University of Benghazi
Faculty of Education Almarj

جامعة بنغازي
كلية التربية – المرج
ISSN 2518-5845

Global Libyan Journal

المجلة الليبية العالمية

العدد السبعون / ديسمبر / 2023

Cardioprotective Effects of Rutin and Mesenchymal Stem Cells on Acetaminophen Induced Cardiac Enzymatic Damage in Rats

Dr. Rabihah E. A. Elduob , Dr. Eda M. A. Alshailabi , Dr. Samia M. Efkeren

Zoology Department, Omar Al-Mukhtar University, El Beida, Libya.

eda.muftah@omu.edu.ly

University of Benghazi
Faculty of Education Almarj

جامعة بنغازي
كلية التربية – المرج
ISSN 2518-5845

Global Libyan Journal

المجلة الليبية العالمية

العدد السبعون / ديسمبر / 2023

Cardioprotective Effects of Rutin and Mesenchymal Stem Cells on Acetaminophen Induced Cardiac Enzymatic Damage in Rats

Abstract:

The aims of study to determine the cardioprotective effects of rutin and mesenchymal stem cells on acetaminophen induced cardiac enzymatic damage in rats. seventy male rats were divided into two studies. One: twenty young male rats were used as a source of bone marrow-derived MSCs. Two: fifty adult male rats were divided into five groups: G1: control group; G2: rats were given of PARA (750 mg/kg b.w.) every 72h orally for 21 days, then left for 30 and 60 days without any treatment; G3: rats were given of PARA then, administrated of rutin (25mg/kg b.w/d) for 30 and 60 days; G4: rats were given of PARA then, injected by BM-MSCs (1.5x 106 cells in 0.5 PBS) in the tail vein for 30 and 60 days, and G5: rats were given of PARA then, injected by BM-MSCs in the tail vein, and then administrated of rutin orally for 30 and 60 days. Treated by the PARA showed, a significant increased the CK-MB and LDH levels when compared with normal rats at 30 and 60 days. While, the PARA+MSCs+RTN group showed, a significant decrease in the CK-MB and LDH levels as compared with other groups rats at 30 and 60 days. Rats treated with both MSCs and RTN after treated by PARA showed, a markedly improved in cardiac enzymes activity.

Keywords: rutin; heart; stem cells; acetaminophen; rats.

التأثيرات الوقائية القلبية للروتين والخلايا الجذعية المتوسطة على الأسيتامينوفين المحدث للضرر الأنزيمي القلبي في الجرذان

د. رابحة عيسى الدعب ، د. عيدة مفتاح الشيلابي ، د. سامية محمد إفكيرين.

المستخلص:

تهدف الدراسة إلى تحديد التأثيرات الوقائية للروتين والخلايا الجذعية المتوسطة على الضرر الأنزيمي القلبي الناجم عن الأسيتامينوفين في الجرذان. تم تقسيم سبعين من 1كور الجرذان إلى دراستين. الأولى: تم فيها استخدام عشرين من ذكور الجرذان كمصدر للخلايا الجذعية المتوسطية المشتقة من نخاع العظم. الثانية: تم غيها تقسيم خمسين من ذكور الجرذان البالغين إلى خمسة مجاميع: المجموعة الأولى: المجموعة الضابطة، المجموعة الثانية: أعطيت الجرذان الأسيتامينوفين (750 ملغم/كغم من وزن الجسم) كل 72 ساعة عن طريق الفم لمدة 21 يومًا، ثم تركت لمدة 30 و60 يومًا دون أي معاملة، المجموعة الثالثة: تم إعطاء الجرذان الأسيتامينوفين، ثم تم إعطاؤها الروتين (25 ملغم/كغم من وزن الجسم/اليوم) لمدة 30 و60 يومًا، المجموعة الرابعة: تم إعطاء الجرذان الأسيتامينوفين

بعد ذلك، تم حقنها بواسطة الخلايا الجذعية المتوسطية (1.5 × 106 خلية في 0.5 محلول فوسفات البوتاسيوم) في الوريد الذيلي لمدة 30 و60 يومًا، والمجموعة الخامسة: تم إعطاء الجرذان الأسيتامينوفين بعد ذلك، تم حقنها بواسطة الخلايا الجذعية المتوسطية في الوريد الذيلي، ثم أعطيت الروتين عن طريق الفم لمدة 30 و60 يومًا. أظهرت المعاملة بالأسيتامينوفين زيادة كبيرة في مستويات الكرياتين كيناز القلبي واللاكتات ديهيدروجينيز بالمقارنة مع الجرذان الطبيعية لمدة 30 و60 يومًا. بينما أظهرت مجموعة المعاملات الثلاثة للمجموعة الخامسة انخفاضًا ملحوظًا في مستويات الانزيمات مقارنة بالجرذان في المجموعات الأخرى عند 30 و60 يومًا. أظهرت الجرذان التي عولجت بكل من الخلايا الجذعية المتوسطية والروتين معا بعد معاملتها بالأسيتامينوفين، تحسنًا ملحوظًا في نشاط إنزيمات القلب.

الكلمات المفتاحية: روتين؛ قلب؛ الخلايا الجذعية؛ أسِيتامينُوفين؛ الجرذان.

Introduction:

The heart, as the pump organ, plays a key role in the pathophysiology of septic shock [1]. Acetaminophen (N-acetyl-para-amino-phenol) (PARA) is sparingly soluble in water, freely soluble in alcohol, very slightly soluble in ether and in methylene chloride [2]. However, it is a commonly used analgesic and antipyretic drug and is safe at therapeutic levels, but overdose can lead to potentially damage of organs [3]. Moreover, at overdose APAP is metabolized in the liver by cytochrome P450 (CYP) into reactive metabolite N-acetyl-p-benzoquinone imine (NAPQI) [4, 5]. NAPQI is normally rapidly conjugated with reduced glutathione (GSH) and is excreted eventually as the cysteinyl conjugate or the corresponding mercapturic acid [6]. Furthermore, as long as the rate of formation of this toxin is not greater than the maximal rate of synthesis of GSH there will be no damage to the cell or organ [2, 7]. When more NAPQI if formed than can be conjugated to GSH, the unbound NAPQI becomes toxic by binding to macromolecules, including cellular proteins [8, 2]. The production of various free radicals and successive oxidative stress leads to adverse effect on cellular level of an organ [3].

The citrus flavonoid, rutin is made up of disaccharide flavonol and rutinose quercetin with potent pharmacological capabilities and strong antioxidant including, antiviral, anti-angiogenic, immunomodulatory, antidiarrheal, anti-mutagenic, and anti-inflammatory attributes [9, 10]. Rutin are the flavonoids most abundantly found in foods [11]. Rutin (RTN) also called as quercetin-3-Orutinoside, vitamin P, rutoside, rutinum, and sophorin [12]. Moreover, RTN as a bioflavonoid compound (a glycoside derivative of quercetin) [13]. RTN has a wide range of beneficial health properties in multiple organs owing to its various pharmacological effects. Also, it can reduce the level of oxidative stress via reactive oxygen species (ROS)-scavenging mechanisms and protect neurons against severe oxidative stress and attack by ROS [1]. Besides, RTN has been shown in many studies to be able to reduce cardiac hypertrophy and then cardiac remodeling [12]. Furthermore, the RTN has an antioxidant effect, inhibits pro-inflammatory cytokine productions and maintains vascular barrier integrity [14].

Mesenchymal stem cells (MSCs) are the most widely used stem cells due to their easy accessibility in tissues, such as bone marrow aspirate and fat tissue [15]. Moreover, MSCs are pluripotent adult stem cells residing within the bone marrow microenvironment [16]. In

contrast to their hematopoietic counterparts, MSCs have an adherent nature and are expandable in culture [17]. On the other hand, MSCs directly injected into the infarcted heart caused to induce myocardial regeneration and improve cardiac function, which prevents apoptosis of native cardiomyocytes, and regeneration of lost cardiomyocytes [16]. These bone marrow-derived MSCs were later shown to be able to self-renew, form colonies and differentiate into a multitude of mesodermal cell types in vitro [18].

Thus, the purpose of this study was to investigate the cardioprotective effects of rutin and mesenchymal stem cells on acetaminophen induced cardiac enzymatic damage in rats.

Materials and Methods:

Chemicals:

- Acetaminophen (PARA) ($C_8H_9NO_2$) was used. It was purchased from Sigma chemical Company (USA) [19, 20], and was given to rats orally by gavage.
- Rutin (RTN) ($C_{27}H_{30}O_{16}$) the natural antioxidant was purchased from Sigma chemical Company (USA) [21], and was given to rats orally by gavage.
- Bone marrow derived stem cells, one important source of mesenchymal stem cells (MSCs) [22], have been isolated and cultured in Medical Research Center, Aleibbasiuh, Ain shams University. Rats were injected by BM-MSCs in the tail vein.

Experimental animals and procedures:

70 male albino rats (*Rattus norvegicus*) were divided into two studies:

- Study one: 20 young male albino rats' weight 100 g were used as a source of bone marrow-derived MSCs.
- Study two:50 adult male albino rats' weight of (150-160g) were divided into five groups. Rats were obtained from Animal House of El-Salam Farm, Giza-Cairo, Egypt and were acclimatized to the laboratory conditions for 14 days prior to the start of the experiment. They were housed in metabolic cages at temperature of 24-27 °C (12 hours dark/ light cycle), and received standard food and water ad-libitum with fresh daily supplies. The experimental procedures complied with guidelines of the Committee on Care and use of Experimental Animal Resources, Ain Shams University, Cairo, Egypt.

Examination of Bone Marrow Mesenchymal Stem Cells (MSCs):

Young male rats (n = 20) weight 100 g were used as a source of bone marrow-derived MSCs [16]. The rats were injected by BM-MSCs (1.5x 106 cells in 0.5 PBS) [23] in the tail vein [24]. The cultured BM-MSCs were characterized by using NAVIOS flow cytometer by BECKMAN COULTER in Medical Research Center of Ain Shams University [25].

Experimental Design:

Healthy adult male rats (n = 50) were randomized into five groups (10 rats in each):

Group I- Normal control group (NC), animals were left as normal control rats, and given food and water ad lib.

Group II- Acetaminophen (PARA), rats were orally given of PARP at a dose of (750 mg/kg b.w.) every 72h for 21 days, then left for 30 and 60 days without any treatment.

Group III: Acetaminophen + rutin group (PARA+ RTN), rats were given PARA (750 mg/kg b.w.) every 72h orally for 21 days. Then, administrated of RTN orally at a dose of (25mg/kg b.w/d) for 30 and 60 days.

Group IV: Acetaminophen + mesenchymal stem cells group (PARA+MSCs), rats were received of PARA (750 mg/kg b. w.) every 72h for 21 days. Then, injected by BM-MSCs (1.5x 106 cells in 0.5 PBS) in the tail vein for 30 and 60 days.

Group V: Acetaminophen + mesenchymal stem cells + rutin group (PARA+ MSCs+RTN), rats were received of PARA (750 mg/kg b. w.) every 72h for 21 days. Then, injected by BM-MSCs (1.5x 106 cells in 0.5 PBS) in the tail vein, and orally treated by RTN at a dose of (25mg/kg b.w/d) for 30 and 60 days.

Biochemical analysis of serum:

At the end of the experimental period, the rats were overnight fasted after the last dose and blood samples were collected. Blood samples were taken into clean and dry screw capped centrifuge tubes then centrifuged at 3000rpm for 15 minutes in order to separate clear serum samples. They were then stored at -20ºC until used for determination of different biochemical parameters such as lactate dehydrogenase (LDH) and creatine Kinase-myocardial (CK-MB) band of the control group and the experimental groups were performed by the methods of [26].

Statistics analysis:

All the data are presented as (mean ± SEM). The parameters were analyzed using significance by one way ANOVA. Statistical significance of the differences between the treatment's groups were using the Tukey's test at $P < 0.05$ by using (Minitab version 17).

Results: -

Preparation of the creatine kinase-myocardial band (CK-MB):

The mean values of CK-MB level of control and experimental animals were presented in Table (1), it is showed a significant increase ($P < 0.05$) in the mean value of the CK-MB levels in the PARA groups (13.400±0.513 & 28.160±0.803) as compared to the NC groups (1.900±0.114 & 1.90±0.110) after 30 and 60 days. Whereas, there were the PARA+RTN groups showed a significant decrease ($P < 0.05$) in the mean value of the CK-MB levels (8.100±0.433 & 17.460±0.964) after 30 and 60 days when compared to PARA groups. Also, the mean values of the CK-MB levels after 30 and 60 days showed a significant decrease in treated group with the PARA+MSCs groups (5.880±0.116 & 12.520±0.213) when compared with the PARA groups. Moreover, the treated groups by PARA+MSCs+RTN (3.820±0.150 & 5.180±1.616) in the mean value of the CK-MB levels after 30 and 60 days showed a lower significance when compared with PARA, PARA+RTN, and PARA+MSCs groups.

Preparation of the lactate dehydrogenase (LDH):

The mean values of the LDH level of control and experimental rats were presented in Table (1). The data showed a significant elevation ($P < 0.05$) occurred in the mean value of LDH levels after 30 and 60 days in the PARA groups (186.00±4.022 & 345.4±16.41) as compared to the NC groups (28.80±1.466 & 29.0±1.50). While, there was a significant decline ($P < 0.05$) in the PARA+RTN groups (151.80± 3.286 & 189.8±11.97) and PARA+MSCs groups (118.2±10.04 & 147.06±3.973) as compared to the PARA groups in the mean value of LDH levels after 30 and 60 days. In addition, the mean values of the LDH levels after 30 and 60 days showed a lower significance ($P < 0.05$) in the PARA+MSCs+RTN groups (71.40±3.394 &95.60±2.605) when compared with PARA, PARA+RTN, and PARA+MSCs group.

Table 1: Average of mean values of CK-MB and LDH levels in control and experimental groups**.**

Duration	Parameter	NC	PARA	PCM + RTN	PARA + MSCs	PARA+ MSCs+ RTN
30 days	CK-MB (U/L)	1.900± 0.114^{E}	13.400± 0.513^{A}	8.100± 0.433^{B}	5.880± 0.116^{C}	3.820± 0.150^{D}
	LDH (U/L)	28.80± 1.466^{E}	186.00± 4.022^{A}	151.80± 3.286^{B}	118.2± 10.04^{C}	71.40± 3.394^{D}
days 60	CK-MB (U/L)	1.90± 0.110^{E}	28.160± 0.803^{A}	17.460± 0.964^{B}	12.520± 0.213^{C}	5.180± 1.616^{D}
	LDH (U/L)	29.0± 1.50^{E}	345.4± 16.41^{A}	189.8± 11.97^{B}	147.06± 3.973^{C}	95.60± 2.605^{D}

Data are expressed as mean ± SE of rat within each row, means with different superscript (A, B, C, D& E) were significantly different at $P < 0.05$, were means superscripts with the same letters mean that there is no significant difference ($P < 0.05$).

*NC= Normal control, PARA= Acetaminophen treated group, PARA+RTN= Acetaminophen with rutin group, PARA + MSCs =Acetaminophen with mesenchymal stem cells group, and PARA+MSCs+RTN = Acetaminophen with mesenchymal stem cells and rutin group.

Discussion:

PARA passes through many organs through the circulatory system and causes toxic effects by reacting with DNA and various enzymes [27]. The results in this work showed that PARA caused a highly significant increase ($P < 0.05$) found in CK-MB and LDH levels in treated rats that given PARA at a dose of (750 mg/kg b.w.) every 72h for 21 days and left without any treatment for 30 and 60 days as compared to NC group. Similar findings were achieved by [2, 28, 29, 27]. The increase in cystolic LDH activity by PARA may be because of the intracellular collection of Ca^{2+}, which results in initiation of phosphofructo-kinase and anaerobic glycolysis prompting lactate formation [29]. Besides, [30] suggested that the increased of the LDH activity may be due to a cellular damage and leaked of the enzyme from the tissues into the blood. In addition, increased significantly in the CK-MB and LDH levels markers of cardiac damage for the damaged heart tissue [27]. Also, [31] said the serum LDH considered as a prognostic marker for the investigation of tissues injury.

On the other hand, the mean values of the LDH and CK-MB levels after 30 and 60 days showed, a lower significance ($P < 0.05$) in the PARA+MSCs+RTN groups when compared with PARA, PARA+RTN, and PARA+MSCs group. These decreases in the enzyme levels

may be due to RTN is able to balance autophagic response to enhance cardiac cell survival which plays an essential role in cardiac remodeling process [32, 12]. Moreover, [33], [1] suggested that RTN has possesses anti-inflammatory properties. Additionally, [12] found that the treatment with RTN improves cardiac functions. Also, they reported on the cardioprotective mechanisms of RTN, particularly in cardiac hypertrophy. On the other hand, treated with RTN caused inhibiting the apoptosis of cardiomyocytes and improving cardiac function and related to the restoration of the structure and function of myocardial mitochondria [1], reduce cardiomyocyte damage [34], and it may be protecting cardiac tissue from oxidative damage due to the anti-inflammatory effect associated with the inhibition of interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) [14].

The significant decrease of serum CK-MB, and LDH after MSCs injection suggested regression of damage, improvement of heart function and proliferative and anti-apoptotic properties [35]. Broekman et al. [36] said that the MSCs is beneficial for the immune response and increases healing of epithelial cells. Moreover, [37] found a significant decrease in the levels of heart enzymes due to immunomodulatory properties of the MB-MSCs. The MSCs caused improvement in myocardial performance by differentiation into cardiomyocytes and restoring damaged cardiac tissue [16, 38], and it improved cardiac function after acute myocardial infarction through enhancement of angiogenesis and myogenesis [16]. In addition, [39] suggesting an anti-inflammatory effect of MSCs because treated of MSCs improved inflammatory changes and cardiac function in rats with acute myocarditis.

Conclusion:

In the present study, animals treated with both MSCs and RTN after treated by PARA showed, a markedly improved in cardiac enzymes activity by the role of MSCs and rutin in the regeneration and the remodeling of damaged myocardium.

References:

1. Meng, X.L.; Yu, M. M.; Liu, Y. C.; Gao, Y.; Chen, X. S.; Shou, S. T.; Chai, Y. F.2022. Rutin inhibits cardiac apoptosis and prevents sepsisinduced cardiomyopathy. *Frontiers in physiology.* 13:1-10. DOI: 10.3389/fphys.2022.834077.
2. Farghaly, H. S.; Hussein, M. A. 2010. Protective effect of curcumin against paracetamol-induced liver damage. *Australian Journal of Basic and Applied Sciences*. 4(9): 4266-4274.
3. Bharali, M. K.; Konya, H.; Chetry, L. B. 2014. Protective effect of *Oroxylum indicum* on acetaminophen induced liver injury in rat. *International Current Pharmaceutical Journal*. 3(2): 223-227.
4. James, L. P.; Mayeux, P. R.; Hinson, J. A. 2003. Acetaminophen-induced hepatotoxicity. *Drug Metabolism and Disposition*. 31: 1499–1506. DOI: 10.1124/dmd.31.12.1499.
5. Su, G. L.; Gong, K. Q.; Fan, M. F.; Kelley, W, M.; Hsieh, J.; Sun, J. M.; Hemmila, M. R.; Saman Arbabi, S.; Remick, D. G.; C. Wang, S. C. 2005. Lipopolysaccharide-binding protein modulates acetaminophen-induced liver injury in mice. *Hepatology.* 41(1): 187-195. DOI: 10.1002/hep.20533.
6. Kanchana, N.; Sadiq, A. M. 2011. Hepatoprotective effect of *Plumbago zeylanica* on paracetamol induced liver toxicity in rats. *International Journal of Pharmacy and Pharmaceutical Sciences*. 3(1):151-154.
7. García, N. P.; Lores, O. F.; Fuentes, D. P.; Martínez, D. T.; Hernandez, J. P.; Rivas, L. P.; Martin, O. P.; Batista-Duharte, A. 2014. Reduction of hepatotoxicity induced by acetaminophen overdoses in a mouse model of inflammation induced by freund´s adjuvants. *Journal of Allergy and Therapy.*5: 183. DOI:10.4172/2155-6121.1000183.
8. Cheung, C.; Yu, A. M.; Ward, J. M.; Krausz, K. W.; Akiyama, T. E., Feigenbaum, L.; Gonzalez, F. J. 2005. The cyp2e1-humanized transgenic mouse: role of cyp2e1 in acetaminophen hepatotoxicity. *Drug Metabolism and Disposition*. 33(3):449-57. DOI: 10.1124/dmd.104.002402.
9. Abarikwu, S. O.; Njoku, R. C.; Lawrence, C. J.; Charles, I. A.; Ikewuchi, J. C. 2017. Rutin ameliorates oxidative stress and preserves hepatic and renal functions following

exposure to cadmium and ethanol. *Pharmaceutical Biology*. 55(1):2161–2169. DOI: 10.1080/13880209.2017.1387575.

10. Abarikwu, S. O.; Njoku, R. C.; John, I. G.; Amadi, B. A.; Mgbudom-Okah, C. J.; Onuah, C. L. 2022. Antioxidant and anti-inflammatory protective effects of rutin and kolaviron against busulfan-induced testicular injuries in rats. *Systems Biology in Reproductive Medicine.* 68 (2): 151-161. DOI: 10.1080/19396368.2021.1989727.
11. Erdogan, E.; Ilgaz, Y.; Gurgor, P. N.; Oztas, Y.; Topa,T.; Oztas, E. 2015. Rutin ameliorates methotrexate induced hepatic injury in rats. *Acta Cirúrgica Brasileira*. 30 (11): DOI:10.1590/S0102-865020150110000009.
12. Siti, H. N.; Jalilb, J.; Asmadid, A. Y.; Kamisah, Y. 2020. Roles of rutin in cardiac remodeling. *Journal of Functional Foods*. 64: 1-12. DOI: 10.1016/j.jff.2019.103606.
13. Lopez-Revuelta, A.; Sanchez-Gallego, J. I.; Hernandez-Hernandez, A.; Sanchez-Yague, J. L.; lanillo, M. 2006. Membrane cholesterol contents influence the protective effects of quercetin and rutin in erythrocytes damaged by oxidative stress. *Chemico-Biological Interactions.* 161(1):79- 91. DOI: 10.1016/j.cbi.2006.03.004.
14. Coskun, R.; Celik, A. I.; Suleyman, Z.; Cimen, F. K.; Cankaya, M. 2019.The preventive effects of rutin on immobilization stress-induced cardiac damage. *Acta Poloniae Pharmaceutica Drug Research*. 76 (6): 1079-DOI: 10.32383/appdr/112366.
15. Lee, R. H.; Kim, B.; Choi, I.; Suh, K.; Bae, Y. C.; Jung, J. S. 2004. Characterization and expression analysis of mesenchymal stem cells from human bone marrow and adipose tissue. *Cellular Cell Physiology and Biochemistry.* 14 (4-6): 311–324. https://doi.org/10.1159/000080341.
16. Nagaya, N.; Fujii, T.; Iwase, T.; Ohgushi, H.; Itoh, T.; Uematsu, M.; Yamagishi, M.; Mori, H.; Kangawa, K.; Kitamura, S. 2004. Intravenous administration of mesenchymal stem cells improves cardiac function in rats with acute myocardial infarction through angiogenesis and myogenesis. *American Journal of Physiology-Heart and Circulatory Physiology.* 287: 2670 –2676. DOI:10.1152/ajpheart.01071.2003.
17. Reyes, M.; Dudek, A.; Jahagirdar, B.; Koodie, L.; Marker, P. H.; Ver-faillie, C. M. 2002. Origin of endothelial progenitors in human postnatal bone marrow. *Journal of Clinical Investigation.* 109(3):337-46. DOI: 10.1172/JCI14327.

18. Sheng, G. 2015. The developmental basis of mesenchymal stem/stromal cells (MSCs). *BMC Developmental Biology.* 15 (44): 1-8. DOI 10.1186/s12861-015-0094-5.
19. Anbarasu, C.; Rajkapoor, B.; Kalpana, J. 2011. Protective effect of *Pisonia aculeata* on paracetamol induced hepatotoxicity in rats. *Journal of Experimental and Integrative Medicine* 1(3):167- 172. DOI:10.5455/jeim. 040511.or.008.
20. Alshailabi, E. M. A.; Abdalally, O. A.; Majeed, S. F. 2021. Histopathological study on the protective effect of vitamin C against paracetamol-induced acute hepatic damage in rat. *Global Libyan Journal.* 53: 1-15.
21. Shenbagam, M.; Nalini, N. 2011. Dose response effect of rutin a dietary antioxidant on alcohol-induced prooxidant and antioxidant imbalance-a histopathologic study. *Fundamental and Clinical Pharmacology.* 25: 493–502. DOI: 10.1111/j.1472-8206.2010.00861.x.
22. El-Nahrawy, A. M.; Abu Zahra, F. A.; EL-Dab, R. E. A. 2017. Ameliorative role of bone marrow mesenchymal stem cells and rutin against acetaminophen induced hepatotoxicity in adult male rats. *International Journal of Advanced Research.* 5(9): 450-459. https://doi.org/10.21474/IJAR01/5349.
23. Oskouei, B. N.; Lamirault, A. G.; Joseph, B. C.; Treuer, A. A. V.; Landa, A. S.; Da Silva, A. J.; Hatzistergos, A. K.; Dauer, M.; Balkan, A. W.; Mcniece, A. I.; Harea, A. J. M. 2012. Increased potency of cardiac stem cells compared with bone marrow mesenchymal stem cells in cardiac repair. *Stem Cells Translational Medicine*.1(2): 116–124, DOI: 10.5966/sctm.2011-0015.
24. Jiang, W.; Ma, A.; Wang, T.; Han, K.; Liu, Y.; Zhang, Y.; Zhao, X.; Dong, A.; Du, Y.; Huang, X.; Wang, J.; Lei, X.; Zheng, X. 2006. Intravenous transplantation of mesenchymal stem cell improves cardiac performance after acute myocardial ischemia in female rats. *Translation and Interpreting*. 19(7): 570–580. https://doi.org/10.1111/j.1432-2277.2006.00307.x
25. Krishan, A.; Krishnamurthy, H.; Totey, S. 2011. *Applications of flow cytometry in stem cell research and tissue regeneration.* Science. 183.

26. Vennila, L.; Pugalendi, K. V. 2010. Protective effect of sesamol against myocardial infarction caused by isoproterenol in Wistar rats. *Redox Report.* 15: 36-42. DOI: 10.1179/174329210X12650506623168.
27. Karakuyu, N. F.; Aşcı, H.; Savran, M.; Gülle, K. 2023. Protective role of selenium on the heart of rats exposed acrylamide. *Medical journal of Suleyman Demirel University Suleyman Demirel University.* 30(1): 9-17. DOI: 10.17343/sdutfd.1159213.
28. Lebda, M. A.; Taha, N. M.; Korshom, M. A.; Mandour, A. E. A. 2013. Ginger (*Zingiber officinale*) potentiate paracetamol induced chronic hepatotoxicity in Rats. *Journal of Medicinal Plants Research.* 7 (42):3164–3170. DOI:10.5897/JMPR2013.5252.
29. Abd El Fadil, H.; Edress, N.; Khorshid, N.; Amin, N. 2019. Protective impact of curcumin against paracetamol-induced hepatotoxicity in rats. *International Journal of Pharmaceutical Research and Allied Sciences*. 8(1):84-94.
30. Taha, M. E.; Kamal, A. M.; Ibrahim, D. R. 2020. Possible protective effect of olive leaves extract on paracetamol induced hepatotoxicity in male albino rats. *Bioscience journal Uberlândia*. 36(1): 245-255. DOI:10.14393/BJ-v36n1a2020-49960.
31. Adil, M.; Kandhare, A. D.; Ghosh, P.; Venkata, S.; Raygude, K. S.; Bodhankar, S. L. 2016. Ameliorative effect of naringin in acetaminophen-induced hepatic and renal toxicity in laboratory rats: role of FXR and KIM-1. *Renal Failure.* 38(6): 1007-1020. DOI: 10.3109/0886022X.2016.1163998.
32. Khoynezhad, A.; Jalali, Z.; Tortolani, A. J. 2007. A synopsis of research in cardiac apoptosis and its application to congestive heart failure. *Texas Heart Institute Journal*. 34(3): 352–359.
33. Enogieru, A. B.; Haylett, W.; Hiss, D. C.; Bardien, S.; Ekpo, O. E. 2018. Rutin as a potent antioxidant: Implications for neurodegenerative disorders. *Oxidative Medicine and Cellular Longevity*.2018: 1-17. DOI:10.1155/2018/6241017.
34. Elma, B.; Mammadov, R.; Süleyman, H.; Gündoğdu, B.; Yurt, Ş.; Bilgin, Y.; Çoban, A. 2022. The effect of rutin on experimentally induced acute heart contusion in rats: Biochemical and histopathological evaluation. *Ulusal Travma ve Acil Cerrahi Dernegi*. 28 (8):1073-1081. DOI: 10.14744/tjtes.2021.97760.

35. Soliman, N. A.; Abd-Allah, S. H.; Hussein, S.; Eldeen, M A. 2017. Factors enhancing the migration and the homing of mesenchymal stem cells in experimentally induced cardiotoxicity in rats. *International Union of Biochemistry and Molecular Biology life*. 69(3):162-169. DOI 10.1002/iub.1600.
36. Broekman, W.; Amatngalim, G. D.; de Mooij-Eijk, Y.; Oostendorp, J.; Roelofs, H.; Taube, C.; Stolk, J. Hiemstra, P. S. 2016. TNF-α and IL-1β-activated human mesenchymal stromal cells increase airway epithelial wound healing in vitro via activation of the epidermal growth factor receptor. *Respiratory Research*.17:3 DOI 10.1186/s12931-015-0316-1.
37. Gaafar, T.; Attia, W.; Mahmoud, S.; Sabry, D.; Abdel Aziz, O.; Rasheed, D.; Hamza, H. 2017. Cardioprotective effects of wharton jelly derived mesenchymal stem cell transplantation in a rodent model of myocardial injury. *International Journal of Stem Cells*. 10 (1): 48- 59. DOI:10.15283/ijsc16063.
38. Pulavendran, S.; Thiyagarajan, G.; Ramakrishnan, V. 2010. Antioxidant and anti-inflammatory action of stem cells in cardiac disease. *Journal of Advanced Laboratory Research in Biology*.1(2): 1-8. October 2010
39. Ohnishi, S.; Nagaya, N. 2007. Prepare cells to repair the heart: mesenchymal stem cells for the treatment of heart failure. *American Journal of Nephrology*. 27:301–307 DOI: 10.1159/000102000.