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ORIGINAL ARTICLE

Cichorium intybus attenuates streptozotocin induced diabetic cardiomyopathy via inhibition of oxidative stress and inflammatory response in rats

Manju SHARMA [1,4], Aiman AFAQUE [1], Shridhar DWIVEDI [2], Zeeba S. JAIRAJPURI [3], Yasmeen SHAMSI [5], Mohd Faiyaz KHAN [6], Mohd Ibrahim KHAN [7], Danish AHMED [7]

[1] *Department of Pharmacology, Faculty of Pharmacy, Hamdard University, New Delhi, India*
[2] *Department of Medicine and Preventive Cardiology, Hamdard Institute of Medical Sciences &Research, Hamdard University, New Delhi, India*
[3] *Department of Pathology, Hamdard Institute of Medical Sciences &Research, Hamdard University, New Delhi, India*
[4] *Department of Pharmacology, Hamdard Institute of Medical Sciences &Research, Hamdard University, New Delhi, India*
[5] *Department of Mahiyatul Amraz, Faculty of Medicine (Unani), Hamdard University, New Delhi , India*
[6] *Department of Clinical Pharmacy, College of Pharmacy, Prince Sattam Bin Abdulaziz University, Al-Kharj, Kingdom of Saudi Arabia*
[7] *Department of Pharmaceutical Sciences, Sam Higginbottom University of Agriculture, Technology & Sciences, (SHUATS), Allahabad, India*

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ABSTRACT

The aim of the present study was to investigate the effects of *Cichorium intybus* on lipid peroxidation activities of both enzymatic and non-enzymatic antioxidants, inflammatory mediators, myocardial enzymes and histopathology of cardiac tissues in experimental diabetic cardiomyopathy (DCM). DCM was induced by single intraperitoneal injection of streptozotocin (STZ) (40 mg/kg) combined with high energy intake in rats. Seed extract of *Cichorium intybus* (CIE) (250 mg/kg & 500 mg/kg) was administered orally once a day for 3 weeks. Phytochemical investigations of seed extract revealed presence of some active ingredients such as alkaloids, tannins, saponin, phenols, glycosides, steroids, terpenoids and flavonoids. Seed extract of *Cichorium intybus* confirmed a significant potency towards restoring the blood glucose, an elevation of the levels of aspartate aminotransferase (AST), lactate dehydrogenase (LDH), superoxide dismutase (SOD), thiobarbituric acid reactive substances (TBARS), blood glutathione (GSH), TNF-α and IL-6 and a reduction in the levels of catalase (CAT) was observed following the STZ treatment. Oxidative stress was accompanied by myocardial degeneration as evidenced by histopathological examination of cardiac tissues. Administration of CIE reduced the lipid peroxides level in heart. Serum levels of AST, GSH, LDH and SOD were brought down to physiological levels by CIE in STZ induced DCM rats. CIE also markedly down-regulated serum TNF-α and IL-6 levels. Catalase that was reduced in serum was brought back to near normal level. The extensive necrotic changes of cardiac tissue by STZ was minimized to normal morphology upon CIE administration. The study demonstrates the cardioprotective effect of CIE via inhibition of oxidative stress and pro-inflammatory cytokines.

KEY WORDS: *Cichorium intybus*; diabetic cardiomyopathy, streptozotocin

Introduction

India is pertinently considered as the diabetic capital of the world with the 60 million of diabetics when contrasted with the quantity of diabetic patients around the globe which is increasing quickly and is expected to achieve 439 million by 2030 (Shaw *et al.,* 2010). The long term complications of diabetes are of great concern. However, there is an increasing recognition that diabetes patients suffer from an additional complication termed diabetic cardiomyopathy (DCM). Diabetic cardiomyopathy, as an independent diabetic cardiovascular complication, is characterized by hypertension or valvular heart disease, the myocardial dysfunction in the absence of coronary artery disease. Past investigations reported that the pathophysiology of DCM results in increment of lipid accumulation, hyperglycemia, excessive generation of reactive oxygen species, cardiac inflammation and accumulation of cardiac fibrosis (Westermann *et al.,*

Correspondence address:
Danish Ahmed
Department of Pharmaceutical Sciences, Faculty of Health Science
Sam Higginbottom University of Agriculture Technology and Sciences, SHUATS
Post. Agriculture Institute, Naini, Allahabad - 211007, (U.P.), India.
TEL.: +91-9580001578 • E-MAIL: danish.ahmed@shiats.edu.in

2009; Falcão-Pires & Leite-Moreira, 2012). The existence of oxidative stress has been postulated in patients with diabetes. Lipid peroxidation (LPO) has been implicated in the pathogenesis of naturally occurring or induced diabetes (Baynes & Thorpe, 1996). Oxidative stress may be increased in diabetes owing to hyper-production of reactive oxygen species (ROS), such as $O_2^{\bullet}$, $OH^{\bullet}$ & H_2O_2 and/or a deficiency in the antioxidant defense system (Ahmed *et al.,* 2015). The cytotoxic action of STZ is allied with the generation of reactive oxygen species causing oxidative damage (Szkudelski, 2001). Antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and reduced glutathione (GSH) provides support in scavenging the free radicals by breaking down the ROS (Kim JK *et al.,* 2009). Past investigation have reported a flaw in the scavenging machinery in diabetic patients as compared to the healthy control (Anwer *et al.,* 2012; Anwer *et al.,* 2007).

It has been revealed after extensive investigations within the last two decades that inflammation itself mediated most chronic illnesses such as cancer, neurological, autoimmune, diabetes and cardiovascular diseases. Series of genes expressed gets stimulated by activated macrophages during inflammation in host defense, which conclude the release of different inflammatory mediators, cyclooxygenase-2, nitric oxide, pro inflammatory cytokines etc. (Yoon *et al.,* 2009). Therefore, to prevent delay or treat the above ailment, inflammation should be suppressed. (Begum *et al.,* 2015).

In India, plants, either medicinal or non-medicinal, have been a vital source for treatment of various ailment along with diabetes. *Cichorium intybus*, usually called as Kasni, is used in Unani and Ayurvedic traditional system of medicine to treat hyperglycemia as well as other diseases in India. The genus *Cichorium (Asteraceae)* is made up of six species and it is a wild plant with major geographical native in Europe and is common in Australia, North America and China. Chicory, a common name to *Cichorium intybus,* is well known as a coffee substitute and also widely used medicinally to treat various diseases ranging from diabetes to common wounds. In Eurasian countries and some parts of Africa, *Cichorium intybus* is most commonly used medicinal plant. In spite of its traditional use, chicory is not described in the European Pharmacopoeia or in any official Pharmacopoeia of a European Union member state (European Medicines Agency, 2013). However, due to its ubiquitous distribution several parts of the plant have been used globally in traditional medicines (Süntar *et al.,* 2012).

With its wide therapeutic index different preparations of this plant are employed to treat various diseases. The juice extract is said to be a traditional remedy for tumors and for uterus cancer (Judžentienė & Būdienė, 2008). In South Africa, chicory syrup is used as a purifying medicine and tonic for infants (Van Wyk *et al.,* 1997) and stems, leaves, weed, and roots are made into a tea to treat jaundice. In Turkey, an ointment is made for wound healing from the leaves of chicory (Sezik *et al.,* 2001). The flowers of the chicory plant are used as an herbal treatment of everyday complaint such as appetite defects, sinus problems, cuts and bruises, and treatment of gallstones and gastroenteritis (Judžentienė & Būdienė, 2008). According to the European monograph, utilization of chicory roots includes the relief of symptoms related to mild digestive disorders like flatulence, slow digestion, abdominal fullness etc. and temporary loss of appetite (European Medicines Agency, 2010). The anti-malarial compounds, light-sensitive sesquiterpene lactones lactucin and lactucopicrin, were isolated and identified from chicory roots (Bischoff *et al.,* 2004, Pieroni, 2000). In India, various categories of folk medicine from chicory plant are used. Folkloric use of *c. intybus* has been well documented acting as a hepato-protectant. In a commercial Unani product of India, chicory seeds are one of the main ingredients of Jigrine, used for the treatment of various diseases of the liver (Ahmed *et al.,* 2003). Liv-52, a traditional Indian tonic used widely for hepato-protection (Huseini*et al.,* 2005) is also one of the herbal components of Ayurvedic system of Indian medicine.

Cichiroum intybus seed consist of Chicoric acid which is a major compound in methanolic extracts of chicory. Aliphatic compounds and their derivatives represent the major fractions while minor constituents of the plant comprised of terpenoids. The flowers of chicory comprise flavonoids, saccharides, essential oils, methoxy-coumarin cichorine etc. (Judžentienė & Būdienė, 2008). Numerous studies on *Cichiroum intybus* plant indicated that it possesses antimalarial, antiinflammatory, antimicrobial, antihelmintic, analgesic activity, antiallergic activity, antioxidant, tumor-inhibiting activity, gastro and hepatoprotective effects besides its positive influence in diabetes (Ahmed *et al.,* 2003; Huseini*et al.,* 2005; Nandagopal & Kumari, 2007; Miller *et al.,* 2011; Gürbüz *et al.,* 2002; Cavin *et al.,* 2005; Wesołowska *et al.,* 2006; Heimler *et al.,* 2009; Conforti *et al.,* 2008; Kim *et al.,* 1999; Pushparaj *et al.,* 2007). The antidiabetic effect of the aqueous seed extract of *Cichiroum intybus* has also been investigated in early-stage diabetic rats, chicory treatment led to the increase in insulin levels pointing toward the insulin-sensitizing action of chicory (Ghamarian *et al.,* 2012). However, there is a lack of experimental evidence on the favorable role of *Cichorium intybus* in STZ induced diabetic cardiomyopathy. In view of all the above reports we chose to evaluate chicory for possible beneficial actions in STZ induced diabetic cardiomyopathy.

Materials and methods

Experimental animals

Healthy Albino Wistar rats (8–10 weeks old), weighing about 150–180 g were procured from the Central Animal House Facility, Hamdard University, New Delhi, India. The animals had free access to standard laboratory food and water *ad libitum*, and they were housed in a natural light-dark cycle (12 h each). The experimental protocol was approved by the Institutional Animal Ethics Committee and the care of laboratory animal was taken as per the

guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

Drugs and chemicals

The seeds of *Cichorium intybus* were procured from Hamdard Wakf Laboratories, India and was authenticated by chief scientist, Raw Material Herbarium and Museum, CSIR- NISCAIR (Ref. no. NISCAIR/RHMD/Consult/2014/2538/117). streptozotocin (STZ) was purchased from Sigma Aldrich, St. Louis, USA. The AST, LDH, SOD and CAT diagnostic kits were purchased from Span diagnostics, Surat, India. Rat TNF-α and IL-1β cytometric assay kits were purchased from BD Biosciences, India. All the other biochemicals and chemicals used in the present study were of analytical grade.

Induction of experimental diabetes

Experimental diabetic rats were induced by feeding with high fat diet (HFD) during the whole experimental period. HFD was prepared by adding 20% sucrose (w/w) and 20% lard (w/w) into normal pellet diet. After 8 weeks of dietary manipulations, rats were intraperitoneally injected with STZ (40 mg/kg) dissolved in 100 mM citrate buffer pH 4.5 bolus of STZ. Control rats were administered an equivalent volume of citrate buffer (Wang *et al.,* 2007). Blood glucose levels were measured 72 h after STZ injection and rats which had blood glucose values ≥11.6 mmol/l were used in the groups III–V.

Preparation of extract and preliminary phytochemical studies

Chicory seeds were cleaned and powdered using an electric mill. Every 200 g was soaked in 1 l of distilled water and refluxed for 20 minutes in a boiling water bath to make a 20% solution. The solution was allowed to cool at room temperature before being vacuum filtered through Whatman No. 1 filter paper. The filtrate was lyophilized and stored at –20 °C; every 100 g of powdered seed yielded about 8.2 g of lyophilized substance. The extract was suspended in distilled water and was administered orally to the animals using feeding tubes in doses of 250 mg/kg and 500 mg/kg. Different secondary metabolites are present in plant materials which exhibit various pharmacological activities. Crude extracts were subjected to phytochemical analysis for identification of alkaloids, cardiac glycosides, steroids, tannins, and saponins following standard protocol (Trease & Evans, 2002).

Experimental design

The rats were divided into five groups comprising of six animals in each group as follows:

- Group I: Healthy control, received citrate buffer (1 ml/kg, i.p.)
- Group II: *per se,* received only CIE (500 mg/kg, p.o.)
- Group III: Diabetic cardiomyopathy (DCM), STZ single dose (40 mg/kg, i.p.) + HFD
- Group IV: STZ + HFD + CIE (250 mg/kg, p.o.)
- Group V: STZ + HFD + CIE (500 mg/kg, p.o.)

Diabetic animals were treated with chicory seed extract (250 mg/kg and 500 mg/kg, p.o.) for 3 weeks. On the last day of experiment, blood samples were collected by retro-orbital puncture for biochemical estimations and animals were sacrificed by cervical decapitation under light chloroform anesthesia and organs were collected for histopathological examination.

Measurements of glycemic level in serum

By using digital glucometer (Gluco One) blood glycemic level was determined using serum.

Measurements of myocardial enzymes and inflammatory cytokines in serum

Aspartate Aminotransferase (AST) and Lacate Dehydrogense (LDH) were determined by colorimetric analysis using a spectrophotometer with the associated detection kits (Reitman & Frankel, 1957; McLauchlan DW *et al.,* 1988). TNF-α and IL-6 in serum were determined by cytometric method following the manufactures instructions (Brouckaert *et al.,* 1993).

Estimation of Superoxide Dismutase (SOD) and Malondialdehyde (MDA) in heart tissue

SOD activity was measured according to the method of Marlund and Marklund (Marklund & Marklund, 1974). The enzyme activity was expressed in unit/mg of protein and 1 unit of enzyme is defined as the enzyme activity that inhibits autoxidation of pyrogallol by 50%. The concentration of MDA was measured in heart using the method of Ohkawa *et al.* 1979 (Ohkawab *et al.,* 1979). Briefly, the heart tissues collected soon after sacrificing the rats, were suspended in 150 mM KCl and homogenized in Teflon homogenizer. The concentration of thiobarbituric acid reactive substances (TBARS) was expressed as nmol of MDA/mg of protein using 1,1,3,3,-tetraethoxypropane as standard. The protein were estimated by the method of Lowry *et al.* 1951 (Lowry *et al.,* 1951).

Determination of reduced and total glutathione (GSH)

Freshly collected heart tissues were weighed and 10% (w/v) homogenates were made in 1.15 M KCl using a motor driven Teflon-pestle. GSH was estimated using the method of Sedlak and Lindsay 1968 (Sedlak & Lindsay, 1968) that uses 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB). The total glutathione in blood was estimated by the method of Beutler *et al.*1963 (Beutler *et al.,* 1963). The absorbance was read at 412 nm.

Determination of CAT

CAT activity was estimated following the method of Claiborne (1985) based on a decrease in absorbance at 240 nm due to consumption of hydrogen peroxide (H_2O_2).

Determination of SGOT

Recombinogen kit was used for SGOT estimation. 2 ml of blood samples was collected in centrifuge tube and left to stand for 1 hour at 37 °C and further samples was cooled in refrigerator for 3 hours. The clot formed was then removed and serum samples were decanted out. These serum samples were then centrifuged at 3000 rpm

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Table 1. Isolated and identified compounds from the extraction of *Cichorium intybus* seed.

S.No.	Compound
1.	11,13-Dihydrolactucopicrin
2.	11β,13-Dihydrolactucin
3.	Caffeic acid
4.	Chicoric acid
5.	Chlorogenic acid
6.	Cichorioside
7.	Cichorioside B
8.	Cyanidin
9.	Kaempferol-3-O-glucoside
10.	Kaempferol-3-O-glucosyl-7-O-(6″-O-malonyl)-glucoside
11.	Kaempferol-7-O-glucoside
12.	Lactucin
13.	Lactucopicrin
14.	Malic acid
15.	Pelargonidin-3-O-monoglucuronide
16.	Quercetin-3-O-glucuronide-7-O-(6″-O-malonyl)-glucoside
17.	Quercetin-7-O-(6″-O-acetyl)-glucoside
18.	Quercetin-7-O-galactoside
19.	Tricin-3-O-glucoside
20.	β-Sitosterol

Table 2. Effect of *Cichorium intybus* seed extract on blood glucose of rats.

S.No.	Treatment	Glucose (mg/dl)
1.	Control (1 ml/kg. i.p.)	100.67±6.19
2.	Per se (500 mg/kg, p.o.)	116.33±2.4
3.	STZ Single dose (40 mg/kg, i.p.)+HFD	306.33±10.18*
4.	STZ+HFD+CIE (250 mg/kg, p.o.)	121.33±4.01**
5.	STZ+HFD+CIE (500 mg/kg, p.o.)	106.67±3.26**

Data shown as means±SEM from 6 animals. All comparisons of group IV and V with group III by student's t test (*p<0.001). All comparisons of group I, II, IV and V with group III by ANOVA (**p<0.001).

Table 3. Effect of *Cichorium intybus* seed extract on glutathione in blood and heart muscle of rats.

Groups	Treatment	Whole Blood glutathione (mg %)	Tissue GSH (µmole/g. wt of tissue)
I	Control (1 ml/kg. i.p.)	1.09±0.053	1.89±0.082
II	Per se (500 mg/kg, p.o.)	1.14±0.107**	1.97±0.077**
III	STZ Single dose (40 mg/kg, i.p.)+HFD	2.10±0.071**	3.77±0.047**
IV	STZ+HFD+CIE (250 mg/kg, p.o.)	1.61±0.064**, *	3.28±0.098**,*
V	STZ+HFD+CIE (500 mg/kg, p.o.)	1.56±0.033***	2.87±0.08**, *

Data shown as means ± SEM from 6 animals. All comparisons of group IV and V with group III by student's t test (*p<0.001). All comparisons of group I, II, IV and V with group III by ANOVA (**p<0.001).

for 15 minutes. The supernatant after centrifugation was the serum sample used for analysis.

Histopathology

10% neutral buffered formalin fixed hearts were dehydrated in ascending grades of isopropyl alcohol embedded in paraffin wax and 5 µm sections were stained by haematoxylin and eosin stain.

Statistical analysis

Data were expressed as the mean ±SEM. For a statistical analysis of the data, group means were compared by one way ANOVA with *post hoc* analysis. The Tukey-Karmer *post hoc* test was applied to identify significance among groups; p<0.05 was considered to be statistically significant.

Results

Preliminary phytochemical screening

The results of the preliminary phyto-chemical screening of *cichorium intybus* seeds extract (CIE) showed the presence of alkaloids, phenolics, flavonoids, tannins, steroids, anthraquinones and saponin glycosides.

Chemical constituents

Various compounds from the extraction of *Cichorium intybus* have been isolated and identified and a summary of isolated and identified chemicals are listed below in (Table 1). (Street *et al.* 2013)

Effect of CIE on blood glucose in STZ diabetic rats

In group III (Diabetic group) the blood glucose was at highest level and after the administration of chicory decrease of level of blood glucose was observed. (Table 2).

Effect of CIE on myocardial enzymes in STZ diabetic rats

Myocardial enzymes SGOT and LDH are biochemical indicators of myocardial injury. A significant increase (p<0.01) (Figures 1–2) in the levels of these enzymes was observed in animals treated with STZ. Administration of CIE at doses 250 mg/kg and 500 mg/kg significantly reduced (p<0.01) the level of these enzymes when compared to STZ treatment alone.

Effect of CIE on STZ induced LPO

Freshly prepared heart homogenates were studied for the concentration of malondialdehyde (MDA). Rats treated with STZ alone had a significant increase (p<0.001) in the level of MDA. Simultaneous treatment of CIE at two concentrations reduced the level of MDA in heart (p<0.01) as shown in (Figure 3).

Effect of CIE on STZ induced changes in GSH contents

The glutathione levels in blood and reduced glutathione in heart homogenates are shown in (Table 3). The concentration of glutathione in animals treated with STZ was significantly increased both in blood and in

homogenates of heart (p<0.001) as compared to control rats. Co-administration of CIE extract in two doses (250 mg/kg and 500 mg/kg) decreased the concentration of total and reduced glutathione in blood and heart (p<0.001). However, the values remained at higher than physiological levels at the time of estimation.

Effect of CIE on STZ-induced changes in activity of antioxidant enzymes

Figure 4 and 5 demonstrate the activities of SOD and CAT in cardiac tissue of healthy control, CIE only treated rats, diabetic control, and CIE treated diabetic rats. Only CIE treatment did not register any significant change in the activities of antioxidant enzymes when compared with healthy control rats. A significant (p<0.001) decrease in the activity of CAT and a significant increase in the activity of SOD activity was a notable manifestation of STZ toxicity. Administration of CIE significantly (p<0.001) improved the activities of these enzymes in diabetic rats when compared with diabetic control rats.

Effect of CIE on inflammatory biomarkers in STZ diabetic rats.

To further elucidate the mechanism of action of CIE in myocardial inflammation, pro-inflammatory cytokines such as TNF-α and IL-6 were determined using Cytometric Bead Array technique. The serum level of TNF-α and IL-6 in STZ induced diabetic group was significantly (p<0.001) increased compared with the healthy control group as shown in (Figure 6–7). However, the levels were significantly lowered in animals treated concomitantly with two doses of CIE (p<0.001). No significant difference in IL-6 level was found at the lower dose of CIE.

Effect of CIE on STZ induced histological changes on heart.

Intraperitoneal injection of STZ along with HFD, induced myocardiopathy, characterized by extensive myocardial necrosis associated with massive leucocytic infiltrations predominantly lymphocytes with occasional polymorphs (Figure 8B). The changes were predominantly observed in the ventricular myocardium and subendocardial myocardium. CIE at the dose of 500 mg/kg had remarkable cardioprotective effect as evidenced by minimal myocardial

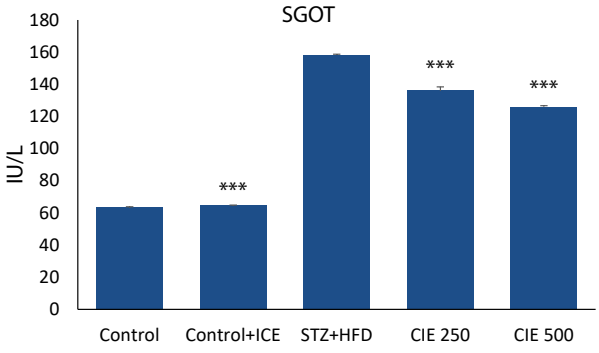

Figure 1. Effect of *Cichorium intybus* treatment on SGOT level in rats. Values are expressed as mean ± SEM (n=6). Significant differences are indicated by ***p<0.001; **p<0.01; *p<0.05 vs. group III.

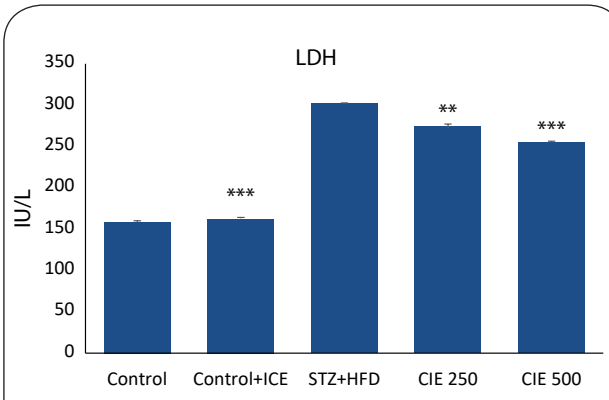

Figure 2. Effect of *Cichorium intybus* treatment on LDH level in rats. Values are expressed as mean ± SEM (n=6). Significant differences are indicated by ***p<0.001; **p<0.01; *p<0.05 vs. group III.

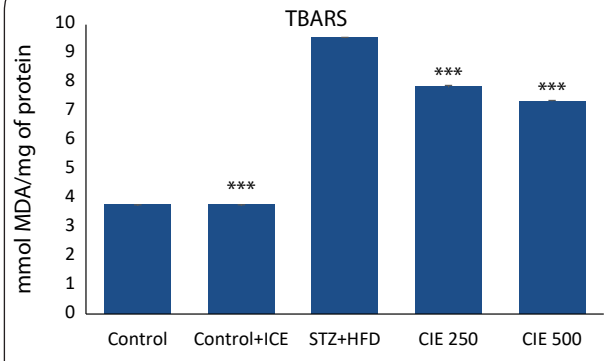

Figure 3. Effect of *Cichorium intybus* treatment on TBARS in the heart of rats. Values are expressed as mean ± SEM (n=6). Significant differences are indicated by ***p<0.001; **p<0.01; *p<0.05 vs. group III.

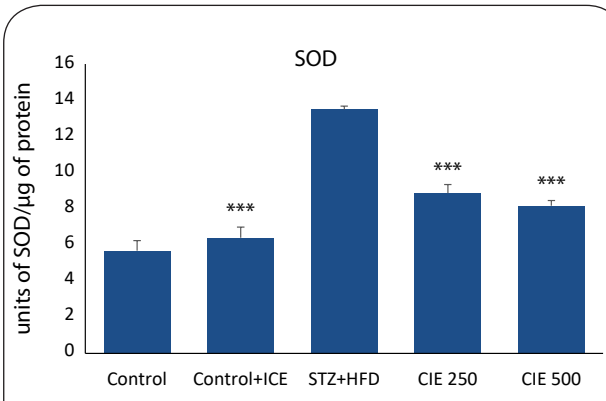

Figure 4. Effect of *Cichorium intybus* treatment on SOD in the heart of rats. Values are expressed as mean ± SEM (n=6). Significant differences are indicated by ***p<0.001; **p<0.01; *p<0.05 vs. group III.

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necrosis and infiltrative changes (Figure 8D). CIE at dose 250 mg/kg was less effective in providing the protective effect on heart from STZ induced necrosis.

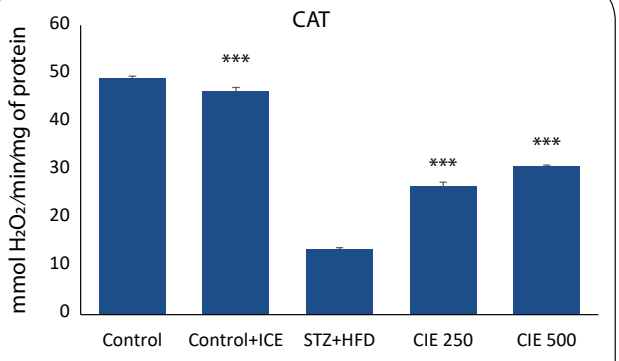

Figure 5. Effect of *Cichorium intybus* treatment on CAT in the heart of rats.Values are expressed as mean ± SEM (n=6). Significant differences are indicated by ***p<0.001; **p<0.01; *p<0.05 vs. group III.

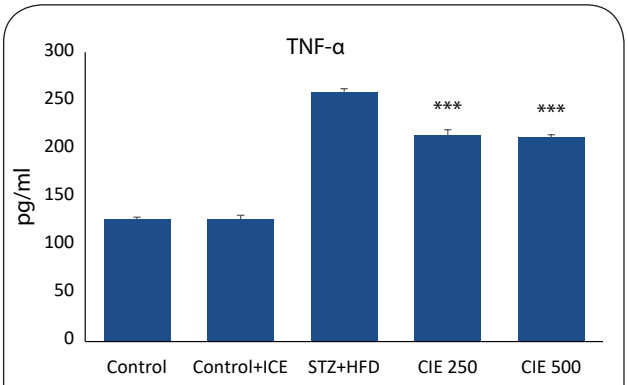

Figure 6. Effect of Cichorium intybus treatment on TNF-α levels in the heart of rats. Values are expressed as mean ± SEM (n= 6). Significant differences are indicated by ***p<0.001; **p<0.01; *p<0.05 vs. group III.

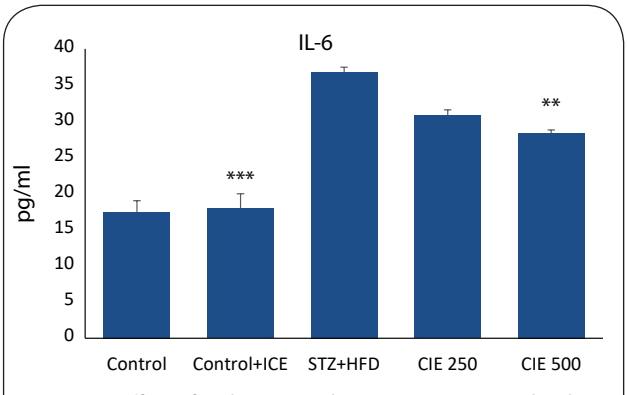

Figure 7. Effect of *Cichorium intybus* treatment on IL-6 level in the heart of rats. Values are expressed as mean ± SEM (n=6). Significant differences are indicated by ***p<0.001; **p<0.01; *p<0.05 vs. group III.

Discussion

The present study was undertaken to evaluate the protective effect of *cichorium intybus* on STZ induced diabetic cardiomyopathy in rats. Experimental diabetes produced by low dose of STZ combined with high energy intake is regarded as a general strategy to obtain type-2 diabetes animal model, since it stimulates the real course of human type-2 DM (Wang *et al.,* 2007; Ti *et al.,* 2011). The high energy intake induces insulin resistance at first and subsequently an injection of low dose STZ makes partial dysfunction of beta cells to suppress insulin secretion which works as a compensation to insulin resistance.

The cytotoxic action of STZ is associated with generation of ROS causing oxidative damage (Szkudelski, 2001). Disturbances of antioxidant defense systems in diabetes have been demonstrated, including alterations in the activities of antioxidant enzymes such as SOD, CAT and impaired glutathione metabolism (Maritim & Sanders, 2003). Chemicals with antioxidant properties and free radical scavengers may help in the regeneration of beta cells and protect pancreatic islets against cytotoxic effects of STZ (Alvarez *et al.,* 2004). Decreased LPO and improved antioxidant status may be one mechanism by which dietary treatment contributes to the prevention of diabetic complications (Armstrong *et al.,* 1996). Myocardial necrosis produced by STZ is also the consequence of increase LPO due to generation of free radicals. *Cichorium* seed extract protects the myocardium from peroxidative damage.

Chicory has reported antidiabetic activity (Pushparaj *et al.,* 2007; Ghamarian *et al.,* 2012) and as evidenced in the present study chicory seed extract possesses a strong antioxidant property. Decreased LPO and improved antioxidant status may be one mechanism by which dietary treatment contributes to the prevention of diabetic complications. The glucose lowering capacity of chicory has been attributed to its chemical composition including antioxidant compounds (Hassan & Yousef, 2010). Chicoric acid, a compound isolated from chicory, is also a new potential antidiabetic agent exhibiting both insulin-sensitizing and insulin-secreting properties (Tousch *et al.,* 2008).

The LDH and AST levels, which were significantly increased following myocardial injury by STZ, were reduced to physiological level in serum upon administration of CIE in two doses. However, the 500 mg/kg dose of chicory had better protective effect than the 250 mg/kg dose group. An elevation of reduced GSH in heart muscle and total glutathione in whole blood was observed in STZ diabetic rats. The demonstration of increased myocardial levels of total and reduced glutathione is consistent with an activation of the glutathione-peroxidase redox pathway secondary to the generation of and subsequent detoxification of STZ induced free radicals (Jackson *et al.,* 1984). Increase in cardiac muscle GSH suggest that the myocardial detoxification system is not overwhelmed as has been suggested. The presence of myocyte damage, as demonstrated histologically, suggests that injury has

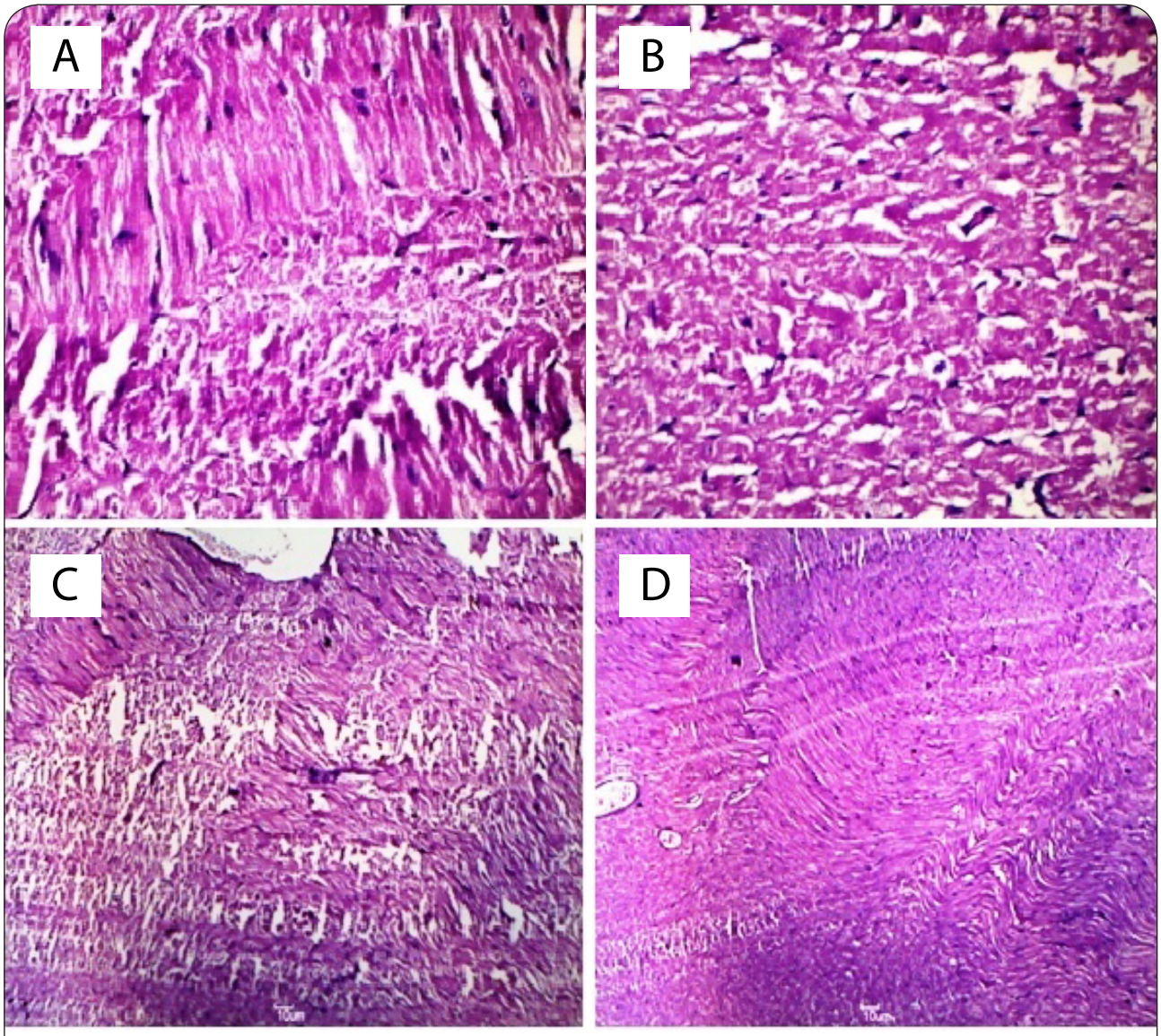

Figure 8. Histopathological changes in heart of rat and in animal treated with *Cichorium intybus*. Figure A- Normal rat heart shows normal morphology of cardiomyocyte. Figure B- Degenerative changes in many myocardial fibres and massive leucocytic infilterations are seen in heart of STZ diabetic rats. Figure C- CIE-250mg/kg treated heart shows mild peripheral necrosis and edema with occasional polymorphs. Figure D- A few myocardial fibres show degenerative changes and necrosis in CIE-500mg/kg treated rats and a minimal infilterative changes. Myocardial tissue sections stained with hematoxylin and eosin (magnification=400X).

occurred despite the elevation of GSH level. If the rise in GSH is in response to free radicals, it would appear that the system is able to cope adequately with their production indicating contributory effect of other mechanisms in cardiac damage by STZ. The lowering of the level of GSH by chicory suggests scavenging effect of chicory on free radicals responsible for the elevation of GSH in heart muscle. The role of chicory in this context requires further study.

Furthermore, cardiac inflammation, characterized by increased levels of pro-inflammatory cytokines, was suppressed by chicory as well. Pro-inflammatory cytokines such as TNF-α and IL-6 are chief mediators of inflammatory reaction and critically participate in the manifestation of DCM. Chicory has been used as an anti-inflammation agent for generations (Cha *et al.,* 2011). TNFα plays several important roles in inflammation based on its appearance at the inflammatory site and ability to induce certain mechanisms including activation and chemotaxis of leukocytes, expression of adhesion molecules on neutrophils and endothelial cells, and regulation of the secretion of other pro-inflammatory cytokines (Collins & Grounds, 2001). The TNF-α stimulates hyperlipidemia and hepaticlipogenesis simultaneously reducing the sensitivity to insulin in muscle tissues and finally the necrosis of target organs (Khanra *et al.,* 2015).

Biochemical observations were in keeping with the morphological changes in the heart. Supplementation with CIE prevented the disorganization of cell plates and restored the cardiac cytoarchitecture nearly similar to

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that of normal rats. The extensive necrotic and abundant infiltrative changes in heart, which were consistently observed in STZ diabetic animals, were reduced to minimum with chicory co-treatment and further substantiated the protective effect of chicory in heart.

In summary, the present study shows that the administration of chicory extract shows remarkable hyperglycemic effect as well as reduces the tissue specific marker enzymes, lipid peroxides, GSH and pro-inflammatory cytokines which are elevated in response to acute administration of STZ and thereby demonstrates its cardioprotective effect. Analytical studies determining the active component of chicory responsible for cardioprotective effect are yet to be isolated and confirmed. Detailed mechanistic action of chicory, particularly the flavonoids present in CIE, on different free radicals requires further study.

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