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Pharmacological effects of *Crocus Sativus* (Saffron) on memory and anxiety in the zebrafish species Danio rerio Ava Azizi, Dornu Biragbara, Lori McGrew, PhD DeBusk College of Osteopathic Medicine, Lincoln Memorial University, Harrogate, TN 37724

Abstract

The Crocus Sativus flower gives rise to the Saffron spice which contains five active ingredients known as crocin, crocetin, picrocrocin and safranal. The rich antioxidant containing components of Saffron have been shown to cross the blood brain barrier by passive transcellular diffusion, making them effective in relation to treating neurological disorders.

In order to test the effects of Saffron on memory and anxiety, Zebrafish Danio Rerio were placed into two groups and put through a series of assessments including a threecompartment memory tank and a dive tank. Zebrafish continue to be at the forefront of behavioral and pharmacological studies due to their ability to display real time effects of substances in the environment. Many strong similarities have been drawn between zebrafish's neuroanatomy and physiology in comparison to mammals, making them a reliable tool in assessing environmental toxicity. Zebrafish have shown to be a great asset in drug screenings and preclinical trials due to their sensitivity to pharmacological stimulants and fast phenotypic responses.

Saffron was administered to a test group via skin absorption, while the control group was transported directly from their habitat to the assessment tanks. The three-compartment memory tank consisted of a middle, right, and left chamber separated by movable dividers. Subjects were initially placed in the center compartment and given a choice of swimming either towards the right or left sections. The two sides of the tank contained visual cues which included photos of shapes taped to the wall. The choice of swimming towards the correct compartment was rewarded by the subject being reunited with their tank mates; while making the incorrect decision led to adverse stimuli such as reduction of swim area. Subject memory was tested by their ability to promptly swim towards the intended side during each trial.

It was hypothesized that subjects in the test group will learn to swim in the correct compartment significantly faster than the control group. The anxiety evaluation was conducted by individually placing subjects from the control and test groups into a dive tank. The dive tank utilizes vertical distribution in a novel environment to measure the behavioral response in Zebrafish. Once placed in the tank, the subjects will combat their inherent nature of seeking protection in an open environment. During each trial, subject movements, behavior, and activity were tracked and analyzed by the Noldus Ethovision software. It was hypothesized that Saffron dosed Zebrafish will show a reduction in anxiety by increasing their vertical swimming and exploration significantly faster than those in the control groups.

Introduction

The Saffron spice has been a vital part of culinary culture in countries such as Iran for thousands of years (3). This unique flavoring agent has also historically been used medicinally due to its many therapeutic qualities (1). Recent clinical trials revealed the effectiveness of saffron in producing similar effects on treating cognitive impairments such as Alzheimer's disease in comparison to traditional medications (2). Findings have shown Saffron's active ingredients constituted antidepressant and anti-anxiety properties in pre-clinical and clinical trials (4). Saffron is considered to be efficacious in treating psychological disorders while simultaneously producing fewer reported side effects in comparison to current antidepressant medication including citalopram, fluoxetine, and imipramine.

Results Cumulative percentage of time in upper zone 10 ug/ml saffron 1 ug/ml saffron Treatment



Fig 1. Zebrafish spent significant more time in upper zone under less anxiogenic dose of lug/ml Saffron







Fig 3. Saffron decreased mobility of Zebrafish at 1ug/ml and 10ug/ml





Fig. 4 Heat map Imaging



Our findings show the opposite of anticipated effects as the treatment of Saffron in both lug/ml and 10 ug/ml resulted in subjects spending less time in the upper dive tank quadrant, in addition to decreasing mobility and velocity. The less than anticipated data may result from subjects' failure to adequately absorb medication. Our study utilized saffron treatment via skin absorption whilst other studies with significant findings utilized injections. The efficacy of Saffron skin absorption versus injection is not yet fully understood, however, future directions may lead to further investigation. Another aspect of our treatment methodology which may have affected results, is the usage of water. Saffron contains traces of lipids which may not dilute in the water holding solution. The effects of solely using water to dilute treatment may be further explored in future directions. Overall, we believe more testing is needed to adequately measure the effects of saffron on memory and anxiety. Time constraints created limitations in our ability to analyze various dosages. Future directions include experimenting with multiple dosages and adjusting time used to absorb treatments.





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Methods

Zebrafish Danio Rerio control groups were individually placed in a holding tank containing system water before transfer to the three-compartment memory tank and dive tank for behavioral assessments. The test subjects were individually treated with a diluted concentration of saffron via skin absorption within the holding tank before placement into assessment apparatuses. Noldus Ethovision software recorded and traced movements of subjects to determine experienced levels of anxiety and overall effects of Safranal





Fig. 5 Zebrafish housing

Fig. 6 Dive tank

Discussion