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Evidence of neuroprotective effects of saffron and crocin in a Drosophila model of parkinsonism

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ABSTRACT

Evidence suggests that saffron and its major bioactives exhibit significant neuromodulatory effects in various animal models. However, specific data related to their efficacy to attenuate oxidative stress and neurotoxicity in animal models of Parkinson's disease (PD) are limited. Hence, we investigated the neuroprotective efficacy of saffron methanolic extract (SME) and its active constituent, crocin (CR) employing a Drosophila model of parkinsonism. We focussed on attenuation of Rotenone (ROT)-induced locomotor phenotype, oxidative stress, mitochondrial dysfunction and neurotoxicity in this model. SME and CR-enrichment significantly reduced ROT (500 μM) induced mortality, rescued the locomotor phenotype and diminished the enhanced levels of oxidative stress markers in head/body regions of flies. The reduced levels of reduced glutathione (GSH) and total thiols (TSH) resulting from ROT exposure were significantly restored with concomitant enhancement of the antioxidant enzymes activities. Further, ROT-induced mitochondrial dysfunctions (MTT reduction, activities of SDH and NADH-Cyt C reductase (complexes I–III) enzymes) were markedly attenuated by SME/CR enrichment. While ROT elevated the activity of acetylcholinesterase (AChE) in head/body regions, both the treatments caused marked diminution of AChE activity and restored the dopamine levels suggesting their effectiveness to mitigate cholinergic function. Interestingly, SME/CR enrichment significantly delayed the onset of locomotor deficits and extended life span of flies among ROT (50 μM)-stressed flies. In a satellite study, flies provided with SME/CR prophylaxis exhibited marked resistance to an acute Paraquat (PQ) challenge as evidenced by the lower incidence of lethality and improved locomotor phenotype. Taken together, the neuroprotective effects of saffron and crocin in the fly model may be largely attributable to its antioxidant action. Based on our findings, we propose that saffron may be exploited as a supplementary therapeutic agent in PD and other oxidative stress mediated neurodegenerative conditions.

1. Introduction

Crocus sativus L. (Family: Iridaceae) (saffron), a stem less perennial herb is widely cultivated in several countries of mild and dry climate (such as Iran, India, Greece, Italy, etc.). Saffron is the world's most expensive spice and is very well renowned since centuries for its multiple medicinal properties (Schmidt et al., 2007). The significant components of stigmas of *C. sativus* are carotenoids (e.g., crocetin, Crocins, α-carotene, lycopene, zeaxanthin), monoterpene aldehydes (e.g., picrocrocin and safranal), monoterpenoids (e.g., crocusatines), isophorones, and flavonoids (Nassiri-Asl and Hosseinzadeh, 2015). Saffron has been used in traditional medicine in several countries for various purposes such as analgesic, sedative, fever reducer, expectorant, antispasmodic, aphrodisiac, digestive and carminative (Hosseinzadeh et al., 2012; Hosseinzadeh and Nassiri-Asl, 2013). In recent years, various beneficial effects of saffron have been demonstrated in models of neuronal, cardiovascular, respiratory and other disorders (Bathaie and Mousavi, 2010).

Parkinson's disease (PD) is the second most common neurodegenerative movement disorder, afflicting 1–2% of the population above the age of 60 years. The pathological hallmarks of PD include selective loss of dopaminergic neurons in the substantia nigra and the presence of Lewy bodies in surviving dopamine neurons (Cannon and Greenamyre, 2010). Lewy bodies are cytoplasmic inclusions composed mainly of alpha-synuclein, which are

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believed to disrupt the brain's normal functioning in PD. Although, it is well known that environmental exposures and individual genetic susceptibility may determine the onset of PD symptoms, the precise cellular and molecular mechanism(s) responsible for the neurodegeneration processes remain unknown. The majority of cases of PD appear to be sporadic, likely to be caused by a combination of genetic and environmental risk factors, the most apparent being increasing age (Chinta et al., 2013). Currently, the involvement of oxidative stress, mitochondrial dysfunction, and abnormal protein aggregation is well appreciated in the pathogenesis of several NDD including PD (Cicchetti and Drouin-Ouellet, 2009; Goldman et al., 2012; Sanders and Greenamyre, 2013). Accordingly, attenuation of oxidative damage by natural constituents has been considered as an attractive complementary proposition to delay or prevent the development/progression of several NDD including PD.

In recent times, the modulatory role of several nutraceuticals to attenuate endogenous redox status has been considered as an effective approach to achieve neuroprotection (Jimenez-Del-Rio et al., 2010; Dumont and Beal, 2011; Prasad and Muralidhara, 2012). Saffron extracts and its bioactive constituents have been demonstrated to possess excellent antioxidant properties (Hosseinzadeh et al., 2009; Karimi et al., 2010; Mashmoul et al., 2013). Further, their memory and learning enhancing properties in animal models have been attributed to their antioxidant action (Papandreou et al., 2006, 2011; Amin and Hosseinzadeh, 2015). During the past decade, extensive research is in progress to unravel the neuroprotective effects of saffron and its bioactive component crocin employing various animal models (Hosseinzadeh et al., 2012; Nassiri-Asl and Hosseinzadeh, 2015). Studies have shown that saffron can reduce depression in animals, humans and reduce signs of Alzheimer's disease during the early phase (Akhondzadeh et al., 2010). Interestingly, aqueous extract of saffron was shown to enhance the brain dopamine levels in a dose-dependent manner in a rat model (Ettehadi et al., 2013). Further, pre-treatment of mice with saffron (through drinking water) was reported to offer significant neuroprotection to nigral, and retinal dopaminergic neurons in MPTP-treated mice (Purushothuman et al., 2013). However, to the best of our knowledge, the efficacy of saffron and crocin in experimental models of PD have not been investigated.

Given the well-known antioxidant attributes of saffron—its bioactives and the increasing evidence emerging on their neuroprotective propensity, we hypothesized that saffron is likely to alleviate locomotor phenotype oxidative stress and neurotoxicity in an idiopathic Drosophila PD model. The advantages of employing the fly model to understand the neuroprotective potential of phytochemicals as well as pharmacological agents has been well appreciated in the recent past (Celotto and Palladino, 2005; Hosamani and Muralidhara, 2009; Sudati et al., 2013; Girish and Muralidhara, 2012; Laurent et al., 2013; Phom et al., 2014). In this context, utilizing Drosophila as a model system, we investigated the neuroprotective efficacy of saffron methanolic extract (SME) and its active ingredient, Crocin (CR) focussing on aspects related to their potential to abrogate rotenone (ROT)-mediated neurotoxic outcome. Initially, we determined whether SME and CR-enriched medium (for 7 days) could modulate the endogenous levels of oxidative markers in flies. Further, employing a co-exposure paradigm, their efficacy to alleviate ROT-induced locomotor deficits, oxidative impairments in head/body regions of flies, cholinergic function, and dopamine levels were determined. We further assessed their prophylactic potential following an acute paraquat (PQ) challenge in terms of lethality and locomotor phenotype in an oxidative stress bioassay (Hosamani and Muralidhara, 2013). Furthermore, we also investigated their ability to delay the development of locomotor deficits and extend the longevity of flies under chronic ROT exposure.

2. Materials and methods

2.1. Chemicals

Rotenone (ROT), Paraquat (PQ), dopamine, Crocin (CR) and other fine chemicals were procured from M/s Sigma Chemical Co. (St. Louis, USA). All other chemicals were of analytical grade and procured from Sisco Research Laboratory Chemicals (Mumbai, India).

2.2. Preparation of saffron methanolic extract (SME)

Stigmas of saffron, *Crocus sativus* purchased from local market were finely cut, dried and macerated with methanol (1:50 w/v) in dark under continuous stirring for 72 h. The extract was centrifuged and filtered through 0.2 μm filter and evaporated to dryness using a rotary evaporator. The dry residue was stored at −20 °C until use. The final yield was 25%.

2.3. Quantification of saffron constituents in SME

The quantification was carried out by Shimadzu HPLC LC-10 AD VP system accessorized with an UV–vis detector, using HPLC column (Make: 'Discovery' Supelco Sigma–Aldrich; C-18, 25 cm–4.6 mm, 5 mm) and an isocratic mobile phase of acetonitrile: water (76:24%) at a flow rate of 1.2 ml/min.

2.4. Drosophila culture

Drosophila melanogaster, wild type (Oregon K) was originally procured from the National Stock Facility, Manasagangotri, University of Mysore, Karnataka, India. Flies were maintained and cultured at the fly laboratory of our research institute under standard conditions (22 °C, 70–80% relative humidity) on a standard wheat flour-agar diet with yeast granules as the protein source (Hosamani and Muralidhara,2009). For all the studies, age synchronized adult (9–10 days old) male flies (50 per replicate; 3 replicates per group) were introduced into glass vials with 2 ml medium containing the test compounds.

2.5. Experimental design

2.5.1. Study 1: effect of SME and CR on endogenous levels of oxidative markers

For this study, adult male flies ($n = 50$/replicate; 3 replicates per group) were maintained on either SME (0.05 and 0.1%) or CR (10 and 25 μM)-enriched medium for 7 days to assess their ability to modulate the endogenous levels of oxidative markers and antioxidant enzymes in head and body regions of flies. The criteria of selected concentrations were based on our preliminary findings.

2.5.2. Study 2: neuromodulatory effect of SME and CR against ROT exposure

In this study, employing a co-exposure paradigm, the propensity of SME and CR to attenuate ROT-induced mortality response, locomotor dysfunction, oxidative stress and neurotoxicity were determined in independent experiments. The concentration of ROT (500 μM, 7 days) used was selected based on our previous findings in flies. Flies were monitored regularly for the prevalence of mortality and locomotor deficits (negative geotaxis assay). For all the studies, SME (0.05 and 0.1%) and CR (10 and 25 μM) were used at two concentrations only. Terminally, various biochemical measurements were made in head and body regions separately.