Egypt. J. Chem. **Vol. 65**, No. 5 pp. 179 - 186 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Cardioprotective Effect Of Cyanidin-3-O-Glucosidein Ischemic Heart Is Mediated Via Inhibition Of Autophagy

M. Khedr[a], M. El Desouky[a*], A. Shafei[b], R. Mostafa[c], A. Hasanin[d], E. Habib[e], H. Abdelhakim[a], M. Mitboli[f]

[a] *Biochemistry Department. Faculty of Science, Cairo University, Egypt.*
[b]*Faculty of Medicine MTI University, Egypt.*
[c]*Clinical physiology Department, Benha college of Medicine, Egypt.*
[d]*Clinical pharmacology department. Ain shams University, Faculty of Medicine, Egypt.*
[e]*Anatomy and Embryology Department, Faculty of Medicine, Galala University, Egypt.*
[f]*Biochemistry department Ain shams faculty of medicine, Egypt.*

Abstract

It is elevated proves that the polyphenolic compounds of natural origin, such as anthocyanins, being cyanidin-3-O-glucoside (Cy3G) one of the most widely distributed anthocyanins, may control ischemia/reperfusion (I/R) injury although how much sheltering occurs is not obvious. Here, the role of cyanidin-3-O-glucoside pretreatment of cardiac ischemia and dysfunction was studied. Cyanidin-3-O-glucoside in 3 doses (5, 10, and 15 mg/kg body weight), was introduced in vivo 5 min before a 45 min closure of the left anterior descending artery, followed by a 120 min reperfusion in male Wistar rats. Blood samples and cardiac tissue specimens were obtained for studying the cardiac markers and the expression of lncRNA-CTC-448F2.4, miR1273a, and mRNA-DCN (Decorin), Specimens of left ventricles were obtained and prepared for histopathological criteria. Specimens of pretreated rats showed minimal cardiac edema, hemorrhage, cellular inflammatory infiltration, and fibrosis in cardiac tissue samples. Notably, this protective administration of cyanidin-3-O-glucoside also produced a dose-dependent downregulation in lncRNA- CTC-448F2.4 and mRNA-DCN (Decorin) with elevation in miR-1273a expressions. Cyanidin-3-O-glucoside pretreatment leads to improvements in cardiac function tests, enzymes, and myocytes. In conclusion, Cyanidin-3-O-glucoside is promising for the protection of the heart from injury following ischemia-reperfusion in the rat examination.

Keywords: Cyanidin-3-O-glucoside; epigenetic ;ischemia/reperfusion (I/R) injury; Rat model

1. Introduction

One of the leading causes of morbidity across the world is acute myocardial infarction (AMI). Myocardial reperfusion is known to be an effective therapeutic choice against AMI [1]Dramatic reduction in mortality from acute myocardial infarction has occurred during the last decades. However, there is still an increasein the incidence of post-infarction heart failure[2].In cardiovascular diseases, ischemic heart disease is the main cause of death, and it is believed that the blood supply restoration, such as reperfusion therapy, is the most effective treatment of cardiac ischemia [3] Nevertheless, an irreversible damage may be caused or even exacerbated during reperfusion of ischemic myocardium with oxygen and substrate-richblood, leading to a deterioration of heart function, which is called an ischemia–reperfusion (I/R) injury[4].Following (I/R)treatment, an occurrence of myocardial ischemia/reperfusion (I/R) injury usually shows in patients with cardiovascular diseases, such as strokes, coronary heart diseases and myocardial infarction[5]. During myocardial (I/R)injury , metabolic disorders and vascular structural damage may present in patients who suffer from (I/R) injury [6] (I/R) injury is induced by reperfusion of blood flow through different complex processes, including disruption of mitochondrial membrane potential, ion accumulation, the formation of reactive oxygen species, etc. Autophagy is one of the processes

*Corresponding author e-mail: meldesouky@sci.cu.edu.eg ; (El Desouky, Mohamed Ali).
Receive Date: 13 September 2021, Revise Date: 20 September 2021, Accept Date: 26 September 2021
DOI: 10.21608/ejchem.2021.95695.4494

activated in response to (I/R)injury [7], It offers a representation of a highly evolutionary conserved process. Through this process, macromolecules and cytoplasmic material are degraded into lysosomes and recycled for biosynthetic or energetic purposes. There is an association between dysfunction of the autophagic process and the onset and development of many human chronic pathologies, such as cancer, as well as metabolic, cardiovascular, and neurodegenerative diseases[8]. Autophagy is the "double-edged weapon" in the pathology of (I/R)injury, and there is a controversy about whether autophagy is beneficial or detrimental. It was targeted as a therapeutic strategy by many studies based on the autophagy impact and regulation on myocardial (I/R)injury [1]. The role of decorin in cardiovascular diseases was recently reviewed by Vu et al., they summarized the role and function of decorin in cardiovascular diseases. Decorin interacting via cell surface receptor regulates plenty of cellular functions (reduces TGF-β dependent fibrosis, proliferation of myofibroblasts and endothelial cells, renal and neuronal protection from ischemic assaults) but these processes are dependent on cell types and pathological circumstances[9]. Myocardial adaptation to oxidative/reductive stress is an essential mechanism that has been evolved for the survival of heart from different disorders. Mitochondria targeting antioxidants have an impact indeed in preventing the detrimental effects of myocardial ischemia/reperfusion injury and in other rodent models of cardiovascular diseases [10]. Complex and variable changes in miRNA expression occur before, during, and after myocardial ischemia and reperfusion. Many studies have pinpointed miRNAs' significant role in the modulation of the cascade of myocardial ischemia-reperfusion (I/R)injury, and the effect of certain therapies on miRNA expression[11]. Competing endogenous RNAs (ceRNAs) form regulatory networks may be the orchestrator and the main factor in pathological processes. The miRNAs are the key factor of the ceRNA network and may contributes in control of the expression of lncRNA"[12]. Bioinformatics analysis of TargetScan and miRWalk databases predicted that miR-1273a had the matched binding base with DCN (Supplementary). In the present study, Cyanidin significantly decrease cardiac enzymes after (I/R)injury, It is accompanied with significant good histopathological appearance of heart muscles and significant protection of the cardiac myocytes destruction,Cyanidin-3-O-glucoside presents many properties, such as anti-inflammatory and anti-tumor effects[13]. Cyanidin-3-O-glucoside protects from the decrease in diameter of order two arterioles. Moreover, at the end of the time-period of blood flow recovery, higher dosage Cyanidin-3-O-glucoside induced a vasodilatationby 40.5% of baseline in order two arterioles, these vasodilatation were associated with preservation of capillary blood supply, significant reduction in venular leakage as well as in leukocyte adhesion to venular walls which may be due to the reduction in reactive oxygen compounds formation induced by Cyanidin-3-O-glucoside, because reactive oxygen compounds is a key player, play an important role in the regulation of vessel wall permeability and adhesion of leukocyte to venular walls[14].

2. Experimental.

2.1. Experimental animals.

Sixty male Wistar rats weighing 200−250 g were obtained from the animal house at Nile Pharmaceuticals Company, (Cairo, Egypt). In an animal room, animals were housed with a 12 h light/dark cycle and access to tap water and normal rat chow ad libitum. All animal procedures were conducted in accordance with the National Institute of Health guideline for the care and use of laboratory animals (NIH Publication No. 85-23, revised 1996), and had the approval of the Institutional Animal Ethics Committee at Ain Shams University, Faculty of Medicine (Approval no. 149/2020).

2.2. Induction of ischemia/reperfusion via ligation of anterior descending coronary artery.

Ligation

Male Wistar rats were anesthetized with urethane (1.2 mg/kg body weight). Using a rodent ventilator at 70–80 breaths/ min, intubation was established, followed by exposure of the heart through median sternotomy and placement of a ligature around the left anterior descending coronary artery with the use of 5-0 polyethylene suture. A noose around a rubber band that was placed flat on the myocardium was formed with the use of the free ends of the ligature. Coronary occlusion was induced by the noose tightening around the rubber band for 45 min [14] This was confirmed by immediate blanching of the infarcted area. Releasing the ligature for 120 min achieved the reperfusion. For confirming myocardial ischemia, Electrocardiogram tracing in lead II was obtained before and after ligation. The sham animals were exposed to the same procedure but without ligation.

2.3. Animal groups

There was a random assignment of animals to five groups (12 rats/group):

Sham group: coronary ligature was positioned without being tied, ischemia-reperfusion (I/R)injury,

pretreated groups in three doses;Cyanidin-3-O-glucoside-5mg/kg body weight (Cy3G-5), Cyanidin-3-O-glucoside-10mg/kg body weight (Cy3G-10), Cyanidin-3-O-glucoside-15mg/kg body weight (Cy3G-15)group. The Cyanidin-3-O-glucosidedoses were administered intraperitoneally, 5 min prior to coronary ligation. In each group, half of the rats were allocated for the measurement of cardiac function and molecular assay, while the other half were allocated for histopathological examination.

2.4. Blood sample collections and measurement of cardiac markers

In order to obtain the Blood for the assessment of serum cardiac markers, cTnT,CK–MB, and LDH centrifugation of the retro-orbital blood samples was performed at 1200×g for 10 min. A measurement of serum LDH activities was conducted spectrophotometrically, with a quantification of serum cTnTand CK-MB by the use of a commercial ELISA kit, Rat CKMB (Creatine Kinase MB Isoenzyme) ELISA Kit, based on the manufacturer's instructions.

2.5. Tissue preparation for histological microscope analysis of cardiac tissues

Specimens from both ventricles were fixated in 10 % PBS- buffered formalin overnight, then placed in 70 % ethanol and processed using paraffin block. Routine hematoxylin-eosin (H&E) and Masson's trichrome stain for detecting collagen fibers, staining were performed on the 3 μm tissue sections, There was an examination of all the tissue sections for morphological evidence of cell death and fibrosis.

2.6. Molecular assays

Quantification of the RNA panel using real-time PCR (qPCR)

Total RNA and miRNA extraction and purification: 1. total and miRNAs were extracted using a miRNeasy Mini Kit; cat no: 217004 (Qiagen, Hilden, Germany) according to the manufacturer's protocol. 2. Reverse transcription: A synthesis of cDNA was conducted by reverse transcription reaction using miScript II RT Kit; cat no: 218161; (Qiagen, Hilden, Germany). 3. miRNA gene expression analysis: The quantification of miRNA-1273a level was performed by amplification , using Hs_miR-1273a miScript Primer Assay; cat no: 218300, ID: MS00031339 and the miScript SYBR Green PCR Kit, cat no: 218073. A normalization of the gene expression was conducted by the Hs_SNORD68_11 miScript Primer Assay, cat no:218300, ID: MS00033712 as a housekeeper gene 4. The quality of total RNA was detected b spectrophotometer A260 to A280 ratio and only specimens with RNA/protein ratio more than 2 included in this study.

The quantification of Lnc-CTC- 448F2.4 expression level was performed by amplification , using RT² qPCR Primer Assay for HumanCTC448F2.4, Primer Assay; cat no: 330701; ID: LPH25249A and the RT² qPCR SYBR Green/ROX Master Mix Kit, cat no:330520. A normalization of the gene expression was conducted by the Hs_ACTB_1_SG QuantiTect Primer Assay, cat no:249900, ID: QT00095431 as a housekeeper gene 5. mRNA gene expression analysis: The quantification of mRNA-DCN expression level was performed by amplification , using the Hs_DCN _1_SG QuantiTect Primer Assay, cat no:249900, ID: QT00032459 and the QuantiTect SYBR Green PCR Kit cat no: 204141 (Qiagen, Germany). There was a use of the Primer sequence Hs_ACTB_1_SG QuantiTect Primer Assay, cat no:249900, ID: QT00095431 as housekeeper gene. After amplification, melting curve analysis was performed to confirm the specificities of the amplicon for SYBR® Green dye–based PCR amplification according to its specific melting temperature(Tm).An analysis of all samples was conducted with the use of the 5 plex Rotor-Gene PCR Analyzer (Qiagen, Germany).Gene Expression Calculation: The 2ΔΔCt method was conducted for analyzing gene expression levels as an endogenous reference control for normalization purposes.(Livak, K. J., &Schmittgen, T. D. (2001) Cardiac markers results and molecular results in a supplementary excel sheet (Results sheet).

2.7. Statistical Analysis

Graphpad Prism version 7 was used for carrying out results' analysis, with data expressed as mean ± SD. The study groups were compared by using analysis of variance (ANOVA), followed by Tukey's test. The Spearman correlation test was used to conduct a correlation between Lnc-CTC-448F2.4, miR-1273a, and DCN. $P < 0.05$ was considered to be statistically significant.

3. Results and discussion

3.1. Effects on cardiac enzymes, CK-MB, LDH and cTnT

Induction of ischemia in the second group; the (I/R)injury group cause a significant upregulation in cardiac markers cTnT, CK-MB,LDH when compared to the sham group, all the pretreated groups revealed a significant reduction in cardiac markers ;CK-MB,LDH compared to the (I/R)injury group, Study for dose dependence shows that while the Pretreated group with low dose; the (Cy3G-5mg/kg body weight) was unable to significantly down-regulate the cTnT level. The pretreatment groups with higher dose (Cy3G-10/kg body weight) and (Cy3G-15/kg body weight) significantly down-regulate the cTnT level in both groups. while there is no significant

deference in between (Cy3G-5/kg body weight) and (Cy3G-10/kg body weight) groups , only the pretreated group with highest dose (Cy3G-15/kg body weight) rats group showed a significant down-regulation in their CK-MB, LDH, cTnT values; in comparison to ischemic group and both pretreated groups with lower dose(Cy3G-5/kg body weight) and (Cy3G-10/kg body weight) (Table 1).

Table (1) The effects on cardiac enzymes: LDH, CK-MB and cardiac troponin-T (cTnT))

The effects on cardiac enzymes:	CardiacTroponin-T (cTnT).	CK-MB (U/L)	LDH (U/L)
Sham	0.0±0.0	43.0±2.94	131.0±9.1
(I/R) injury	0.95±0. 037*	86.50±4.5*	237.3±26.97*
Cy3G-5mg/kg body weight	0.87±0.035	70.50±6.76#	181.3±9.43#
Cy3G-10mg/kg body weight	0.82±0.039#	60.5±6.03#	166.0±4.97#
Cy3G-15mg/kg body weight	0.62±0.031#,a,b	52.5±2.08#,a,b	158.5±2.89#

Values are mean ± SD; number of animals = 6 rats/each group.
One-way ANOVA followed by Tukey's multiple comparison test. LDH= Lactate dehydrogenase, CKMB= Creatine Kinase MB, (Cy3G-5)= Cyanidin-3-O-glucoside, IR= ischemia reperfusion.
* P < 0.001 compared to sham group. # P < 0.001 compared to (I/R) injurygroup. a P < 0.001 compared (Cy3G-5mg). b P < 0.001 compared to (Cy3G-10mg).

3.2. Effect of Cyanidinon the expression of cardiacLnc-CTC- 448F2.4, miR-1273a , DCN mRNA based on relative quantity (RQ) among the study groups

It was revealed by one-way ANOVA with P-value < 0.0001, followed by Tukey's multiple comparison test of (I/R) injury induction and Cyanidin-3-O-glucoside pretreatment that was an effect on the expression of cardiac miR-1273a, lncRNA-CTC-448F2.4,mRNA-DCN based on relative quantity (RQ). In (I/R)injury group a significant down-regulation of miR-1273a expression compared to sham group while by pretreatment with by Cyanidin-3-O-glucoside, a significant up regulation of (RQ) miRNA to 32 fold compared to (I/R)injury (fig A). Furthermore, a 6, 26, 65 fold up regulation in (RQ) miRNA-1273a was noticed compared to the ischemic group in Cy3G-5mg/kg body weight, Cy3G-10mg/kg body weight, Cy3G-15mgkg body weight group .(fig D).

Up regulation mRNA-DCN expression in (I/R)injury group compared to sham group was found, while by pretreatment with by Cyanidin-3-O-glucoside, a significant down-regulation of (RQ) mRNA to -19.7 fold compared to (I/R)injury(fig B).Furthermore, a -9, -28, -149 fold down regulation in (RQ) miRNA-1273a was noticed in comparison to the ischemic group in Cy3G-5mg/kg body weight, Cy3G-10mg/kg body weight, Cy3G-15mgkg body weight group respectively.(fig E).

A significant up regulation of lncRNA-CTC-448F2.4 expression in (I/R)injury group compared to sham group was found while by pretreatment with by Cyanidin-3-O-glucoside, a significant down-regulation of (RQ) miRNA to -5 fold compared to (I/R)injury was detected (fig C).Furthermore, a -2.7, -7.9, -11.5 fold down regulation in (RQ) lncRNA-CTC-448F2.4 can be noticed compared to the ischemic group in Cy3G-5mg/kg body weight, Cy3G-10mg/kg body weight, Cy3G-15mgkg body weight group respectively which prove the effect of pretreatment with Cyanidin-3-O-glucosidein a dose-dependent manner(fig F).

3.3. Correlation between RNA network expressions in cardiac tissues

A significant negative correlation can be noticed between miR-1273a expression and expression of mRNA-DCN. Moreover, there was a significant negative correlation between miR-1273a expression and expression of lncRNA-CTC-448F2.4. Additionally, there was a significant positive correlation between expression of mRNA-DCN and lncRNA-CTC-448F2.4. These statistical association findings correspond with the in-Silico data concerned with the expression of miR-1273a, mRNA-DCN and lncRNA-CTC-448F2.4 IR model. (figure 7)

For this study, we used a variety of public microarray databases and computation algorithms for the selection of genetically and functionally linked RNAs involved in autophagy process related (I/R)injury after myocardial ischemia, at both genetic and epigenetic levels. We evaluated the role of cyanidin in the myocardial IR injury animal model via modulating lncRNA (CTC-448F2.4) - miR (HSA-MIR-1273a) - mRNA DCN. It has substantial interest to clinical medicine owing to its antifibrotic, antiinflammatory, and anticancer effects, An emerging concept that multiple proteases, especially those produced by inflammatory cells, are capable of cleaving DCN elevated the expression level of TGF βRII, increased the phosphorylation level of TGF βRI, enhanced the expression of p15, and finally inhibited the proliferation of HepG2 cells[15].our results agree with[16]revealed that isolated rat hearts are protected by oxidative stress, increased in several cardiovascular diseases, after treatment with cyanidin[17]. Also anthocyanins were able to

counteract microvascular changes such as arteriolar vasoconstriction, increase of microvascular permeability and leukocyte adhesion [18, 19] In agreement with previous observations by Di Giacomo et al. who have observed the effects of Cyanidin-3-Oglucoside injection before the bilateral common carotid artery occlusion and during reperfusion. Their data indicate that cyanidin is able to reduce the lipid hydroperoxides and the expression of neuronal and inducible NOS and to increase the expression in endothelial nitric oxide synthase (eNOS) [16]. In the present study, Light microscopy examination of the cleared out ventricle of the sham group appeared longitudinally organized cardiac muscle filaments, which were branched, round and hollow in shape with a uniform breadth(fig 1), induction of ischemia/reperfusion in rat model via ligation of anterior descending coronary artery cause myocardial destruction and massive infarction which was widespread throughout the left ventricle and papillary muscle, widespread necrosis, focal vacuolation and massive interstitial edema (fig 2 and 3), that was

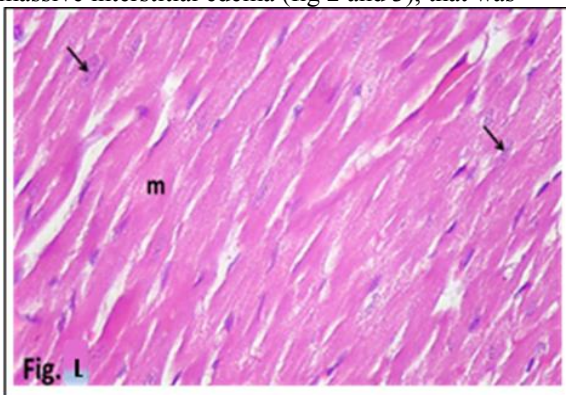

Figure 1: Photomicrographs of H&E stained sections of left ventricular of control group showing branching and anastomosing cylinder cardiac muscle fibers (m) with central elongated vesicular nuclei (↓)

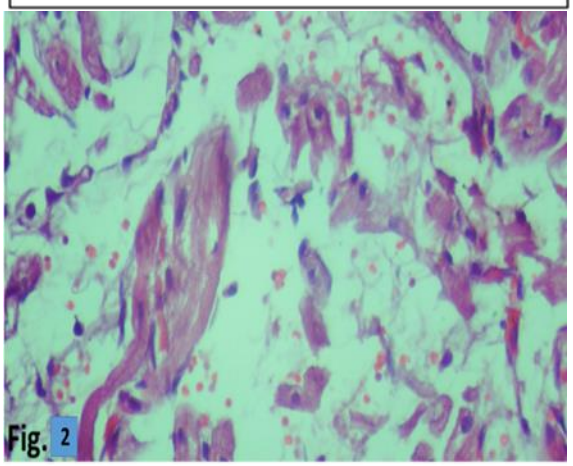

Figure 2: Photomicrographs of H&E stained sections of left ventricular of ischemic reperfusion group show massive areas of necrosis with obvious interstitial edema and hemorrhage.(magnification: x400)

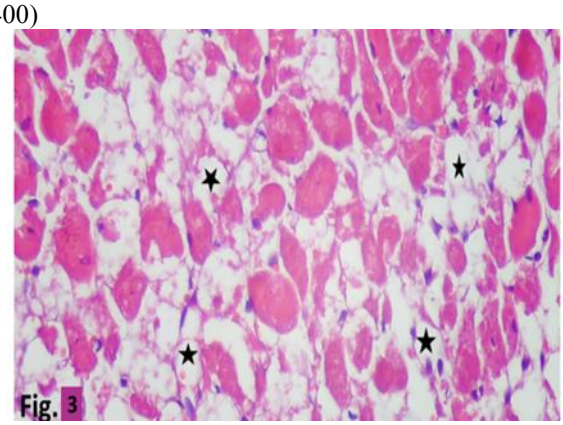

Figure 3: Photomicrographs of H&E stained sections of left ventricular of ischemic reperfusion group show massive vacuolization and interstitial edema (magnification: x400)

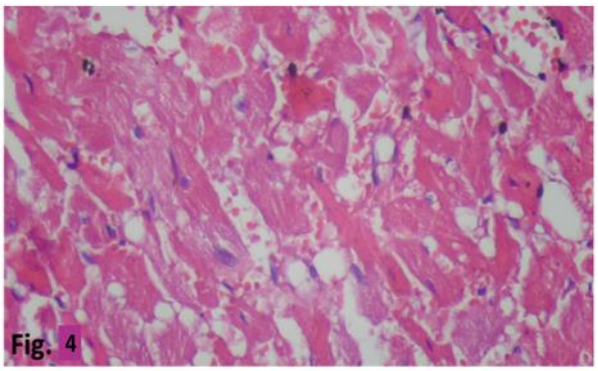

Figure 4 : Photomicrographs of H&E stained sections of cardiac muscles of rat groups received a low dose of Cy3G-5mg show Mild interstitial edema, sporadic myofiber vacuolization (magnification: x400).

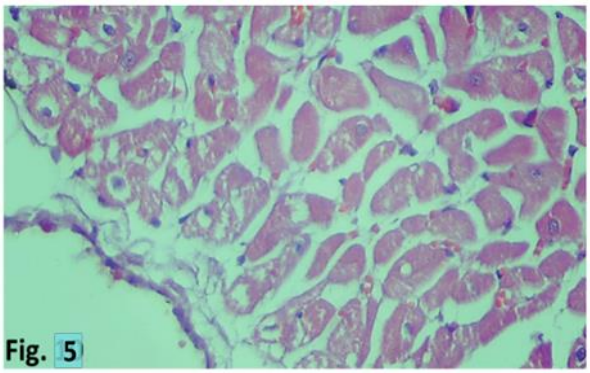

Figure 5. Photomicrographs of H&E stained sections of cardiac muscles of rat groups received dose of Cy3G-10mgshow only mild interstitial edema. No vacuolization of cardiomyocytes (magnification: x400)

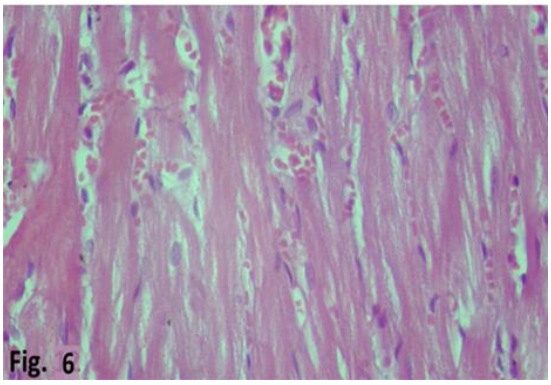

Figure 6 :Photomicrographs of H&E stained sections of cardiac muscles of rat groups received high dose of Cy3G-15mg.No vacuolization of cardiomyocytes or necrotic areas is detected (magnification: x400)

Associated with increase of both lncRNA-CTC-448F2.4 and mRNA DCN expression and decrease in miR-1273a expression in the (I/R)injury model. Also cardiac enzymes and Cardiac Troponin-T (cTnT) levels was elevated .Administration of Cyanidin-3-Oglucoside in a low dose (5,10 mg/kg) leads to a significant protection of both groups compared to IR group, alteration of both lncRNA-CTC-448F2.4 and mRNA DCN expression to be downlevel, also miR-1273a expression altered to elevated level(fig 4 and 5). Administration of Cyanidin-3-O-glucoside (15 mg/kg) showed the greatest protective effect and minimal pathological changes of cardiac muscle fibers (fig 6). We hypothesize that Cyanidin-3-O-glucosidecaused a decrease in expression of both lncRNA-CTC-448F2.4 and mRNA DCN in the heart in association with increase in miR-1273a expression in the (I/R) injury model. The effect of Cyanidin-3-O-glucosideconfirmed by both histopathological study and decreased level of cardiac markers and Cardiac Troponin-T (cTnT) levels, Cyanidin-3-Oglucoside of a dose (5&10mg/kg body weight) showed mild cardiomyocytes disarray, which was more evident in the left ventricular wall. Mild interstitial edema, individual myofiber swelling with hyalinization, sporadic myofiber vacuolization, and hypercontraction band formation were random and multifocal throughout the left ventricle. Administration of high dose Cyanidin-3-Oglucoside -15mgshowed marked improvement of histopathological features of cardiac muscle. Most of examined areas showed normal histological architecture of cardiac muscle, only mild interstitial edema. No vacuolation of cardiomyocytes or necrotic areas was detected. The present results agrees with [20]who reported that modulation of autophagy by Cyanidin-3-Oglucoside in animal model may due to impact of DCN which has a role in the process of autophagy, decorin is capable of inducing autophagy [4]. The present findings provide a rationale for designing novel therapeutic approaches through combining the conventional strategies with Cyanidin-3-Oglucoside mediated autophagy. Cyanidin-3-Oglucosidein (Cy3G-15/kg body weight)dose has the potential to alleviate cardiac injury pathologically, improve cardiac function following ischemia-reperfusion in a rat model of (I/R)injury. Consequently, Cyanidin-3-Oglucosidemight offer a role to unlock (I/R) injury.

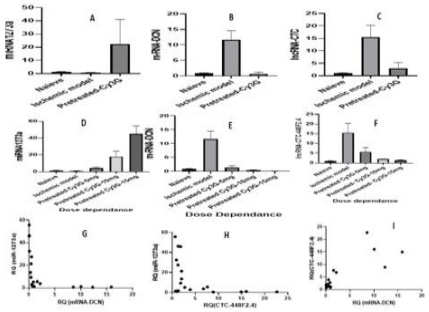

Fig. (A–C): Effects of IR and cyanidin-3-O-glucoside treatment on A: RQ of cardiac miR-1273a , B: RQ of cardiac RQ of cardiac DCN mRNA, C: RQ of cardiac lnc RNA-CTC-448F2.4. Fig. (D–F)cyanidin-3-O-glucoside pretreatment dosedependane on D: RQ of cardiac miR-1273a , E: RQ of cardiac RQ of cardiac DCN mRNA, F:RQ of cardiac lnc RNA-CTC-448F2.4.Values are mean ± SD; number of animals = 6 rats/each group. **P < 0.001, *P < 0.01 compared to sham group. #P < 0.001 compared to IR group, aP< 0.05 compared to Cy3G-5mg.
bP< 0.05 compared to Cy3G-10mg. One-wayANOVA followed by Tukey's multiple comparison test. IR= ischemia reperfusion, cyanidin-3-O-glucoside. Fig. (G–I): Spearman correlation coefficient between D: DCN mRNA and miR-1273a, E: lnc RNA-CTC-448F2.4 and miR-1273a, F: DCN mRNA and lnc RNA-CTC-448F2.4. P < 0.05 is considered significant.

4. Conclusion

Cyanidin-3-Oglucosidein (Cy3G-15/kg body weight)dose has the potential to alleviate cardiac injury pathologically, improve cardiac function following ischemia-reperfusion in a rat model of (I/R)injury. Consequently, Cyanidin-3-Oglucosidemight offer a role to unlock (I/R) injury.

5. Conflicts of interest

Authors declare that there is no conflict of interest.

6. Findings sources

None

7. Acknowledgment

Thanks to the Chemistry department, Faculty of Science, Cairo University, Egypt.

8. References

1. Aghaei M., Motallebnezhad M., Ghorghanlu S., Jabbari A., Enayati A., Rajaei M., ... & Khori V. Targeting autophagy in cardiac ischemia/reperfusion injury: a novel therapeutic strategy. *Journal of Cellular Physiology*, *234*(10), 16768-16778(2019).
2. Bencsik P., Gömöri K., Szabados T., Sántha P., Helyes Z., Jancsó G., ... & Görbe A., Myocardial ischaemia reperfusion injury and cardioprotection in the presence of sensory neuropathy: Therapeutic options. *British Journal of Pharmacology*, *177*(23), 5336-5356(2020).
3. Di Giacomo C., Acquaviva R., Santangelo R., Sorrenti V., Vanella L., Volti G. L., ... &Galvano F. Effect of treatment with Cyanidin-3-O-β-D-Glucoside on rat ischemic/reperfusion brain damage. *Evidence-based Complementary and Alternative Medicine: eCAM*, *2012(2012)*.
4. Di Giacomo C., Acquaviva R., Santangelo R., Sorrenti V., Vanella L., Volti G. L., ... & Galvano F. Effect of treatment with Cyanidin-3-O-β-D-Glucoside on rat ischemic/reperfusion brain damage. *Evidence-based Complementary and Alternative Medicine: eCAM*, *2012 (2012)*.
5. Sedighi M., Sewell R. D., NazariA., Abbaszadeh S., Cheraghi M., Amini A., ... & Rafieian-Kopaei M. A review on the most important medicinal plants effective in cardiac ischemia-reperfusion injury. *Current pharmaceutical design*, *25*(3), 352-358(2019).
6. Levonen A. L., Hill B. G., Kansanen E., Zhang J., & Darley-Usmar V. M. Redox regulation of antioxidants, autophagy, and the response to stress: implications for electrophile therapeutics. *Free Radical Biology and Medicine*, *71*, 196-207(2014).
7. Chen G., Wu D. I., Guo W., Cao Y., Huang D., Wang H., ... & Ning Q. Clinical and immunological features of severe and moderate coronavirus disease 2019. *The Journal of clinical investigation*, *130*(5), 2620-2629(2020).
8. Liu Y., Wang X., Wang Z., Ju, W., & Wang D. Decorin inhibits the proliferation of HepG2 cells by elevating the expression of transforming growth factor-β receptor II. *Experimental and Therapeutic Medicine*, *12*(4), 2191-2195(2016).
9. Gáspár R., Gömöri K., Kiss B., Szántai Á., Pálóczi J., Varga Z. V., ... & Görbe A. Decorin protects cardiac myocytes against simulated ischemia/reperfusion injury. *Molecules*, *25*(15), 3426(2020).
10. Mastantuono T., Starita N., Battiloro L., Di Maro M., Chiurazzi M., Nasti G., ... & Lapi D. Laser speckle imaging of rat pial microvasculature during hypoperfusion-reperfusion damage. *Frontiers in Cellular Neuroscience*, *11*, 298(2017).
11. Mony V. K., Benjamin S., & O'Rourke E. J. A lysosome-centered view of nutrient homeostasis. *Autophagy*, *12*(4), 619-631(2016).
12. Olivas-Aguirre F. J., Rodrigo-García J., Martínez-Ruiz N. D. R., Cárdenas-Robles A. I., Mendoza-Díaz S. O., Álvarez-Parrilla E., ... & Wall-Medrano A. Cyanidin-3-O-glucoside: Physical-chemistry, foodomics and health effects. *Molecules*, *21*(9), 1264(2016).
13. Walters J. W., Amos D., Ray K., &Santanam N. Mitochondrial redox status as a target for cardiovascular disease. *Current opinion in pharmacology*, *27*, 50-55(2016).
14. Wang S., Li, Y., Song X., Wang X., Zhao C., Chen A., &Yang P. Febuxostat pretreatment attenuates myocardial ischemia/reperfusion injury via mitochondrial apoptosis. *Journal of Translational Medicine*, *13*(1), 1-11(2015).
15. Xue Q., He, N., Wang Z., Fu, X., Aung L. H. H., Liu Y., ... & Yu, T. Functional roles and mechanisms of ginsenosides from Panax ginseng in atherosclerosis. *Journal of ginseng research*, *45*(1), 22-31(2021).
16. Tabima D. M., Frizzell S., & Gladwin M. T. Reactive oxygen and nitrogen species in pulmonary hypertension. *Free Radical Biology and Medicine*, *52*(9), 1970-1986(2012).
17. Yang L., Wang B., Zhou Q., Wang Y., Liu X., Liu Z., & Zhan Z. MicroRNA-21 prevents excessive inflammation and cardiac dysfunction after myocardial infarction through targeting KBTBD7. *Cell death & disease*, *9*(7), 1-14(2018).
18. Zhao D., Yang J., &Yang L. Insights for oxidative stress and mTOR signaling in

myocardial ischemia/reperfusion injury under diabetes. *Oxidative Medicine and Cellular Longevity*, *2017(2017)*.

19. Zhou T., Chuang C. C., & Zuo L. Molecular characterization of reactive oxygen species in myocardial ischemia-reperfusion injury. *BioMed research international*, *2015*(2015).
20. Mastantuono T., Di Maro M., Chiurazzi M., Battiloro L., Muscariello E., Nasti G., ... & Lapi D. Rat pial microvascular changes during cerebral blood flow decrease and recovery: Effects of cyanidin administration. *Frontiers in Physiology*, *9*, 540(2018).