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Anti-Fibrotic Effect of Tinospora Cordifolia and Trandolapril Combination in the Isoproterenol-Induced Cardiac Fibrosis in Rats

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ABSTRACT

The increase of extracellular matrix (ECM) in the cardiac tissue causes myocardial fibrosis, which is a heart problem. Because cardiac fibrosis is a primary cause of heart failure, preventing and treating it is one of the most important goals in the treatment of heart failure. The goal of this study was to see how effective the combination of Tinospora cordifolia and trandolapril was at treating Isoproterenol (ISO)-induced cardiac fibrosis in rats. Isoproterenol 5 mg/kg/day (s.c.) for 15 days was used to promote cardiac fibrosis. ST-segment elevation confirms cardiac fibrosis. Tinospora cordifolia (100 and 200 mg/kg, p.o.) and trandolapril (3 mg/kg, p.o.) were then given to these proven cardiac fibrotic rats for two weeks.Tinospora cordifolia and trandolapril therapy significantly reduced ST-segment elevation, heart rate, weight, LVWI, RVWI, and soluble collagen levels. In comparison to the isoproterenolgroup, Tinospora cordifolia and trandolapril treatment restored oxidative stress by increasing the levels of superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH) and lowering lipid peroxidation (LPO). Tinospora cordifolia and trandolapril, both as monotherapy and in combination, were found to have a cardioprotective effect against heart fibrosis in this investigation. The cardioprotective effect could be attributed to a reduction in oxidative stress and collagen deposition, as well as inhibition of Ang II, which obliquely regulates TGF- β overexpression. The results provided by this study give evidence that Tinospora cordifolia and trandolapril are useful against cardiac fibrosis.

KEYWORDS: Cardiac fibrosis, Isoproterenol, Tinospora cordifolia, Trandolapril.

I. INTRODUCTION

Cardiac fibrosis is characterised by abnormal heart conditions caused by pathological ECM remodelling. Cardiac fibrosis is part of a group of diseases known as fibrosis, which is defined by tissue hardening and scarring.[1,2] The fibrillar collagen network is in close contact with all types of cardiac cells, and thus plays a critical role in the maintenance of ventricular form, size, and function.[3] Cardiac fibrosis is caused by the activation of fibroblasts into myofibroblasts, which create higher levels of ECM proteins that build fibrous tissue and affect the normal breakdown of ECM. Both processes result in collagen deposition, which has an impact on systolic and diastolic functioning. Cardiac fibrosis worsens over time, causing a steady decline in heart function.[4] Stress and damage produce mediators that induce fibroblast activation and differentiation, such as hormones, cytokines, and proteins, which eventually contribute to cardiac fibrosis.[5]Many compounds have been discovered to play a role in the evolution of cardiac fibrosis. The renin-angiotensin system (RAS), TGF-β, and endothelin (ET) are all key components of this process, according to numerous studies. When the heart is wounded, macrophages and fibroblasts create renin and angiotensin-converting enzyme (ACE), which then make angiotensin II (Ang II). The Ang II type 1 (AT1) receptor binds to Ang II, causing hypertrophy, fibroblast proliferation, and increased collagen production. MMP-1,-2,-3,-9, and -14 are matrix metalloproteinases that are found in human cardiomyocytes and are influenced by a variety of pro-inflammatory and pro-fibrotic factors.[6] TGF-β is a major fibrosis mediator. TGF-β_1 regulates the development of fibroblasts. TGF-β_1 increases collagen gene expression, which enhances fibrillar collagen synthesis.[7]

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Tinospora cordifolia has been shown to have antioxidant, antihypertensive, and anti-inflammatory properties in many studies, although it has not been researched extensively for its usefulness in myocardial fibrosis.[8] Trandolapril is an ACE inhibitor (angiotensin-converting enzyme inhibitor). It inhibits the conversion of ANG I to ANG II in the renin-angiotensin-aldosterone system, which leads to more controlled cardiac functioning.[9]

In order to target the pathophysiological processes of cardiac fibrosis, researchers used Tinospora cordifolia and trandolapril in isoproterenol-induced cardiac fibrosis in rats to target Ang II, collagen deposition, and oxidative stress.

II. MATERIALS AND METHODS

Plant material:

Tinospora cordifolia stems were collected from Nashik's locality and authenticated by the AyurvedMahavidyalaya Nashik (Voucher no. 476/2020/2021). Using ethanol as a solvent, the dried stem powder of the plant Tinospora cordifolia was collected after a 7-day maceration process. The presence of flavonoids, saponins, tannins, alkaloids, and glycosides was determined in early phytochemical studies.

Chemicals:

As gift samples,received trandolapril (Hetero Healthcare Ltd., India) and captopril (Torrent Pharmaceuticals, India). The trandolapril and captopril formulations were made with distilled water. All of the chemicals were bought from standard suppliers. All of the chemicals were of the highest quality.

Acute oral toxicity study of Tinospora cordifolia:

Tinospora cordifolia was tested for acute toxicity in Wistar rats at a maximum dose of 2000 mg/kg in accordance with OECD (Organization for Economic Co-operation and Development) guidelines No. 420. At a dose of 2000 mg/kg, the LD50 value and gross observational effects were detected. The doses of 100 and 200 mg/kg of extract were chosen for the study based on the literature review and acute toxicity study.

Experimental animals:

Wistar rats (180-220 g) of either sex (n = 6) were utilised in the investigation. Animals were housed in polypropylene cages and kept under typical laboratory settings, including a temperature of 252°C, a 12:12 h L:D cycle, and humidity of 50.5%, with free access to water and food. Before the experiment, the animals were acclimated to standard laboratory circumstances. During the light period, the experiment was conducted (08:00-16:00 h). The experiment was carried out in accordance with the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi(India). The study protocol was approved by the Institutional Animal Ethical Committee of M.V.P.S. College of Pharmacy, Nashik-02, with approval number (IAEC/2020/04).

Induction of cardiac fibrosis in rats:

Rats were given isoproterenolat a dose of 5 mg/kg/day, s.c. for15 days. ECG recording (ST-segment elevation) with the PowerLab Data Acquisition System (AD Instruments, Australia) verified the development of cardiac fibrosis.[10,11]

Experimental design:

Rats were randomly separated into seven groups to study isoproterenol-induced cardiac fibrosis. There are six animals in each group. Group I (Control) - vehicle (normal saline; 5 ml/kg, p.o.), Group II (fibrotic)- isoproterenol(5 mg/kg/day, s.c., for 15 days), Group III - Tinospora cordifolia + isoproterenol (100 mg/kg, p.o. + 5 mg/kg/day, s.c., for 15 days), Group IV - Tinospora cordifolia + isoproterenol (200 mg/kg, p.o.+ 5 mg/kg/day, s.c., for 15 days a week), Group V - trandolapril + isoproterenol (3 mg/kg, p.o. + 5 mg/kg/day, s.c., for 15 days), Group VI - Tinospora cordifolia + trandolapril + isoproterenol (200 mg/kg, p.o. + 3 mg/kg, p.o. + 5 mg/kg/day, s.c., for 15 days respectively), Group VII - Captopril + isoproterenol (50 mg/kg, p.o. + 5 mg/kg/day, s.c., for 15 days respectively). The treatment of drug was given daily for two weeks after the induction of cardiac fibrosis.

Methods:

All of the therapies began two weeks after cardiac fibrosis was induced. All of the animals' body weights were measured before and after the weekly medication treatments. Every week, the ECG and heart rate of each animal were recorded before and after therapy. Animals were killed by cervical dislocation at the end of the treatment programme, and hearts were removed by dissection. The excised heart is cleaned in ice-cold saline and blotted on filter paper individually to calculate the left ventricular weight index (LVWI) and right ventricular weight index (RVWI). SOD, CAT, GSH, soluble collagen estimate, and malondialdehyde (MDA) levels were determined by homogenising a tiny portion of the heart. Histopathological examinations were conducted on the leftover cardiac tissue from each group.

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Measurement of ECG and heart rate:

During the experimental period, rats were anesthetized with ketamine at 75 mg/kg, i.p., and xylazine at 15 mg/kg, i.p. to measure heart rate and ECG. The leads of the instrument were attached to the dermal layer of both front paws and hind legs and connected to the PowerLab data acquisition system (AD Instrument, Australia) for the measurement of ECG and heart rate (HR).[12]

Left Ventricular (LVWI) and Right Ventricular Weight Indices (RVWI):

The rats were killed at the end of the treatment period. The heart was removed quickly and cleaned in ice-cold normal saline. The right and left ventricles were separated and weighed separately. The weight indices for the left and right ventricles were computed by dividing the left and right ventricular free wall mass (mg) by the body mass (g).[13]

Biochemical estimation:

Dissection and homogenization:

For further research, animals were euthanized through cervical dislocation. The heart was removed and weighed after being cleaned in isotonic saline. In a saline solution, a 10% (w/v) tissue homogenate was created. The supernatant was produced by centrifuging the tissue homogenate (Remi–C-30, Remi Industries Ltd., Mumbai, India). The following assay procedure was carried out using a microplate reader. Other enzyme tests were centrifuged at 12000 g for 60 minutes at 4°C. An ElicoTM BL 200 bio spectrophotometer was used for further experiments.

Hydroxyproline-Sirius red assay:

Collagen levels were determined using hydroxyproline and the Sirius red assay (0.1 percent each of fast green FCF and direct red 80 in picric acid, sigma). The cardiac tissue supernatant (100 µl) from each sample was mixed with 900 µl of Sirius red dye on a rotator for 30 minutes at room temperature. The mixture was then centrifuged for 10 minutes at 14,000 rpm. The resulting supernatant was then gently drained off without disturbing the particle. The particle was gently shaken for 10 minutes after being resuspended in 500 µl of 0.5 N NaOH. A microplate reader was used to measure the absorbance of a sample (100 µl) at 550 nm (Nano Spectra Max 250, BMG Lab Tech, Germany). In each set of studies, the collagen content was calculated using a hydroxyproline standard curve.[14,15]

Catalase activity (CAT):

For the measurement of H_2O_2 breakdown, the Luck method was used to determine catalase activity at 240 nm. A test mixture of 3 ml H_2O_2 in phosphate buffer (0.0125 M H_2O_2) and 0.05 ml supernatant of cardiac tissue homogenate was used for this procedure. The millimolar extension coefficient of H_2O_2 was used to calculate catalase activity (0.07). It was measured in micromoles of H_2O_2 decomposed per milligramme of protein per minute.[16]

Estimation of reduced glutathione (GSH):

The amount of reduced glutathione (GSH) in cardiac tissue homogenate was determined using Ellman's (1959) technique. A 0.75 ml sample of homogenate was allowed to precipitate with 0.75 ml of 4 % sulphosalicylic acid and centrifuged at 1,200 xg for 15 minutes at 4°C. In a 0.1 M phosphate buffer with a pH of 8.0, the assay mixture contains 0.5 ml supernatant and 4.5 ml of 0.01 M 5,5'- dithiobis (2-nitrobenzoic acid) (DTNB). The mixture's absorbance was measured at 412 nm, and the amount of GSH detected was calculated in micromoles of GSH per milligramme of proteins.[17]

Superoxide dismutase activity (SOD):

The superoxide dismutase activity was measured using the Kono (1978) method. Superoxide dismutase prevented the reduction of nitroblue tetrazolium chloride (NBT). A spectrophotometer was used to measure the absorbance at 516 nm. NBT and a portion of homogenate make up the reaction mixture. By adding hydroxylamine hydrochloride to the reaction mixture, the reaction was started. The findings were expressed as a percentage inhibition of NBT reduction.[18]

Lipid peroxidation assay (LPO):

The quantitative analysis of malondialdehyde in heart tissue homogenate was done using Wills' (1966) lipid peroxidation method. 0.2 ml of 8% SLS, 1.5 ml of 20% acetic acid, 1.5 ml of 0.8 percent aqueous solution of thiobarbituric acid (TBA), and 0.1 ml of tissue homogenate make up the reaction mixture. With distilled water, the volume was increased to 4 ml, after which the combination was heated in a water bath at 95°C for 60 minutes and cooled under tap water. Then, with vigorous shaking, 5 mL of n-butanol:pyridine (15:1 by volume) was added to the reaction mixture. Using the chromophore's molar extension coefficient (1.56 105 M-1 cm-1)[19], the absorbance was measured at 532 nm and the results were represented as nanomoles of MDA per milligramme of protein.

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Statistical analysis:

The data was presented as a mean standard error of the mean (SEM). The study's data was subjected to a one-way ANOVA, followed by a Dunnett's test, with a significance threshold of $p<0.05$.

III. RESULTS AND DISCUSSION

Tinospora cordifolia ethanolic extract contains flavonoids, saponin, and tannins. The preliminary phytochemical analysis confirms this. The acute toxicity research of Tinospora cordifolia ethanolic extract found that a dose of 2000 mg/kg, p.o. did not cause any mortality in rats within 24 hours. The animals were then examined for another 14 days to see if any major behavioural changes had occurred. The LD50 of the Tinospora cordifolia extract was determined to be larger than 2000 mg/kg based on the findings. The rats weighed an average of 202.23g before being infected with cardiac fibrosis. A drop in body weight (169.46 g) was found after induction of cardiac fibrosis. Tinospora Cordifolia, trandolapril, and their combination treatment significantly increased body weight ($p<0.001$) compared to the ISO group. Captopril, when administered as a normal medicine, raised body weight considerably. When compared to the control group, the ISO-treated group had a higher heart rate. In comparison to the ISO group, Tinospora Cordifolia, trandolapril, and their combination dramatically improved heart rate. The standard, captopril, also helped to restore heart rate (Table 1). When compared to the control group, the ISO-treated group has considerably higher LVWI and RVWI. When compared to the ISO group, the treatments dramatically reduced LVWI and RVWI. Captopril also lowered the indices of the left and right ventricles (Table 1).

Table 1: Effect of Tinospora cordifolia and trandolapril on heart rate, left and right ventricular weight indices

Groups	Heart Rate (beats/min)	LVWI	RVWI
Group I (Control)	350.8±1.815	0.0023±0.00011	0.0020±0.00009
Group II (Isoproterenol (5 mg/kg/day, s.c.), for 15 days)	427.5±2.24$^{###}$	0.0052±0.00039$^{###}$	0.0026±0.00010$^{###}$
Group III (Tinospora cordifolia (100 mg/kg p.o.) + Isoproterenol)	390.7±2.15***	0.0028±0.00057***	0.0023±0.00007***
Group IV (Tinospora cordifolia (200 mg/kg, p.o.) + Isoproterenol)	385±1.93***	0.0029±0.00012***	0.0023±0.00013***
Group V (Trandolapril (3 mg/kg p.o.) + Isoproterenol)	375±2.73***	0.0027±0.00016***	0.0024±0.00018***
Group VI (Tinospora cordifolia (200 mg/kg, p.o.) + Trandolapril (3 mg/kg, p.o.) + Isoproterenol)	360.2±0.94***	0.0026±0.00014***	0.0022±0.00022***
Group VII (Captopril (50 mg/kg p.o.) + Isoproterenol)	354±1.06***	0.0026±0.00008***	0.0020±0.00010***

Note: Each value represents mean ± S.E.M. (n=6). Group II compared with Group I. Group III, IV, V, VI, VII compared with group II. $^{\#,\ *}p < 0.05$, $^{\#\#,\ **}p < 0.01$, $^{\#\#\#,\ ***}p < 0.001$, ^{ns} non-significant (One way ANOVA followed by Dunnett's test).

Captopril lowered the ST segment (Table 2). The ISO-treated group had a shorter PR, QRS, and RR section and a longer QT segment than the control group.

Table 2: Effect of Tinospora cordifolia and trandolapril on electrocardiographic measurements

Groups	ST-segment elevation (mv)	PR segment (ms)	QRS complex (ms)	QT interval (ms)	RR interval (ms)
Group I (Control)	0.180±0.00026	32±03651	432±0.3651	68±0.3416	164±0.4472

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Group II (Isoproterenol (5 mg/kg/day, s.c.), for 15 days)	0.196±0.00037***	31±0.3651ns	431±0.5774***	67±0.4216*	163±0.2454ns
Group III (Tinospora cordifolia (100 mg/kg p.o.) + Isoproterenol)	0.195±0.00037***	31±0.3651ns	431±0.2582***	67±0.3651*	163±0.3651ns
Group IV (Tinospora cordifolia (200 mg/kg, p.o.) + Isoproterenol)	0.197±0.00037***	31±0.3651ns	431±0.5164***	67±0.2582*	163±0.5774ns
Group V (Trandolapril (3 mg/kg p.o.) + Isoproterenol)	0.198±0.00037***	31±0.3651ns	431±0.5164***	67±0.3651*	163±0.4472ns
Group VI (Tinospora cordifolia (200 mg/kg, p.o.) + Trandolapril (3 mg/kg, p.o.) + Isoproterenol)	0.193±0.00037***	31±0.3651ns	431±0.5774***	67±0.3651*	163±0.3651ns
Group VII (Captopril (50 mg/kg p.o.) + Isoproterenol)	0.192±0.00035***	31±0.3641ns	431±0.5174***	67±0.3651*	163±0.3641ns

Note: Each value represents mean ± S.E.M. (n=6). Group II compared with Group I. Group III, IV, V, VI, VII compared with group II. #, * $p < 0.05$, ##, ** $p < 0.01$, ###, *** $p < 0.001$, ns non-significant (One way ANOVA followed by Dunnett's test).

Tinospora Cordifolia with trandolapril therapy restored the PR, QRS, QT, and RR segments when compared to the ISO group (Fig. 1).

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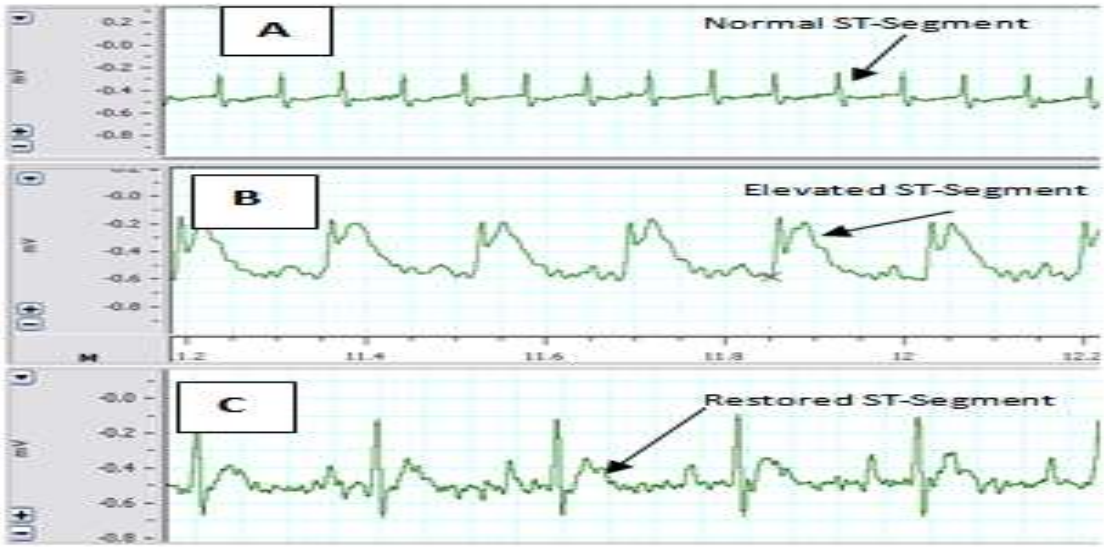

Fig 1: Effect of Tinospora cordifolia and Trandolapril combination on Electrocardiograph recording

(A) Vehicle control-treated; (B) Isoproterenol (5 mg/kg) and (C) Tinospora cordifolia and Trandolapril combination on post-treated isoproterenol (5 mg/kg).

Changes in ECG segments were restored as a result of the treatment, indicating cardioprotection. The ECG pattern was normalised after using captopril (Table 2). The standard curve of absorbance vs. concentration of the hydroxyproline standard is shown in Table 3.

Table 3: Effect of Tinospora cordifolia and trandolapril on soluble collagen level

Groups	Concentration (µg)
Group I (Control)	5.5±0.011
Group II (Isoproterenol (5 mg/kg/day, s.c.), for 15 days)	8.3±0.015$^{###}$
Group III (Tinospora cordifolia (100 mg/kg p.o.) + Isoproterenol)	6.6±0.042***
Group IV (Tinospora cordifolia (200 mg/kg, p.o.) + Isoproterenol)	5.9±0.013***
Group V (Trandolapril (3 mg/kg p.o.) + Isoproterenol)	5.8±0.007***
Group VI (Tinospora cordifolia (200 mg/kg, p.o.) + Trandolapril (3 mg/kg, p.o.) + Isoproterenol)	5.7±0.008***
Group VII (Captopril (50 mg/kg p.o.) + Isoproterenol)	5.6±0.008***

Note: Each value represents mean ± S.E.M. (n=6). Group II compared with Group I. Group III, IV, V, VI, VII compared with group II. $^{\#,\ *}p < 0.05$, $^{\#\#,\ **}p < 0.01$, $^{\#\#\#,\ ***}p < 0.001$, ^{ns}non-significant (One way ANOVA followed by Dunnett's test).

On the data points, the line equation was derived using least-squares linear regression analysis. The absorption for the collagen sample in (Table 3) is 0.03429 [hydroxyproline] + 0.6828, and the line equation is A = 0.03429c + 0.6828. The ISO-treated group of rats had a considerable increase in heart tissue soluble collagen compared to the control group due to increased collagen deposition. Tinospora Cordifolia and trandolapril therapies for two weeks, as well as their combination treatments, resulted in a considerable reduction in soluble collagen levels. Furthermore, the amounts of tissue soluble collagen increased late in the experiment, indicating an increase in collagen synthesis. Both Tinospora cordifolia and trandolapril inhibit the formation of soluble collagen.[20] The amount of soluble collagen in the body was reduced by captopril (Table 3).

A group of rats given ISO showed cardiotoxicity (owing to the production of oxidative stress) as compared to the control group, implying a significant decrease in SOD, CAT, and GSH enzyme levels and an increase in MDA levels. TGF- β expression has been proven in the literature to protect against cardiac damage, as its

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cytoprotective effect has been defined via an interaction with the redox-balancing proteins SOD and CAT, preventing post-ischemic damage in the heart and lowering inflammation and apoptosis.[21] Treated animals with Tinospora Cordifolia, trandolapril, or their combination showed a significant rise in SOD, CAT, and GSH enzymes, as well as a reduction in MDA, when compared to-treated animals (Table 4).

Table 4: Effect of Tinospora cordifolia and trandolapril on oxidative stress indices

Groups	**SOD (% inhibition of reduction of NBT)**	**CAT (µmole of H_2O_2 decomposed/ mg protein/min**	**GSH (µmole of GSH/mg protein)**	**LPO (nmole of MDA/mg protein)**
Group I (Control)	83.52±0.60	10.26±0.044	9.13±0.015	19.17±0.47
Group II (Isoproterenol (5 mg/kg/day, s.c.), for 15 days)	59.63±0.77$^{###}$	7.11±0.037$^{###}$	8.06±0.011$^{###}$	25.5±0.22$^{###}$
Group III (Tinospora cordifolia (100 mg/kg p.o.) + Isoproterenol)	63.04±0.53***	7.20±0.025^{ns}	8.22±0.0073***	23.17±0.30**
Group IV (Tinospora cordifolia (200 mg/kg, p.o.) + Isoproterenol)	70.2±0.54***	7.65±0.062***	8.42±0.0076***	20.83±0.30***
Group V (Trandolapril (3 mg/kg p.o.) + Isoproterenol)	73.77±0.44***	7.90±0.041***	8.39±0.0049***	22.17±0.60***
Group VI (Tinospora cordifolia (200 mg/kg, p.o.) + Trandolapril (3 mg/kg, p.o.) + Isoproterenol)	75.3±0.41***	8.08±0.052***	8.68±0.0049***	22.17±0.60***
Group VII (Captopril (50 mg/kg p.o.) + Isoproterenol)	77.74±0.40***	8.34±0.032***	8.70±0.0049***	19.33±0.66***

Note: Each value represents mean ± S.E.M. (n=6). Group II compared with Group I. Group III, IV, V, VI, VII compared with group II. $^{\#, *}p < 0.05$, $^{\#\#, **}p < 0.01$, $^{\#\#\#, ***}p < 0.001$, ^{ns}non-significant (One way ANOVA followed by Dunnett's test).

A histology slice of the heart of the control-treated rat revealed normal tissue architecture. Isoproterenol (5 mg/kg)-induced cardiac fibrosis had aberrant tissue architecture with hard and scar tissues, indicating myocardial fibrosis. A post-isoproterenol (5 mg/kg) produced cardiac fibrosis histological section of Tinospora cordifolia and trandolapril demonstrated minor hard and scar tissue, indicating that myocardial tissue architecture had been repaired. Captopril also improved heart tissue shape (Fig. 2).

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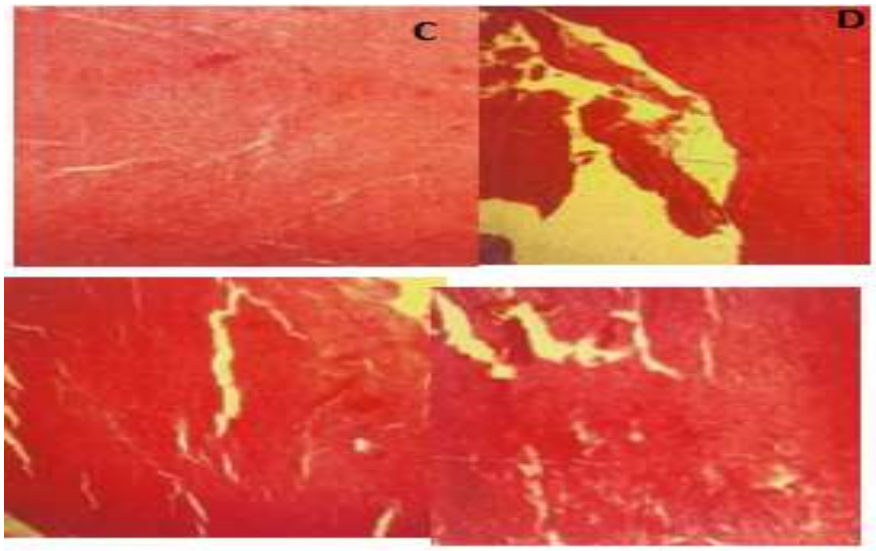

Fig 2: Effect of Tinospora cordifolia and Trandolapril combination on cardiomyocyte cross-sectional area (A) Vehicle control-treated; (B) Isoproterenol (5 mg/kg); (C) Tinospora cordifolia and Trandolapril combination on post-treated isoproterenol (5 mg/kg) and (D) Captopril on post-treated isoproterenol (5 mg/kg).

In humans, cardiac fibrosis and heart failure is characterized by activation of the sympathetic nervous system leadingtohighcirculatingnoradrenalineconcentrations. Thusnoradrenaline or a catecholamine mimicking its action like isoproterenol would only be relevantto study cardiac fibrosis in experimental animals. Cardiac remodeling is seen in isoproterenol(ISO) infused rats with severe myocardial fibrosis accompanied by myocardial injury and β-adrenoceptormediatedapoptosishasalsobeendemonstratedincardiomyocytes.Increasedoxidative stress resulting from an increased cardiac generation of reactive oxygen species (ROS)is implicated in the progression of cardiac fibrosis and heart failure in ISO mediated hypertensive model.Antihypertensive, hypolipidemic, anti-inflammatory, antioxidant, antibacterial, antidiabetic, hepatoprotective, anticancer, and anti-HIV effects have been discovered in Tinospora cordifolia.[22,23]The fundamental motivation for focusing on herbal medications is that developments in modern drug discovery methodologies and the search for new chemical diversity have piqued interest in discovering new leads from natural sources. Because of their low toxicity and high efficacy, plant-based medications are frequently used. Herbs and medications together have the potential to replicate or increase the effects of either component. Trandolapril is an ACE inhibitor that is used to treat hypertension, heart failure, and improve heart attack survival. It's converted to trandolaprilat, a physiologically active diacid form, by the liver.

Trandolaprilat inhibits the enzyme ACE, which transforms angiotensin I to angiotensin II.[24] Through interactions with Ang II receptor type 1 (AT1) receptors, Ang II induces hypertrophy, fibroblast proliferation, and collagen formation. Myocardial injury, reactive oxygen species, Ang II, excessive hyperglycemia, pH changes, and certain proteases stimulate TGF- β, a cardiac cytokine. TGF- β promotes ECM production while inhibiting ECM degradation.

The goal of this study is to see how well Tinospora cordifolia and trandolapril work together to cure cardiac fibrosis. For some conditions, a combination of medications rather than monotherapy would be more effective. As a result, employing isoproterenol-induced cardiac fibrosis, the cardioprotective effects of both medicines were studied in this investigation. This study emphasises the effect of Tinospora cordifolia and trandolapril in preventing cardiac fibrosis in treated animals.

ER stress is connected to cardiotoxicity, which causes cell damage, according to various studies. ER stress is generated in the hearts of transgenic mice during a chronic inflammatory insult, resulting in an increase in MCP-1 protein levels and heart failure.[25] ER stress, on the other hand, has been found to protect the heart during myocardial injury and even promote myocardial hypertrophy in other studies.[26,27] Our findings, on

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the other hand, confirm the first hypothesis, implying that using ISO as an MF inducer produces myocardial damage and that the development of cardiac fibrosis is closely linked to ER stress. Tinospora cordifolia extract at 100 and 200 mg/kg/day were utilised in the trial, as well as trandolapril at 3 mg/kg/day, which is within the standard range of this drug's use. Finally, by reducing oxidative stress and lowering collagen deposition levels, Tinospora cordifolia, trandolapril, and their combination may be a preventive factor against cell death caused by necrosis/apoptosis, which is caused by ISO treatment. Overall, the data demonstrated that ER stress activation is involved in ISO-induced cardiac fibrosis.

Tinospora cordifolia,trandolapril, and their combination inhibit collagen release and cardiomyocyte loss. Tinospora cordifolia, trandolapril, and their combination have been demonstrated to protect against myocardial fibrosis and have anti-fibrotic characteristics. This protection is due to the regulation of oxidative stress and the level of soluble collagen. Suppressing oxidative stress, Tinospora cordifolia, trandolapril, and their combination may lower endoplasmic reticulum stress, preventing cardiac fibrosis. Tinospora cordifolia and trandolapril's anti-fibrotic properties could be attributed to antioxidant defence enzyme regulation.

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