Saffron (Crocus sativus) intake provides nutritional preconditioning against myocardial ischemia-reperfusion injury in wild type and Apo-E(-/-) mice: involvement of Nrf2 activation

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Background: Saffron is an antioxidant herbal derivative with potential anti-atherosclerotic and cardioprotective properties. However, its efficacy as a nutritional preconditioning agent is not yet fully elucidated. Purpose: We investigated the cardioprotective properties of a standardized saffron aqueous extract (SFE) against ischemia/reperfusion injury in Wild-Type and Apo-E(-/-) mice and the underlying molecular mechanisms. Methods: Male Wild-Type and Apo-E (-/-) mice following normal-diet were subjected to 30 min ischemia followed by 2 h reperfusion, with the following interventions: 1) Control Group (n=9): receiving WFI for 4 weeks 2) Saffron Group (n=8), receiving SFE per
os for 4 weeks at a dose of 60 mg/kg. 3) Apo-E(-/-) Control Group (n=6): receiving WFI for 4 weeks. 4) Apo-E(-/-) Saffron Group (n=6), receiving SFE per os for 4 weeks at a dose of 60 mg/kg. Ischemic area/area at risk (I/R%) ratio was measured. Furthermore, blood samples and ischemic myocardial tissue were collected at the 10th min of reperfusion for assessment of MDA and Nitrotyrosine p-eNOS, eNOS, p-Akt, Akt, p-p42/p-p44, p42/p44, p-GSK3-β, GSK-3β, IL-6 and Nrf-2 expression respectively. Moreover, we assessed SFE effect on Nrf-2 expression in vitro on HEK 293T transfected cells, via luciferase assay. 

**Results:** SFE reduced infarct size both in Wild-type (15.50±3.27 vs Control 42.00±2.73, *p<0.05) and Apo-E(-/-) mice (16.13±1.47 vs Control 46.69±1.56, *p<0.05) vs Control group, while eNOS, Akt, GSK-3β and p-44/p-42 phosphorylation, reduced IL-6 expression was observed in the same group. Moreover, MDA and Nitrotyrosine levels were decreased in both SFE treated Wild-Type and Apo-E(-/-) mice. Moreover SFE induced Nrf-2 expression both in Wild-Type and Apo-E(-/-) mice, while saffranal induced Nrf-2 expression in vitro in dose-dependent manner.

**Conclusions:** SFE exerted infarct-limiting effects both in Wild-Type and Apo-E (-/-) mice through activation of Akt/eNOS/ERK1/2/GSK3-β/Nrf-2 pathway, bestowing anti-apoptotic and antioxidant protection against ischemia-reperfusion injury.