Myricetin (3,3′,4′,5,5′,7-Hexahydroxyflavone) Prevents 5-Fluorouracil-Induced Cardiotoxicity

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ABSTRACT: 5-Fluorouracil (5-FU) is a strong anti-cancer drug used to manage numerous cancers. Cardiotoxicity, renal toxicity, and liver toxicity are some of the adverse effects which confine its clinical use to some extent. 5-FU-induced organ injuries are associated with redox imbalance, inflammation, and damage to heart functioning, particularly in the present study. Myricetin is an abundant flavonoid, commonly extracted from berries and herbs having anti-oxidative and anti-cancer activities. We planned the current work to explore the beneficial effects of myricetin against 5-FU-induced cardiac injury in Wistar rats through a biochemical and histological approach. Prophylactic myricetin treatment at two doses (25 and 50 mg/kg) was given to rats orally for 21 days against cardiac injury induced by a single injection of 5-FU (150 mg/kg b.wt.) given on the 20th day intraperitoneally. The 5-FU injection induced oxidative stress, inflammation, and extensive cardiac damage. Nevertheless, myricetin alleviated markers of inflammation, apoptosis, cardiac toxicity, oxidative stress, and upregulated anti-oxidative machinery. The histology of heart further supports our biochemical findings mitigated by the prophylactic treatment of myricetin. Henceforth, myricetin mitigates 5-FU-induced cardiac damage by modulating oxidative stress, inflammation, and cardiac-specific markers, as found in the present study.

1. INTRODUCTION

Anti-cancer therapies have shown great promise in improving the quality of life and survival rate of cancer patients. In the recent past, tremendous progress has been made in anti-cancer drug discovery, along with the addition of an abundance of literature revealing the underlying mechanisms of cancer metastasis.1 Drug therapies targeting signaling pathways to regulate the cell cycle and check the proliferative processes are effective. In the past few decades, the use of fluoropyrimidines and anthracycline chemotherapy has significantly upgraded the outcomes of cancer treatment.2

5-FU belongs to the class of fluoropyrimidines which constitute an essential group of different chemotherapeutic regimens used to treat various types of malignancies.3,4 Anti-cancer compounds such as 5-FU and related pyrimidines are associated with drug toxicities, with cardiotoxicity being one of the many manifestations.5,6 5-FU is recognized as the second most commonly used anti-cancer drug causing cardiotoxicity. Different mechanisms have been proposed with regard to cardiotoxicity of 5-FU; few of them include endothelial damage, followed by coagulation, oxidative stress-induced direct toxicity, activation of the inflammatory cascade, mitochondrial membrane damage, and thrombogenicity.7−10 5-FU after activation is converted to different nucleotide forms crucial for inducing cardiotoxicity is 5-fluoro-2'-deoxyuridine-5'-monophosphate (FdUMP). This FdUMP tends to inhibit thymidylate synthase via competitive binding, which ultimately leads to decreased cell growth and "thymine-less death" in the presence of folates. Other metabolites of 5-FU also induce cytotoxicity: fluorodeoxyuridine triphosphate by incorporating in DNA and fluorouridine-5'-triphosphate 5-fluorocytosine by interfering with RNA. These metabolites also affect the mitochondrial phosphate metabolism, induce the oxidative damage, and promote the release of catecholamines and histamine.9,10

Flavonoids constitute an important class of phytochemicals belonging to plant secondary metabolites with polyphenols as their structural components. They are primarily present in honey, fruits, vegetables, and some beverages.11−13 Flavonoids have been known to have some exceptional pharmacological properties such as anti-oxidant, anti-cancer, anti-inflammatory,
anti-aging, and so forth. Previous reports suggest that flavonoids function and modulate apoptosis and inflammatory processes affected by the anti-cancer drug.

Myricetin (3,3′,4′,5,5′,7-hexahydroxyflavone) is usually obtained from berries, herbs, vegetables, and so forth in abundance and is a flavonoid mainly by the members of myricaceae, anacardiaceae, polygonaceae, pinaceae, and primulaceae families (Figure 1). Myricetin is poorly soluble in water but melts swiftly and in some organic solvents and basic media in deprotonation otherwise. The estimated intake of myricetin has been reported to be 1.1 mg per day for males and 0.98 mg per day for females. Myricetin has been recognized to have adequate pharmacological properties such as anti-oxidant, anti-microbial, anti-inflammatory, anti-cancer, neurodegeneration prevention, and cardioprotective and epigenetic modulations. Its role in bone remodeling, wound healing, non-alcoholic fatty liver disease, cellular autophagy, diabetic eye disorders, and gastric injury has been documented recently. The role of myricetin in protecting cardiotoxicity in other known models of cardiotoxicity is well established.

In the current project, we propose investigating the role of myricetin in protecting 5-FU-induced cardiotoxicity by influencing a critical cellular inflammatory cascade, oxidative damage, and other cardiotoxicity markers.

2. MATERIALS AND METHODS

2.1. Chemicals. 5-Fluorouracil (5-FU) and, if not otherwise specified, chemicals were acquired by Sigma-Aldrich, USA.

2.2. Preparation of 5-FU. SFU was melted in a sterile 0.2 μm syringe filter was used to filter under sterile conditions.

2.3. Animals Treatment. Experimental animals were divided into four groups having six animals each. Group I was the control group; it was administered with 5% sodium carboxymethyl cellulose (CMC-Na) for 20 days with a single intraperitoneal injection of 0.9% normal saline on the 20th day. The toxicant group/group II was treated with a single injection of 5-FU (150 mg/kg b.wt.) on the 20th day intraperitoneally. Group III was given myricetin (25 mg/kg b.wt.) for 20 days orally and a single injection of 5-FU (150 mg/kg b.wt.) on the 20th day intraperitoneally. Group IV was treated with myricetin orally (50 mg/kg b.wt.) for 20 days and a single injection of 5-FU (150 mg/kg b.wt.) on the 20th day intraperitoneally. All the animals were sacrificed on the 21st day, and the heart tissue was obtained after perfusion.

sacrifice, blood was drawn under mild anesthesia, and serum was collected. Biochemical and immunological assays were performed with the heart tissue. The excised tissue was washed with 0.85% ice-cold saline, and a homogenate was prepared using a homogenizer in phosphate buffer (0.1 M, pH 7.4 at 40 °C) and KCl (1.17%). For histopathological studies, a part of the heart tissue was stored in 10% buffered formalin.

2.4. Preparation of Post-mitochondrial Supernatant. The heart was set instantly to get perfused with cold saline. It was homogenized in 0.1 M chilled phosphate buffer with 7.4 pH (10% w/v) at 700g centrifugation in a cooling centrifuge for 10 min to disperse the nuclear debris. The post-mitochondrial supernatant (PMS) thus obtained was used for performing a range of enzyme estimations.

2.5. Estimation of Anti-oxidant Machinery. 2.5.1. Superoxide Dismutase Activity. The superoxide dismutase (SOD) activity was evaluated by the Marklund and Marklund method. The reaction mixture consisted of 3 mL with 100 μL of PMS, 2.875 mL of Tris-hydrochloric acid buffer having 50 mM 8.5 pH, and pyrogallol of 24 mM in 10 mM HCl. The SOD activity was measured as units per milligram protein and measured at 420 nm.

2.5.2. Catalase Activity. The catalase (CAT) activity was evaluated as mentioned by Claiborne. The total reaction consisted of 3 mL with 1.95 mL (0.1 M, pH 7.4) of phosphate buffer, 1 mL of H2O2 (0.10 mM), and 0.05 mL of 10% PMS. nmol H2O2 consumed per min per milligram protein was used to calculate the CAT activity, and the absorbance was measured at 240 nm.

2.5.3. Glutathione. Glutathione (GSH) was evaluated as described by Rashid et al. The total volume of the reaction mixture was 3 mL having 0.4 mL of supernatant and 2.2 mL (0.1 M having pH 7.4) of phosphate buffer with 400 μL of di-thio-bis-2-nitrobenzoic acid (4 mg/mL). Also, 1.0 mL of 4% sulfosalicylic acid was combined with 1 mL of 10% PMS. nmol H2O2 consumed per min per milligram protein was used to calculate the GSH activity, and the absorbance was measured at 412 nm and calculated as nmol GSH conjugates per gram tissue.

2.5.4. GSH Reductase Activity. The GSH reductase (GR) activity was calculated as explained by Rashid et al. The total reaction of 2 mL which consisted of 1.65 mL (0.1 M having pH 7.6) of phosphate buffer, 0.1 mL of EDTA of 0.5 mM, 0.05 mL of 1 mM GSH, 0.1 mL of NADPH with 0.1 mM, and 0.1 mL of 10% PMS. The absorbance was measured at 340 nm and calculated as nmol NADPH oxidized per min per milligram protein.

2.5.5. Quinone Reductase and Xanthine Oxidase Activity. Quinone reductase (QR) and xanthine oxidase (XO) activities were evaluated as described by Rehman et al.

2.6. Evaluation of Nitric Oxide. The nitric oxide (NO) produced was assessed as described in Rehman et al. by measuring the level of nitrate (an indicator of NO) in the supernatant using a colorimetric reaction with the Griess reagent. In brief, 100 μL of supernatants from different groups was mixed with 100 μL of Griess reagent [0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5% H3PO4]. After incubation at room temperature in the dark for 10 min, the total nitrates were measured spectrophotometrically at 540 nm. The concentration of nitrite in the sample was determined from a NaNO2 standard curve.

2.7. Assessment for Hydrogen Peroxide (H2O2). H2O2 was calculated by the Pich and Mizel method. Microsomes (2

Figure 1. Structure of myricetin.
**Table 1. Myricetin Treatment Effects on Antioxidant Enzymes and Lipid Peroxidation in 5-FU-Induced Cardiac Damage**

| Parameter                        | group I       | group II      | group III     | group IV      |
|----------------------------------|---------------|---------------|---------------|---------------|
| LPO (nmol MDA formed/g tissue)   | 2.716 ± 0.10  | 5.476 ± 0.55*** | 4.238 ± 0.58#  | 3.18 ± 0.42### |
| SOD (U/mg protein)               | 54.72 ± 6.31  | 10.96 ± 1.15*** | 39.51 ± 4.41#  | 23.26 ± 2.01### |
| H₂O₂ (nmol of H₂O₂/g tissue)     | 171.3 ± 18.4  | 350.9 ± 26.7*** | 262.4 ± 21.4### | 217.4 ± 20.3### |
| CAT (nmol/min/mg protein)        | 85.27 ± 9.75  | 27.13 ± 3.26*** | 56.05 ± 3.94#  | 41.76 ± 4.26### |
| GR (nmol/min/mg protein)         | 242.8 ± 28.1  | 113.6 ± 16.0*** | 203.4 ± 19.9### | 151.8 ± 11.5### |
| GSH (nmol/mgprotein)             | 271.0 ± 23.3  | 127.4 ± 11.6*** | 218.4 ± 15.1### | 152.3 ± 12.4### |

*Values of these parameters were expressed as mean ± SD (n = 6). Significant differences were indicated by ***p < 0.001 when compared to group II, and #p < 0.01, ##p < 0.01, and ###p < 0.001 when compared with group II. Group I: normal saline (10 mL/kg b.w), group II: 5-FU, group III: 5-FU + myricetin (25 mg/kg b.wt.), and group IV: 5-FU + myricetin (50 mg/kg b.wt.).

mL) were suspended at 37 °C for 1 h incubation in 1 mL reaction concoction having phenol red of 0.28 nm, horseradish peroxidase, dextrose of 5.5 nm, and phosphate buffer of 0.05 M with pH 7.0. The reaction was stopped with 10 μL of NaOH of 10 N and then centrifuged at 800 g for 5 min. OD was measured at 610 nm, and the amount was measured as nmol H₂O₂/h/g tissue.

**2.8. Assessment for Lactate Dehydrogenase Activity.**

The lactate dehydrogenase (LDH) activity was evaluated in the serum as done by Rashid et al. The total assay mixture was 3 mL volume in total with 0.1 mL of 0.02 M NADH, 1.1 mL of phosphate buffer of 0.1 M (pH 7.4), 200 μL of serum, 100 μL of 0.01 M sodium pyruvate, and distilled water. LDH was measured as nmol NADH oxidized per min per milligram protein, and OD was measured at 340 nm.

**2.9. Assessment for MPO Measurement.**

MPO was estimated with a commercially available kit (Jiancheng Bioengineering Institute, A044, Nanjing, PRC) and is used to screen the parenchymal infiltration of neutrophils and macrophages.

**2.10. ELISA-Based Assays.**

Following are the ELISA kits used for tumor necrosis factor alpha (TNF-α), caspase-3 (cat no: E-EL-RO160, Elabscience, Beijing, China), MCP-1 (cat no: ab2194045, Abcam, Cambridge, UK), CK-MB (cat no: abx15346, Abcam, Cambridge, UK), BAX (cat no: LS-F35417, LSBio, WA, USA), NF-κB (cat no: LS-F69373, LSBio Inc. WA, USA), TNF-α (cat no: 88-7340-22, Invitrogen, Thermo Fischer, USA), IL-1β (cat no: 670.040.096, Diaclone SAS, France), and IL-10 (cat no: 670 020, Diaclone SAS, France), and the assay was carried out as per the instructions of the manufacturer.

**2.11. Histology.**

The heart was taken out fast from animals’ chest and put in 10% neutral buffered formalin for further slicing of tissue. The heart tissue was embedded in paraffin wax, and microtome was used to section it. Then, the tissue was stained with hematoxylin and eosin on the glass slides and is ready for observation under the microscope.

**2.12. Ethical Report.**

All the measures for the use of experimental rats were put in place with ethical clearance obtained from the Institute’s Animal Ethics Committee at College of Pharmacy, King Saud University, Riyadh, Saudi Arabia (approval no: KSU-SE-20-60).

**2.13. Statistical Analysis.**

Statistical analysis was done on the data obtained from animals by software such as SPSS 20.0, and the results are shown as mean ± SE. Tukey–Kramer’s multiple comparisons test was used to find the alterations between groups using analysis of variance. Statistical significance was set at p < 0.05 for all comparisons as a minimum criterion.

**3. RESULTS**

**3.1. Effect of Myricetin on Different Biochemical Parameters against 5-FU-Induced Cardiac Damage.**

**3.1.1. Reduced GSH and Its Dependent Enzymes such as GR.**

We observed a considerable (***p < 0.001) decline in GSH and GR levels of group II rats compared to group I.

Figure 2. (A,B) Effect of myricetin treatment on QR and XO in 5-FU-induced cardiotoxicity in Wistar rats. (A) The level of XO was found to be significantly elevated (**p < 0.001) in group II treated with 5-FU in comparison to that in the control group (group I). However, myricetin treatment significantly reduced the XO level in group III (#p < 0.05) and group IV (##p < 0.01) compared to that in group II. (B) Significantly depleted levels of QR (***p < 0.001) in group II treated with 5-FU in comparison to that in the control group (group I). However, myricetin treatment significantly restored the QR level in both the groups (#p < 0.05 and ##p < 0.01). The data obtained was presented as mean ± SD (n = 6).
Administration of 5-FU Induced Cardiac Damage. Significant (##p < 0.01) increase in GSH levels was seen in group III that received myricetin (25 mg/kg), while an extensive increase in GSH and GR (###p < 0.001) levels was seen in rats administered with myricetin (50 mg/kg) compared to levels in the positive control group that had received 5-FU only (group II) (Table 1), demonstrating a boost in antioxidant machinery.

3.1.2. Lipid Peroxidation (MDA). We observed a considerable (***p < 0.001) upsurge in MDA in group II compared to the negative control group. There is a remarkable decrease (#p < 0.05 and ###p < 0.001) of MDA in group III and IV rats that had received myricetin (25 and 50 mg/kg) at two doses as compared to the levels in the toxic group that had got 5-FU only (group II) (Table 1), indicating a decrease in oxidative stress.

3.1.3. Superoxide Dismutase Activity. There is a considerable (***p < 0.001) decrease in the SOD activity of positive control compared with negative control. There was an elevation in the SOD activity (#p < 0.05 and ###p < 0.001) in group III and IV rats that had received myricetin (25 and 50 mg/kg) at two doses as compared to the SOD activity of the toxic group that had got 5-FU only (group II) (Table 1), indicating an alleviation in oxidative stress and augmentation of the anti-oxidant armory.

3.1.4. Hydrogen Peroxide (H$_2$O$_2$) and Catalase (CAT). It was found that the antioxidant enzyme activity such as the CAT activity was significantly decreased in the 5-FU-treated group compared with the control group (***p < 0.001). There was huge production of H$_2$O$_2$ in the 5-FU group than in the control group (###p < 0.001) due to the metabolic activities and catabolism of CAT. However, myricetin treatment significantly restored the activity of CAT (#p < 0.05, ##p < 0.01) and reduced the production of H$_2$O$_2$ (###p < 0.001) (Table 1) at both doses (25 and 50 mg/kg) significantly, demonstrating mitigation in ROS production.

3.2. Effect of Myricetin on QR and XO in 5-FU-Induced Cardiac Damage. Administration of 5-FU decreased the activity of QR and increased the activity of XO significantly (***p < 0.001) in group II compared to the negative control group. However, myricetin treatment significantly increased the QR activity (#p < 0.05 and ###p < 0.01) at both the doses and decreased the activity of XO in group III and IV rats (#p < 0.05 and ##p < 0.01) significantly than group II (Figure 2), demonstrating the beneficial outcome of myricetin treatment.

3.3. Effect of Myricetin on Myeloperoxidase and NO Levels in 5-FU-Induced Cardiac Damage. There is a significant elevation in myeloperoxidase (MPO) and NO (**p < 0.01) levels in the 5-FU-administered group II than the negative control. However, myricetin (25 mg/kg) in group III and IV group (50 mg/kg) attenuated MPO (##p < 0.01 and ###p < 0.001) and NO (##p < 0.05 and ####p < 0.001) activities, respectively, as compared to group II (Figure 3), indicating a decrease in neutrophil infiltration and hence inflammation.

3.4. Effect of Myricetin on Inflammatory Intermediaries (NF-κB, TNF-α, IL-6, IL-10, and IL-1β) in 5-FU-Induced Cardiac Damage. Administration of 5-FU-augmented (**p < 0.001) inflammatory mediators such as NF-κB, TNF-α, IL-6, IL-10, and IL-1β analyzed in our study than the negative control. However, myricetin treatment at

Table 2. Effect of Myricetin on Inflammatory Markers in 5-FU-Induced Cardiotoxicity*#

|               | group I | group II | group III | group IV |
|---------------|---------|----------|-----------|----------|
| NF-κB [pg/mL] | 413.0 ± 41.62 | 1140.1 ± 115.4*** | 918.21 ± 77.01# | 703.8 ± 62.41### |
| TNF-α [pg/mL] | 213.52 ± 19.2 | 755.83 ± 65.8*** | 589.62 ± 49.9# | 301.47 ± 33.7### |
| IL-6 [pg/mL]  | 724.53 ± 84.6 | 1516.5 ± 119.2*** | 1316.5 ± 115.3## | 923.64 ± 88.2### |
| IL-10 [pg/mL] | 702.21 ± 48.7 | 1317.2 ± 114.4*** | 1045.3 ± 84.1# | 987.3 ± 108.3### |
| IL-1β [pg/mL] | 798.10 ± 63.8 | 1402.1 ± 132.4*** | 1198.6 ± 89.4## | 914.5 ± 82.6### |

*Values of these parameters were expressed as mean ± SD (n = 6). Significant differences were indicated by ***p < 0.001 when compared to group II and #p < 0.01, ##p < 0.01, and ###p < 0.001 when compared with group II. Group I: normal saline (10 mL/kg b.w), group II: 5-FU, group III: 5-FU + myricetin (25 mg/kg b.w), and group IV: 5-FU + myricetin (50 mg/kg b.w).
Figure 4. (A–D) Effect of myricetin on cardiotoxicity markers (LDH, MCP, CK-MB, and cTn-1) in 5-FU-induced cardiac damage. (A) Significantly elevated levels of LDH were observed (***p < 0.001) in 5-FU-treated group II in comparison to that in the control group (group I). Treatment with myricetin markedly reduced the LDH levels in the respective groups (#p < 0.05 and ###p < 0.01). (B) The level of MCP was also found to be significantly elevated (***p < 0.001) in group II treated with 5-FU in comparison to that in the control group (group I). However, myricetin treatment markedly reduced the MCP levels in group III (#p < 0.05) and group IV (##p < 0.01) compared to that in group II. (C) The level of another cardiotoxicity marker CK-MB was found to be significantly elevated (***p < 0.001) in group II treated with 5-FU in comparison to that in the control group (group I). However, myricetin treatment significantly reduced the CK-MB levels in group III (##p < 0.01) and group IV (###p < 0.001) compared to that in group II. (D) Another important marker cardiac troponin cTn-1 showed a steep increase after treatment in the 5-FU-treated group (group II). Myricetin treatment was successful in bringing down the elevated levels of cTn-1 at both the doses analyzed (##p < 0.01 and ###p < 0.001).

Figure 5. (A,B) Effect of myricetin on apoptotic marker proteins (BAX and caspase-3) in 5-FU-induced cardiac damage. (A) The level of BAX was found to be significantly elevated (***p < 0.001) in 5-FU-treated group II in comparison to that in the control group (group I). However, both the doses of myricetin treatment markedly reduced the BAX levels in the respective groups (#p < 0.05 and ##p < 0.01). (B) The level of caspase-3 was also found to be significantly elevated (***p < 0.001) in group II treated with 5-FU in comparison to that in the control group (group I). However, myricetin treatment markedly reduced the caspase-3 level in group III (##p < 0.01) and group IV (###p < 0.001) compared to that in group II.
both the doses in group III (25 mg/kg) and group IV (50 mg/kg) ameliorated inflammatory mediators NF-κB (#p < 0.05 and ###p < 0.001), TNF-α (#p < 0.05 and ###p < 0.001), IL-6 (###p < 0.01 and ####p < 0.001), IL-10 (#p < 0.01 and ####p < 0.001), and IL-1β (#p < 0.01 and ####p < 0.001) significantly (Table 2) compared to the S-FU-administered only group, deciphering alleviation in the inflammatory process.

3.5. Effect of Myricetin on Cardiac Toxicity Markers in S-FU-Induced Cardiac Damage. Administration of S-FU upregulated the known toxicity markers such as MCP, CK-MB, cTn-I, and LDH significantly (###p < 0.001) in group II as compared to the negative control. However, myricetin treatment significantly downregulated MCP (#p < 0.05 and ###p < 0.001), CK-MB (####p < 0.01 and ####p < 0.001), c-Tn-I (###p < 0.01 and ####p < 0.001), and LDH (#p < 0.05 and ##p < 0.01) at both the doses in group III and group IV rats significantly as compared to group II (Figure 4), demonstrating beneficial effect of myricetin treatment.

3.6. Effect of Myricetin on Apoptotic Marker Proteins in S-FU-Induced Cardiac Damage. Administration of S-FU upregulated Bax (pro-apoptotic) and caspase-3 (executioner caspase) significantly (###p < 0.001) in group II than in group I. However, myricetin treatment significantly downregulated Bax (#p < 0.05 and ###p < 0.001) and caspase-3 (###p < 0.01 and ####p < 0.001) at both the doses in group III and group IV rats significantly as compared to that in group II, demonstrating cell survival and decrease in organ damage (Figure 5).

3.7. Histological Examination of Myricetin in S-FU-Induced Cardiac Damage. Histological examination of cardiac sections revealed in group I that no deformity in cardiac cells was observed. Arrows indicated faintly striated eosinophilic sarcoplasm. Fibers exhibited oval vesicular central nuclei (n) and narrow interstitial space (s) (Figure 6). In group II, double arrows (11) represent wide interstitial spaces; triangle indicates perivascular cellular infiltration and myocyte degeneration, deeply stained nuclei are represented by (d). In group III treated with a lower dose of myricetin, myofibers appear close to normal with central nuclei represented by (d), interstitial spaces were narrower (s). In group IV, higher dose myricetin ameliorated nuclear degeneration; normal myofibers with central nuclei can be represented by (n) and regular narrow interstitial spaces (s) (Figure 6).

4. DISCUSSION

We have studied the involvement of crucial cellular inflammatory cascade, oxidative stress, and apoptosis in S-FU-induced cardiotoxicity in current communication. Natural compounds with antioxidant properties have been gaining much attention in reducing organ toxicities induced by various etiological insults in the recent past. The present study was conducted to explore the effect of myricetin in S-FU-induced cardiotoxicity.

Chemotherapy is one of the vital strategies currently followed for cancer treatment. However, it is associated with untoward toxic side effects, limiting its therapeutic usage. S-FU is a potent anti-neoplastic drug, but at the same time, its vast adverse effects such as hepatotoxicity, nephrotoxicity, and cardiotoxicity delimit its clinical usage. S-FU-induced cardiotoxicity is very well documented, but there are still some gaps regarding the exact mechanisms involved in cardiotoxicity. The most widely accepted mechanism of toxicity of S-FU is via the activation of reactive oxygen species (ROS), which creates a discourse of oxidative and nitrosative stress, cell membrane damage, inflammation, and apoptosis in cardiac tissue. The imbalance in the oxidative–antioxidative mechanism leads to architectural abnormalities in cardiac tissue-like sarcoplasmic vacuolization, hemorrhagic infarction, edema of myocytes, and focal necrosis with prominent infiltration of neutrophils. Elevation in cardiac inflammation markers such as TNF-α and NF-κB, oxidative stress markers (NO, XO, GPX, SOD, etc.), and apoptotic enzymes (BAX, caspases, BCL, etc.) are persistent in S-FU-induced cardiotoxicity. S-FU-induced oxidative stress leads to disruption of cell membranes, dysregulation of inflammatory gene expressions, and induction of apoptosis cascade leading to cell death. ROS synthesis is critical in inducing cardiac damage by contributing to systemic inflammatory response during S-FU therapy by significantly increasing the levels of inflammatory mediators such as IL-6 and TNF-α and causing lipid, protein, and nucleic acid peroxidations. In the present study, S-FU induced a significant increase in LPO as evident in increased malonaldehyde levels in cardiac tissue, which agrees with previous studies. However, myricetin treatment ameliorated MDA significantly and dose dependently, possibly by scavenging ROS as reported previously. Very recently, Lin et al. (2020) have reported that myricetin significantly decreases MDA levels in cardiopathic rats by upregulating HSP-72. Myricetin has well been reported to substantially inhibit ROS generation with simultaneous activation of anti-oxidative enzymes in H2O2-induced cell damage.

Various enzymatic and non-enzymatic antioxidants remove free radicals in biological systems, acting as an effective defense against ROS. GSH and its oxidized counterpart, GSSG, are the essential anti-oxidative agent in the body. GSH, a tripeptide, is an essential antioxidant that interacts directly with its SH group with free radicals. In this study, GSH reservoirs were depleted after S-FU administration due to depletion of thiol in scavenging ROS, which is in line with the previous studies. Nevertheless, myricetin administered...
prophylactically replenished GSH in treatment groups III and IV, as reported previously.57

ROS generation is associated with the depletion of anti-oxidative enzymes in the cell. The depot of antioxidant enzymes SOD, GPx, GR, CAT, and QR will significantly decrease in the current study deciphering the role of ROS and free radical generation in the pathophysiology of 5-FU-induced cardiotoxicity. Catalase is associated with H₂O₂ removing enzymes constituting the principal antioxidant in the body, having byproducts such as H₂O₂ and O₂⁻. H₂O₂ and other ROS are further catalyzed to H₂O and O₂ by CAT, GR.63 SOD helps convert superoxide anion free radicals to hydrogen peroxide (H₂O₂), and H₂O₂ is then eliminated by catalase or GSH-Px.63 QR is a phase-II detoxifying enzyme involved in catalyzing the two-electron lessening catalyzed by cytochromes P₄₅₀ in xenobiotic metabolism, thereby shielding cells from ROS and redox imbalance-induced injury. 5-FU influences SOD and GSH-Px by lowering them significantly in the myocardial tissues of guinea pigs.65 Similarly, in our study, all the anti-oxidative enzymes, viz., SOD, CAT, GPx, GR, and QR, were decreased significantly in the 5-FU group, indicating the generation of oxidative stress due to loss of anti-oxidative enzyme depot. However, SOD, GPx, GR, CAT, and QR were replenished by myricetin treatment, possibly by scavenging peroxy radicals, superoxide radicals, peroxide, and singlet oxygen demonstrating its antioxidant potential.60

Some pro-oxidative enzymes in the body enhance oxidative stress by increasing free radicals, and hence, a balance of pro-oxidative and anti-oxidative profiles is necessary to maintain cell homeostasis. XO is a pro-oxidant that produces oxidative stress by reducing oxygen (O₂) to the superoxide anion radical (O²⁻). Recently, it has been investigated that myricetin significantly ameliorated cisplatin-induced decrease in XO levels in colon tissue in Wistar rats.66 Similarly, in our study, XO was found to be increased in the positive control group by 5-FU administration which was alleviated by myricetin treatment in both treatment groups.

There is a cross-talk between oxidative stress and inflammation in chemotherapy-induced organ toxicities, particularly S-FU. S-FU administration results in oxidative damage and activates TNF-α with subsequent ROS and RNS production, causing organ damage and apoptosis. After its activation, TNF-α binds to its receptor-1, instigating the traditional stimulation of NF-κB and forming a portion of the innate immune system in reaction to various forms of strains upregulating the gene expression needed to regulate infection and injury. NF-κB is a redox-sensitive transcription factor that regulates differentiation, cellular proliferation, inflammation, and so forth, in biological cells. NF-κB is typically associated with its repressor inhibitory protein IκB in the cytoplasm. However, when the cell undergoes any stress or is exposed to any hazardous agent, cytoplasmic IκB kinase (IKK) phosphorylates and degrades IκB, resulting in the increased nuclear translocation of NF-κB, where it activates transcription of downstream genes such as pro-inflammatory mediators including TNF-α and cytokines such as IL-10, IL-1B, IL-6, various chemokines MCP-1, IL6, IL1β, IL10, and adhesion molecules resulting in subsequent tissue injury.5,10,36,35,48 The role of pro-inflammatory cytokines in the pathology of 5-FU-induced cardiac damage is still not known precisely. Cytokine secretion is considered one of the mediators of inflammation and therefore contributes to the pathology of tissue damage. 5-FU administration has been reported to elevate pro-inflammatory cytokines in serum IL1β, TNF-α, and IL-6 levels.46,51 Similarly, in our study, S-FU-activated NF-κB, which triggered transcriptional upregulation of downstream genes such as TNF-α and pro-inflammatory cytokines such as IL-10, IL-1β, and IL-6, which agrees with previous studies.67-70 It has been well documented that myricetin regulates the deteriorated levels of the inflammatory molecular markers such as NF-κB, IL-6, and TNF-α in cisplatin-induced toxicities.57 Our results showed that myricetin treatment at both doses inhibited NF-κB activation and other downstream inflammatory genes, consistent with previous reports.70,71

The elevated levels of pro-inflammatory cytokines further lead to the synthesis of other pro-oxidant species such as NO, which enhances the inflammation process in the cell. Therefore, it is imperative to inhibit NO formation to mitigate further downstream cytokine storm activation. NO reacts with superoxide radical (O²⁻), leading to the formation of peroxynitrite, which is a cytotoxic molecule. As the inflammatory reactions proceed, there is a simultaneous flow of neutrophils, the central inflammatory cells, to the affected area. MPO measures neutrophil infiltration, an essential milestone in acute inflammation to induce damage by forming reactive oxygen metabolites and cytokotoxic proteins such as MPO and proteases into the extracellular fluid.65 Recently, it has been concluded that myricetin decreases NO production by downregulating the NO producer (NOS₂) in hydrogen peroxide induced in an in vitro culture of ARPE-19 cells.72 In the current work, both MPO and NO were raised in the 5-FU-directed group compared to that in the control group. However, myricetin treatment at both doses diminished MPO, XO, and NO, demonstrating alleviation of oxidative insult by myricetin, thereby decreasing ROS and redox imbalance following previous studies.66

Diagnostic markers used for the diagnosis of myocardial infarction are present in high concentrations in the myocardium, which get released into extracellular fluid upon any cardiac injury. These markers are elevated in the plasma due to oxidative stress and subsequent inflammatory reactions.73 These essential and sensitive markers are troponins and MB isoenzyme of creatine kinase (CK-MB). Cardiac troponin-I (cTnI) is a cell structural protein composed of striated muscles that regulate contraction. cTnI is recognized as a marker of myocardial damage rather than the feature of just myocardial infarction. It is considered the gold standard for drug-induced cardiotoxicity and acute myocardial infarction.74,75 In the present study, serum CK-MB and cTnI levels were significantly elevated, signifying 5-FU-induced necrotic damage in the myocardium and changes in the integrity and permeability of the plasma membrane, as reported previously. However, treatment with myricetin at both doses alleviated serum CK-MB and cTnI levels, demonstrating membrane integrity and decreased myocardial injury by myricetin.76

The mitochondrial membrane permeability depends on the mitochondrial transmembrane potential. Various pro-apoptotic proteins such as BAX and anti-apoptotic protein such as Bcl-2 and their ratio in mitochondria govern the amount of caspase-3 activation by releasing cytochrome C.77 Oxidative stress activates caspases which are cysteine-dependent enzymes. Caspase-3, an executioner caspase, activates intrinsic and extrinsic apoptosis pathways by DNA disintegration and leakage of explicit cellular proteins such as PARP, actin, and laminin. S-FU-triggered apoptosis is a caspase-dependent course.
involving stimulation of the originator BAX and caspase-3.\cite{46,78} However, very recently, myricetin treatment has been known to significantly decrease the activity of caspase-3 in in vitro studies on Cd-induced neurotoxicity, increasing PC12 cell line viability.\cite{77} In the present study, BAX and caspase-3 activities are significantly upregulated in the 5-FU-administered group and treatment with both doses of myricetin prophylactically attenuated caspase-3 and BAX activities demonstrating blockade of apoptosis.\cite{40}

Histopathological findings further corroborated our results. Polk et al. (2014) showed that the myocardium of rats intoxicated with 5-FU showed multifocal hemorrhages, myofibrillar necrosis, perivascular infiltration of inflammatory cells displaying pericarditis, and vasculitis with ruptured vessel walls and micro thrombosis.\cite{8} In our experiment, 5-FU-induced histopathological alterations such as predominant vacuolization, enlarged intercellular spaces, myocardial edema, constricted heart vessels, multifocal diffused areas of necrosis, and perivascular cell infiltrations throughout the affected areas. On the other hand, myricetin treatment led to a significant decrease in cell infiltration and correction of histomorphological abnormalities occurring in myocardial infarctions in the cardiac tissue of rats induced with different toxicants.\cite{42,81} In our study, myricetin treatment alleviated histological inflammatory evaluations generated by 5-FU with predominant restoration at higher doses (write dose and some observed changes). Liao (2017) recently demonstrated that myricetin could significantly attenuate the cardiac tissue changes induced by streptozotocin, such as disordered myocardium and enlarged cardiomyocytes, which agrees with our study.\cite{82}

5. CONCLUSIONS

The present study deciphers the cross-talk of various signaling mechanisms such as ROS, inflammatory, and apoptosis pathways, enhancing redox imbalance, inflammation, and cell death in the 5-FU-induced cardiotoxicity. However, myricetin treatment mitigates these pathways and provides cardioprotective potential against 5-FU-induced injury, further supported by histopathological examination and cardiac function tests. Also, the model used in this study is different from what occurs clinically, and therefore, this article provides proof that myricetin may be beneficial for 5-FU-induced cardiotoxicity. Still, it needs to be confirmed in a more clinically relevant model.

5.1. Limitations. The small sample size and the single-dose use of 5-FU are the limitations of our study. This study should be considered a preliminary study, and the conclusion of this study should be interpreted carefully. More studies with many experimental animals and multiple doses of 5-FU should be considered to reveal the underlying mechanism of action. Additionally, human studies must use myricetin to elucidate their role in 5-FU toxicity prevention.

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Notes

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# REFERENCES

(1) Choudhari, A. S.; Mandave, P. C.; Deshpande, M.; Ranjekar, P.; Prakash, O. Phytochemicals in Cancer Treatment: From Preclinical Studies to Clinical Practice. Front. Pharmacol. 2020, 10, 1614.

(2) Zitvogel, L.; Apetoh, L.; Ghiringhelli, F.; Kroemer, G. Immunological aspects of cancer chemotherapy. Nat. Rev. Immunol. 2008, 8, 59–73.

(3) Greim, J. L. 5-Fluorouracil: forty-plus and still ticking. A review of its preclinical and clinical development. Invest. New Drugs 2000, 18, 299–313.

(4) Sar, J. D.; Kaur, J.; Khodadadi, R.; Rehman, M.; Lobo, R.; Chakraborti, S.; Herrmann, J.; Lerman, A.; Grothey, A. 5-fluorouracil and cardiotoxicity: a review. Ther. Adv. Med. Oncol. 2018, 10, 1758835918780140.

(5) Stewart, T.; Pavlakis, N.; Ward, M. Cardiotoxicity with 5-fluorouracil and capectabine: more than just vasospastic angina. Intern. Med. J. 2010, 40, 303–307.

(6) Anand, A. J. Fluorouracil cardiotoxicity. Ann. Pharmacother. 1994, 28, 374–378.

(7) Muhammad, R. N.; Saltam, N.; El-Abhar, H. S. Activated ROCK/Akt/eNOS and ET-1/ERK pathways in 5-fluorouracil-induced cardiotoxicity: modulation by simvastatin. Sci. Rep. 2020, 10, 14693.

(8) Polk, A.; Vistisen, K.; Vaage-Nilsen, M.; Nielsen, D. L. A systematic review of the pathophysiology of 5-fluorouracil-induced cardiotoxicity. BMC Pharmacol. Toxicol. 2014, 15, 47.

(9) Saif, M. W.; Shah, M. M.; Shah, A. R. Fluoropyrimidine-associated cardiotoxicity: revisited. Expet Opin. Drug Saf. 2009, 8, 191–202.

(10) Alter, P.; Herzum, M.; Soufi, M.; Schaefer, J.; Maisch, B. Cardiotoxicity of 5-Fluorouracil. Cardiovasc. Hemalot. Agents Med. Chem. 2006, 4, 1.

(11) Panche, A. N.; Diwan, A. D.; Chandra, S. R. Flavonoids: an overview. J. Nutr. Sci. 2016, 5, No. e47.
(12) Burak, M.; Imen, Y. Flavonoids and their antioxidant properties. Turk. Klin. Tip Bilimleri Derg. 1999, 19, 296–304.

(13) Jucu, M. M.; Cynse Filho, F. M. S.; de Almeida, J. C.; Mesquita, D. D. S.; Barriga, J. R. M.; Dias, K. C. F.; Barbosa, T. M.; Vasconcelos, L. C.; Leal, L. K. A. M.; Ribeiro, J. E.; Vasconcelos, S. M. M. Flavonoids: biological activities and therapeutic potential. Nat. Prod. Res. 2020, 34, 692–705.

(14) Fideles, L. d. S.; de Miranda, J. A. L.; Martins, C. D. S.; Barbosa, M. L. L.; Pimenta, H. B.; Pimentel, P. V. d. S.; Teixeira, C. S.; Scafuri, M. A. S.; Façanha, S. d. O.; Barreto, J. E. F.; Carvalho, P. M. d. M.; Scafuri, A. G.; Araújo, J. L.; Rocha, J. A.; Vieira, I. G. P.; Ricardo, N. M. P. S.; da Silva Campbell, M.; Ribeiro, M. E. N. P.; da Castro Brito, G. A.; Cerqueira, G. S. Role of Rutin in S-Fluorouracil-Induced Intestinal Mucositis: Prevention of Histological Damage and Reduction of Inflammation and Oxidative Stress. Molecules 2020, 25, 2786.

(15) Navarro-Hortal, M. D.; Varela-López, A.; Romero-Márez, J. M.; Rivas-Garcia, L.; Speranza, L.; Battino, M.; Quiles, J. L. Role of flavonoids against adriamycin toxicity. Food Chem. Toxicol. 2020, 146, 118120.

(16) de Miranda, J. A. L.; Martins, C. D. S.; Fideles, L. d. S.; Barbosa, M. L. L.; Barreto, J. E. F.; Pimenta, H. B.; Freitas, F. O. B.; Pimentel, P. V. d. S.; Teixeira, C. S.; Scafuri, A. G.; Dos Santos Luciano, M. C.; Araújo, J. L.; Rocha, J. A.; Vieira, I. G. P.; Ricardo, N. M. P. S.; da Silva Campbell, M.; Ribeiro, M. E. N. P.; da Castro Brito, G. A.; Cerqueira, G. S. Troxerutin Prevents S-Fluorouracil Induced Morphological Changes in the Intestinal Mucosa: Role of Cyclooxygenase-2 Pathway. Pharmaceuticals 2020, 13, 10.

(17) Rashid, S.; Nafees, S.; Siddiqi, A.; Vafa, A.; Afzal, S. M.; Parveen, R.; Ali, N.; Hasan, S. K.; Barnwal, P.; Shahid, A.; Sultan, S. Partial protection by 18β-Glycyrrhetinic acid against Cisplatin induced oxidative intestinal damage in wistar rats: possible role of NFkB and caspases. Pharmacol. Rep. 2017, 69, 1007–1013.

(18) Taheri, Y.; Sulieria, H. A. R.; Martín, M.; Sytar, O.; Belayti, A.; Yeskaliyeva, B.; Seitzmanna, G.; Salehi, B.; Semwal, P.; Painini, S.; Kumar, A.; Azini, E.; Martorell, M.; Setzer, W. N.; Maroyi, A.; Sharifi-Rad, J. Myricetin bioactive effects: moving from preclinical evidence to potential clinical applications. BMC Complementary Med. Ther. 2020, 20, 241.

(19) Lau-Cam, C. A.; Chan, H. H. Flavonoids from Comptonia peregrine. Phytochemistry 1973, 12, 1829.

(20) Jones, J. R.; Lebar, M. D.; Jinwil, U. A.; Abisambra, J. F.; Koren, J.; Blair, L.; O’Leary, J. C.; Davey, Z.; Trotter, J.; Johnson, N. A. G.; Weeber, E.; Eckman, C. B.; Baker, B. J.; Dickey, C. A. The diarylheptanoid (+)-αR,11S-myricanol and two flavones from bayberry (Myrica cerifera) destabilize the microtubule-associated protein tau. J. Nat. Prod. 2011, 74, 38–44.

(21) Umadevi, I.; Daniel, M.; Sabnis, S. D. Chemotaxonomic studies on some members of Anacardiaceae. Proc. Plant Sci. 1988, 98, 205–208.

(22) Abd El-kader, A. M.; El-Readi, M. Z.; Ahmed, A. S.; Nafady, A. M.; Wink, M.; Ibraheem, Z. Z. Polyphenols from aerial parts of Polygonum bellardii and their biological activities. Pharm. Biol. 2013, 51, 1026–1034.

(23) Hergert, H. L. The flavonoids of lodgepole pine bark. J. Org. Chem. 1956, 21, 534–537.

(24) Chua, L. S.; Latiff, N. A.; Lee, S. Y.; Lee, C. T.; Sarmidi, M. R.; Chua, L. S.; Latiff, N. A.; Lee, S. Y.; Lee, C. T.; Sarmidi, M. R.; Hergert, H. L. The flavonoids of lodgepole pine bark. J. Org. Chem. 1956, 21, 534–537.

(25) Yao, Y.; Lin, G.; Xie, Y.; Ma, P.; Meng, L.; Wu, T. Preformulation studies of myricetin: A natural antioxidant flavonoid. Pharmazie 2014, 69, 19–26.

(26) Lin, J.; Zhang, S. M.; Wu, K.; Willett, W. C.; Fuchs, C. S.; Giovannucci, E. Flavonoid intake and colorectal cancer risk in men and women. Am. J. Epidemiol. 2006, 164, 644–651.

(27) Jiang, M.; Zhu, M.; Wang, L.; Yu, S. Anti-tumor effects and associated molecular mechanisms of myricetin. Biomed. Pharmacother. 2019, 120, 109506.
kidneys of Wistar rats.

Ahmad, S. B. Piperine Regulates Nrf-2/Keap-1 Signalling and Exhibits Anti-oxidative and Anti-inflammatory Effects on 5-fluorouracil-Induced Oxidative Stress, Inflammatory Response, and Goblet Cell Mucositis in a rat model. *Antioxid. Redox Signal.* 2013, 16, 734.

Ahmad, S.; Ali, N.; Nafees, S.; Ahmad, S. T.; Hasan, S. K.; Buyukkocak, S.; Ozturk, H. S. Reduced antioxidant defense capacity in myocardial tissue from guinea pigs treated with 5-fluorouracil. *J. Toxicol. Environ. Health, Part A* 2000, 59, 585–589.

Atiq, A.; Shal, B.; Naveed, M.; Khan, A.; Ali, J.; Zeeshan, S.; Al-Sharari, S. D.; Kim, Y. S.; Khan, S. Diadzein ameliorates 5-fluorouracil-induced intestinal mucositis by suppressing oxidative stress and inflammatory mediators in rodents. *Eur. J. Pharmacol.* 2019, 843, 292–306.

B. D.; Ruchi, B. S.; Sandra, C.; Álvaro, V. Myricetin: A Dietary Molecule with Diverse Biological Activities. *Nutrients* 2016, 8, 90.

Chang, D. K. Ursodeoxycholic acid attenuates 5-fluorouracil-induced cardiomyopathy. *Cureus* 2019, 11, No. e1562.

Cho, B. O.; Yin, H. H.; Park, S. H.; Byun, E. B.; Ha, H. Y.; Jiang, S. I. Anti-inflammatory activity of myricetin from Diospyros lotus through suppression of NF-kB and STAT1 activation and Nrf2-mediated HO-1 induction in lipopolysaccharide-stimulated RAW264.7 macrophages. *Biosci. Biotechnol. Biochem.* 2016, 80, 1520–1530.

Chen, M.; Chen, Z.; Huang, D.; Sun, C.; Xie, J.; Chen, T.; Zhao, X.; Huang, Y.; Li, D.; Wu, B.; Wu, D. Myricetin inhibits TNF-$\alpha$-induced inflammation in AS49 cells via the SIRT1/NF-kB pathway. *Palm. Pharmacol. Ther.* 2020, 65, 102000.

Arumugam, B.; Palanisamy, U. D.; Chua, K. H.; Kuppusamy, U. R. Protective effect of myricetin derivatives from Syzygium malaccense against hydrogen peroxide-induced stress in ARPE-19 cells. *Mol. Vis.* 2019, 25, 47.

Sheybani, A.; Crum, K.; Raucci, F. J.; Burnette, W. B.; Markham, L. W.; Soslow, J. H. Duchenne muscular dystrophy patients: troponin leak in asymptomatic and implications for drug toxicity studies. *Pediatr. Res.* 2021, 1–8.

Allison, J. D.; Tanavin, T.; Yang, Y.; Birnbaum, G.; Khalid, U. Various Manifestations of 5-Fluorouracil Cardiotoxicity: A Multi-center Case Series and Review of Literature. *Cardiovasc. Toxicol.* 2020, 20, 437–442.

Khudhair, A. R.; Numan, I. T. Investigating the Possible Cardio-Protective Effects of Telmisartan against 5-Fluorouracil-Induced Cardiotoxicity in Wistar Rats. *Technol. Innov. Pharmaceut. Res.* 2021, 7, 1–8.

Taha Mohamed, E.; Mohamed, G.; Mohamed, S. Evaluation of cardioprotective activity of Lepidium sativum seed powder in albino rats treated with 5-fluorouracil. *Beni-Seif Univ. J. Basic Appl. Sci.* 2016, 5, 208–215.

Hassoun, R.; Budde, H.; Zhayzkybayeva, S.; Herwig, M.; Sieme, M.; Delat, S.; Mostafi, N.; Gömöri, K.; Tangos, M.; Jarkas, M.; Pabel, S.; Bruckmüller, S.; Skrygan, M.; Lodi, M.; Jaquet, K.; Sequeira, V.; Gambichler, T.; Remedios, C. D.; Kovács, A.; Mannherz, H. G.; Mügge, A.; Sossalla, S.; Hamdani, N. Stress activated signalling impaired protein quality control pathways in human hypertrophic cardiomyopathy. *Int. J. Cardiol.* 2021, 344, 160–169.

Filgueiras, M. d. C.; Morrot, A.; Soares, P. M. G.; Costa, M. L.; Mermelstein, C. Effects of 5-fluorouracil in nuclear and cellular morphology, proliferation, cell cycle, apoptosis, cytoskeletal and caveolar distribution in primary cultures of smooth muscle cells. *PLoS One* 2013, 8, No. e63177.
Aminzadeh, A.; Salarinejad, A. Effects of myricetin against cadmium-induced neurotoxicity in PC12 cells. *Toxicol. Res.* 2021, 10, 84−90.

Shiga, T.; Hiraide, M. Cardiotoxicities of 5-Fluorouracil and Other Fluoropyrimidines. *Curr. Treat. Options Oncol.* 2020, 21, 27.

Sheta, A.; Elsakkar, M.; Hamza, M.; Solaiman, A. Effect of metformin and sitagliptin on doxorubicin-induced cardiotoxicity in adult male albino rats. *Hum. Exp. Toxicol.* 2016, 35, 1227−1239.

Liao, H.-h.; Zhu, J. X.; Feng, H.; Ni, J.; Zhang, N.; Chen, S.; Liu, H. J.; Yang, Z.; Deng, W.; Tang, Q. Z. Myricetin Possesses Potential Protective Effects on Diabetic Cardiomyopathy through Inhibiting IkBa/NFkB and Enhancing Nrf2/HO-1. *Oxid. Med. Cell. Longev.* 2017, 8370593.