Undergraduate Students, Populations, Health and Wellness

Investigating the Cardiac Effects of New Generation Anti-Diabetic Drug Empagliflozin Using Zebrafish Embryo Model

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Abstract

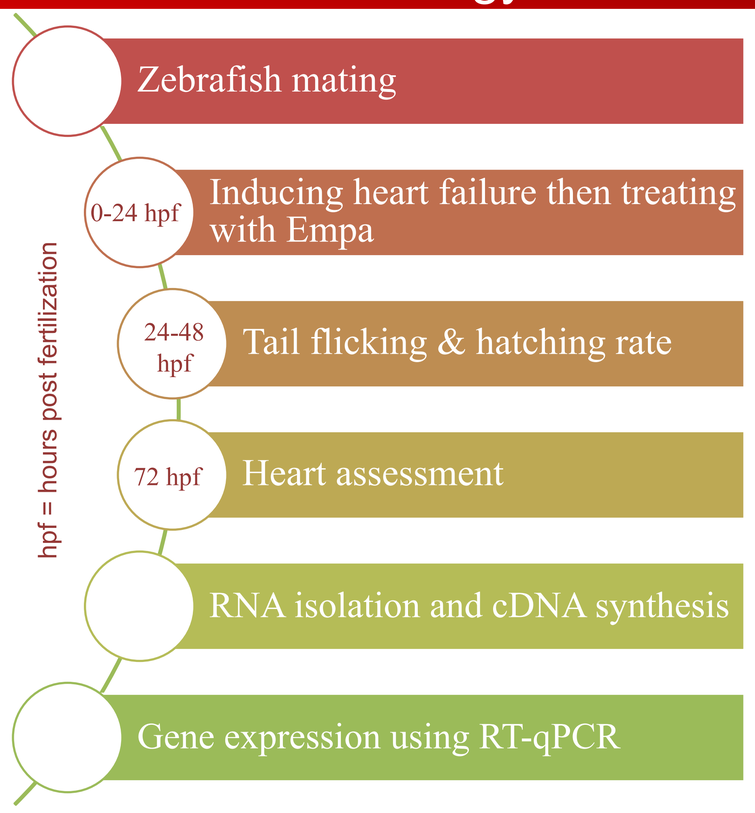
Type 2 diabetes mellitus (T2DM) affects >16% of adults in Qatar. Newly emerging class of anti-diabetic drugs focuses on SGLT inhibition were observed to reduce CVDs risks in diabetic patients. Up to date, the mechanism contributing to the CV benefits remains unrevealed. Zebrafish embryos were treated with Aristolochic Acid to induce heart failure then treated with Empagliflozin to determine its beneficial effect. Furthermore the expression of SGLT1 & 2 were determined in the hearts of zebrafish. SGLT2 was expressed more then SGLT1 in the heart and whole embryo. Empa significantly improved the zebrafish embryo's cardiac health after induction of heart failure.

Introduction

Empagliflozin was shown nto improve the cardiovascular (CV) complication. Yet, the exact mechanism of Empagliflozin on CV tissues remains unclear. Therefore, this research aims to:

- Determine the expression level of SGLT1 and SGLT2 in zebrafish.
- Determine the toxicity of Empa on zebrafish.
- Analyze the impact of Empagliflozin in improving the cardiac function in zebrafish.

Methodology



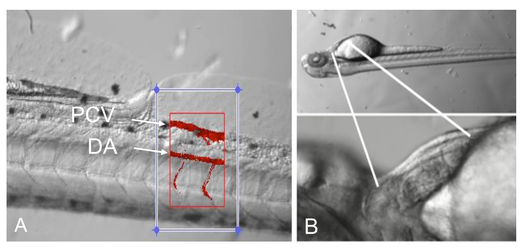


Figure 1. (A) Heart assessment using PCV and DA, (B) zebrafish heart location.

Results

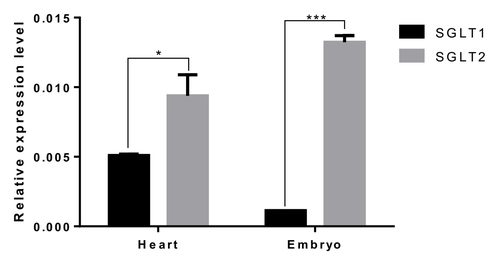


Figure 1: SGLT-1 and SGLT-2 Expression in Zebrafish Using qPCR. RNA was isolated from hearts of zebrafish embryos and whole embryos then reverse transcribed to cDNA and subjected to QPCR analysis. Analysis was by one-way-ANOVA with sidak post hoc test to compare groups. Data is presented as mean ± SEM. N = 3 per group.

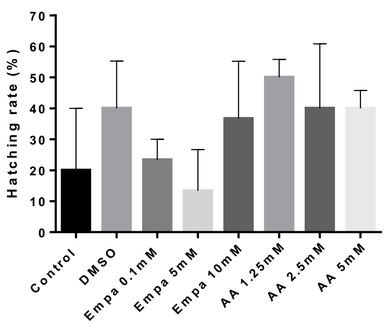


Figure 2: Hatching Rate measured at 48 hpf for Toxicity Study.
Embryos were treated at 0 hour post fertilization (hpf) with PTU water as control, vehicle control containing 0.1% DMSO (DMSO), Empa and AA.

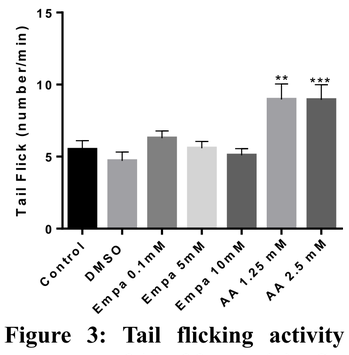


Figure 3: Tail flicking activity of embryos at 24 hpf for Toxicity Study
Embryos were treated at 0 hour post fertilization (hpf) with PTU water as control, vehicle control containing 0.1% DMSO (DMSO), Empagliflozin and AA.

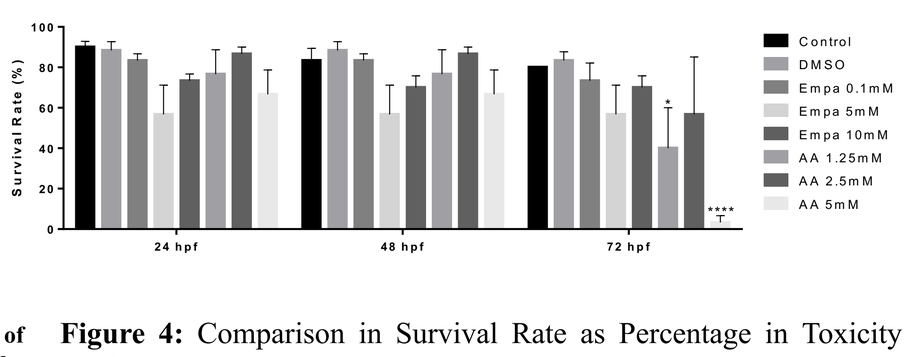


Figure 4: Comparison in Survival Rate as Percentage in Toxicity Study
Embryos were treated at 0 hour post fertilization (hpf) with PTU water as control, vehicle control containing 0.1% DMSO (DMSO), and Empa and AA concentrations.

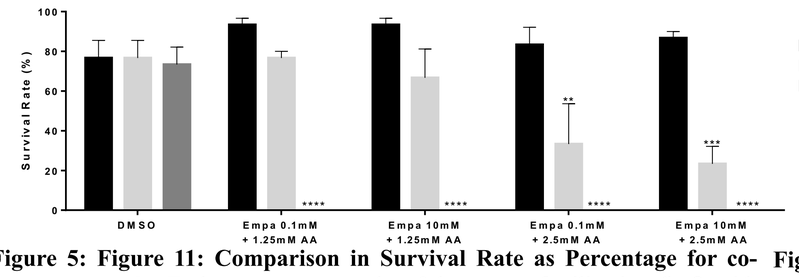


Figure 5: Figure 11: Comparison in Survival Rate as Percentage for co-treatment study. Embryos were treated at 0 hour post fertilization (hpf) with vehicle control containing 0.1% DMSO (DMSO), Empagliflozin (Empa) at 0.1 or 5 mM for 8 hours followed by aristolochic acid (AA) at 1.25 or 2.5 mM for 24 hours at 24, 48 and 72 hrs. Treatment with 0.1 mM Empa + 2.5 mM AA caused a significant decrease in survival rate. All treatments cause a death in all embryos at 72 hrs.

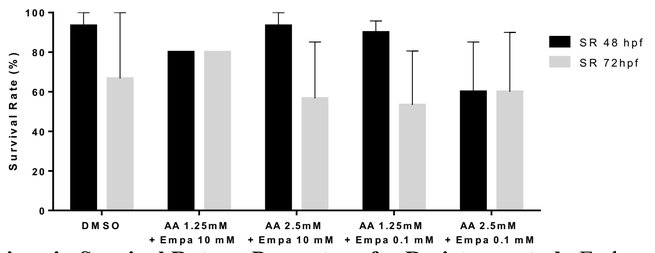


Figure 7: Comparison in Survival Rate as Percentage for Resistance study Embryos were treated at 0 hour post fertilization (hpf) with vehicle control containing 0.1% DMSO (DMSO), aristolochic acid (AA) at 1.25 or 2.5 mM for 24 hours followed by Empagliflozin (Empa) 0.1 or 5 mM after 24 hrs for 32 hours. analysis was done at 48 and 72 hrs post treatment. No significant difference was observed.

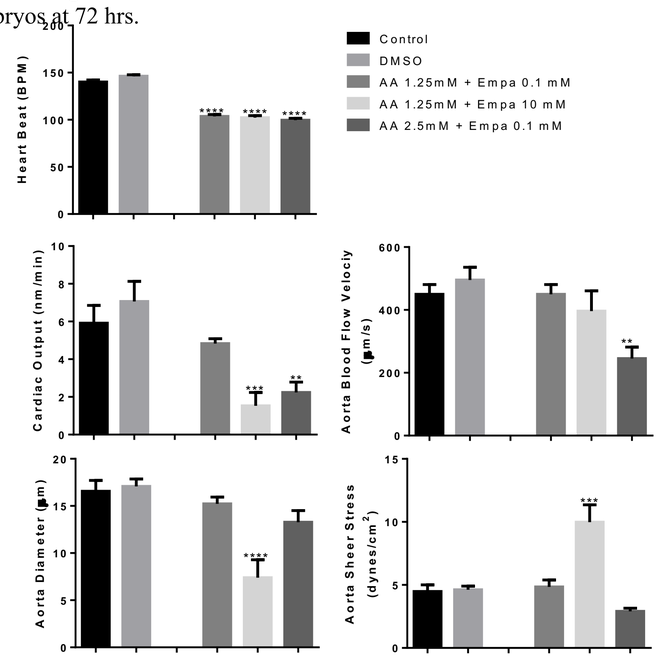


Figure 8: Empagliflozin Effect on Cardiac Injury Markers. Embryos were treated at 0 hour post fertilization (hpf) with vehicle control containing 0.1% DMSO (DMSO), aristolochic acid (AA) at 1.25 or 2.5 mM followed by Empagliflozin (Empa) 0.1 or 5 mM after 24 hrs. Analysis was done at 72 hrs post treatment. Heartbeat was significantly lower in all treated groups.

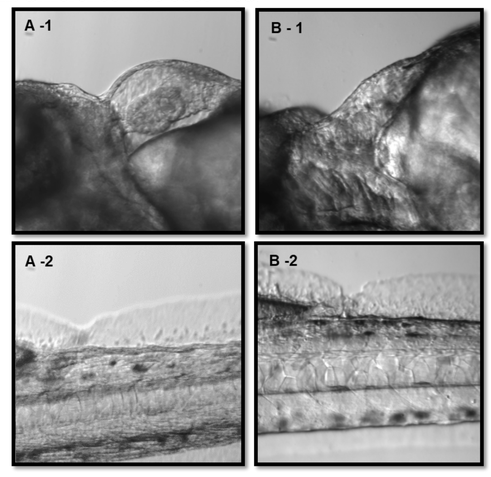


Figure 9: Phenotypic Changes of Heart and Tail of Zebrafish Embryos. A-1. Heart of diseased embryo in Aristolochic Acid 1.25 mM. A-2. Tail of Diseased embryo in Aristolochic Acid 1.25 mM. B-1. Heart of diseased embryo with Aristolochic Acid 1.25 mM then treated with Empagliflozin 0.1mM. B-2. Tail of embryo diseased with Aristolochic Acid 1.25 mM then treated with Empagliflozin 0.1mM.

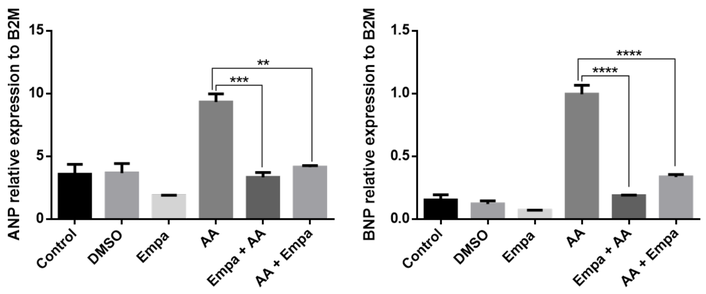


Figure 10: Cardiac Parameters Assessment – ANP and BNP Relative Expression to B2M. RNA was extracted from embryos then reversed transcribed and subjected to QPCR. B2M, a reference gene, showed similar expression levels across all studies groups; control treated with PTU, DMSO as a vehicle control, 0.1 mM Empagliflozin (Empa), 1.25 mM Aristolochic Acid (AA), 8 hrs of Empa followed by 24 hrs of AA, and 24 hrs of AA followed by 32 hrs of Empa. Combination treatment caused a significant decreased in both ANP and BNP.

Conclusion

In conclusion, this study showed that Empagliflozin is effective in protecting or treating the cardiac function from cardiomyopathies in the zebrafish. This was evident by the improvement of the cardiac parameters after injury as well as the expression level of cardiac injury markers. The study showed that SGLT2 is highly expressed in the hearts which could mean that the effect of Empa in the cardiac tissue is direct through its target SGLT2.

Resources

1. Chen J, Williams S, Ho S, Loraine H, Hagan D, Whaley JM, et al. Quantitative PCR tissue expression profiling of the human SGLT2 gene and related family members. Diabetes Ther. 2010;1(2):57-92.
2. Zakaria ZZ, Benslimane FM, Nasrallah GK, Shurbaji S, Younes NN, Mraiche F, et al. Using Zebrafish for Investigating the Molecular Mechanisms of Drug-Induced Cardiotoxicity. Biomed Res Int. 2018;2018:1642684.
3. Yalcin HC, Amindari A, Butcher JT, Althani A, Yacoub M. Heart function and hemodynamic analysis for zebrafish embryos. Dev Dyn. 2017;246(11):868-80.