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COMBINATIONAL THERAPY OF METFORMIN AND ELLAGIC ACID REDUCES INFARCT AREA AND MYOFIBRIL DAMAGE IN ISOPROTERENOL-INDUCED MYOCARDIAL INFARCTION IN WISTAR RATS

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ABSTRACT

Isoproterenol is a β-adrenoreceptor agonist widely used as a toxicant in preclinical cardio toxic studies. Metformin is an oral biguanide which has shown cardioprotective effect in various models of myocardial infarction. Ellagic acid is a potent antioxidant which possesses free radical scavenging and also cardioprotective properties. The cardioprotective effect of these drugs in combination, however, has not been tested so far. Hence in this study we have tried to see the combinational effect of ellagic acid and metformin in isoproterenol challenged rats. Thirty six Wistar rats were randomly divided into six groups; Normal control, Toxic control, Ellagic acid alone, Metformin alone and Ellagic acid + Metformin, respectively. Histopathology and infarct size measurement was performed to assess the potential cardioprotective role of the test drugs. Ellagic acid, metformin and their combination groups showed substantial reversal in the injury, however, the effect of combination group was found to be better than the two drugs alone.

KEYWORDS: Ellagic acid, Metformin, Isoproterenol, Myocardial Infarction, Oxidative stress.

INTRODUCTION

Cardiovascular diseases (CVDs) are estimated to be the leading cause of mortality and hospital admissions worldwide.[1] Myocardial Ischemia (MI) is the most common representation of CVDs which receives ongoing attention from healthcare researchers. MI occurs due to the oxygen shutdown and corresponding reperfusion in the specific tissue of heart.[2] Clinically, myocardial ischemia can be diagnosed from the patient's history, ECG and Echocardiography. The development of medical science remarkably lowers the extent of morbidity but it still needs further evolution.

Isoproterenol (ISO), a synthetic catecholamine and a β-adrenoceptor agonist, has been found to have cardio toxic activity on the myocardium. ISO causes generation of highly cytotoxic free radicals through the auto oxidation of catecholamines which eventually results in imbalance between production of free radicals and antioxidant defense system.[3] This free radical-mediated peroxidation of membrane phospholipids and subsequent variation in membrane permeability is the leading target responsible for cardiotoxicity.[4] Hence, ISO is widely used as toxicant to produce the condition mimicking the MI.

Metformin is a most prescribed first-line drug for the management of type 2 diabetes. Metformin is known to lowers the blood glucose by suppressing the hepatic glucose production, reducing intestinal glucose absorption and improving the glucose uptake and utilization.[5] Besides its blood glucose lowering ability, metformin is further investigated for new indications. One of the most beneficial effects of metformin is cardiovascular protection in diabetic patients.[6] Various animal and clinical studies also showed the beneficial effects of metformin in CVDs.[7] The activation of AMP-activated protein kinase by metformin is found to improve the left ventricular function and survival in heart failure.[8]

Ellagic acid is a polyphenol that occur naturally in raspberries, strawberries, grapes and pomegranates.[9] Ellagic acid possesses the antioxidant, anti-inflammatory, antifibrotic properties and known to inhibit lipid peroxidation. A recent study showed that pretreatment with ellagic acid protects the rat heart in oxidative stress model of MI.[10]

Metformin and Ellagic acid has already been evaluated in the ISO challenged rats but their combination effects have not been examined so far. In the purview of above facts, we planned to evaluate the cardioprotective potential of ellagic acid and metformin in combination in isoproterenol-induced myocardial infarction in rats.

MATERIALS AND METHODS

Experimental animals

All the experimental procedures involving the use of laboratory animals were approved by the Institutional Animal Ethics Committee, Hamdard University (India). Wistar albino male rats weighing 180-250 grams were procured from Animal House Facility, Hamdard University, New Delhi (India). The animals were housed in polypropylene cages for one week to acclimatize to the standard conditions. (12 h light: dark cycle; temperature, 23±2 ºC; RH 60±5 %). The standard pellet diet and free access to the tap water was provided to the animals.

Drugs and chemicals

Ellagic acid and ISO were purchased from Himedia, India and SigmaChemical, St. Louis, MO, USA, respectively. Metformin API was a gift sample from International Testing Centre, Haryana, India. All other chemicals used were of the highest analytical grade.

Induction of experimental myocardial infarction

ISO (85mg/kg body weight) was dissolved in saline and injected subcutaneously into rats at an interval of 24hrs for two days to induce MI.

Experimental design

After acclimatization, the rats were divided into six groups (n=6) and treated as follows:

Group 1 (Normal control)

Normal control rats were given 2 ml of oral saline daily for ten days and 0.1ml normal saline subcutaneously on 11^{th} and 12^{th} of the experiment.

Group 2 (Toxic control)

Normal rats were treated with 2ml normal saline orally for ten days and given ISO (85mg/kg s.c.) on 11^{th} and 12^{th} day of the experiment.

Group 3 (Ellagic acid alone)

Rats were pretreated with ellagic acid (15mg/kg oral) for ten days and given ISO (85mg/kg s.c.) on the 11^{th} and 12^{th} day of the experiment.

Group 4 (Metformin alone)

Normal rats were treated with 2ml of saline for ten days and given metformin (100mg/kg orally) and ISO (85mg/kg s.c.) on 11^{th} and 12^{th} day of the experiment.

Group 5 (Ellagic acid with metformin)

Normal rats were pretreated with ellagic acid (15mg/kg body weight) for ten days and given metformin (100mg/kg orally) and ISO (85mg/kg s.c.) on 11^{th} and 12^{th} day of the experiment.

Group 6 (Ellagic acid with metformin *per se*)

Normal rats were treated with ellagic acid (15mg/kg orally) for ten days and given metformin (100mg/kg orally) on 11^{th} and 12^{th} day.

After 24 hrs of the last dose, rats were sacrificed using ether anesthesia. The hearts were then removed and infarct size measurement and histopathology was performed.

Estimation of infarct size

The hearts were sectioned into six slices each with 2 mm thickness, immersed in 2% triphenyl tetrazolium chloride (TTC) dye for 30 minutes at 37^{o} C. The heart was differentiated according to white-colored infarct area and red-purple non-infarct area. The slices were placed on a glass plate in a row, and the images were captured using a digital camera. Image J Software version 1.49 (NIH, Bethesda, MD) for the measurement of infarction. The percentage of the area of infarction was calculated as [(white or yellow area/total area) X 100].[11]

Histopathology

The heart tissue preserved in formalin was impregnated in paraffin wax and then sliced into thin sections. These sections were stained with Eosin-Haematoxylin dye to determine the extent of tissue damage by the free radicals and the influence of the test drug, metformin and ellagic acid as well as their combination in reversing the damage. The slides were evaluated with Mieji microscope enabled with Lumenera camera.

STATISTICAL ANALYSIS

Data were expressed as mean ± SEM (standard error mean) of six rats per group. Means were compared by one-way analysis of variance (ANOVA) with post hoc analysis. The Tukey-Kramer post hoc test was applied to identify the significance among different groups. $P<0.05$ was considered statistically significant. Graph Pad Software, Inc. version (5.01) was used for statistical analysis.

RESULTS

Effect of ellagic acid and metformin on infarct size

Figure 1 represents that there was a significant increase in the infarct size in the toxic group when compared to control group ($p<0.001$). All the pretreatment groups showed significant decrease in infarct size ($p<0.001$). The drug alone treated group showed non-significant changes when compared with the normal control.

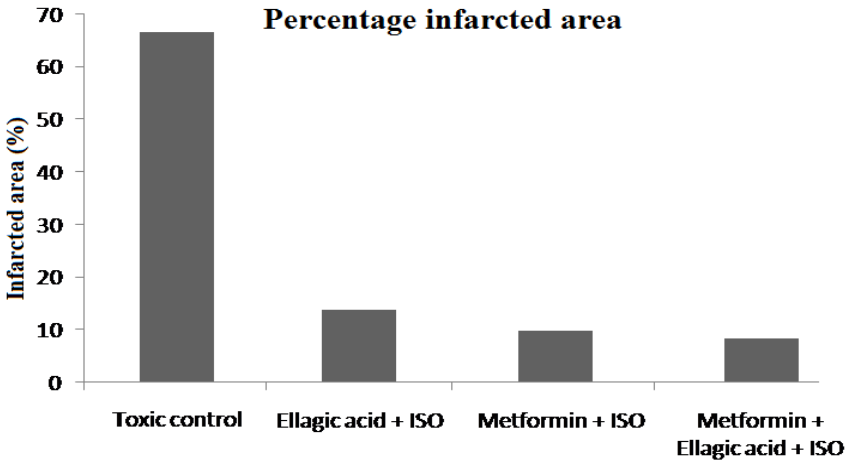

Figure 1: Represents percentages of infarcted area in various groups

Histopathology

Figure 2 shows photomicrographs of histological slides of various groups at 10X. The microscopic section of normal control group shows normal architecture of cardiac muscle. The ISO treated group shows necrosis, pyknotic nucleus and separated cardiac muscle fibers. Pretreatment with ellagic acid (15 mg/kg oral) and metformin (100 mg/kg, oral) shows decrease in separation of muscle fibers and necrosis. The combination group shows better cellular morphology than the two drug alone. *Per se* group shows normal architecture and morphology of cardiac tissue.

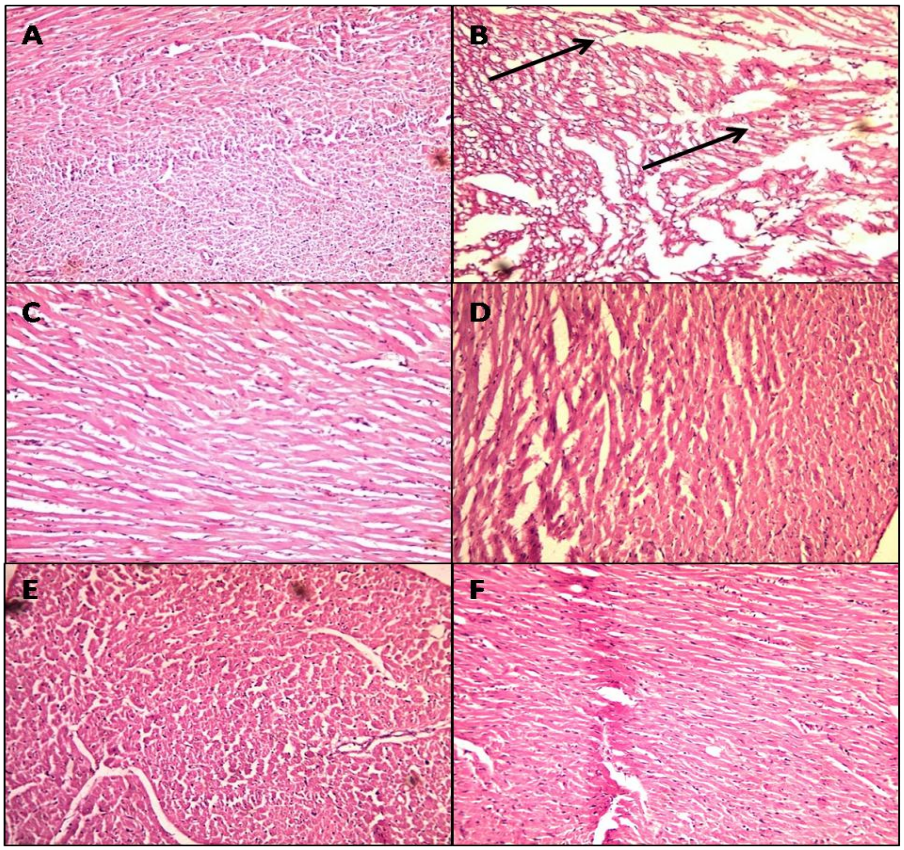

Figure 2: Showing histopathology of the heart. (A) Microscopic section of normal control group rat heart showing normal architecture of cardiac muscle. (B) Microscopic section of isoproterenol-induced group showing necrosis, pyknotic nucleus and separated cardiac muscle fibers. (C, D) Microscopic section of ellagic acid (15 mg/kg oral) and isoproterenol (85 mg/kg s.c); metformin (100 mg/kg, oral) and isoproterenol (85 mg/kg, s.c) treated group showing decrease in separation of muscle fibers and lesser necrosis. (E) Microscopic section of combination group: ellagic acid (15 mg/kg, oral) plus metformin (100 mg/kg, oral) and isoproterenol (85 mg/kg, s.c) treated group showing no necrosis and separation in muscle fibers. (F) Microscopic section of ellagic acid (15 mg/kg, oral) and metformin (100 mg/kg, oral) *per se* group showing normal architecture.

DISCUSSION

The results of our study with short-term oral administration of metformin and ellagic acid in ISO challenged rats showed considerable protection against myocardial infarction and cellular damage. When we estimated the infarcted area of the heart in ISO treated animals, we observed around 66% infarction in the apical region which was considerably reduced with the ellagic acid, metformin and their combinational treatment.

Histopathological examination of the myocardium of these rats when examined under 10X. We found considerable disorientation of myofibril, formation of pyknotic nucleus, fiber separation and increased myofibril thickness along with vacuole formation.

Although the pathogenesis of cardiac dysfunction is not fully understood, studies on ß-adrenergic stimulation induced cardiac damage provide a good insight into this pathology and clearly indicate the involvement of oxidative stress and necrotic changes as explained above.

Metformin and ellagic acid prevented infarction as evident by the decrease in infarct area by them. Histological examination of the myocardium in the test drugs treated groups also shown the reversal of cellular integrity and maintained normal architecture.

When we compared the changes concerning the metformin plus ellagic acid treated group (combination group) we found that the performance of combination group was better than metformin and ellagic acid alone treated groups.

CONCLUSION

Thus, we can conclude that metformin, ellagic acid, and combination groups all have maintained the cellular integrity and reduced the infarcted area, however combination group was found better than metformin and ellagic acid as a separate test drug.

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