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Co-Administration of Naringenin with Isoproterenol Alters Cardio-Hepatic Bioactivity in Experimental Animal Models

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ABSTRACT

Isoproterenol is a beta-1 adrenergic agonist that has over time been used in the management of cardiovascular cases. Above optimal dosage, it may lead to altered liver function and cardiac activity shown by variations in heart and liver biomarkers. This study aimed at investigating the effect of co-administration of isoproterenol and naringenin on cardio-hepato activity and when administered at separate treatment periods. Female Wistar rats were randomly divided into four groups of five rats. Group A (Control) received normal saline, group B received 18mg/kg body weight of Isoproterenol in saline (given subcutaneously, for one week), Group C received 18mg/kg of isoproterenol and naringenin (40mg/kg orally), Group D received Isoproterenol (18mg/kg) for one week and supplemented with naringenin for two weeks. Treatment lasted for 21days. Blood samples were collected for biochemical analyses, heart and liver were collected for histological analyses. Plasma Creatine kinase, Troponin, Lactate dehydrogenase, Malondealdehyde, liver enzymes, all increased in isoproterenol administration alone; dropped with co-treatment with naringenin except for ISO+NRG, but increased in activity at 1week isoproterenol treatment and then followed by two weeks naringenin treatment. Histological alterations were prominent in heart and liver of rats in all treatment groups showing moderate aggregate of myocardial inflammation with loss of cardiac tissues and also infiltration of inflammatory hepatic cells with periductal fibrosis. This results suggest that co-administration of isoproterenol with naringenin has in vivo efficacy and has the potential to improve cardiac-hepatic function, but may fail to ameliorate tissue injury at separate treatment periods.

Keywords: Isoproterenol, Troponin, Naringenin, Creatine kinase, liver enzymes, heart.

INTRODUCTION

Isoproterenol ([1-(3-4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride)] is a synthetic catecholamine and a non-selective β-1 adrenergic agonist that is used in the management of cardiovascular disease. However, adverse effect has also been reported especially in cases of overdose and acute toxicity (1). The adverse effect range from severe stress in the myocardium resulting in compromised cardiac integrity and expression of infarct like-necrosis [2] to subsequent generation of cytotoxic free radicals via autoxidation of cathecholamines [3]. In event of overdose, contraindications or side effect occur such as Hepatotoxicity and glycogenolysis, activation of renin angiotensin –aldosterone system in the Kidney[4,5] intracellular depletion of ATP , elevated levels of calcium which activates beta-1 adrenergic receptor, increased myocardial cAMP and altered membrane permeability[6 ,7]

with a resultant increase in cardiac-specific troponins, troponin I (cTnI) and troponin T (cTnT) considered as biochemical markers in myocardial infarction, and unstable angina[8,9]

Generally, experimental findings have demonstrated that the actions of Isoproterenol (ISO) via stimulation of the adrenergic receptors brings to bare the main index of the pathogenesis of myocardial hypertrophy. This has been partly attributed to enhanced protein synthesis and cardiac mass and exposes such vulnerable heart to ischemic injury [1]. However, in order to ameliorate toxicity often caused by drugs and their negative physiological impact in the body, the use of plant/herbal product is gradually being considered as an alternative medicine. The high-profile phytochemical components in such plants and their antioxidant and anti-inflammatory activity [10,11] mediates their beneficial responses and essential for their protective function. Naringenin, a naturally-occurring flavonoid of the flavanone subclass is predominantly found in citrus fruits and tomatoes [12,13] and because of its dietary antioxidant property, it has received a special attention. Naringenin exhibits various pharmacological and therapeutic properties including anti-mutagenic, anti-inflammatory and free radical scavenging activity [14,15]. The aim of this research work therefore, was to find out the bioactivity of naringenin in isoproterenol-induced cardio-hepatic toxicity in experimental animal models.

EXPERIMENTAL DESIGN & METHODS

Animals

Animals were obtained from the Department of Physiology, Faculty of Basic Medical Sciences, Cross River University of Technology, Calabar, Nigeria. The animals were kept in standard cages and under standard conditions with 12-h light/dark cycle, at 36°C ± 4°C. All the rats had free access to food and water ad libitum. Ethical approval for the study and use of Laboratory Animals was obtained from the Faculty of Basic Medical Science Animal Research Ethical Committee of Cross River University of Technology, Calabar, Nigeria. ***Approval number*** (CRUTECH/FBMS/NIG/REC./2023/25).

Experimental Design

Acclimatization period for the female Wistar rats was one week after which the animals (weighing between 180-200g) were randomly selected and put into four cages containing five rats each. Group A was Control. Group B received 18mg/kg body weight of Isoproterenol (subcutaneously) only (ISO). Group C received 18mg/kg of Isoproterenol + 40mg/kg of naringenin orally (ISO + NRG) (co-administration). Group D received 18mg/kg of ISO for 1week and then NRG 40mg/kg body weight for two weeks (ISO 1wk + NRG 2wks). Isoproterenol hydrochloride at 18mg/kg dissolved in normal saline (0.9 NaCl) was administered subcutaneously for one week. The animals in group D were treated with isoproterenol for one week after which NRG (40 mg/kg orally) was supplemented. After the last treatment of ISO and NRG, animals were anesthetized and blood was immediately collected by cardiac puncture into blood sample bottles and centrifuged at 1000 rpm for 10 minutes. The serum obtained was refrigerated at 4°C and used for various biochemical evaluations.

Biochemical Analysis

Blood samples collected by cardiac puncture were put in plain bottles while the organs collected were stored in tissue sample bottles and stored at 10^{o} C and later used for biochemical analyses.

Troponin, CK-MB and lactate dehydrogenase as well as liver enzymes such as Aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alanine phosphatase (ALP), were determined using commercially available kits.

Serum Cardiac Marker Enzymes

The activity of serum cardiac marker enzymes like cardiac troponin C (cTnC), lactate dehydrogenase (LDH) and creatine kinase-MB (CK-MB) were assessed to check the cardiac function and integrity. Serum levels of myocardial-damage enzyme markers were measured from blood samples collected by cardiac puncture from the rat at the time of euthanasia. Creatine phosphokinase (CK) (EC2.7.3.2) and its heart isoenzyme (CK-MB), as well as Troponin were determined using conventional diagnostic kits. Aspartate aminotransferase (AST, EC 2.6.1.1), other liver enzymes and lactic dehydrogenase (LDH, EC1,1,1,27) were measured by the method of (16,17).

Malondialdehyde:

Plasma MDA was estimated by method of (18). After the reaction of thiobarbituric acid with malondialdehyde, the reaction product was extracted in butanol and was measured.

Histological Studies Tissue Processing for Histology

Following fixation, paraffin embedding and 4μm tissue sections (heart and liver) were obtained with the Reichert Jung 2050 rotary microtome, followed by Haematoxylin and Eosin staining procedures. A light microscope was used to examine the slides, and photomicrographs taken with a digital camera (19). The slides were viewed at magnification of X 400 and photomicrographs were taken.

STATISTICAL ANALYSIS

Statistical analysis was performed using Graph pad version 5.0. Results obtained are expressed as mean±SEM. Comparisons between multiple groups were assessed using one-way analysis of variance (ANOVA) and a post hoc test using Bonferroni multiple range test to detect significant differences between groups. Statistical significance was accepted at the level of $P<0.05$.

DISCUSSION

The use of isoproterenol ([1-(3-4-dihydroxyphenyl)-2-isopropylaminoethanol hydrochloride)] in the treatment of myocardial infarction and other related cardiovascular issues has being on for years. It is a synthetic catecholamine, a βeta-1 adrenoceptor agonist found predominantly in the heart. Apart from its role in the treatment of cardiovascular disease, such as congestive heart failure and cardiogenic shock [1], our interest was to examine the possible ameliorative effect of naringenin on the possible damage often caused by the drug (isoproterenol) in the heart and liver in adverse dosages as well as alterations in cardiac enzyme activity and hepatic biomarkers that occur in cardiovascular damage.

In this study, we have shown that high dose administration of isoproterenol induced cell oxidative stress. Usually, persistent tissue stress triggered by disoriented mitochondrial membrane and catecholamine auto-oxidation [20,21,22] results in a geometric generation of free radicals or reactive oxygen species which induce tissue injury and may lead to the initiation of pathologic alterations in the myocardium, including inflammation and cell death. This

eventually leads to the development of cardiac dysfunction [23,24]. One of the well known mechanism by which this occurs is through the activation of NF-κB and may trigger the stimulation of pro-apoptotic factors in cardiomyocytes as well as the hepathocyes, mediating cell death [25]. This was evident in this study by the increased activity of malondealdehyde. To mitigate against oxidative damage therefore, there has to be certain approaches to protect the myocardium and hepatocytes from isoproterenol induced damage. In the light of the above, this study showed that concurrent treatment of the animals with ISO and Naringenin greatly attenuated oxidative stress and promoted the antioxidant defense system in the myocardium and hepatocytes.

Furthermore, from our results, we noted a significant increase in troponin, creatine kinase (Ck-MB), and lactate dehydrogenase activity with isoproterenol administration. Various experimental reports have shown that all of these endogenous myocardial biomarkers stand out as evidences of cardiac damage and serves as surrogate markers for heart or cellular injury [26, 27]. Results obtained in this study agrees with an earlier reported work that documented that high dose of ISO injection would trigger excessive generation of free radicals [28], induce cardiac tissue injury evidenced by increased serum creatine kinase-MB (CK-MB), cardiac troponin I (cTnI), and lactate dehydrogenase (LDH), along with several histopathological changes [29,30] .From this study, co-administration of isoproterenol (a beta-1 adrenergic agonist) and naringenin (an anti-inflammatory flavonoid) remarkably reduced the severity of peroxidation, creatine kinase-MB and lactate dehydrogenase activity. Some studies have shown that treatment with naringenin has the potentials to significantly decrease the production of pro-inflammatory cytokines like IL-1β, TNF-α, IL-6 by attenuating the activation of the Nrf2/NF-κb signaling pathway in ISO-induced toxicity in animals [31,32, 33,34,35] which is usually associated with cardiac dysfunction, oxidative tissue injury and inflammatory response as well as reverting the increased serum cardiac marker enzymes such as LDH, CK-MB and troponin to almost near normal due to anti-lipid peroxidation and cardioprotective activity[36,37]. The issue of utmost concern in this study was that, administration of isoproterenol for one week followed by dietary supplementation of naringenin for the next two weeks caused an increase in the activity of CK-MB, MDA, Troponin and LDH. This result was further substantiated by the prominent histological alterations observed in the liver and heart of rats in all treatment groups showing moderate aggregate of myocardial inflammation with loss of cardiac tissues and also infiltration of inflammatory hepatic cells with periductal fibrosis. This is revealing and suggest that use of naringenin following established cases of cardiac and hepatic injuries in isoproterenol toxicity over time may not be ameliorative.

Similarly, changes in liver enzyme activity were observed mostly mainly alanineaminotransferase (ALT), aspartate-aminotranseferase (AST) and alanine phosphatase (ALP). Reports have shown that impairement of cardiac function may lead to hepatic dysfunction because the heart and the liver coexist and as such heart failure often provokes liver damage due to cardio-hepatic interactions. Typically, a transient increase in serum AST, ALT and LDH indicates liver injury [38,39]. When the ratio of serum ALT to LDH is less than 1.5 in the course of liver injury it is an indication of cardiogenic injury. The changes in liver enzyme level observed following isoproterenol administration in this study agrees with an earlier documented work [39, 40]. A common cause of the loss of integrity of the organs or tissues is usually autoxidation of the tissues resulting in the generation of free radicals. In an event of a

dislodgement in balance between the antioxidant system and the generated free radicals, the defense system collapses and tissue damage occurs. The issue of concern in this study therefore is the failure of naringenin with the reported high phytochemical content and extensive biological activities including anti-inflammatory, antioxidant properties exhibited by scavenging or quenching free radicals to ameliorate the damaging effect of isoproterenol in the liver and heart.

CONCLUSION

We conclude that naringenin has an in vivo efficacy of counteracting the isoproterenol induced cardio-hepatic dysfunction with combined therapy, but may fail to ameliorate tissue injury at separate treatment periods after established cases of isoproterenol toxicity.

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Declaration of Conflicting of Interests

The authors declare that there is no conflicts of interest with respect to the research authorship, and/or publication of this article.

Ethical Approval: Ethical approval for the study was obtained from the Faculty of Basic Medical Science Animal Research Ethical Committee of Cross River University of Technology, Calabar, Nigeria. ***Approval number: CRUTECH/FBMS/REC/NIG/2023/025***

Institutional Review Board Statement: The animals handling, and experimental protocol conformed to the guidelines of the National Institutes of Health and approved by ethical committee of animal experiments at Cross River University of Technology.

Data Availability Statement: Data analyzed or generated during this study are included in this manuscript.

Consent to participate and to publish. All authors agreed to participate and to publish this article.

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