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Cichorium intybus Inhibits Doxorubicin- Induced Myocardial fibrosis in Wistar Rats Via Inflammatory Markers and Collagen Repression

Vishwadeep Shelke[1*], Ghanshyam Jadhav[1], Amol Mhaske[2], Vidya Shirsath[1], Sagar Kamble[1] and Awais Sayyad[1]

[1]Department of Pharmacology, Nashik District Maratha Vidya Prasarak Samaj's College of Pharmacy, Nashik- 422002, India.
[2]Department of Pharmaceutical Analysis, Nashik District Maratha Vidya Prasarak Samaj's College of Pharmacy, Nashik- 422002, India.

Authors' contributions

This work was carried out in collaboration with all authors. Author V Shelke designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript, and managed the analyses of the study. Authors V Shelke, V Shirsath, AM, SK, AS and GJ managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Many allopathic medicines demand to remedy for myocardial fibrosis but fail to fulfill the purpose, because of side effects and high cost. Herbal medicine has fewer side effects and natural herbs are considered safe when compared with synthetic medicines. The present study aimed to study the effect of extract of *Cichorium intybus* on experimentally induced myocardial fibrosis in Wistar rats. The objective of the present study was to find out the possibilities of the use of *Cichorium intybus* as a supportive/ protective medicine and to explore the possible toxicities of *Cichorium intybus* in these models. Myocardial fibrosis was induced by Doxorubicin (2.5 mg/kg, i.p. Thrice a week) rats. Blood pressure, Collagen levels, Left ventricle weight index were effectively reduced in animal groups treated with the extract. The effect of extracts was studied on various oxidative stress markers like SOD, CAT, LOP, and NO. Extract of *Cichorium intybus* was shown significantly

**Corresponding author: E-mail: vishwadeepshelke@gmail.com;*

decreased in blood pressure and significantly shows the antioxidant effect when compared to the hypertensive control group.

Keywords: Cardiac fibrosis; Cichorium intybus; antioxidant; blood pressure; collagen; doxorubicin; heart rate.

1. INTRODUCTION

Fibrosis describes the development of fibrous connective tissue as a mending response to injury or damage. Areas of the heart that have become defaced due to myocardial infarction may go through fibrosis. This can increase the risk of heart failure. Fibrosis can involve several myopathic diseases involving hypertrophy, ischemic, hypertensive, restrictive cardiomyopathy & also radiation-induced cardiac myopathy. Cardiovascular disease responsible for 31% of all deaths and the main reason for deaths worldwide. Ischemia & IHD (Ischemic Heart Disease), end myocardial fibrosis are a primary cause of heart disease. Each year CVD (Cardiovascular Disease) causes 3.9 million deaths in European Union. Cardiac fibrosis is the major reason for the progression of heart failure, so its prevention and treatment are the main aim for curing heart failure [1]. According to the American heart association 7.0 million Americans >20 years of age self-report having a stroke, where the risk of atrial fibrillation recently has been estimated to be 1 in 3 whites & 1 in 5 blacks in the United States [2].

Recently some experimental studies explored the role of natural extracts over myocardial infarction and cardiac fibrosis. At normal conditions, collagen synthesis by fibroblasts and degradation by MMPs (Matrix metalloproteases) occur in symmetry. In cardiac fibrosis, this balance gets disturbed which leads to ECM (Extracellular matrix) and collagen accumulation. Interestingly there is more than one complex pathway involved in this kind of deposition and degradation which promotes challenges to find out proper treatment for cardiac fibrosis.

From the beginning, the natural compounds have shown the ability to rehabilitate various CVS (Cardiovascular System) and CNS (Central nervous system) diseases. *Cichorium intybus* possess tremendous pharmacological effects such as improvement in metabolism, antioxidant effect, and radioactive effect [3]. Several studies show that it has potent effects in the treatment of Hypertension, Liver cirrhosis, Malaria, obesity, diabetes, cardiovascular, renal failure, cancer, and neurological disorders [4-6]. It contains Esculetin (6, 7-dihydroxy derivative of coumarin), Cichorygenin which could be effective against cardiac fibrosis [7]. Esculetin can be used as a precursor to making synthetic Quercetin. This bioflavonoid is safely and effectively used in Asian countries for treating Hypertension, heart inflammatory diseases and for promoting a healthy immune system. According to Kadakol et al. [7], Esculetin attenuates alteration in the renin-angiotensin system, oxidative stress (Keap1) and cell proliferation (Ki67), Esculetin treatment restored the normal level of permissive PTHMs and H2A/H2B ubiquitination in IR (Ischemic Reperfusion) and diabetic heart which might be the basic mechanism behind its cardioprotective role [7]. Similarly, Palanivel Karthika et al. show that Esculetin prevents lipid peroxidation and antioxidants in myocardial infarction conditions [8]. The above potential results concerned to *Cichorium intybus* generates eagerness about this super plant to explore in fibrosis diseases. These findings collectively support the interpretation that *Cichorium –intybus* extract may be beneficial in protecting the myocardial structure against excessive ECM and collagen production in cardiac fibrosis, as there is no one has been working on the effect of *Cichorium intybus* in the treatment of cardiac fibrosis.

In the present study, we investigated the potential effect of *Cichorium intybus* extract to attenuate experimentally induced cardiac fibrosis in rats. Here we used Doxorubicin as a chemical inducer for myocardial fibrosis [9,10]. Doxorubicin (Adriamycin) is used mainly for chemotherapy and acts as a topoisomerase II blocker. This Anthracycline representative shows side effects on the heart by generating free radicals through mitochondrial redox cycling in cardiomyocytes which leads to left ventricular dysfunction. The effects of the extract on the collagen level were also investigated to gain mechanistic insight into its cardioprotective effects.

2. MATERIALS AND METHODS

2.1 Chemicals, Reagents, and Drugs

5,5'-Dithiobis (2- nitro benzoic acid) (DTNB), Nitrobluetetrazolium Chloride (NBT) were

procured from Alfa Aesar, A Johnson Mathey Company, Chennai, India. Ethylenediamine tetra-acetic acid and 2-Thiobarbituric acid procured from Research Lab, Fine Chem., Mumbai, India. Potassium- sodium tartrate, Potassium chloride, Calcium Chloride, and Sodium chloride were procured from the Fine chem industry, Mumbai, India. Green fast FCF and Direct red 80 were procured from Sigma-Aldrich Co. USA. Potassium- sodium tartrate, Copper sulfate, and Folin-ciocalteu reagent were procured from the Research lab, Mumbai, India. Doxorubicin was procured from Neon Labs, Mumbai, India. Captopril was procured from Torrent Pharmaceuticals, India.

2.2 Plant Materials

Cichorium intybus plant is mostly found in Western ghats of India. The plant was collected from the local area in Nashik and collected and authenticated from the Pharmacognosy department at Nashik District Maratha Vidya Prasarak Samaj's College of Pharmacy, Nashik, India. The plant was shade dried and powdered for further extraction. Dried plant powder of *Cichorium intybus* (500 gms) was extracted using Ethanol (1500) for 8 hours using the Soxhlet apparatus. The total extract was collected and dried using an evaporator and stored in an airtight container at 4ºC and the weight of ethanolic extract obtained was 35 gms and used for studies [11].

2.3 Estimation of Total Flavonoid Content

The Flavonoids content was determined by using colorimetric determination. The standard calibration curve was made by using rutin. 10 milligrams of rutin were dissolved in 80% ethanol and then diluted to 10, 20, and 50µg/ml. The diluted standard solutions (0.5 ml) were separately mixed with 1.5 ml of 95% ethanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate, and 2.8 ml of distilled water. The same concentrations of *Cichorium intybus* extract were also prepared. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a bio spectrophotometer. The total flavonoid content of UPE was calculated from the standard calibration curve equation [12].

2.4 Estimation of Total Phenolic Content

Total soluble phenolic compounds present in the extract were determined with the Folin-Ciocalteu reagent. The calibration curve was prepared by preparing gallic acid (GA) solutions at concentrations 0, 50, 100, 150, 250, 500 µg/ml in ethanol. To 0.1 mL of extract (1mg/ml in distilled water) 1 ml of Folin-Ciocalteu reagent was added. After 3 minutes, 3 ml 2 % Na2CO3 was added. Subsequently, the mixture was shaken for 2 h at room temperature and the absorbance was measured using a bio spectrophotometer at 760 nm. All tests were performed in triplicate. Total phenol values were expressed in terms of gallic acid equivalent (mg/g of dry mass) [13].

2.5 Experimental Animals

All experiments were performed according to the international ethical standards and as per the guidelines of the Committee for the Purpose of Control and Supervision of Animals (CPCSEA) New Delhi, India, and the Institutional Animal Ethical Committee (IAEC) approved protocol of this study (IAEC/2019/04). Male Wistar rats weighing 180-220g were acquired from Wockhardt limited, Wockhardt Research Centre D-4 MIDC Area, Chikhalthana, Aurangabad/ India for the experiment. They were kept in polypropylene cages with husk bedding, which was renewed every week. Animals were housed in 12-hour light and 12-hour dark cycle conditions. The temperature was maintained at about 25±5℃. Rats were fed with commercial pellet rat chow and given water *ad libitum.* After the quarantine period animals were divided into six groups for further experiment.

2.6 Acute Toxicity

Acute toxicity test (LD_{50}) of *Cichorium intybus* was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines [14]. The extract was administered with the minimum dose to individual animals and observed for the next 24 h. If no mortality was seen then, the dose was increased by 50, 100, 200, 500, 800, 1000, 1500, and 2000 mg/kg, p.o.

2.7 Doxorubicin Induced Myocardial Fibrosis

Wistar strain rats were treated with Anthracycline derivative DOX (Doxorubicin), DOX (2.5 mg/kg, i.p. thrice a week) to achieve cumulative dose i.e. 15 mg/kg in 2 weeks. The animals were divided into 6 groups (Group I- Vehicle control, Group II- Disease control, Group III- *CI* extract 100 mg/kg, Group IV- *CI* extract 200mg/kg, Group V- *CI*

extract 300mg/kg, and Group VI- Captopril) and 7 animals in each group. To compare with the treatment group Captopril was taken (50 mg/kg by oral gavage) (Attia et al. 2018). (Table 1). Progression of myocardial fibrosis was confirmed by ECG recording (Elevation of ST-segment). The rats in the treatment groups were orally administered with Extract of *Cichorium intybus* 100 mg/kg/d, 200 mg/kg/d, and 300mg/kg/d respectively for 3 weeks till good results come out.

2.8 Recording Electrocardiogram and Heart Rate in Experimental Animals

On the 14th day, the animals were anesthetized with sodium pentobarbital (30 mg/kg. i.p.). The leads were attached to the dermal layer of both front paws and hind legs for the measurement of ECG and heart rate (HR) on Power lab data acquisition system (AD Instrument, Australia) [15,16]. The procedure followed accordingly is mentioned in the brochure of the Power lab data acquisition system (AD Instrument, Australia).

2.9 Left Ventricular Weight Index (LVWI) and Right Ventricular Weight Index (RVWI)

The left ventricle and right ventricle were separated quickly after the heart weight measurement.

The index calculated as the left and right ventricular free wall mass (mg) divided by body mass (g), respectively [17].

2.10 Dissection and Homogenization

Right after two weeks of experimental animals were euthanized by using the decapitation method according to CPCSEA guidelines. The hearts were removed and weighed rinsed with isotonic saline. A 10% (w/v) tissue homogenate was prepared in saline solution. The supernatant was obtained by centrifugation (Remi – C - 30, Remi Industries Ltd. Mumbai, India) of the homogenate. A microplate reader was used for the subsequent assay method.

2.11 Hydroxyproline Assay

Collagen in heart tissue was measured with a Hydroxyproline and Sirius Red assay (reagents from Sigma; 0.1% each of Fast Green FCF and Direct Red 80 in Picric Acid). Tissue supernatant liquid (100 µl) from each sample was combined with 900 µl of Sirius red dye on a rotator at room temperature for 30 minutes. The blend was then centrifuged at 14,000 rpm for 10 minutes, after which the supernatant was poured out carefully without disturbing the pellet. The pellet was resuspended in 500 µl of 0.5 N NaOH, and shaken gently for 10 minutes. The sample (100 µl) absorbance was read at 550 nm on a microplate reader (BMG Lab Tech, Germany) [18,19].

2.12 Catalase Activity CAT

Method of Luck was used for assay of catalase activity. The H_2O_2 breakdown is measured at 240nm. 3 ml of 0.01M H_2O_2 phosphate buffer pH 7 was added with 0.05 ml of tissue homogenate supernatant (10%), the change in absorbance was measured after 1 minute at 240 nanometers. The millimolar extinction coefficient of H_2O_2 (0.071) was used to calculate the enzyme activity. The results were expressed as the micromoles of hydrogen peroxide decomposed /minute/mg of protein [20,21].

2.13 Estimation of Reduced Glutathione GSH

Reduced glutathione (GSH) in the heart is estimated according to the method of Elman. A 0.75 ml sample of the homogenate is precipitated with 0.75 ml of 4 % sulphosalicylic acid. The samples are centrifuged at 1200 × g for 15 min at 4 ºC. The assay mixture contained 0.5 ml supernatant and 4.5 ml of 0.01 M DTNB [5,5-Dithiobis (2-nitrobenzoic acid)] in 0.1 M phosphate buffer (pH 8.0). The yellow color developed is read immediately at 412 nm. The results are expressed as µM of GSH per milligram of protein [22].

2.14 Estimation of Superoxide Dismutase SOD

Superoxide dismutase activity (SOD) was carried as per the procedure described by Kono. SOD inhibited the reduction of nitro blue tetrazolium chloride (NBT). The absorbance was measured at 560 nm. The reaction was started by addition of 0.1 ml of 1 mM hydroxylamine hydrochloride to the reaction mixture containing 0.1 ml of 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.1 ml of 24 µM NBT, 0.1 ml of 0.03% v/v Triton X100 reagent and homogenate to make 1 ml final volume. Incubation of reaction mixture at 37℃ for 20 minutes was carried out and absorbance was taken at 560 nm. Results expressed as the % inhibition of reduction of NBT [22].

2.15 Estimation of Malondialdehyde (Lipid Peroxidation Assay)

This determination was performed as per the procedure described by Wills.

The results were given as Nano moles of MDA per mg of protein using the molar extension coefficient of the chromophore ($1.56 \times 105\ M^{-1}cm^{-1}$). The constituents of the reaction of were tissue homogenate 0.1ml, 8% sodium lauryl sulphate (SLS) 0.2ml, 20% acetic acid 1.5ml, 0.8% thiobarbituric acid solution 1.5ml. The reaction mixture was heated on a water bath at 95℃ for 1 hour. After that n-butanol and pyridine were mixed in the ratio 15:1 and 5ml of this mixture was added to the reaction mixture and shaken vigorously. At 2200 rotation per minute reaction mixture was centrifuged for 5 minutes. The upper organic layer was taken for absorbance and it was measured at 532 nm [20].

2.16 Data Analysis

Statistical analysis was performed with Prism software version 8.4.3 (Graph Pad Software, San Diego, CA, USA). Results are expressed as mean ± SEM. Statistical significance was evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test. A value of $p < 0.05$ was considered to be statistically significant.

3. RESULTS

3.1 Total Flavonoid and Phenolic Content from the *Cichorium intybus* Extract

The total flavonoid content of CI extract in terms of rutin equivalent was found to be 14.21 mg/g of dry weight (r2=9877). The phenolic content of CI extract in terms of Gallic acid equivalent was found to be 37.1 mg/g of dry weight (r2=0.9966).

3.2 Effect on ECG Parameters (ST-segment)

DOX administered group of animals showed significant ($p < 0.01$) ST-segment elevation as compared to the control group. The ST-segment elevation represents conduction block and the consequent loss of myocardial cell membrane function due to alteration of autorhymicity. Treatment with E.E. *of C. I.* (100, 200, 300 mg/kg) showed a significant ($p < 0.01$) cardioprotective effect with more than 50% decline in ST-segment elevation of ECG as compared to the DOX group. Treatment with E.E. *of C.I.* showed a cardioprotective effect against DOX by inhibition of ST-segment alteration (Fig 1).

3.3 Effect on Heart Rate

DOX (2.5 mg/kg) treated rats showed significant ($p < 0.001$) increase in heart rate compared to control (water for injection) treated group during treatment schedule. *Cichorium intybus* extract (100, 200, 300 mg/kg/day) in DOX (2.5 mg/kg) induced animals showed significant ($p < 0.001$) decrease in heart rate compared to DOX (2.5 mg/kg) (Group II) treated rats during treatment schedule (Fig.2).

3.4 Effect on Right Ventricle (RVWI) and left ventricle weight indexes (LVWI)

DOX treated rats showed a significant ($p < 0.001$) increase in the LVWI compared to vehicle-treated group. *Cichorium intybus* extract (100, 200, 300 mg/kg) post-treated DOX (2.5 mg/kg) rats showed significant ($p < 0.001$) decrease in LVWI compared to DOX (2.5 mg/kg) treated rats. There is no significant difference in change in RVWI (Table 2).

3.5 Effect of *Cichorium intybus* Extract on Level of Soluble Collagen (Hydroxyproline Assay)

DOX administered group of animals showed an increase in myocardial tissue soluble collagen as compared to the control group due to an increase in collagen deposition. Treatment with *Cichorium intybus* extracts (100, 200, 300 mg/kg) showed a significant decrease in soluble collagen level (Fig. 4). The equation of the line was determined by performing a least-squares linear regression analysis on the data points. For the collagen estimation the equation of the line is $A = 0.03429c + 0.6828$(Fig. 4).

3.6 Effects on CAT and GSH levels

A marked decrease in levels of GSH and CAT enzymes were observed after DOX administration as compared to control group, indicating induction of cardiotoxicity in rats. Treatment with *Cichorium intybus* extracts (100, 200, 300 mg/kg) showed a significant ($p < 0.001$) rise in levels of GSH and CAT as compared to animals treated with DOX (Table 3).

3.7 Effects on SOD

Significant (p < 0.001) decrease in the level of SOD enzymes was observed after Doxorubicin administration as compared to the vehicle-treated group, indicating induction of cardiotoxicity in rats. Treatment with *Cichorium intybus* extract (100,200,300 mg/kg) showed a significant (p< 0.001) rise in the level of SOD as compared to rats treated with Doxorubicin.

3.8 Effects on LPO

Levels of MDA were significantly increased in Doxorubicin treated group, as compared to the vehicle treated group; while administration of *Cichorium intybus* extract (100,200,300 mg/kg) significantly (p< 0.001) lowered levels of LPO as compared to the Doxorubicin treated group. (Table 3).

4. DISCUSSION

The result of the present study distinctly indicated the cardioprotective role of *Cichorium intybus* in the treatment of myocardial fibrosis. In doxorubicin treated animals, body weight decreased over 2 weeks of a study indicating the change in body weight after the

Table 1. Experimental design of DOX-induced myocardial fibrosis (Each group containing seven animals)

Group (n=7)	Treatment Schedule
Group I	Control Group
Group II	Disease control (Doxorubicin (2.5mg/kg/day, i.p.) 3 times a week)
Group III	DOX + *CI* 100 mg/kg
Group IV	DOX + *CI* 200 mg/kg
Group V	DOX + *CI* 300 mg/kg
Group VI	DOX + Captopril

Where, DOX, Doxorubicin, C.I., Cichorium intybus, i.p., Intraperitoneal

Table 2. Effect of *Cichorium intybus* extract on left ventricle weight indexes (LVWI) and Right ventricle (RVWI)

Treatment groups	LVWI	RVWI
Control Group	0.00248±0.009	0.00197±0.0073
Disease control	0.00312±0.0089##	0.00210±0.0087##
DOX + *CI* 100 mg/kg	0.00291±0.0088**	0.00210±0.0088**
DOX + *CI* 200 mg/kg	0.00249±0.0089**	0.00198±0.0087**
DOX + *CI* 300 mg/kg	0.00249±0.0097**	0.00198±0.0088**
DOX + Captopril	0.00248±0.0085**	0.00198±0.0080**

Each column represents mean ± S.E.M. (n = 7).
Group II compared with Group I. Group III, IV, and V compared with group II.
$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p < 0.001$, ^{ns}non significant (One way ANOVA followed by Dunnett's test).

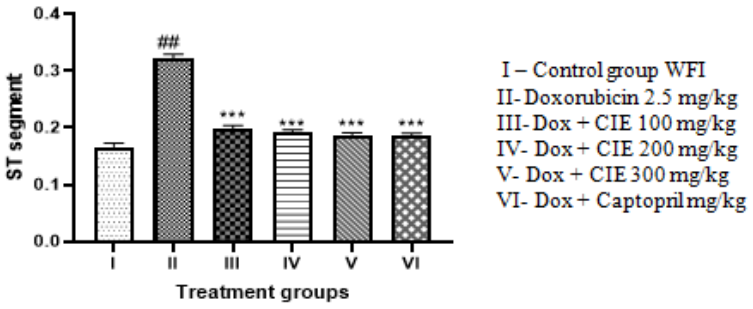

Fig. 1- Effect of *Cichorium intybus* extracts on ST-segment elevation in DOX (2.5 mg/kg) treated rats

Each column represents mean ± S.E.M. (n = 7).
Group II compared with Group I. Group III, IV, and V compared with group II.
$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p < 0.001$, ^{ns}non significant (One way ANOVA followed by Dunnett'stest)

Table 3. Effect of *Cichorium intybus* extract on biochemical parameters in rat heart

Treatment Groups	Catalase activity (µMole of H2O2 decomposed/ mg Protein/min)	SOD level (% inhibition of reduction of NBT)	GSH levels (µMole of GSH/mg protein)	Lipid Peroxidation (nmole of MDA/mg protein)
Control Group	10.88±0.421	82.58±1.02	9.13±1.02	19.4±0.630
Disease Control (Doxorubicin)	7.66±0.390##	60.362±0.932##	8.94±0.04##	25.2±0.583##
DOX + *CI* 100 mg/kg	6.578±0.29**	72.75±0.381**	9.066±0.13**	22±0.489**
DOX + *CI* 200 mg/kg	8.14±0.23**	71.116±0.318**	9.116±0.22**	21.4±0.328**
DOX + *CI* 300 mg/kg	9.46±0.41**	70.13±0.213**	9.118±0.31**	20±0.311**

Each column represents mean ± S.E.M. (n = 7)
Group II compared with Group I. Group III, IV and V compared with group II
**p<0.05, **p <0.01, ***p < 0.001, nsnon significant (One way ANOVA followed by Dunnett'stest)*

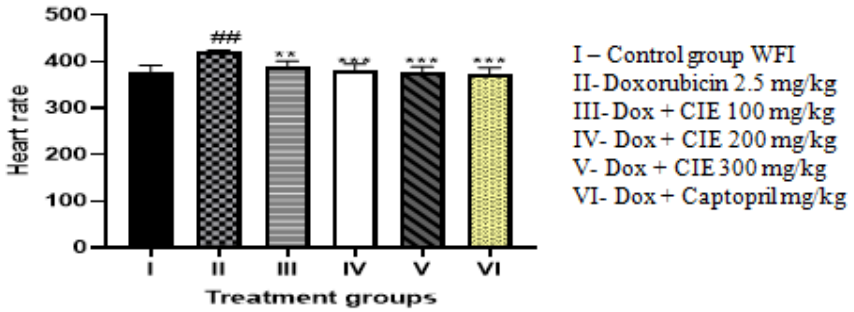

Fig. 2. Effect of *Cichorium intybus* extract on heart rate
Each column represents mean ± S.E.M. (n = 7)
Group II compared with Group I. Group III, IV, and V compared with group II
**p<0.05, **p <0.01, ***p < 0.001, nsnon significant (One way ANOVA followed by Dunnett'stest)*

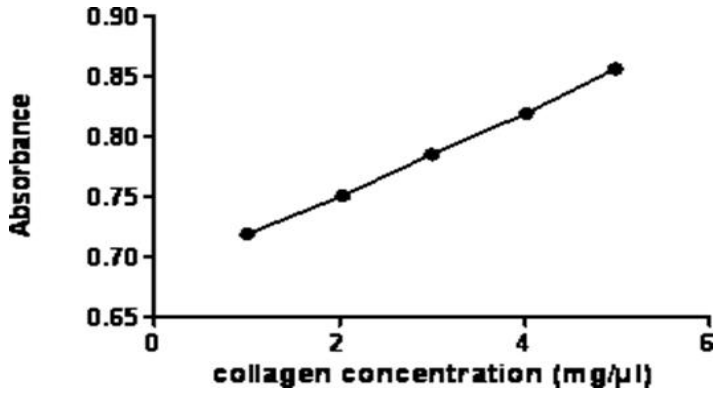

Fig. 3. The concentration dependence of the absorbance of collagen solution of the control group in ringer solution at 650 nm. (Hydroxyproline assay)

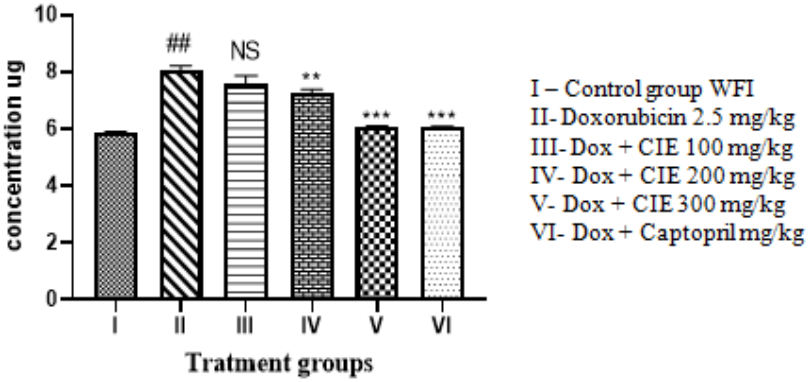

Fig. 4. Effect of *Cichorium intybus* extract on level of soluble Collagen in DOX (2.5 mg/kg) treated rats.

Each column represents mean ± S.E.M. (n = 7)

Group II compared with Group I. Group III, IV, and V compared with group II

$^{*}p<0.05$, $^{**}p<0.01$, $^{***}p<0.001$, ^{ns}non significant (One way ANOVA followed by Dunnett'stest)

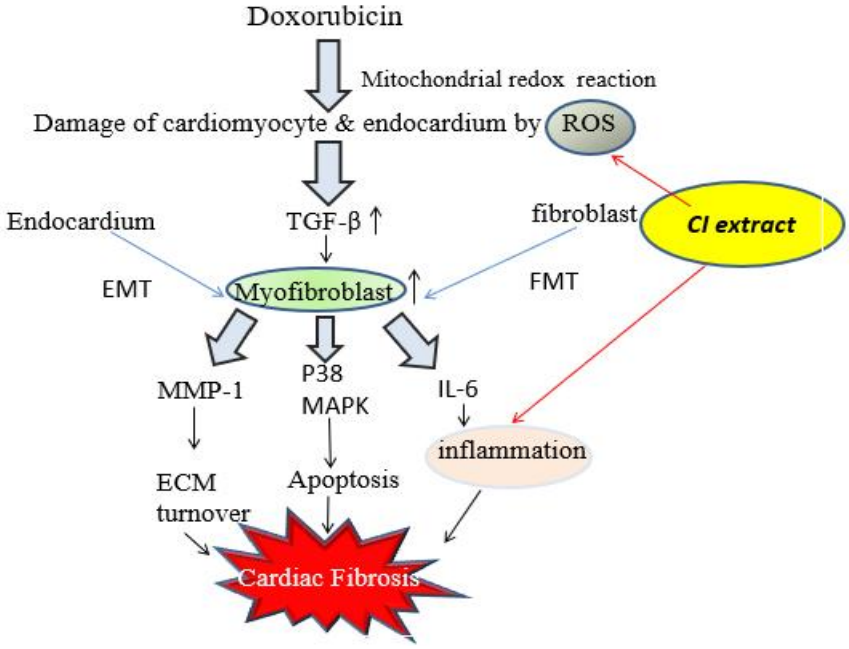

Fig. 5. The mechanism by which Doxorubicin induces Cardiac fibrosis and *Cichorium intybus* action in Doxorubicin-induced Cardiac fibrosis

administration of doxorubicin. Ethanolic extract of *Cichorium intybus* did not show any toxic effect up to 2000mg/kg oral dose. Rats were administered up to the maximum possible dose. The DOX-treated rats receiving *Cichorium intybus* and Captopril for 2 weeks did not show any decline in body weight throughout the study indicating a protective effect. The ECG is always considered the most important initial clinical test for diagnosing myocardial ischemia, infarction, and fibrosis. Its correct interpretation is usually the basis for the Study of *Cichorium intybus* in cardiac fibrosis. Electrocardiograph abnormalities such as ST-segment elevation could be due to the consecutive loss of cell membrane in the injured myocardium. In the present study, the ECG pattern of vehicle-treated rats in both animal models of cardiac fibrosis was normal indicating normal cardiac electrical activity. In Doxorubicin-treated groups, significant (p <

0.001) elevation in ST-segment was observed compared to normal rats indicating myocardial fibrosis. *Cichorium intybus* at the dose of 300 mg/kg successfully minimized the elevated ST-segment indicating its cardioprotective properties against myocardial fibrosis in DOX-induced myocardial fibrosis animals.

Non-significant increases in heart rate observed in DOX-induced animals indicate attenuation of R-R interval during ECG recording whereas no significant change in heart rate was observed after the treatment with *Cichorium intybus*.

Treatment with *Cichorium intybus* 100,200,300 mg/kg and Captopril in DOX treated rats decreased the index of heart weight to body weight ratio and prevented cardiac hypertrophy. The present study showed that doxorubicin-induced marked left ventricular structural remodeling as indicated by raised wall volume of left ventricle and septum under stereological investigation.

Treatment with *Cichorium intybus* 200,300 mg/kg in DOX treated rats showed that the left ventricle mitigated this consequent alteration of the architecture of the heart reducing the process of remodeling, where 100 mg/kg dose shows some amount of reduction in remodeling. The reactive expansion of the extracellular matrix components, especially through collagen formation and angiogenesis, represents important processes related to the cardiac remodeling leading to the increased mass of the wall of the left ventricle and septum.

A significant increase in soluble collagen level was reported in DOX- treated group of animals indicating a deposition of collagen which leads to the generation of fibrotic condition. After the entire study period, there was no significant increase in collagen level in *Cichorium intybus* and Captopril treated animal groups which indicate cardioprotective effect in myocardial fibrosis.

In rats treated with DOX, an antioxidant system in cardiac tissue was found to be compromised as seen by significant ($p < 0.001$) reduction in SOD, GSH, and CAT levels compared to normal rats. An extensive rise in the level of LPO was observed in DOX-treated rats indicating the presence of oxidative stress in the heart. Several previous studies clearly state that oxidative stress is associated with the exacerbation of the condition of cardiac hypertrophy and fibrosis in the subject of study. In the present study, DOX-treated rats along with *Cichorium intybus* treatment maintained the SOD, CAT, and GSH level in cardiac tissue, and LPO was found to be significantly lower than DOX-treated rats which are consistent with previous reports. (Fig. 5).

However, cardiac collagen deposition was reduced to different degrees by long-term treatment with *Cichorium intybus* and Captopril. Our data show that there is a positive correlation between the amount of type I collagen and oxidative parameters. We observed that morphological changes in the rat heart were correlated with biochemical parameters, which strengthen the relevance of *Cichorium intybus* treatment for attenuating the severity of change caused by this disease.

5. CONCLUSION

In the case of *Cichorium intybus*, it exhibited a remarkable effect on histone deacetylase enzyme during collagen synthesis. The action is credited to its ability to direct inhibition of histone deacetylase enzyme which controls the collagen synthesis and thus collagen deposition at the site of fibrosis. *Cichorium intybus* antihypertensive effect as well as it shows its antioxidant activity. It inhibits endoplasmic reticulum stress-induced free radical generation thus inhibit myocardial cell death. Hence *Cichorium intybus* is the drug of choice for the treatment of myocardial fibrosis by targeting different pathways.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All experiments were performed according to the international ethical standards and as per the guidelines of the Committee for the Purpose of Control and Supervision of Animals (CPCSEA)

New Delhi, India and the Institutional Animal Ethical Committee (IAEC) approved protocol of this study (IAEC/2019/04).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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