ZITA MEDICAL MANAGEMENT

www.hsmc.gr/en/pharmakeftiki-journal/

PHARMAKEFTIKI, 37, 2S, 2025 | 363-366 RESEARCH

https://doi.org/10.60988/p.v37i2S.230

Esculetin improves gut dysbiosis in isoproterenol-induced myocardial infarction in male rats

Zahraa Jabbar Mujbel[1,*], Gaith Ali Jasim[2], Huda Jaber Waheed[1]

[1]Department of Pharmacology & Toxicology, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq
[2]Al-Bayan University, Baghdad, Iraq

KEY WORDS:
esculetin; isoproterenol; myocardial infarction; intestinal dysbiosis; rat

ARTICLE INFO:
Received: December 29, 2024
Revised: February 21, 2025
Accepted: February 21, 2025
Available online: October 10, 2025

*** CORRESPONDING AUTHOR:**
Zahraa Jabbar Mujbel, Department of Pharmacology & Toxicology, College of Pharmacy, Mustansiriyah University, Baghdad, Iraq; e-mail: zahraa1996j@gmail.com

ABSTRACT

Myocardial infarction (MI), a prevalent form of cardiovascular disease, has been associated with alterations in gut microbiota composition. Esculetin, a coumarin derivative, possesses multiple therapeutic properties. This study aimed at investigating the potential protective role of esculetin against isoproterenol (ISO)-induced myocardial toxicity in male rats, with a particular focus on intestinal dysbiosis and histopathological changes. The experimental protocol spanned 21 days, during which rats were randomly assigned to five groups (n=6 per group): (i) group 1 (normal control) received normal saline, (ii) group 2 (positive control) was administered ISO at 100 mg/kg, subcutaneously, every 24 h for two consecutive days, and (iii) groups 3, 4, and 5 were similarly administered ISO (100 mg/kg, subcutaneously, every 24 h for two days), followed from day 3 by oral esculetin at doses of 20, 40, and 60 mg/kg once daily, respectively. ISO administration significantly elevated serum creatine kinase-MB (CK-MB) levels, indicating myocardial injury. Esculetin treatment markedly reduced CK-MB levels in a dose-dependent manner. ISO also significantly decreased sodium–potassium adenosine triphosphatase (Na^+,K^+-ATPase) activity (a key marker of cellular membrane integrity and ion transport) while esculetin administration restored its activity. Histopathological examination of the small intestine in ISO-treated rats revealed severe enteritis characterized by villous damage, epithelial sloughing, vascular congestion, and pronounced leukocyte infiltration. Esculetin treatment ameliorated these changes, with the highest dose (60 mg/kg) yielding the most notable improvements, including restoration of normal villous architecture, enterocyte integrity, and intestinal gland morphology. These findings suggest that esculetin exerts protective effects against ISO-induced MI by mitigating cardiac toxicity, reversing intestinal dysbiosis, and improving histopathological outcomes.

PHARMAKEFTIKI, 37, 2S, 2025 | 363-366

1. Introduction

Cardiovascular diseases encompass a range of disorders affecting the circulatory system, with myocardial infarction (MI) remaining a leading cause of global mortality and morbidity. Obstruction of the coronary arteries compromises myocardial perfusion, resulting in ischemic necrosis of cardiac tissue.

The induction of MI using isoproterenol (ISO), a synthetic catecholamine, is a widely accepted, non-invasive method in experimental models. ISO induces myocardial stress primarily through free radical generation, leading to irreversible damage to cardiac membranes. Its administration elevates heart rate, oxygen demand, intracellular calcium load, and cyclic adenosine monophosphate levels in myocardial cells[1].

Recent studies underscore the role of gut microbiota in modulating the pathogenesis of coronary artery disease. Notably, Emoto *et al.*[2] have demonstrated a correlation between gut microbiota composition and the severity of MI in rats[2]. Gut dysbiosis – defined as an imbalance in the composition, diversity, and abundance of intestinal microorganisms – has been strongly linked to increased cardiovascular risk. Alterations in microbial populations can compromise the intestinal barrier, suggesting a mechanistic connection between cardiac injury and gut microbiota integrity[3].

Esculetin (6,7-dihydroxycoumarin), a plant-derived coumarin isolated from *Artemisia capillaris* and *Citrus limonia*, is a low molecular weight phenolic compound with diverse pharmacological properties. It has demonstrated efficacy in managing thromboembolic events, including stroke and MI. Given its safety profile and therapeutic potential, esculetin is a promising candidate for mitigating MI and associated intestinal dysbiosis.

This study investigates the protective effects of esculetin against ISO-induced MI and gut dysbiosis, with particular emphasis on its influence on myocardial lysosomal and membrane-bound enzymes and associated inflammatory responses in rats.

2. Methodology

Thirty untreated male albino Wistar rats (200–250 g) were procured from the Animal House of the College of Pharmacy, Mustansiriya University. The animals were housed in large cages with unrestricted access to food and water, and acclimatized for 3 weeks under controlled environmental conditions, including air vacuum systems. MI was induced by a subcutaneous injection of ISO at a dose of 100 mg/kg, dissolved in normal saline, administered over two consecutive days. The total study duration was 3 months, encompassing biochemical and histological analyses.

The treatment phase lasted 21 days, during which the rats were randomly assigned to five groups (n=6 per group): (i) group 1 (negative control) received water and a standard diet, (ii) group 2 (positive control) received ISO (100 mg/kg) subcutaneously, every 24 h for two days, (iii) group 3 received ISO as above, followed from day 3 by esculetin (20 mg/kg in 1 mL of normal saline) administered orally once daily, (iv) group 4 received ISO followed by esculetin (40 mg/kg in 1 mL of normal saline) orally once daily, and (v) group 5 received ISO followed by esculetin (60 mg/kg in 1 mL of normal saline) orally once daily.

On day 22, rats were fasted and sedated using ketamine (100 mg/kg) and xylazine (10 mg/kg). Blood samples were collected from the right ventricle and centrifuged for biomarker analysis. Cardiac biomarkers, including creatine kinase-MB (CK-MB) and sodium–potassium adenosine triphosphatase (Na^+,K^+-ATPase), were quantified using enzyme-linked immunosorbent assay (ELISA). Intestinal tissues underwent histopathological processing: fixation, dehydration, clearing, embedding, and haematoxylin and eosin staining.

Ethical approval was granted by the Mustansiriya University College of Pharmacy Ethics Committee (approval number: 49; date: 12/2/2025). Statistical analysis was performed using the GraphPad Prism 5.0 software. Results were expressed as mean ± standard error of the mean (SEM), and analysed *via* analysis of variance (ANOVA) followed by Dunnett's *post hoc* test. A p-value <0.05 was considered statistically significant.

3. Results and Discussion

Serum CK-MB activity was significantly elevated

PHARMAKEFTIKI, 37, 2S, 2025 | 363-366

Table 1. The effect of esculetin on serum cardiac enzymes sodium–potassium adenosine triphosphatase (Na^+,K^+-ATPase) and creatine kinase-MB (CK-MB). Statistical analysis was evaluated using the least significant difference test; statistical significance between tested means is represented by the letters a, b, c, and d, with "a" denoting the highest level of significance and "d" the lowest. Identical letters indicate no statistically significant difference between the corresponding means.

Groups	**CK-MB activity** (IU/L)	**Na^+,K^+-ATPase activity** (IU/L)
Group 1 (negative control)	1.302 ± 0.323 [a]	5.336 ± 1.356 [d]
Group 2 (positive control; isoproterenol)	7.177 ± 1.409 [c]	0.463 ± 0.178 [a]
Group 3 (esculetin; 20 mg/kg)	3.914 ± 1.499 [b]	2.790 ± 0.963 [b]
Group 4 (esculetin; 40 mg/kg)	2.563 ± 0.581 [b]	3.147 ± 0.694 [b]
Group 5 (esculetin; 60 mg/kg)	1.318 ± 0.532 [a]	4.116 ± 0.481 [c]

(p<0.05) in the ISO-induced group (7.177 ± 1.409 IU/L) compared to the control group (1.302 ± 0.323 IU/L). Esculetin treatment at 20 mg/kg reduced CK-MB activity levels to 3.914 ± 1.499 IU/L (p<0.05), with further reductions observed at 40 mg/kg (2.563 ± 0.581 IU/L) and 60 mg/kg (1.318 ± 0.532 IU/L). The highest dose showed no significant difference from the control group (Table 1).

Similarly, Na^+,K^+-ATPase activity was significantly suppressed (p<0.05) in the ISO group (0.463 ± 0.178 IU/L) relative to controls (5.336 ± 1.356 IU/L). Esculetin restored enzyme activity in a dose-dependent manner: 20 mg/kg (2.790 ± 0.963 IU/L), 40 mg/kg (3.147 ± 0.694 IU/L), and 60 mg/kg (4.116 ± 0.481 IU/L), with near-complete recovery at the highest dose (Table 1).

Histopathological findings were as follows: group 1 exhibited normal intestinal villi, enterocytes, and glands; group 2 showed severe enteritis with villous damage, epithelial sloughing, vascular congestion, and leukocyte infiltration; group 3 displayed thickened villi, increased cellularity in the villus core, enterocyte and goblet cell hyperplasia, and intact glands; group 4 demonstrated normal intestinal architecture; group 5 showed predominantly normal villi and glandular structures.

ISO-induced MI was associated with significant cardiac injury and intestinal dysbiosis, primarily mediated by inflammatory mechanisms. CK-MB, a reliable marker of myocardial necrosis, was markedly elevated in ISO-treated rats, consistent with prior findings[4]. ISO mimics infarct-like cardiac damage comparable to human MI. Esculetin reduced CK-MB levels in a dose-dependent fashion, with the 60 mg/kg dose restoring levels to near-control values.

ISO-induced oxidative stress impaired Na^+,K^+-ATPase activity, compromising membrane integrity. Esculetin stabilized myocardial and lysosomal membranes, preventing enzyme leakage and reducing necrosis. All esculetin-treated groups showed significant recovery of Na^+,K^+-ATPase activity[5].

Histopathological analysis confirmed dose-dependent improvements in intestinal morphology. At 60 mg/kg, esculetin nearly resolved cardiac injury and restored normal villous architecture. ISO-treated rats exhibited severe intestinal damage, including villous thickening, epithelial sloughing, leukocyte infiltration, and hyperplasia; findings consistent with those of Zhao *et al.*[6] who have reported deterioration of the intestinal barrier in MI models. Collectively, these results affirm the dual therapeutic potential of esculetin in ameliorating cardiac and intestinal damage induced by ISO.

4. Conclusion

Esculetin significantly attenuated ISO-induced MI and gut dysbiosis by enhancing cardiac enzyme activity and restoring intestinal architecture in a

PHARMAKEFTIKI, 37, 2S, 2025 | 363-366

dose-dependent manner. The 60 mg/kg dose yielded the most pronounced improvements, suggesting that esculetin is a promising candidate for the treatment of MI and its associated gastrointestinal complications.

Acknowledgements

None.

Conflicts of interest

None exist.

ORCIDs

0009-0008-1462-0012 (Z.J. Mujbel); 0000-0001-5153-4094 (G.A. Jasim); 0000-0001-8491-8334 (H.J. Waheed)

References

1. Eltobshy S.A.G., Hussein A.M., Elmileegy A.A., Askar M.H., Khater Y., Metias E.F., *et al.* Effects of heme oxygenase-1 upregulation on isoproterenol-induced myocardial infarction. *Korean J. Physiol. Pharmacol.* 23(3), 203–217, 2019. DOI: 10.4196/kjpp.2019.23.3.203
2. Emoto T., Yamashita T., Sasaki N., Hirota Y., Hayashi T., So A., *et al.* Analysis of gut microbiota in coronary artery disease patients: a possible link between gut microbiota and coronary artery disease. *J. Atheroscler. Thromb.* 23(8), 908–921, 2016. DOI: 10.5551/jat.32672
3. Sun L., Jia H., Li J., Yu M., Yang Y., Tian D., *et al.* Cecal gut microbiota and metabolites might contribute to the severity of acute myocardial ischemia by impacting the intestinal permeability, oxidative stress, and energy metabolism. *Front. Microbiol.* 10, 1745, 2019. DOI: 10.3389/fmicb.2019.01745
4. Pullaiah C.P., Nelson V.K., Rayapu S., G V N.K., Kedam T. Exploring cardioprotective potential of esculetin against isoproterenol induced myocardial toxicity in rats: *in vivo* and *in vitro* evidence. *BMC Pharmacol. Toxicol.* 22(1), 43, 2021. DOI: 10.1186/s40360-021-00510-0
5. Lobo R.O., Chandrasekhar Sagar B.K., Shenoy C.K. Bio-tea prevents membrane destabilization during Isoproterenol-induced myocardial injury. *J. Microsc. Ultrastruct.* 5(3), 146–154, 2017. DOI: 10.1016/j.jmau.2016.09.001
6. Zhao J., Zhang Q., Cheng W., Dai Q., Wei Z., Guo M., *et al.* Heart-gut microbiota communication determines the severity of cardiac injury after myocardial ischaemia/reperfusion. *Cardiovasc. Res.* 119(6), 1390–1402, 2023. DOI: 10.1093/cvr/cvad023

HOW TO CITE:
Mujbel Z.J., Jasim G.A., Waheed H.J. Esculetin improves gut dysbiosis in isoproterenol-induced myocardial infarction in male rats. *Pharmakeftiki* 37(2s), 363-366, 2025. https://doi.org/10.60988/p.v37i2S.230