DOI: https://doi.org/10.53350/pjmhs22167643

ORIGINAL ARTICLE

The Effect of Rutin on some of the Cardiac Biomarkers in White Male Rats Exposed to Oxidative Stress

MUHAMMAD FADHEL AL HUSSANI[1], ASEEL NAJAH SABOUR[2]
[1-2]University of AL-Qadisiyah, AL-Qadisiyah, Iraq
Correspondence to: Muhammad Fadhel Al Hussani, Email: sci.bio.mas.20.21@qu.edu.iq

ABSTRACT

The current study was designed to investigate the effect of Rutin on some physiological parameters related to the heart in male white rats exposed to oxidative stress with hydrogen peroxide. The study included 48 adult male white rats, their weights ranged from (220-280) g, and their ages ranged between (8-12) weeks. The rats were randomly distributed into six groups of 8 rats for each group, and as follows: The first group, group 1, was considered a negative control group G1 that was supplied with water and the diet for the duration of the experiment which amounted to 30 days and the second group, group 2, was considered a positive control group that was given drinking water containing hydrogen peroxide at a concentration of 1% by means of special drinking bottles and the diet for 30 days, the third group G3 was orally dosed with Rutin at a concentration of 60 mg/kg of body weight in addition to drinking water containing hydrogen peroxide at a concentration of 1% for 30 days . The fourth group G4 also dosed orally with a solution of Rutin at a concentration of 30 mg/kg bw in addition to drinking water containing hydrogen peroxide at a concentration of 1% for 30 days. fifth group G5 dosed orally with a Rutin only at a concentration of 30 mg / kg bw for 30 days. the sixth group G6 was also dosed orally with Rutin only at a concentration of 30 mg / kg bw for30 days. After the end of the experiment, the animals were anesthetized then blood was drawn from the heart directly for the purpose of obtaining serum to measure the level of Troponin, LDH, CK-MB and Myoglobin. The results showed a significant increase ($p < 0.05$) in the level of Troponin, LDH and Ck-MB in the positive group compared to the negative control group, while a significant decrease in the levels of these enzymes was observed in the third and fourth groups compared to the positive control group, while in the two groups The fifth and sixth recorded a significant decrease compared to the positive control group. After obtaining these results, it became clear that Rutin compound has a positive effect in preventing oxidative stress and heart muscle damage.

Keywords: Rutin, hydrogen peroxide, Rats.

INTRODUCTION

Medicinal plants have a pivotal role in the treatment of diseases affecting humans, as many active substances and medicines are extracted from natural sources [1]. Bioactive compounds are derived in the form of secondary metabolites that have pharmacological or toxic effects on humans and animals [2]. These products are represented by alkaloids, phenolics, and terpenoids [3]. Flavonoids contain a group of polyphenolic compounds known to have anti-inflammatory and anti-free radical properties and inhibition of hydrolytic and oxidative enzymes [4], due to these properties of flavonoids, have attracted the attention of researchers in the medical field recently [5].

Rutin or RTN, also known as vitamin P, is a nutritional compound from the group of flavonoids consisting of quercetin bound with the disaccharide Rutinose. The cells have anti-cancer and anti-aging properties [6], [7], and it also has anti-platelet aggregation, anti-viral, anti-hypertensive properties, as well as supporting and strengthening capillaries [8]. Rutin is found in a wide range of plants from citrus fruits such as oranges and lemons. It is found in tomatoes as well as buckwheat, berries, apricots, and cherries [9].

some studies reported on the plant Styphnolobium japonicum, whose dried flowers and flower buds are used as herbal medicine in traditional Chinese medicine, where those buds are rich in Rutin, whose level can reach 16.5% [10], [11]. Also, these flowers were used in the treatment of high blood pressure, arteriosclerosis, conjunctivitis, and hemorrhagic disease [12]. Data and research that dealt with the study of some foods rich in flavonoids indicate the association of these diets with a lower incidence of cardiovascular diseases [13], [14]. The researchers believed that the cause of endothelial dysfunction is a cellular reduction and oxidative stress [15], [16].

Oxidative stress refers to the pathological state of reactive oxygen species (ROS) accumulation resulting from excessive production of oxygen radicals or impairment of the intracellular antioxidant defense system [17]. Oxidative stress also has an important role in regulating the functioning of the heart and blood vessels and has become an important target for the prevention and treatment of cardiovascular diseases. Oxidative stress can cause severe functional damage to endothelial cells and cardiomyocytes [17]. In addition, oxidative stress contributes to the pathogenesis of hypertension, ischemic injury to the myocardium, dysperfusion, atherosclerosis, and other associated diseases by regulating inflammation and stimulating vascular smooth muscle hypertrophy [18], [19].

The data of several epidemiological and clinical studies showed a direct relationship between the development of cardiovascular diseases and the low consumption of diets rich in fruits and vegetables [20], [21]. Numerous scientific studies indicate that flavonoid-rich fruits, vegetables, and nuts have many cardiovascular health benefits through anti-oxidant and free-radical activities that cause oxidative stress, anti-inflammatory, and anticoagulant through complex and multiple mechanisms. [20]–[23].

MATERIALS AND METHODS

Animal of the study: The experiment was conducted in the animal house of the college of science / University of Al-Kufa,48 male rats that were purchased from the animal house of the college the ages ranged between (16-18) weeks, and their weight ranged between (220-280mg) the experimental animal was placed in a plastic cage, which was 50*35*15 cm in size, with a metal cap,8 rats in each cage in a room of 3*4 meters. All animals were exposed to the same conditions, from a temperature range of 20-25c, organized by an air conditioner and the lighting hours were 13 hours of light, against 11 hours of darkness,

Experimental Design:

Each group contains eight male rats:

1. First group G1: represents the negative control were rats given water and food for the duration of the experiment, which lasted for one month
2. Second group G2: represents the positive control were rats dosed Orally with hydrogen peroxide H2O2 at concentration 1% in drinking water for one month.
3. Third group G3: Rats orally dosed with Hydrogen peroxide 1% in drinking water and Rutin compound 60 mg/kg of body weight for one month
4. Fourth group G4: Rats orally dosed with Hydrogen peroxide 1% in drinking water and Rutin 30 mg/kg of body weight for one month.
5. Fifth group G5: Rats Orally dosed with Rutin compound 60 mg/kg of body weight for one month. .

6. Sixth group G6:Rars Orally dosed with Rutin 30 mg/kg for one month **.**

Specimens' collection: After the end of the 30-day trial period and 24 hours after the last day, animals were anesthetized by injection of ketamine and xylazine dissected and blood samples were obtained from the abdominal vein in a non-heparinized tube to perform the serological tests which include LDH, CK-MB, Myoglobin and Troponin. The sampling technique was applied to respect the recommendations of (21,22).

Statistical Analysis: ANOVA analysis and LSD test were used according to (SPSS version 18) to find the mean for all treatments at the ($p \leq 0.05$) (SPSS 2011).

RESULTS

The changes in level of troponin: The results of the study shown in the table (1) showed a significant increase ($p < 0.05$) in the level of troponin in the positive control (G2) that was treated with hydrogen peroxide at a concentration of 1%compared to the first group G1, the negative control

Also the results showed a significant decrease in the level of troponin for the third group (G3) treated with hydrogen peroxide and Rutin at a concentration of 60 mg/kg and fourth group (G4) treated with hydrogen peroxide and Rutin 30 mg/kg body weight, compared to the positive control group (G2), no significant difference was observed between (G3) and (G4) .

As for fifth group (G5) and sixth group (G6) treated with rutin at a concentration of 60 mg/kg and rutin at a concentration of 30 mg/kg respectively, no difference was observed in the level of troponin compared to the negative control (G1), in addition, no significant difference was observed between (G5) and (G6).

Changes in the level of the enzyme LDH: The changes in the Level of Lactate dehydrogenase

The results of the statistical analysis shown in table (1) showed a significant increase ($p<0.05$) in the level of LDH enzyme in the positive control group (G2) treated with hydrogen peroxide compared to the negative control group (G1) .There was a significant decrease ($p < 0.05$) In the level of LDH enzyme in the group (G3) and group (G4) compared to the positive control group (G2), the group (G3) recorded a significant decrease in the level of LDH enzyme compared to group (G4).

While the fifth group (G5) did not show a significant difference ($p < 0.05$) compared to the negative control group (G1), while the group (G6) show a significant increase ($p < 0.05$) compared to the group (G5) and Negative control (G1).

The changes in the Level of CK-MB: The results of this study shown in the table (1), recorded a significant increase($p < 0.05$) in the level of CK-MB in the positive control group (G2) treated with hydrogen peroxide at a concentration of 1% compared to the negative control group (G1), and the results recorded a significant decrease in the level of CK-MB in the third group (G3) treated with hydrogen peroxide and Rutin at a concentration of 60 mg / kg and fourth group (G4) treated with hydrogen peroxide and Rutin at a concentration of 30 mg / kg compared to the positive control group (G2) at a significance level ($p < 0.05$), there was no significant difference between the groups (G3) and (G4) . There was a significant decrease ($p < 0.05$) in the fifth group (G5) and G6 compared to the negative control group (G1). (G5) show a significant increase ($p < 0.05$) in the level of CK-MB compared with the group (G6).

Changes in the level of myoglobin: The results of the statistical analysis shown in table (1) showed a significant increase ($p < 0.05$) in the level of Myoglobin in the positive control group (G2) compared to the negative control group (G1), and there was a significant decrease ($p < 0.05$) in the level of myoglobin for the group (G3) and fourth group (G4) compared to the positive control group (G2), while no significant differences were recorded between the two groups (G3). and (G4). The group (G5) did not show any significant differences when compared to the negative control (G1), while the group (G6) recorded a significant increase at the level of significance ($p<0.05$) compared to the negative control (G1). no significant differences were recorded between the two groups (G5) and (G6).

Table 1: Effect of one mouth treatment with Hydrogen Peroxide and Rutin on some of cardiac biomarkers in White male rats compared to control.

Cardiac biomarkers				
Group	LDH (U / l)	CK-MB (ng/ mL)	Myoglobin (ng/ mL)	TnI (ng/ mL)
Control G1	346.3±17.4D	67.54±4.59B	53.5±3.39B	0.09±0.02B
H_2O_2 G2	910.5±182.5A	105.7±4.47A	161.3±23.5A	1.16±1.06A
H_2O_2 + Rutin (60mg/kg) G3	615.6±24.5 B	41.7±6.17E	64.7±12.2B	0.108±0.11B
H_2O_2 + Rutin (30mg/kg) G4	359.5 ±79.8D	36.3±5.53E	38.03±5.96B	0.066±0.02B
Rutin (60mg/kg) G5	336.7±52.8D	58.5±9.9C	45.7±3.07B	0.42±±0.72B
Rutin (30mg/kg) G6	488.2±6.19C	50.8±3.14D	80.4±19.5B	0.39±0.44B
L.S.D 0.05	85.9	6.87	78.4	0.561

Value represents the mean ± the standard error

Different vertical letters in one column indicate significant differences ($P <0.05$) between the groups.

Similar vertical letters within the same column indicate that there is no significant difference between the groups.

DISCUSSION

Troponin I, CK-MB, LDH, and Myoglobin levels are considered an important prognostic factor for cardiac activity called Cardiac biomarkers, as increased levels of each of these enzymes in the positive control group treated with 1% hydrogen peroxide was evidence of oxidative damage to the myocardium. and ROS-induced stress, which may increase the risk of or lead to cardiomyopathic cardiomyopathy [24]. Troponin I, CK-MB, LDH, and Myoglobin are also leaked from cardiac muscle tissue into the blood due to the damage muscle cells plasma membrane, where this damage is induced by hydrogen peroxide. Therefore, leakage of these enzymes is an important sign of experimental myocardial infarction [25]. Hydrogen peroxide stimulates the production of nitric oxide radicals $ONOO^-$, which leads to oxidative modifications in the nucleic acids of cardiac cells, which leads to myocardial structural remodeling and thus leads to myocardial functional impairment [26].

Hydrogen peroxide affects by causing severe effects of proteins, lipids and nucleic acids inside the mitochondria, in particular the process of lipid peroxidation in the inner membrane of cardiac mitochondria, which is important for energy metabolism, which leads to the dissolution of cytochrome C and thus the production of ATP decreases [27].

H_2O_2 damages mitochondrial DNA, mtDNA, and thus leads to the ROS production cycle that leads to a loss of the function of mitochondrial enzymes to transfer electrons and thus occurs programmed cell death. This effect is known as the “toxic effect of oxidative stress” [28].

After oral administration, rutin is metabolized to quercetin and a disaccharide by the enzyme β-glucosidase and then to its final metabolites in the form of phenolic acids such as (3,4-dihydroxybenzoic, 3,4-dihydrophenylacetic acid, 4-hydroxyl benzoic acid) [29], these products possess a dihydroxyphenyl

group that gives the ability of phenols to inhibit iron and copper ions that can begin to form active free oxygen radicals, thus removing heavy metals and scavenging free radicals.

Therefore, the significant decrease in the levels of cardiac biomarkers Troponin I, CK-MB, LDH and Myoglobin in the (G3) and (G4) groups (dose with hydrogen peroxide and Rutin at a concentration of 60 mg/kg and 30 mg/kg, respectively) compared with the control group show the positive role of this substance in terms of the ability to protect the heart and resist oxidative stress. In a study of the effect of rutin on the cell death caused by hydrogen peroxide in vivo and in vitro in the cell line derived from the heart tissue of rats with myocardial ischemia it was found that rutin significantly reduced oxidative myocardial damage caused by oxidative stress [30]. Also, the results of our study agreed with [31], who showed that rutin has a protective effect on the heart and blood vessels by preventing oxidative damage in the lining cells of the aorta. The results of the current study also showed the cardiac enzymes of the two groups (G5) and (G6) that were dosed orally with rutin compound only at a concentration of 60 mg / kg and a concentration of 30 mg / kg, respectively, at moderate and close levels than the levels in the negative control group (G1) and thus rutin proves that it is non-toxic bioflavonoid compound that does not have a negative effect when used, and this is one of the most important reasons for its common use in human medicine [32].

REFERENCES

1 M. J. Balunas and A. D. Kinghorn, “Drug discovery from medicinal plants,” Life Sci, vol. 78, no. 5, pp. 431–441, Dec. 2005, doi: 10.1016/J.LFS.2005.09.012.

2 A. Bernhoft, H. Siem, E. Bjertness, M. Meltzer, T. Flaten, and E. Holmsen, “Bioactive compounds in plants–benefits and risks for man and animals,” The Norwegian Academy of Science and Letters, Oslo, 2010.

3 J. N. Kabera, E. Semana, A. R. Mussa, and X. He, “Plant secondary metabolites: biosynthesis, classification, function and pharmacological properties,” J. Pharm. Pharmacol, vol. 2, no. 7, pp. 377–392, 2014.

4 E. Frankel, “Nutritional benefits of flavonoids. International conference on food factors: Chemistry and Cancer Prevention,” Hamamatsu, Japan, Abstracts C-2, 1995.

5 E. Mohammad F., W. A. Hasan, and E. G. Mohamed, “Natural antioxidant flavonoids in formalin-induced mice paw inflammation; inhibition of mitochondrial sorbitol dehydrogenase activity,” J Biochem Mol Toxicol, vol. 31, no. 7, p. N/A-N/A, Jul. 2017, doi: 10.1002/JBT.21896.

6 C. la Casa, I. Villegas, C. Alarcón De La Lastra, V. Motilva, and M. J. Martín Calero, “Evidence for protective and antioxidant properties of rutin, a natural flavone, against ethanol induced gastric lesions,” J Ethnopharmacol, vol. 71, no. 1–2, pp. 45–53, Jul. 2000, doi: 10.1016/S0378-8741(99)00174-9.

7 P. Lakhanpal and D. K. Rai, “Quercetin: a versatile flavonoid,” Internet Journal of Medical Update, vol. 2, no. 2, pp. 22–37, 2007.

8 R. Guo, P. Wei, and W. Liu, “Combined antioxidant effects of rutin and Vitamin C in Triton X-100 micelles,” J Pharm Biomed Anal, vol. 43, no. 4, pp. 1580–1586, Mar. 2007, doi: 10.1016/J.JPBA.2006.11.029.

9 J. Ashraf, J. Siddique, N. Mirani, and A. Rub, “Protective effect of rutin against carbon tetrachloride-induced hepatotoxicity in mice,” International Journal of Drug Development and Research, vol. 4, no. 2, p. 0, 2012.

10 Z. Gan, Q. Chen, Y. Fu, and G. Chen, “Determination of bioactive constituents in Flos Sophorae Immaturus and Cortex Fraxini by capillary electrophoresis in combination with far infrared-assisted solvent extraction,” Food Chem, vol. 130, no. 4, pp. 1122–1126, Feb. 2012, doi: 10.1016/J.FOODCHEM.2011.08.018.

11 J. Xi and S. Luo, “Pressure-enhanced solid–liquid extraction of rutin from Chinese scholar-tree flower: Kinetic modeling of influential factors,” Sep Purif Technol, vol. 156, pp. 809–816, Dec. 2015, doi: 10.1016/J.SEPPUR.2015.11.006.

12 T. Kimura, P. P. H. But, J.-X. Guo, and C. K. Sung, “International Collation of Traditional and Folk Medicine,” International Collation of Traditional and Folk Medicine, Apr. 1996, doi: 10.1142/3041.

13 L. Hooper et al., “Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials,” Am J Clin Nutr, vol. 88, no. 1, pp. 38–50, Jul. 2008, doi: 10.1093/AJCN/88.1.38.

14 M. M. Dohadwala and J. A. Vita, “Grapes and Cardiovascular Disease,” J Nutr, vol. 139, no. 9, pp. 1788S-1793S, Sep. 2009, doi: 10.3945/JN.109.107474.

15 T. M. Paravicini and R. M. Touyz, “Redox signaling in hypertension,” Cardiovasc Res, vol. 71, no. 2, pp. 247–258, Jul. 2006, doi: 10.1016/J.CARDIORES.2006.05.001/2/71-2-247-FIG3.GIF.

16 S. R. Thomas, P. K. Witting, and G. R. Drummond, “Redox Control of Endothelial Function and Dysfunction: Molecular Mechanisms and Therapeutic Opportunities,” https://home.liebertpub.com/ars, vol. 10, no. 10, pp. 1713–1765, Aug. 2008, doi: 10.1089/ARS.2008.2027.

17 J. L. dos Santos, A. S. de Quadros, C. Weschenfelder, S. B. Garofallo, and A. Marcadenti, “Oxidative Stress Biomarkers, Nut-Related Antioxidants, and Cardiovascular Disease,” Nutrients 2020, Vol. 12, Page 682, vol. 12, no. 3, p. 682, Mar. 2020, doi: 10.3390/NU12030682.

18 J. Suen et al., “Effect of Flavonoids on Oxidative Stress and Inflammation in Adults at Risk of Cardiovascular Disease: A Systematic Review,” Healthcare 2016, Vol. 4, Page 69, vol. 4, no. 3, p. 69, Sep. 2016, doi: 10.3390/HEALTHCARE4030069.

19 P. Pignatelli, D. Menichelli, D. Pastori, and F. Violi, “Oxidative stress and cardiovascular disease: New insights,” Kardiologia Polska, vol. 76, no. 4. Via Medica, pp. 713–722, Apr. 10, 2018. doi: 10.5603/KP.a2018.0071.

20 G. Y. Tang, X. Meng, Y. Li, C. N. Zhao, Q. Liu, and H. bin Li, “Effects of Vegetables on Cardiovascular Diseases and Related Mechanisms,” Nutrients 2017, Vol. 9, Page 857, vol. 9, no. 8, p. 857, Aug. 2017, doi: 10.3390/NU9080857.

21 C. N. Zhao et al., “Fruits for Prevention and Treatment of Cardiovascular Diseases,” Nutrients 2017, Vol. 9, Page 598, vol. 9, no. 6, p. 598, Jun. 2017, doi: 10.3390/NU9060598.

22 J. Tomé-Carneiro and F. Visioli, “Polyphenol-based nutraceuticals for the prevention and treatment of cardiovascular disease: Review of human evidence,” Phytomedicine, vol. 23, no. 11, pp. 1145–1174, Oct. 2016, doi: 10.1016/J.PHYMED.2015.10.018.

23 A. Cassidy et al., “Habitual intake of flavonoid subclasses and incident hypertension in adults,” Am J Clin Nutr, vol. 93, no. 2, pp. 338–347, Feb. 2011, doi: 10.3945/AJCN.110.006783.

24 S. B. Nimse and D. Pal, “Free radicals, natural antioxidants, and their reaction mechanisms,” RSC Adv, vol. 5, no. 35, pp. 27986–28006, 2015.

25 M. Padmanabhan and P. S. M. Prince, “Preventive effect of S-allylcysteine on lipid peroxides and antioxidants in normal and isoproterenol-induced cardiotoxicity in rats: A histopathological study,” Toxicology, vol. 224, no. 1–2, pp. 128–137, Jul. 2006, doi: 10.1016/j.tox.2006.04.039.

26 S. Kumar and S. Gupta, “Thymosin Beta 4 Prevents Oxidative Stress by Targeting Antioxidant and Anti-Apoptotic Genes in Cardiac Fibroblasts,” PLoS One, vol. 6, no. 10, p. e26912, Oct. 2011, doi: 10.1371/JOURNAL.PONE.0026912.

27 H. F. Galley, “Oxidative stress and mitochondrial dysfunction in sepsis,” BJA: British Journal of Anaesthesia, vol. 107, no. 1, pp. 57–64, Jul. 2011, doi: 10.1093/BJA/AER093.

28 M. W. Fariss, C. B. Chan, M. Patel, B. van Houten, and S. Orrenius, “Role of mitochondria in toxic oxidative stress,” Mol Interv, vol. 5, no. 2, p. 94, 2005.

29 T. M. Alice, H. Yves, and S. Jin, “Biotransformation of Rutin to Quercetin by Human Gut Bacteria and Its Effect on Rutin Bioavailability,” 2018. [Online]. Available: www.researchinventy.com

30 J. J. Jeong et al., “Rutin from Lonicera japonica inhibits myocardial ischemia/reperfusion-induced apoptosis in vivo and protects H9c2 cells against hydrogen peroxide-mediated injury via ERK1/2 and PI3K/Akt signals in vitro,” Food and Chemical Toxicology, vol. 47, no. 7, pp. 1569–1576, Jul. 2009, doi: 10.1016/J.FCT.2009.03.044.

31 D. W. Kim et al., “Germinated buckwheat extract decreases blood pressure and nitrotyrosine immunoreactivity in aortic endothelial cells in spontaneously hypertensive rats,” Phytotherapy Research: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives, vol. 23, no. 7, pp. 993–998, 2009.

32 M. Ghiasi, A. Azadnia, M. Arabieh, and M. Zahedi, “Protective effect of rutin (vitamin p) against heme oxidation: A quantum mechanical approach,” Comput Theor Chem, vol. 996, pp. 28–36, Sep. 2012, doi: 10.1016/J.COMPTC.2012.07.009.