Myocardial fibrosis is a major global health issue that is linked to practically all types of heart disease. Inhibiting the TGF-β signalling pathway is currently of great interest due to its well-established role in cardiac fibrosis. To avoid heart failure, it's essential to prevent cardiac fibrosis. However, there is currently no effective therapeutic approach. The objective of the present study was to evaluate the effect of Nintedanib (tyrosine kinase inhibitor) and Pirfenidone (TGF-β inhibitor) in the treatment of cardiac fibrosis. Cardiac fibrosis was induced by Doxorubicin (2.5 mg/kg/day, i.p. 3 times a week). Cardiac fibrosis was confirmed by ST-segment elevation. Confirmed cardiac fibrotic rats were treated with Nintedanib (30 and 50 mg/kg, p.o.) and Pirfenidone (50 and 100 mg/kg, p.o.) for 2 weeks. The treatment of Nintedanib and Pirfenidone remarkably minimized ST-segment elevation, decreased heart rate, heart weight, LVWI, RVWI and increased blood pressure. Combination treatment of Nintedanib and Pirfenidone normalized oxidative stress with a significant (p<0.0001) rise in the level of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) and reduced lipid peroxidation (LPO) as compared to the doxorubicin group. The present study revealed that Nintedanib and Pirfenidone, as monotherapy as well as in combination, have an anti-fibrotic effect against cardiac fibrosis. The anti-fibrotic effect could be attributed to a reduction in oxidative stress and collagen deposition, as well as inhibition of TGF-β overexpression. The results provided by this study give evidence that Nintedanib and Pirfenidone are useful against cardiac fibrosis.
INTRODUCTION:
Cardiovascular disease (CVD), a group of disorders affecting the heart or cardiovascular system, is the leading cause of death globally, accounting for 31% of all deaths \[1\]. Cardiac fibrosis is defined by an excess of extracellular matrix in the myocardium and is present in almost all cardiac pathologies \[2\]. The most significant fibrotic remodelling of the ventricle is found in disorders linked with abrupt cardiomyocyte loss that's because the mature mammalian heart has minimal regenerating capacity. Following an acute myocardial infarction, the death of a high number of cardiomyocytes causes an inflammatory response, which eventually results in the replacement of the dead myocardium by a collagen-based scar \[3\]. The accumulation of ECM in the cardiac tissue causes myocardial fibrosis, which is an aberrant heart condition. Cardiac fibrosis is a pathological ECM remodelling that results in abnormal cardiac diseases. Excess extracellular matrix retention in cardiac muscles causes structural and electrical alterations that raise the risk of arrhythmias, heart failure, and ischemia in patients. Because it is a primary cause of heart failure, preventing and treating it is critical to curing heart failure. It is part of the fibrosis group of illnesses, which refers to the hardening and scarring of tissue \[4,5\].

Numerous chemicals have been recognised as being crucial in the development of cardiac fibrosis. Numerous studies have demonstrated the importance of the renin-angiotensin system (RAS), TGF-β, and endothelin (ET) in this process. Renin and angiotensin-converting enzyme (ACE), which is produced by macrophages and fibroblasts when the heart is damaged, produces angiotensin II (AngII). When Ang II binds to the Ang II type 1 (AT1) receptor, hypertrophy, fibroblast proliferation, and collagen synthesis are stimulated. Human cardiomyocytes include the matrix metalloproteinases MMP-1,-2,-3,-9, and -14, which are controlled by a number of pro-inflammatory and pro-fibrotic stimuli \[6\]. TGF-β is the main fibrosis mediator. TGF-β1 controls fibroblasts' ability to grow. TGF-β1 increases collagen gene expression, which enhances the creation of fibrillar collagen \[7\]. In preclinical lung fibrosis disease models, nintedanib has anti-fibrotic and anti-inflammatory effects. Nintedanib (NIN) decreases human lung fibroblast proliferation, migration, and fibroblast-to-myofibroblast transition in IPF patients. Various studies show pirfenidone (PIRF) show anti-inflammatory and anti-fibrotic effect. Pirfenidone is approved for the treatment of mild to moderate IPF. This anti-fibrotic activity is seen not just in the lungs, but also in the kidneys and the liver.

MATERIALS AND METHODS

Drugs and Chemicals
For the study, the drug captopril was obtained from Torrent Pharmaceuticals, India, nintedanib from Glenmark Pharmaceuticals, and pirfenidone from Lupin Limited. In distilled water, nintedanib, pirfenidone and captopril were prepared as a formulation. All of the chemicals were obtained from standard manufacturers. The compounds were all of the analytical grade.

Experimental animals
Laxmi Biopharm PVT. LTD. Alephata, Dist: Pune, India provided the Wistar rats of either sex, each weighing 180-220 g, which were used in the experiment. The animals were housed in polypropylene cages with husk bedding, which were changed every 48 hours, and were kept at a temperature of 25±2ºC throughout a 12:12 h light-dark cycle. They received free access to water and commercial pellet rat feed. The Institutional Animal Ethical Committee (IAEC) of M.V.P’s College of Pharmacy, Nashik-02, approved the study (IAEC/2021/01) and the examinations were conducted in line with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Induction of cardiac fibrosis in rats
Rats were given doxorubicin i.p. three times per week at a dose of 2.5 mg/kg/day. ECG recording (ST-segment elevation) using the PowerLab Data Acquisition System...
(AD Instruments, Australia)[8,9] was used to confirm the development of cardiac fibrosis.

**Experimental design**

Rats were randomly assigned to one of eight groups to study doxorubicin-induced cardiac fibrosis. Each group has six different animals. Group I (Control) - vehicle (normal saline; 5 ml/kg, p.o.), Group II (fibrotic)- DOX (2.5 mg/kg/day, i.p., for 3 times a week), Group III – DOX + NIN (2.5 mg/kg/day, i.p. + 30 mg/kg, p.o), Group IV - DOX + NIN (2.5 mg/kg/day, i.p. + 50 mg/kg, p.o), Group V - DOX + PIRF (2.5 mg/kg/day, i.p. + 50 mg/kg, p.o), Group VI - DOX + PIRF (2.5 mg/kg/day, i.p. + 100 mg/kg, p.o), Group VII - DOX + NIN + PIRF (2.5 mg/kg/day, i.p. + 50 mg/kg, p.o + 100 mg/kg, p.o respectively), Group VIII - DOX + Captopril (2.5 mg/kg/day, i.p. + 50 mg/kg, p.o). After the induction of cardiac fibrosis, a drug for treatment was administered everyday for 15 days.

**METHODS**

After 15 days, following the induction of cardiac fibrosis, all treatments were started. Body weight of each animal was recorded both before and after their drug treatments. Every week, the heart rate and ECG of every animal were recorded before and after the treatment. Animals were euthanized using a euthanasia chamber provided with carbon dioxide, at the conclusion of the treatment plan. Then hearts were removed by dissection. The left and right ventricular mass (mg) divided by the body mass (g), respectively, were used to determine the left and right ventricular weight indices.[11]

**Biochemical estimation**

**Dissection and homogenization**

For other experimental tests, the heart was removed, washed with isotonic saline, and weighed. A tissue homogenate 10% (w/v) was prepared in ice-cold 0.1 M phosphate buffer (pH 7.4). The post-nuclear fraction for catalase assay was produced by using centrifugation (Remi–C-30, Remi Industries Ltd. Mumbai, India) of the homogenate at 1000 rpm for 20 min (at 4°C); for another remaining assay of an enzyme, 12,000 rpm of centrifugation was done at 60 min and 40°C. A Ultra-Violet spectrophotometer (UV-2450 Shimadzu) was used for subsequent assay.

**Catalase activity (CAT)**

For the purpose of measuring the breakdown of H2O2, the catalase activity at 240 nm was determined using the Luck method. A mixture of 3 ml of H2O2 in phosphate buffer (0.0125 M H2O2) and 0.05 ml of cardiac tissue homogenate supernatant was used for this assay. In order to calculate the catalase activity, the millimolar extension coefficient of H2O2 was used (0.07). It was measured in terms of the amount of H2O2 that was broken down per minute per mg of protein.[12]

**Estimation of reduced glutathione (GSH)**

Reduced glutathione (GSH) was measured in cardiac tissue homogenate using Ellman's method (1959). A 0.75 ml sample of homogenate was centrifuged at 1,200 x g for 15 min at 4°C with the assistance of 0.75 ml of 4% sulphasalicylic acid. A 0.1 M phosphate buffer with a pH of 8.0, 0.5 ml of supernatant, and 4.5
ml of 0.01 M 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) comprise the test combination. The amount of GSH detected was measured as micromoles of GSH per milligram of proteins and the absorbance was measured at 412 nm [13].

**Superoxide dismutase activity (SOD)**
The superoxide dismutase activity was determined using the Kono (1978) method. Superoxide dismutase prevents nitroblue tetrazolium chloride (NBT) from being reduced. In order to determine the absorbance was measured at 516 nm. NBT and some homogenate make up the reaction mixture. Hydroxylamine hydrochloride was added to the reaction mixture to initiate the reaction. The result were expressed in terms of the percentage inhibition of NBT reduction [14].

**Lipid peroxidation assay (LPO)**
Malondialdehyde in cardiac tissue homogenate was quantitatively measured using Wills' (1966) lipid peroxidation method. The reaction mixture consists of 0.1 ml of tissue homogenate, 1.5 ml of 20% acetic acid, 1.5 ml of 0.8% thiobarbituric acid (TBA) aqueous solution, and 0.2 ml of 8% SLS. With distilled water, the volume was adjusted to 4 ml. This mixture was then heated at 95 °C for 60 minutes in a water bath and cooled under running tap water. After vigorously shaking, 5 ml of n-butanol:pyridine (15:1 by volume) was added to the reaction mixture. The results were represented as nanomoles of MDA per milligram of protein using the molar extension coefficient of the chromophore (1.56 105 M-1 cm-1) and the absorbance was measured at 532 nm [15].

**Statistical analysis**
Mean±SEM was used to express the results. The data from the study were subjected to a one-way ANOVA, followed by Dunnett's test, with the significance level set at p<0.05.

**RESULTS**

**Effect of nintedanib, pirfenidone, and their combination on body weight**
Before induction of cardiac fibrosis, the rats had a mean body weight of 199.2±3.763 g. After induction of cardiac fibrosis, a decrease in body weight (142.2±3.936 g) was observed. Treatment with NIN, PIRF, and their combination were significantly (p<0.0001) able to increase the body weight as compared to DOX group. Captopril used as a standard drug also significantly (p<0.0001) increased body weight (Table 1).

**Effect of nintedanib, pirfenidone, and their combination on heart weight.**
Before induction of cardiac fibrosis, the rats had a mean heart weight of 0.588±0.02774g. After induction of cardiac fibrosis, an increase in heart weight (1.025±0.1144 g) was observed. Treatment with NIN, PIRF, and their combination was significantly (p<0.0001) able to decrease the heart weight as compared to DOX group. Captopril used as a standard drug also significantly (p<0.0001) increased body weight (Table 1).

**Effect of nintedanib, pirfenidone, and their combination on heart rate**
DOX administration causes a decrease in heart rate as compared to control group. Treatment with NIN, PIRF, and their combination show significant (p<0.0001) increase in heart rate in comparison to DOX. Captopril, standard also increased heart rate significantly (p<0.0001) (Table 1).

**Effect of nintedanib, pirfenidone, and their combination on blood pressure**
DOX administration causes decrease in blood pressure as compared to control group. Treatment with NIN, PIRF, and their combination show significant (p<0.0001) increase in BP in comparison to DOX. Captopril, standard also increased BP significantly (p<0.0001) (Table 1).
Table 1: Effect of nintedanib and pirfenidone on body weight, heart weight, heart rate and blood pressure in doxorubicin-treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight (g)</th>
<th>Heart weight (g)</th>
<th>Heart rate (beats/min)</th>
<th>Blood pressure (mmHg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>199.2±3.763</td>
<td>0.5883±0.02774</td>
<td>266.9±0.3270</td>
<td>119.3±2.261</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg</td>
<td>142.2±3.936</td>
<td>1.025±0.114</td>
<td>238.5±5.359</td>
<td>84.43±5.229</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + NIN 30 mg/kg</td>
<td>189.8±5.522</td>
<td>0.7450±0.03622</td>
<td>371.4±1.578</td>
<td>109.4±7.507</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + NIN 50 mg/kg</td>
<td>188.2±2.750</td>
<td>0.6783±0.04936</td>
<td>275.4±0.8135</td>
<td>122.3±1.745</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + PIRF 50 mg/kg</td>
<td>176.7±3.499</td>
<td>0.6950±0.03879</td>
<td>370.0±2.150</td>
<td>119.6±3.562</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + PIRF 100 mg/kg</td>
<td>185.8±4.976</td>
<td>0.6800±0.02966</td>
<td>369.4±0.3615</td>
<td>116.2±4.553</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + NIN 50 mg/kg + PIRF 100 mg/kg</td>
<td>201.3±5.451</td>
<td>0.7117±0.03351</td>
<td>303.0±0.4472</td>
<td>108.5±6.758</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + Captopril 50 mg/kg</td>
<td>194.0±3.055</td>
<td>0.6817±0.02587</td>
<td>360.5±7.460</td>
<td>113.2±5.423</td>
</tr>
</tbody>
</table>

Each column represents mean ± SEM. (n=6). DOX 2.5 mg/kg treated group compared with control. NIN 30 mg/kg, NIN 50 mg/kg, PIRF 50 mg/kg, PIRF 100 mg/kg, NIN 50 mg/kg + PIRF 100 mg/kg, and Captopril 50 mg/kg treated groups compared with DOX 2.5 mg/kg. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns non-significant (One way ANOVA followed by Dunnett’s test).

Effect of nintedanib, pirfenidone, and their combination on LVWI and RVWI

LVWI and RVWI are significantly increased in DOX treated group as compared to control group. Treatments with NIN, PIRF, and their combination significantly (p<0.0001) decreased LVWI and RVWI as compared to the DOX group. Captopril also reduced left and right ventricle indices significantly (p<0.0001) (Table 2).

Table 2: Effect of nintedanib and pirfenidone on heart weight to body weight ratio (HW/BW), left ventricle weight index (LVWI) and right ventricle weight index (RVWI) in doxorubicin-treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>Heart weight to body weight ratio (HW/BW)</th>
<th>LVWI</th>
<th>RVWI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.003383±0.0001612</td>
<td>0.002400±0.0001300</td>
<td>0.002167±0.0001145</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg</td>
<td>0.006178±0.0006646</td>
<td>0.005000±0.0001098</td>
<td>0.003583±0.0001195</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + NIN 30 mg/kg</td>
<td>0.003715±0.0002316</td>
<td>0.004133±0.0001308</td>
<td>0.003000±0.0001211</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + NIN 50 mg/kg</td>
<td>0.003403±0.0002564</td>
<td>0.002967±0.0001202</td>
<td>0.002383±0.0002123</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + PIRF 50 mg/kg</td>
<td>0.003880±0.0003281</td>
<td>0.003883±0.0002915</td>
<td>0.002950±0.0001335</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + PIRF 100 mg/kg</td>
<td>0.003542±0.0001911</td>
<td>0.002600±0.0001915</td>
<td>0.002467±0.0001230</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + NIN 50 mg/kg + PIRF 100 mg/kg</td>
<td>0.003547±0.0002042</td>
<td>0.002667±0.0001111</td>
<td>0.002300±0.0001354</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + Captopril 50 mg/kg</td>
<td>0.003687±0.0002326</td>
<td>0.002500±0.0001815</td>
<td>0.002300±0.0001100</td>
</tr>
</tbody>
</table>
Each column represents mean ± SEM. (n=6). DOX 2.5 mg/kg treated group compared with control. NIN 30 mg/kg, NIN 50 mg/kg, PIRF 50 mg/kg, PIRF 100 mg/kg, NIN 50 mg/kg + PIRF 100 mg/kg, and Captopril 50 mg/kg treated groups compared with DOX 2.5 mg/kg. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns non-significant (One way ANOVA followed by Dunnett’s test).

Effect of nintedanib, pirfenidone, and their combination on ECG parameters ST-segment evaluation

DOX administered group of animals showed significant ST-segment elevation as compared to control group. The ST-segment elevation represents conduction block and the consequent loss of myocardial cell membrane function. Treatment with NIN, PIRF, and their combination showed significant (p<0.0001) cardio-protective effect by reducing ST-segment elevation of ECG as compared to DOX group (Figure 1 and 2). Captopril showed reduction in ST-segment significantly (p<0.0001).

![Fig. 1: Effect of nintedanib and pirfenidone on ST-segment in doxorubicin-treated rats.](image)

(A) Control; (B) DOX-treated (2.5 mg/kg); (C) DOX + NIN 30 mg/kg; (D) DOX + NIN 50 mg/kg; (E) DOX + PIRF 50 mg/kg; (F) DOX + PIRF 100 mg/kg; (G) DOX + NIN 50 mg/kg + PIRF 100mg/kg; (H) DOX + Captopril 50 mg/kg.

Segment PR, QRS, QT, and RR evaluation

The data of the experimental study such as PR segment, QRS complex, QT interval, and R-R interval are shown in Figure 2. DOX treated group showed reduction of PR, QRS, and RR segment and prolongation of QT segment as compared to control group. The treatment with NIN, PIRF, and their combination showed restoration of PR, QRS, QT, and RR segment as compared to DOX group. Thus, treatment showed cardioprotection by restoration of changes in ECG segments. Captopril normalized ECG pattern.
Fig. 2: Effect of nintedanib and pirfenidone ECG parameters in doxorubicin-treated rats.

(A) Effect on ST-segment (B) Effect on PR interval (C) Effect on QT interval (D) QRS interval
Each column represents mean ± SEM. (n=6)
(A) Control; (B) DOX-treated (2.5 mg/kg); (C) DOX + NIN 30 mg/kg; (D) DOX + NIN 50 mg/kg;
(E) DOX + PIRF 50 mg/kg; (F) DOX + PIRF 100 mg/kg; (G) DOX + NIN 50 mg/kg + PIRF
100mg/kg; (F) DOX + Captopril 50 mg/kg. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001,
ns non-significant (One way ANOVA followed by Dunnett’s test).

Effects on change in SOD, CAT, and GSH levels
Group of animals with DOX administration showed cardiotoxicity (by the generation of oxidative stress) when compared to control group, indicating significant decrease in levels of SOD, CAT, and GSH enzymes and increased in level of MDA. However, animals subjected to treatment with NIN, PIRF, and their combination showed significant increase in levels of SOD, CAT, and GSH enzymes with reduction of MDA as compared to animals treated with DOX (Table 3).
Table 3: Effect of nintedanib and pirfenidone on biochemical parameters in doxorubicin-treated rats.

<table>
<thead>
<tr>
<th>Groups</th>
<th>SOD (μM)</th>
<th>CAT (μM)</th>
<th>GSH (μM)</th>
<th>LPO (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>82.55±0.8913</td>
<td>10.67±0.7507</td>
<td>8.831±0.3463</td>
<td>17.64±0.3497</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg</td>
<td>51.16±1.868****</td>
<td>6.203±0.3674****</td>
<td>6.465±0.4536****</td>
<td>26.98±0.5128****</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + NIN 30 mg/kg</td>
<td>55.83±1.510**</td>
<td>8.312±0.3889*</td>
<td>8.367±0.1175****</td>
<td>19.06±0.7445****</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + NIN 50 mg/kg</td>
<td>67.29±1.988****</td>
<td>9.514±0.4848****</td>
<td>8.343±0.2099****</td>
<td>16.37±1.279****</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + PIRF 50 mg/kg</td>
<td>59.10±3.702ns</td>
<td>8.309±0.4401*</td>
<td>8.293±0.07489***</td>
<td>17.53±1.197***</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + PIRF 100 mg/kg</td>
<td>62.58±2.289**</td>
<td>10.25±0.2913****</td>
<td>8.296±0.1177****</td>
<td>15.16±0.5664****</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + NIN 50 mg/kg + PIRF 100 mg/kg</td>
<td>67.49±1.447****</td>
<td>10.01±0.5363****</td>
<td>8.842±0.09373****</td>
<td>15.29±0.7708****</td>
</tr>
<tr>
<td>DOX 2.5 mg/kg + Captopril 50 mg/kg</td>
<td>67.36±1.842****</td>
<td>9.737±0.3845****</td>
<td>8.850±0.1389****</td>
<td>18.69±0.6935****</td>
</tr>
</tbody>
</table>

Each column represents mean ± SEM. (n=6). DOX 2.5 mg/kg treated group compared with control. NIN 30 mg/kg, NIN 50 mg/kg, PIRF 50 mg/kg, PIRF 100 mg/kg, NIN 50 mg/kg + PIRF 100 mg/kg, and Captopril 50 mg/kg treated groups compared with DOX 2.5 mg/kg. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, ns non-significant (One way ANOVA followed by Dunnett’s test).

Histopathological analysis

Histological section of the control-treated rat’s heart showed normal tissue architecture. Doxorubicin (2.5 mg/kg) induced rat’s heart showed abnormal tissue architecture with shard and scar tissues suggesting myocardial fibrosis.

Histological section of NIN, PIRF, and their combination of post-treated doxorubicin (2.5 mg/kg) induced rat’s heart showed mild hard and scar tissue, revealing restoration of myocardial tissue architecture. Captopril also showed restoration of myocardial tissue architecture (Figure 3).

Fig. 3: Effect of nintedanib and pirfenidone on cardiomycocyte cross sectional area in doxorubicin-treated rats.
DISCUSSION
According to the results of this study, a combination of nintedanib and pirfenidone is the most effective treatment for experimentally induced cardiac fibrosis in Wistar rats. Nintedanib is a tyrosine kinase inhibitor and can be used orally which has been evaluated in treatment of cardiac fibrosis in rats. Nintedanib exerted potent inhibitory effect on fibrosis by reducing oxidative stress. It also inhibits the proliferation of fibrotic tissue and avoids further damage to cardiac tissue. Additionally, at a dose of 30mg/kg and 50mg/kg nintedanib attenuates ECM deposition. Nintedanib binds to the intracellular ATP binding pocket of fibroblast growth factor receptors (FGFRs), platelet-derived growth factor receptors (PDGFRs), and vascular endothelial growth factor receptors (VEGFRs), and transforming growth factor-β preventing auto-phosphorylation and downstream signalling which play important role in pathogenesis of cardiac fibrosis. Pirfenidone exhibit both anti-inflammatory and antifibrotic effects. Pirfenidone's anti-fibrotic action is achieved in part by a reduction in oxidative stress. Pirfenidone at a dose of 50mg/kg inhibits the growth of fibroblasts, suppresses collagen formation stimulated by transforming growth factor beta and inhibits fibrogenic mediators such as transforming growth factor beta synthesis. Pirfenidone has also been proven to suppress the production of inflammatory mediators like TNF-alpha and IL-1. Nintedanib and pirfenidone protect the heart from oxidative free radical production, apoptosis, and collagen deposition, according to the findings of this study. The effects are linked to the capacity to reduce cardiac hypertrophy and fibrotic changes caused by reactive oxygen species. Nintedanib and pirfenidone reduce the activity of reactive oxygen species (ROS), which reduces lipid peroxidation activity. In clinical practice, cardiac hypertrophy and fibrosis are found to be a precursor to heart failure, deadly arrhythmias, and myocardial infarction. As a result, nintedanib and pirfenidone might be regarded as first-line treatments for myocardial fibrosis, preventing additional cardiac problems.

When compared to the DOX group, the combination therapy of nintedanib and pirfenidone demonstrated a rise in body weight, an increase in heart rate and blood pressure, and a decrease in heart weight, LVWI, and RVWI. In comparison to the DOX-treated group, normal ECG was also recorded. Nintedanib and pirfenidone have normal antioxidant activity against DOX-induced cardiac fibrosis, according to the findings. As a result, the current study shows thata combination of nintedanib and pirfenidone was helpful in treating cardiac fibrosis.

CONCLUSION
According to the results of this study, treatment of Nintedanib and Pirfenidone is the most effective treatment for experimentally induced cardiac fibrosis in Wistar rats. In the present study, the results proved that the treatment of Nintedanib and Pirfenidone showed increased in body weight, heart rate, and blood pressure and a decrease in heart weight, LVWI, and RVWI as compared to DOX and treated groups. Also, normal ECG were obtained as compared to DOX-treated groups. The data of the study showed that Nintedanib and Pirfenidone exerted normal antioxidant activity against DOX-induced myocardial fibrosis. Hence the present study provides evidence that the combination treatment of Nintedanib and Pirfenidone was effective in cardiac fibrosis in rats.

Conflicts of interest
The author reported no conflict of interest. The authors alone are responsible for the content and writing of the article.

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