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Original Article

Pleiotropic attenuating effect of *Ginkgo biloba* against isoprenaline-induced myocardial infarction via improving Bcl-2/mTOR/ERK1/2/Na^+, K^+-ATPase activities

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ABSTRACT

Objective: Myocardial infarction (MI) is linked to an imbalance in the supply and demand of blood oxygen in the heart muscles. Beta-blockers and calcium antagonists are just two of the common medications used to treat MI. However, these have reportedly been shown to be either ineffective or to have undesirable side effects. Extract of *Ginkgo biloba* leaves (GBE), a Chinese herbal product offers special compatibility benefits in therapeutic settings relating to inflammatory diseases and oxidative stress. In order to better understand how GBE affects MI in rats insulted by isoprenaline (ISO), the current study was designed.

Methods: The heart weight index, serum lipid profile, cardiac marker enzymes, endogenous antioxidants [catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), nitrites and malondialdehyde (MDA)], inflammatory mediators [tumour necrosis factor alpha (TNF-α) and interleukin-6 (IL-6)], immunohistochemical expressions of B-cell lymphoma factor-2 (Bcl-2), extracellular signal-regulated kinase (ERK1/2), and mammalian target of rapamycin (mTOR) and histopathological analysis were used to assess the cardioprotective properties of GBE.

Results: The findings showed that GBE effectively attenuated myocardial infarction by boosting the body's natural antioxidant defense system and reducing the release of inflammatory cytokines as well as heart injury marker enzymes. The expression of Bcl-2, ERK1/2 and mTOR was increased while the histomorphological alterations were reversed.

Conclusion: The cardioprotective effects of GBE may be due to a mechanism involving increased Bcl-2/mTOR/ERK1/2/Na^+, K^+-ATPase activity.

1. Introduction

Cardiovascular disease (CVD) has been reported to be the leading course of death worldwide constituting about seven million deaths which comprises of 12.8% of all mortality cases in the year 2020 (Qin et al., 2022; Teo & Rafiq, 2021; Virani et al., 2020). Myocardial injury (MI), also known as myocardial ischemia injury, was one of the pathophysiology of CVD and it was brought on by the restriction of blood and oxygen supply to the heart (Redline, Azarbarzin, & Peker, 2023). An imbalance between the demands of coronary blood and myocardial oxygen caused MI injury, which was irreversible progression of cardiac muscle tissue destruction (Liu et al., 2021). The onset of symptoms, its associated impairment

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and its recurrence endangered the patients' quality of life and increased their mortality rate, particularly sudden death, which was the most common pattern of harm brought on by MI (Lin et al., 2021). Studies on the pathophysiology of MI have recently shown that oxidative stress and inflammation played significant roles in the pathogenesis of MI (Deng et al., 2021). The cardiac cell experienced a number of aberrant metabolic and biochemical variations as a result of an increased inflammatory response (Wang et al., 2020). The interaction between the ERK1/2 signalling pathway had received little attention despite decades of in-depth study (Zhu et al., 2022). By lowering the expression of inflammatory cytokines, activation of ERK1/2 reduced the severity of acute myocardial infarction in rats (Khalifa et al., 2022). According to research by Shen et al. (2023), the MAPK/ERK1/2 signalling pathway both exacerbated and defended against cardiac damage. Additionally, early research offered strong support for a potential function for the mammalian target of rapamycin (mTOR) signalling pathway in the regulation of lifespan in invertebrates and this data had recently been extended to mammals (Mota-Martorell et al., 2022). According to Lotfollahzadeh et al. (2023), unregulated mTOR signalling has been linked to metabolic diseases like obesity. On the other hand, little was known about the function of mTOR in cardiovascular disorders. The lack of oxygen prevented oxidative phosphorylation, which caused the mitochondrial membrane to become depolarized, ATP decreased and cardiac contractile performance was limited (Zhou et al., 2021). Aerobic glycolysis was replaced by anaerobic glycolysis when there was a sustained lack of oxygen which consequently resulted to lactic acid builds up and a decrease intracellular pH (Souto-Carneiro et al., 2020). However, free radicals and reactive oxygen species (ROS) were activated by the acidic conditions present during ischemia (Sadiq, 2023). The inflammatory response was then triggered by an accumulation of ROS, which increased contractile failure, hypertrophy and fibrosis as well as cell death (Xie et al., 2021). These anomalies may have function to significantly trigger the development of cardiac injury as a whole (Chen et al., 2021). Numerous investigations have shown that anti-inflammatory treatments may stop the occurrence of these harmful incidence to lessen MI and its associated dysfunctions (Asiwe et al., 2024). Indeed, intriguing results have came from a number of treatment studies employing animal models. But none of the pre-clinical phases were practical or useful in extensive human clinical practice (Milic et al., 2022). It was decided to use *Ginkgo biloba* L. in this case because of its unique properties of antioxidative and anti-inflammatory properties.

G. biloba, also referred to as the "living fossil", was said to be indigenous to China, Japan and Korea, and had experienced very few evolutionary changes over the course of 200 million years (Wang et al., 2023). According to Wang et al. (2020), it was thought to have originated in the isolated mountain valleys of Zhejiang Province in eastern China. Both its leaves and its nuts were now widely farmed (Shahrajabian et al., 2022). For more than 5 000 years, traditional Chinese medicine had employed medicinal preparations of the dried leaves for a variety of uses. Only lately the full pharmacological potential of the *Ginkgo* leaf had been recognized (Feodorova et al., 2020; Ge et al., 2021). Studies on the biological activity of various *Ginkgo* leaf components first began with the development of contemporary scientific methodologies about 20 years ago. *Ginkgo* leaves' amazing vitality had led to its use as food, vitamins and medicine whether fresh or dried form (Bommakanti et al., 2023). The predominant pharmacologically active constituents were identified to be flavonols (kaempferol, quercetin, myricetin, apigenin, isorhamnetin, luteolin and tamarixetin) and terpene trilactones (ginkgolide A, ginkgolide B, ginkgolide C, ginkgolide J, ginkgolide M, ginkgolide K, ginkgolide L and bilobalide) (Liu et al., 2021; Obrenovich et al., 2022). The leaves of *G. biloba* were recently used to isolate two novel ginkgolides (ginkgolide P and Q) (Hébert et al., 2022). Numerous studies have shown that *G. biloba* extract (GBE) helped to treat a variety of illnesses, including type 2 diabetes, atherosclerosis (Asiwe et al., 2024; Liu, Gao, Li, Jiang, & Chen, 2022), hypertension (Asiwe et al., 2023a, 2023b, 2023c) and dementia (Shareena & Kumar, 2022; Adebayo et al., 2023). GBE was frequently utilized to treat oxidative stress-related disorders since it was high in antioxidants (Adebayo et al., 2022; Asiwe et al., 2023a; Barbalho et al., 2022; Ben-Azu et al., 2022). With this background, we investigated the mechanism by which *G. biloba* extract attenuated the cardiomyocyte damage caused by isoprenaline exposure in a Wistar rat model.

2. Materials and methods

2.1. Materials

The Chinese company Nanjing Jiancheng Bioengineering Institute provided the chemicals Na^+, K^+-ATPase, lactate dehydrogenase (LDH) and gamma-glutamyl transferase (GGT). Cardiac troponin-I (cT-I) and creatine kinase-MB (CK-MB) were purchased from Roche Diagnostics Elecsys 2010 (Berlin, Germany). The kits for measuring total cholesterol (TC), triglycerides (TG), low density lipoprotein-cholesterol (LDL-C) and high density lipoprotein-cholesterol (HDL-C) were supplied by Randox Laboratories Ltd (Crumlin, Ireland). Isoprenaline was purchased from Sigma-Aldrich (Saint Louis, USA) while Mason Vitamins (Miami Lakes, Florida, USA) provided the standardised *G. biloba* extract. The analytical grade was used for all other substances used in this study.

2.2. Animals

Male Wistar rats, aged 10–14 weeks and weighing an average of 150 g, were housed within the established laboratory norms of temperature (25 °C) and humidity (45%–55%), and were allowed free access to fresh water and food. To reduce animal suffering, all research was conducted after receiving ethical approval from the Faculty of Basic Medical Science Research Ethics Committee (ethical approval number: RBC/FBMC/DELSU/23/182). However, prior to the start of the experiments, all animals underwent a seven-day acclimatization period.

2.3. Research design and treatment regimen

Animals were randomly selected into four groups after 7 d of acclimating to the laboratory setting. The dose of isoprenaline and GBE was adapted from the study of Asiwe et al. (2023a) and treated as follows: Group 1 received normal saline for 28 d (10 mL/kg b.wt); Group 2 received isoprenaline (150 mg/kg b.wt) for day 1 and 2 consecutively; Group 3 received GBE (50 mg/kg b.wt) after 2 d exposure to isoprenaline; Group 4 received GBE (50 mg/kg b.wt) for 28 d.

Following the 28-day experiment, the animals were weighed and sedated using ketamine (70 mg/kg) after overnight (12 h) fasting from food. Blood samples were then taken through the retro-orbital sinus into plane bottles for a serum biochemical analysis (Fig. 1). The remaining heart tissues were fixed in 10% phosphate buffered formalin for histological and immunohistological examinations, while a portion of the heart tissues were excised, degreased of blood and fat, weighed and homogenized for additional biochemical tests. In phosphate-buffered saline as Asiwe et al. (2021) have already explained, heart tissues were homogenized. The homogenates were then centrifuged for 15 min at 3 000 r/min in a 4 °C-cooled centrifuge. After the supernatants

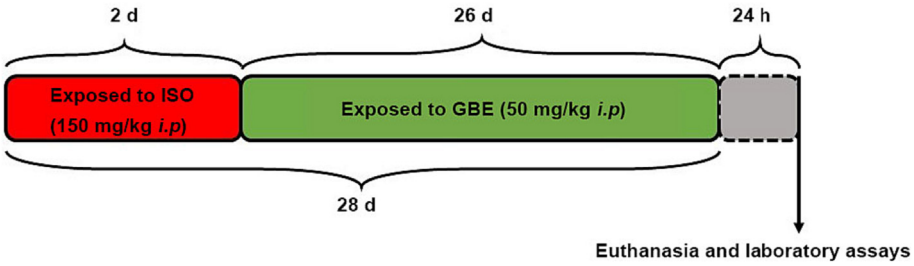

Fig. 1. Flow chart of research design.

were decanted, CK-MB, cT-I, Na^+, K^+-ATPase and antioxidant activity were all assayed.

2.4. Estimation of cardiac injury markers

Using their respective ELISA kits from Nanjing Jiancheng Bioengineering Institute (Nanjing, China), the levels of cT-I and CK-MB in the heart supernatant were measured. The operation was carried out in accordance with the manufacturer's instruction.

2.5. Assay of lipid profile, lactate dehydrogenase (LDH) and gamma glutaryl transferase (GGT)

According to the techniques described by Reitman and Frankel (1957), Randox Laboratories Ltd. in Crumlin, UK, provided the commercial kits. These kits were employed to calculate the levels of total cholesterol (TC), triglyceride (TG) and high density lipoprotein-cholesterol (HDL-C). This formula was used to determine low density lipoprotein-cholesterol (LDL-C): TC – TG/5 – HDL-C = LDL-C. Also, following the manufacturer's instructions, the levels of LDH and GGT activities in the serum were measured using their respective commercial ELISA kits.

2.6. Measurement of Na^+, K^+-ATPase activity

The Na^+, K^+-ATPase activities were evaluated in accordance with Dai et al.'s (2021) instructions. The cardiac tissue supernatant was incubated at 37 °C with 0.2 mL of buffer, 0.2 mL of $CaCl_2$, 0.2 mL of ATP and 0.2 mL of water. This mixture was then incubated with 2.0 mL of 20% trichloroacetic acid (TCA) for 10 min at 37 °C, and the absorbance was measured at 650 nm.

2.7. Assay of cardiac antioxidant activities

Commercial diagnostic kits and established techniques were used to determine the cardiac superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), nitrites, and malondialdehyde (MDA) status. In summary, the McCord and Fridovich (1969) approach was used to identify SOD activity. The amount of enzyme that stopped pyrogallol oxidation by half was assumed to equal one unit of SOD. SOD activity was represented as U/mg protein. The CAT assay was carried out in accordance with Beers and Sizer's (1957) guidelines. The enzyme quantity designated as one unit of CAT was determined to be required to break down 1 mol of hydrogen peroxide (H_2O_2)/min. As mg/protein, the CAT enzyme activity was expressed. According to Góth (1991), the reduced GSH concentration was found by combining the solutions, which included 2 mL of syringe, 8 mL of Milli-Q water and 100 μL of 1.0 mol/L NaCl with pH adjusted to 4.7 using 0.1 mol/L of HCl. At 400 nm, the absorbance spectrum measurements were made. A method developed by Ohkawa et al. (1979) was used to determine the amount of MDA in the myocardium of the heart. A total of 1 mL of 10% TCA, 0.5 mL of saline, and 0.5 mL of tissue homogenate were combined after being centrifuged at 3 000 r/min for 20 min. The protein-free supernatant was combined with 0.25 mL of TBA reagent to make 1 mL, which was then heated for an hour at 95 °C. After bringing the tubes to room temperature and immersing them in running water, the absorbance at 532 nm was measured. The Griess method was used to assess nitrite, a product of nitric oxide (NO) generation according to Asiwe et al. (2022a). The Griess reagent was freshly made with 0.1% *N*-(1-napththyl) ethylene diamine dihydrochloride and 1% sulphanilamide in 5% phosphoric acid. The cardiac supernatant was transferred to a microtiter plate together with 50 μL of distilled water for diluting it and 100 μL of Griess reagent for a 10-min incubation period at room temperature and in the dark. The standard curve's reference point was sodium nitrite. The absorbance was measured at 540 nm with a microplate reader (MICRO READ 1000, Namur, Belgium).

2.8. Histology and immunohistochemistry

Standard methods were used to embed 10% phosphate-buffered formalin-fixed heart tissue in paraffin blocks, it was segmented into 4–5 μm sections, deparaffinized and then rehydrated. After that, the slices were H&E-stained for cytoarchitectural alterations. More specifically, immunohistochemistry was carried out on paraffin-embedded, 5-μm-thick cardiac fixed-tissue slices. We examined the immunoreactivity of Bcl-2, mTOR and ERK1/2 using rabbit polyclonal antibodies from Elabscience Biotechnology (Philadelphia, USA), and the Avidin-Biotin Complex (ABC) technique described by Kolawole et al. (2022) and Asiwe et al. (2023c) was adopted.

2.9. Statistical evaluation

The mean and standard error of mean (SEM) for all results were presented. One-way ANOVA was used to analyse the results and Bonferroni multiple comparison test was used in the post-hoc analysis using GraphPad Prism software version 8.3.5. Statistical significance was defined as a difference with $P < 0.05$.

3. Results

3.1. G. biloba reversed cardiac hypertrophic phenotype

Isoprenaline induced an increase in heart weight [F (3, 16) = 43.29, $P < 0.000\ 1$, $R^2 = 0.890\ 3$] when compared to the control group. However, treatment with GBE significantly reduced cardiac weight index compared to the ISO group as shown in Fig. 2.

3.2. G. biloba attenuated hyperlipidaemia

There was a significant increase in TC [F (3, 16) = 36.25, $P < 0.000\ 1$, $R^2 = 0.871\ 7$], TG [F (3, 16) = 28.83, $P < 0.000\ 1$,

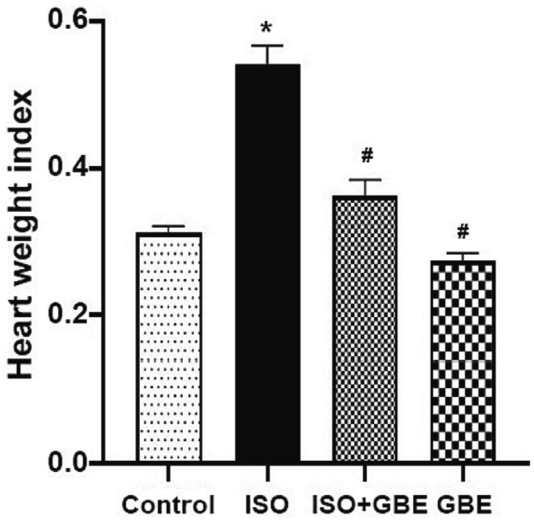

Fig. 2. *G. biloba* reversed cardiac hypertrophic phenotype (Mean ± SEM, n = 5). $^{*}P < 0.05$ *vs* control group, $^{\#}P < 0.05$ *vs* ISO group. ISO, isoprenaline; GBE, *Ginkgo biloba* extract.

R^2 = 0.843 9] and LDL-C [F (3, 16) = 37.83, P < 0.000 1, R^2 = 0.876 4] in isoprenaline exposed animals when compared to the control group. HDL-C [F (3, 16) = 50.80, P < 0.000 1, R^2 = 0.905 0] was significantly reduced in ISO-group when compared to control group. However, treatment with GBE significantly abated ISO-induced dyslipidaemia by increasing HDL-C and reducing TC, TG as well as LDL-C (Fig. 3).

3.3. G. biloba modulated cardiometabolic activities

Isoprenaline induced cardiometabolic disturbances that resulted in significantly increase in LDH [F (3, 16) = 117.5, P < 0.000 1, R^2 = 0.956 6], GGT [F (3, 16) = 32.41, P < 0.000 1, R^2 = 0.858 7], CK-MB [F (3, 16) = 7.959, P = 0.001 8, R^2 = 0.598 8] and troponin-1 [F (3, 16) = 24.00, P < 0.000 1, R^2 = 0.818 2]. However, GBE significantly abated cardiometabolic disturbances by significantly reducing LDH, GGT, CK-MB as well as troponin-I as shown in Fig. 4.

3.4. G. biloba abated oxidative disturbances

Acute adrenergic stimulation caused by isoprenaline induced in the heart oxidative disturbances by significantly reducing GSH [F (3, 16) = 30.74, P < 0.000 1, R^2 = 0.852 1], SOD [F (3, 16) = 15.79, P < 0.000 1, R^2 = 0.747 5] and catalase [F (3, 16) = 16.43, P < 0.000 1, R^2 = 0.754 9] which consequently significantly increased cardiac nitrite [F (3, 16) = 15.42, P < 0.000 1, R^2 = 0.743 0] and MDA [F (3, 16) = 8.653, P = 0.001 2, R^2 = 0.618 7] levels when compared with control group. However, treatment with GBE ameliorated ISO-induced oxidative disturbances by significantly increasing GSH, SOD and catalase while significantly reducing the cardiac nitrite and MDA level as shown in Fig. 5.

3.5. G. biloba inhibited inflammatory responses

There was a significant release of pro-inflammatory cytokines (TNF-α and IL-6) in isoprenaline exposed animals when compared to control group. Following treatment with GBE significantly inhibited the release of TNF-α [F (3, 16) = 10.40, P = 0.000 5, R^2 = 0.661 0] and IL-6 [F (3, 16) = 20.04, P < 0.000 1, R^2 = 0.789 8] when compared with ISO-treated group as presented in Fig. 6.

3.6. G. biloba inhibited apoptosis

Acute exposure to isoprenaline induced cardiac apoptosis by significantly suppressing the cardiac Bcl-2 [F (3, 16) = 33.97, P < 0.000 1, R^2 = 0.864 3] expression relative to the control group. However, treatment with GBE significantly inhibited apoptosis by stimulating the expression of Bcl-2 when compared to ISO-exposed animals as shown in Fig. 7.

3.7. G. biloba protected cardiac structural integrity by enhancing ERK1/2 and mTOR expression

As presented in Figs. 8 and 9, isoprenaline significantly inhibited the expression of cardiac ERK1/2 [F (3, 16) = 22.90, P < 0.000 1, R^2 = 0.811 1] and mTOR [F (3, 16) = 45.87, P < 0.000 1, R^2 = 0.875 8] when compared with control group. However, treatment with GBE significantly enhanced the cardiac expression of ERK1/2 and mTOR relative to ISO-treated group.

3.8. G. biloba modulated cardiac Na^+, K^+-ATPase activities

Sodium-potassium ATPase enzyme [F (3, 16) = 23.23, P < 0.000 1, R^2 = 0.813 3] was significantly reduced in isoprenaline exposed animals when compared with control group. However, treatment with GBE significantly enhanced the activities of Na^+, K^+-ATPase enzyme relative to ISO-treated group as shown in Fig. 10.

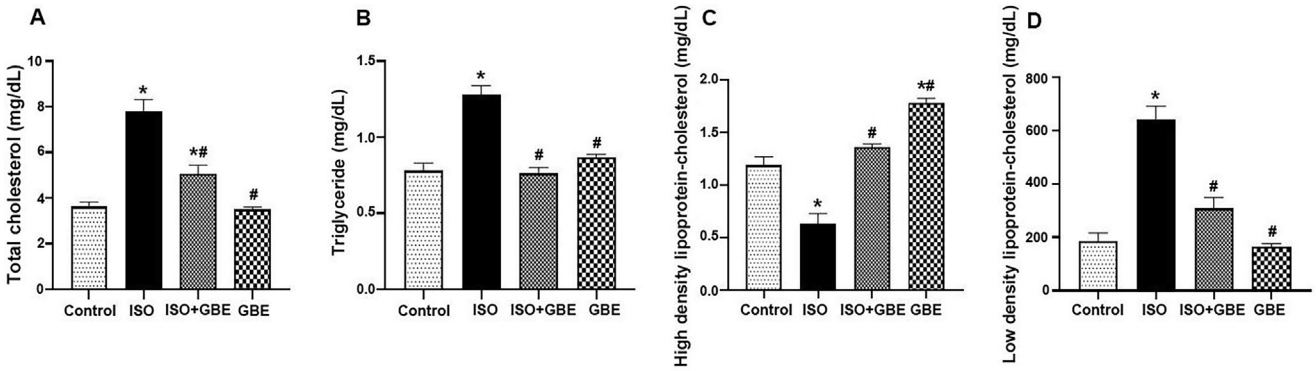

Fig. 3. *G. biloba* attenuated hyperlipidaemia (Mean ± SEM, n = 5). (A) Total cholesterol (TC), (B) Triglyceride (TG), (C) High density lipoprotein-cholesterol (HDL-C), (D) Low density lipoprotein-cholesterol (LDL-C). $^{*}P < 0.05$ *vs* control group, $^{\#}P < 0.05$ *vs* ISO group. ISO, isoprenaline; GBE, *Ginkgo biloba* extract.

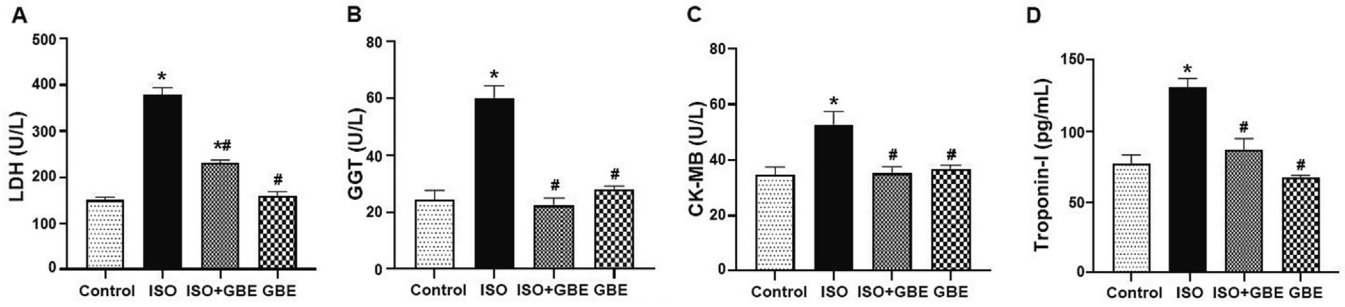

Fig. 4. *G. biloba* modulated cardiometabolic activities (Mean ± SEM, n = 5). (A) lactate dehydrogenase (LDH), (B) gamma glutaryl transferase (GGT), (C) creatine kinase-MB (CK-MB), (D) troponin-I. $^{*}P < 0.05$ *vs* control group, $^{\#}P < 0.05$ *vs* ISO group. ISO, isoprenaline; GBE, *Ginkgo biloba* extract.

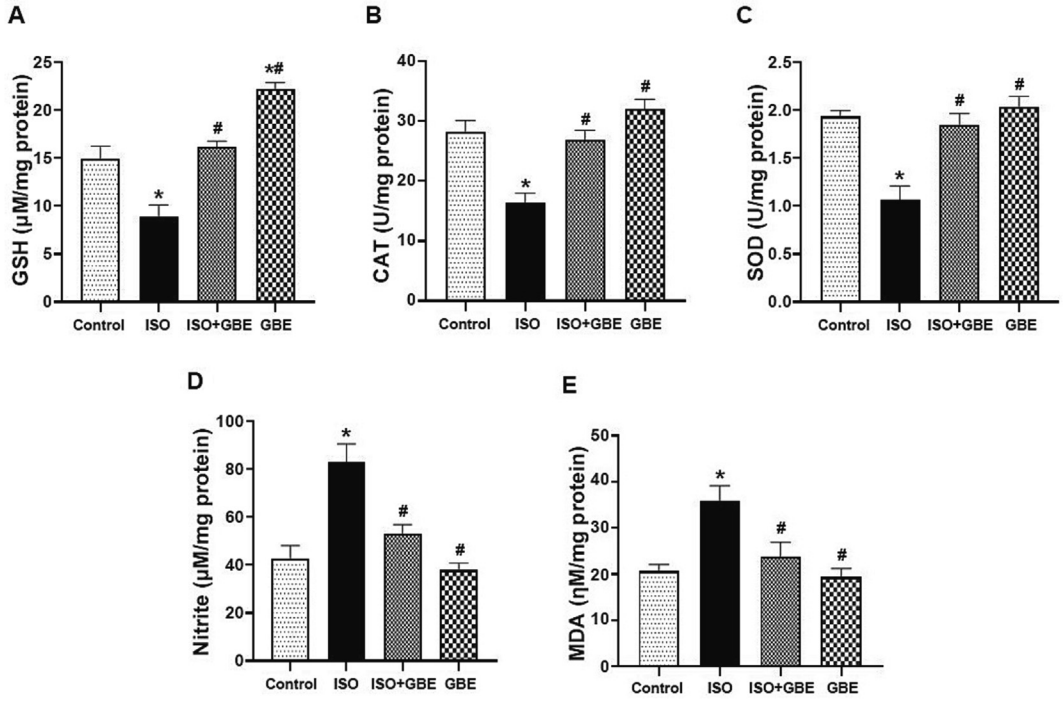

Fig. 5. *G. biloba* abated oxidative disturbances (Mean ± SEM, n = 5). (A) reduced glutathione (GSH), (B) catalase (CAT), (C) superoxide dismutase (SOD), (D) nitrite, (E) malondialdehyde (MDA). $^{*}P < 0.05$ *vs* control group, $^{\#}P < 0.05$ *vs* ISO group. ISO, isoprenaline; GBE, *Ginkgo biloba* extract.

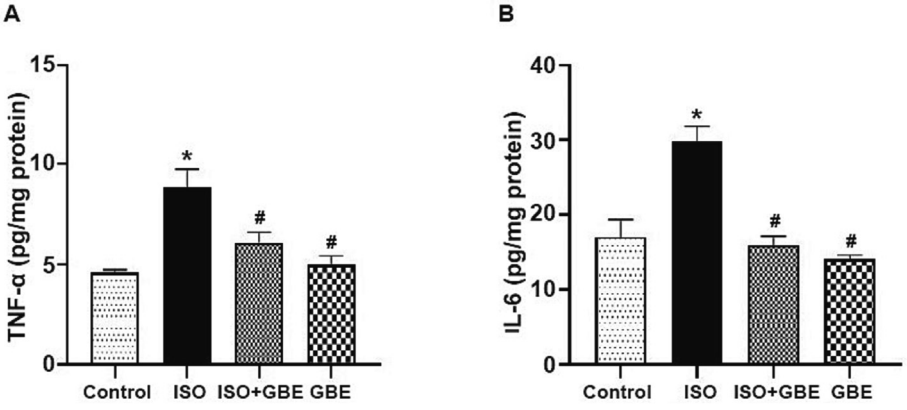

Fig. 6. *G. biloba* inhibited inflammatory responses (Mean ± SEM, n = 5). (A) tumor necrosis factor (TNF-α), (B) interleukin-6 (IL-6). $^{*}P < 0.05$ *vs* control group while $^{\#}P < 0.05$ *vs* ISO group. ISO, isoprenaline; GBE, *Ginkgo biloba* extract.

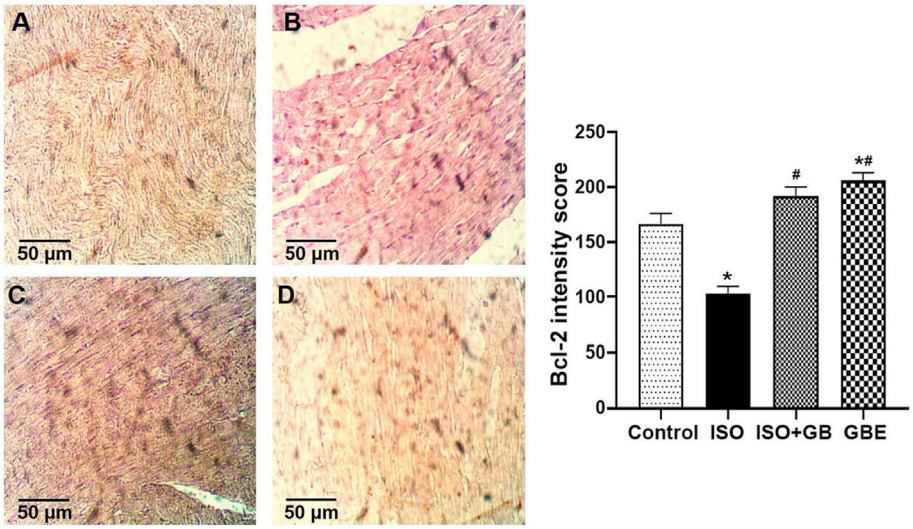

Fig. 7. *G. biloba* inhibited apoptosis (Mean ± SEM, $n = 5$). (A) control (×400), (B) isoprenaline (ISO) (×400), (C) *G. biloba* supplement (GBS) (×400), (D) *G. biloba* extract after isoprenaline (×400). $^{*}P < 0.05$ *vs* control group, $^{\#}P < 0.05$ *vs* ISO group. ISO, isoprenaline; GBE, *Ginkgo biloba* extract.

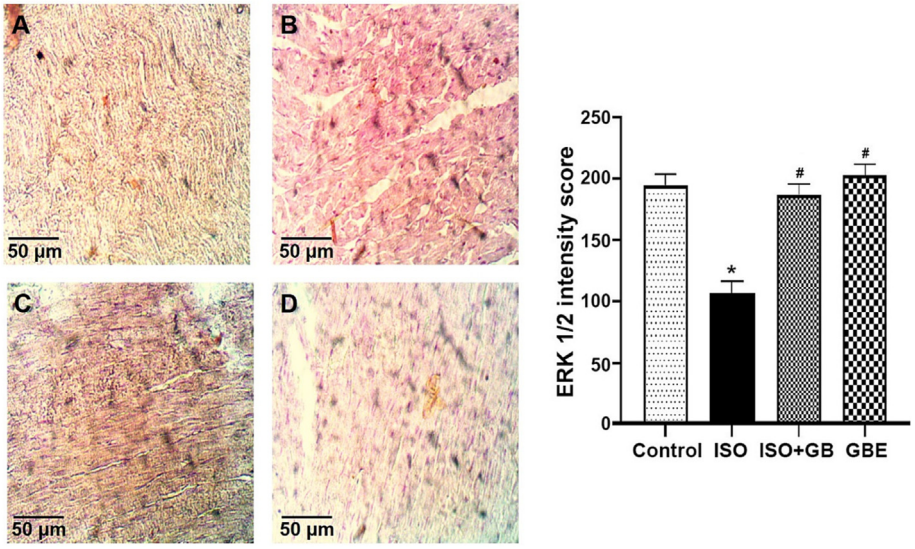

Fig. 8. *G. biloba* protected cardiac structural integrity by enhancing ERK1/2 expression (Mean ± SEM, $n = 5$). (A) control (×400), (B) isoprenaline (ISO) (×400), (C) *G. biloba* supplement (GBS) (×400), (D) *G. biloba* extract after isoprenaline (×400). $^{*}P < 0.05$ *vs* control group, $^{\#}P < 0.05$ *vs* ISO group. ISO, isoprenaline; GBE, *Ginkgo biloba* extract.

3.9. G. biloba reversed ISO-induced histoarchitectural alteration

Acute adrenergic stimulation by isoprenaline induces histoarchitectural alterations in the heart when compared to control group. However, following treatment with *G. biloba*, there were complete recovery and the slides showed normal histoarchitecture as shown in in Fig. 11.

4. Discussion

For the treatment of neurological disorders, GBE had been known and utilized in China for about 5000 years (Das et al., 2022). Recent research suggested that GBE activities were mediated via anti-oxidant and anti-inflammatory capabilities (Asiwe et al., 2022b, 2023a, 2024). However, there were questions about the precise mechanism that might be in charge of its cardioprotective effects. Therefore, the cardioprotective effect of GBE against isoprenaline-induced myocardial damage was investigated. Our results showed that GBE attenuated isoprenaline-induced myocardial damage via regulation of B-cell lymphoma factor 2 (Bcl-2), mammalian target of rapamycin (mTOR), extracellular signal-regulated kinase (ERK1/2) and sodium–potassium ATPase activities in addition to the previously reported modulation of antioxidant and anti-inflammatory mediators.

Low levels of HDL-C, high levels of LDL-C and high triglyceride levels were characteristics of dyslipidaemia, which was usually linked to modifications in lipid metabolism (Deprince et al., 2020). In line with the hallmarks of hyperlipidaemia or dyslipidaemia, our study found that rats exposed to ISO had significantly higher total cholesterol, triglycerides, LDL-C, as well as a significantly lower level of HDL-C. These findings were in line with earlier research that demonstrated abnormal cholesterol deposition in the myocardium during isoprenaline-induced myocardial infarction due to increased mobilization of LDL-C from the blood into the myocardial membranes (Sivasangari et al., 2021; Lin et al., 2022). The quantity of free fatty acids released from adipose tissue that

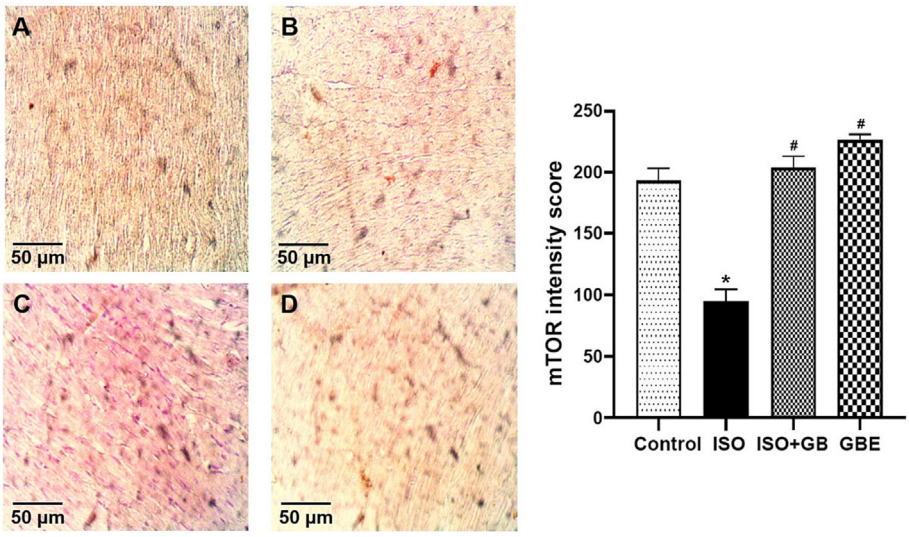

Fig. 9. *G. biloba* protected cardiac structural integrity by enhancing mTOR expression (Mean ± SEM, n = 5). (A) control (×400), (B) isoprenaline (ISO) (×400), (C) *G. biloba* supplement (GBS) (×400), (D) *G. biloba* extract after isoprenaline (×400). $^{*}P < 0.05$ *vs* control group, $^{\#}P < 0.05$ *vs* ISO group. ISO, isoprenaline; GBE, *Ginkgo biloba* extract.

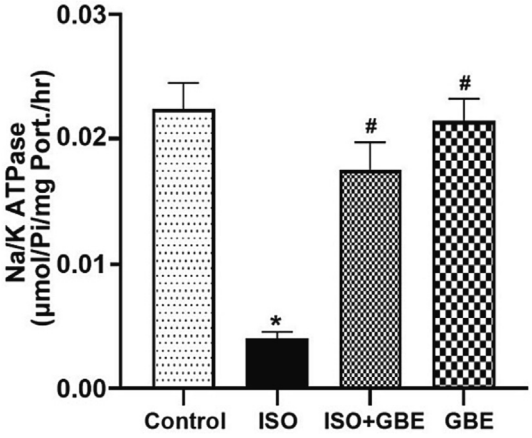

Fig. 10. *G. biloba* modulates cardiac Na^{+}, K^{+}-ATPase activities (Mean ± SEM, n = 5). $^{*}P < 0.05$ *vs* control group, $^{\#}P < 0.05$ *vs* ISO group. ISO, isoprenaline; GBE, *Ginkgo biloba* extract.

entered the myocardium fluctuated depending on the quantity of free fatty acids that were present in the coronary sinus (Iacobellis, 2022). The excess free fatty acids can be used by the body to make triglycerides, leading to hypertriglyceridemia, even though the heart can use them as source of energy (Heeren & Scheja, 2021). In the current investigation, GBE therapy greatly reduced the rise in total cholesterol, triglycerides, and LDL-C that isoprenaline-induced elevation caused. In the past, Banin et al. (2021) demonstrated that GBE ingestion can reduce plasma total cholesterol, triglycerides and LDL-C, while also increase HDL-C levels in experimental animals. According to Ganjikunta et al. (2022), the cardioprotective action of GBE was likely connected to its capacity to prevent the increased accumulation of lipids in the myocardium by virtue of its antilipidemic function which was attributed to its saponin contents.

According to general clinical observations, ISO induction in animal models might result in varying degrees of heart damage (Angelovski et al., 2023). Increased heart weight index, which indicated an enlarged heart, had been linked to increased protein and water content, edematous intramuscular space and inflammatory cells infiltrating injured areas (Ottone et al., 2022). According to a previous study, an increase of 1% in myocardial water content may result in a 10% decline in ventricular function (Connelly et al., 2020). This was demonstrated by a related study that found that in rats exposed to ISO, ischemic cardiac tissue injury caused the heart weight index to increase. This ISO-induced cardiac weight index, however, was significantly restored by the GBE therapy, indicating the protective actions of GBE on the cell membrane. The release of cytosolic enzymes into the blood stream happened when myocardial lesions occured because isoprenaline made cardiac muscle cells more permeable (Rong et al., 2023). ATP was a phosphate energy storage form that can be produced via LDH, GGT and CK-MB (Khalil, 2022). Compared to the control group, the ISO-induced animals in this study had significantly higher levels of LDH, GGT, and CK-MB, indicating that ISO had triggered a number of cardiac lesions. These signs, however, markedly lowered by GBE, suggesting that GBE might be able to lessen the cardiotoxicity brought on by isoprenaline-induced acute adrenergic stimulation. This result was consistent with the information presented by Marchetti et al. (2020). A crucial diagnostic of myocardial injury, particularly in myocardial infarction, was cT-I, a protein with contractile characteristics unique to cardiac muscle (Chaulin, 2022). In our study, GBE considerably reduced the amount of cT-I, showing that it had the potential to reduce cardiac damage.

The induction of inflammatory responses was one of the crucial elements in MI (Dutka et al., 2020). According to studies, transcription of nuclear factor kappa-B (NF-κB) translocation from the cytosol to the nucleus had a critical role in producing the inflammatory responses that resulted in TNF-α and IL-6 and triggered oxidative stress, which in turn impaired cardiac function (Qi et al., 2020). Anti-inflammatory drugs were therefore essential for reducing inflammatory reactions (Gao et al., 2020). Here, the findings demonstrated that ISO stimulation increased cardiac TNF-α and IL-6 production and release. Following treatment with GBE in ISO-provoked animals, the status of these markers were noticeably decreased, suggesting that GBE had cardioprotective effects against MI animals. This finding was in line with earlier studies that had shown GBE to be a potent natural herbal anti-inflammatory product (Gupta et al., 2021; Asiwe et al., 2023a, 2023b, 2023c, 2024).

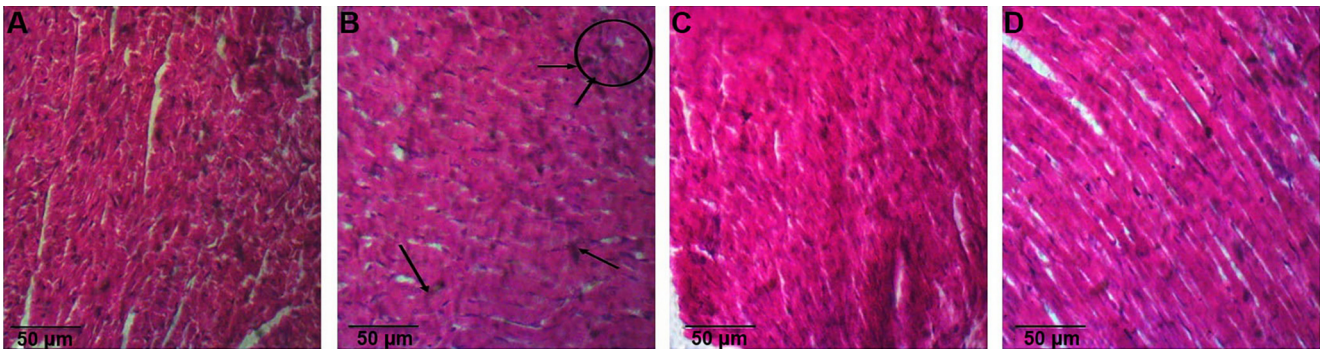

Fig. 11. *G. biloba* reverses ISO-induced histoarchitectural alteration. (A) control, (B) isoprenaline (ISO), (C) *G. biloba* supplement (GBS), (D) *G. biloba* extract after isoprenaline. The tissues were stained with H&E and ×400 magnification was used to capture the slides with light microscope. Arrows indicated significant lesion.

Myocardial injury was associated with an excessive production of reactive oxygen species (ROS), which may damage cell membrane proteins and lipids and affect the structure and functionality of heart muscle (Zhao et al., 2022). As a result of the depletion of antioxidants caused by the production of free radicals, lipid peroxidation occurs (Asiwe et al., 2023b). The permeabilization of the mitochondrial and cytoplasmic membranes caused by severe lipid peroxidation may release MDA, a marker of lipid peroxidation into the bloodstream (Ishimov et al., 2021; Mohideen et al., 2021). Significantly, ISO-exposed animals had higher level of cardiac MDA whereas receiving GBE treatment resulted in a notable reduction in lipid peroxidation, thus enhancing the cardio protective function. The findings of our study also demonstrated a significant increase in cardiac nitrite levels in ISO-treated rats, indicating a dysregulation of nitric oxide signalling that may further contributed to the animals' poor cardiovascular performance (Srivastava et al., 2022). Accordingly, NO was thought to mediate glutamatergic excitotoxicity via synergistic and cooperative release of glutamate and inflammatory cytokines like TNF-α by cardiac cells, thereby creating a favourable environment that exacerbated cardiac damage and heart failure (Ben-Azu et al., 2022; Asiwe et al., 2022b, 2024). On the other hand, GBE treatment significantly lowered cardiac nitrite, thereby reinstating the normal nitric oxide signalling and inhibition of peroxynitrite damage. An endogenous antioxidant enzyme served vital roles in preserving the tissues' homeostasis by preventing tissue or cellular damage brought on by free radicals (Anik et al., 2022). All live cells produce glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and other vital antioxidants. Oxidative stress is brought on by the shortage of oxygen in the heart blood flow as a result of the ischemic damage brought on by acute adrenergic activation. Fortunately, the myocardium is protected from damage by the endogenous antioxidants present in the cell (Tan et al., 2023). As anticipated, ISO-induced animals had considerably lower levels of SOD, CAT and GSH, which may have indicated oxidative stress in the heart. In ISO-induced rats, this antioxidant content in the heart was raised by GBE therapy. This supported GBE's scavenging actions and its ability to prevent myocardial damage. Consistent with several research, glycyrrhizic acid's antioxidant activities diminished oxidative stress and lipid peroxidation brought on by ISO-induced heart damage in rats (Chu et al., 2021; Yildiz et al., 2022).

Adenosine triphosphatase (ATPase), a protein that uses energy from sodium, potassium, calcium and magnesium translocation, aids in the contraction and relaxation of the heart muscle. The normal intracellular sodium and calcium status in heart muscles is maintained by ATPase activities (Zhang et al., 2021). A change in the ATPases' enzyme activity indicated a pathological adaptation of the membrane (Ojo et al., 2023). In the biopsies of patients with heart failure, for instance, the Na^+, K^+-ATPase activity was much lower. The aldosterone antagonist was still another factor in the regulation of Na^+, K^+-ATPase after a heart attack since it lowered hyperaldosteronism and decreased Na^+, K^+-ATPase activity (Obradovic et al., 2023). In this study, it was found that ISO-exposed animals had decreased cardiac Na^+, K^+-ATPase activity, which may be related to increased lipid peroxidation and membrane damage. In rats with GBE treatment there was an observed increased in the activity of Na^+, K^+-ATPase. However, similar evidence was also reported by Ojo et al. (2023) in MI-animals that were ISO-challenged. Asiwe et al. (2023c) earlier stated that the membrane stabilizing effect of GBE may also be partly responsible for this behaviour.

The interaction between antioxidant, anti-inflammatory and anti-apoptotic molecules had been reported to play important role in the activation of survival signals in order to maintain homeostasis (Chauhan et al., 2022; Asiwe et al., 2023a). Nevertheless, an insult to this signalling cascade had been demonstrated to distort normal physiology and consequently myocardial injury (Muraleedharan & Dasgupta, 2022; Asiwe et al., 2023a). An abnormality in the ERK1/2 signalling pathway, one of the traditional MAPK signalling pathways, can result in cardiovascular diseases like coronary atherosclerosis and myocardial damage as well as myocardial hypertrophy. It also played a critical role in the proliferation and differentiation of myocardial cells (Ghodrat et al., 2021; Mallick et al., 2021). The ERK1/2 signalling pathway can be activated in response to stress which significantly increased the level of phosphorylation of the ERK1/2 protein, thereby, affecting the expression of the downstream target protein NF-κB and regulation of cell proliferation and apoptosis (Jayachandran et al., 2020; Wen et al., 2020). In this study, it was discovered that the level of ERK1/2 expression clearly decreased in the myocardial cells of rats exposed to isoprenaline. Moreso, in animal cardiomyocytes exposed to ISO, it was noticed that the expression of B-cell lymphoma factor 2 (Bcl-2), a powerful regulator of the apoptotic process, significantly decreased which suggested cardiomyocyte apoptosis. However, the expression of ERK1/2 and Bcl-2 in myocardial cells increased dramatically after GBE treatment, demonstrating that GBE had shortened the interplay between inflammation and apoptosis in causing myocardial damage to mediate cardioprotection. Consistent with previous report, stimulation of the ERK1/2 signalling pathway can control endothelial cell proliferation and migration while encouraging cardiac angiogenesis (Zhai et al., 2021). Autophagy is a well-conserved self-degradative process with several linkages to human disorders due to its important role in cellular stress response, equilibrium, survival and overall organism development (Zhou et al., 2022). Recent research found that

chronic heart ischemia hindered cardiomyocyte autophagy, which worsened the effects of acute myocardial injury on cardiac remodelling (Wei et al., 2021; Mao et al., 2022). However, inhibiting apoptosis solely, according to some studies, may not always result in meaningful recovery of cardiomyocyte survival or function (Ikeda et al., 2022; Mao et al., 2022; Asiwe et al., 2023a). In this study, in addition to confirming the cardioprotective mechanism of GBE, we paid close attention to how it affected autophagy. Mammalian target of rapamycin (mTOR) is a key player in the regulation of a number of vital cellular functions, including protein synthesis, cell growth, proliferation, autophagy, lysosomal function as well as metabolism (Ikeda et al., 2022; Querfurth & Lee, 2021). According to Simpson et al. (2020), the mTOR pathway controlled both healthy and pathological processes in the cardiovascular system. This study found that isoprenaline induction greatly reduced the expression of cardiomyocyte mTOR which greatly suggested that autophagy and apoptosis in cardiac cells had been stimulated. Consistent with earlier studies, *G. biloba* may boost cell survival by directly activating mTOR signalling. However, therapy with GBE greatly increased the expression of mTOR suggesting that GBE could play a role in autophagy. Notably, we examined if the cardioprotection of GBE involved this cascade of protein signalling given that the mTOR/ERK1/2/Na^+, K^+-ATPase pathway was known to have an anti-inflammatory, anti-apoptosis and autophagy regulatory function in cardiac diseases (Barangi et al., 2023). As was predicted, the GBE increased the expression of these protein enzymes, suggesting that it controled the mTOR/ERK1/2/Na^+, K^+-ATPase activities to lessen the effects of isoprenaline on the myocardium. Additionally, the study's histology findings supported our hypothesis that GBE reduced myocardial damage because complete recovery from isoprenaline-induced histological changes was observed in rats treated with GBE, which was in line with earlier report by Singh et al. (2020), Alawode et al. (2021) and Asiwe et al. (2023a).

5. Conclusion

The results of the present investigation, taken together, indicated that therapy with GBE shielded rats' myocardium from isoprenaline-induced myocardial injury. Along with the previously reported antioxidant and anti-inflammatory benefits, GBE's capacity to regulate mTOR/ERK1/2/Na^+, K^+-ATPase activities may be the reason for its attenuating characteristics. However, the lack of evidence for apoptotic gene profiling and inflammatory molecules such as NF-κB that could be required to further adjudge the impacts of GBE on MI-related condition, was highlighted as some of the possible limitations of the study. Notwithstanding, this did not in any way invalidate our findings as biochemical and immunohistochemical evidences from this study had provided enough support to our claim.

CRediT authorship contribution statement

Jerome Ndudi Asiwe: Conceptualization, Data curation, Funding acquisition, Methodology, Project administration. **Abodunrin Adebayo Ojetola:** Data curation, Funding acquisition. **Nwoke Enekabokom Ekene:** Data curation, Funding acquisition, Resources.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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