Comparison the toxicity of saffron extract(*Crocus sativus L.*) and tartrazine in zebrafish model

Mahdieh Kiannezhad[1], Jalal Hassan[2], Mohammad Kazem Koohi[3], Amir Moghaddam Jafari*[1]

1. Department of Toxicology, Faculty of Veterinary Medicine, Ferdowsi University of Mashhad, Mashhad, Iran.
2. Department of Comparative Biosciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.
3. Department of Toxicology, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

ABSTRACT

1. INTRODUCTION

Tartrazine (TTZ) is used as colorant in food products, medicines, and cosmetics. This is also used as a substitute for saffron (*Crocus sativus L.*) due to the color similar to saffron, low cost, ease of access and stability. Tartrazine is a monoazoprazolone dye, recently researches on its potential teratogenic properties in pregnant women and the possibility of passing the dye from the placenta to the fetus were often conducted on mice and confirmed the creation of teratogenic effects in mice. zebrafish is an excellent model that be comparable to the first trimester of development of human embryos and within 72 hours, early development of zebrafish is complete and most of the internal organs, including the cardiovascular system, liver and kidney, have developed, which can be comparable to the first trimester of development of human embryos, so in this study, toxicity of tartrazine and soffron extract on zebrafish larvae has been investigated.

Table 1. *CAS Number, FD&C Number, Purity, Solubility, and Test Concentrations of the Azo Dye.*

	Tartrazine
CAS number	1934-21-0
FD&C number	FD&C Yellow No. 5
Purity	>85%
Concentrations, %	(0, 0.0001, 0.001, 0.01, 0.1, 1, 5 and 10%)
Water solubility	260 g/L (30°C)

2. MATERIALS AND METHODS

The embryos were randomly distributed in 24-well plates at a density of 20 embryos/well, containing 1 mL of exposure media per well with the various test concentrations of each dye. The test concentrations were selected following the OECD Test Guidelines (2013) and methods of Joshi and Katti.1() All experiments were done in triplicate, and each assay was repeated 3 times. An azo dye, tartrazine, has been compared saffron extract, and their developmental toxicity on zebrafish (*Danio rerio*) from gastrulation stage (5.25 hours postfertilization [hpf]) until hatching and developmental trajectory was traced up to day 5. *Danio rerio* embryos in the laboratory (n= 20/concentration) exposed to graded dilutions of tartrazine and saffron extract (0, 0.0001, 0.001, 0.01, 0.1, 1, 5 and 10%). median lethal concentration (LC50), median effective concentration (EC50), teratogenic index (TI) for each two dyes were calculated at 96 hours after postfertilization, the scoring of lesions was done at 120 hours after fertilization.

3. RESULTS

The results showed that exposure to 1% of each dye was completely embryo lethal. the exposure of the embryos from 0.0001% to 0.1% tartrazine can cause hatching problems and developmental abnormalities such as edema of the cardiac area and yolk sac, spinal defects including spinal curvature and tail deviation, malformation of facial areas, malformation of pectoral fins and body fins and caudal fins, also can cause non-formation or malformation of somites, but no morphological defects was seen in embryos exposed to saffron extract like control group (0%). Embryos exposed to 1% concentration of tartrazine extract was coagulated within 24 hours, the rate of mortality was higher than saffron. the fetus had the same growth as the 24-hour fetus in the control group like saffron group. For saffron extract, LC50 was 0.017% and the individual EC50 value for hatching was at 0.487% and TI ratio was of 0.034%. For tartrazine, the LC50 was 0.009% and the individual EC50 value for hatching was at 0.354% and the TI ratio was of 0.025, and the individual EC50 for edema of the cardiac and yolk sac was at 0.153% and the TI ratio was of 0.058.

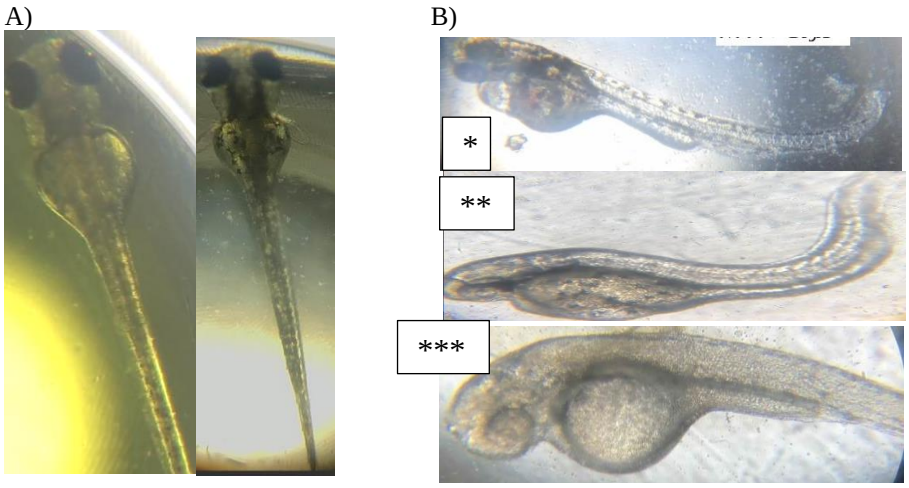

Fig. 1. *Developmental abnormalities in zebrafish embryos exposed to 2 dyes at 120 hours postfertilization (hpf), including cardiac edema, yolk sac edema and spinal defects including spinal curvature and tail distortio.* A) *exposed to Saffron extract and like control group without any morphological defect. B) * exposed to 0.0001% Tartrazine; ** exposed to 0.001% Tartrazine; *** exposed to 0.01% Tartrazine.*

Table 2. *LC50, EC50, and TI value at 96 hpf of Zebrafish Embryos Exposed to 2 Dyes.*

	SAFFRON	TARTRAZINE
LC_{50},(%)	0. 017	0.009
Individual EC50, %		
Hatching	0.487	0.354
TI (LC_{50}/EC_{50})	0.034	0.025
Individual EC50 Cardiac and Yolk sac edema, %	-	0.153
TI (LC_{50}/EC_{50})	-	0.058

4. CONCLUSION

Food additives (FAs) are the substances/chemicals supplemented during the processing of food either to enhance the nutritive value or quality or to enhance taste, flavor, freshness, and appearance (decoration/presentation). However, the safety limits and post consumption health hazards associated with these FAs are of apprehension. Therefore, in recent years, there is an increasing concern about the impacts of FAs on human. This study showed that tartrazine is more toxic than saffron extract. Saffron does not cause developmental toxicity in the same concentrations as tartrazine. Tartrazine and saffron are not teratogenic for zebrafish embryos up to a dose level of 0.1%.

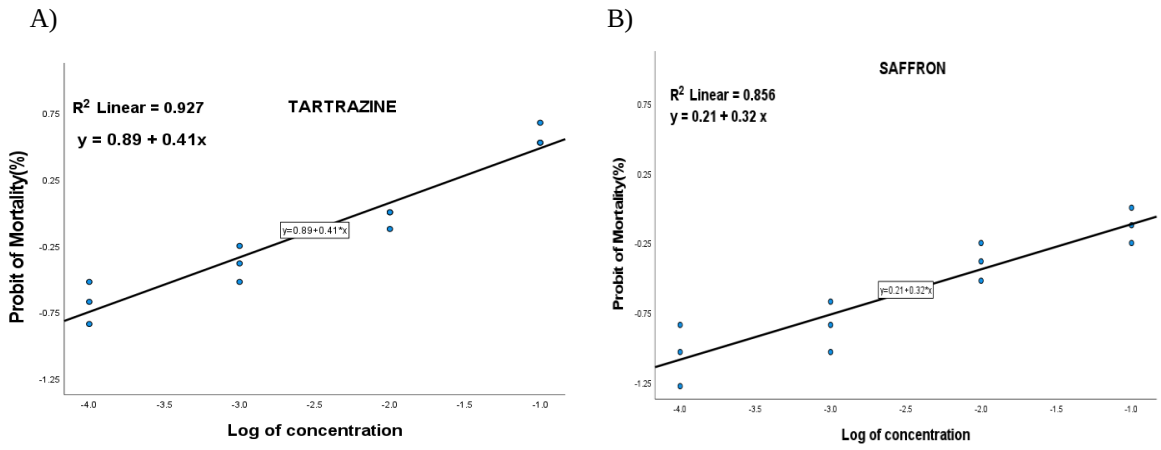

Fig. 1. *Mortality rate of zebrafish embryos exposed to two dyes at different concentrations at 96 hours postfertilization (hpf). (A) tartrazine; (B) Saffron.*

Keywords: Saffron, Tartrazine, Zebrafish, Developmental toxicity, Food colors

REFERENCES

(1) Nishimura Y, Inoue A, Sasagawa S, et al. Using zebrafish in systems toxicology for developmental toxicity testing. *Congenit Anom*. 2016;56(1):18-27. 15.
(2) Lantz-McPeak S, Guo X, Cuevas E, et al. Developmental toxicity assay using high content screening of zebrafish embryos. *J Appl Toxicol*. 2015;35(3):261-272
(3) Lantz-McPeak S, Guo X, Cuevas E, et al. Developmental toxicity assay using high content screening of zebrafish embryos. *J Appl Toxicol*. 2015;35(3):261-272
(4) Joshi V, Katti P. Developmental toxicity assay for food additive tartrazine using zebrafish (*Danio rerio*) embryo cultures. *International Journal of Toxicology*. 2018;37(1):38-44.
(5) OECD. Environment Directorate Joint Meeting of the Chemicals Committee and the Working Party on Chemicals, Pesticides and Biotechnology. 2013.
(6) Gupta R, Ranjan S, Yadav A, Verma B, Malhotra K. Toxic effects of food colorants erythrosine and tartrazine on zebrafish embryo development. *Current Research in Nutrition and Food Science Journal*. 2019;7(3):876-85.