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Research Article
Modulatory Effect of *Cassia alata* Leaf Extract on Isoproterenol-Induced Myocardial Inflammation and Fibrosis in Male Albino Wistar Rats

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Abstract

Background and Objective: Cardiovascular problems are the leading cause of death worldwide. Myocardial infarction (MI) is a severe health problem since it kills more people than any other type of cardiac disease. The current study investigated the preventive effect of *Cassia alata* (*C. alata*) leaf methanol extract on isoproterenol (ISO)-induced MI in rats. **Materials and Methods:** A total of 24 male Wistar rats were divided into 4 groups (n = 6). Group I rats received a standard pellet diet, Group II rats were treated with *C. alata* leaf extract (100 mg kg^{-1}) for 14 days and Group III rats received a subcutaneous injection of ISO (85 mg kg^{-1}) for the last two consecutive days (13th and 14th day) and Group IV rats received *C. alata* (100 mg kg^{-1}) for 14 days with ISO for last 2 days. **Results:** Cardiac serum markers, lipid peroxidative, inflammatory and fibrotic markers were increased and decreased cellular antioxidants in ISO-induced rats. At the same time, the pre-treatment of *C. alata* leaf extract reduced the activities of cardiac marker enzymes and lipid peroxidative markers and enhanced the antioxidant status in ISO-induced rats. Further, *C. alata* leaf extract pre-treatment inhibited inflammatory markers and downregulated the fibrosis markers in ISO-induced rats. **Conclusion:** The findings of the present study reveal that *C. alata* leaf extract attenuates cardiotoxicity through the modulation of oxidative stress and inflammation in ISO-induced MI rats. The antioxidant, free radical scavenging and anti-inflammatory properties of *C. alata* leaf extract have been connected to its cardioprotective properties.

Key words: *Cassia alata* leaf extract, isoproterenol, cardiotoxicity, cardiac markers, lipid peroxidation, antioxidant status, inflammation

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Myocardial infarction (MI) is a significant public health issue and the major cause of death in developed and developing countries[1]. Despite advances in clinical care, increased public awareness and widespread adoption of healthcare technologies, myocardial infarction continues to be the leading cause of mortality worldwide[2]. The MI is a severe form of myocardial necrosis caused by an imbalance in coronary blood flow and myocardial demand[3]. Blood pressure, electrocardiograph changes, loss of ventricular functioning and increased serum expression of cardiac-related proteins can all be used to corroborate a clinical diagnosis of MI[4]. In General, MI is linked with a range of pathophysiological changes in the myocardial, including free radical damage, thrombosis and hyperlipidemia[5].

Isoproterenol (ISO), a β-adrenergic agonist, has generated considerable stress in the myocardium, leading to infarct-like necrosis of the heart muscle[6]. The ISO-induced myocardial necrosis leads to fluctuating membrane permeability and structural and functional loss of the membrane and tissues that comprise the myocardium[6]. The necrotic condition of myocardial tissues is characterized by an increase in cardiac enzymes, changes in ECG, lipid peroxide formation and abnormal cardiac function[7]. This eventually causes a decrease in myocardial cell conductivity and a reduction in the typical electrical efficiency of the heart[8]. The pathophysiological and morphological changes of the heart in such a myocardial necrosis model in rats are similar to those seen in a human MI event[9]. Inflammation is a common occurrence in the etiology of MI and sustained inflammation in the myocardium plays an essential role in the progression of the disease[10]. Nuclear factor-kappa B (NF-κB) is a crucial regulator of inflammation and apoptosis[11]. Phosphorylation of NF-κB increases the production of pro-inflammatory cytokines such as tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β), as well as other inflammation-related proteins, resulting in a variety of pathophysiological changes in MI[12].

The usage of herbal remedies has achieved widespread acceptance worldwide and its efficacy is directly related to the active substances contained inside. *Cassia alata* (*C. alata*) has been used as a traditional medicine to treat various diseases, especially skin diseases. Previous research suggests that *C. alata* has some pharmacological activity like anti-allergic, anti-inflammatory, antioxidant, anticancer, anti-diabetic and antifungal properties and its other medicinal uses[13]. According to scientific studies, *C. alata* leaf extract contains a variety of flavones, flavonols, flavonoids glycosides, alatinone, alanonal and β-sitosterol-β-D-glucoside[14]. It has been discovered that *C. alata* leaf extract had significant antioxidant activity against streptozotocin-induced diabetic rats[15]. The ISO induced substantial oxidative stress in the heart, resulting in an antioxidant imbalance. Hence, the present study evaluated the myocardial production of *C. alata* leaf extract against ISO-induced MI in Wistar rats.

MATERIALS AND METHODS

Study area: The present study was carried out in the Department of Basic Theory of Traditional Chinese Medicine, Shandong University of Traditional Chinese Medicine, Jinan, China, from June to December, 2021.

***Cassia alata* leaf extract preparation:** Fresh leaves of *C. alata* were collected from the local market, dried to a constant weight at 50°C and ground into a coarse powder. The powdered leaf material was defatted with an appropriate millilitre of methanol for 3 hrs and then extracted by maceration for 6 hrs. The methanolic extract was concentrated to dryness using a rotary evaporator attached to a vacuum pump and stored at 4°C until use.

Experimental rats: The Institutional Animal Ethics Committee of (IAEC), Shandong University of Traditional Chinese Medicine, approved this work and the study was carried out following the institutional ethical standards. This research uses 24 male albino Wistar rats at 150-180 g weight. All animals were housed in a clean, air-conditioned environment, kept at a temperature of 25°C, 50% humidity and a 12:12 hrs dark/light cycle. All animals have consumed a standard pellet diet and drinking water was provided *ad libitum*.

After 1 week of accumulation, all experimental animals were divided into four groups, each with six animals. Group 1 (Normal): The rats were treated with a standard pellet diet. Group II (*C. alata* control): The rats were treated orally with *C. alata* leaf extract for 14 days. Group III (ISO): ISO (85 mg kg^{-1} dissolved in 1 mL of normal saline) was subcutaneously (s.c.) injected into rats for the last two consecutive days (13th and 14th days) to induce experimental MI. Group IV (*C. alata* + ISO): The rats received oral administration of *C. alata* leaf extract (100 mg kg^{-1}) using an intragastric tube for 14 days and ISO for the last two consecutive days (13th and 14th days). The total experimental duration was 14 days. The animals were anaesthetized by intramuscular doses of ketamine hydrochloride and sacrificed on the 15th day. The blood was gathered after sacrificing through the internal jugular vein. The blood was centrifuged at 2000 rpm and collect the serum

was for the study of cardiac markers. The heart tissue was immediately removed, washed with physiological saline and homogenated with the appropriate buffer used for biochemical estimation.

Activities of cardiac parameters: The serum Cardiac Troponin-T (cTnT) (catalog No: MBS285604) and I (cTnI) (catalog No: MBS1601650) levels, Creatine kinase (CK) (catalog No: MBS173707), CK-MB (catalog No: MBS173056) and Lactate Dehydrogenase (LDH) (catalog No: MBS269777) activities were estimated using the commercial kit obtained from MyBioSource International, San Diego, CA, USA.

Estimation of lipid peroxidative markers and antioxidants assay: The levels of Thiobarbituric Acid Reactive Substances (TBARS) and lipid hydroperoxide (LOOH) in the heart tissue of experimental rats were determined and the supernatant of heart tissue was used to determine superoxide dismutase (SOD) activity, catalase (CAT), glutathione peroxidase (GPx) and reduced Glutathione (GSH) activities. According to the earlier procedure, all these oxidative stress markers were estimated[16].

Estimation of inflammatory cytokine markers: The ELISA kits for the serum levels of inflammatory cytokines such as TNF-α (catalogue No: ab236712), IL-6 (catalogue No: ab234570) and NF-κB (catalogue No: ab176648) were obtained from Abcam Scientific Company. The reaction mixture contained 50 µL of the sample and 50 µL of antibody cocktail in the experimental wells and was incubated at 37°C for 1 hr. The exploratory wells were washed with TMB substrates (100 µL) and set for 10 min. End of the reaction 100 µL stop solution was added to the selected wells and then coloured developed was read OD at 450 nm using an ELISA reader.

Inflammatory signalling and cardiac fibrotic gene expression by PCR array: The total RNA was isolated from the experimental heart tissues using an RNeasy mini kit (Qiagen, China) according to the manufacturer's instruction. The cDNA was reverse transcribed from RNA using the First Strand cDNA Synthesis Kit (Qiagen reverse transcriptase kit). The relative expression pattern of inflammatory genes such as TNF-α, IL-6, NF-κB, COX-2 and fibrotic genes like MMP-2, MMP-9, TGF-β1, Fibronectin, α-SMA, collagen-I, collagen-III, Smad-2, Smad-3 were analyzed by PCR array using RT^2 real-time SYBR Green PCR master mix (Qiagen). The fold changes of gene expression were plotted as a heatmap using heat mapper online software.

Assessment of histopathological changes: After sacrificing, the heart tissues (left ventricle) were immediately removed and fixed in 10% formalin then dehydrated on treatment with a series of different concentrations of ethanol and embedded in paraffin wax. Then the slides were stained with Hematoxylin-Eosin (H&E) dyes. The slides were evaluated with a light microscope (40x). The pathologist examined all histopathological changes.

Statistical analysis: All the results were calculated as the Mean±Standard Deviation (SD). The results were statistically analyzed (SPSS software package) using One-way ANOVA followed by Duncan's Multiple Range Test (DMRT). A value of $p<0.05$ was considered statistically significant.

RESULTS

Effect of *C. alata* leaf extract on level of cardiac markers in serum: Figure 1a shows the effect of *C. alata* leaf extract on serum cardiac marker enzymes cTnT and cTnI in experimental animals. The activity of cTnT (1.18±0.09 ng mL^{-1}) and cTnI (0.6±0.04 ng mL^{-1}) were significantly elevated ($p<0.05$) in the ISO group compared to control group. Pretreatment with *C. alata* leaf extract could significantly lower ($p<0.05$) the activities of cTnT (0.72±0.04 ng mL^{-1}) and cTnI (0.41±0.01 ng mL^{-1}) compared with ISO group.

Figure 1b shows the activities of cardiac marker enzymes CK (208.65±15.23 IU L^{-1}), CK-MB (135.36±6.57 IU L^{-1}) and LDH (310±20 IU L^{-1}) significantly ($p<0.05$) increased in the ISO group when compared to the control group. Oral administration of *C. alata* leaf extract had significantly reduced the activities of CK (168.17±8.67 IU L^{-1}), CK-MB (102.17±3.56 IU L^{-1}) and LDH (240±18 IU L^{-1}) compared to the ISO group. *C. alata* leaf extract alone and control did not show any significant effects.

Effect of *C. alata* leaf extract on lipid peroxidation products and antioxidant enzymes in experimental rats: Figure 2a-b shows the levels of lipid peroxidation products such as TBARS (1.68±0.09 mmol/100 g wet tissue) and LOOH (110.62±10.63 mmol/100 g wet tissue) were significantly increased ($p<0.05$) in ISO-induced rats compared with control rats. Whereas, rats pretreated with *C. alata* leaf extract for 14 days significantly reduced ($p<0.05$) the TBARS (0.75±0.05 mmol/100 g wet tissue) and LOOH (67.86±4.75 mmol/100 g wet tissue) levels compared to ISO induced group.

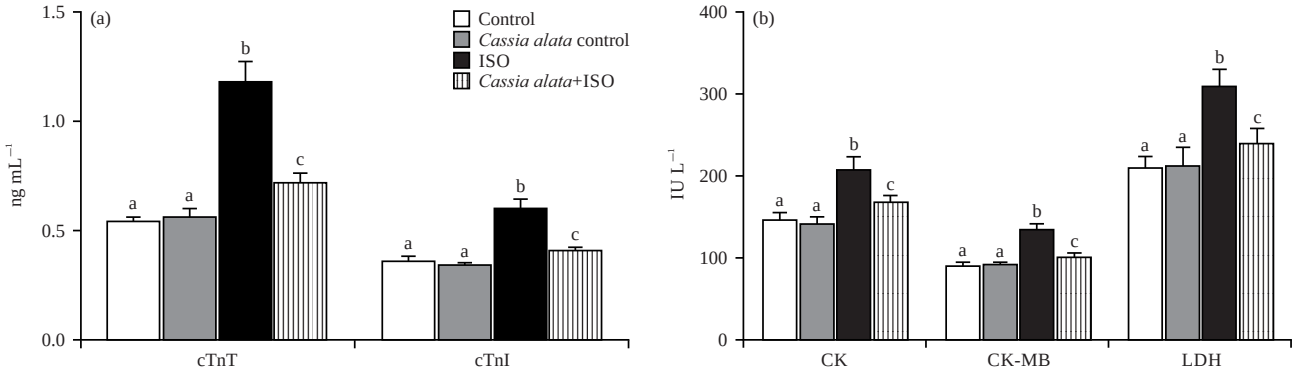

Fig. 1(a-b): Effect of *Cassia alata* methanolic extract on cardiac markers in the serum of ISO-induced rats, (a) Activities of cTnT and cTnI and (b) Activities of CK, CK-MB and LDH

Values are given as Mean±SD of six experiments in each group, values not sharing a common marking superscript are significantly different at $p \leq 0.05$ (DMRT), cTnT: Cardiac troponin-T, CK: Creatine kinase and LDH: Lactate Dehydrogenase

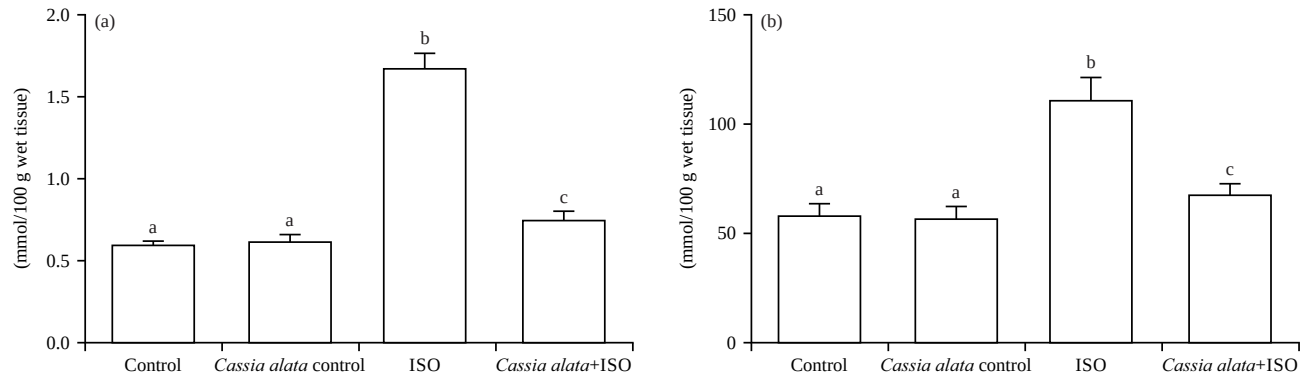

Fig. 2(a-b): Effect of *Cassia alata* methanolic extract on the levels of lipid peroxidation products in the heart tissue of ISO-induced rats, (a) TBARS (thiobarbituric acid reactive substances) level in the heart tissue and (b) LOOH (lipid hydroperoxide) level in the heart tissue

Values are given as Mean±SD of six experiments in each group, values not sharing a common marking superscript are significantly different at $p \leq 0.05$ (DMRT)

Table 1: Effect of *Cassia alata* methanolic extract on antioxidant status in the heart tissue of ISO-induced rats

Groups	Control	*Cassia alata* control	ISO	*Cassia alata* +ISO
SOD (U^1 mg^{-1} protein)	6.98±0.48^a	6.95±0.52^a	3.26±0.18^b	6.18±0.26^c
Catalase (U^2 mg^{-1} protein)	43.17±2.17^a	44.50±2.07^a	28.46±1.09^b	39.59±1.93^c
GPx (U^3 mg^{-1} protein)	6.61±0.31^a	6.58±0.34^a	3.53±0.13^b	6.19±0.26^c
GSH (µg mg^{-1} protein)	5.04±0.04^a	5.10±0.12^a	3.08±0.15^b	4.95±0.29^c

Values are given as Mean±SD of six experiments in each group, values not sharing a common marking superscript are significantly different at $p \leq 0.05$ (DMRT). U^1: Enzyme concentration required for 50% inhibition of nitroblue tetrazolium reduction in one minute, U^2: µmole of hydrogen peroxide consumed per minute and U^3: µmole of glutathione consumed per minute

Table 1 indicated the activities of cardiac antioxidant enzymes SOD (3.26±0.18 U mg^{-1} protein), CAT (28.46±1.09 U mg^{-1} protein), GPx (3.53±0.13 U mg^{-1} protein and GSH (3.08±0.15 µg mg^{-1} protein) decreased significantly ($p<0.05$) in rats treated with ISO. However, pre-treatment with *C. alata* leaf extract significantly raised the activities/levels of these antioxidants SOD (6.18±0.26 U mg^{-1} protein), CAT (39.59±1.93 U mg^{-1} protein), GPx (6.19±0.26 U mg^{-1} protein) and GSH (4.95±0.29 µg mg^{-1} protein) when compared to the ISO values.

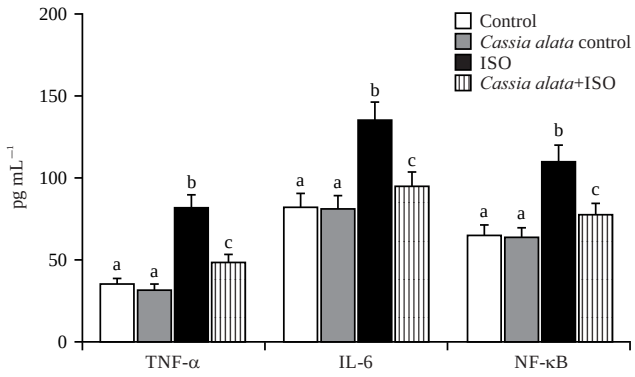

Fig. 3: Effect of *Cassia alata* methanolic extract on the levels of TNF-α, IL-6 and NF-κB in the serum of ISO-induced rats
Values are given as Mean±SD of six experiments in each group, Values not sharing a common marking superscript are significantly different at $p \leq 0.05$ (DMRT)

Effect of *C. alata* leaf extract on ISO-induced inflammatory cytokines: The levels of inflammatory markers TNF-α, IL-6 and NF-κB in ISO-induced rats as shown in Fig. 3. The TNF-α (82.07±7.62 pg mL^{-1}), IL-6 (135.81±10.47 pg mL^{-1}) and NF-κB (110.28±9.63 pg mL^{-1}) levels were considerably higher ($p<0.05$) in the ISO group compared to the control group, while pretreatment with *C. alata* leaf extract to ISO-induced groups significantly reduced TNF-α (49.29±4.08 pg mL^{-1}), IL-6 (95.47±8.36 pg mL^{-1}) and NF-κB (78.24±6.42 pg mL^{-1}) levels compared to ISO group.

Effect of *C. alata* leaf extract on inflammatory and fibrotic gene expression profile in heart tissue: Figure 4 shows the heatmap analysis of PCR array results for inflammation and fibrotic gene expression in *C. alata* leaf extract and ISO-treated rats. The inflammatory genes such as TNF-α, IL-6, NF-κB, COX-2 and fibrotic genes like MMP-2, MMP-9, TGF-β1, Fibronectin, α-SMA, Collagen-I, collagen-III, Smad-2, Smad-3 were upregulated in ISO-induced rats. Pre-treatment with *C. alata* leaf extract markedly decreased the levels of both these cytokines and fibrotic genes markers.

Effect of *C. alata* leaf extract on heart tissue by histopathological examinations: The heart tissues of control rats have normal architecture without cellular necrosis, oedema and inflammation in Fig. 5a. Similarly, control rats treated with *C. alata* leaf extract have normal architecture without cellular necrosis, oedema and inflammation in Fig. 5b. The ISO-induced group showed myocardial cellular oedema, degeneration and the appearance of inflammatory cells in heart tissues in Fig. 5c. Pretreatment with *C. alata* leaf extract heart architecture showed significantly decreased inflammatory cells and near-normal architecture of the myocardium in Fig. 5d.

DISCUSSION

The ISO-induced MI is a widely used in vivo animal model for the experimental evaluation of cardioprotective medicines because it closely mimics the clinical characteristics of human MI[17]. The current study demonstrated that the methanolic extract of *C. alata* leaf protects cardiomyocytes against ISO-induced MI by inhibiting lipid peroxidation, activating the endogenous antioxidant defence system and preserving the histological integrity of cardiomyocytes as indicated by decreased leakage of myocyte injury marker enzymes. Additionally, *C. alata* leaf extract protects the heart from inflammation and fibrosis caused by ISO.

Cytosolic enzymes such as CK, CK-MB and LDH, can also diagnose MI[18]. When myocardial cells are disrupted or destroyed by necrosis, the heart membrane becomes porous or ruptures, allowing enzymes to leak[19]. These enzymes enter the circulation, increasing their concentration in the serum[20]. In this work, an elevated CK, CK-MB and LDH in the serum of ISO-induced rats was found. Our report was similar to those of previous reports[21,22].

Interestingly, pre-treatment with *C. alata* leaf extract restored the ISO-induced elevation of serum levels of these diagnostic marker enzymes, implying that the efficiency of *C. alata* leaf extract lowers the degree of ISO-induced necrotic damage to the cardiac membrane. Furthermore, compared to ISO-treated rats, *C. alata* leaf extract pre-treatment followed by ISO significantly reduced pathological alterations such as loss of myofibrillar alignment, severe cytoplasmic vacuolization, myocardial degeneration and inflammatory cell infiltration (Fig. 4). The results of the current study showed the cardioprotective effect of *C. alata* leaf extract.

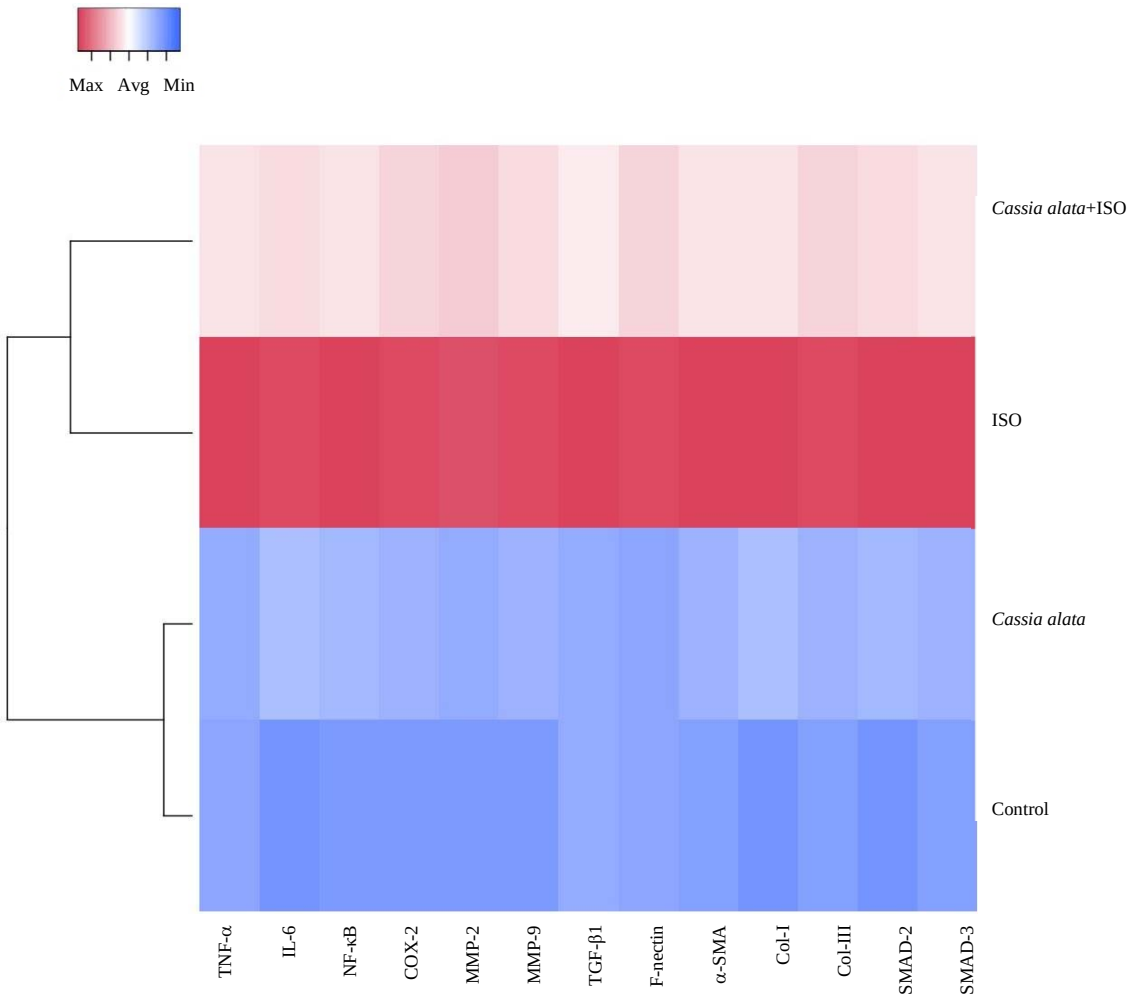

Fig. 4: Heat map analysis of PCR array results for inflammation and fibrotic gene expression in *Cassia alata* leaf extract and ISO-treated rats

Brown colour represents the upregulated expression, the blue colour represents the down-regulated expression and the white/brown colour represents a median expression of the given genes. The heat map results of three (n = 3) independent experiments were analyzed using the online tool

Cardiac troponin (I and T) levels are a highly sensitive and specific biochemical marker of myocardial cell injury, reflecting even on microscopic zones of myocardial necrosis[23]. In clinical studies, an increased cTnI and cTnT concentration, have been related to a four to five fold increase in the risk of MI[24]. The current study found serum cTn-I and T activity higher in ISO-induced rats than in normal control rats. The observed increase in troponin levels could be attributed to ISO-induced cardiac damage. Pre-treatment with *C. alata* leaf extract lowered serum cTn-I and T activity in ISO-induced rats. This decreased activity of these enzymes in ISO-induced animals could be attributed to *C. alata* leaf extract's protective action on the cardiac membrane, limiting the leakage of these suggestive enzymes.

Peroxidation of endogenous lipids may contribute significantly to the cytotoxic character of an excessive dose of ISO and has been associated with pathogenic outcomes such as myocardial necrosis and the formation of lipid hydroperoxides, indicating cardiac muscle injury[25]. As a result of isoproterenol autoxidation, many reactive oxygen species are produced[26]. They can have Polyunsaturated Fatty Acids (PUFAs) inside the membranes to be damaged, leading to the development of peroxyl radicals[26]. In the current investigation, ISO treatment resulted in a significant increase in lipid peroxidation, as measured by TBARS and LOOH levels, indicating oxidative stress. *Cassia alata* leaf extract pre-treatment resulted in a considerable reduction in TBARS and LOOH attributed to the *C. alata* leaf extract with a

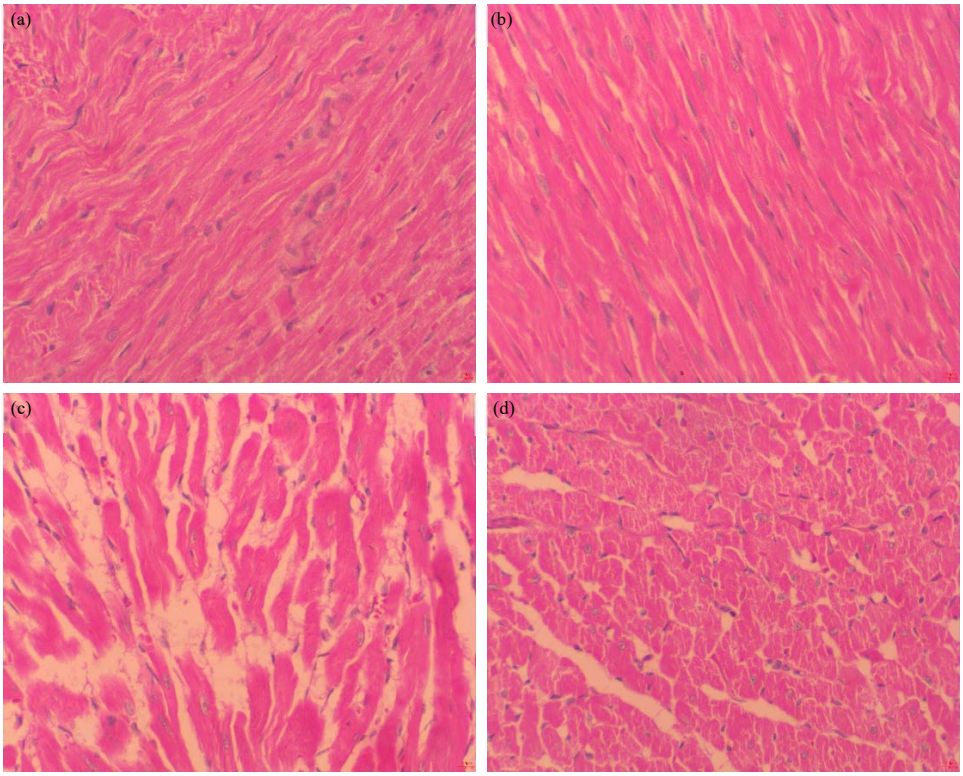

Fig. 5(a-d): Histopathological analysis of heart tissues from different treatment groups, (a) Control, (b) *Cassia alata* control, (c) ISO and (d) *Cassia alata* +ISO

cardioprotective effect by reducing oxidative stress in tissue. The presence of antioxidant substances in *C. alata* leaf extract is the key component that protects the organism from oxidative stress caused by free radicals[27].

The SOD, CAT, GPx, GR and GSH function as the first line of defence against oxidative damage by degrading O_2 and H_2O_2 before their interaction and formation of the more deadly hydroxyl radical[28]. It is essential to balance enzymatic antioxidants and free radicals when removing oxidative stress from intracellular organelles[29]. However, in pathological conditions such as MI, the formation of ROS can severely disrupt this equilibrium, increasing the load on the antioxidant defence system[30]. In this investigation, the enzymes SOD, CAT, GPx and GSH activities have significantly reduced in the heart of ISO-administered rats accommodating to the previous studies[31,32]. Increased use of these enzymes for scavenging ROS and inactivation by high ISO oxidants could explain the decline in their activity. In earlier research, *C. alata* leaf extract has significant free radical scavenging properties[13]. Likewise, pre-treatment with *C. alata* leaf extract increased SOD, CAT and GPx activity in the current study in ISO-injected rats. According to these findings, *C. alata* leaf extract could significantly boost cellular antioxidative defence against oxidative stress. It could function as a preventive antioxidant by scavenging superoxide anions or as a chain-breaking antioxidant by eliminating lipid-free radicals.

Oxidative stress increased the synthesis of pro-inflammatory cytokines, establishing a mechanistic connection between ROS overproduction and inflammation[33]. The production of reactive oxygen species (ROS) during MI increased the levels of pro-inflammatory mediators and induced NF-κB-mediated inflammation. ISO activates NF-κB via a β-Adrenergic receptor-dependent mechanism. The NF-κB is a crucial regulator of inflammation and apoptosis[34]. In patients with MI, phosphorylation of NF-κB results in the production of pro-inflammatory cytokines such as TNF-α, IL-6 and COX-2. Pre-treatment with *C. alata* leaf extract to ISO administered rats demoted NF-κB and TNF-α mRNA levels[35]. A considerable decrease in these inflammatory mRNA levels was observed with *C. alata* leaf extract in ISO-treated rats for the entire experimental period. The principal mechanism of cardio-protection is inhibition of inflammation by *C. alata* leaf extract, which may be attributed to anti-inflammatory, anti-atherogenic and anti-oxidant actions of *C. alata* leaf extract. It has been observed that the antioxidant activity of *C. alata* leaf extract is displayed in streptozotocin-induced diabetic rats by modulating the antioxidant activity[15].

The MMPs are proteases that are involved in the regulation and control of the extracellular matrix (ECM). They have been shown to accelerate the breakdown of the ECM, hasten the development of atheromatous plaque and produce unstable plaque[36,37]. Previously, it was observed that elevated MMP-2 and -9 expression during heart failure may result in cardiac myocyte death due to apoptosis, resulting in MI and heart failure[38]. Study observed in this study that treatment with *C. alata* leaf extract lowered MMP-2 and -9 expression via its antioxidant impact[39]. The preventive effect of *C. alata* leaf extract against MI may limit the expression of MMP-2 and -9, which attenuates MI and thereby maintains the normal functioning of the heart.

The TGF-β1 primarily communicates with cells via Smad proteins found in the cytoplasm. Smads translocate to the nucleus following phosphorylation, where they act as transcription factors and induce fibrous tissue deposition. Smad2/3 are required for the production of TGF- β1-induced genes in adult fibroblasts[40]. The TGF-β1 mRNA expression is enhanced in scars after myocardial infarction. It is associated with increased collagen I/III expression and Smad2/3 phosphorylation, implying that the TGF-β1/Smad2/3 signalling pathway may substantially influence myocardial fibrosis. We demonstrated that isoproterenol treatment increased TGF- β1 levels significantly, followed by an increase in Smad2/3 phosphorylation and collagen-I/III production. Pretreatment with *C. alata* leaf extract lowered TGF-β1 expression and prevented isoproterenol-induced increases in Smad2/3 phosphorylation and collagen I/III expression. These findings revealed that the pharmacological effects of *C. alata* leaf extract on isoproterenol-induced cardiac fibrosis and heart failure were mediated through modulation of the TGF-β1/Smad/Collagen pathway.

CONCLUSION

Pretreatment of *C. alata* leaf extract for 14 days improves ISO-induced cardiac inflammation and fibrosis. The pharmacologic actions of *C. alata* leaf are related to regulating the cardiac markers, cardiac antioxidant activity, inflammation and MMPs and TGF-β1/Smad/Collagen signalling pathways. These findings give us more information about the molecular mechanisms that make *C. alata* protective against MI.

SIGNIFICANCE STATEMENT

The above data indicate that the cardioprotective effects of *C. alata* leaf extract are mostly related to increased activity and suppression of the inflammatory response. This conclusion of this experiment would contribute to developing a novel cardioprotective drug that might be prescribed to treat MI patients in conjunction with a regular MI therapy regimen.

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