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BRIEF COMMUNICATION

# Long-term administration of fisetin was not as effective as short term in ameliorating IR injury in isolated rat heart

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## Abstract

The current study aims to determine the comparative efficacy of fisetin in reducing myocardial ischemia–reperfusion injury (IR) in isolated rat hearts when the drug was given either oral or intraperitoneal (ip) for short-term and long-term administration. Rats treated with fisetin (20 mg/kg-oral/ip) for short (30 min prior to surgery) and long (15 days prior to surgery followed by 1-day washout) duration were subjected to myocardial IR using Langendorf perfusion system. Hemodynamics, cardiac injury, mitochondrial functional assessment, and fisetin levels were estimated. Unlike the long-term administration of fisetin, the short-term treated-rat heart exhibited significant cardioprotection, measured via hemodynamic indices (RPP in mmHg × beats/min × 10 ^ 4: IR — 4 ± 0.1, FIPS — 2.49 ± 0.18, FIPL — 1.87 ± 0.14), reduced infarct size (in % area of infarct: IR — 38 ± 5, FIPS — 17 ± 1, FOS — 14 ± 2), improved mitochondrial ETC enzyme activity (NQR activity in IFM: FIPS — 0.25 ± 0.016, FIPL — 0.20 ± 0.02), and declined oxidative stress (GSH in IFM: FIPS — 1.52 ± 0.14, FIPL — 1.25 ± 0.22). However, no significant difference in the protection was observed between the animals treated with oral or intraperitoneally administered fisetin. Single dose of fisetin administration before IR protocol was more effective than 15 days of fisetin-treated drug followed by 1-day washout, thus may not be suitable for long-term dietary supplement for post-surgical cardiac rehabilitation.

**Keywords** Fisetin · Myocardial ischemia–reperfusion · Isolated rat heart · Short term vs long term · Cardioprotection

## Abbreviations

| | |
|---|---|
| ANOVA | Analysis of variance |
| CK | Creatine kinase |
| CPCSEA | Committee for the Purpose of Control and Supervision of Experiments on Animals |
| COX | Cyclooxygenase |
| DCPIP | 2,6-Dichlorophenolindophenol |
| ETC | Electron transport chain |
| GSH | Glutathione |
| GSSG | Glutathione disulfide |
| HR | Heart rate |
| IR | Ischemia-reperfusion |
| IFM | Interfibrillar mitochondria |
| LDH | Lactate dehydrogenase |
| LVDP | Left ventricular developed pressure |
| LVEDP | Left ventricular diastolic pressure |
| NQR | Rotenone-sensitive |
| NADH | Oxidoreductase |
| QCR | Ubiquinol cytochrome C reductase |
| RPP | Rate pressure product |
| SOD | Superoxide dismutase |
| SQR | Succinate decylubiquinone |
| DCPIP | Reductase |
| SSM | Subsarcolemmal mitochondria |
| TTC | Triphenyl tetrazolium chloride |

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## Introduction

Fisetin is a naturally occurring flavonoid known to attenuate myocardial ischemia–reperfusion injury which was established in two experimental models, namely the isolated rat heart model and left anterior coronary artery (LAD) ligation model (Long et al. 2020; Shanmugam et al. 2021a). The comparative evaluation of the reported cardioprotection from these studies suggested that fisetin given prior to 30 min of IR protocol was more effective. Recently, fisetin is under the investigation of 2 different clinical trials where the duration of fisetin administration was different. One study evaluates whether short-term (days 0, 1 and days 8, 9) oral treatment with fisetin (20 mg/kg) can reduce the rate of death and long term complications related to COVID-19 (https://clinicaltrials.gov/ct2/show/NCT04771611). Another study tests the efficacy of the fisetin as a supplement (20 mg/kg/day, orally for 2 consecutive days) in reducing inflammatory factors in blood in elderly adults and to test the efficacy of the drug in reducing frailty and markers of inflammation, insulin resistance, and bone resorption in elderly adults (https://clinicaltrials.gov/ct2/show/NCT03675724).

Early studies have reported that fisetin is rapidly metabolized in the liver, and thus the bioavailability of fisetin in the target organ is underscan. Besides, the route of administration of fisetin is also a detrimental factor for bioavailability (Shia et al. 2009). In the present study, we explored the efficacy of fisetin in ameliorating ischemia–reperfusion injury in isolated rat hearts by preadministrating the animal with fisetin for 15 days (1 dose/day) and 30 min (single dose) before IR protocol. In these experiments, we administered fisetin via two routes (ip and oral) for the comparative evaluation. By using an isolated rat heart model, we negate the influence of the neurohormonal axis in regulating cardiac physiology during IR events.

## Materials and methods

### Animals

Male Wistar rats of 200–250 g used for the study after prior approval from the institutional animal ethical committee (CPCSEA Approval No.: 552/SASTRA/IAEC/RPP) were segregated randomly into 7 groups ($N = 6$/group) as follows: (1) Normal (*N*), (2) ischemia–reperfusion (IR), (3) fisetin control (FC — fisetin oral for 15 days followed by 1-day washout), (4) fisetin ip 30 min before IR protocol (FIPS), (5) fisetin oral 30 min before IR protocol (FOS), (6) fisetin ip for 15 days followed by 1-day washout before IR (FIPL), and (7) fisetin oral for 15 days followed by 1-day washout before IR (FOL). Fisetin at a concentration of 20 mg/kg was used in the study. IR was induced by the Langendorff system (30-min ischemia + 60-min reperfusion), and the hemodynamic parameters were measured as described previously [2]. The cardiac injury was assessed by using TTC staining, measuring the activities of caspase 3, lactate dehydrogenase (LDH) and creatine kinase (CK) (Shanmugam et al. 2021a).

### Mitochondrial electron transport chain complex activities and oxidative stress assessment

Mitochondrial subpopulations namely interfibrillar mitochondria (IFM) and subsarcolemmal mitochondria (SSM) were isolated, according to the procedure mentioned previously; ETC complex enzyme activities in IFM and SSM were measured spectrophotometrically [2]. In addition, reduced and oxidized glutathione (GSH and GSSG, respectively) and enzyme activities such as superoxide dismutase (SOD) and catalase were estimated in IFM and SSM fractions as per standard procedures (Shanmugam et al. 2021a).

### Estimation of fisetin concentration

The fisetin concentration in the myocardial tissue was analyzed by the fluorescence method as mentioned previously (Shanmugam et al. 2021b).

### Statistical analysis

The data were expressed at mean ± S.D. and analyzed using GraphPad Prism. All the data were subjected to one way ANOVA and Dunnet's comparison post-tests, as appropriate. *P* level of $< 0.05$ was considered to be statistically significant.

## Results

### Fisetin provides cardiac protection towards IR independent of the route of administration

According to Table 1, the isolated rat heart shows reduced cardiac performance when subjected to I/R which was evident from the declined LVDP and RPP (63% and 66% respectively), compared with the normal. Fisetin when given as either ip or oral route 30 min prior to the IR protocol, the decline in the hemodynamic indices were low (FIPS: LVDP — 31%, RPP — 24%; FOS-LVDP — 19%, RPP — 20%).

The infarct size analysis by TTC (Figure 1) indicated that the fisetin treatment via oral and ip route was effective in reducing the I/R associated myocardial injury (in % area of infarct: IR — 38 ± 5, FIPS — 17 ± 1, FOS — 14 ± 2). Further evaluation by cardiac marker enzyme activity (LDH: IR

**Table 1** Hemodynamics measurement and estimated fisetin level at the end of reperfusion

| | *N* | IR | FC | FIPS | FOS | FIPL | FOL |
|---|---|---|---|---|---|---|---|
| LVDP (×10 mmHg) | 11.6 ± 1.7* | 4 ± 0.1 | 10.3 ± 0.3* | 7.6 ± 0.9* | 8.9 ± 2.0* | 6.3 ± 2.0 | 5.6 ± 0.9 |
| HR (beats/min) | 305 ± 28 | 293 ± 18 | 325 ± 20 | 327 ± 16 | 297 ± 28 | 297 ± 28 | 227 ± 16 |
| RPP (mmHg × beats/min × 10 ^ 4) | 3.5 ± 0.15* | 1.09 ± 0.43 | 3.23 ± 0.87* | 2.49 ± 0.18* | 2.64 ± 0.14* | 1.87 ± 0.14 | 1.27 ± 0.18 |
| Conc. of fisetin (μM)/mg of tissue | 0 | 0 | 549 ± 37 | 2115 ± 33 | 2308 ± 45 | 386 ± 26$^{\#}$ | 272 ± 14$^{\#}$ |

Data were represented as mean ± SD of 6 individual experiments

*LVDP* left ventricular developed pressure, *HR* heart rate, *RPP* rate pressure product, *dp/dt* rate of rise of left ventricular pressure

$^{*}p < 0.05$ vs IR, $^{\#}p < 0.05$ vs FIPS/FOS

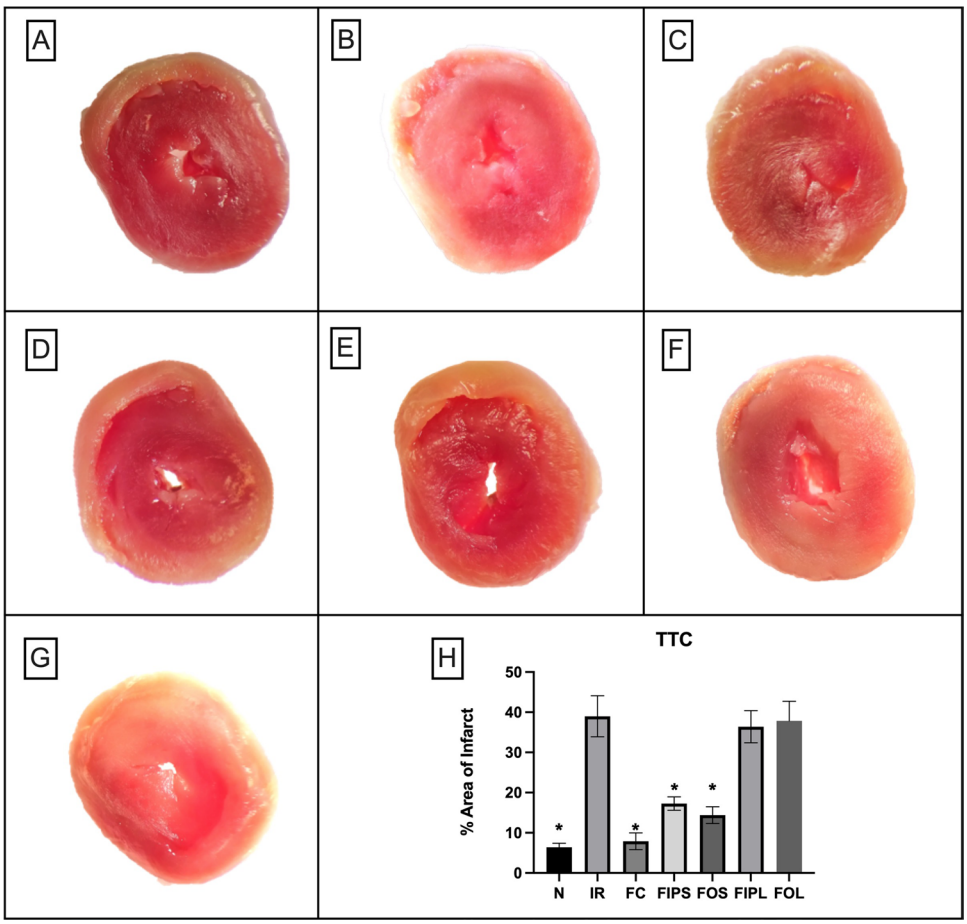


**Fig. 1** Representative infarcts of different groups stained with TTC (**A**) *N* (*n* = 3), (**B**) IR (*n* = 3), (**C**) FC (*n* = 3), and (**D**) FIPS (*n* = 3), (**E**) FOS (*n* = 3), (**F**) FIPL (*n* = 3), (**G**) FOL, (**H**) percentage infarct size measurement. *$p < 0.05$ vs IR

— 0.90 ± 0.08, FIPS — 2.62 ± 0.04, FOS — 2.47 ± 0.03; CK: IR — 12.9 ± 1.1, FIPS — 17.2 ± 1.6, FOS — 17.4 ± 2.1; caspase 3: IR — 50711 ± 219, FIPS — 42251 ± 554, FOS — 42772 ± 695) in the heart and coronary perfusate (LDH: IR — 6.5 ± 0.12, FIPS — 3.0 ± 0.08, FOS — 2.3 ± 0.1; CK: IR — 11.8 ± 1.3, FIPS — 6.1 ± 1.2, FOS — 6.9 ± 1.0)were in coherence with the hemodynamic data (Figure 2A–E). Fisetin treatment to the IR-challenged heart significantly improved the mitochondrial ETC complex activities, like NQR, SQR, QCR, and COX and increased the antioxidant levels (Figure 2F–L). In short, with respect to the overall improvement of IR rat heart by fisetin, oral administration provides a slight advantage over the ip route of administration.

## Short term fisetin pretreatment provides better cardioprotection towards IR injury than long-term treatment

We measured the hemodynamics, infarct size, and cardiac injury markers in the isolated rat hearts subjected to IR from the single and multiple-dose fisetin-treated groups (both ip

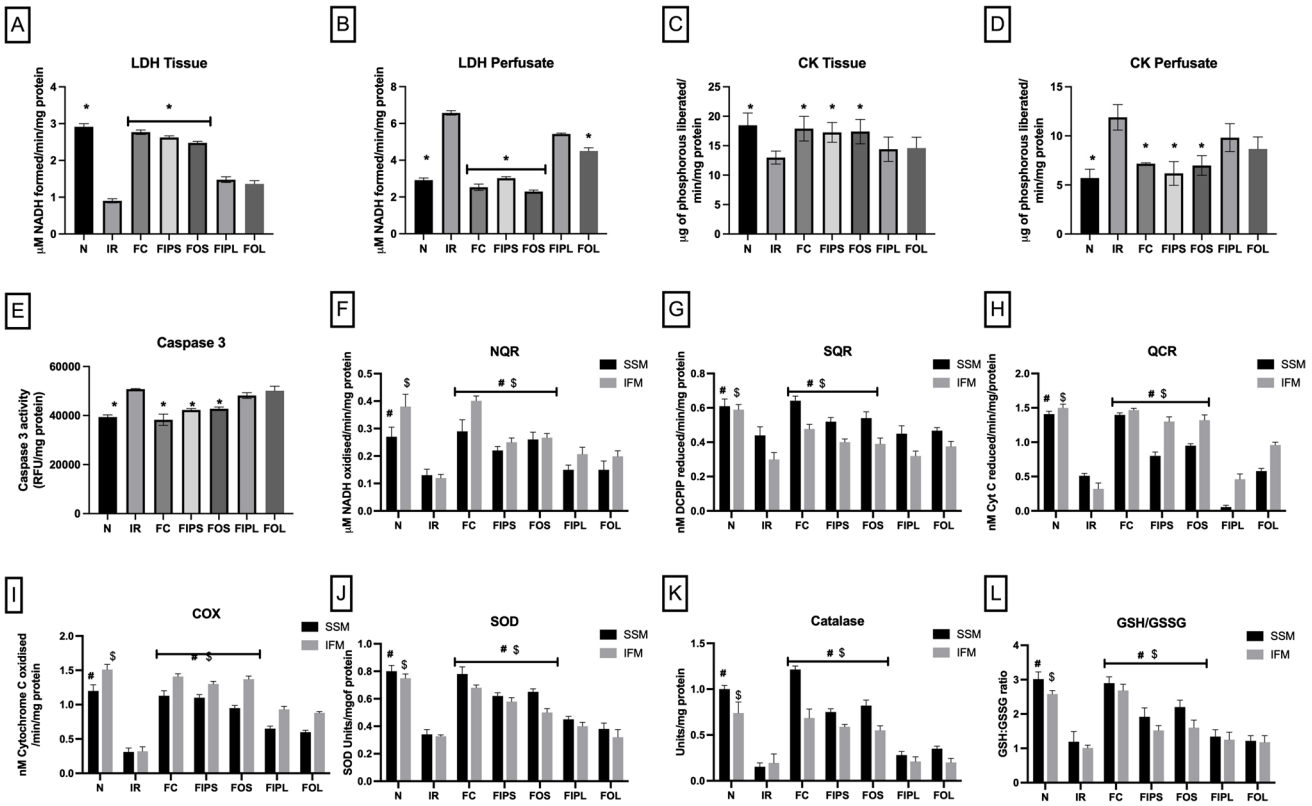


**Fig. 2** Effect of fisetin treatment on IR injury — (**A**) LDH activity in the tissue, (**B**) LDH activity in the perfusate, (**C**) CK activity in the tissue, and (**D**) CK activity in the perfusate. (**E**) Caspase-3 activity in the tissue. $^{*}p < 0.05$ vs IR. Effect of fisetin on mitochondrial enzyme activities — (**F**) NQR, (**G**) SQR, (**H**) QCR, and (**I**) COX activities in the mitochondrial samples isolated from heart tissues. Effect of fisetin on antioxidant defence system of isolated mitochondria — (**J**) SOD activity, (**B**) catalase activity, (**C**) GSH:GSSG ratio in the respective groups. Data were represented as mean ± SD. $^{\#}p < 0.05$ vs IR (SSM); $^{\$}p < 0.05$ vs IR (IFM)

and oral route). The hemodynamic data in Table 1 shows that 30-min short-term treatment shows better hemodynamic recovery (FIPS: LVDP — 47% and RPP — 56 %; FOS — LVDP — 55% and RPP — 58%) than the long-term treatment (FIPL: LVDP — 36% and RPP — 41 %; FOL-LVDP — 28% and RPP — 14%), and infarct size has been reduced significantly ($P < 0.05$) in short-term fisetin-treated hearts than long-term treated hearts subjected to IR (in % area of infarct: IR —38 ± 5, FIPL —36 ± 4, FOL —37 ± 4) (Fig. 1).

Long-term fisetin administration failed to improve the IR associated–declined mitochondrial function. On the other hand, a single dose of fisetin was sufficient to recover IR associated–mitochondrial dysfunction. These observations were similar in both the mitochondrial subpopulations (IFM and SSM). The distinct protective effect of long- and short-term fisetin administration was intact even when the route of administration was different (ip or oral). According to Table 1, the concentration of fisetin in the myocardium is higher in short-term fisetin-pretreated rats (FIPS —2115 ± 33) than the long-term administered rats (FIPL —386 ± 26).

## Discussion

Fisetin was reported to have low solubility (10.45 μg/ml) and relatively poor oral bioavailability (44%), and undergo rapid metabolism (Grynkiewicz and Demchuk 2019). Interestingly, fisetin has high biological activity, but its use as a food supplementation is still rare, even though it is a natural compound present in fruits and vegetables (Grynkiewicz and Demchuk 2019). This raises two fundamental questions to be explored namely, (1) whether a sufficient quantity of fisetin was available in the target organ or its metabolite is biologically active (considering the rapid metabolism) during IR and (2) does the route of administration is critical for the efficacy of fisetin action.

The initial finding of our study suggested that unlike long-term administration of fisetin for 15 days, the drug given 30 min before IR protocol was efficient in attenuating ischemia–reperfusion injury. Irrespective of differences in route of drug administration, the cardioprotective effect of fisetin was similar. This suggested that the bioavailability of fisetin in the heart tissue may be similar even if the route of administration was different. Furthermore, we checked the fisetin content in the myocardium and found similar levels

of fisetin in the tissue from the rat treated with fisetin 30 min prior to IR via ip and oral. On the other hand, we could not find a significant concentration of fisetin in the myocardium of rats treated with fisetin for 15 days followed by a 1-day washout. Corresponding to this data, the IR associated–cardiac injury and compromised hemodynamics were higher in rats treated with fisetin for 15 days either oral or ip route. All these experimental observations indicate that fisetin can exert cardioprotection against IR in the heart. But we cannot rule out the possible presence and activity of fisetin metabolite in the cardioprotection, which need to be further explored.

Myocardial IR injury is characterized by elevated oxidative stress and severe mitochondrial dysfunction that promote injury and compromised physiological function (Perrelli et al. 2011). Previous studies have shown that fisetin is a potent antioxidant agent by acting as a free radical scavenger and also by triggering the production of antioxidants (Shanmugam et al. 2021b). In addition, through our previous publication, we demonstrated the mitochondrial protective effect of fisetin in both in vivo and in vitro models (Shanmugam et al. 2021a). In the present study, we observed fisetin-mediated mitoprotection was low in the long-term administered fisetin-treated rat than the short-term fisetin-treated rats. This may be explained by the presence of a higher concentration of fisetin in the myocardium of short-term treated rats. A similar observation was found with IR associated–oxidative stress in fisetin-administered rats where the fisetin's ability to reduce oxidative stress was observed only in short-term administration animals than the long-term treated group.

## Conclusion

Based on the above results we concluded that irrespective of the route of administration, fisetin renders the cardioprotection towards IR injury. The potential of fisetin to render the IR protection is short-lived, hence, it has less potential to utilize as a nutraceutical agent to enhance cardiac tolerance; rather, it can be used as a therapeutic agent.

**Author contribution** PNP, BS, and SRB have processed the experimental data and performed the analysis. PNP drafted the manuscript, designed the figures and tables, and compiled the literature sources. GAK has contributed to the design and implementation of the research, to the interpretation of the results, and the writing of the manuscript. The authors declare that all the data were generated in-house and that no paper mill was used.

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**Data availability** All the data generated or analyzed during the present study are included in this published article.

## Declarations

**Ethics approval and consent to participate** The present study was performed at SASTRA Deemed University, and all the animal experiment procedures performed in this research were approved by the Institutional Animal Ethical Committee (IAEC), SASTRA University held on July 28th 2018 (CPCSEA approval number: 552/SASTRA/IAEC/RPP).

**Consent for publication** Not applicable.

**Competing interests** The authors declare no competing interests.

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