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Evaluation of Cardiomyopathic Effect of Naringin and Sinapinic Acid

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KEYWORDS

Sinapinic Acid, Naringin, Cardiomyopathy, Streptozotocin, Creatine kinase.

ABSTRACT:

Introduction: Diabetic cardiomyopathy is the result of diabetes-induced cardiac injury, which is intimately linked to the production of oxidative stress and inflammation. Both Naringin (NA) and Sinapinic Acid (SA) have anti-inflammatory and antioxidant properties. It is unknown, nevertheless, if NG and SA can prevent diabetic cardiomyopathy by controlling inflammation and oxidative stress.

Objectives: This study used an analysis of type 1 diabetic mice induced with streptozotocin (STZ) to examine the effects of NG and SA on diabetic cardiomyopathy.

Methods: In this technique, Wistar rats each weighing 150 to 200 gm of body weight were utilized for performing the experiment. Experimental animals were segregated into five groups, each group containing six animals. 30 minutes prior to the treatment of Naringin, SA and Glibenclamide, all the experimental animals were administered with 2 g/kg of glucose substrate solution through oral route of administration. After 28th day, heart was separated from all the animals, the tissue was homogenated and then extract the supernatant liquid by employing centrifugation process. Supernatant liquid was utilized for estimating Total Creatine kinase (CK), Creatine kinase isomerize (CK-MB) and Troponin-I enzymes. These enzymes were estimated employing commercial kits (Biosystems) in semi-auto analyzer. For Histological analysis, the animal cardiac tissue was isolated and thoroughly rinsed with normal saline solution and immersed in formalin (10%). The excised specimens were then embedded in liquid paraffin and were appropriately sectioned into 5 µm thickness. The fixed myocardial sections were stained by employing eosin and Heamatoxylin.

Results: Diabetic cardiomyopathy was specifically characterized primarily by diastolic and then by systolic dysfunction along with intracellular potassium loss and calcium and sodium ions retention. STZ showed increased Total CK, Troponin-I and CK-MB when correlated with normal values. Increased levels of enzymatic biomarkers such as Total CK, Troponin-I and CK-MB was observed in STZ administered experimental rats when correlated with normal animal groups. Histopathological analysis of Naringin and SA against diabetes induced by STZ in rat myocardium was studied. STZ induced diabetes in experimental rats showed a large infarct area with predominant lymphocytic fibrosis and infiltration. Treatment with Naringin and SA dose-dependently reversed these morphological manifestations. Control group animal cardiac tissue demonstrated a regular cardiac cells arrangement.

Conclusions: Naringin at a dose of 100 and 200 mg/Kg showed significant reduction of cardiac biomarkers in STZ induced diabetes in experimental rats. SA at a dose of 150 and 300 mg/Kg showed significant reduction of cardiac biomarkers in STZ induced diabetes in experimental rats.

1. Introduction

One of the most frequent consequences of diabetes mellitus is heart failure, and diabetes itself is a separate risk factor for heart failure. According to the Framingham research, the risk of developing heart failure is five times higher in women with diabetes and two

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times higher in men with diabetes compared to those without the disease [1]. Conversely, 30–40% of people with diabetes also have complications [2], indicating that diabetes and heart failure are closely related.

In addition to the complication of ischemic heart disease, metabolic disorders such as glucose toxicity and lipotoxicity based on insulin resistance, vascular endothelial dysfunction, microcirculatory disorder, and capillary failure are the basis of the relationship between diabetes and heart failure. There are several systems at work. "Diabetic cardiomyopathy" is the term that has been suggested to describe cardiac dysfunction when there is no major valvular heart disease, hypertension, or coronary artery disease [3,4,5, 6]. Its existence has been contested for a long time, nevertheless [4,7,8]. The 2013 Heart Failure Guidelines from the American College of Cardiology (ACC) and American Heart Association (AHA) include information on diabetic cardiomyopathy [9]. However, diabetic cardiomyopathy has not been proven to be a distinct clinical entity, and further research is anticipated, according to the most recent guidelines on diabetes and cardiovascular disease published by the European Society of Cardiology (ESC) and the European Society of Diabetes (EASD) [2]. In patients without diabetes, the illness may be referred to as lipotoxic cardiomyopathy or obesity-related cardiomyopathy because patients with obesity, insulin resistance, and dyslipidemia exhibit similar heart dysfunction [10].

Numerous naturally occurring flavonoids have demonstrated strong cardioprotective effects [11, 12]. Numerous lines of evidence have indicated the beneficial impact that grapefruit's rich flavonoids, SA and NG, play in the cardiovascular system. It has been demonstrated that NG and SA lessen heart damage after ischemia-reperfusion injury by preventing damage from mitochondrial oxidative stress [13]. NG and SA prevented septic cardiac failure in the mouse sepsis model by reducing NF-κB-dependent cardiac inflammation [14]. Additionally, in rat models, NG and SA therapy reduced cardiac cell death caused by ischemia-reperfusion injury [15]. These findings imply that the cardioprotective mechanism of NG and SA may entail anti-inflammatory and antioxidant characteristics.

Thus, using STZ-induced diabetic mice as a model, this work examined the effects of NG and SA on diabetic cardiomyopathy and associated signaling pathways.

2. Objectives

To compare the experimental results of anti-hyperglycemic potential of selected phytochemical compounds in streptozotocin induced diabetic rats and to assess the protective efficacy of selected phytochemical compounds for diabetes associated cardiomyopathy.

3. Methods

Evaluation Cardiomyopathic Effect

Experimental procedure for hypoglycemic effect of Naringin and SA on glucose loaded rats

Adiga,Shalini (2010) proposed an experimental procedure for hypoglycemic activity in the rats loaded with glucose substrate [16]. In this technique, Wistar rats each weighing 150 to 200 gm of body weight were utilized for performing the experiment. Experimental animals were segregated into five groups, each group containing six animals. 30 minutes prior to the treatment of Naringin, SA and Glibenclamide, all the experimental animals were administered with 2 g/kg of glucose substrate solution through oral route of administration. Blood samples were collected by puncturing the tail vein and the blood glucose level was estimated at various time points like 0, 10, 30, 60, 120 and 240 minutes correspondingly after glucose substrate administration.

Experimental animal groups and substance administered with were mentioned below:

Groups Treatment

Group I : Control

Group II : Glucose substrate solution (2gm/Kg)

Group III : Glibenclamide (10 mg/kg)

Group IV : Naringin(100 mg/kg)/SA(150 mg/kg)

Group V : Naringin(200 mg/kg)/SA(300 mg/kg)

The percentage of glucose level change in every group was determined with reference to initial value and was calculated using

% glucose change = G0 – Gt X 100/Go

Whereas Go represents 0-hour glucose value and Gt represents glucose level at the treatment end.

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Cardiomyopathic effect of Naringin and SA in STZ induced diabetic rats

After completing estimation of antihyperglycemic activity at 28th day, heart was separated from all the animals, the tissue was homogenated and then extract the supernatant liquid by employing centrifugation process. Supernatant liquid was utilized for estimating Total Creatine kinase (CK), Creatine kinase isomerize (CK-MB) and Troponin-I enzymes. These enzymes were estimated employing commercial kits (Biosystems) in semi-auto analyzer.

Histological analysis of cardiomyopathic effect of Naringin and SA on STZ induced diabetic rats

After 28th day, the experimental animals were euthanized humanely through cervical dislocation technique [17]. The animal cardiac tissue was isolated and thoroughly rinsed with normal saline solution and immersed in formalin (10%). The excised specimens were then embedded in liquid paraffin and were appropriately sectioned into 5 µm thickness. The fixed myocardial sections were stained by employing eosin and Heamatoxylin.

4. Results

Evaluation of Cardiomyopathic Effect

Cardiomyopathic effect of Naringin on STZ induced diabetes in experimental rats

The cardiomyopathic effect of Naringin on STZ induced diabetes in experimental rats was performed in accordance with the method specified in chapter 3.

Table 1. Effect of Naringin on cardiac Total CK, CK-MB and Troponin-I levels

S.No	Treatment	Troponin-I (ng/mL)	CK-MB (U/L)	Total CK (U/L)
1	Control	0.18±0.02	329 ±9.8	64±3.4
2	STZ (60 mg/kg)	1.32±0.01	469 ± 12.4	160±7.4
3	Naringin (100 mg/kg)	1.05±0.01	409 ±13.2	98±4.5
4	Naringin (200 mg/kg)	1.02±0.02	402 ±19.3	89±4.8

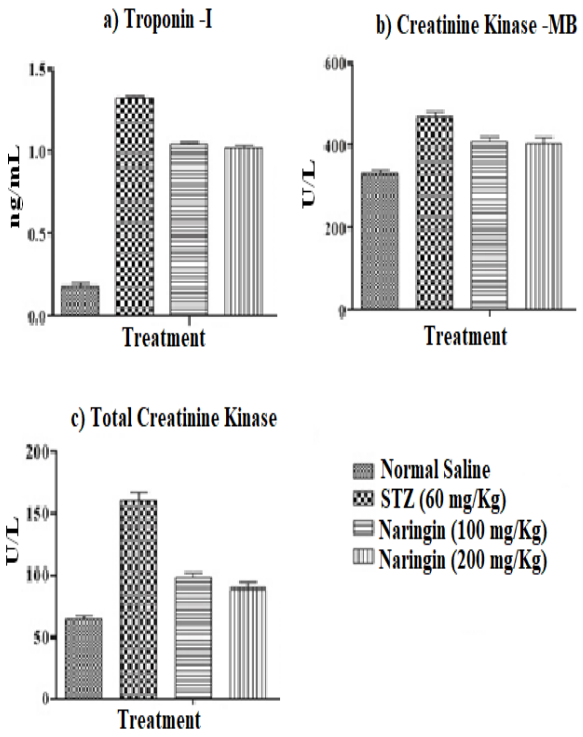

Figure 1. a) Indicates Troponin-I levels, after administering 2 different doses of Naringin on Streptozotocin (STZ) induced diabetes in experimental rats. On the other hand, Creatinine Kinase-Mb and Total Creatinine Kinase were presented as b and c correspondingly. Naringin was loaded at doses 100 and 200 mg/Kg to the respective groups. The obtained values were demonstrated as Mean ± SEM; By using Dunnett's test, one-way ANOVA significance was calculated. The obtained results were compared with the values of diabetic rats.

Histological analysis of cardiomyopathic effect of Naringin on STZ induced diabetes in experimental rats

Histopathological analysis of cardiomyopathic effect of Naringin on STZ induced diabetes in experimental rats was performed in accordance with the method specified in chapter 3.

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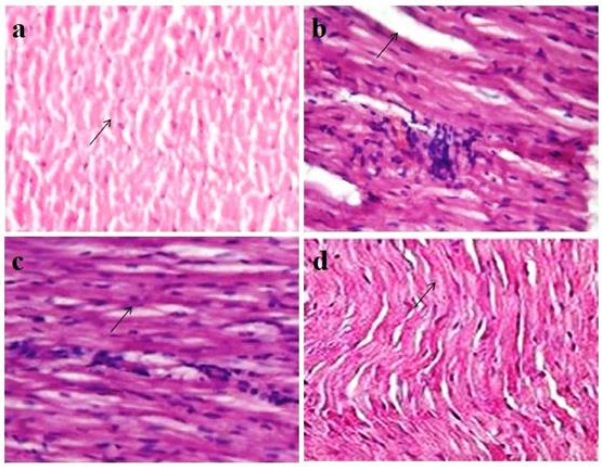

Figure 2. Histopathological analysis of Naringin on STZ induced diabetes in experimental rat's myocardium using H & E staining technique. Morphological study of myocardium was studies and pictorial representation were given as a for control and b for 60 mg/Kg streptozotocin induced diabetic animals. Administration of Naringin at 2 different doses 100 and 200 mg/Kg was presented as c and d.

Cardiomyopathic effect of SA on STZ induced diabetes in experimental rats

The cardiomyopathic effect of SA on STZ induced diabetes in experimental rats was performed in accordance with the method specified in chapter 3.

Table 2. Effect of SA on cardiac Total CK, CK-MB and Troponin-I levels

S.No	Treatment	Troponin-I (ng/mL)	CK-MB (U/L)	Total CK (U/L)
1	Control	0.18±0.02	329 ±9.8	64±3.4
2	STZ (60 mg/kg)	1.32±0.01	469 ± 12.4	160±7.4
3	SA (150 mg/kg)	1.01 ± 0.02	402 ± 19.2	97 ± 8.3
4	SA (300 mg/kg)	1.02 ± 0.01	401 ± 20.9	86 ± 7.5

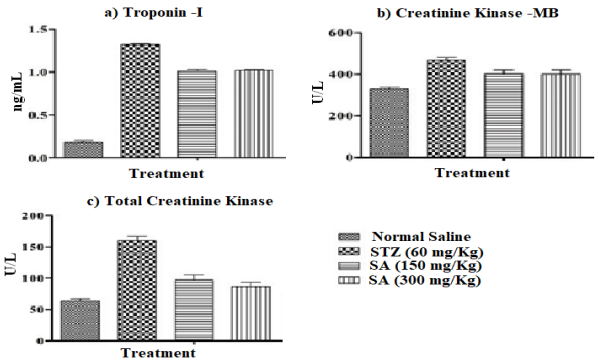

Figure 3. a) Indicates Troponin-I levels, after administering 2 different doses of SA on Streptozotocin (STZ) induced diabetes in experimental rats. On the other hand, Creatinine Kinase-Mb and Total Creatinine Kinase were presented as b and c correspondingly. SA was loaded at doses 150 and 300 mg/Kg to the respective groups. The obtained values were demonstrated as Mean ± SEM; By using Dunnett's test, one-way ANOVA significance was calculated. The obtained results were compared with the values of diabetic rats.

Histological analysis of cardiomyopathic effect of SA on STZ induced diabetes in experimental rats

Histopathological analysis of cardiomyopathic effect of SA on STZ induced diabetes in experimental rats was performed in accordance with the method specified in chapter 3.

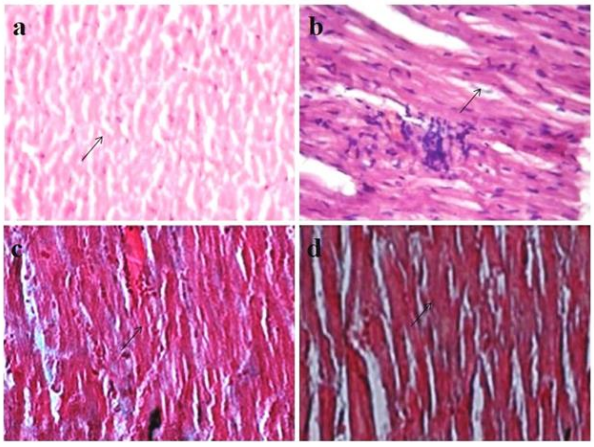

Figure 4. Histopathological analysis of SA on STZ induced diabetes in experimental rats myocardium using H & E staining technique. Morphological study of myocardium was studies and pictorial representation were given as a for control and b for 60 mg/Kg streptozotocin induced diabetic animals. Administration

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of SA at 2 different doses 150 and 300 mg/Kg was presented as c and d.

5. Discussion

Diabetic cardiomyopathy was specifically characterized primarily by diastolic and then by systolic dysfunction along with intracellular potassium loss and calcium and sodium ions retention. Lack of glucose supply and oxygen supply can cause severe damage to myocardium which leads to increased permeability and then causes rupturing, so that the biological enzymes get leaked out. These biological enzymes were also referred as specific biomarkers, could be used to estimate the level of damage. STZ showed increased Total CK, Troponin-I and CK-MB when correlated with normal values. Earlier research studies suggested that the cardiomyopathy induced by STZ was one among the reliable experimental animal models for the screening of cardiomyopathy. Increased levels of enzymatic biomarkers such as Total CK, Troponin-I and CK-MB was observed in STZ administered experimental rats when correlated with normal animal groups. Histopathological analysis of Naringin and SA against diabetes induced by STZ in rat myocardium was studied. STZ induced diabetes in experimental rats showed a large infarct area with predominant lymphocytic fibrosis and infiltration. Treatment with Naringin and SA dose-dependently reversed these morphological manifestations. Control group animal cardiac tissue demonstrated a regular cardiac cells arrangement. In the same manner, there were alterations in myocardium tissue parameters examined after inducing diabetes on 28th day, indicating cardiomyocyte cellular damage which was caused by hyperglycaemia. Treatment of Naringin and SA for 28 days orally, significantly decreased cardiomyopathic effect in STZ induced diabetes experimental rats.

Additionally, cardiac tissue biomarkers such as Total CK, Troponin-I and CK-MB levels were estimated. These parameters were very much controlled at high dose of Naringin (200 mg/Kg). Experimental animals with STZ induced diabetes showed increased levels of Total CK, Troponin-I and CK-MB when correlated with normal control group. These specific tissue biomarkers such as Total CK, Troponin-I and CK-MB levels were reduced from 160 to 89 U/L, 1.32 to1.02 ng/mL and from 469 to 402 U/L correspondingly after administrating 200 mg/Kg Naringin when correlated with diabetic experimental rats.

Concurrently, cardiac tissue biomarkers such as Total CK, CK-MB and Troponin-I levels were reduced from 160 to 89 U/L, 469 to 402U/L and 1.32 to1.02 ng/mL correspondingly after administering 300 mg/Kg SA when correlated with control group. These specific biomarkers were noticed with great decrease in non-treated diabetic experimental rats. Treatment with 300 mg/Kg SA significantly reduced Total CK, CK-MB and Troponin-I levels when correlated with diabetic control group.

Haematoxylin and Eosin (H&E) stained myocardium sections of control group rats demonstrated well-arranged cardiac cellular structure and normal striations. Myocardial section of STZ induced diabetic experimental rats depicted disorganized cardiac cellular structures along with destroyed striations. Myocardial sections of experimental rats exhibited much better striations in a dose-dependent manner after treatment with 200 mg/Kg and 300 mg/Kg Naringin and SA correspondingly.

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