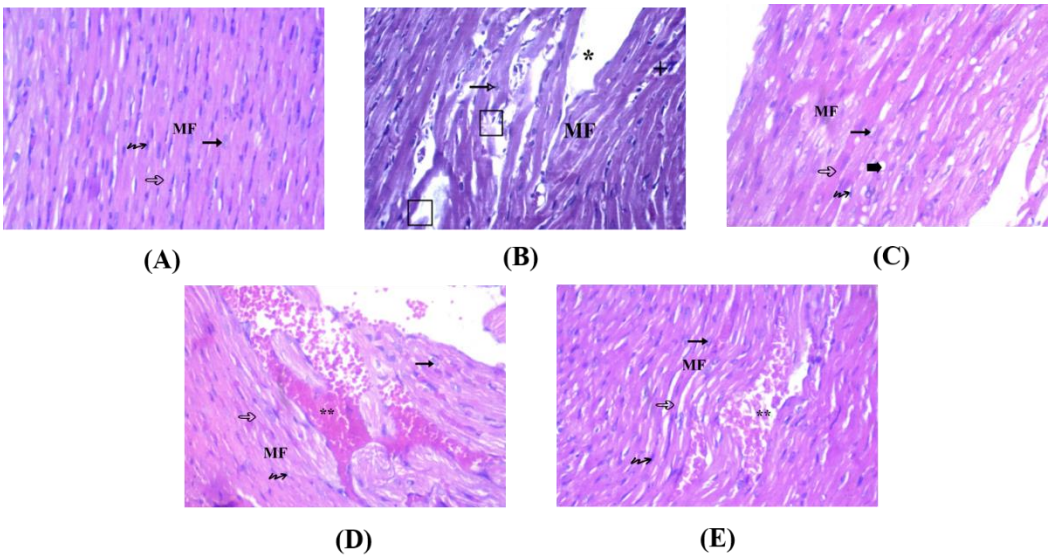

Fig. 6: Histopathological images of the rodent cardiac sections. A) normal control group showing: branching and anastomosing longitudinal muscle fibres (MF), acidophilic sarcoplasm (hollow arrow), oval vesicular nuclei (arrow), interstitial cells (wavy arrow), B) doxorubicin (20mg/kg) showing: inflammation (+), loss of muscle fibers (*), absence of striations (square), C) liraglutide (0.2 mg/kg/day) showing: branching muscle fibers (MF), ovoid vesicular nuclei (arrow), sarcoplasm (hollow arrow), interstitial cells (wavy arrow), wavy muscle fibres (^), mild vacuolation (bold arrow), (D) pravastatin (20 mg/kg/day) showing: longitudinal muscle fibres (MF), vesicular nuclei (arrow), sarcoplasm (hollow arrow), interstitial cells (wavy arrow), moderate congestion (**), (E) combination of liraglutide and pravastatin showing: long muscle fiber aligned longitudinally (MF), oval shaped nuclei (arrow), interstitial cells (wavy arrow), mild congestion (**), sarcoplasm (hollow arrow).

4. Discussion

Doxorubicin-induced cardiotoxicity (DIC) represents a major long-term challenge for cancer survivors, typically manifesting within the first year of doxorubicin treatment.[51] The high incidence of cardiotoxicity is an alarm for the urgent need to optimize chemotherapeutic regimens to enhance patient outcomes.[52,53]

Over the past decades, a growing understanding of the molecular mechanisms underlying cardiac injury has opened new avenues for further investigations. Recent studies have focused on different mediators as gasotransmitters (nitric oxide, carbon monoxide, and hydrogen sulfide), as well as endogenous peptides. In parallel, personalized medicine has emerged recently in the cardio-oncology field and takes into consideration the patient's metabolic and immunological response.[54] Several cardioprotective strategies have been explored to mitigate DIC, including dexrazoxane, the only FDA-approved cardioprotective agent, which acts by chelating iron and reducing ROS formation. Additionally, mitochondrial-targeted antioxidants such as MitoQ and SS-31 have shown promise in preserving mitochondrial integrity and function.[55–57] In a lipopolysaccharide-induced

myocardial injury rodent model, overexpression of glutathione S transferase kappa 1, a novel cardioprotective agent, attenuates mitochondrial dysfunction, inflammatory responses, and pyroptosis.[58]

Mitochondrial quality control mechanisms (fusion, fission, degradation, and biogenesis) play a crucial role in maintaining cardiac function and cellular homeostasis.[59] Besides these processes, targeting mitochondrial oxidative stress has also emerged as a therapeutic strategy. In 5—flurouracil induced cardiotoxicity rodent model, Tambe et al demonstrated that enhancing mitochondrial antioxidant defences mitigated oxidative perturbations. Their study assessed mitochondrial functional status, apoptotic cell death, and oxidative stress, highlighting the protective potential of modulating mitochondrial redox balance.[60]

These approaches, alongside metabolic modulators such as GLP-1 receptor agonists and statins, highlight the growing interest in targeting mitochondrial dysfunction as a therapeutic strategy.

In the present study, we investigated whether pravastatin alone and in combination with liraglutide (GLP-1 agonist) could restore mitophagy/biogenesis equilibrium and redox status during doxorubicin exposure.

Echocardiographic, histopathological, and biochemical analysis demonstrated that Dox significantly reduced left ventricular ejection fraction and fractional shortening, while markedly elevated cardiac injury biomarkers, CK-MB, and Troponin-T.[61] Treatment with liraglutide and pravastatin noticeably preserved cardiac tissue architecture and showed a significant reduction in cardiac injury markers (CK-MB and Troponin-T). These findings were corroborated by improvements in cardiac ultrastructure and histopathological features.[62,63]

Previous scientific studies have shown that DIC arises from the accumulation of Dox and its reactive metabolites in cardiac mitochondria.[6,18] Within the cardiac mitochondria, doxorubicin undergoes redox cycling, leading to oxidative stress and disruption of mitochondrial dynamics.[64] This imbalance between the intrinsic detoxifying system and ROS production was shown by elevated MDA and a decline in superoxide dismutase (SOD) levels.[65,66] Liraglutide exhibited potent antioxidant effects, as shown in a robust decline in MDA and concurrent increase in SOD levels[67–69], thereby supporting energy metabolism and alleviating oxidative stress to preserve mitochondrial architecture. Urbano et al. demonstrated that pravastatin was able to restore the oxidative stress/antioxidant defense system equilibrium, thereby mitigating ROS-mediated mitochondrial damage. Pravastatin has also been shown to preserve cardiac tissues from oxidative radicals following ischemic injury, as evidenced by a significant reduction in MDA and improved SOD activity.[26,70]

The overwhelming oxidative stress induced by doxorubicin results in extensive damage to mitochondrial macromolecules and aggravates mitochondrial membrane damage. The dysfunctional mitochondria accumulate due to the inhibition of the mitochondrial autophagic system, mitophagy. Consistent with previous reports, the decline in the canonical mitophagy markers PINK1 and Parkin levels following Dox administration contributes significantly to cardiotoxicity.[15,19] Activation of the mitochondrial degradation pathway by GLP-1R analogue facilitates the removal of dysfunctional mitochondria and prevents the formation of mega-mitochondria. Liraglutide has been shown to upregulate PINK1 and Parkin, thereby protecting myocytes, as well as neuronal and hepatocytes, from dysfunctional mitochondrial accumulation.[71,72]

Chen and colleagues further proved that doxorubicin administration disturbs the orchestration between intertwined stages of mitochondrial biogenesis and degradation in the cardiotoxic rat models.[73,74] The decline in PGC-1α levels and its downstream factors impairs mitochondrial biogenesis signalling pathways and produces a vicious cycle of oxidative damage.[75–77] Elkhoely showed that liraglutide preserved mitochondrial dynamics homeostasis via replenishing the expression of the mitochondrial biogenesis coactivator PGC-1α.[78] In line with this, upregulation of PGC-1α and its downstream regulators in the substantia nigra protects dopaminergic neurons and motor functions, while its downregulation following lentivirus injection worsens neurotoxicity and mitochondrial

dynamics.[34,79] Although this study focused on key regulators of mitophagy and mitochondrial biogenesis (PINK1/Parkin/PGC-1α), downstream signaling pathways such as NRF1/2 and Akt/mTOR were not assessed. These pathways play critical roles in mitochondrial transcriptional regulation and cellular survival [80,81] and should be explored in future investigations.

Beyond GLP-1 agonist, accumulating preclinical evidence indicates that statin therapy decreases vulnerability to DIC through antioxidant, anti-apoptotic properties, and preserves mitochondrial dynamics.[7,66,82] The present study confirms, for the first time, that pravastatin administration significantly enhances the orchestration between mitophagy/biogenesis, with a remarkable elevation in PINK1/Parkin, PGC-1α levels, supporting its protective role in mitochondrial quality control. Most notably, we demonstrated here for the first time that the combination of liraglutide and pravastatin produces an enhanced cardio-protective effect by restoring mitochondrial dynamics and cardiac oxidative status. While the combination therapy exhibited superior cardioprotective effects compared to monotherapy, formal pharmacological synergy was not evaluated. Future studies employing dose–response matrices and combination index modeling are warranted to distinguish additive from synergistic interactions. A limitation of this study is the relatively small sample size, which may affect statistical power and generalizability. Upcoming studies should investigate the value of incorporating liraglutide and pravastatin into modified chemotherapeutic protocols to maximize treatment efficacy and improve patient compliance in oncology practice. Although both liraglutide and pravastatin are clinically approved drugs, translation of these findings requires caution due to interspecies differences in pharmacokinetics and pharmacodynamics. Long-term safety, optimal dosing, and potential drug–drug interactions in cancer patients remain to be elucidated.

5. Conclusion

Cardiac mitochondrion loaded with Dox shift their balance toward mitochondrial dysfunction and cardiac damage. Proper regulation of mitophagy and biogenesis proteins is the cornerstone for regulating the well-structured mitochondrial population in cardiac tissues following chemotherapeutic regimens. In summary, liraglutide and pravastatin additively protect against Dox-induced cardiotoxicity by restoring mitochondrial quality control via PINK1/Parkin/PGC-1α signaling. Targeting mitophagy–biogenesis equilibrium may provide a novel cardioprotective strategy during chemotherapy. Identifying the molecular pathway of liraglutide alongside pravastatin's ability to purge damaged mitochondria may open new avenues for managing other disorders associated with mitochondrial dysfunction.

Ethical Approval

Ethical approval of the experiment was obtained by the Institutional Animal Care and Use Committee at Cairo University (Permit number: PT3459).

Authors' Contribution

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