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PERSPECTIVE

Neuroprotective properties of dietary saffron: more than just a chemical scavenger?

There is increasing evidence that consumption of saffron, a spice derived from the flower of the *Crocus savitus* plant, has various therapeutic effects, including protection of the central nervous system. These beneficial effects have been widely attributed to the strong antioxidant properties of saffron and its key constituents, although data showing that these antioxidative properties are potentiated by the presence of living cells (Ordoudi et al., 2009) suggest actions that extend beyond direct chemical scavenging. One recent study (Skladnev et al., 2016) has provided evidence that dietary saffron can modulate the brain transcriptome, up-regulating specific stress-inducible pathways – coupled with the observation that saffron exhibits a non-linear dose-response relationship, these findings suggest that saffron, like other phytochemicals, might exert its neuroprotective effects by acting as a mild biological stressor.

Saffron as a neuroprotective intervention: Whilst medical advances have allowed many of us to reach the sixth and seventh decades of life and beyond, one undesirable consequence of this lifespan extension has been a rapid rise in the prevalence of age-related neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. Current medical treatments can partly ward off some of the devastating symptoms of these diseases but do little to slow or prevent the underlying degeneration.

The lack of success in pharmaceutically targeting proposed disease-specific processes, despite considerable research and investment, has spawned a divergent field of research focused on addressing the common destructive processes (*e.g.*, oxidative stress, inflammation, mitochondrial dysfunction) upon which all neurodegenerative diseases converge. One potential intervention that has shown efficacy in mitigating these destructive processes is the ingestion of certain phytochemicals – natural compounds derived from plants (Murugaiyah and Mattson, 2015). One source of phytochemicals earning increasing scientific attention is the spice saffron.

Saffron has a long history of medicinal use, being purported to hold healing properties since at least the 7th century BC and continuing to play a part in modern traditional medicines. Compositional analysis of saffron has revealed over 150 compounds, making attribution of therapeutic benefits to particular components difficult, however most studies aiming to identify the bioactive molecule(s) have focused on the three major metabolites: crocin, picrocrocin and safranal.

Although early research pointed to the anticancer properties of saffron (Abdullaev, 2002), it was not until the pioneering work of Maccarone and colleagues in models of retinal degeneration that saffron was repurposed for use in combating neurodegenerative disease. The pre-clinical results were promising, with as little as two days of saffron pre-conditioning mitigating retinal damage induced by bright light exposure (Maccarone et al., 2008). Falsini and colleagues extended these observations of saffron-induced neuroprotection to patients with early stage age-related macular degeneration, showing that 20 mg saffron supplementation per day improved retinal function (Falsini et al., 2010; Piccardi et al., 2012).

Extending these findings to the brain, Ahmad and colleagues demonstrated that 7 days of pre-treatment with crocetin (a key component of saffron) could protect against midbrain neuron degeneration in a 6-hydroxydopamine rat model of parkinsonism (Ahmad et al., 2005). Furthermore, work in our laboratory showed that 5 days of pre-conditioning with whole saffron, ground and delivered in drinking water, could provide neuroprotection to mice exposed to the parkinsonian neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) (Purushothuman et al., 2013).

The mechanisms underlying these neuroprotective effects have been largely ascribed to the strong antioxidant properties of several constituents of saffron (in particular crocetin), which in turn mitigate downstream consequences such as oxidative stress and apoptosis. However, the observation that the antioxidant effects of saffron extract and purified crocetin are greatly potentiated by the presence of living cells (Ordoudi et al., 2009) suggests either that additional cell-mediated metabolism is required to enhance saffron's direct scavenging capacity or that saffron acts by stimulating cells to upregulate their endogenous antioxidant defenses.

One mechanism by which endogenous defenses might be activated is in response to stress, which raises the important question of whether saffron, like other phytochemicals (Murugaiyah and Mattson, 2015), confers its neuroprotective effects by acting as a mild stressor, conditioning cells against more severe subsequent insults. To our knowledge, this possibility has not been widely considered. Evidence for this hypothesis would rely on (at least) the following two conditions being met: (i) the relationship between saffron dose and degree of neuroprotection would be biphasic rather than linear, and (ii) upregulation of stress-inducible molecules and pathways would be observed upon exposure to saffron.

Evidence to this effect was uncovered by a recent study from our group (Skladnev et al., 2016) that sought to understand both the dose-response relationship of saffron and its mechanisms of action in the context of neuroprotection. Adopting the saffron administration protocol of Purushothuman (2013), whereby saffron was delivered as an aqueous extract in drinking water (0.01% (w/v)), we pre-conditioned mice with saffron for 2, 5 or 10 days, before administering the parkinsonian neurotoxin MPTP and allowing mice to survive for 7 days. Using immunohistochemistry to assess two main outcome measures in brain sections – number of functional dopaminergic cells in the substantia nigra pars compacta (SNc) and neuronal activity in the caudate-putamen complex (which is innervated by dopaminergic projections from the SNc) – we found that 5 days of pre-conditioning was more effective in providing neuroprotection 2 or 10 days pre-conditioning. Although more extensive validation studies using a broader range of doses (including variations in both the daily dose and period of pre-conditioning) will be required to confirm these observations, our data are suggestive of biphasic dose-response relationship. While we have not encountered other studies that report inhibitory effects at higher saffron doses, it has been shown that saffron is toxic at very high doses, with an LD50 in mice of > 600 mg/kg (Nair et al., 1991), supporting the notion that the dose-response relationship is non-linear.

Our study also aimed to explore the molecular networks that are regulated by saffron, and thus provide insights into its mechanisms of action, by assessing the brain transcriptome following saffron pre-conditioning. Importantly, this analysis was conducted in the absence of an insult (*e.g.*, MPTP), in order to dissect the molecular systems that are stimulated or depressed as a direct response to saffron treatment from systems that are

perturbed by the insult and restored by the neuroprotective intervention. Despite observing an overall down-regulation of broad, generic pathways relating to cell damage and death, the results suggested significant up-regulation of specific stress-inducible pathways, such as Fas and TNFR1 signalling. In addition to concerted changes across molecular networks, we also observed various gene expression changes that would usually be associated with a stress response.

Together, these findings provide evidence in support of the idea that saffron might indeed act as a mild stressor, and that this mechanism of action might play an important role in its capacity to induce a neuroprotective response. The biphasic dose-response relationship we observed is reminiscent of the phenomenon of hormesis, whereby a stimulus that is ineffectual or even damaging at a particular dose is beneficial at a lower dose. Similar observations have been made for other phytochemicals, for example phenolics and alkaloids, as reviewed elsewhere (Murugaiyah and Mattson, 2015). Further research is now required to determine whether saffron activates similar signalling pathways to other neurohormetic phytochemicals.

If further evidence supports the hypothesis that saffron induces a cellular stress response, dosage recommendations may need to be given more careful consideration. Clinical trials of saffron for treating early stage age-related macular degeneration have demonstrated efficacy with 20 mg of saffron supplementation per day (Falsini et al., 2010; Piccardi et al., 2012; Marangoni et al., 2013), equating to daily dose of < 0.5 mg/kg, far lower than the estimated 14 mg/kg/d delivered in the study by Skladnev et al. (2016). While this suggests that saffron may have a relatively broad therapeutic window, it remains unclear exactly what concentrations of saffron or its many constituents reach different target tissues. For example, how efficiently does saffron or its constituents penetrate the blood-brain barrier? Although recent studies using an *in vitro* model have demonstrated that crocetin may be able to cross the blood-brain barrier (Lautenschlager et al., 2015), this has yet to be confirmed *in vivo*. It will also be important to better delineate the contributions of, and interactions between, the many compounds that comprise saffron, in order to optimize dosage or design purified extracts to circumvent any antagonistic relationships that might exist.

Conclusions: In summary, increasing evidence points towards the potential neuroprotective actions of saffron and other dietary phytochemicals in the context of neurodegenerative disease. Previous explanations for this beneficial effect have focused largely on the strong antioxidant capabilities of saffron, but there is now evidence to suggest that saffron can act by modulating the brain transcriptome in the absence of an insult.

While most previous studies have assumed a relatively linear dose-response relationship for saffron, whereby increasing the dose or duration of treatment would be associated with greater protection, our recent results instead point to the possibility of a biphasic dose-response relationship. When coupled with the observation of an up-regulation of stress-inducible molecular pathways upon saffron treatment, these findings suggest a hormesis-like phenomenon, placing saffron amongst a variety of other dietary phytochemicals thought to confer beneficial effects by acting as mild biological stressors.

Although further research is needed to better elucidate the optimal treatment regime for eliciting neuroprotection and the mechanisms underlying this effect, the many advantages of saffron consumption as an intervention (*e.g.*, non-invasive, safe at therapeutic doses, easy to administer) provide strong impetus to further develop this line of therapy.

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Nicholas V. Skladnev, Daniel M. Johnstone*
Bosch Institute, University of Sydney, NSW, Australia; Discipline of Physiology, University of Sydney, NSW, Australia
***Correspondence to:** *Daniel M. Johnstone, Ph.D., Daniel.Johnstone@sydney.edu.au.*
***Accepted:** 2017-01-18*
***orcid:** 0000-0003-2876-3299 (Daniel M. Johnstone)*

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