

THE POTENTIAL OF *HIBISCUS SABDARIFFA* LINN. (ROSELLE) POLYPHENOL-RICH EXTRACT AS A CARDIOPROTECTIVE AGENT IN MYOCARDIAL INFARCTION MODEL

Siti Balkis Budin, Nor Anita Sharifuddin, Fatin Farhana Jubaidi, Satirah Zainalabidin*

Programme of Biomedical Science, Centre of Health and Applied Sciences, Faculty of Health Sciences, Universiti Kebangsaan Malaysia, Jalan Raja Muda Abdul Aziz, 50300 Kuala Lumpur, Malaysia

Article history

Received
24 January 2019
Received in revised form
24 May 2019
Accepted
30 May 2019
Published online
26 August 2019

*Corresponding author
satirah@ukm.edu.my

Graphical abstract



Abstract

Myocardial infarction (MI) is a common cause of death due to interrupted blood supply to the heart. Roselle calyx (*Hibiscus sabdariffa* Linn.) is rich in polyphenols and has a potential in alleviating the risk of cardiovascular disease through its antioxidant activity. This study was aimed to investigate the effects of *Hibiscus sabdariffa* Linn. or roselle polyphenol-rich extract (HPE) supplementation in oxidative stress and cardiac injury biomarkers as well as cardiac histological changes following isoprenaline (ISO)-induced myocardial infarction (MI). Twenty-one male rats were randomly divided into three groups: control, MI, and HPE+MI. Normal saline or HPE (20 mg/kg BW) was given to MI and HPE+MI groups, respectively, for 14 consecutive days via force feeding. On the 15th and 16th day, 85 mg/kg body weight of ISO was administered subcutaneously to induce MI. Control group was only given normal saline throughout this 16-day duration of study. The results showed that HPE reduced the oxidative stress markers malondialdehyde (MDA) and nitrite oxide (NO) in HPE+MI group when compared with MI group ($p < 0.05$) while increased reduced glutathione (GSH) level reflexes the improvement in antioxidant status. Cardiac injury biomarkers analysis showed no significant difference in HPE+MI group when compared to MI group. Histological study showed that HPE managed to reduce cardiac muscle fibre damage and infiltration of inflammatory cells in ISO-induced MI rats. In conclusion, HPE has the potential in protecting the heart against ISO-induced MI by reducing the oxidative stress and increasing antioxidant status.

Keywords: Heart, isoprenaline, oxidative stress, polyphenol, roselle

Abstrak

Infarksi miokardium (MI) merupakan antara punca utama kematian yang disebabkan oleh gangguan pada bekalan darah kepada jantung. Kaliks rosel (*Hibiscus sadariffa* Linn.) kaya dengan polifenol dan mempunyai potensi dalam mengurangkan risiko penyakit kardiovaskular melalui aktiviti antioksidanya. Kajian ini bertujuan untuk mengkaji kesan suplementasi ekstrak kaya polifenol kaliks rosel (HPE) terhadap tekanan oksidatif, enzim jantung dan kerosakan histologi jantung selepas aruhan isoprenalin (ISO). Tikus jantan dibahagikan kepada 3 kumpulan; kawalan, MI dan HPE+MI. Salin normal atau HPE (20 mg/kg berat badan) masing-masing diberikan kepada kumpulan MI dan HPE+MI selama 14 hari melalui paksaan oral. Pada hari ke-15 dan ke-16, ISO diberikan kepada tikus sebanyak 85 mg/kg berat badan secara subkutan untuk mengaruh MI. Kumpulan kawalan hanya diberikan salin normal sepanjang 16 hari kajian. Hasil kajian menunjukkan suplementasi HPE berupaya memulihkan penanda-penanda tekanan oksidatif malondialdehid (MDA) dan nitrit oksida (NO) pada tikus kumpulan HPE+MI berbanding kumpulan MI serta peningkatan aras glutathion terturun (GSH) ($p < 0.05$). Analisis biopenanda kecederaan jantung pula menunjukkan tiada perbezaan yang signifikan antara kumpulan

HPE+MI apabila dibandingkan dengan kumpulan MI. Kajian histologi menunjukkan HPE berupaya mengurangkan kerosakan serat otot jantung dan infiltrasi sel-sel inflamasi pada jantung tikus aruhan MI. Kesimpulannya, HPE mempunyai potensi untuk melindungi jantung daripada MI aruhan ISO melalui penurunan tekanan oksidatif dan peningkatan status antioksidan.

Kata kunci: Isoprenalin, jantung, polifenol, roselle, tekanan oksidatif

© 2019 Penerbit UTM Press. All rights reserved

1.0 INTRODUCTION

Myocardial infarction (MI) is an acute necrosis in the myocardium due to the imbalance state between blood supply and oxygen demand [1]. Accumulation of reactive oxygen species (ROS) in the ischemic tissue causes oxidative injuries in lipid membrane, protein and DNA. Consequently, this will lead to the alteration in function, structural and biochemical characteristics of the affected cardiac tissues [2].

Isoprenaline (ISO) has been widely used in previous studies to induce MI in rat models [3]. ISO is a synthetic catecholamine drug and beta-adrenergic agonist, capable to induce oxidative damage on the myocardium resulting in focal necrosis of the heart muscle [4]. There are several mechanisms involved including increased oxygen demand, poor oxygen utilization, increased calcium overload and accumulation. In addition, ISO also promotes extensive inflammation, increased myocardial cyclic adenosine monophosphate (cAMP) levels, altered membrane permeability, and increased levels of lipid peroxides [5, 6]. All these processes lead to alterations in cardiac enzymes and consequently results in cardiac dysfunctions. These pathophysiological and morphological changes in ISO-induced MI in rat model are similar to those observed in MI patients.

Hibiscus sabdariffa Linn. or locally known as roselle is a herbaceous plant that has been proven to possess high antioxidant activity [7]. Polyphenol-rich extract of roselle (HPE) is a collection of phenolic compounds extracted from the dried roselle calyx. Based on previous phytochemical screening by our own laboratory, roselle contains numerous types of secondary metabolites including kaempferol, chlorogenic acid, hibiscus acid and quercetin [8]. Phenolic content in roselle shown ability to inhibit oxidative stress by eliminating free radicals, acting as agent for anti-inflammatory, antihypertensive [9-11], hypoglycaemic, hypolipidemic as well as rich with antioxidants properties [12-13].

To our best of knowledge, the potential of HPE in protecting against MI is still not known. Therefore, this study was conducted to investigate the effect of HPE supplementation prior to ISO-induced MI in rat model.

2.0 METHODOLOGY

2.1 Roselle Polyphenol Extract (HPE) Preparation

Hibiscus sabdariffa calyces (specimen voucher: UKMB 40308, authenticated by Universiti Kebangsaan Malaysia's Herbarium) were collected from a local plantation in Terengganu, Malaysia. HPE preparation was carried out according to method by Lim *et al.* [8] in which 5 g of roselle calyx powder was extracted with 50 ml HPLC grade methanol and heated at 60°C for 30 minutes. Extract was filtered with Whatman filter paper No. 4 and the resulting residue was extracted twice in the same steps as previously mentioned. The extract was collected and evaporated until dry and the resulting residue was dissolved in 5% dimethyl sulfoxide (DMSO) with 10 ml of distilled water. The resulting aqueous solution of roselle was divided successively three times with 10 ml n-hexane (3 x 10 ml) and 10 ml of ethyl acetate (3 X 10 ml). Ethyl acetate soluble fraction was then evaporated, dried with rotary evaporator and the extracts were stored at -20°C.

2.2 Experimental Animal

Sprague-Dawley male rats (220-250 g) supplied by Animal Unit of Universiti Kebangsaan Malaysia were used in this study. Research protocols and the usage of animals were approved by the Universiti Kebangsaan Malaysia Animal Ethics Committee (UKMAEC). The rats were allowed to adapt for a week before starting the experiment. The rats were placed in a room that has 12 hours dark and light cycle, with a good ventilation system and allowed to a standard food and water *ad libitum* throughout the experiment.

2.3 Study Design

Sprague-Dawley male rats were randomized divided into three groups (n=7 in each group); Control, MI and HPE+MI. The MI and HPE+MI groups were given normal saline or HPE (20 mg/kg BW) [12] respectively for 14 days via force-feeding and followed by 85 mg/kg BW (s.c.) of ISO (Sigma Aldrich, USA) on the 15th and 16th day [15]. The control rats were given normal saline for 14 days via force-feeding before being administered with normal saline at 1 ml/kg

(s.c.) on the 15th and 16th day. On the 17th day, the rats were put under anaesthesia. Blood was taken through orbital sinus, centrifuged at 4000 rpm for 10 minutes to obtain serum for cardiac enzyme level determination. The heart was removed and cleaned with a cold 1.15% potassium chloride (KCl) solution and weighed. The heart was then incised, and part of the left ventricle were cut for histological evaluation. The remaining heart tissue was weighed and cut into pieces, added with 1.15% KCl solution at the ratio of 10 ml/g (w/v) and homogenized using Ultra Turax T25 homogenizer. The homogenates were centrifuged at 8000 rpm for 20 min at 4°C and the supernatant were collected for oxidative stress and antioxidant status evaluation. The heart homogenate was prepared based on the method described by Ramalingam *et al.* [11].

2.4 Oxidative Stress and Antioxidant Enzyme Evaluation

Malondialdehyde (MDA) level was determined based on calorimetric protocol by Stocks and Dormandy [16]. Nitrite concentration was measured as an index of nitric oxide (NO) production, which was determined based on calorimetric assay reaction by Miles [17]. Meanwhile, reduced glutathione (GSH) level was determined by using Ellman's method [18] and superoxide dismutase (SOD) activity determination was done according to Beyer & Fridovich's method [19].

2.5 Measurement of Cardiac Enzyme Level

Measurement of aspartate amino transferase (AST), alanine aminotransferase (ALT) and creatine kinase (CK) enzyme level were determined by using an automated chemistry analyzer (Olympus AU400) based on guidelines by the International Federation for Clinical Chemistry (IFCC). The enzyme levels were measured by kinetic technique (IFCC method). Troponin T (cTnT) levels were measured quantitatively by using sandwich ELISA commercial kit (CTN-T Elisa Kit, Cusabio).

2.6 Histological Study

The left ventricle part of the heart was fixed in 10% formalin, dehydrated in graded concentration of ethanol before being embedded in paraffin wax. The tissue was then sectioned at a mean thickness of 5 µm. The tissue was then stained by Hematoxylin and Eosin (H&E) for histological evaluation.

2.7 Statistical Analysis

The data were expressed as mean (standard deviation (SEM)). Statistical analysis was carried out by using Prism GraphPad version 6. Statistical significance was determined by one-way ANOVA followed by Post-hoc Benferroni test with $p < 0.05$ considered as significant between groups.

3.0 RESULTS AND DISCUSSION

Nutraceutical agents such as roselle represent a promising approach for management of heart diseases. Roselle calyces are rich with natural polyphenols which possess potent antioxidants properties as well as blood pressure lowering and anti-atherosclerotic effects [11, 13-14]. Therefore, we use HPE to study its protective effect against myocardial infarction in rat model.

3.1 Body Weight of Rats

Table 1 shows the body weight of rats after 16 days of study. There were no significant differences in body weight between the rats in the control, MI and HPE+MI groups.

Table 1 Body weight of rats

Study Groups	Body weight (g)
Control	300.85
MI	285.86
HPE + MI	292.57

Statistical analysis was performed using one-way ANOVA followed by Benferroni post-hoc test. Results were expressed as mean \pm SEM. $n=7$ in all groups. There was no significant difference in body weight between all groups

3.2 Heart/Body Weight Ratio of Rats

Figure 1 shows the effect of HPE on heart/body weight ratio in different experimental groups. The heart/body weight ratios in MI group and HPE+MI group were significantly higher ($p < 0.05$) compared to the control group. However, there was no significant difference between the MI group and the HPE + MI group.

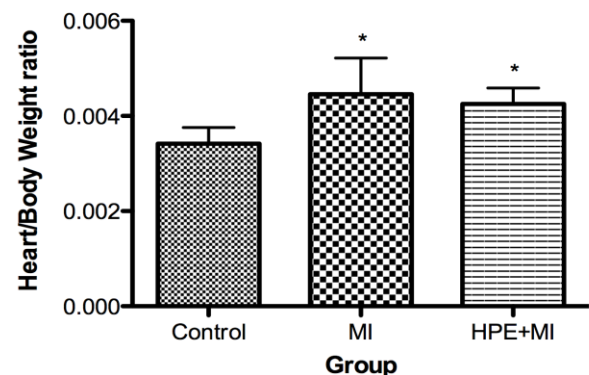


Figure 1 Heart/Body weight ratio of rats. Effect of HPE on heart/body weight ratio. Results were expressed as mean \pm SEM. Statistical analysis was performed using one-way ANOVA followed by Benferroni post-hoc test. $n=7$ in all groups. * $p < 0.05$ vs control group

Our findings showed that there were no significant differences in the rat body weight between the groups. This is consistent with a study by Goyal and colleagues [20] whereby ISO induction did not affect the amount of food intake among the rats. However, the heart/body weight ratio showed an increment in both MI and HPE-treated groups. According to Gupta and colleagues [6], this manifestation probably due to development of myocardial oedema and presence of inflammatory cells resulting from the inflammatory condition induced by ISO.

3.3 Oxidative Stress and Antioxidant Enzyme Evaluation

Figure 2 shows the effect of HPE on MDA, nitrite and GSH level as well as SOD enzyme activity in the heart homogenate. The MDA and nitrite concentration level were significantly higher ($p < 0.05$) in the MI and HPE+MI groups when compared to the control group. HPE supplementation has significantly reduced the MDA and NO level ($p < 0.05$) in HPE+MI group when compared to the MI group (Figure 2a~b). ISO administration caused a significant reduction ($p < 0.05$) in the SOD enzyme activity and GSH level in MI group (Figure 2c~d). There was significant difference in GSH level between group MI and HPE+MI group ($p < 0.05$), however this could not be seen in SOD enzyme activity.

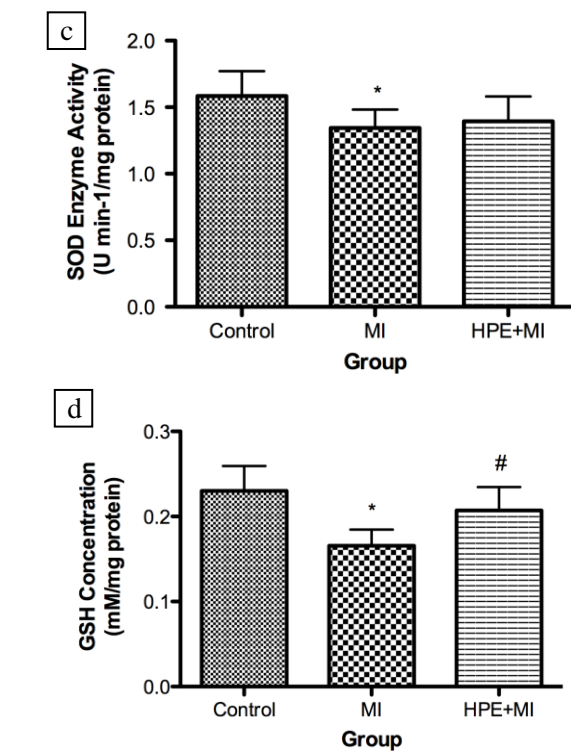
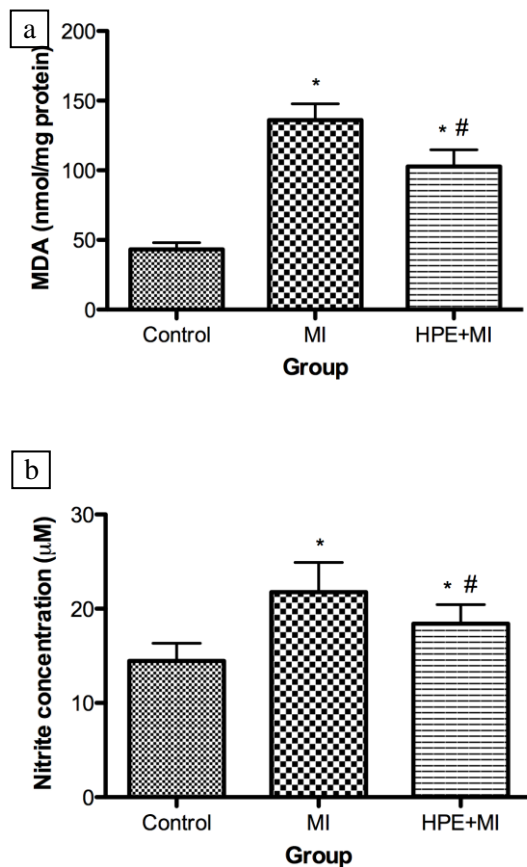


Figure 2 Oxidative Stress and Antioxidant Enzyme Evaluation. Effect of HPE on levels of (a) malondialdehyde, (b) nitrite, (c) SOD enzyme activity and (d) GSH. Results were expressed as mean \pm SEM. Statistical analysis was performed using one-way ANOVA followed by Benferroni post-hoc test. $n=7$ in all groups. * $p < 0.05$ vs control group. # $p < 0.05$ vs MI group

There was an increase in MDA level in the MI group. However, the raise in MDA level was successfully reduced in HPE treated group. Earlier study by Kao *et al.* [10] showed a decrease in MDA level of rat heart with lipopolysaccharide-induced liver damage when treated with HPE. Similarly, Zainalabidin *et al.* [13] also reported that HPE treatment was able to reduce MDA level in nicotine induced rat heart. This is probably contributed by the antioxidant activity of the phenolic compounds in HPE that inhibits oxidative stress via elimination of free radicals and termination of free radicals' chain.

Free radical-trapping antioxidants such as SOD and GSH are the first line of cellular defence against oxidative injury [21]. Reduction in SOD activity and level of GSH in MI group is consistent with earlier studies [2, 14, 22]. Interestingly, only GSH content was preserved but not SOD activity in HPE treated group. Lim *et al.* [8] had identified the main constituents of HPE which are anthocyanin, chlorogenic acid, hibiscus acid and flavonoids. All these compounds are rich with antioxidant properties and free radical scavenging activity. Therefore, this could contribute to the high GSH level in HPE treated group. Previous study showed that superoxide scavenging activity of HPE was due to chlorogenic acid (CA), which have a

SOD-like activity [23]. Our result is in contrary probably due to low CA content based on our own laboratory findings [8]. Furthermore, SOD activity may not precisely indicate superoxide level in the heart as superoxide is highly reactive and has short half-life [24] which could explain the insignificant increase of SOD activity in the HPE treated rats.

Cardiac nitrite measured as a marker of nitrite oxide (NO) in this study is often considered as indicator for inflammation. NO is usually generated by inducible nitric oxide synthase (iNOS) in setting of myocardial injury and is crucial for activation of pro-inflammatory and anti-inflammatory molecules such as macrophage inflammatory protein 2 (MIP-2) and monocyte chemoattractant protein 1 (MCP-1) [25-26]. Consistent with this, the nitrite level in the MI group was elevated. However, HPE supplementation significantly blunted increase nitrite generation in the MI+HPE group. Reduction of NO levels in HPE supplementation group could be due to HPE's ability to inhibit expression of iNOS through action anti-inflammatory activity. This finding is in line with previous studies which proved that HPE can reduce nitrite levels in rats induced by lipopolysaccharides (LPS) with reduced activation of iNOS in Kupffer cells [10, 27]

ISO induction generates free radicals through the mechanism of β -adrenoceptor, which affects the metabolism of cells to produce free radicals that are cytotoxic and cause necrosis of the myocardium [22]. ISO is oxidized into isoproterenol-o-quinone, which cyclizes and oxidizes to isoprenochrome. From this auto-oxidation process, it gives rise to the production of superoxide anions and, subsequently, hydrogen peroxide [28]. This could lead to form highly reactive hydroxyl radicals and causes protein, lipid, and DNA damage and increased infarction size. In addition, excessive free radicals' activity may result in the loss of function and integrity of myocardial membranes. These free radicals may attack polyunsaturated fatty acids (PUFAs) within the membranes, forming peroxy radicals. These radicals could then attack adjacent fatty acids, causing a chain reaction of lipid peroxidation [5]. Therefore, the plasma membranes become fragile and porous, causing the release of cardiac enzymes into the bloodstream.

3.4 Enzyme Marker for Cardiac Injury

Table 2 shows that there were significant increases ($p < 0.05$) in the levels of ALT, AST, CK and cTnT in the MI and HPE+MI groups compared with the control group. However, supplementation of HPE in MI group shows no significant difference in all cardiac enzymes level compared with MI group.

Table 2 Enzyme Markers for Cardiac Injury

Enzyme	Control	MI	HPE+MI
ALT (U/L)	52.80 \pm 5.87	82.40 \pm 10.1 ^a	72.14 \pm 7.73 ^a
AST (U/L)	124.86 \pm 8.59	238.83 \pm 23.9 ^a	220.12 \pm 10.4 ^a
CK (U/L)	200.71 \pm 18.3	417.43 \pm 18.0 ^a	395.67 \pm 22.8 ^a
cTnT (pg/ml)	99.57 \pm 5.97	397.29 \pm 14.9 ^a	375.88 \pm 5.96 ^a

Effects of HPE on cardiac enzyme levels. Results were expressed as mean \pm SEM. Statistical analysis was performed using one-way ANOVA followed by Benferroni post-hoc test. n=7 in all groups. ^a $p < 0.05$ vs control group.

ALT = Alanine transaminase. AST = Aspartate transaminase. CK = Creatine Kinase. cTnT = Cardiac troponins.

As expected, there was an increase of serum AST, ALT, CK and cTnT levels in MI group, which is consistent with previous studies [15, 29]. HPE supplementation in MI rats showed a decrease in all of the enzymes although not significant. The low dose of HPE and duration of treatment is most probably not sufficient enough to provide a protective effect against ISO-induced MI. Based on study by Adetutu & Owoade [30], HPE treatment of 50 mg/kg in carbon tetrachloride (CCl₄)-induced rats for 7 days was unable to reduce AST and ALT levels which indicate inability of HPE to protect against cell membrane damage. It is probable that HPE could give its protective effect by maintaining the structure and function of plasma membrane integrity if given at higher doses.

3.5 Histological Study of Heart

Figure 3 shows the histological examination in heart tissue. The cardiac tissue for the control group (Figure 3A) exhibits normal morphological features. On the other hand, the cardiac tissue of MI group (Figure 3B) showing features of myocardial necrosis with infiltration of inflammatory cells and damage to the cardiac muscle fibres. Histological structure of heart tissue for HPE + MI group (Figure 3C) shows a reduction in the inflammatory cell infiltration and less of necrotic cells seen.

Based on the tissue histological studies of MI rats, signs of necrosis were visible with cardiac muscle fibres separation and damage in the striated muscle which has been proven in previous study [2].

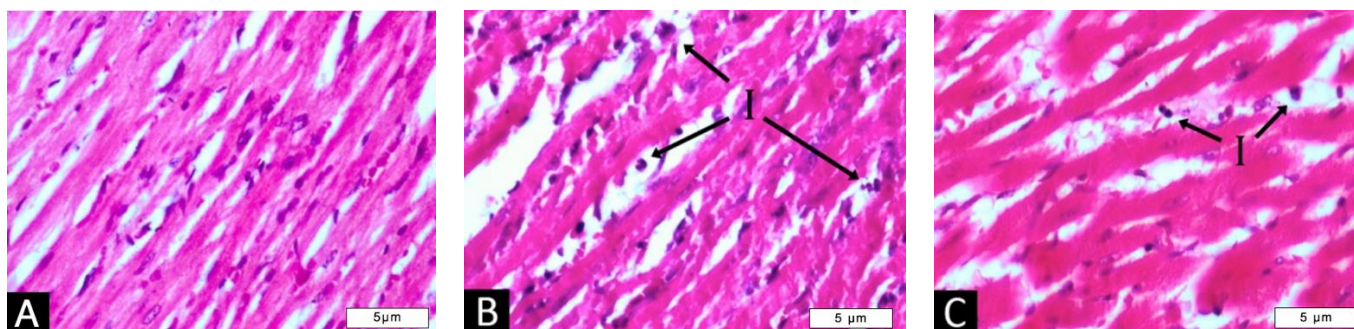


Figure 3 Histological examinations of the myocardial tissue in all experimental rats. (A) The control group shows normal morphological features of cardiac muscle fibres; (B) MI group shows obvious inflammatory cell infiltration (I), with degenerative changes of cardiac muscle fibres. (C) HPE + MI group shows a reduction of inflammatory cell infiltration (I), and minimum number of necrotic cells. Magnification: $\times 400$

ISO-induced MI leads to inflammation process [31] and this is evidenced by the presence of inflammatory cell infiltration in the heart tissue of MI group. Presence of neutrophil in the ischemic myocardial tissue is believed to be the main source of free radicals [2]. Supplementation of HPE in MI rats showed a decrease in myocardial necrosis, infiltration of inflammatory cells and cardiac muscle fibre damage. This demonstrates the ability of HPE in protecting cardiac tissue from damage caused by MI. The histopathological finding in the HPE treated group is consistent with the reduction in oxidative stress and status which indicated by the low levels of MDA and NO. In addition, the increase in GSH level could also protect the cell membrane from the free radicals damaging activity.

4.0 CONCLUSION

In conclusion, HPE shows a potential in protecting cardiac cells from ISO induced MI proven by lowering the level of oxidative stress markers, normalizing the GSH antioxidant enzyme and protecting the structural damage of heart tissues. The phenolic content in HPE probably trapped the free radicals and reduced the lipid peroxidation formation induced by ISO.

Acknowledgement

We gratefully acknowledge the financial support (GUP-2017-018) from Universiti Kebangsaan Malaysia. We thank Miss Nur Liyana Mohammed Yusof and Miss Shafreena Shaukat Ali for the statistical analysis assistance.

References

- [1] Upaganlawar A, Balaraman R. 2010. Protective Effects of Lagenaria Siceraria (Molina) Fruit Juice in Isoproterenol Induced Myocardial Infarction. *International Journal of Pharmacology*. 6: 645-651. DOI: <http://dx.doi.org/10.3923/ijp.2010.645.651>.
- [2] Wang, S., Tian, S., Yang, F., Yang, H. G., Yang, X. Y. and Du, G. H. 2009. Cardioprotective Effect of Salvianolic Acid A on Isoproterenol-induced Myocardial Infarction in Rats. *European Journal of Pharmacology*. 615(1-3): 125-132. DOI: <http://dx.doi.org/10.1016/j.ejphar.2009.04.061>.
- [3] Abdel-Reheim, E. M. 2016. Cardioprotective Efficacy of Taurine on Lipid-metabolism of Isoproterenol-induced Myocardial Infarction. *International Journal of Pharmacology and Pharmaceutical Sciences*. 8(12): 135-141. DOI: <http://dx.doi.org/10.22159/ijpps.2016v8i12.14966>.
- [4] Karthick, M. and Prince, P. S. 2006. Preventive Effect of Rutin, a Bioflavonoid on Lipid Peroxides and Antioxidants in Isoproterenol-induced Myocardial Infarction in Rats. *Journal of Pharmacy and Pharmacology*. 58: 701-707. DOI: <https://doi.org/10.1211/jpp.58.5.0016>.
- [5] Khalil, M. I., Ahmed, I., Ahmed, R., Tanvir, E. M., Afroz, R., Paul, S., Gan, S. H. and Alam, N. 2015. Amelioration of Isoproterenol-induced Oxidative Damage in Rat Myocardium by Withania somnifera Leaf Extract. *BioMedical Research International*. Article ID 624159. DOI: <http://dx.doi.org/10.1155/2015/624159>.
- [6] Gupta, S. K., Mohanty, I., Talwar, K. K., Dinda, A., Joshi, S., Bansal, P., Saxena, A. and Arya, D. S. 2004. Cardioprotection from Ischemia and Reperfusion Injury by Withania Somnifera: A Hemodynamic, Biochemical and Histopathological Assessment. *Molecular and Cellular Biochemistry*. 260: 39-47. DOI: <https://doi.org/10.1023/B:MCBI.0000026051.16803.03>.
- [7] Cissouma, A. I., Tounkara, F., Niko, M., Yang, N. and Xu, X. 2013. Physico Chemical Properties and Antioxidant Activity of Roselle Seed Extracts. *Advance Journal of Food Science and Technology*. 5(11): 1483-1489. DOI: <http://dx.doi.org/10.19026/ajfst.5.3371>.
- [8] Lim, Y. C., Budin, S. B., Othman, F., Latip, J. and Zainalabidin, S. 2017. Roselle Polyphenols Exert Potent Negative Inotropic Effects Via Modulation of Intracellular Calcium Regulatory Channels in Isolated Heart. *Cardiovascular Toxicology*. 17 (3): 251-259. DOI: <https://doi.org/10.1007/s12012-016-9379-6>.

- [9] El-Shafey, A. A. M., Seddek, M. N. E., El-Ezaby, M. M., Seliem, M. M. E. and Abs El-Maksoud, M. A. E. 2013. Protective Effect of Garlic "Allium sativum" and Karkada "Hibiscus sabdariffa" on Acrylamide Treated Male Albino Rats. *Egyptian Journal of Experimental Biology (Zoology)*. 9(1): 101-107.
- [10] Kao, E. S., Hsu, J. D., Wang, C. J., Yang, S. H., Cheng, S. Y. and Lee, H. J. 2009. Polyphenols Extracted from Hibiscus sabdariffa L. Inhibited Lipopolysaccharide-induced Inflammation by Improving Antioxidative Conditions and Regulating Cyclooxygenase-2 Expression. *Bioscience, Biotechnology and Biochemistry*. 73(2): 385-390. DOI: <https://doi.org/10.1271/bbb.80639>.
- [11] Ramalingam, A., Budin, S. B., Lim, Y. C., Si, L. Y. N. and Zainalabidin, S. 2016. Dietary UKMR-1 Roselle Supplementation Prevents Nicotine-Induced Cardiac Injury by Inhibiting Myocardial Oxidative Stress. *Sains Malaysiana*. 45(7): 1131-1137.
- [12] Peng, C. H., Chyau, C. C., Chan, K. C., Chan, T. H., Wang, C. J. and Huang, C. N. 2011. Hibiscus Sabdariffa Polyphenolic Extract Inhibits Hyperglycemia, Hyperlipidemia, and Glycation-oxidative Stress while Improving Insulin Resistance. *Journal of Agriculture and Food Chemistry*. 59(18): 9901-9909. DOI: <https://doi.org/10.1021/jf2022379>.
- [13] Zainalabidin, S., Shahidin, S. N. F. S. N. and Budin, S. B. 2016. Hibiscus Sabdariffa Linn (Roselle) Protect against Nicotine-induced Heart Damage in Rats. *Sains Malaysiana*. 45(2): 207-214.
- [14] Velioglu, Y. S., Mazza, G., Gao, L. and Oomah, B. D. 1998. Antioxidant Activity and Total Phenolics in Selected Fruits, Vegetables and Grain Products. *Journal of Agriculture and Food Chemistry*. 46: 4113-4117. DOI: <https://doi.org/10.1021/jf9801973>.
- [15] Panda, V. S. and Naik, S. R. 2008. Cardioprotective Activity of Ginkgo Biloba Phytosomes in Isoproterenol-induced Myocardial Necrosis in Rats: A Biochemical and Histoarchitectural Evaluation. *Experimental and Toxicologic Pathology*. 60: 397-404. DOI: <https://doi.org/10.1016/j.etp.2008.03.010>.
- [16] Stocks, J. and Dormandy, T. L. 1971. The Autoxidation of Human Red Cell. Lipid Reduced by Hydrogen Peroxide. *British Journal of Haematology*. 20: 95-111. DOI: <https://doi.org/10.1111/j.1365-2141.1971.tb00790.x>.
- [17] Miles, A. M., Wink, D. A., Cook, J. C. and Grisham, M. B. 1996. Determination of Nitric Oxide using Fluorescence Spectroscopy. *Methods in Enzymology*. 268: 105-120. DOI: [https://doi.org/10.1016/S0076-6879\(96\)68013-6](https://doi.org/10.1016/S0076-6879(96)68013-6).
- [18] Ellman, G. 1959. Tissue Sulphydryl Groups. *Archives of Biochemistry and Biophysics*. 32: 70-77. DOI: [https://doi.org/10.1016/0003-9861\(59\)90090-6](https://doi.org/10.1016/0003-9861(59)90090-6).
- [19] Beyer, W. F. and Fridovich, I. 1987. Assaying for Superoxide Dismutase Activity: Some Large Consequences for Minor Changes in Conditions. *Analytical Biochemistry*. 161: 559-566. DOI: [https://doi.org/10.1016/0003-2697\(87\)90489-1](https://doi.org/10.1016/0003-2697(87)90489-1).
- [20] Goyal, S., Arora, S., Mittal, R., Joshi, S., Nag, T. C., Ray, R., Kumari, S. and Arya, D. S. 2009. Myocardial Salvaging Effect of Telmisartan in Experimental Model of Myocardial Infarction. *European Journal of Pharmacology*. 619: 75-84. DOI: <https://doi.org/10.1016/j.ejphar.2009.07.026>.
- [21] Sawyer, D. B., Siwik, D. A., Xiao, L., Pimentel, D. R., Singh, K. and Colucci, W. S. 2002. Role of Oxidative Stress in Myocardial Hypertrophy and Failure. *Journal of Molecular and Cellular Cardiology*. 134: 379-388. DOI: <https://doi.org/10.1006/jmcc.2002.1526>.
- [22] Kavitha, P. and Sowmia, C. 2017. Antioxidant Effect of Polyherbal Formulation in Isoprenaline Hydrochloride Induced Myocardial Infarction in Rats. *International Journal of Pharmacy and Pharmaceutical Sciences*. 9(10): 273-275. DOI: <https://doi.org/10.22159/ijpps.2017v9i10.20310>.
- [23] Fernández-Arroyo, S., Herranz-López, M., Beltrán-Debón, R., Borrás-Linares, I., Barrión-Catalán, E., Joven, J., Fernández-Gutiérrez, A., Segura-Carretero, A. and Micol, V. 2012. Bioavailability Study of a Polyphenol-enriched Extract from Hibiscus Sabdariffa in Rats and Associated Antioxidant Status. *Molecular Nutrition and Food Research*. 56(10): 1590-1595. DOI: <https://doi.org/10.1002/mnfr.201200091>.
- [24] Halliwell, B. 1977. Generation of Hydrogen Peroxide, Superoxide and Hydroxyl Radicals during the Oxidation of Dihydroxyfumaric Acid by Peroxidase. *Biochemical Journal*. 163: 441-448. DOI: <http://dx.doi.org/10.1042/bj1630441>.
- [25] Zaafan, M. A., Zaki, H. F., El-Bairay, Al. and Kenawy, S. A. 2013. Protective Effects of Atorvastatin and Quercetin on Isoprenaline-induced Myocardial Infarction in Rats. *Bulletin of Faculty of Pharmacy, Cairo University*. 51: 35-41. DOI: <https://doi.org/10.1016/j.bfopcu.2013.03.001>.
- [26] Akiyama, K., Kimura, A., Suzuki, H., Takeyama, Y., Gluckman, T. L., Terhakopian, A., Katagiri, T., Suh, K. Y., Roseto, J. and Bing, R. J. 1998. Production of Oxidative Products of Nitric Oxide in Infarcted Human Heart. *Journal of the American College of Cardiology*. 32(2): 373-379. DOI: [https://doi.org/10.1016/S0735-1097\(98\)00270-8](https://doi.org/10.1016/S0735-1097(98)00270-8).
- [27] Rajab, N. F., Musa, S. M., Ahmad Munawar, M., Leong, L. M., Heng, K. Y., Ibrahim, F. W. and Chan, K. M. 2016. Anti-neuroinflammatory Effects of Hibiscus sabdariffa Linn. (Roselle) on Lipopolysaccharides-induced Microglia and Neuroblastoma Cells. *Malaysian Journal of Health Sciences*. 14(2): 111-117. DOI: <http://dx.doi.org/10.17576/JSKM-2016-1402-13>.
- [28] Remião, F., Carvalho, M., Carmo, H., Carvalho, F. and Bastos, M. L. 2002. Cu²⁺-Induced Isoproterenol Oxidation into Isoprenochrome in Adult Rat Calcium-Tolerant Cardiomyocytes. *Chemical Research in Toxicology*. 15(6): 861-869. DOI: <http://dx.doi.org/10.1021/tx025518q>.
- [29] Amran, A. Z., Jantan, I., Dianita, R. and Buang, F. 2015. Protective Effects of the Standardized Extract of Zingiber Officinale on Myocardium against Isoproterenol-induced Biochemical and Histopathological Alterations in Rats. *Pharmaceutical Biology*. 53(12): 1795-1802. DOI: <https://doi.org/10.3109/13880209.2015.1008147>.
- [30] Adetutu, A. and Owoade, A. 2013. Hepatoprotective and Antioxidant Effect of Hibiscus Polyphenol Rich Extract (HPE) Against Carbon Tetrachloride (CCl₄) Induced Damage in Rats. *British Journal of Medicine and Medical Research*. 3(4): 1574-1586. DOI: <https://doi.org/10.9734/BJMMR/2013/3762>.
- [31] Yoshikawa, T. and Naito, Y. 2002. What is Oxidative Stress? *Journal of the Japan Medical Association*. 45(7): 271-276.