Role of carnitine in regulation of hypoglycemia-induced hypertension and cardiac hypertrophy

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Abstract

Background: Cardiovascular disease is a leading cause of death in diabetic patients. Hyperglycemia and iatrogenic hypoglycemia exacerbate several pathogenic mechanisms underlying hypertension and heart diseases. Carnitine is a potent endogenous antioxidant and cellular fatty acid transporter for antioxidative stress and energy production in the cardiovascular system. In the present study, we aimed to find the role of carnitine in the regulation of hypoglycemia-induced hypertension and cardiac hypertrophy. Methods: Albino Wistar male rats were divided into six groups. The, first three groups received saline (n= 5), D-carnitine (n= 5) at dose of 500mg/kg/day, intraperitoneal for carnitine depletion, or acetyl-L-carnitine (n= 5) at a dose of 300mg/kg/day, intraperitoneal for carnitine supplementation for 15 days. In the last three groups, rats had pretreatment conditions for 5 days with saline, D-carnitine or acetyl-L-carnitine. Then, they were treated with insulin glargine (InG) for 10 days with saline (n= 6), D-carnitine (n= 5) or acetyl-L-carnitine (n= 6), respectively. Results: Our results showed that carnitine deficiency provoked hypoglycemia-induced hypertension. Mean arterial pressure was elevated from 78.16 ± 4.67 to 100 ± 2.09 mmHg in InG treated group, and from 78.2 ± 3.8 to 123.4 ± 12.6 mmHg in InG+D-carnitine treated group. Acetyl-L-carnitine resisted the elevation in blood pressure in all hypoglycemic animals and kept it within the normal values (68.33 ± 6.7 mmHg). In heart, acetyl-L-carnitine increased myocardial carnitine content leading to the attenuation of hypoglycemia-induced oxidative stress, which was evaluated through measurement of the oxidative stress biomarkers such as inducible nitric oxide synthase, NAD(P)H quinone dehydrogenase-1, heme oxygenase-I, and glutathione transferase. Moreover, acetyl-L-carnitine prevented induction of cardiac hypertrophy during hypoglycemic conditions, which was assessed via the evaluation of mRNA expression of the cardiac hypertrophy biomarkers, α-myosin heavy chain and β-myosin heavy chain. Conclusions: These findings demonstrate that carnitine plays an essential role in providing energy and antioxidants to the cardiovascular system leading to the prevention of hypoglycemia-induced hypertension and cardiac hypertrophy.

Background

In diabetic patients, hypoglycemia is the most frequent side effect of diabetes management, often arising from attempts to achieve a state of euglycemia. Previous studies have shown that intensive glycemic control in type1 diabetes (T1D) increases hypoglycemic episodes to ten episodes per a week, and induces mortality in 2–4% of subjects [1, 2]. In type2 diabetes (T2D), insulin and sulphonylurea-dependent patients have a higher frequency of hypoglycemic episodes among diabetic subjects [3]. In both types, repetitive hypoglycemic episodes in insulin dependent diabetic patients lead to a defect in counterregulation mechanisms, causing a syndrome called hypoglycemia unawareness (HU). Diabetic patients with HU experience longer durations of hypoglycemia due to a reduction in catecholamine-induced autonomic symptoms that usually alert them to take actions [4].

Clinical studies have shown a correlation between long durations of hypoglycemia and functional disturbance in different organ systems like the brain, heart and kidneys leading to neuronal failure, cardiac dysfunction and renal damage, respectively [5, 6, 7]. Recently, the link between hypoglycemia and

cardiovascular disease (CVD) has been highlighted by different clinical studies that showed a positive correlation between hypoglycemia and CVD-linked morbidity and mortality [6]. Following severe hypoglycemia, many severe pathophysiological pathways reduce endogenous vasodilators and promote the release of vasoconstrictor and inflammatory agents, leading to endothelial damage, oxidative stress, inflammation and arterial dysfunction-induced CVD [6]. Different harmful factors have been involved in the activation of signal transduction pathways causing oxidative stress, vasoconstriction and endothelial damage in the cardiovascular system during hypoglycemic conditions [8, 9, 10]. One of these factors is angiotensin II (AngII), which is a potent endogenous vasoconstrictor agent that can induce hypertension and cardiovascular diseases [9]. AngII has been implicated to cause hypertension and cardiac injury in both hyperglycemia and hypoglycemia [9, 10, 11]. Previously, we found that insulin-induced hypoglycemia elevated AngII levels in blood stream, kidney and heart leading to free radical formation (superoxide and peroxynitrite) through increased expression of NADPH oxidase enzymes causing renal and cardiac oxidative stress [9]. In previous studies, it has been demonstrated that free radical formation induces functional disturbances, leading to organ damage during hypoglycemia and other metabolic diseases [12]. Starvation and insulin-induced hypoglycemia have been suggested to cause depletion of a wide range of antioxidants such as glutathione peroxidase, glutathione S-transferase, and superoxide dismutase, leading to oxidative stress organs like the brain, kidneys and liver [12]. Together, these factors promote cardiovascular injury, which may cause organ dysfunction in diabetic patients.

Carnitine is an amino acid derived from multiple compounds, including L-carnitine, acetyl-L-carnitine (ALCAR), and propionyl-L-carnitine, which have an important role in fatty acid metabolism for energy production [13]. In the 1960s, nutritional and pharmacological supplements of carnitine were promoted to prevent human diseases caused by carnitine deficiency [14]. Carnitine promotes energy balance in the cell via improvement of energy metabolism in tissues that mainly depend on fatty acid-induced energy production, such as skeletal and cardiac muscles [15]. Later, carnitine was shown to be involved in many disorders such as CVD, diabetes mellitus, neuronal disorders, liver and kidney diseases [13]. In both types of diabetes, carnitine supplementation attenuates chronic complications of diabetes, such as neuropathy, retinopathy, renal and cardiovascular complications. In contrast, carnitine deficiency has been linked to exacerbation of these complications, in diabetic patients [16]. Previous studies showed that carnitine supplementation is beneficial for hypertension management in various hypertensive animal models and diabetes-induced hypertension [17]. Consistently, animal studies suggest that the antihypertensive properties of carnitine are related to its antioxidant activities, which increase nitric oxide availability and reduce components of the renin angiotensin system (RAS) [17]. Moreover, carnitine treatment has been involved in many strategies for the prevention and treatment of mild to severe cardiac diseases [18]. As reported in previous studies, carnitine treatment provides protection against cardiac hypertrophy through alteration in oxidative metabolism, glycolysis and ATP production in damaged cardiac tissues [19]. However, the link between cardiovascular performance, carnitine accumulation and carnitine depletion is still not fully understood.

Taken together, carnitine supplementation appears to provide cardiovascular protection in various diseases, such as heart disease and hyperglycemia-induced CVD. To our knowledge, no studies have

investigated the role of carnitine in the regulation of hypoglycemia-induced cardiovascular disturbances. Therefore, in the present study, we evaluated the effect of carnitine accumulation and carnitine depletion in the prevention of cardiac oxidative stress, hypertension, and cardiac hypertrophy in a chronic hypoglycemic rat model.

Methods

Animals

Male Wistar albino rats weighing 190-220 g (n= 47) were obtained from the Animal Care Center, College of Pharmacy, King Saud University (Riyadh, Saudi Arabia). Animals were fed with a standard chow pellet diet and had free access to water under controlled conditions (25◦C and a 12 h light/dark cycle).

Developing a hypoglycemic-hypertensive rat model

Healthy male rats group (n= 15) was divided into five groups (n= 3 for each group) and treated with several doses of insulin glargine (InG) (Lantus SoloSTAR®) (0, 10, 15, 20, 25units/kg/day, subcutaneous) to produce a hypoglycemic-hypertensive rat model for further investigation of hypoglycemia-induced CVD. Blood glucose (BG) levels and systemic hemodynamic functions were monitored using a glucometer (ACCU-Chek Performa) and BP-2000 Blood Pressure Analysis System™ (Visitech Systems), respectively.

Treatment

For chronic treatment, we had six groups of male rats. The, first three groups received saline (n= 5), D-carnitine (DC, Sigma, St Louis, MO, USA) (n= 5) at dose of 500mg/kg/day, intraperitoneal (*i.p.*) for carnitine depletion as mentioned earlier [20], or acetyl-L-carnitine (ALCAR, Sigma, St Louis, MO, USA) (n= 5) at a dose of 300mg/kg/day, *i.p.* for 15 days. In the last three groups, rats had pretreatment conditions for 5 days with saline, DC (500mg/kg/day, *i.p.*), or ALCAR (300mg/kg/day, *i.p.*). Then, they were treated with InG for 10 days with saline (InG+saline) (n= 6), DC (InG+DC) (n= 5) or ALCAR (InG+ALCAR) (n= 6), respectively. At the day of surgery, all rats were anesthetized with ketamine and xylazine (ketamine 100mg/kg and xylazine 10mg/kg, *i.p.*). Then, rat's chest was opened by aseptic and sharp surgical scissors and forceps. Blood samples were collected directly from the heart. Rats were euthanized by exsanguination from the heart. Throughout the treatment course, BG levels, blood pressure and heart rate were monitored before (day 0) and during treatment (day 3, 6 and 9).

Measurement of carnitine levels in the heart

Following previous analytical technique [21], total carnitine levels was assessed in cardiac tissues through the interaction between carnitine and acetyl-CoA with carnitine acetyltransferase enzyme leading to the production of acetyltransferase and CoA. The free CoA reacted with 5,5-dithiobis-(2-nitrobenzoic

acid) (DTNB, Sigma, St Louis, MO, USA) and produced thiophenolate ions that was measured spectrophotometrically at 412 nm.

Real-time quantitative polymerase chain reaction (RT-PCR) assay

At the day of analysis, total RNA was extracted from the cardiac tissues of all treated groups using TRIzol reagent (Invitrogen®, USA), as per the manufacturer's instructions [22]. Following RNA isolation, cDNA was synthesized using the High Capacity cDNA synthesis reverse transcription kit (Applied Biosystems®, USA) and RT-PCR was performed using SYBR® Green PCR master mix (Applied Biosystems®, USA) as described before [22]. mRNA levels of cardiac hypertrophy markers [α-myosin heavy chain (α-MHC) and β-myosin heavy chain (β-MHC)] and oxidative stress (iNOS) were measured on the Applied Biosystems 7500 Real-Time PCR system (Applied Biosystems®, USA) [22]. All the primers were designed using PubMed database and purchased from Integrated DNA technologies (IDT, Coralville, IA) (Table 1). The data are shown as the fold change in mRNA expression levels normalized to β-actin as a loading control.

Table 1
Primers for Real Time PCR experiments.

Gene	Forward primer	Reverse primer
α-MHC	TCCTTTATCGGTATGGAGTCTG	TGATCTTGATCTTCATGGTGCT
β-MHC	ATCAAGGGAAAGCAGGAAGC	CCTTGTCTACAGGTGCATCA
iNOS	CCCTTCCGAAGTTTCTGGCAGCAGC	GGGTGTCAGAGTCTTGTGCCTTTGG
β-actin	CCAGATCATGTTTGAGACCTTCAA	GTGGTACGACCAGAGGCATACA

Western blot analysis

Cardiac proteins were extracted using lysis buffer as described before [23]. Briefly, cardiac tissue was minced and homogenized in ice-cold lysis buffer mixed with a protease inhibitor cocktail. After lysate collection, protein concentration was identified using the Direct Detect® spectrometer (EMD Millipore, USA). The western blot technique was used to determine protein expression as described in our previous study [23]. We analyzed 25-50 µg on a 10% SDS-polyacrylamide gel electrophoresis (SDS-PAGE) for protein separation. All proteins were transferred from the gel to Immun-Blot® LV PVDF membrane (BioRad, USA) and blocked overnight at 4°C. The blots were incubated with anti-iNOS, anti-NAD(P)H Quinone Dehydrogenase-1 (NOQ1), Anti-Heme Oxygenase-I (HO-I) and Anti- Glutathione transferase (GSH) antibodies (Santa Cruz Biotechnology Inc., CA, USA) at 4°C overnight and then incubated with appropriate peroxide-conjugated secondary antibodies (Santa Cruz Biotechnology Inc., CA, USA). β-actin was used as a protein loading control.

Statistics

Data were expressed as mean ± SEM and analyzed by one-way or two-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test when appropriate (GraphPad Prism 7). ($P < 0.05$) was considered statistically significant.

Results

Hemodynamic changes in InG-treated groups

All InG treated groups (10, 15, 20, 25U/kg) showed hypoglycemic conditions (BG levels < 60 mg/dl) (Fig. 1) (n= 3 for each group). A significant elevation in systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) was shown at doses (20, 25U/kg) with regular heart rate (HR) compared with the control group (Fig. 2). InG (20U/kg) led to increased MAP from 72.83 ± 0.86 mmHg (control group) to 93.5 ± 2.9 mmHg with lower mortality compared with InG (25U/kg) (Fig. 2c). However, InG at dose (20U/kg/day, *s.c.*) was selected for our further studies of hypoglycemia-induced hypertension and cardiac damage.

Hemodynamic changes during hypoglycemia with DC and ALCAR treatment

In the presence or absence of carnitine, blood pressure and heart rate were monitored to identify carnitine function in attenuation of hypoglycemia-induced hypertension. InG (20U/kg) decreased BG in all treated groups (InG+Saline, InG+DC and InG+ALCAR) to levels that were considered hypoglycemic (BG levels < 50 mg/dl) (Fig. 3). MAP was continuously increased in the InG+Saline group (100 ± 2.09 mmHg) compared with the control group (MAP= 78.4 ± 8.7 mmHg) (Fig. 4a). During InG+DC treatment, we found that hypoglycemia-induced hypertension was exaggerated dramatically with DC treatment (MAP= 123.4 ± 12.6 mmHg) (Fig. 4a). ALCAR attenuated the elevation in blood pressure during hypoglycemia and successfully kept MAP within a normal range (68.33 ± 6.7 mmHg) compared with the control group (Fig. 4a). We observed the same scenario with SBP, which was dramatically increased during DC treatment compared with the InG+Saline group and ALCAR resisted the elevation in SBP during hypoglycemia in InG+ALCAR group (Fig. 4b). Heart rate was normal in all treated groups during the study (Fig. 5).

Carnitine levels in cardiac tissues

Measurement of total carnitine in the heart was used to assess the effect of chronic hypoglycemia on cardiac carnitine levels. Ten days of hypoglycemia decreased cardiac carnitine content compared with the control group. DC with hypoglycemia promoted the reduction of carnitine content in comparison with hypoglycemia alone group. ALCAR treatment increased total carnitine levels during hypoglycemic conditions to reach normal levels compared with the control group (Fig. 6).

α-MHC and β-MHC genes levels

The link between hypoglycemia and carnitine deficiency in the cardiac tissues was guided us to investigate whether carnitine has a role in attenuation of cardiovascular risk of hypoglycemia or not. α-MHC and β-MHC genes were assessed as cardiac hypertrophy biomarkers to determine carnitine function

in the prevention of hypoglycemia-induced cardiac hypertrophy. As expected, the carnitine supplement (ALCAR) prevented induction of cardiac hypertrophy during 10 days of severe hypoglycemia and kept α-MHC and β-MHC genes expression within normal levels (Fig. 7). α-MHC mRNA level was ~40-fold decreased in InG+Saline and InG+DC groups compared with euglycemic groups. ALCAR totally prevented the reduction of α-MHC mRNA level in the hypoglycemic group compared with the InG+Saline and InG+DC groups (Fig. 7a). β-MHC mRNA level was ~7-fold increased during hypoglycemia with and without DC treatment compared with the euglycemic groups (Fig. 7b). ALCAR significantly attenuated the elevation of β-MHC mRNA levels during hypoglycemic conditions compared with the InG+Saline and InG+DC groups (Fig. 7b). However, carnitine has a critical role in overcoming the risk of hypoglycemia-induced cardiac hypertrophy.

Inducible Nitric Oxide Synthase (iNOS) levels

Since carnitine is known as a potent endogenous antioxidant, we planned to evaluate its role in the attenuation of hypoglycemia-induced oxidative stress. In cardiac tissues, we measured levels of iNOS as a biomarker of oxidative stress. Hypoglycemic conditions significantly increased iNOS expression, and DC treatment exaggerated iNOS mRNA and protein expression during hypoglycemia (Fig. 8). ALCAR blocked the induction of iNOS during hypoglycemia compared to the euglycemic groups (Fig. 8).

NQO1, HO-I and GSH levels

For further investigation into the role of carnitine in the prevention of hypoglycemia-induced cardiac oxidative stress, we measured protein levels of NQO1, HO-I, and GSH as biomarkers of oxidative stress. We found that NQO1 and GSH were dramatically induced in the InG+ALCAR group compared with the InG+Saline and InG+DC groups (Fig. 9). HO-I was induced during hypoglycemic conditions and inhibited with ALCAR treatment as compared with control group (Fig. 9). However, we confirmed that hypoglycemia provokes cardiac oxidative stress through reducing carnitine content in the heart.

Discussion

In the present study, the hypoglycemic protocol was adopted to study hypoglycemia-induced hypertension and cardiac damage, which was confirmed through continuous monitoring of blood pressure and gene expression of cardiac hypertrophy biomarkers, respectively. Clinical studies emphasized the severity of severe hypoglycemia in causing CVD through activation of inflammation, endothelial dysfunction, blood coagulation abnormality and impaired cardiovascular autonomic function [24]. Thus, we investigated whether carnitine deficiency and/or supplementation play a role in the attenuation of cardiovascular complications during chronic severe hypoglycemia.

Our results confirm what has been shown in previous studies of hypertension and cardiac injury during hypoglycemic conditions [9, 10]. Recently, cardiovascular injuries related to hypoglycemia have been linked to depletion of energy production and/or hyperinsulinemia and induction of pathological mechanisms such as oxidative stress and RAS in brain, heart, and kidneys [9, 25, 26, 27]. In the current

study, we approved that hypoglycemia-induced hypertension was exaggerated with D-carnitine treatment due to carnitine depletion, as D-carnitine is the inactive isomer of L-carnitine [20]. However, L-carnitine (the active form) was exchanged with D-carnitine from the intracellular to the extracellular space, causing a reduction in L-carnitine cellular content [20]. Depletion of carnitine motivated hypoglycemia-induced hypertension might be due to an elevation of oxidative stress as indicated in our results via assessment of the iNOS, NQO1, HO-I, and GSH levels in the heart. Our results demonstrate that the presence of carnitine can reduce cardiovascular complications of hypoglycemia through its antioxidant properties and effect on energy supply. Carnitine supplementation maintained the systemic hemodynamic function within the normal values and prevented hypoglycemia-induced oxidative stress through the augmentation of antioxidants like NQO1 and GSH and attenuation of oxidants including HO-I.

At the cellular level, carnitine, carnitine transporters and mitochondrial enzymes (carnitine acylcarnitine translocase, carnitine palmitoyltransferase-1 and carnitine palmitoyltransferase-2) are responsible for uptake fatty acid chains through the mitochondrial membrane [35]. This step is essential for fatty acid breakdown to increase energy production to skeletal muscle and heart during prolonged fasting [35]. However, patients with repetitive hypoglycemia and carnitine deficiency may suffer from severe complications due to the absence of compensatory processes in energy provision. Carnitine deficiency was linked, in previous studies, to deficiency in several essential factors such as ascorbic acid, niacin and ferrous ion [13]. Carnitine depletion has been defined in pathologies related to dysfunction of sodium-dependent plasma membrane carnitine transporters, including OCTN2, and long term use of pivalate-conjugated antibiotics [36, 37]. Our data demonstrates that chronic hypoglycemia and/or hyperinsulinemia reduces endogenous carnitine levels in the cardiac tissues, promoting the increased incidence of hypertension and cardiotoxicity. This link between hypoglycemia and carnitine insufficiency has been described in a previous report of hypoglycemic encephalopathy [34]. In this report, they found systemic carnitine deficiency in a child was suffering from hypoketotic hypoglycemia with seizures that were managed in the early stages of treatment [34]. The mechanism of hypoglycemia and/or hyperinsulinemia-induced carnitine deficiency is still unclear.

However, observation of carnitine deficiency and cardiovascular complications of intensive insulin treatment prompted us to treat hypoglycemic animals with carnitine supplementation (ALCAR). ALCAR increased the myocardial carnitine content providing cardiovascular protection against hypertension and cardiac hypertrophy during long-term episodes of hypoglycemia and/or hyperinsulinemia. ALCAR has been approved for the regulation of diabetic complications such as insulin resistance and hypertension following euglycemic clamp and carnitine supplementation studies in rodents and human [28, 29]. Clinically, ALCAR treatment (1 g twice daily) ameliorated impaired glucose tolerance, hypoadiponectinemia, insulin resistance and hypertension, in T2D [28]. In high-fat diet-fed mice, carnitine supplements enhanced insulin function in management of blood glucose levels without changes in food intake and body weight [30]. The protective role of ALCAR may be based on its potent antioxidant properties, as shown in previous investigations. These demonstrated that ALCAR confers antioxidant effects through augmentation of the NQO1-dependent antioxidant signaling pathways, which protects endothelial function and prevents induction of hypertension [31, 32]. NQO1 production modulates

spontaneous hypertension via inhibition of acetylation of endothelial nitric oxide synthase-derived nitric oxide in a hypertensive animal model [32]. Recently, L-carnitine was reported to preserve cardiac function through NQO1 activation in radiation-induced cardiovascular injury [33]. As shown in the current and previous studies, a strong relationship between oxidative stress and heart disease, and the prevention of free radical formation is essential in regulating of cardiac hypertrophy and hypertension.

Conclusions

In summary, our study demonstrates the essential role of carnitine in the cardiovascular system, through the provision of energy and antioxidants, leading to the prevention of hypertension and cardiac hypertrophy during long-term hypoglycemia. For further investigations, future studies will be conducted to investigate the role of carnitine in the control of renin angiotensin-II system-induced hypertension and cardiac injury during hypoglycemia.

Abbreviations

T1D: Type1 Diabetes; T2D: Type2 Diabetes; HU: Hypoglycemia Unawareness; CVD: Cardiovascular Disease; AngII: Angiotensin II; ALCAR: Acetyl-L-Carnitine; RAS: Renin Angiotensin System; InG: Insulin Glargine; BG: Blood Glucose; i.p.: Intraperitoneal; DC: D-Carnitine; DTNB: 5,5-Dithiobis-(2-Nitrobenzoic Acid); RT-PCR: Real-Time Quantitative Polymerase Chain Reaction; α-MHC: α-Myosin Heavy Chain; β-MHC: β-Myosin Heavy Chain; iNOS: Inducible Nitric Oxide Synthase; SDS-PAGE: SDS-Polyacrylamide Gel Electrophoresis; NOQ1: NAD(P)H Quinone Dehydrogenase-1; HO-I: Heme Oxygenase-I; GSH: Glutathione Transferase; ANOVA: Analysis of Variance; SBP: Systolic Blood Pressure; DBP: Diastolic Blood Pressure; MAP: Mean Arterial Pressure; HR: Heart Rate

Declarations

Ethics approval and consent to participate

All procedures performed on animals were approved by the Institutional Animal Care and Use Committee (IACUC) at King Saud University, (Riyadh, Saudi Arabia).

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

All authors declare that there are no conflicts of interest.

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Authors' Contributions

WA designed the study. WA, NO, FI, MA1 and NA performed the animal study and samples analysis. WA, AH, AA, FA and MA2 analyzed the results and wrote the manuscript. All authors read and approved the final manuscript.

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Figures

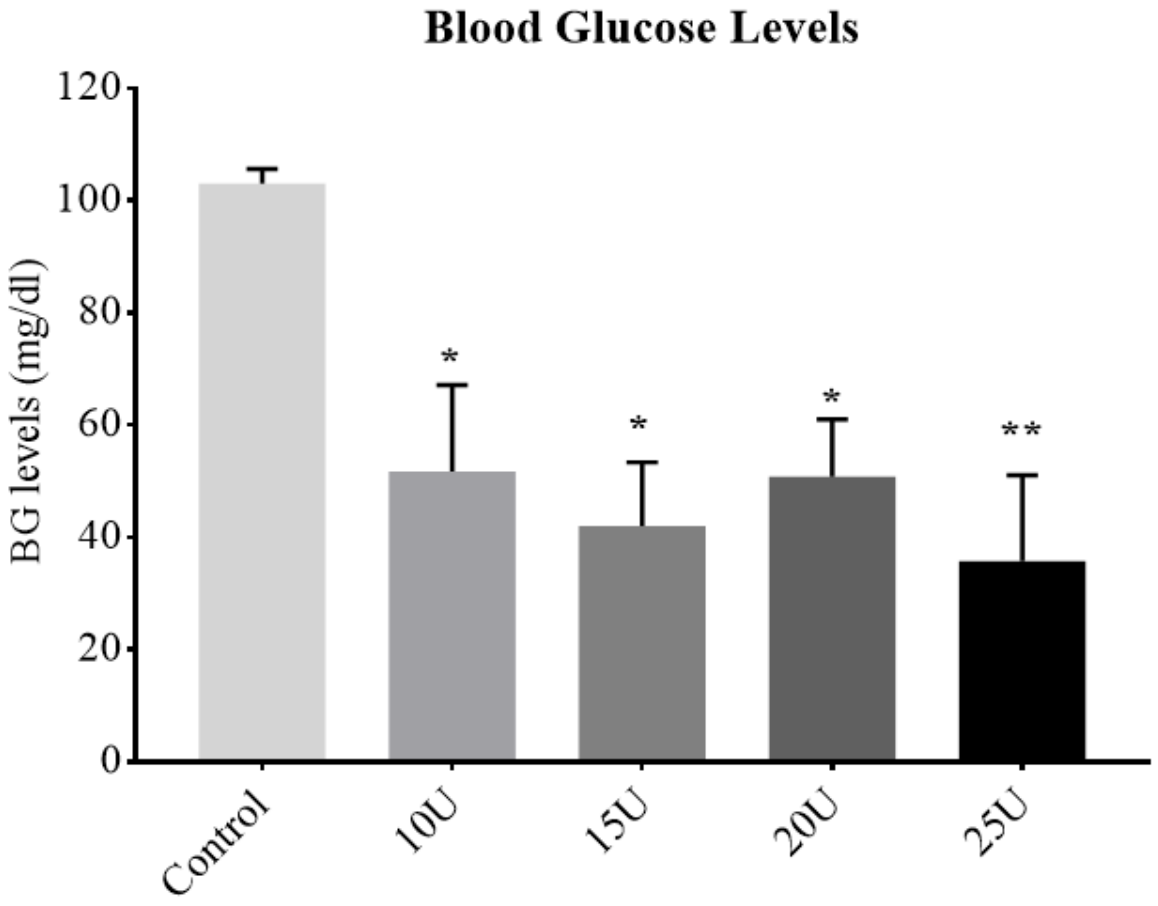

Figure 1

Measurement of blood glucose levels in all groups treated with saline, 10, 15, 20 and 25U/kg/day of InG. Values are expressed as mean ± SEM, (n= 3). *P< 0.05 and **P< 0.01 for control vs 10, 15 & 20U and control vs 25U, respectively (by one-way ANOVA followed by Tukey-Kramer posttests).

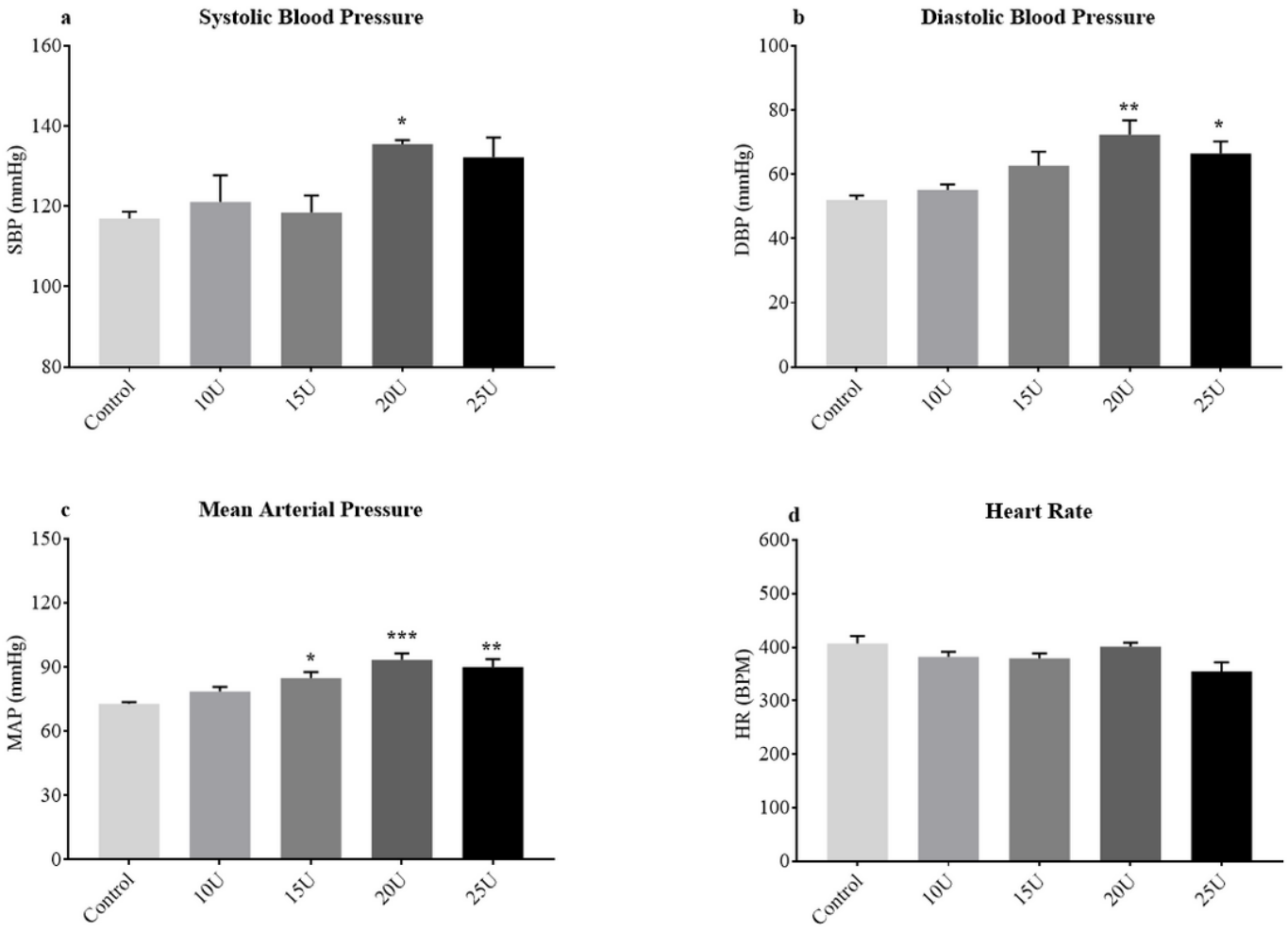

Figure 2

Measurement of hemodynamic functions in all groups treated with saline, 10, 15, 20 and 25U/kg/day of lnG. Values are expressed as mean ± SEM, (n= 3). a Systolic blood pressure was significantly increased in the 20U treated animals compared with the control group (*P< 0.05). b Diastolic blood pressure was significantly increased in the 20 & 25U treated animals compared with the control group (*P< 0.05 & **P< 0.01). c Mean arterial pressure was significantly increased in the 15, 20 & 25U treated animals compared with the control group (*P< 0.05, **P< 0.01 & ***P< 0.001). d Heart rate showed no significant changes in all treated groups. Analyses were performed using one-way ANOVA followed by Tukey-Kramer posttests.

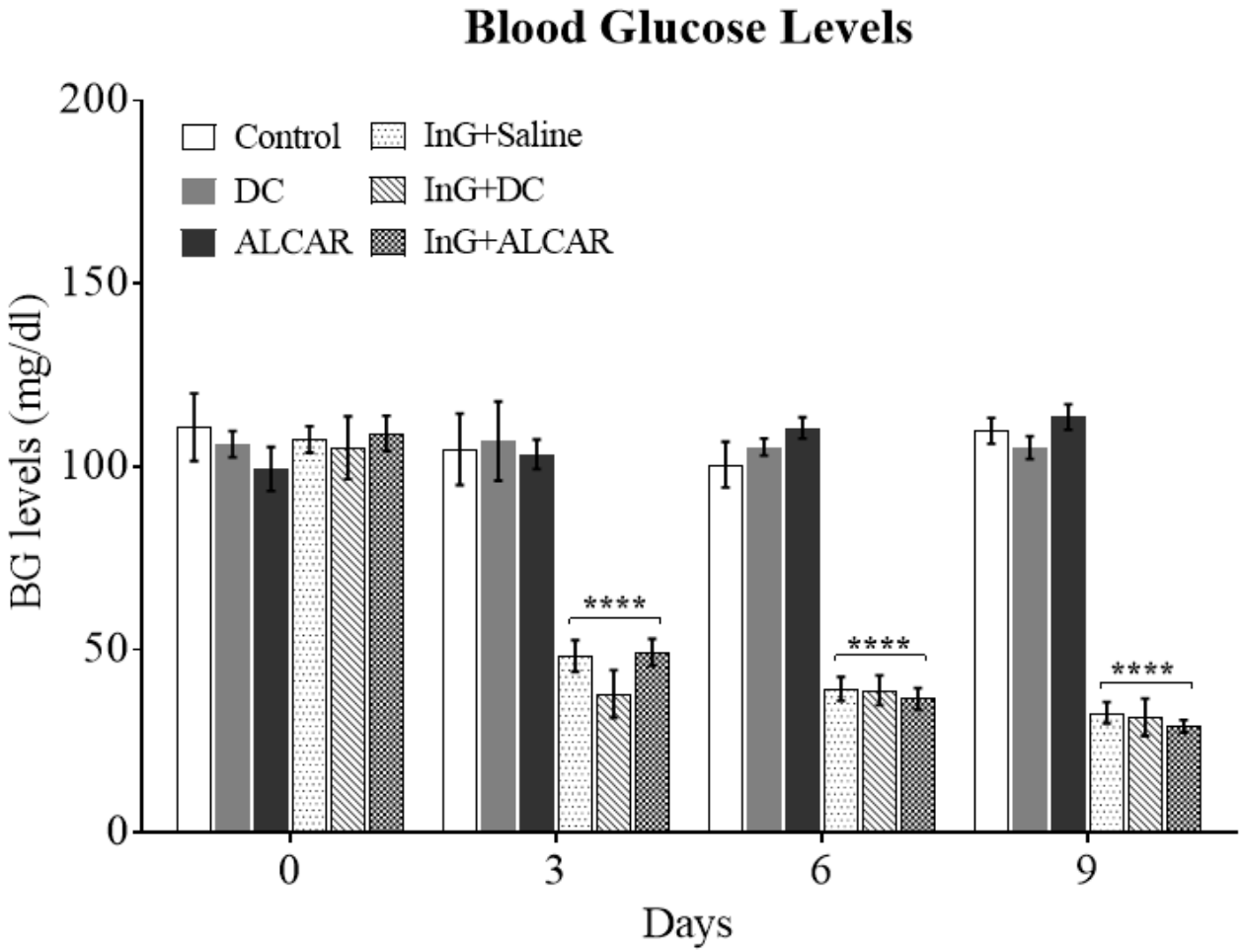

Figure 3

Measurement of blood glucose levels in all groups treated with saline, DC, ALCAR, InG+Saline, InG+DC and InG+ALCAR at day 0, 3, 6 and 9. Values are expressed as mean ± SEM, (n= 5-6). ****P< 0.0001 for InG+Saline, InG+DC and InG+ALCAR vs control (by two-way ANOVA followed by Tukey-Kramer posttests).

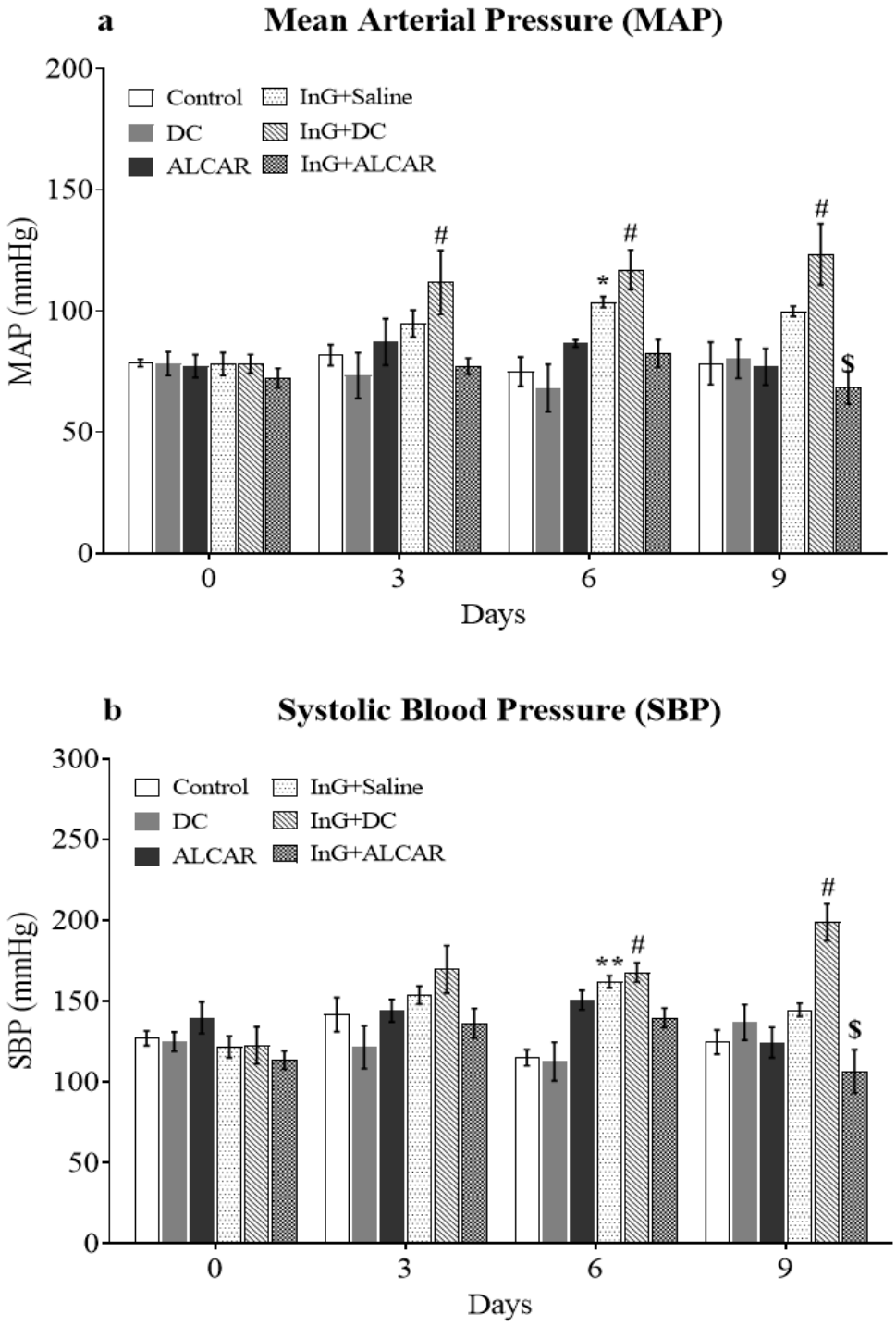

Figure 4

Measurement of blood pressure in all groups treated with saline, DC, ALCAR, InG+Saline, InG+DC and InG+ALCAR at day 0, 3, 6 and 9. a Mean arterial pressure and, b Systolic blood pressure were significantly increased in InG+Saline and InG+DC compared with the control group. MAP & SBP were significantly decreased in InG+ALCAR compared with InG+Saline and InG+DC. Values are expressed as mean ± SEM, (n= 5-6). *P< 0.05 & **P< 0.01 for InG+Saline vs control, #P< 0.05 for InG+DC vs control, and $P< 0.05 for

InG+Saline and InG+DC vs InG+ALCAR. Analyses were performed using two-way ANOVA followed by Tukey-Kramer posttests.

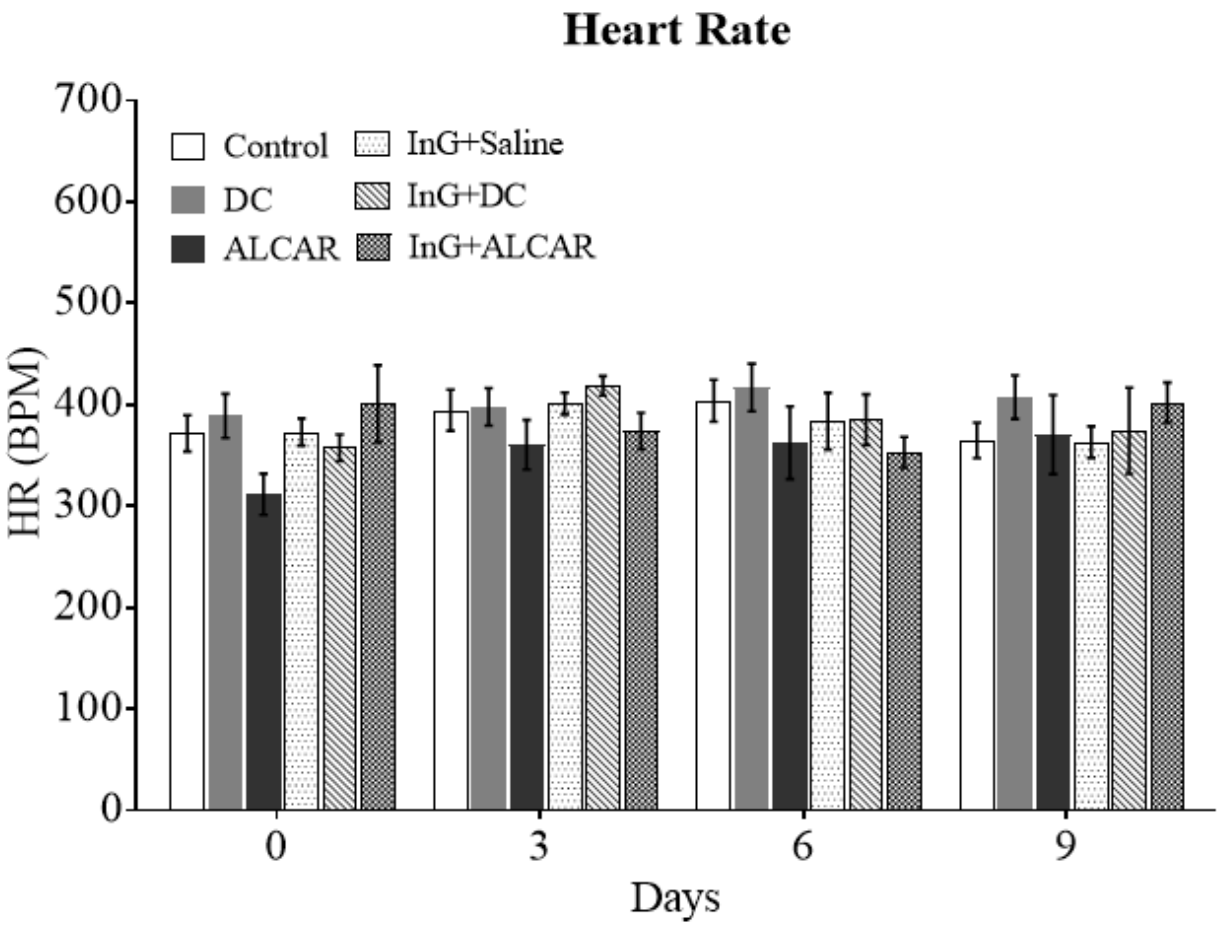

Figure 5

Heart rates measurement in all groups treated with saline, DC, ALCAR, InG+Saline, InG+DC and InG+ALCAR at day 0, 3, 6 and 9. Heart rate showed no significant changes in all treated groups. Values are expressed as mean ± SEM, (n= 5-6). Analyses were performed using two-way ANOVA followed by Tukey-Kramer posttests.

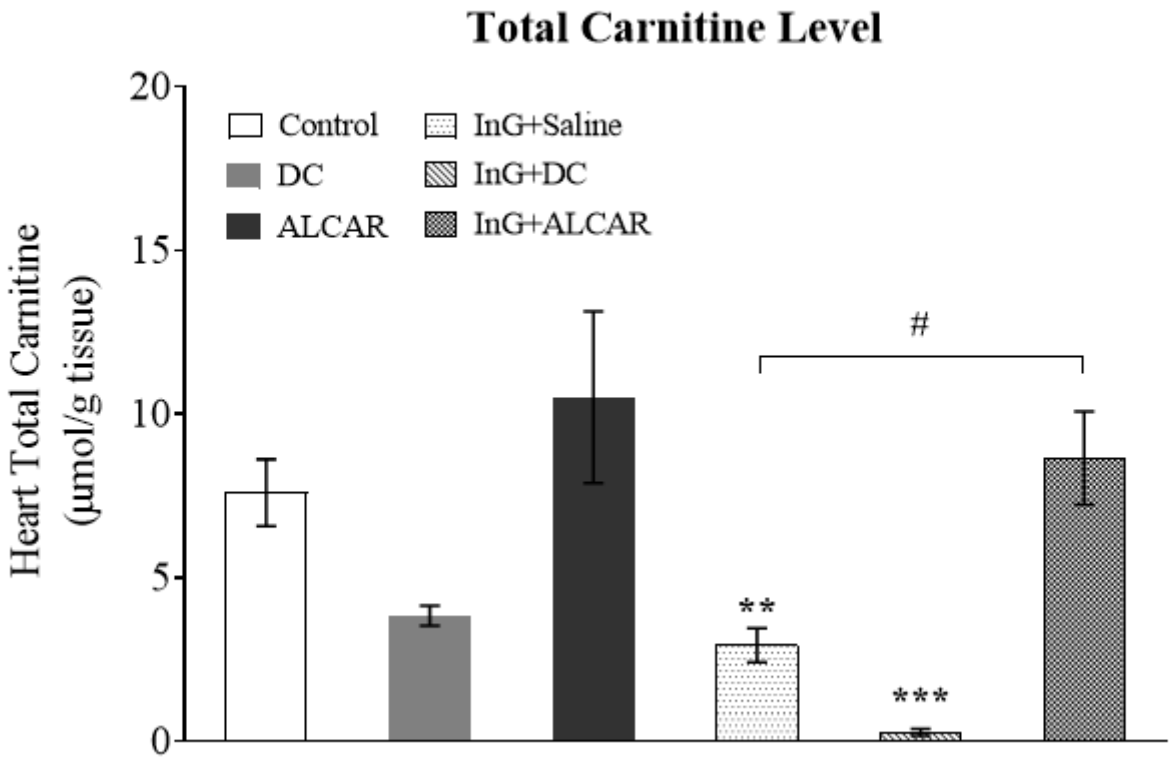

Figure 6

Total carnitine levels in cardiac tissues in all groups treated with saline, DC, ALCAR, InG+Saline, InG+DC, and InG+ALCAR. Carnitine levels were significantly decreased in InG+Saline and InG+DC compared with the control group. In InG+ALCAR, carnitine levels restored to the normal levels and increased significantly compared with InG+Saline and InG+DC. Values are expressed as mean ± SEM, (n= 5-6). **P< 0.01 for InG+Saline vs control and ***P< 0.001 for InG+ALCAR vs control. #P< 0.01 for InG+Saline and InG+DC vs InG+ALCAR. Analyses were performed using one-way ANOVA followed by Tukey-Kramer posttests.

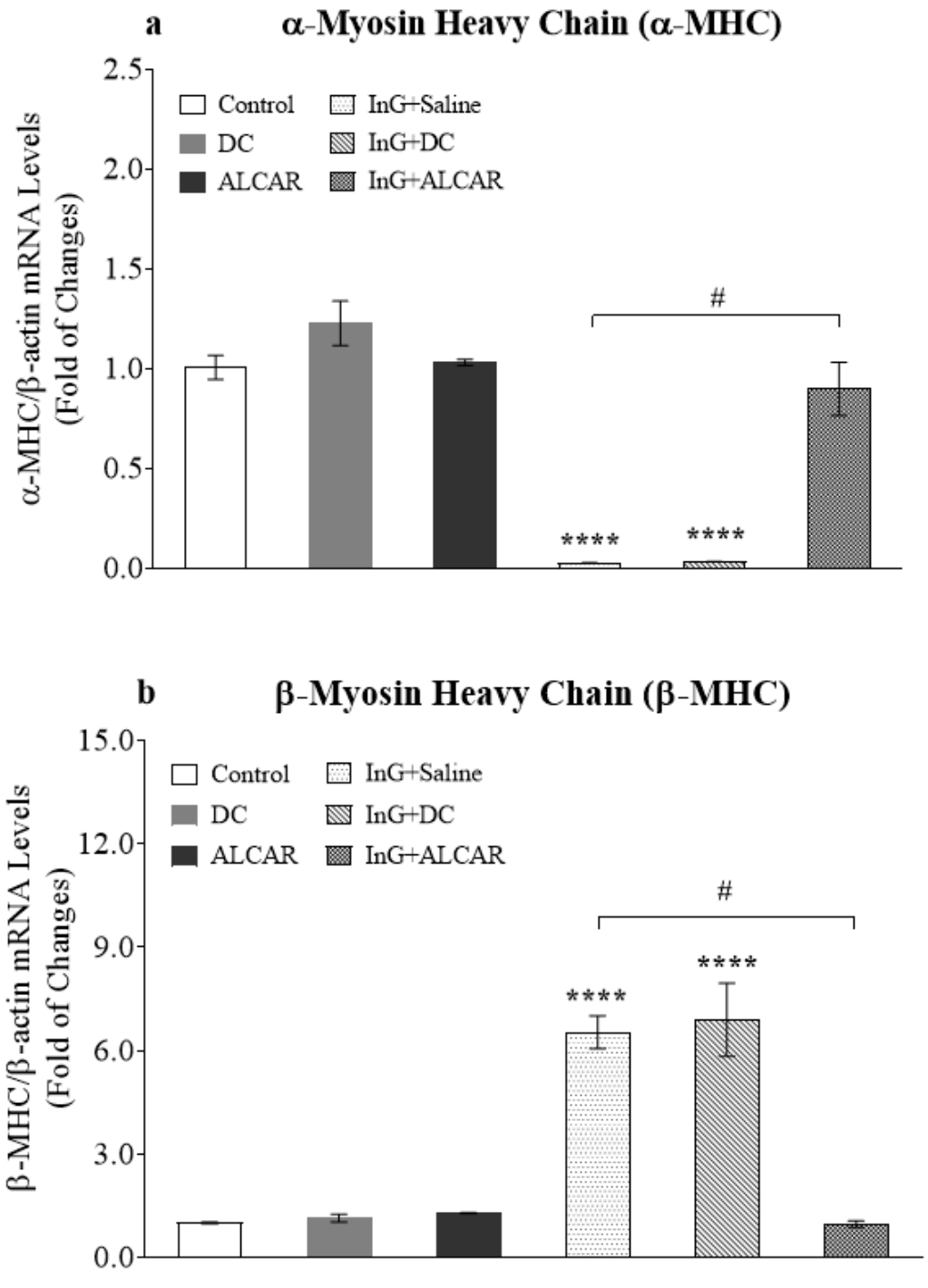

Figure 7

mRNA levels of cardiac hypertrophy biomarkers in all groups treated with saline, DC, ALCAR, InG+Saline, InG+DC, and InG+ALCAR, a α-myosin heavy chain (α-MHC) was significantly decreased in InG+Saline and InG+DC compared with the control. α-MHC was increased in InG+ALCAR compared with InG+Saline and InG+DC. b β-myosin heavy chain (β-MHC) was significantly increased in InG+Saline and InG+DC compared with the control. β-MHC was decreased in InG+ALCAR compared with InG+Saline and InG+DC.

Values are expressed as mean ± SEM, (n= 5-6). ****P< 0.0001 for InG+Saline and InG+DC vs control, #P< 0.0001 for InG+Saline and InG+DC vs InG+ALCAR. Analyses were performed using two-way ANOVA followed by Tukey-Kramer posttests.

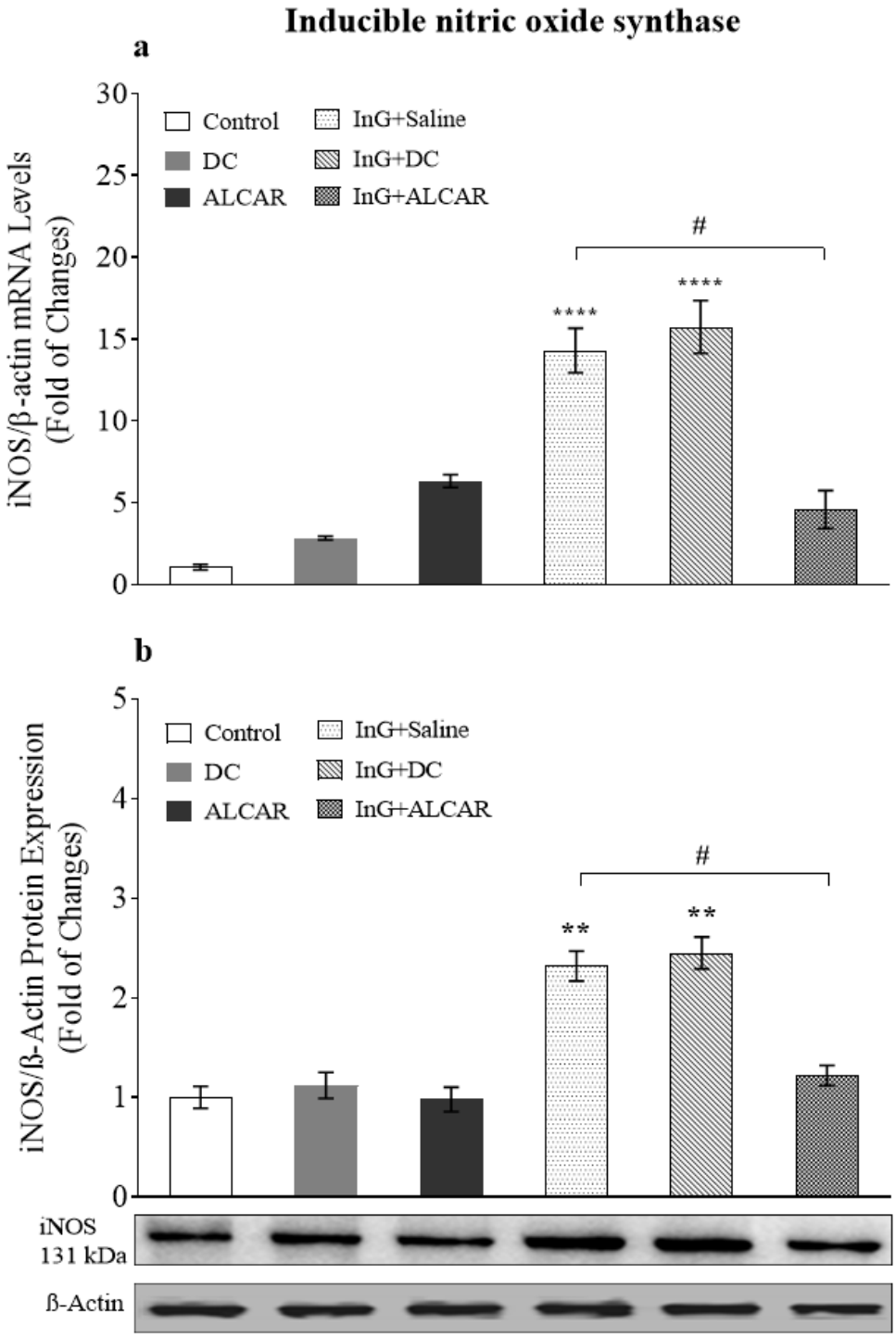

Figure 8

Measurement of inducible nitric oxide synthase (iNOS) in all groups treated with saline, DC, ALCAR, InG+Saline, InG+DC and InG+ALCAR. a mRNA levels of iNOS was significantly increased in InG+Saline

and InG+DC compared with the control. iNOS was decreased in InG+ALCAR compared with InG+Saline and InG+DC. b At the protein level, iNOS was also significantly increased in InG+Saline and InG+DC compared with the control. iNOS protein level was decreased in InG+ALCAR compared with InG+Saline and InG+DC. Values are expressed as mean ± SEM, (n= 5-6) ****P< 0.0001 for control vs InG+Saline and InG+DC. a #P< 0.0001 for InG+Saline and InG+DC vs InG+ALCAR. b **P< 0.01 for InG+Saline and InG+DC vs control, and #P< 0.01 for InG+Saline and InG+DC vs InG+ALCAR. Analyses were performed using two-way ANOVA followed by Tukey-Kramer posttests.

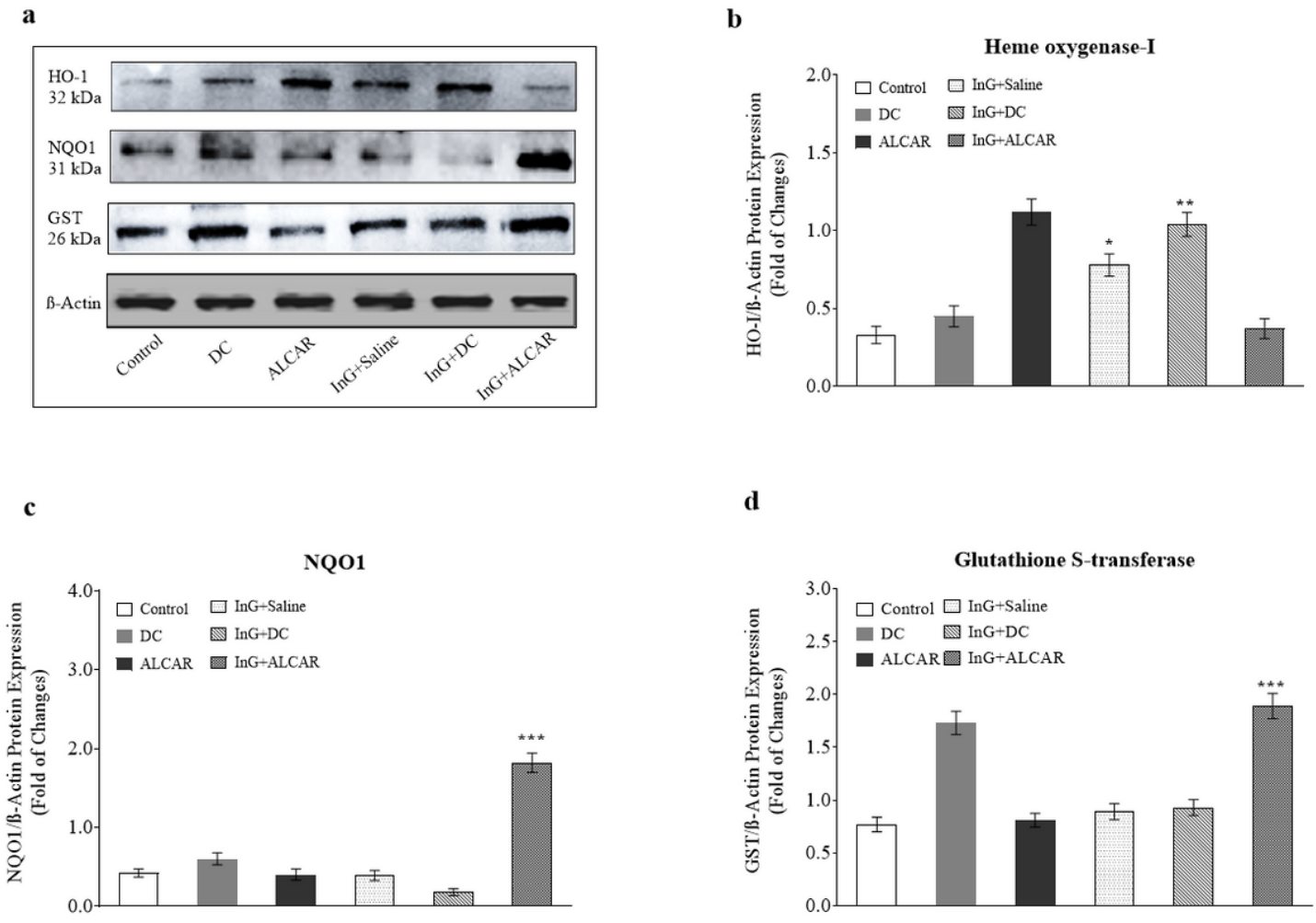

Figure 9

Measurement of NAD(P)H quinone dehydrogenase-1 (NQO1), heme oxygenase-I (HO-I) and glutathione transferase (GSH) in all groups treated with saline, DC, ALCAR, InG+Saline, InG+DC and InG+ALCAR. b HO-I was induced in InG+Saline and InG+DC compared with the control. c, d NQO1 and GSH protein level was significantly increased in InG+ALCAR compared with control, InG+Saline, and InG+DC. Values are expressed as mean ± SEM, (n= 5-6). b *P< 0.05 & **P< 0.01 for InG+Saline & InG+DC vs control. c, d ***P< 0.001 for InG+ALCAR vs control. Analyses were performed using two-way ANOVA followed by Tukey-Kramer posttests.

Supplementary Files

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