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Original Paper

Investigation of PPARγ Involvement in Crocin Effects on Apoptosis

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Background: Apoptosis is an important form of cell death, which is involved in cardiovascular disorders. We investigated the effects of combined crocin and PPARγ antagonist on the expression of regulatory genes involved in apoptosis. Also, we explored whether PPARγ is involved in the protective effect of crocin on apoptosis after myocardial ischemia.

Methods: The experimental rats were divided into five groups: control group, Isoprenaline (ISO) group, GW9662 as PPARγ antagonist+ISO, crocin+ISO group, and GW9662+crocin+ISO group. Isoprenaline was injected to induce myocardial ischemia.

Results: Findings showed that ISO and GW9662 increased *Bax* and caspase-3 gene expression and cardiac Creatine Phosphokinase (CPK) marker but decreased Bcl2. Crocin alleviated the expression of *Bax* and caspase-3 while increased Bcl2. It also reduced CPK levels in serum. However, GW9662 reversed these effects.

Conclusion: According to results, crocin may regulate apoptosis through PPARγ activation.

Keywords: Bax, Caspase-3, Creatine Phosphokinase (CPK), Crocin, Myocardial ischemia, GW9662

Introduction

Necrosis and apoptosis play an essential role in the process of tissue damage which leads to myocardial infarction (Krijnen et al., 2002). Apoptosis is recognized as a process that regulates and requires energy. It is known as a programmed cell death which does not promote inflammatory responses (Saraste & Pulkki 2000). As apoptosis is involved in myocardial infarction and cell damage, understanding this process has therapeutic implications.

Several studies reported that the apoptotic myocytes were abundant in the infarcted zone (Abbate et al., 2000; Olivetti et al., 1996). The regulatory proteins such as Bcl2 (B-cell lymphoma 2), Bax (Bcl2 associated x, apoptosis regulator), and caspase-3 are involved in apoptosis. Bcl2 is an inhibitor of apoptosis expressed in the infarcted area, while Bax is recognized as a pro-apoptotic protein that triggers caspase cascade events (Ortiz et al., 2003).

Isoproterenol (ISO) is an agonist of the β-adrenergic receptor that results in cell death and heart failure (Zaugg et al., 2000). ISO damages the myocardium by activating apoptotic genes and oxidative stress. Furthermore, ISO triggers cardiomyocytes' apoptosis through Adenosine Monophosphate Kinase (AMPK) inactivation and endoplasmic reticulum stress (Zhuo et al., 2013).

Antioxidants play some important roles in tissue and organ damages. Crocin is a natural carotenoid compound and water-soluble color pigment and one of the saffron ingredients that has antioxidant (Yaribeygi et al., 2018), anti-apoptotic (Razavi et al., 2016), and antitumor (Sun et al., 2013) effects. Crocin could improve diabetes and metabolic disorders (Abou-Hany et al., 2018; Algandaby, 2020). It exerts protection against lung injury and inflammation (Elsherbiny et al., 2016; Dianat et al., 2018).

GW9662 is known as a Peroxisome Proliferator-Activated Receptor-gamma (PPARγ) antagonist that is used in many research studies. PPARγ is a subtype of nuclear receptors that has several roles in metabolic disorders and also cardiac damages (Ahmadian et al., 2013; Byeon & Chung 2017). According to studies, PPARγ activation protects against apoptosis-induced oxidative stress by increasing anti-apoptotic gene and protein

expression in cardiomyocytes (Ren et al., 2009). Furthermore, the activation of PPARγ is linked to AMPK and Protein kinase B (AKT) signaling pathways and could inhibit apoptosis (Liu et al., 2016). In this study, we explore the effects of crocin on ISO-induced apoptosis through PPARγ.

Materials and Methods

Male Wistar rats weighing 200-230 g (3 months old) were used in this study. The animals were housed in an air-conditioned colony room at 23±2°C and under a 12-12 h light-dark cycle and a standard pellet diet and free access to water. The experiments were performed in accordance with the instructions defined by the committee for control and supervision of experiments on animals and approved by the Ethics Committee for Animal Experiments at Jundishapur University of Medical Sciences (IR.AJUMS.ABHC.REC.1397.005).

The rats were divided into five groups: the control group; the ISO group, which received isoprenaline (100 mg/kg) subcutaneously on two terminal days to induce infarction; the G+ISO group, which received GW9662 at a dose of 1 mg/kg intraperitoneally (Mahajan et al. 2017; Agrawal et al. 2014), and then ISO (100 mg/kg) subcutaneously to induce infarction; the C+ISO group, which received crocin (40 mg/kg) (Yaribeyg et al., 2018; Wang et al. 2018) via gavage and then ISO (100 mg/kg) subcutaneously; and the G+C+ISO group, which received crocin (40 mg/kg) and GW9662 (1 mg/kg) and then ISO (100 mg/kg). The rats were treated for 4 weeks.

The serum level of Creatine Phosphokinase (CPK) was measured using an appropriate commercial kit (Okinaka et al., 1961).

Quantitative Real-Time PCR for Apoptotic Genes Expression

The total RNA from the homogenized tissues was extracted using RNeasy Plus Mini Kit, and then complementary DNA was synthesized. A LightCycler PCR (Roche, Diagnostics) was used to assess the caspase-3, *Bax, Bcl2*, and housekeeping genes levels (Table 1). The gene-specific primers (Bioneer, Daejeon, South Korea) are shown in Table 1. The gene expression level was normalized against the *GAPDH* expression.

Statistical Analysis

All data were presented as means±SEM. The data analyzed using one-way ANOVA followed by the Tukey post hoc test. P values less than 0.05 were considered statistically significant.

Results

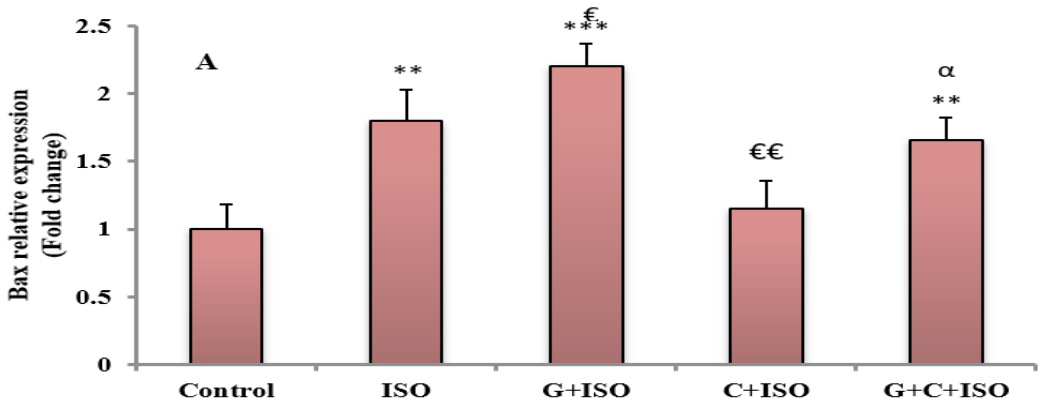
As shown in Figure 1A, the expression of *Bax* was significantly increased in both ISO and G+ISO groups as compared to control (P<0.01, P<0.001, respectively). However, the rising *Bax* expression was more in the G+ISO group. As compared to the ISO group, crocin administration in the C+ISO group significantly reduced *Bax* expression (P<0.01). But, GW9662 significantly reversed the crocin-induced decrease in expression of (P<0.05). As shown in Figure 1B, the caspase expression level was greatly enhanced in the ISO group compared to the control. Although the level of this gene was higher in the G+ISO group than the ISO group, there was no significant difference. Crocin treatment significantly ameliorated caspase-3 compared to the ISO group (P<0.01); however, GW9662 inhibited the crocin effect.

Moreover, *Bcl2* was significantly decreased in both ISO and G+ISO groups as compared to control (P<0.01, P<0.001, respectively) (Figure 1C). Although in the G+ISO group, the reduction was more. Crocin administration significantly augmented this gene expression level compared to the ISO (P<0.01); however, PPARγ antagonist significantly blocked crocin effects.

As shown in Figure 2, CPK was increased in serum of both ISO and G+ISO groups compared to the control. Nevertheless, crocin significantly reduced this marker (P<0.01) compared to the two mentioned groups and reached this factor approximately to the control level, but GW9662 showed the opposite effect of crocin.

Table 1. Sequences of primers

mRNA	Reverse Primer	Forward Primer
Bax	**TGAGGTTTATTGGCACCTCC**	**TTTTCCTGGGATGAATGGGG**
Bcl2	**ATCTCCAGTATCCCACTCGTA**	**TGGTACCTGCAGCTTCTTTC**
Caspase-3	**ACAAGCCCATTTCAGGGTA**	**GAGCTTGGAACGCGAAGA**
GAPDH	**CGGAGATGATGACCCTTTTG**	**TGCTGGTGCTGAGTATGTCGTG**



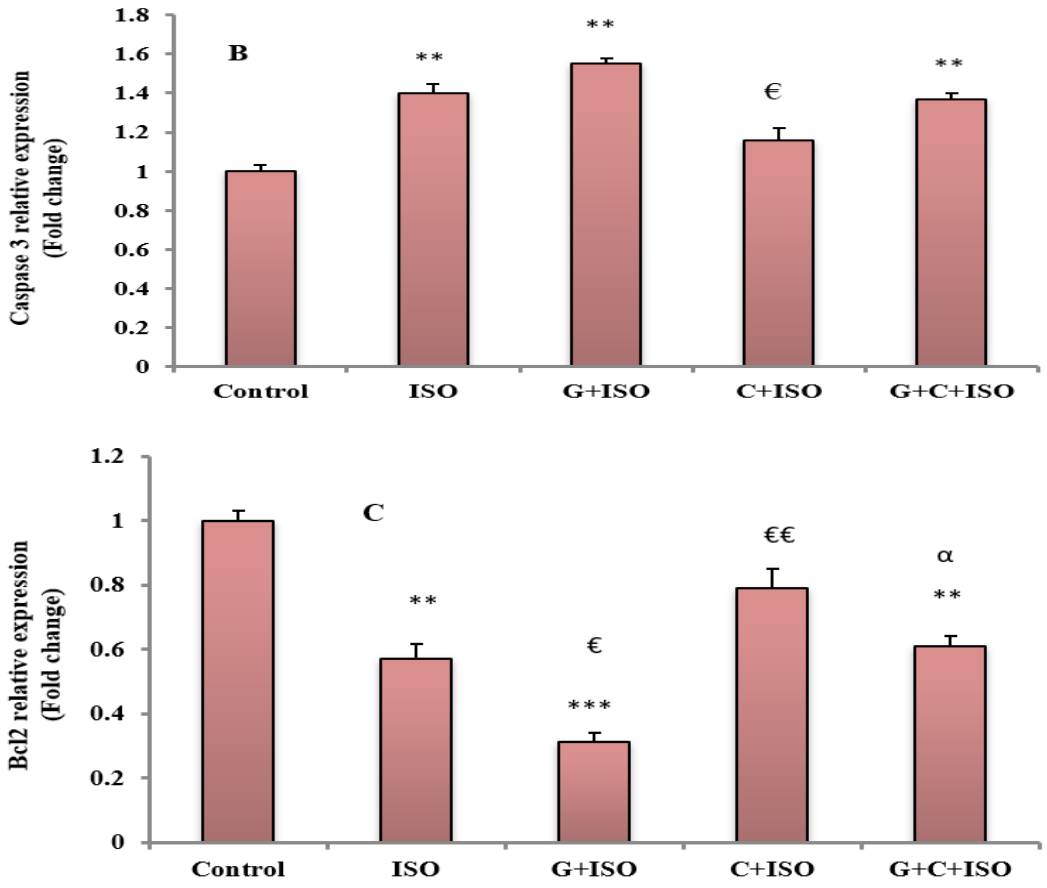


Figure 1: Effect of crocin and GW9662 on the Bax, caspase-3, and Bcl2 genes expression levels.
The data are expressed as the mean±SEM (n=8).
****P<0.01, ***P<0.001 vs. control; €P<0.05, €€P<0.01 vs. ISO; αP<0.05 vs. C+ISO using one-way ANOVA followed by the Tukey post hoc test.**

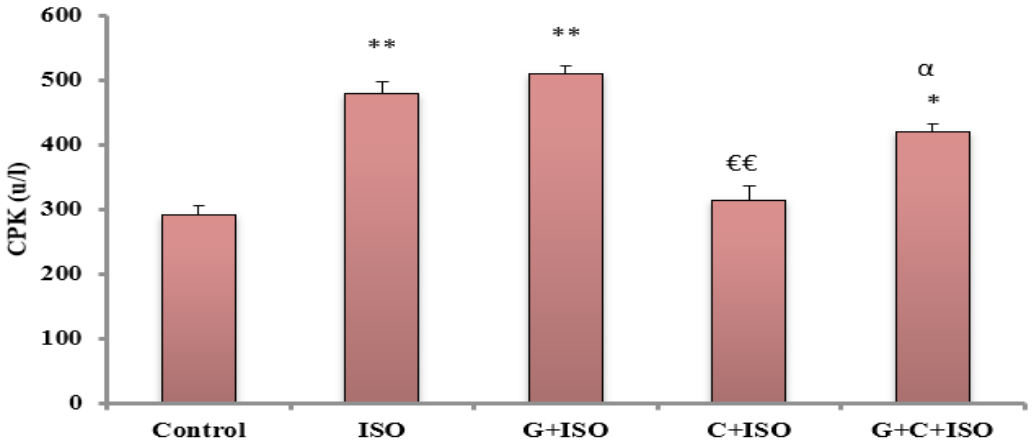


Figure 2: Effect of crocin and GW9662 on CPK content
*P<0.05, **P<0.01 vs. control; $^{€€}$P<0.01 vs. ISO; ^{α}P<0.05 vs. C+ISO using one-way ANOVA followed by the Tukey post hoc test.
The data are expressed as the mean±SEM (n=8).

Discussion

This study was performed to determine whether the PPARγ activation is involved in crocin's protective effects in apoptosis after myocardial infarction. The results support that crocin could enhance *Bcl2* and reduced *Bax* and caspase expression levels.

Apoptosis is known as programmed cell death, which involves a series of cellular regulatory proteins and signals transduction cascade (Fan et al., 2005). Persistent stimulation of β-adrenergic receptors results in mitochondrial dysfunction and oxidative stress that leads to apoptosis. Reactive Oxygen Species (ROS) play an essential role in apoptosis. Cellular redox change is also a part of the signal transduction pathway during apoptosis. Antioxidants can inhibit or delay apoptosis (Amin et al., 2011;

Feng et al., 2020). By preventing oxidant-mediated damage, antioxidants may play a protective role in apoptosis (Zeisel, 2004). They also suppress apoptosis by inhibiting AMPK signaling in cells (Meng et al., 2020).

Because several cellular proteins, including Bax, Bcl2, and caspase, are involved in apoptosis, we measured their expressions. Our study showed that ISO increased expression of Bax and caspase-3 as compared to control, and their expression levels were higher in the G+ISO group than ISO group; crocin treatment significantly reduced Bax and caspase-3 levels. However, in the G+C+ISO group, their expression levels increased compared to the C+ISO group. Bax and caspase 3 are pro-apoptotic proteins. Previous studies reported that Bax expression increased in myocardial infarction (Krijnen et al., 2002). It has been reported that β-adrenergic receptors trigger apoptosis in adult rat ventricular myocytes through ROS/ JNK (c-Jun N-terminal Kinases)-dependent activation of the mitochondrial death pathway (Remondino et al., 2003). Similar to our results, a previous study showed that crocin decreased Bax and caspase-3 in cardiotoxicity induced by diazinon (Razavi et al., 2016). It has also been demonstrated that crocin attenuated Bax/ Bcl2 ratio and caspase in diabetic cardiomyopathy caused by Streptozotocin (STZ) (Feidantsis et al., 2018). Contrary to our results, crocin stimulated apoptosis by enhancing the Bax and caspase activation in human gastric adenocarcinoma, which could be due to the anti-cancer properties of crocin (Hoshyar et al., 2013). Furthermore, the current study indicated that the expression of Bcl2 was decreased in both ISO and G+ISO groups, while crocin administration significantly enhanced this gene. *Bcl2* is recognized as an apoptotic inhibitor. According to our findings, a recent research reported that crocin potentiated *Bcl2* expression while ameliorated Bax and caspase expressions in cardiotoxicity induced by arsenic trioxide (Liang et al., 2020). Crocin has also been shown to improve retinal ischemia injury-induced apoptosis via PI3K (Phosphoinositide 3-Kinase)/AKT signaling pathway and it enhanced the Bcl2/ Bax ratio (Qi et al., 2013). PPARγ agonists have been shown to improve apoptosis. Rosiglitazone is an agonist of PPARγ that protects cardiac tissue against apoptosis by increasing Bcl2 and reducing caspase in vivo and in vitro studies. It has been demonstrated that cardiomyocytes expressing PPARγ exhibited 3 fold increase in Bcl2 (Ren et al., 2009; Zhang et al., 2020). Also, it has been reported that inactivation of PPARγ causes apoptosis and reduces antioxidants capacity in renal tubular cells (Wen et al. 2016). Also, PPARγ activation has been shown to increase Bcl2 and reduce Bax in a diabetic cardiomyopathy model (Rani et al., 2016). In addition, pioglitazone as PPARγ agonist prevented apoptosis in endothelial cells (Gensch et al., 2007). Therefore, based on several studies, PPARγ has as an anti-apoptotic factor.

Furthermore, CPK was measured to confirm myocardium damage. CPK is a cardiac injury marker that releases in serum during myocardium ischemia. Our results demonstrated that CPK increased in serum of rats in ISO and G+ISO groups while crocin improved this marker's level. Nevertheless, the PPARγ antagonist showed the opposite effect of crocin in the C+G+ISO group. Thus, according to our and other studies, crocin may exert protective effects through PPARγ activation. It has been shown that crocin decreased CPK in cardiotoxicity induced by patulin (Boussabbeh et al., 2015). Our previous study showed that crocin enhanced *PPARγ* gene expression level in diabetic rats (Badavi et al., 2020).

Conclusion

Based on the findings, crocin could improve apoptosis by increasing Bcl2 and alleviating Bax and caspase-3. As GW9662 reversed these effects of crocin, therefore, crocin may exert its effects by involving PPARγ.

Acknowledgments

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Ethical Permission

This study was approved by the Ethics Committee for Animal Experiments at Jundishapur University of Medical Sciences (IR.AJUMS.ABHC.REC.1397.005).

Conflict of Interests

The authors declared no conflict of interest.

References

1. Abbate A, Melfi R, Patti G, Baldi F, Ambrosio A.D, et al (2000). Apoptosis in recent myocardial infarction. Clin Ter , 151: 247, https://doi.org/10.1186/s12967-017-1191-y.
2. Abou-Hany H.O, Atef H, Said E, Elkashef H.A and Salem HA (2018). Crocin mediated amelioration of oxidative burden and inflammatory cascade suppresses diabetic nephropathy progression in diabetic rats. Chem Biol Interact, 284: 90-100, DOI: 10.1016/j.cbi.2018.02.001.
3. Agrawal Y. O, Sharma P.K, Shrivastava B, Ojha S, Upadhya H.M, et al (2014). Hesperidin produces cardioprotective activity via PPAR-gamma pathway in ischemic heart disease model in diabetic rats. PloS one, 9: e111212, DOI: 10.1371/ journal.pone.0111212
4. Ahmadian M, Myoung Suh J, Hah N, Liddle C, Atkins A.R, et al (2013). PPAR[gamma] signaling and metabolism: the good, the bad and the future. Nat Med, 99: 557-66, DOI: 10.1038/nm.3159.
5. Algandaby Mardi M (2020). Crocin prevents metabolic syndrome in rats via enhancing PPAR-gamma and AMPK. Saudi J Biol Sci, https://doi.org/10.1016/j.sjbs.2020.01.004.
6. Amin P, Singh M and Singh K (2011). Adrenergic Receptor-Stimulated Cardiac Myocyte Apoptosis: Role of 1 Integrins. J. Signal Transduct, doi:10.1155/2011/179057, https://doi.org/10.1155/2011/179057.
7. Badavi M, Mard S.A, Dianat M and Dashtbozorgi N (2020). Crocin attenuates oxidative stress and inflammation in myocardial infarction induced by isoprenaline via PPARγ activation in diabetic rats. J. Diabetes Metab. Disord, https://doi.org/10.1007/s40200-020-00686-y.
8. Boussabbeh M, Salem I.B, Neffati F, Najjar M.F, Bacha, H, et al (2015). Crocin prevents patulin-induced acute toxicity in cardiac tissues via the regulation of oxidative damage and apoptosis. J Biochem Mol Toxicol, 29: 479-88, https://doi.org/10.1002/jbt.21718.
9. Byeon S.H and Chung H (2017). Deep Retinal Capillary Nonperfusion Is Associated With Photoreceptor Disruption in

Diabetic Macular Ischemia?. Am J Ophthalmol, 174: 179-80, DOI: 10.1016/j.ajo.2016.05.002.

10. Dianat M, Radan M, Badavi M, Mard S.A, Bayati V, et al (2018). Crocin attenuates cigarette smoke-induced lung injury and cardiac dysfunction by anti-oxidative effects: the role of Nrf2 antioxidant system in preventing oxidative stress. Respir Res, 19: 58, DOI: 10.1186/s12931-018-0766-3.
11. Elsherbiny N.M, Salama M.F, Said E, El-Sherbiny M and Al-Gayyar MMH (2016). Crocin protects against doxorubicin-induced myocardial toxicity in rats through down-regulation of inflammatory and apoptic pathways. Chem Biol Interact, 247: 39-48, DOI: 10.1016/j.cbi.2016.01.014.
12. Fan T.J., Han L.H, Cong R.S and Liang J (2005). Caspase family proteases and apoptosis. Acta Biochim. Biophys. Sin, 37: 719-27, DOI: 10.1111/j.1745-7270.2005.00108.x.
13. Feidantsis K, Mellidis K, Galatou E, Sinakos Z and Lazou A (2018). Treatment with crocin improves cardiac dysfunction by normalizing autophagy and inhibiting apoptosis in STZ-induced diabetic cardiomyopathy. Nutr Metab Cardiovasc Dis, 28: 952-61.
14. Feng D, Zhou H, Jin X, Wei J, Zhang Q, et al (2020). Electroacupuncture Pretreatment Alleviates LPS-Induced Acute Respiratory Distress Syndrome via Regulating the PPAR Gamma/NF-Kappa B Signaling Pathway. Evid Based Complement Alternat Med, DOI: 10.1155/2020/4594631.
15. Gensch C, Clever Y.P, Werner C, Hanhoun M., Böhm M, et al (2007). The PPAR-γ agonist pioglitazone increases neoangiogenesis and prevents apoptosis of endothelial progenitor cells. Atherosclerosis,192:67-74.
16. Hoshyar R, Bathaie S.Z and Sadeghizadeh M (2013). Crocin triggers the apoptosis through increasing the Bax/Bcl-2 ratio and caspase activation in human gastric adenocarcinoma, AGS, cells. DNA Cell Biol, 32: 50-57, DOI: 10.1089/dna.2012.1866.
17. Krijnen P.A.J, Nijmeijer R, Meijer CJLM, Visser C.A, Hack C.E and Niessen HWM (2002). Apoptosis in myocardial ischaemia and infarction. J Clin Pathol, 55: 801-11, DOI: 10.1136/jcp.55.11.801
18. Liang Y, Zheng B, Li J, Shi J, Chu L, et al (2020). Crocin ameliorates arsenic trioxide-induced cardiotoxicity via Keap1-Nrf2/HO-1 pathway: Reducing oxidative stress, inflammation, and apoptosis. Biomed & Pharmacother, 131: 110713, https://doi.org/10.1016/j.biopha.2020.110713.
19. Liu H.J, Liao H.H, Yang Z and Tang Q.Z (2016). Peroxisome proliferator-activated receptor-γ is critical to cardiac fibrosis. PPAR research, DOI: 10.1155/2016/2198645.
20. Mahajan U. B, Chandrayan G, Patil C.R, Arya D.S, Suchal K, et al (2017). The Protective Effect of Apigenin on Myocardial Injury in Diabetic Rats mediating Activation of the PPAR-gamma Pathway. Int J Mol Sci, 18(4):756, DOI: 10.3390/ijms18040756.
21. Meng D.F, Guo L.L, Peng L.X, Zheng L.S, Ping Xie, et al (2020). Antioxidants suppress radiation-induced apoptosis via inhibiting MAPK pathway in nasopharyngeal carcinoma cells. Biochem Biophys Res Commun, 527: 770-77, https://doi.org/10.1016/j.bbrc.2020.04.093.
22. Okinaka S, Kumagai H, Ebashi S, Sugita H, Momoi H, et al (1961). Serum creatine phosphokinase: Activity in progressive muscular dystrophy and neuromuscular diseases. Arch Neurol, 4: 520-25, DOI: 10.1001/archneur.1961.00450110050006.
23. Olivetti G, Quaini F, Sala R, Lagrasta C, Corradi D, et al (1996). Acute myocardial infarction in humans is associated with activation of programmed myocyte cell death in the surviving portion of the heart. J. Mol. Cell. Cardiol, 28: 2005-16, DOI: https://doi.org/10.1006/jmcc.1996.0193.
24. Ortiz A, Justo P, Sanz A, Lorz C and Egido J (2003). Targeting apoptosis in acute tubular injury. Biochem. Pharmacol, 66: 1589-94, https://doi.org/10.1016/S0006-2952(03)00515-X.
25. Qi Y, Chen L, Zhang L, Liu W.B, Chen X, et al (2013). Crocin prevents retinal ischaemia/reperfusion injury-induced apoptosis in retinal ganglion cells through the PI3K/AKT signalling pathway. Exp. Eye Res, 107: 44-51, DOI: 10.1016/j.exer.2012.11.011.
26. Rani N, Bharti S, Bhatia J, Nag T.C, Ray R et al (2016). Chrysin, a PPAR-γ agonist improves myocardial injury in diabetic rats through inhibiting AGE-RAGE mediated oxidative stress and inflammation. Chem Biol interact, 250: 59-67, DOI: 10.1016/j.cbi.2016.03.015
27. Razavi B.M, Hosseinzadeh H, Abnous K, Khoei A and Imenshahidi A (2016). Protective effect of crocin against apoptosis induced by subchronic exposure of the rat vascular system to diazinon. Toxicol Ind Health, 32: 1237-45, https://doi.org/10.1177/0748233714554941.
28. Remondino A, Kwon S.H, Communal C, Pimentel D.R, Sawyer D.B, et al (2003). β-Adrenergic receptor–stimulated apoptosis in cardiac myocytes is mediated by reactive oxygen species/c-Jun NH2-terminal kinase–dependent activation of the mitochondrial pathway. Circ Res, 92: 136-38, DOI: 10.1161/01.res.0000054624.03539.b4.
29. Ren, Y., Sun, C., Sun, Y., Tan, H., Wu, Y., Cui, B and Wu, Z (2009). PPAR gamma protects cardiomyocytes against oxidative stress and apoptosis via Bcl-2 upregulation. Vascul. Pharmacol, 51: 169-74, DOI: 10.1016/j.vph.2009.06.004.
30. Saraste, A and Pulkki, K (2000). Morphologic and biochemical hallmarks of apoptosis. Cardiovasc. Res, 45: 528-37, DOI: 10.1016/S0008-6363(99)00384-3.
31. Sun Y, Xu H.J, Zhao Y.X, Wang L.Z, Sun L.R, et al (2013). Crocin exhibits antitumor effects on human leukemia HL-60 cells in vitro and in vivo. Evid-Based Complement Alternat Med, DOI: 10.1155/2013/690164.
32. Wang Y, Wang Q, Yu W and Du H (2018). Crocin attenuates oxidative stress and myocardial infarction injury in rats. Int Heart J, 17-114, DOI: 10.1536/ihj.17-114
33. Wen L.L, Li C.Y, Chou H.C, Chang C.C, Lo H.Y, et al (2016). Perfluorooctanesulfonate mediates renal tubular cell apoptosis through PPARgamma inactivation. PloS one, 11(5):e0155190, DOI: 10.1371/journal.pone.0155190.
34. Yaribeygi, H, Mohammadi, M.T and Sahebkar, A (2018). Crocin potentiates antioxidant defense system and improves oxidative damage in liver tissue in diabetic rats. Biomed & Pharmacother, 98: 333-337, DOI: 10.1016/j.biopha.2017.12.077.
35. Zaugg M, Xu W, Lucchinetti E, Shafiq S.A, Jamali N.Z, et al (2000). β-Adrenergic receptor subtypes differentially affect apoptosis in adult rat ventricular myocytes. Circulation, 102: 344-50, DOI: 10.1161/01.cir.102.3.344.
36. Zeisel S.H (2004). Antioxidants suppress apoptosis. J Nutr, 134(11):3179S-3180S, DOI: 10.1093/jn/134.11.3179S
37. Zhang L, Wu P., Zhang L, SreeHarsha N, Mishra A, et al (2020). Ameliorative effect of rosiglitazone, a peroxisome proliferator gamma agonist on adriamycin-induced car-

dio toxicity via suppressing oxidative stress and apoptosis. IUBMB Life, 72: 607-15, DOI: 10.1002/iub.2190.

38. Zhuo X.Z, Wu Y, Ni Y.J, Liu J.H, Gong M, et al (2013). Isoproterenol instigates cardiomyocyte apoptosis and heart failure via AMPK inactivation-mediated endoplasmic reticulum stress. Apoptosis, 18(7):800-10 , DOI: 10.1007/s10495-013-0843-5.