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ORIGINAL ARTICLE

Steroidal Saponin Diosgenin from *Dioscorea bulbifera* Protects Cardiac Cells from Hypoxia-reoxygenation Injury Through Modulation of Pro-survival and Pro-death Molecules

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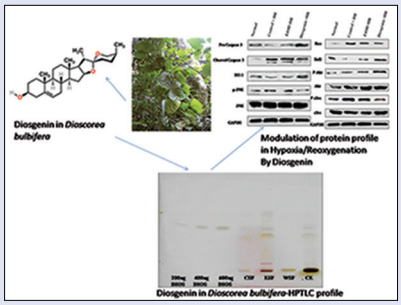
ABSTRACT

Background: Diosgenin, a steroidal saponin from plants, exhibits many biological potentials. Herein, the cardioprotective role of diosgenin is studied. **Materials and Methods:** The effect of diosgenin, isolated from *Dioscorea bulbifera*, was studied on hypoxia-reoxygenation (HR) in H9c2 cardiomyoblast cells. The amount of diosgenin in the plant extract was analyzed by high-performance thin layer chromatography using a solvent system comprising of chloroform:methanol:acetic acid:formic acid (13:4.5:1.5:1). Cardioprotection was checked by (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. Further, the release of lactate dehydrogenase, an enzyme released during cell death was checked. The proteins responsible for cell death (Bax) and cell survival (Bcl-2, hemeoxygenase-1 and Akt) were analyzed using Western blot to check the cardioprotective role of diosgenin. **Conclusion:** Supplementation of diosgenin mitigates HR injury, thereby exhibiting cardioprotective potential.

Key words: Cardioprotection, diosgenin, H9c2, hypoxia, reoxygenation

SUMMARY

- The cardioprotective effect of Diosgenin was evidenced from the improved cell survival after hypoxia-reoxygenation injury demonstrated through MTT cell survival assay.
- The release of lactate dehydrogenase, an enzyme released during cell death was decreased by Diosgenin.
- Diosgenin upregulated the pro-survival molecules like B-cell lymphoma 2 (Bcl-2), heme oxygenase-1 and the phosphorylation of ATK (at serine 473); and at the same time pro-death molecules like Bax was downregulated.
- Thus, Diosgenin as a plant based steroidal saponin is confirmed to mitigate ischemic reperfusion injury.



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INTRODUCTION

Ineffici t vascular supply and the resultant reduction in tissue oxygen tension often lead to neovascularization in order to satisfy the needs of the tissue. The reliance of many cells on aerobic respiration as a mandatory energy source requires a variety of responses to oxygen lack or "hypoxia."[1] Both hypoxia (lack of oxygen relative to metabolic needs) and reoxygenation (reintroduction of oxygen to hypoxic tissue) are important in human pathophysiology because they occur in a wide variety of important clinical conditions. Prominent examples of tissue hypoxia that predispose to injury during reoxygenation include circulatory shock, myocardial ischemia, stroke, and transplantation of organs.[2-5] At the cellular level, hypoxia activates numerous major signaling pathways, resulting in changes in gene expression, which influence the cellular ability to survive or die. Severe hypoxia, occurring at partial pressure of oxygen below 20 mm Hg, impairs cellular energy production and ion homeostasis, leading to cell injury and cell death. In contrast, a lower degree of hypoxia, defi ed as between 50 and 100 mm Hg, may activate mechanisms that could produce cellular phenotype more resistant to acute severe oxidative stress.[6,7] Th s phenomenon is one of the most important components of different forms of ischemic heart diseases which include myocardial infarction also. As diseases due to ischemia (e.g. myocardial infarction and stroke) are exceedingly common causes of morbidity and mortality which involves production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), understanding the role of ROS and RNS in reoxygenation injury has the potential to lead to therapies that could improve public health. Cellular models of hypoxia-reoxygenation (HR) have provided useful tools for the study of reactive species mediated mechanisms of cellular dysfunction in ischemia-reperfusion injury.[8] H9c2 cells are derived from embryonic rat heart and are generally accepted to be a good model for cardiomyoblast cells. These cells have been successfully implemented to study mechanisms of cellular and cardiac protection.[9,10]

Dioscorea bulbifera commonly known as air potato belonging to a class of yam contains a large number of polyphenols including organic acids, some of which may be potential antioxidants, furthermore, *D. bulbifera*

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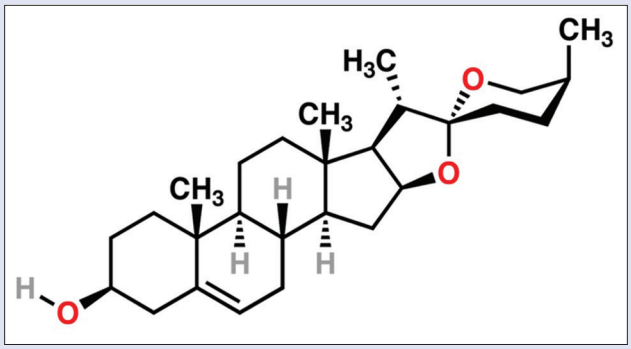

Figure 1: Structure of diosgenin

yam also contains the steroidal saponin diosgenin, the principal material for the synthesis of corticosteroids, estrogen, and contraceptives.[11] Diosgenin is among the ten most important sources of steroids and the most often prescribed medicine of plant origin.[12] Among the steroidal saponins, diosgenyl saponins are the most abundant and exert a large variety of biological functions.[13] Diosgenin is the primary furostanol saponin [Figure 1] found in several plants, including *Dioscorea* species (yams), fenugreek, and *Costus speciosus*. Th s compound has been shown to be useful for the maintenance of healthy blood cholesterol levels,[14] it has been reported that diosgenin retarded the progression of osteoporosis[15] and possessed anti-inflammatory effects in rats.[15] Also, diosgenin glycosides altered myocardial mechanical activities, probably, through the modulation of extracellular calcium (Ca^{2+}) flux across the plasma membrane.[16] Another study demonstrated the vasodilator effect of diosgenin in rat superior mesenteric artery.[17] Diosgenin has been found to ameliorate myocardial infarction by its lipoperoxidative activity.[18] Our earlier study on the hydroalcoholic extract of *D. bulbifera* on myocardial ischemic reperfusion injury[19] further kindled us to study the quantity of diosgenin in the various fractions of *D. bulbifera* and study its role in HR injury in *in vitro* condition.

In the present study, we quantifi d the amount of diosgenin present in *D. bulbifera* by high-performance thin layer chromatography (HPTLC) profile. Also, we report that in H9c2 rat myocardium-derived cardiomyoblast, apoptosis is inhibited in conditions of simulated ischemia, represented by HR and are prevented by diosgenin, from *D. bulbifera*. The protective effect of diosgenin correlates with a marked down-regulation of pro-apoptotic protein Bax and death signaling kinases (phospho-c-Jun N-terminal kinases and phospho-Src), with an up-regulation of cell survival molecules (p-Akt, and hemeoxygenase-1 [HO-1]) and anti-apoptotic protein Bcl-2 in H9c2 cardiomyoblast cells.

MATERIALS AND METHODS

Collection of plant material and processing

D. bulbifera Linn. tuber was purchased from Ayurmed Biotech Pvt. Ltd., Mumbai, and it was authenticated at the Centre for Advanced Research in Indian System of Medicine (CARISM) by qualifi d botanists. The tubers were cut into pieces of 5–8 mm thickness, and it was air-dried. The air-dried tubers were coarsely pulverized.

Extraction and fractionation of *Dioscorea bulbifera* Linn. tuber

The extraction and fractionation procedures were carried out as described by Gao *et al.*, with slight modifi ations.[20] About 2 kg of the coarsely powdered tuber was extracted with 5 L of 85% ethanol by cold maceration process for 72 h with occasional shaking. The extracts were drained and this process was repeated thrice. The obtained extract was pooled and concentrated *in vacuo* to obtain the crude extract (CE). The total ethanolic CE was suspended in water, and it was subjected to partitioning using chloroform and ethyl acetate to yield chloroform, ethyl acetate and water-soluble fraction. The individual fractions were concentrated *in vacuo*, and they were stored in a desiccator to free from moisture for further analysis.

High-performance thin layer chromatography fingerprinting analysis of alcoholic extract of *Dioscorea bulbifera*

The fi gerprinting analysis of the hydroalcoholic extract and the different fractions were carried out at the CARISM, SASTRA University, Thanjavur using HPTLC, CAMAG, Switzerland.

Preparation of sample

Two hundred milligram extract of *D. bulbifera* was dissolved in 10 ml of 99.9% ethanol, and it was filtered through Whatmann filter paper 1 and the filtrate was used for analysis. Similarly, the different solvent fractions were dissolved in their respective solvents, and the filtrate was used for the analysis.

Chromatographic condition

Sample: Hydroalcoholic extract, choloroform fraction, ethyl acetate fraction,

acqeous fraction,

Solvent system: Chloroform:methanol:acetic acid:formic acid (13:4.5:1.5:1)

Application: Linomat V

Temperature: 25°C

Saturation time: 15 min

Volume for application: 10 and 20 µl

Plate: Silica gel GF 254

Plate size: 10 cm × 10 cm

Application position: 15 mm

Solvent front: 80 mm.

Quantification of diosgenin using high-performance thin layer chromatography

Purifi d diosgenin isolated from *Dioscorea* species was purchased from Sigma Aldrich (St. Louis, MO, USA) and was used as the standard. Diosgenin content in the alcoholic extract and fractions were estimated using a modifi d procedure of Suthar and Mulani.[21] Silica gel G 60 F254 precoated plates were used with chloroform: Acetone (80:20) as solvent system. CE and the different fractions were spotted on precoated TLC plates. The ascending mode was used for the development of thin layer chromatography. TLC plates were developed up to 8 cm. After development, the plate was derivatized dipping in 10% methanolic sulfuric acid reagent (10 ml concentrated sulfuric acid added in 90 ml of methanol with cooling and the reagent must be freshly prepared), heated at 110°C for 2–3 min and brought to room temperature. Brownish spot with Rf >>0.54–0.57 was visible and scanned under visible light at 535 nm.

Standard curve of diosgenin was also run simultaneously at concentration of 500–1500 ng. The results are expressed as percentage of diosgenin present in CE, chloroform soluble fraction, ethyl acetate soluble fraction and water-soluble fraction.

Hypoxia-reoxygenation injury in H9c2 cells

Cell culture

Rat myocardium-derived H9c2 cardiac myoblast cell line was purchased from the American Type Culture Collection (ATCC; Manasses, VA, USA). Cells were grown on Dulbecco's modifi d Eagle's medium (DMEM, Invitrogen, Grand Island, NY, USA) containing 4 mM L-glutamine, 4.5 g/L glucose, and 10% fetal bovine serum (Invitrogen) and incubated at 37°C in a humidifi d chamber with 5% CO_2. To prevent the loss of differentiation potential, cells were not allowed to become confluent.

Induction of hypoxia

H9c2 culture plates were subjected to either 30 min. of hypoxia or 30 min of hypoxia followed by 1 h of reoxygenation (H/R) or hypoxic adaptation, where the cells were subjected to three cyclic episodes of 5 min hypoxia and 5 min reoxygenation, followed by H/R. Hypoxia was given by placing the cultured plates into an air-tight hypoxic chamber placed in a humidifi d 37°C CO_2 incubator, and passing the mixture of 95% nitrogen and 5% CO_2. Reoxygenation was given by placing back the cultured plates in a humidifi d 37°C CO_2 incubator saturated with air.[22]

Cell survival assay

At the end of the experimentation, cells were washed with phosphate buffered saline. The viability of cells was studied using (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) cell proliferation assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) as described by the manufacturer. Since diosgenin was dissolved in ethanol, a separate group treated with ethanol was also subjected to HR.

Cell death assay

Cell death analysis was performed with the spent culture medium as substrate with lactate dehydrogenase (LDH) cytotoxicity assay kit (Cayman Chemical Company, Ann Arbor, MI, USA) as mentioned by the manufacturer.

Western blot analysis

The respective cytosolic or nuclear proteins were separated by SDS-PAGE and transferred to nitrocellulose filters. Filters were blocked in 5% nonfat dry milk and probed with primary antibody overnight.[22] Primary antibodies such as HO-1, Akt, phospho-Akt (Ser 473), glyceraldehyde-6-phosphate dehydrogenase (GAPDH) were obtained from Santa Cruz Biotechnology, Santa Cruz, CA, USA; whereas Bax, Bcl-2, Src and phospho-Src (Tyr416), were obtained from Cell Signaling Technology, Beverly, MA, USA. All primary antibodies were used at a dilution of 1:1000. Protein bands were identifi d with horseradish peroxidase conjugated secondary antibody (1:2000 dilutions) and Western blotting luminol reagent (Santa Cruz Biotechnology). GAPDH was used as loading control for cytosolic and nuclear fraction. The resulting blots were digitized, subjected to densitometric scanning using a QuantiOne software (Bio-Rad), and normalized against loading control.

Statistical analysis

Results are expressed as the mean ± standard error of the mean (SEM), and Bonferroni's correction was also performed. The results were considered to be signifi ant if $P < 0.05$. Western blotting was performed with QuantiOne software with H9c2 cell lysate for the expression of protein markers mean ± SEM $^{*}P = 0.05$ versus normal, ethanol (EtOH) + HR and diosgenin + HR; $^{\#}P = 0.05$ versus HR.

RESULTS

Quantification of diosgenin

Figure 2a shows the fi gerprinting of the various fractions of *D. bulbifera* Linn. which shows the presence of diosgenin in the different fractions, whereas [Figure 2b] chromatogram profile of the various fractions and the CE showing the peak area of diosgenin in the different fractions. The percentage of diosgenin quantifi d from crude and different fractions is given in Table 1. The ethyl acetate fraction possesses the maximum quantity of diosgenin of 0.66% whereas the crude ethanolic extract used for the study contains 0.069%.

Table 1: Percentage of diosgenin in crude and different fractions of *Dioscorea bulbifera* Linn.

Extract or fraction	% of diosgenin
Total ethanolic extract (CE)	0.069±0.003
Chloroform soluble fraction (CSF)	ND
Ethyl acetate soluble fraction (ESF)	0.66±0.01
Water soluble fraction (WSF)	ND

Results are given as mean±S.D. of triplicate samples

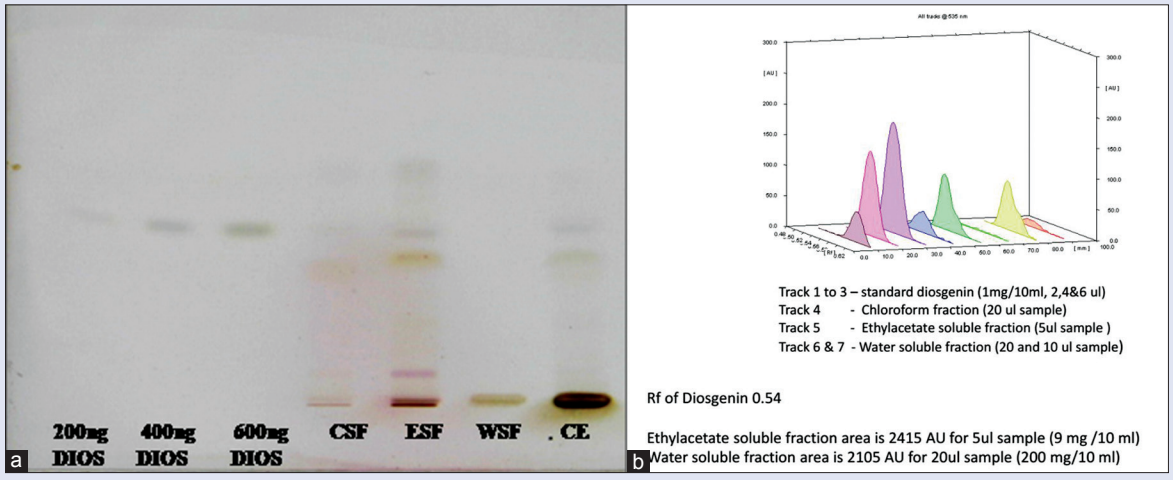

Figure 2: (a) High-performance thin layer chromatography profile of the various fractions of *Dioscorea bulbifera* Linn. for the estimation of diosgenin (b) High-performance thin layer chromatography of various fractions of *Dioscorea bulbifera* Linn. showing diosgenin peak area

Diosgenin prevents H/R-induced cell death and increases cell viability

HR injury in H9c2 cardiac myoblast cells increased cell death as assessed by quantitative MTT assay [Figure 3]. The cytoprotective effect of the saponin diosgenin was evidenced from the improved cell survival [Figure 3]. We also measured the release of LDH in the culture media to determine the extent of the leakage of the cytosolic components during oxidative insult. Hypoxia and H/R resulted in a signifi ant increase in LDH level compared with the normal cells [Figure 4].

Anti-apoptotic effect of diosgenin phosphorylates Akt

To verify whether Akt is involved, the Akt phosphorylation pattern was examined in normal, H/R control, EtoH + H/R as well as in diosgenin treated groups. It was explored that diosgenin-treated cells show an upstream effector's pattern of phosphorylated Akt during HR [Figure 5].

Effect of Akt overexpression on Bcl-2/Bax

Bax and Bcl-2 play an important role in the regulation of apoptosis; so in order to ascertain whether the anti-apoptotic effects conferred by hypoxia were due to altered expression of Bcl-2 family proteins, the Bcl-2/Bax ratio was studied. Akt blocked the expression of mitochondrial Bcl-2 family protein, Bax and its overexpression inhibited H/R-induced loss of Bcl-2 protein on treating with diosgenin [Figure 5].

Effect of diosgenin on mitogen-activated protein kinase family protein, c-Jun N-terminal kinases

The role of c-Jun N-terminal kinases (JNK), a mitogen-activated protein kinase (MAPK) family protein in HR condition, has been controversial with studies, suggesting both a defensive and detrimental facet to JNK activation. Because of the extreme clinical importance of this family of proteins, their regulation by oxidative stress has been under intense study for many years. In order to reveal its role in H/R, we examined JNK and p-JNK in normal, Control hypoxia, EtoH + H/R and diosgenin + H/R groups. It was observed that there was a down-regulation of JNK in diosgenin + H/R group [Figure 5].

Effect of diosgenin on Src kinases

Src kinases are key upstream mediators of MAPK signaling pathways and have been shown to have important roles in cell proliferation, migration and survival. It was observed that the Src kinase activity was augmented in the control H/R group which was seen attenuated in the diosgenin + H/R group [Figure 5].

Effect of diosgenin on hemeoxygenase-1

HO-1, a stress-inducible protein and Phase II enzyme, is an important cytoprotective agent in various models of HR injury and was found to be up regulated in diosgenin treated group during HR [Figure 5].

DISCUSSION

Diosgenin, a steroidal saponin isolated from *D. bulbifera*, matches a standard obtained from Sigma Aldrich (purity 93%) [Figure 1]. Sapogenins are aglycones of glycosidic saponins; the latter are so called because they have the property of forming soapy lather in water. The steroidal sapogenins have 27 carbons and are internal ketals of 16, 26-dihydroxy-22-keto-steroids. Diosgenin, chemically named as (25R)-spirost-5-en-3 β-ol, is prominent among the sapogenins used in steroid industry. Diosgenin has a parent steroid (cyclopentano perhydro phenanthrene [CPPP]) ring structure and it consists of two components namely the hydrophilic sugar moiety and a hydrophobic steroid (CPPP) unit. The structural conformation has revealed that the carbons at 5 and 25 are responsible for Diosgenin's biological activity.[23] Based on its chemical nature to bind to various target proteins, it was identifi d that diosgenin mediates pro-survival and pro-death protein molecules thereby alleviating HR injury hypoxia followed by re-oxygenation (H/R) is associated with both apoptotic and necrotic cell death, whereas hypoxia contributes to only a minimal amount of cell death.[24] The mechanisms by which the ischemic insult induces apoptosis are not well understood. Furthermore, only little knowledge exists concerning pharmacological agents that target the hypoxia-induced apoptotic pathways in the heart and thereby exerting a cardioprotective effect. Therapeutic reperfusion is currently performed

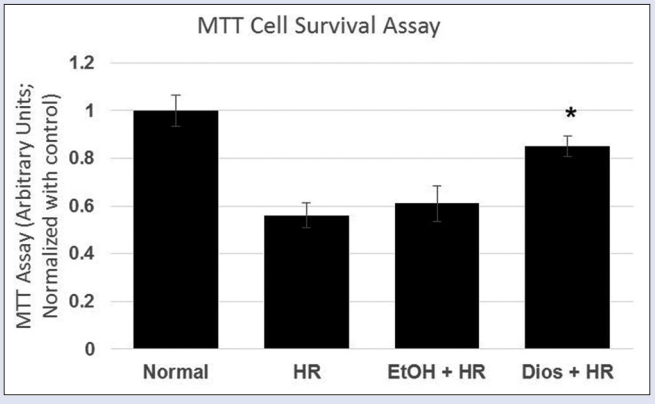

Figure 3: Diosgenin induces cell survival. H9c2 cells were cultured in (1 × 10^4 in a 96-well plate). H9c2 cells were treated with 30 μM of diosgenin followed by hypoxia and reoxygenation. (3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide cell survival assay was performed with (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide Cell Proliferation Assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). Results are expressed as mean ± standard error of the mean *P = 0.05 versus normal, ethanol + hypoxia-reoxygenation and Dios + hypoxia-reoxygenation; #P = 0.05 versus hypoxia-reoxygenation

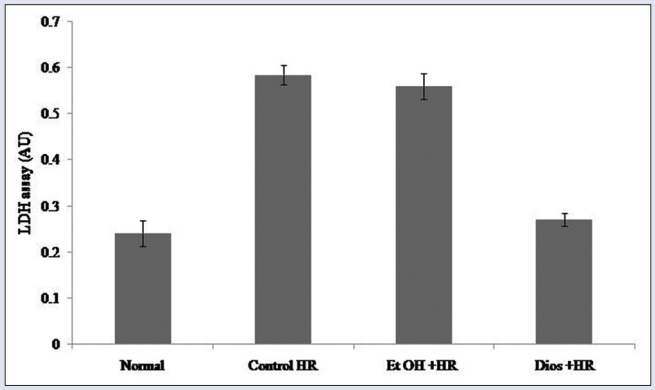

Figure 4: Diosgenin induces cell survival. H9c2 cells were cultured in (1 × 10^4 in a 96-well plate). H9c2 cells were treated with 30 μM of diosgenin, followed by hypoxia and reoxygenation. The release of lactate dehydrogenase as a marker of cell death was performed with lactate dehydrogenase cytotoxicity assay kit (Cayman Chemical Company, Ann Arbor, MI, USA). Results are expressed as mean ± standard error of the mean *P = 0.05 versus Normal, ethanol + hypoxia-reoxygenation and Dios + hypoxia-reoxygenation; #P = 0.05 versus hypoxia-reoxygenation

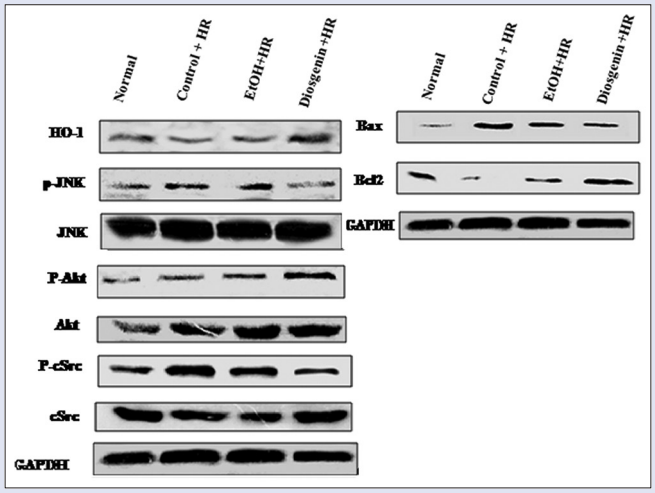

Figure 5: Diosgenin induces cell survival signals and attenuates death protein levels. H9c2 cells were cultured in (3×10^5 in a 10 cm dish). H9c2 cells were treated with 30 μM of diosgenin, followed by hypoxia and reoxygenation. Western blotting was performed with QuantiOne software with H9c2 cell lysate for the expression of protein markers mean ± standard error of the mean $^*P = 0.05$ versus Normal, ethanol + hypoxia-reoxygenation and Dios + hypoxia-reoxygenation; $^{\#}P = 0.05$ versus hypoxia-reoxygenation

without any measure to protect the myocardium from apoptosis during the treatment of myocardial infarction.[25] In the present study, we show that HR induces apoptosis in H9c2 myoblast cells that is accompanied by Bax up-regulation, down-regulation of Bcl-2 and cell survival molecules (p-Akt and HO-1), activation of death signaling kinases (p-JNK and p-Src). Our study demonstrates that reduced apoptosis and death signaling kinases through Akt-mediated signaling molecules may be a possible mechanism in diosgenin treatment during HR.

The Akt signaling pathway plays a central role in mediating protection in HR conditions. There is abundant evidence that activated Akt prevents cardiomyocyte apoptosis induced by multiple pathological insults including ischemia/reperfusion, pressure overload, hypoxia, hypoglycemia, or cardiotoxic drugs.[26-31] Pharmacological activation of Akt has been demonstrated to provide protection against various stressors by providing mitochondrial integrity and function. Akt activation has also been shown to decrease apoptosis, as well as to reduce infarct size and to improve cardiac function after H/R.[32,33] These fi dings have described mechanisms by which the Akt pathway promotes cell proliferation and inhibits cell death. Activated Akt phosphorylates various substrates, activating anti-apoptotic factors and inactivating pro-apoptotic factors.[33] A signifi ant expression of phosphorylated Akt was observed in the diosgenin treated cells during HR. Comparison between diosgenin + H/R and EtOH + H/R revealed that diosgenin displayed signifi ant protection compared with EtOH.

The anti-apoptotic role of Bcl-2 is well documented in the myocardium. It was established that genetic modifi ation of the myocardium with the anti-apoptotic human Bcl-2 gene conferred myocardial protection against HR-induced apoptosis.[34,35] Furthermore, several studies have reported a reduction of apoptosis by H/R via altering the expression of Bcl-2 and/or Bax protein, thus increasing the ratio of Bcl-2/Bax protein prevents the progression of apoptosis in myocardium after HR.[36,37] Akt has also been reported to phosphorylate and inhibit Bax. Recently we have shown that *Dioscorea bulibifera* extract ameliorates rat ischemia/reperfusion injury with down regulated expression of Bax activity resulting in considerable reduction in the Bax/Bcl-2 ratio.[19] In the present study, it was shown that apoptosis occurred in H9c2 cardiomyoblast cells subjected to 30 min of hypoxia followed by 60 min of reoxygenation. Treatment with cells pretreated with 30 μM of diosgenin and subjected to hypoxia (30 min) and reoxygenation (1 h) stimulates phosphorylation of Akt, a kinase directly downstream of PI-3 kinase. Phosphorylated Akt activates Bcl-2, an intracellular anti-apoptotic membrane protein. Up-regulation of Bcl-2 has an inverse relationship with Bax, which shows a signifi ant down-regulation. The MAPK pathways are also known to play both pro and anti-apoptotic roles in cardiomyocyte cell death. There are three principal MAPK terminal effector kinases: The extracellular signal-regulated protein kinases, p38, and the JNK. p38, MAPK and JNK are stress-activated protein kinases that are known to regulate cell viability and are phosphorylated by MAPK kinases MKK3/6 and MKK4/7, respectively, in response to several cellular stresses including ischemia (p38), reperfusion (JNK), as well as UV radiation and cytokines.[38] Bax-dependent activation of the mitochondrial pathway of apoptosis is mediated by early JNK activation.[39] It is possible that the Akt pathway down regulates the JNK pathway, as reported in some studies.[40,41] JNK is strongly induced by oxidative stress in cultured cardiac myocytes and in intact hearts[42,43] and may be a critical determinant of ischemic injury in the myocardium as well as the brain.[44-47] Our results obtained were in accordance with the previous literature that exhibited pronounced activation of JNK after HR which seemed to be well prevented by diosgenin treatment during the same.

Among the large number of tyrosine kinases, in the present study, the Src family of tyrosine kinases was focused as a candidate responsible for triggering or mediating ischemic preconditioning.[48] c-Src has been implicated in mechanisms of cell survival and death, which was regulated by complex signal transduction processes. Several studies show the increased activity of c-Src in case of cellular damage.[49] The present study probed that distinct activation of c-Src after HR was well prevented by diosgenin compared to that of the control as well as EtOH + H/R treated cells.

The Phase II enzyme HO-1 is an inducible isoform of the fi st and rate-limiting enzyme of the degradation of heme into iron, carbon mono oxide, and biliverdin, the latter being subsequently converted into bilirubin. Several positive biological effects exerted by this enzyme have gained attention such as anti-inflammatory, anti-apoptotic, angiogenic, and cytoprotective functions and are attributable to carbon monoxide and/or bilirubin. Thus, the physiological induction of HO-1 may be an adaptive and benefic al response to several possibly noxious stimuli, including heme itself, suggesting a potentially autoprotective and autodefensive role in several pathophysiological states including acute coronary syndromes and stroke 9.[50] The results of our study showed an up-regulation of HO-1 in diosgenin treated H9c2 cardiomyoblast cells.

CONCLUSION

Altogether, the results of the present study substantiate that diosgenin, a steroidal saponin present in *D. bulbifera*, modulates HR injury in H9c2 cells via Akt-mediated signaling pathway. Cardioprotection by diosgenin was further demonstrated through MTT cell viability assay and LDH release assay thereby contributing to the protective role of *D. bulbifera* in experimental HR. At this instance, it is interesting to identify that dioscin, which is the glycoside form of diosgenin, promotes autophagy in lung tumors.[51] Likewise, cardioprotection by adaptation to ischemia augments autophagy.[22] Hence, further studies are worth studying to study the role of diosgenin in *D. bulbifera* as a potential functional food ingredient.

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Nil.

Conflicts of interest

There are no confli ts of interest.

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