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ORIGINAL ARTICLE

Hecogenin Attenuates Isoproterenol-Induced Myocardial Infarction through Nuclear Factor-Kappa B-Mediated Signaling Pathway in Rats

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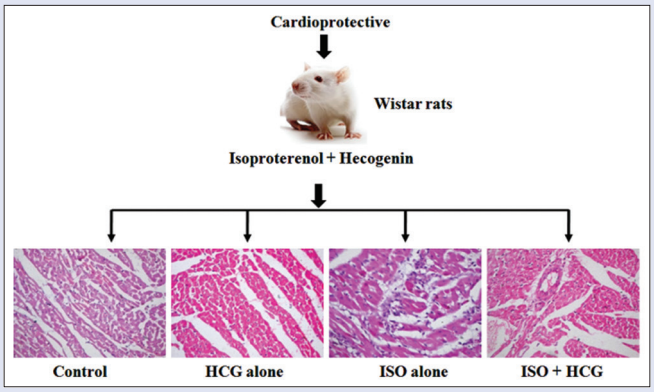
ABSTRACT

Background: Cardiac-related problems accounts for about 10%–35% of all deaths. Medicinal plants and their derivatives are a rich source of effective cardioprotective agents. Hecogenin (HCG) is a sapogenin with a wide spectrum pharmacological property. In this study, we aimed to evaluate the cardioprotective effect of HCG against isoproterenol (ISO)-induced myocardial infarction (MI) in rats. **Materials and Methods:** The animals were divided into four groups. Group I: Control group consisted ofrats fed onstandard pellet (23 days); Group II: HCG group, HCG 50 μg/kg body weight (BW), oral administration, (P. O) on 21 days; Group III: ISO group, 60 mg ISO/kg BW; subcutaneous (sc) administered on days 22 and 23; Group IV: HCG (50 μg/kg BW. P. O) (21 days) + ISO (days 22 and 23). After sacrificing the animals, we analyzed thebiochemical and molecular markers. **Results:** Our results showed that there were no significant differences in the BW of rats; however, the HCG and ISO groups showed a significant reduction in heart weight. When compared with ISO group, hepatic and cardiac biomarkers were low in HCG + ISO group. Furthermore, compared to the ISO group, the level of lipid peroxidation products was restored to their optimal level in the HCG + ISO group. According to the histopathological findings, animals from HCG group demonstrated restoration of their tissue architecture. Immunohistochemistry demonstrated that animals in the HCG group showed reduced expression levels of nuclear factor kappa B (NF-κB) and p53, which was similar to the control and HCG group. This demonstrates cardioprotective effects of HCG. **Conclusion:** HCG attenuated ISO-induced MI via inhibition of activation of NF-κB and p53 signaling pathway. Overall, the findings recommended that HCG is a promising therapeutic agent for the treatment of MI.

Key words: Antioxidants, hecogenin, isoproterenol, myocardial infarction, nuclear factor kappa B, p53

SUMMARY

- Effects of HCG on antioxidant and lipid peroxidation activity on isoproterenol (ISO) treated rats
- HCG suppressed inflammatory marker on ISO-induced myocardial infarction rats
- HCG induced increased expression of apoptosis p53 protein in ISO-induced rats.



Abbreviations used: HCG: Hecogenin; ISO: Isoproterenol; MI: Myocardial infarction; CHD: Coronary heart disease; NF-κB: Nuclear factor-kappa B; HW: Heart weight; BW: Bodyweight; TBARS: Thiobarbituric acid reactive substances; CD: Conjugated dienes; PUFA: Polyunsaturated fatty acids; LHPs: Lipid hydroperoxides; CAT: Catalase; SOD: Superoxide dismutase; H and E: Haematoxylin and eosin; DMRT: Duncan's multi-range test.

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INTRODUCTION

Cardiovascular diseases (CVDs) are increasing at an alarming rate in both developed and undeveloped countries. The prevalence rate of coronary heart disease accounts for about 10%–35% of all deaths worldwide.[1] It is expected that mortality rate due to CVDs will increase upto 23.6 million in 2030.[2] Myocardial infarction (MI) is the ischemic cardiac condition in which there is an irregular and an increased demand for blood supply leading to the necrosis of cardiomyocytes.[3] The necrotic condition in cardiac tissue is attributed to the changes in the level of cardiac enzymes, lipid peroxides, and electrocardiogram (ECG).[4]

Isoproterenol (ISO)-induced myocardial necrosis experimental model is an ideal and well-studied model to assess cardiac dysfunction.[5] It belongs to the group of synthetic catecholamines (β-adregenic agonist), which may cause severe necrosis of the cardiac muscle by increasing oxidative stress and generation of free radicals which in turn causes irreversible changes to the myocardium.[6,7] Many conventional medicines are in the class of antagonists of Ca^{2+} and β-blockers,

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which have been considered prior for the prevention and treatment of CVD. Numerous CVD drugs have reported adverse effects such as dysrhythmia and toxicity.[8,9] Thus, plant-derived phytochemicals serve as a potential source of therapeutic modalities for various diseases that can further exploit MI treatment.

Hecogenin (HCG) isolated from the *Agave* genus belongs to steroidal sapogenin, which possesses potential pharmacological activities, including cardioprotective, anti-hypertensive, anti-nociceptive, anti-cancer, and anti-fungal properties.[10,11] However, to the best of our knowledge, there are no studies conducted on the cardioprotective effect of HCG against ISO-induced MI. Therefore, in this study, we aim to explore the cardioprotective effect of HCG in rats induced with MI and analyzed the biochemical, cardiac, and molecular markers.

MATERIALS AND METHODS

Chemicals

HCG (CAS: 467-55-0 Purity ≥98%) and ISO (CAS: 51-30-9, purity: ≥=98%) were procured from Sigma Aldrich (USA). Kits were procured from Roche Diagnostics (Risch, Switzerland). All other chemicals used in this study were obtained from Sigma Aldrich (USA).

Animals

As per the Institutional Animal Ethics committee norms, the study was approved by the Xi'an Children's Hospital Animal Ethical Committee (approval No. XCH202106726A). About 180–220 g, male Wistar rats (6–7 weeks old) were maintained under controlled humidity (65% ± 5%) and temperature (25°C ± 2°C). They were kept under alternating 12 h light and dark cycles. They were provided with standard pellet diet and fresh water *ad libitum* during the study.

Experimental design and induction

ISO (60 mg/kg body weight [BW]) was freshly prepared in 0.9% NaCl saline for induction of MI. The solution was administered subcutaneously to the rats in the thigh for 2 days.[12] Animals were segregated into four groups each containing 5 rats. The doses were fixed, which isbased on aprevious literature.[10]

Group I: Control rats with standard pellet (23 days).

Group II: ISO (60 mg/kg BW) administered subcutaneously (sc) on days 22 and 23.

Group III: HCG (50 mg/kg BW per os (p. o)) (21 days) + ISO (days 22 and 23).

Group IV: HCG (50 mg/kg BW, p. o)-orally administered (21 days).

During the experimental period, BW of the animals was measured every week. After the final day of experimentation, the animals were sacrificed by cervical dislocation between 8:00 a. m. and 9:00 p. m. after the final ISO injection, and the blood samples were collected from the jugular vein. Serum samples were then obtained by centrifuging the blood at 2000 rpm at 4°C for 20 min. After blood collection, the heart was collected, washed (using saline), dried (on a filter paper), and weighed. The formula for determining the ratiois as follows:

$$ratio(\%) = \frac{H.W.(g)}{B.W.(g)} \times 100$$

Where heart weight (H. W.) is the HW and B. W. is the BW.

Tissue homogenate was prepared by homogenizing the tissue in phosphate buffer (pH 7.4). The homogenate was centrifuged at 2000 rpm for 10 min and the supernatant was collected for biochemical analysis. The excised heart tissue was subjected to histopathological analysis.

Effect of HCG on hepatic and cardiac markers

The serum levels of hepatic and cardiac marker enzymes were assessed using commercially available kits (Thermo Fisher Scientific, USA). The serum activity of creatine kinase (CK) and CK-MB (isoenzyme of CK present in the heart tissue), lactate dehydrogenase (LDH), aspartate transaminase (AST), and alanine transaminase (ALT), and cardiac troponin T and I (cTnT, and cTnI respectively) were assessed using chemiluminescence kits (Roche diagnostics Risch, Switzerland).

Effect of HCG on lipid peroxidation products

Estimation of thiobarbituric acid reactive substances

The levels of thiobarbituric acid reactive substances (TBARS) formed in the blood and cardiac tissue[13] were quantified using the previously described procedure.[14] Briefly, 0.2 ml of serum or tissue homogenate, 0.2 ml of 8.1% SDS, 1.5 ml of acetic acid (20%), and 1.5 ml of thiobarbituric acid (8%) were added. The mixture was added up to 4 ml with distilled water and was heated for 60 min in a water bath at 95°C. After incubation, the tubes were cooled to room temperature and the final volume was increased to 5 ml in each tube. The butanol: pyridine (15: 1) mixture (5 ml) was added and the contents were vortexed thoroughly for 2 min. After centrifugation (3000 rpm) for 10 min, the upper organic layer was aspirated. The MDA reacts with TBARS at high-temperature and acidic conditions to form a pink adduct. This pink color formed can be spectrophotometrically detected at 535 nm against the blank.

Determination of conjugated dienes

The level of CDs formed was determined based on a previously described procedure.[15] Tissue homogenate mixed with 0.1 M Tris–HCl (pH 7.5) and was centrifuged. 1 ml of supernatant was mixed thoroughly with 5 ml of chloroform: methanol (2:1) followed by centrifugation at ×1000 g for 5 min to separate the phases. 3 ml of the lower chloroform layer was removed using a syringe and placed in a test tube and evaporated to dryness in a water bath at 45°C. Dissolved the residue in 1.5 ml cyclohexane and absorbance was noted at 233 nm against cyclohexane as blank. The molar extinction coefficient of conjugated dienes is $2.52 \times 10^4\ M^{-1}$.

Determination of lipid hydroperoxides

The LHPs formed in the plasma and cardiac tissues were estimated using Fe^{2+} oxidation with xylenol orange to form characteristic chromophore which can be detected at 560 nm.[16]

Effect of HCG on enzymatic and non-enzymatic antioxidants

Superoxide dismutase activity

The activity SOD was detected in the myocardial cells based on a previously described procedure.[17] Tissue homogenate was centrifuged and 150 µl supernatant was added to the reaction mixture containing 600 µl of 0.052 mM sodium pyrophosphate buffer (pH 7.0) and 186 mM of phenazine methosulphate (50 µl). To initiate enzymatic reaction 100 µl of 780 µM NADH was added. After 1 min, glacial acetic acid (500 µl) was added to stop the reaction. The color intensity was measured at a wavelength of 560 nm. Results were expressed as units/mg protein.

Catalase activity

The catalase (CAT) activity in the myocardial cells was based on converting dichromate in acetic acid to chromic acetate, with perchromic acid as the intermediate.[17] In short, the CAT reaction solution consists of 625 µl of 50 mM of potassium phosphate buffer (pH 5), 100 µl of 5.9 mM H_2O_2, and 35 µl enzyme extract, a change in absorbance was observed for one minute at a wavelength of 240 nm by spectrophotometer. A change of 0.01 in absorbance for one minute was taken as one unit of CAT activity.

Glutathione peroxidase activity

GPx activity was assessed based on the method of Habig *et al.* (1974).[18] 100 µl supernatant samples were mixed with 100 µl ethylenediaminetetraacetic acid (1 mM), 1.49 ml phosphate buffer (0.1 M; pH 7.4), 100 µl sodium azide (1 mM), 50 µl glutathione (GSH) reductase (1 IU/ml), 50 µl GSH (1 mM), 100 µl NADPH (0.2 mM) and 10 µl H_2O_2 (0.25 mM). The loss of NADPH was recorded at 340 nm at room temperature. The absorbance was noted at 340 nm.

Reduced glutathione activity

GSH was estimated using Ellman's reaction. Using the substrate (1-chloro-2,4-dinitrobenzene) the absorbance was increased at 340 nm which is used for the estimation of GST in cardiac tissue[19] and was expressed as µM GSH/g tissue.

The conversion of ascorbic acid to dehydroascorbic acid in the presence of thiourea and 2, 4-dinitrophenylhydrazine was performed to estimate the level of vitamin C.[20] The level of vitamin E was estimated based on the reduction of ferric ion.[21] The total protein was estimated in both blood and cardiac tissues.[22]

Histopathology

Cardiac tissue was fixed using 10% formaldehyde solution and embedded using paraffin. Then, 3-5 µm sections were cut by using amicrotome, and the sections were stained using hematoxylin and eosin. Histological analysiswas performed using light microscopy.[2]

Immunohistochemistry

For immunohistochemistry, 6 m thick sections of formalin-fixed and paraffin-embedded specimens were used. For deparaffinization and rehydration, sections were delivered over graded series of ethanol to water. These sections were heated in citrate buffer (10 mM, pH 6.0) in a scientific microwave oven at 95°C for 10 min for antigen unmasking. Afterward sections were incubated in H_2O_2 (30%) in CH_3OH for 2 min to prevent intrinsic peroxidase activity and to prevent non-specific binding and then incubated in goat serum for 2 h. Subsequently, the specimen was incubated for 2 h at 4°C with primary antibodies (nuclear factor kappa B [NF-κB] [1:1000] and p53 [1:1000]), followed by 2 hincubation at room temperature with secondary antibodies. Finally, 3,3′-diaminobenzidine was used to generate color. Under a light microscope, the pieces were counterstained with hematoxylin and examined.

Statistical analysis

SPSS version 17.0 (SPSS Inc., Chicago, IL, USA) was used to perform analysis of variance and Duncan's multi-range test. The results are presented as mean ± standard deviation. $P < 0.05$ were regarded as statistically significant.

RESULTS

Effect on the body weight and heart weight

Table 1 shows the changes in the BW and HW of ISO-induced experimental rats. Group III animals showed substantial variation in the HW compared to the controls; however, there was no significant change in the BW ($P < 0.05$) between the groups of experimental rats. HW of HCG group showed a substantial reduction in the HW suggesting the cardioprotective effects of HCG. In contrast, there were no marked variations among control and HCG alone treated groups.

Table 1: Effect of hecogenin on body weight, heart weight and heart weight/body weight ratio in control and isoproterenol induced rats

Groups/ treatments	Body weight (g)	Heart weight (g)	Heart weight/body weight ratio (%)
Control	188.60±14.36	0.670±0.05[a]	0.355±0.03[a]
HCG (50 µg/kg bw)	185.75±14.22	0.640±0.05[a]	0.344±0.03[a]
ISO (60 mg/kg bw)	178.84±13.69	0.890±0.07[b]	0.547±0.04[b]
ISO+HCG (50 µg/ kg bw)	184.65±14.13	0.648±0.05[c]	0.350±0.03[c]

Values are means±SD for six rats. Values not sharing a common superscript differ significantly at [a-c]$P<0.05$ (DMRT). ISO: Isoproterenol; HCG: Hecogenin; DMRT: Duncan's multi-range test; SD: Standard deviation

Effect of HCG on serum cardiac and hepatic marker enzymes

Tables 2 and 3 show the effect of HCG on serum cardiac and hepatic marker enzymes in ISO-induced MI rats. The activities of cardiac and liver marker enzymes were markedly increased in ISO-induced experimental rats. When rats were administered with HCG for 21 days and then induced with ISO, their behaviors and levels of cardiac and hepatic marker enzymes were significantly ($P \leq 0.05$) low when compared with rats in ISO alone group.

Effect on lipid peroxidation products

Table 4 shows the concentrations of CDs, LHPs, and TBARS in experimental rats. As compared to normal rats, the ISO-induced MI rats showed significantly ($P < 0.05$) high levels of lipid peroxidation products in plasma and cardiac tissue. Pre-treatment of ISO-induced rats with HCG (50 µg/kg BW) markedly ($P < 0.05$) reduced the levels of lipid peroxidation products in contrast to ISO alone treated rats. On HCG pre-treatment to the ISO-induced rats reduced nearly 67% TBARS, 54% CDs, 81% LHPs in plasma, whereas in cardiocytes showed 76% TBARS, 81% CDs, 87% LHPs.

Effect of HCG on markers of antioxidants

Table 5 shows the effect of HCG on enzymatic markers of antioxidants in experimental animals. Experimental rats induced with ISO showed a marked reduction in the enzymatic antioxidants compared to the control animals. HCG (50 µg/kg bw) treated groups exhibited normal enzymatic activities compared to the ISO-induced experimental rats.

Table 6 shows the level of GSH and vitamins C and E in the blood and heart tissues of experimental animals. The level of non-enzymatic antioxidants in group III decreased significantly ($P < 0.05$) compared with the control group. HCG (50 µg/kg bw) restored their levels.

Histopathological assessment of myocardial tissues

Figure 1 shows the effect of HCG on cardiac tissues. Experimental rats belonging to the control group and the HCG alone group have shown to exhibit normal tissue architecture of myocardium. Animals in the ISO alone treated group showed collapsed tissue architecture with infiltration of inflammatory cells and necrosis, whereas HCG + ISO-treated animals exhibited reduced ISO-induced myocardial tissue damages and restored cardiocytes architecture.

Immunohistochemistry of nuclear factor kappa B and p53 in myocardial tissues

Figures 2 and 3 depict the immunohistochemistry of NF-κB and p53 in cardiac tissues. Experimental rats belonging to the control and group II animals revealed positive NF-κB cells. Experimental groups treated with ISO alone showed higher number of positive NF-κB cells, whereas the

Table 2: Effect of hecogenin on the activities of aspartate transaminase, alanine transaminase, lactate dehydrogenase, creatine kinase, and CK-MB in the serum of control and isoproterenol induced rats

Groups/treatments	AST (IU/L)	ALT (IU/L)	LDH (IU/L)	CK (IU/L)	CK-MB (IU/L)
Control	69.66±5.30[a]	19.25±1.47[a]	209.78±15.97[a]	145.27±11.06[a]	99.58±7.58[a]
HCG (50 µg/kg bw)	66.58±5.10[b]	18.16±1.39[b]	206.24±15.70[b]	144.47±11.00[b]	97.34±7.45[b]
ISO (60 mg/kg bw)	113.47±8.64[c]	53.06±4.04[c]	296.16±22.55[c]	220.21±16.77[c]	171.38±13.05[c]
ISO+HCG (50 µg/kg bw)	68.49±5.22[d]	19.35±1.47[d]	216.76±16.59[d]	150.68±11.47[c]	107.57±8.19[d]

Values are means±SD for six rats. Values not sharing a common superscript differ significantly at [a-c]$P<0.05$ (DMRT). ISO: Isoproterenol; HCG: Hecogenin; DMRT: Duncan's multi-range test; SD: Standard deviation; AST: Aspartate transaminase; ALT: Alanine transaminase; LDH: Lactate dehydrogenase; CK: Creatine kinase; CK-MB: Creatine Kinase

Table 3: Effect of hecogenin on the levels of cardiac troponin T and cardiac troponin I in the serum of control and isoproterenol-induced rats

Groups/treatments	cTnT	cTnI
Control	0.57±0.04[a]	0.40±0.03[a]
HCG (50 µg/kg bw)	0.55±0.04[a]	0.37±0.03[a]
ISO (60 mg/kg bw)	1.35±0.10[b]	0.66±0.05[b]
ISO+HCG (50 µg/kg bw)	0.59±0.04[c]	0.39±0.03[c]

Values are means±SD for six rats. Values not sharing a common superscript differ significantly at [a-c]$P<0.05$ (DMRT). ISO: Isoproterenol; HCG: Hecogenin; DMRT: Duncan's multi-range test; SD: Standard deviation; cTnT: Cardiac troponin T; cTnI: Cardiac troponin I

HCG + ISO treated groups exhibited down-regulated expression of NF-κB in myocardial tissues. Control group rats and HCG group rats did not reveal p53-positive cells. Animals belonging to ISO alone group showed significantly ($P < 0.05$) higher number of p53-positive cells, whereas the HCG (50 µg/kg bw)-treated groups inhibited the expression of p53 in myocardial tissues.

DISCUSSION

HCG is a steroidal sapon in with a broad spectrum of biological activities.[11] ISO-induced myocardial damage is similar to the damage caused in humans.[23] It can also cause severe myocardial necrosis with increased surge of free radicals, leading to the peroxidation of polyunsaturated fatty acids leading to heart injury, both structural and functional.[24,25] Adrenochrome is a product of ISO oxidation which causes extensive damage to the heart tissue leading to necrosis and loss of contraction.[26] The free radicals oxidize the extracellular proteins including enzymes are destroying their activity.[27]

The BW and HW in experimental rats were measured every week. BW did not show any marked variations between the groups; however, there were remarkable changes in the HW and HW/BW ratio, which may be attributed to the increased levels of water content and necrosis and increased migration of inflammatory cells.[28] HCG + ISO treated animals as showed a significant reduction in the HW signifying the cardioprotective properties of HCG.

Leakage of cytosolic enzymes including CK-MB, LDH, AST, and ALT (which serve as diagnostic markers from the damaged tissue) into the bloodstream may occur when cell membranes become more permeable or rupture. The amounts of these cellular enzymes in the serum reflect the alterations in plasma membrane integrity and/or permeability.[8]

The levels of serum cardiac and hepatic markers in ISO-induced MI rats were assessed as they tend to be considered as marker for diagnostics. Cytosolic enzymes, i.e., AST and ALT are found in the myocardial cells discharged into the blood stream may occur when cell membranes become more permeable or rupture. The amounts of these cellular enzymes in the serum reflect the alterations in the integrity and permeability of the plasma membrane.[29] The other diagnostic markers can be released into the blood due to the disruption of myocardial membrane due to necrosis.[30] In this study, the tissue proteins specific to the myocardium in the case of myocardial injury were studied, namely cTnT and cTnI. They are highly sensitive biomarker for the MI diagnosis.[31] Our results suggest that these two proteins were markedly increased in ISO-induced experimental rats, and HCG significantly lower their levels. Thus, HCG effectively protected the myocardial membrane by reducing the damage and enzyme leakage in the myocardium.

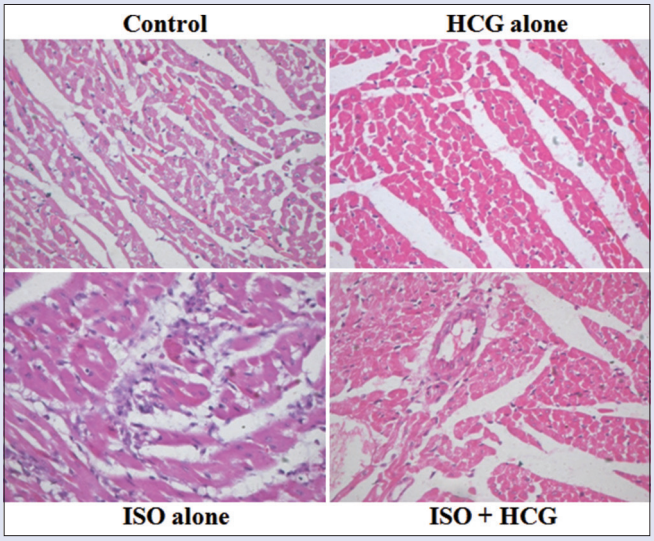

Figure 1: Histopathology of cardiac tissues. Control; HCG (50 mg/kg body weight) alone treated rats showed normal tissue architecture; isoproterenol alone treated rats shows the disruption and necrosis. HCG (50 mg/kg body weight) + isoproterenol treated rats more than 90% recovery of the damaged tissues

Lipid peroxidation causes several diseases, including MI. In ISO-induced rats, we found myocardial necrosis which was the result of peroxidative damage.[32] In ISO alone treated rats, the level of lipid peroxidation products was increased; however, HCG reduced their levels. Enzymatic antioxidants play a crucial role in protecting the cells against oxidative tissue injury. SOD quenches free radicals, and its activity is reduced due to the activity of ISO in rats. Decrease in its activity disrupts its ability to remove the superoxide ions.[33] GST is a detoxifying enzyme alleviating stress-induced by oxidation.[34] This study revealed that the antioxidant enzyme levels were lowered in the ISO-induced rats, whereas HCG increased their levels in the blood and heart indicating its cardioprotective effects.

Non-enzymatic antioxidants including Vitamins C, E and GSH act as the defense mechanisms of scavenging free radicals. They tend to reduce the blood pressure and cholesterol, thereby reducing the cardiac problems.[35] Compared to the control animals, experimental animals injected with

Table 4: Effect of hecogenin on the level of thiobarbituric acid reactive substances, lipid hydroperoxides and conjugated dienes in the plasma and heart tissue of control and isoproterenol-induced rats

Groups/treatments	Control	HCG (50 µg/kg bw)	ISO (60 mg/kg bw)	ISO+HCG (50 µg/kg bw)
TBARS				
Plasma (mmol/dL)	0.78±0.06[a]	0.77±0.06[a]	3.29±0.25[b]	0.80±0.06[c]
Heart (mmol/100 g wet tissue)	0.47±0.04[a]	0.44±0.03[a]	0.95±0.07[b]	0.46±0.04[c]
LHPs				
Plasma (mmol/dL)	11.45±0.87[a]	9.55±0.73[a]	19.09±1.45[b]	10.25±0.78[c]
Heart (mmol/100 g wet tissue)	64.28±4.89[a]	62.20±4.74[a]	114.11±8.69[b]	63.86±4.89[c]
CD				
Plasma (mmol/dL)	0.79±0.06[a]	0.77±0.06[a]	2.08±0.16[b]	0.80±0.06[c]
Heart (mmol/100 g wet tissue)	37.66±2.87[a]	36.08±2.76[a]	71.94±5.48[b]	38.37±2.94[c]

Values are means±SD for six rats. Values not sharing a common superscript differ significantly at [a-c]$P \leq 0.05$ (DMRT). ISO: Isoproterenol; HCG: Hecogenin; DMRT: Duncan's multi-range test; SD: Standard deviation; CD: Conjugated dienes; LHPs: Lipid hydroperoxides; TBARS: Thiobarbituric acid reactive substances

Table 5: Effect of hecogenin on the level superoxide dismutase, catalase, glutathione peroxidase, and glutathione-S-transferase in the erythrocytes and heart of control and isoproterenol-induced rats

Groups/treatments	Control	HCG (50 µg/kg bw)	ISO (60 mg/kg bw)	ISO+HCG (50 µg/kg bw)
SOD				
Erythrocytes (U*/mg Hb)	8.15±0.62[a]	7.40±0.57[a]	3.79±0.29[b]	7.80±0.60[c]
Heart (U*/mg Protein)	10.19±0.78[a]	8.40±0.64[a]	4.90±0.37[b]	9.66±0.74[c]
CAT				
Erythrocytes (U**/mg Hb)	178.88±13.62[a]	176.64±13.52[a]	126.27±9.62[b]	177.60±13.52[c]
Heart (U**/mg protein)	52.41±3.99[a]	51.71±3.96[a]	28.36±2.16[b]	50.96±3.88[c]
GPx				
Erythrocytes (U[@]/mg Hb)	15.83±1.21[a]	13.66±1.05[a]	7.96±0.61[b]	14.57±1.12[c]
Heart (U[@]/mg protein)	9.41±0.72[a]	8.48±0.65[a]	5.19±0.40[b]	8.65±0.66[c]
GST				
Erythrocytes (U[$]/mg Hb)	7.89±0.60[a]	6.50±0.50[a]	3.98±0.30[b]	7.31±0.56[c]
Heart (U[$]/mg protein)	5.99±0.46[a]	5.05±0.44[a]	2.65±0.20[b]	5.54±0.42[c]

Values are means±SD for six rats. Values not sharing a common superscript differ significantly at [a-c]$P<0.05$ (DMRT). U*: Enzyme concentration required for 50% inhibition of NBT reduction/min; U**: mmole of hydrogen peroxide consumed/min; U[@]: mmole of GSH utilized/min; U[$]: mg of CDNB conjugate formed/min. ISO: Isoproterenol; HCG: Hecogenin; DMRT: Duncan's multi-range test; SD: Standard deviation; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; GST: Glutathione-S-transferase

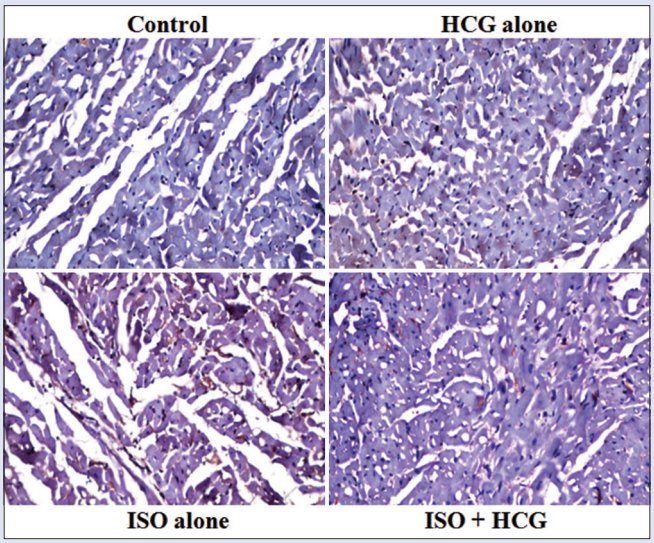

Figure 2: Immunohistochemistry staining patterns of inflammatory marker nuclear factor kappa B protein expression in the control, and experimental rats in each group

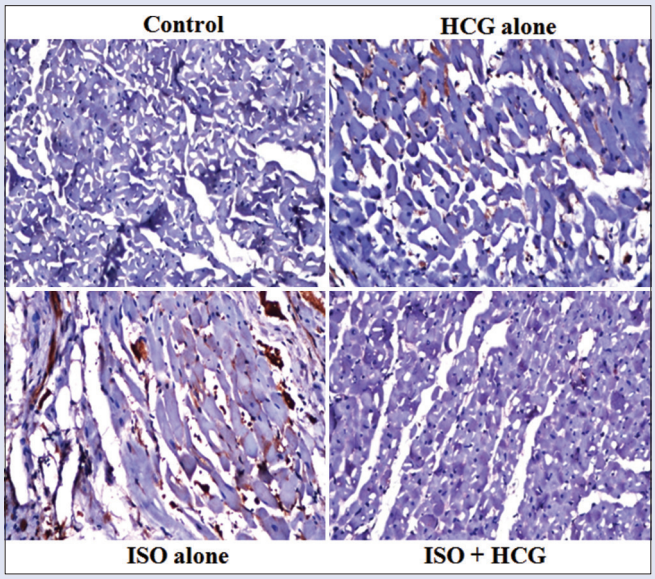

Figure 3: Immunohistochemistry staining patterns of apoptosis marker p53 protein expression in the control, and experimental rats in each group

ISO markedly reduced the levels of non-enzymatic antioxidants. HCG alone administered animals prior to the administration of ISO showed optimal activity similar to control animals.[36] Histopathological analysis suggests that the animals in the experimental group treated with ISO alone had collapsed tissue architecture with infiltration of inflammatory cells and necrosis, whereas the animals in the HCG + ISO treated group revealed normal architecture suggesting the cardioprotective properties of HCG.

Table 6: Effect of hecogenin on the level of Vitamin-C, -E, and glutathione in the plasma and heart of control and isoproterenol-induced rats

Groups/treatments	Control	HCG (50 µg/kg bw)	ISO (60 mg/kg bw)	ISO+HCG (50 µg/kg bw)
Vitamin-C				
Plasma (mg/dL)	2.65±0.20[a]	2.19±0.17[a]	1.09±0.08[b]	2.45±0.19[c]
Heart (µg/mg protein)	0.59±0.04[a]	0.56±0.04[a]	0.27±0.02[b]	0.58±0.04[c]
Vitamin-E				
Plasma (mg/dL)	1.65±0.13[a]	1.55±0.12[a]	1.03±0.08[b]	1.61±0.12[c]
Heart (µg/mgprotein)	2.78±0.21[a]	2.69±0.21[a]	1.88±0.14[b]	2.76±0.21[c]
GSH				
Plasma (mg/dL)	38.76±2.95[a]	37.18±2.85[a]	18.35±1.40[b]	38.25±2.93[c]
Heart (µg/mg protein)	8.76±0.67[a]	8.60±0.65[a]	4.89±0.37[b]	8.71±0.67[c]

Values are means±SEM for six rats. Values not sharing a common superscript differ significantly at [a-c]$P \leq 0.05$ (DMRT). ISO: Isoproterenol; HCG: Hecogenin; DMRT: Duncan's multi-range test; SEM: Standard error of mean; GSH: Glutathione

NF-κB signaling pathway is the focal hub which induces the production of pro-inflammatory cytokines causing severe injury to the heart.[37] Immunohistochemistry of cardiac tissue revealed more number of NF-κB and p53-positive cells in the ISO alone treated rats, whereas the HCG + ISO treated animals showed a reduction in their levels similar to that of the control group animals indicating the suppression of the NF-κB and p53.

CONCLUSION

Our data show that administration of HCG to ISO-induced rats was beneficial in terms of normalizing the activity of enzymatic and non-enzymatic antioxidants, and reduced the levels of cardiac markers. HCG exhibited antioxidant, anti-lipid peroxidative, and free radical scavenging properties. In addition, HCG down regulated the production of NF-κB and p53. This shows that HCG can regulate the defense mechanism and thereby protect the cardiac tissue from MI. We highly recommended further research on HCG with regard to its therapeutic and preventive role against CVDs.

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Conflicts of interest

There are no conflicts of interest.

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