Research Article

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Daidzein Alleviates Isoproterenol-Induced Cardiac Injury in Rats Via NF-kB Pathway Inhibition

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ABSTRACT

The current study set out to explore the anti-inflammatory effects of Daidzein (Dz) on the isoproterenol (Iso)-induced myocardial infarction (MI) in male Wistar rats. Iso (80 mg/kg) was injected subcutaneously into rats to cause MI for two days at intervals of 24 hours. ELISA kits were used to measure the amounts of TNF-α & IL-6 in cardiac tissues. The quantity of NF-kB in the cytosol and nuclei was measured using a western blot approach. H&E staining was used to identify histological cardiac changes. In rats with Iso-induced myocardial infarction, Dz therapy shrunk the size of the infarct and reduced histological anomalies in the myocardium. Dz significantly lowered cardiac pro-inflammatory cytokine levels and prevented NF-kB nuclear translocation in MI-damaged rats, substantially reversing Iso-induced damage. This research shows that Dz treatment *in vivo* reduces Iso-induced myocardial damage by preventing NF-kB activation, which may therefore reduce the release of inflammatory cytokines.

Keywords: Daidzein; isoproterenol; myocardial injury; inflammation

1. Introduction

Cardiovascular disease (CVD) is a leading cause of mortality and morbidity worldwide [1]. Myocardial infarction (MI), also referred to as Angina pectoris occurs when blood circulation ceases or diminishes to a portion of the heart, harming the cardiac muscle. The most rampant symptom is pain or aching in the chest that may radiate to the arm, shoulder, back, neck, or jaw [2]. In 2019, 17 million people died from non-communicable diseases, with 38% of these deaths related to cardiovascular disease, according to the WHO. More than 75% of mortalities worldwide occur in low-income and middle-income nations [3]. The majority of cardiovascular illnesses may be avoided by controlling behavioral factors including smoking, an unhealthy diet that leads to obesity, drinking alcohol, and leading a sedentary lifestyle [4]. The major cause of heart attack and stroke is blood vessel obstruction, either from fatty deposits or thrombus development in the inner layer of blood arteries, which results in ischemia and an increase in blood pressure. There are more underlying causes for the disease to deteriorate as a result of ongoing cardiovascular medication therapy, which encourages CVD [5]. Therefore, treating CVD with a natural subtance that has a helpful role in totally healing the condition is the best option.

Numerous studies have shown the advantages of a polyphenol-rich diet in preventing chronic illnesses, particularly those with a component of inflammatory disease wherein reactive oxygen species are produced [6]. Since soybeans include necessary amino acids, lipids, protein, and advantageous secondary metabolites including phenolic and isoflavones compounds, they are regarded as a complete diet [7]. The possible health advantages of soy eating are assumed to be at least in part due to the isoflavones found in soybeans. According to epidemiological study, dietary soy proteins reduces the incidence of coronary artery disease, cancer, and osteoporosis [8]. When it comes to isoflavones according to current studies, Dz has positive effects on human well-being, including the chemoprevention of cancer and cardiac diseases.Dz (7-hydroxy-3-(four-hydroxyphenyl)-4h-chromen-4-one) is a certainly occurring compound discovered solely in soybeans and different legumes and structurally belongs to a class of compounds referred to as isoflavones.Dz and different isoflavones are produced in flora via the phenylpropanoid pathway of secondary metabolism and are used as sign providers, and defence responses to pathogenic attacks [9]. In human beings, current research has proven the viability of the usage ofDz in medicine for menopausal alleviation, osteoporosis, blood cholesterol, and decreasing the risk of a few hormone-related cancers.Dz and other isoflavones have been shown to mimic the pharmacological actions of the gonadal steroid estrogen with which they have structural similarities. Several studies have looked at the effect of isoflavones in the brain.Dz had a superior neuron-protective effect on lipopolysaccharide-induced dopaminergic neurodegeneration than other isoflavones. Researchers used amyloid-β and LPS to simulate chronic glial cell inflammation and analyzed the changes in the inflammatory cytokine, reactive oxygen species (ROS), and evaluated the effects of a selective estrogen receptor modulator,Dz, on inflammatory responses of primary astroglial cell culture It can also be used in place of estrogen substitute therapy to treat menopausal women who have lost bone density and prevent or treat osteoarthritis and osteoporosis [10].Dz treatment also triggered several morphological changes in SKOV3 cells, which are characteristic of apoptosis.Dz induces the apoptosis of SKOV3 cells. It was observed that Dz increased the DNA fragmentation, observed using DAPI, and led to an increase in orange fluorescence following AO/EB staining, which are changes indicative of apoptosis.Dz showed that treatment led to a significant increase in the apoptotic cell populations of SKOV3 cells. A clinical issue with treatments like coronary bypass surgery, angioplasty, thrombolysis, and transplantation that are frequently used to restore blood flow to the heart and lessen damage from severe myocardial ischemia is ischemic-reperfusion wreckage. The NF-kB (Nuclear factor-kappa B), as per several studies conducted, is crucial in myocardial damage [11]. As a result, the goal of the current work was to know, how Dz altered the activation of NF-kB in cardiac injured rat model. In this work we investigated that Dz inhibits the production of pro-inflammatory cytokines by restricting the nuclear translocation of NF-kB, hence reducing myocardial cell death. From the above literature survey Dz have been promising evidence of its pharmacological effects such as anti-inflammatory, anticarcinogenic and anti-oxidant activity. This study is to overcome the side effects of the current drugs in medical practice and to evaluate the cardio protective activity of Dz.

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2. Materials and methods

2.1 Drugs & Chemicals

We bought Dz and Isoproterenol from Sigma Aldrich Co. in St. Louis, Missouri, in the United States, and TNF-α & IL-6 ELISA kits from R&D Systems (Minneapolis).

2.2 Animals

For the experiments, thirty-two male Wistar-Albino rats, ranging between 180g to 200 g, were used. The rats were acclimatized to the lab environment at 25°C, 50°F, temperature and humidity, and an alternating 12-hour cycle of light and darkness. They were given unhindered admittance to continuous lab feed and water and spent a week acclimating to their surroundings in the animal house before the experiment. The procedure for experimenting complied with the standards set out by the CPCSEA (Committee for the Control and Supervision of Experiments on Animals), New Delhi, India. All the animal experiments were approved Institutional Animal Ethics Committee (IAEC) of JSSCP, Ooty. IAEC approval no. JSSCP/OT/IAEC/06/2019-20.

2.3 Induction of Experimental MI

Rats were subcutaneously administered Iso (80 mg/kg) in saline once every 24 hours for two days to create a MI model. Based on prior research and a fixation pilot trial, the Iso dosage was selected [12]. Abnormalities of electrocardiograph are the highest standards commonly used for definite identification of myocardial injury. The ST- segments signifies the interval between repolarization and ventricular depolarization. This study evaluated a ST-segment elevation after administering Iso.

2.4 Experimental Design

Rats (n=8) were divided into four groups each through random assignment after one-week acclimatization retro, and each group received the following care: Sham: For six weeks, animals were given distilled water (2 mL/kg), and then on the 43rd and 44th day, they were given an injection of regular saline (1 mL). Iso: For six weeks, animals were given distilled water (2 mL/kg), and then on the 43rd and 44th day, they were given a s.i. of Iso (80 mg/kg).Dz10 + Iso: Animals received an injection of Iso (80 mg/kg) on the 43rd and 44th days after receiving an oral pre-treatment of Dz (10 mg/kg) by oral gavage for 6 weeks.Dz20 + Iso: Animals received an injection of Iso (80 mg/kg) on the 43rd and 44th days after receiving an oral pre-treatment ofDz (20 mg/kg) for 6 weeks.

Based on earlier research from the literatures and after performing acute oral toxicity studies as per OECD 423 guidelnes, the Dz dosage was chosen. Rats were sedated and slaughtered after completion of the experiment. 5% isoflurane was used in high dose as for euthanising the animals. The serum was separated by centrifugation technique. The heart tissues were isolated after blood was drawn, and they were then thoroughly rinsed twice in ice-cold phosphate buffer saline (PBS) [13,14]. The samples were homogenized in phosphate buffer (pH 7.4) to provide 10% w/v homogenates. The homogenates were then spun for 10 min at 2000 rpm, and the supernatant was collected and stored at -20 °C for biochemical analysis. Then for histological analysis, 10% formalin was used to preserve a small cardiac sample.

2.5 Histopathology Studies

The cardiac tissue was instantly preserved in 4% formalin, treated with ethanol, and fixed to paraffin wax after the heart tissues were swiftly removed and emptied its content in a falcon tube. Hematoxylin and eosin (H&E) were used to stain the heart tissue. A 100x light microscope was used to see the findings.

2.6 Determination of Pro-Inflammatory Cytokines in Heart

Following the manufacturer's directives, ELISA kits from R&D Systems (MN, USA) were used to do an ELISA of TNF-α & IL-6 in the cardiac homogenate. The absorbance was determined at 450 nm with a multiple modes plate reader (Infinite M200 Pro, Tecan, Switzerland), and the cytokine levels were calculated as pg/ml of tissue.

2.7 Western blotting

Proteins were separated using polyacrylamide electrophoresis (SDS-PAGE), placed on nitrocellulose membranes, and then incubated at 4 °C with monoclonal NF-kB p65 antibodies for a night. A 2° antibody affixed to horseradish peroxidase was used to identify proteins using an improved chemiluminescence approach (5% skim milk powder diluted 1:5000 in TBS-T and incubated for an hour at ambient temperature).

2.8 Statistical Analysis

The one-way ANOVA method was employed to evaluate the datas, which were all reported as mean±SD. A Dunnett's post hoc analysis was performed thereafter to ascertain the statistically significant distinction between the groups. Utilising GraphPad Prism 8 (GraphPad Software, USA), the analysis was completed statistically. Each test was considered significant if the p-value < 0.001.

3. Results

3.1 Effect ofDz on Inflammatory cytokines in Iso-induced Rats

Figure 1 shows the levels of inflammatory cytokines including TNF-α & IL-6 in cardiac tissues. The s.i. of Iso dramatically boosted the secretion of cytokines that trigger inflammation in the heart in contrast to the Sham group. (p< 0.001). Dz therapy significantly (P< 0.001) reduced the Iso-induced raise in cardiac TNF- α & IL-6 in this MI rat model when compared with Iso group (Diseased control).

3.2 Effect ofDz on NF-kB

The NF-kB complex was translocated from the cytoplasm to the nucleus during MI, as seen in Figure 2. However, Dz administration decreased NF-kB translocation inside the nucleus was in a dose-dependent means.

3.3 Histopathological evaluation of cardiac cells

The control group showed normal morphology of cardiac cells as the presence of a lesion and an increase in cardiac muscle fiber destruction and hypertrophy was shown in the Iso group. There were trifling morphological changes detected in the low dose ofDz treated group and remarkable improvements were seen at high doses ofDz shown in Figure 3.

4. Discussion

The major cause of mortality from cardiovascular disease is MI. When a blood clot blocks blood flow to the heart, MI typically results. Without blood, tissues start to lose oxygen and die. Because of population aging, it is anticipated that myocardial infarction would become more common in the future [15]. The overproduction of free radicals brought on by isoproterenol induction can lead to oxidative stress, cell damage, and ultimately myocardial infarction [16]. Extreme dosages of isoproterenol cause myocardial damage, necrosis, fibroblastic hyperplasia, hypoxia, and impaired inhibition of systolic and diastolic function, in addition to other side effects. Myocardial compliance, which closely resembles local myocardial damage, is typically employed to research the effects of ischemia and cardio-protecting medicines on the myocardium [17]. Dz was previously studied for its potential pharmacological effects such as anti cancer activity, neuroprotective, anti oxidant, meno-

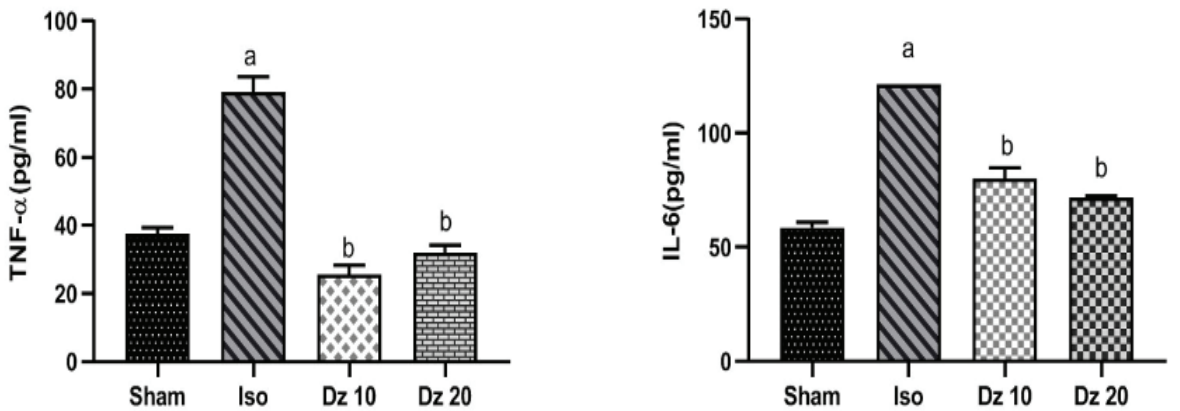

Figure 1. Expression levels of (A) cardiac tumour tissue necrosis factor (TNF-α), and (B) interleukin-6 (IL-6) varied significantly across the groups. The statistical significance were performed using One-way ANOVA followed by Dunnett's test in Graph Pad Prism 8.0 software. Results were presented as mean ± SD, n=8, with a p-value < 0.001, [a] represents Sham group vs Iso group, and [b] represents Iso group vs Dz treated groups.

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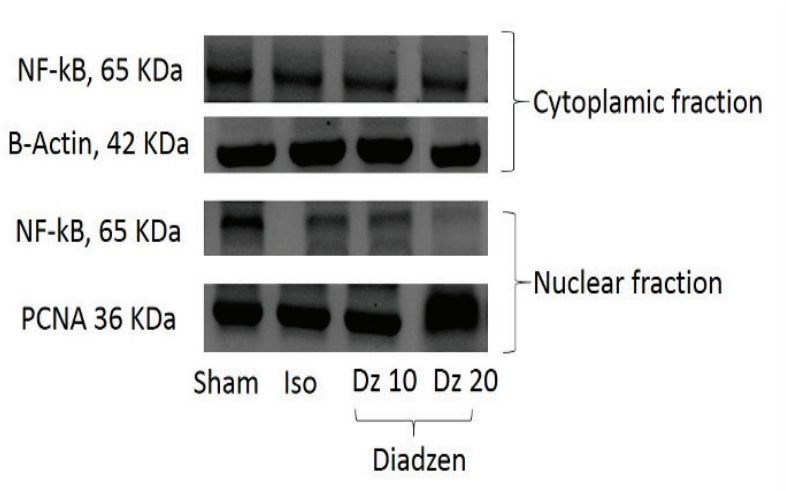

Figure 2. Effect of Dz on MI-induced NF-kB nuclear translocation. Two hours following the start of reperfusion, the nuclear and intracellular fractions of each group's hearts were isolated separately, and NF-kB was discovered as described in the Methods. To verify the quality of the nuclear extracts (NE) and cytoplasmic extracts (CE), gels were once again probed with abs against the cytoplasmic marker β-actin and the nuclear marker PCNA. Blots are pictures of four groups that had the same outcomes.

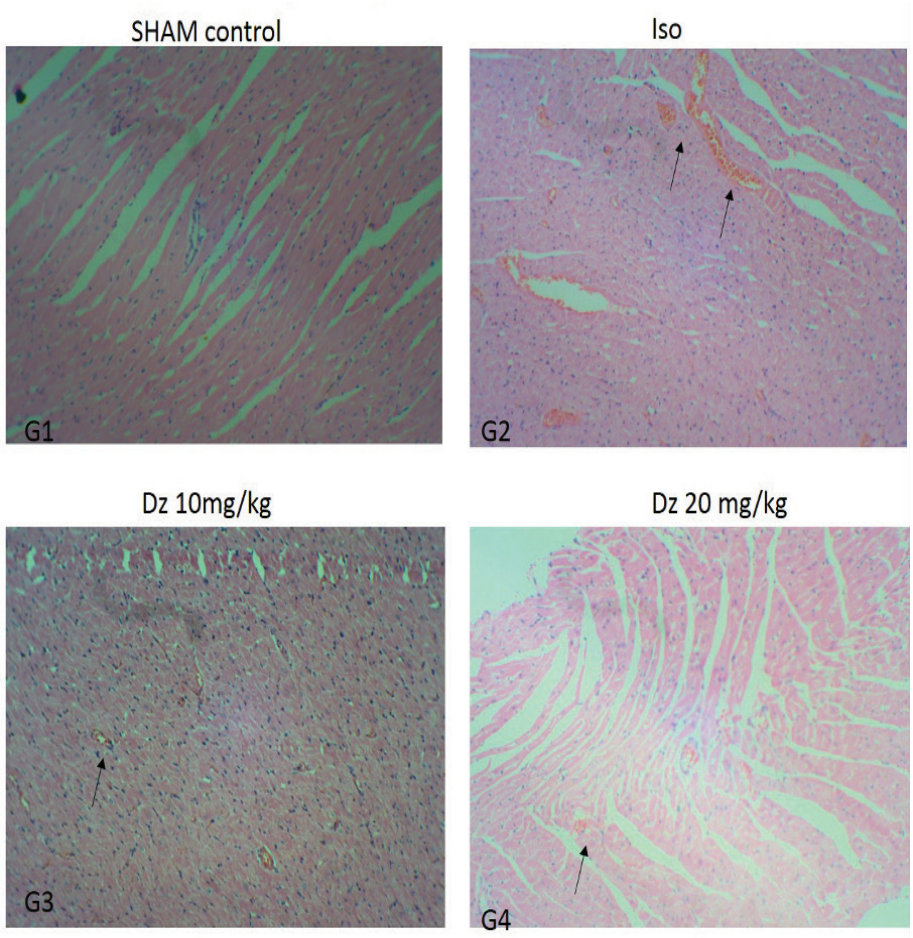

Figure 3. A representative micrograph of the myocardium from MI treated with Iso and with Dz (10 and 20 mg/kg) is displayed. This was done 24 hours after reperfusion was achieved. The Iso group displays edema, hypertrophy, and fibre breakdown in the cardiac muscle fibres, but the Dz (10mg and 20mg/kg)-treated group only displays very minimal histological changes.

pausal alleviation. In the current study, we found that Dz protects against myocardial infarction by lowering NF-kB nuclear translocation, which functionally normalizes levels of inflammatory cytokines. As per previous research low dose of Dz for about 6 weeks was found to be effective in reducing inflammatory responses than giving high dose of Dz for shorter duration [18]. The presence of higher levels of inflammatory cytokines, which have long been linked to acute MI, serves as evidence for our findings. By monitoring the concentrations of these cytokines, one may gauge the extent and severity of the acute MI. As a result, acute MI can be identified by cytokine detection in serum. Infarcts and overexpression of inflammatory mediators can both be detrimental to heart health. According to research,Dz lowers the levels of pro-inflammatory cytokines (TNF-α & IL-6) in rats with MI. The injection of Iso increases the levels of pro-inflammatory cytokines such as TNF-α & IL-6 in group II animals. The cardiac cells in theDz at 10 & 20 mg/kg treated rats significantly recovered from the damage induced by Iso, according to histopathological analysis. Dz treatment links rats with rat MI caused by ISO to Dz's dose-dependent anti-inflammatory action.

5. Conclusion

Dz reduced infarct size and pro-inflammatory cytokine expression, demonstrating its cardioprotective effect. The expression of pro-inflammatory cytokines (TNF-α & IL-6) was also decreased thus had an anti-inflammatory effect. The cardioprotective effect of Dz may be ascribed to a reduction in the cellular levels of NF-kB and suppression of their translocation into the nucleus thereby reducing pro-inflammatory cytokines in cardiac cells. This study is limited with pro inflammatory cytokines, NF-kB levels and histological studies (Approved by IAEC of JSSCP, Ooty. IAEC approval no. JSSCP/OT/IAEC/06/2019-20). Further research can be directed towards estimating cardiac markers like CK-MB, cardiac troponin, toxicity profiling, clinical trials on Dz, formulation of Dz into suitable dosage forms either as transdermal patches or oral dosage forms and check its pharmacokinetic profiles.

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Conflict of Interest

The authors declare no conflict of interest.

Statement of Contribution of Researchers

Concept, Design, Supervision — R.V.; Materials; Data Collection and/or Processing, Writing, Analysis and/or Interpretation — V.S.; Literature Search, Critical Reviews — T.M., R.T., H.M.

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