POLISH JOURNAL OF NATURAL SCIENCES
Abbrev.: Pol. J. Natur. Sc., Vol 35(4): 419–439, Y. 2020

TARTRAZINE INCREASES OXIDATIVE STRESS AND MODULATES THE EXPRESSION OF APOPTOTIC-REGULATORY GENES IN *DROSOPHILA MELANOGASTER*

***Adedoja D. Wusu*[1], *Modupe O. Oyeniran*[2], *Jimoh O. Igbalaye*[3], *Solomon O. Rotimi*[4], *Olusegun K. Afolabi*[5]**

[1] ORCID: 0000-0001-5284-588X
[4] ORCID: 0000-0002-3678-9977
[5] ORCID: 0000-0002-8790-7451
[1–3] Department of Biochemistry, Lagos State University, Lagos, Nigeria
[4] Department of Biochemistry, Covenant University Canaan Land, Ota, Nigeria
[5] Department of Biochemistry, Ladoke Akintola University of Technology, Ogbomosho, Nigeria

Key words: Tartrazine, *Drosophila melanogaster*, food additive, oxidative stress, apoptosis.

Abstract

In this study, the effect of exposure to a food colorant tartrazine was investigated on biomarkers of oxidative stress and apoptosis in *Drosophila melanogaster*. *D. melanogaster* eggs were grown on cornmeal medium containing tartrazine at different concentrations (0, 250, 500, 1000, 2000 mg/kg). Third instar larvae of *D. melanogaster* were harvested. Oxidative stress markers were assayed spectrophotometrically while the level of expression of some apoptosis-regulatory genes were quantified using reverse polymerase chain reaction technique. Tartrazine significantly ($p < 0.05$) increased the levels of hydroperoxides and malondialdehyde in a dose-dependent manner. Interestingly, glutathione level increased among the groups, while the activities of glutathione transferase were reduced across the treatment groups. Tartrazine exposure resulted in misexpression of apoptosis and stress genes with a significant reduction in ANCE, HSP 27 genes. This study suggests that tartrazine induced oxidative stress and could induce apoptosis in *D. melanogaster* larvae, thus a risk factor in developmental toxicity.

Addres: Olusegun Kayode Afolabi, Ladoke Akintola University of Technology, Ogbomosho, Nigeria, phone: +2348062395836, e-mail: okafolabi@lautech.edu.ng

Introduction

Tartrazine (E102) is a synthetic lemon-yellow azo dye commonly used as food, drug and cosmetic colourant (El-Keredy 2017, Khayyat et al. 2017, Tripathy et al. 1989). It is also employed as a dye for wool and silk (Sarikaya et al. 2012, Tripathy et al. 1989). Due to its low cost and stability, tartrazine is widely used in numerous consumables, including food products (Amin et al. 2010, Khayyat et al. 2017, Walton et al. 1999). Some non-food consumables containing tartrazine include cosmetics, food supplements and some specific prescription drugs (Amin et al. 2010). Allowed Dietary Intake (ADI) of tartrazine is 7.5 mg/kg/day (Amin et al. 2010, Walton et al. 1999).

Most producers involved in food industry use food additives to achieve the desired organoleptic characteristics of each product. Conversely, these additives may have adverse health effects as demonstrated in several studies (Bateman et al. 2004, Raposa et al. 2016). As the consumption of these substances increased, the incidence of diseases, notably, eczema, headache, allergic asthma, diarrhoea, hyperactivity and hypersensitiveness also increased (Uysal et al. 2015). The ingestion of tartrazine has also been associated with some behavioural changes in children such as irritability, restlessness, and sleep disturbance (Bateman et al. 2004, Rowe 1988).

Tartrazine toxicity may result directly or indirectly from the biotransformation of its azo linkage (Chequer et al. 2011). Metabolic reduction of tartrazine in the intestine of the animal by the intestinal microflora results in the formation of two metabolites – sulfanilic acid and aminopyrazolone (Chung et al. 1992, Russ et al. 2000). These metabolites of tartrazine can generate reactive oxygen species (ROS), causing oxidative stress, and affect hepatic and renal architectures and biochemical profiles (Himri et al. 2011, Khayyat et al. 2017). Oxidative stress has been implicated in several diseases (Caro and Cederbaum 2004) and the process has also been established as a key pathomechanism of inflammatory, apoptotic and ageing processes (Askari et al. 2018, Hamishehkar et al. 2014, Raposa et al. 2016, Selvakumar et al. 2018).

There are contradictory reports of the toxicity of food colorants in literature. Azo dyes have been reported to be genotoxic, inducing DNA damage in treated animals (Tsuda et al. 2001). Conversely, another research showed no significant increase in the number of micronuclei found even at high dose of tartrazine while investigating for DNA damage in mice. However, tartrazine and azorubine increased the mRNA level of CYP1A1, which are pivotal in the metabolic activation of certain procarcinogenic

substances in the liver of mice (POUL et al. 2009). Also, altered kidney and liver function and oxidative stress biomarkers were observed in male rats after tartrazine and azorubine intake (AMIN et al. 2010). In the same study, alanine aminotransferase, aspartate aminotransferase, total protein, and albumin levels were significantly increased in the treated groups compared with the control. Furthermore, tartrazine exposure reportedly disrupted hepatic oxidative balance in the animals (AMIN et al. 2010). Increased tissue concentrations of enzymes involved in the oxidative mechanisms indicate that these food colorants may intervene in the multi-step process of inflammation and carcinogenesis (RAPOSA et al. 2016). Another comparative *in vivo* toxicity study administered different doses of tartrazine to guinea pigs showed no cytotoxic changes in tissues and organs and reported absence of the development of neoplastic alteration in the experimental animals (RUS et al. 2010).

The common fruit fly, *Drosophila melanogaster*, is a well-studied and highly tractable genetic model organism for understanding the molecular mechanism of human diseases (PANDEY and NICHOLS 2011). The extensive knowledge of the genetics of *Drosophila melanogaster* and the long experimental experience with this organism has made it of unique usefulness in mutation research and genetic toxicology (SARIKAYA et al. 2012). Many fundamental biological, physiological, and neurological properties are conserved between mammals and *D. melanogaster*. Nearly 75% of human disease-causing genes have a recognisable match in the genome of *Drosophila* and 50% of the fly protein sequences have mammalian homologs (PANDEY and NICHOLS 2011, SARIKAYA et al. 2012). Furthermore, *Drosophila* is a widely accepted model organism used in *in vivo* genotoxicity and mutagenicity studies (SARIKAYA et al. 2012, WANG et al. 2018).

It is established that azo dyes, such as, tartrazine are bio-transformed into metabolic intermediates capable of generating copious amount of reactive oxygen species. However, reports regarding the toxicity of tartrazine are inconsistent. Hence, the present study aimed to evaluate the toxicity of tartrazine by examining the biomarkers of oxidative stress and its apoptotic potentials in the 3rd stage instar larvae of *Drosophila melanogaster*.

Materials and Methods

Chemicals

Tartrazine CI19140 was a product of Jinzhou Tianyu Science & Technology Co., Ltd, China. Trizol®, RNAhold® and EasyScript® one-step

RT-PCR kit was obtained from TransBionovo Co., Ltd. Beijing, China. Other chemicals and reagents were of analytical standard and purchased from Sigma-Aldrich.

Raising of Flies

The flies and larvae were cultured on a standard Drosophila diet medium containing agar, fruit concentrate (apple), sugar, yeast, $MgSO_4$ and $CaCl_2$. Additional yeast suspension was provided for healthy growth of the organism. Flies were kept in darkness for the period of egg laying and harvest; otherwise, the flies were cultured in normal light/dark cycle at 25 ± 1^0C (UYSAL et al. 2015). The fruit agar is used to collect the eggs of adult female *Drosophila melanogaster*.

Experimental Design

The experiment was carried out using previously described procedures but with slight modification (RAHUL et al. 2015, UYSAL et al. 2015). The final concentration of tartrazine i.e. 250, 500, 1000 and 2000 mg/kg bw was established in diet of the respective groups, except for the control group, which contains no tartrazine. Vials were prepared and made into five different groups in triplicates. For each group, a specific concentration of sample solution was made in a total volume of 8 ml and feed was added to make up to a total of 80 ml. The prepared feed (25 ml) was placed into vials in each group and 35 µl of PBS-washed harvested *Drosophila melanogaster* eggs was pipetted into each vial. From each of the five groups, 20 developed third stage insta-larva were harvested after five days and placed in labelled 1.5 ml Eppendorf tube which was centrifuged. After that, 300 µl of homogenizing buffer (0.25 M sucrose, 1 mM EDTA, 10 mM HEPES-NaOH, pH 7.4) was added and then homogenized. Homogenizing buffer was added to make 1000 µl. The mixture was put in refrigerated centrifuge at 10000 g for 20 minutes at 4ºC. The supernatant was extracted into Eppendorf tubes and placed on ice until required for use.

Biochemical Assays

RNA Extraction Protocol

TriPure (a brand of Trizol) reagent (RIO et al. 2010) was added into eppendorf tubes containing ~20 3rd stage insta-larvae and homogenized. Thereafter, 200 µl of chloroform was added and vortexed for 30 seconds.

After incubating at room temperature for 10 minutes, the mixture was centrifuged at 11300 rpm for 15 minutes. Two layers appeared after centrifuging, with the top colourless layer containing the RNA. The RNA was transferred to a new labelled eppendorf tubes and 250 µl of iso-propanol was added and then mixed by inversion. This was left to seat at room temperature for 10 minutes, and then centrifuged at 1200 rpm for 10 minutes and the supernatant was collected after centrifuging. After addition of 75% cold ethanol, the resulting solution was properly mixed and then centrifuged at 9100 rpm for 5 minutes. The supernatant was carefully discarded and then pellet dried in a speed vacuum for 20 minutes. The dry pellet was dissolved in RNAfree water and used for gene expression analysis.

Gene Expression Analysis

The expression level of certain apoptotic, DNA methylating and chromatin modifying genes (Table 1) were quantified using relative reverse transcriptase polymerase chain reaction (RT-PCR) techniques as described by CHAUDHRY (2006), with appropriate modifications. In brief, about 500 ng

Table 1

List of genes studied and the sequences of Gene Specific Primers

Gene code	Gene name	Primer sequence (5'->3')
HSP 27	heat shock protein 27	forward: AAAGATGGCTTCCAGGTGTG
		reverse: CCCTTGGGCAGGGTATACTT
DMILP-2	insulin like peptide-2	forward: ACTCCCGCAGAGCCTTCATA
		reverse: GCTCAACGAGGTGCTGAGTA
ANCE	angiotensin converting enzyme	forward: ATATCGCCGGACAGAACGC
		reverse: CAGAAGTCCTGTGGCAGCTT
NC	DM death regulator NEDDE-like caspase	forward: ATTGGAATGCCGAAGAGGCA
		reverse: ATACGACGAGGAGGTCACCA
DCP-1	death caspase-1	forward: CCAAAAAGGGCGCAACAACT
		reverse: TGGCAGTGAAGTAGTGCCAG
DEBCL	death executioner BCL-2	forward: CCCAATCCCTCTAACGGACG
		reverse: TGTTCAGTGCCGGGAAAACT
β-TUB	Tubulin, Beta	forward: ACCAATGCAAGAAAGCCTTG
		reverse: ATCCCCAACAACGTGAAGAC

of RNA was used for the semi-quantitative RT-PCR using the Transgen® *EasyScript*® one-step RT-PCR reagent. Briefly, the cDNA synthesis was carried out at 45ºC for 30 minutes. This was followed by 35 cycles of PCR

amplification, using gene specific primers (GSP) – Table 1, in a C1000 Touch™ Thermal Cycler (BioRad, CA, USA). The cycles consisted of 94ºC for 30 s, 5min at the annealing temperature of GSP and 1min at 72ºC. The level of transcription of the genes relative to *β*-Tubulin was quantified using Image J® software (ABRÀMOFF et al. 2004).

Lipid Peroxidation

Lipid peroxidation was measured by the method of OHKAWA et al. (1979). Briefly, larva homogenate (50 µl) was added to the reaction mixture consisting of 8% SDS (50 µl), 20% acetic acid (40 µl) and 150 µl distilled water. Reaction was initiated by adding 400 µl of 1% TBA and terminated by 10% TCA. After centrifugation at 1000 rpm for 10 minutes, the supernatant was extracted and absorbance was read at 535 nm. MDA content of the sample was calculated by using the extinction coefficient of MDA, which is $1.56 \cdot 10^5$ M^{-1} cm^{-1}.

Glutathione Content Determination

GSH content in the exposed larvae were determined using Ellman's reagent (DTNB), following a previously described procedure (SINGH et al. 2009). The assay mixture consisted of 400 µL thiol reagent, 10 µL 0.01% 5, 5'-Dithiobis-2-nitro benzoic acid (DTNB), 25 µL TCA and 125 µL of the sample. The mixture was centrifuged at 5000 rpm for 5 minutes. The supernatant was collected, and absorbance was read at 412 nm after 5 minutes.

Hydroperoxide Assay

Hydrogen peroxide assay was performed following a previously described procedure (LONG et al. 1999). 50 µL of the sample was mixed with 200 µL of distilled water, immediately followed by the addition of FOX reagent (12.5 µL), thoroughly mixed and then incubated at room temperature for 30 minutes. Absorbance was then read at 560 nm against a water blank.

Acetylcholinesterase (AChE) Assay

Acetylcholinesterase activity was measured by the method of (ELLMAN et al. 1961) as reported by JAHROMI et al. 2013a. The assay reaction mixture consisted of 5 µL acetylcholine chloride, 25 µL DTNB (10 mM), 10 µL sample, 750 µL phosphate buffer (0.1 M, pH 8.0). The reaction mixture was thoroughly mixed and incubated at room temperature for 10 minutes. The change in absorbance was monitored against a water blank at 412 nm for 3 min.

Glutathione-S-Transferase (GST) assay

Glutathione-S-transferase activity was determined following a previously described procedure (RAHUL et al. 2015). The assay reaction mixture consisted of 500 µL of 0.1 M phosphate buffer, 50 µL of 10 mM CDNB, 50 µL of 10 mM reduced glutathione and 50 µL of the sample. The reaction mixture was thoroughly mixed by inversion, followed by immediate reading of absorbance against a buffer blank at 340 nm within 5 minutes.

Thioredoxin Reductase Assay

Thioredoxin reductase activity was determined following a previously described method (HOLMGREN and BJORNSTEDT 1995) with minor modifications (SINGH et al. 2009). The reaction mixture consists of 4 µL NADPH, 316 µL thioredoxin buffer, 40 µL DTNB and 40 µL BSA. The reaction mixture was incubated in the dark, at room temperature for 10 minutes. Thereafter, 25 µL of sample was added and the resulting mixture was left to stay for approximately 3 minutes. Absorbance was then read against a buffer blank at 410 nm at every 30 seconds for 3 minutes.

Chloramine Assay

Chloramine concentrations in larval homogenates were determined to measure the formation of oxidative protein products, using a previously described method (STANLEY et al. 2010). The assay reaction mixture contains 1000 µL DTNB and 50 µL sample. The mixture was then incubated in the dark, at room temperature for 15 minutes. Absorbance was read against a water blank at 412 nm.

Statistical Analysis

Statistical analysis was performed using a software program (GraphPad Prism 8.0.1) (GraphPad Software Inc., San Diego, CA, USA). All data were presented as mean ±S.E.M. One-way ANOVA followed by post-hoc Tukey's test was used to assess the significance of the difference between control and treatment groups.

Results

Enzymatic Antioxidant Activities Following Exposure to Tartrazine

Table 2 depicts the gluthathione transferase (GST), acetylcholinesterase (AChE) and thioredoxin reductase (TR) activities in *Drosophila melanogaster* larvae after 5 days of exposure to different tartrazine concentrations. There was a non-dose dependent decrease in the activity of GST in all treated groups in comparison to the control. However, significant reduction in GST activity was observed only in the groups exposed to 500 mg/kg bw and 2000 mg/kg bw at 0.660 ±0.210 kU/mg protein and 0.372 ±0.020 kU/mg protein respectively in comparison with the control (2.869 ±1.735 kU/mg protein), corresponding to 77% and 87% decrease in GST activity respectively. Also, the tartrazine exposed groups showed a non-dose dependent decrease in AChE activity when compared with the control, though not statistically significant. Meanwhile, all tartrazine exposed groups showed a statistically significant decrease in thioredoxin reductase (TR) activity. The observed TR activity corresponds to 40% (0.003 ±0.000 kU/mg protein), 60% (0.002 ±0.000 kU/mg protein), 40% (0.003 ±0.000 kU/mg protein) and 60% (0.002 ±0.000 kU/mg protein) reduction for 250, 500, 1000 and 2000 mg/kg.bw of tartrazine respectively in comparison with the control (0.005 ±0.000 kU/mg protein).

Table 2

GST, AChE and TR activities in *Drosophila melanogaster* larvae after 5 days of exposure to tartrazine

Group	GST [kU/mg protein]	AChE [kU/mg protein]	TR [kU/mg/protein]
Control	2.869±1.735^{a}	0.411±0.195^{a}	0.005±0.000^{a}
250 mg/kg bw	1.043±0.109^{a,b}	0.288±0.040^{a}	0.003±0.000^{b}
500 mg/kg bw	0.660±0.210^{b}	0.281±0.021^{a}	0.002±0.000^{b}
1000 mg/kg bw	1.264±0.750^{a,b}	0.339±0.019^{a}	0.003±0.000^{b}
2000 mg/kg bw	0.372±0.020^{b}	0.303±0.045^{a}	0.002±0.000^{b}

Each value is mean ±S.E.M for 20 3rd stage larvae. Values within a column with different alphabet superscripts are significantly different from each other at $p < 0.05$.

Non-enzymatic Antioxidants Levels and Oxidative Stress Parameters Following Exposure to Tartrazine

Table 3 depicts glutathione (GSH), malondialdehyde (MDA), hydrogen peroxide (H_2O_2) and chloramine levels in *Drosophila melanogaster* larvae after 5 days of exposure to different tartrazine concentration. There was

increase in GSH levels of the groups exposed to tartrazine. The increased GSH levels is non-dose dependent with statistically significant increase, compared with the control, being observed just in the groups exposed to 500 mg/kg bw and 2000 mg/kg bw at 1.110 ±0.213 nmole/mg protein and 1.247 ±0.138 nmole/mg protein respectively, which corresponds to a respective increase by 84% and 106%. Similarly, increase in MDA levels in the groups exposed to tartrazine was non-dose dependent. The groups exposed to 500 mg/kg bw, 1000 mg/kg bw and 2000 mg/kg bw of tartrazine, compared with the control, showed a statistically significant increase in malondialdehyde levels at (39.773 ±7.619 pmole/mg protein), (54.415 ±0.803 pmole/mg protein) and (52.283 ±0.328 pmole/mg protein), corresponding to 43%, 95% and 88% increase respectively. The highest level of MDA (54.415 ±0.803 pmole/mg protein) was observed at 1000 mg/kg bw of tartrazine concentration. Conversely to preceding observations, there was a dose-dependent increase in H_2O_2 levels across the tartrazine exposed groups. However, only the group exposed to 2000 mg/kg bw of tartrazine showed a statistically significant increase in H_2O_2 level (1.345 ±0.234 nmole/mg protein) when compared with the control (0.703 ±0.127 nmole/mg protein). This is equivalent to about 91% increase. In total deviation from all result patterns observed in this study, trends in chloramine levels across all groups appears irregular and there was no statistically significant effect on chloramine levels in tartrazine exposed groups compared with the control.

Table 3

GSH, MDA, H_2O_2 and Chloroamine levels present in *Drosophila melanogaster* larvae after 5 days of exposure to tartrazine

Group	GSH [nmole/mg protein]	MDA [pmole/mg protein]	H_2O_2 [nmole/mg protein]	Chloramine [pmole/mg protein]
Control	0.604 ± 0.001^{a}	27.812 ± 4.490^{a}	0.703 ± 0.127^{a}	216.545 ± 34.26^{a}
250 mg/kg bw	$0.754\pm0.049^{a,b}$	38.083 ± 3.040^{a}	0.787 ± 0.159^{a}	163.500 ± 74.45^{a}
500 mg/kg bw	$1.110\pm0.213^{b,c}$	$39.773\pm7.619^{a,b}$	$1.056\pm0.060^{a,b}$	313.687 ± 52.04^{a}
1000 mg/kg bw	$0.931\pm0.128^{a,b,c}$	54.415 ± 0.803^{c}	$1.121\pm0.107^{a,b}$	246.290 ± 19.02^{a}
2000 mg/kg bw	1.247 ± 0.138^{c}	$52.283\pm0.328^{b,c}$	1.345 ± 0.234^{b}	183.637 ± 07.63^{a}

Each value is mean ± S.E.M. for 20 *drosophila* larvae. Values within a column with different alphabet superscripts are significantly different from each other at $p < 0.05$

Expression Pattern of Apoptotic and Oxidative Stress genes

Figure 1 depicts the expression patterns of heat shock protein 27 (HSP27), inhibitors of apoptotic protein-like protein 2 (ILP2), negative cofactor 2 (NC2), angiotensin-1 converting enzyme (ANCE) and death caspase (Dcp) genes in *Drosophila melanogaster* larvae after 5 days

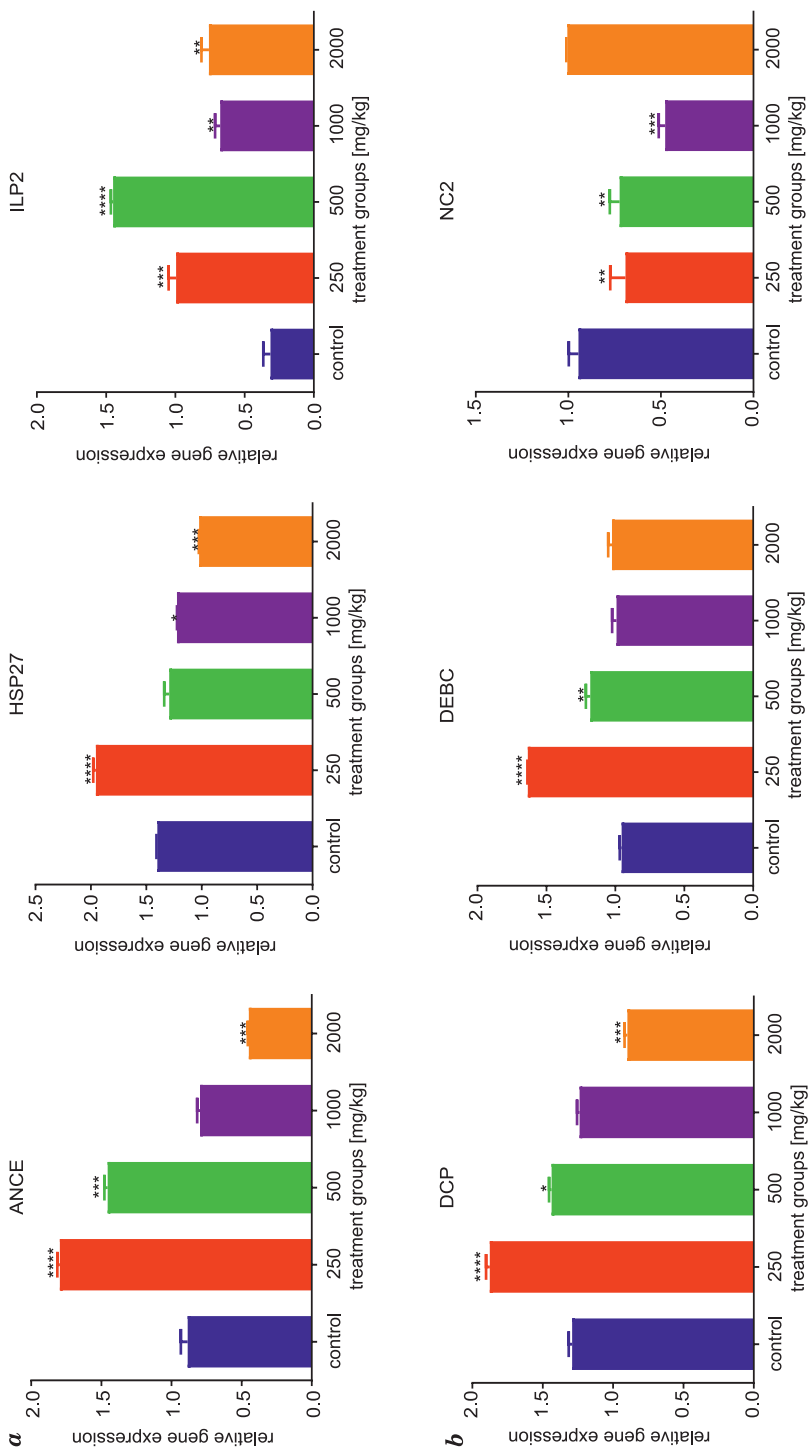

Fig. 1. Expression patterns of: *a* – oxidative stress development related genes (ANCE, HSP27, ILP2); *b* – apoptosis (DCP, DEBC, and NC2) in 3rd stage instar larvae of *Drosphilia melanogaster* after 5 days of exposure to tartrazine. Data represent mean ± S.E.M. * $p < 0.05$; ** $p < 0.01$, *** $p < 0.001$; **** $p < 0.0001$ as compared to control

of exposure to different tartrazine concentration. HSP27 gene expression increased significantly at the dose 250 mg/kg bw, which dropped to a non-significant level at the dose of 500 mg/kg bw. The HSP27 gene expression decreased further to a statistically significant level with increasing concentration of tartrazine at 1000 mg/kg bw and 2000 mg/kg bw as compared with the control. On ILP2 gene expression, an irregular non-dose related increase in the gene expression was observed in all tartrazine exposed groups, though all significantly higher when compared with control. Tartrazine exposure has no statistically significant effect on NC2 gene expression except for the significant reduction observed in the group exposed to 1000 mg/kg bw tartrazine. ANCE expression increased significantly at the dose of 250 mg/kg bw, which reduced slightly at the dose of 500 mg/kg bw, however, still significantly higher when compared with the control. The ANCE gene expression further decreased accordingly with increasing concentration, initially, to almost normal level at 1000 mg/kg bw and then to a statistically significant level at 2000 mg/kg bw dose of tartrazine as compared with the control. Expression pattern of Dcp gene was strikingly similar to that observed in ANCE gene expression. The Dcp gene expression increased significantly at 250 mg/kg bw but steadily reduced and finally to a statistically significant level at 2000 mg/kg bw dose of tartrazine when compared with the control.

Discussion

This present study undertaken to evaluate the toxic outcome of tartrazine administration in *Drosophila melanogaster* has shown definitive oxidative stress and apoptotic responses induced by the additive. Tartrazine elevated the oxidative stress parameters, MDA, H_2O_2, and chloramine contents in *D. melanogaster* larvae while decreasing the activities of the antioxidant enzymes, TR and GST. In addition, tartrazine modulated the expression of apoptotic and oxidative stress genes like HSP27, ILP2, ANCE and Dcp after 5 days of exposure.

Reactive oxidants are generated in biological systems both through endogenous processes and external factors including exposure to drugs, chemicals and pollutants. Due to the high reactivity of these oxidants, damage may occur to these biological systems. Increase in free radicals causes overproduction of MDA, which is a marker of oxidative stress (VAVÁKOVÁ et al. 2015). MDA is one of the final products of polyunsaturated fatty acid peroxidation (lipid peroxidation) in cells, and is usually implicated in the pathogenesis of various diseases such as atherosclerosis,

stroke and Graves' disease (AHMAD et al. 2008, PALIPOCH and KOOMHIN 2015). Tartrazine belongs to the group of azo dye food colorants and metabolized inside the body into aromatic amines by intestinal microflora. The resulting amines are able to generate ROS as part of their metabolism by the interaction of the active amino groups with nitrite or nitrate containing foods (MOUTINHO et al. 2007). In this study, tartrazine-treated groups showed a significant increase in MDA levels which suggests that high concentration of tartrazine might induce lipid peroxidation due to increase ROS, hence oxidative stress in biological system. This is consistent with reports where significant higher concentrations of MDA were observed in animals fed with synthetic dyes, such as tartrazine (EL-DESOKY et al. 2017, AMIN et al. 2010, GAO et al. 2011). In another study, tartrazine-treated rats exhibited elevated levels of MDA and nitric oxide (NO), indicating the manifestation of oxidative stress (KHAYYAT et al. 2017). In the same vein, there was a significant elevation of H_2O_2 level in the group with the highest tartrazine administration. This is an indication that high dose of tartrazine results in the generation of numerous superoxide radicals, resulting in increased oxidative stress. The elevated levels of malondialdehyde (MDA) and hydrogen peroxide (H_2O_2) in this study evidently indicate that tartrazine induces oxidative stress by generating ROS in the *Drosophila melanogaster* larvae.

Antioxidant defence systems constitute mechanisms by which living organisms combat oxidative stress by mopping up generated free radicals. These antioxidants can be enzymatic or non-enzymatic. Superoxide dismutase (SOD) is an enzymatic antioxidant that acts as living organisms first line of defence against ROS by catalysing their conversion to less reactive or inert species (MITTLER et al. 2004). SOD catalyses the dismutation of superoxide to hydrogen peroxide and oxygen (KOHEN and NYSKA 2002). The end product of the dismutation reaction H_2O_2 can be removed by the activity of the enzyme catalase (ONYEKA et al. 2012). The increased concentration of H_2O_2 could be an indication of a breakdown of the enzymatic antioxidant system in the larvae. Thioredoxin reductase (TR) is an important enzymatic antioxidant that catalyses the reduction of thioredoxin in a thiol-like redox system that involves several oxidoreductases including glutathione reductase and lipoamide dehydrogenase among others (MUSTACICH and POWIS 2000). It is a conserved protein that is involved in protecting organisms against various oxidative stress (CHENGA et al. 2018). TR plays important roles in a wide range of cellular processes, such as cell growth, DNA synthesis, apoptosis regulation, and antioxidative defence in organisms (CHO et al. 2001, MUSTACICH and POWIS 2000). It protects cells through redox homeostasis against oxidative stress (CHENGA

et al. 2018, MCCARTY et al. 2015, RAHLFS et al. 2002). This study revealed a significant reduction in TR levels in all the groups administered with tartrazine. This suggested that tartrazine interferes with thioredoxin recycling, thus affecting the availability of its reduced form required for important biological processes including free radical scavenging.

Glutathione (GSH) is one of the most abundant cellular antioxidants, providing protection against reactive species. The antioxidant activity comes from the free thiol group of GSH, which is easily oxidized non-enzymatically by electrophiles and other oxidants. GSH also serves critical roles in detoxification of electrophiles (WU et al. 2004). Furthermore, GSH plays predominant role in regulation of cellular and subcellular redox state, for example through reactions with glutaredoxin and protein disulfide isomerases to organize a proper tertiary structure of proteins through thiol-disulfide exchange (FRANCO et al. 2007). Glutathione S-transferases (GST) are a large and diverse group of enzymes which catalyze the conjugation of electrophilic xenobiotic substrates with the tripeptide glutathione (GSH; g-glu-cys-gly) (MA et al. 2018). Besides, GST can also serve as nonenzymatic binding proteins (known as ligandins) interacting with various chemical compounds that include steroid, thyroid hormones, bile acids, heme, bilirubin and fatty acids (CHO et al. 2001). Also, evidence suggests that GST is involved in cellular defense against diverse groups of toxic agents that may be generated in the environment or within the cell (MA et al. 2018). GST is involved in the metabolism of drugs and xenobiotics with numerous findings suggesting it having a vital role in resistance to stress (CURTIS et al. 2010, HAYES and STRANGE 2000, ROXAS et al. 2000). In this present study, a significant elevated level of GSH and significant reduction in GST activities were observed following a high dose of tartrazine in the 3rd instar larvae of *Drosophila melanogaster*. The observed elevated level of GSH might be an adaptive response of the larvae to the exogenous molecule, tartrazine or a failure of the glutathione utilizing system in the cell. The observed reduction in GST activities is suggestive of the latter contributing to the accumulation of GSH in the cell. The reduction in GST activities could have resulted from its inhibition or depletion following increased ROS generation with subsequent oxidation of the protein. This would effectively compromise the protein activity, eventually contributing to a poor clearance of tartrazine from the system which will further subject the larvae to increased chemical stress. Our results are in consonance with similar study reflecting the damaging effect of tartrazine on antioxidant defense system. The authors reported GSH, SOD and catalase activities were reduced while MDA levels increased in tissue homogenate of rats that consumed high tartrazine. They concluded

that tartrazine adversely affects and alters biochemical markers in liver and kidney at both high and low doses (AMIN et al. 2010).

Acetylcholinesterase catalyses the hydrolysis of neurotransmitters, primarily acetylcholine, into acetate and choline, serving essential neurologic function (LIONETTO et al. 2013). It is the most important biological component of cholinergic function (JAHROMI et al. 2013b). AChE is involved in membrane integrity and changes in membrane permeability occurring for synaptic transmission and conduction (SCHMATZ et al. 2009). This present study found no significant effect of tartrazine on AChE activity in the 3rd instar larvae of *Drosophila melanogaster*. Likewise, there was no significant effect of tartrazine on chloramines levels in the larvae. The reaction of proteins with chloramines and other chlorinated oxidants results in chlorination of amino acid residues and formation of group of products generally classified as advanced oxidation protein products (AOPPs), which are surrogate markers of inflammation (ÇAKATAY et al. 2008, KORKMAZ et al. 2013, MARROCCO et al. 2017). Despite evidence in our study of tartrazine's ability to disrupt antioxidant defense system, its lack of effect on both AChE activity and on chloramines level imply that tartrazine may not be neurotoxic and inflammatory in *D. melanogaster*.

Amidst several changes in cellular activity and physiology, stressed cells remarkably produce a highly conserved set of proteins, the Heat Shock or Stress Proteins (HSPs) and certain non-coding RNAs, like the hsrω transcripts in *Drosophila* and the satellite III transcripts in humans (ARYA et al. 2007, JOLLY and LAKHOTIA 2006, LAKHOTIA 2012). Hence, this study went further to investigate the effect of tartrazine on the level of expressions of certain stress and apoptosis related genes, such as, Hsp27, ILP-2, NC2, ANCE and Dcp genes in the 3rd stage instar larvae of *Drosophila melanogaster*. These genes have been reported to be differentially expressed at different stages of development (DWIVEDI and LAKHOTIA 2016).

Hsp27 is a 27-kDa protein, which represents a key component of the cellular adaptive response that helps maintain cellular homeostasis under stress. It exerts its anti-apoptotic influence by inhibiting cytochrome c and TNF-mediated cell death (ARYA et al. 2007, PAUL et al. 2002). Increased expression of Hsp27 during stress response correlates with better survival from cytotoxic stress (BRUEY et al. 2000, CONCANNON et al. 2003, DWIVEDI and LAKHOTIA 2016, PAUL et al. 2002). Findings from this study reveal a dose-dependent effect of tartrazine on Hsp27 gene expression in 3rd stage instar larvae of *Drosophila melanogaster*. At low dose, a significant increase in Hsp27 gene expression was observed as compared to the control, indicating an adaptive response to the stress induced by tartrazine in the larvae. However, higher doses of tartrazine resulted in significant reduction in

Hsp27 gene expression in the 3rd stage instar larvae of *Drosophila melanogaster*. This reduced expression might be as a result of the overwhelming burden or toxicity of high dose of tartrazine on the adaptive response mechanism and perhaps cellular damage via induction of apoptosis. On the other hand, our results depicted significant increase in the expression level of ILP-2 genes in all 3rd stage instar larvae groups administered with tartrazine. ILP-2 belongs to a protein family known as Inhibitors of Apoptosis Protein (IAP), which inhibits apoptosis via interaction with diverse component of the caspase signaling pathways (KHALILI et al. 2016, RICHTER et al. 2001). The gradual reduction of IL-2 expression after peaking at 500 mg/kg bw tartrazine dose indicates the potential toxicity of tartrazine at higher doses.

NC2 is a protein factor that interacts with TATA-binding protein (TBP) to mediate the initiation of transcription (MASSON et al. 2008). Research data have suggested that the association of NC2 with DNA bound TBP competes with the association of transcription factor IIA (TFIIA) and transcription factor IIB (TFIIB), and thus inhibits transcription initiation (CANG_and PRELICH 2002, KAMADA et al. 2001, XIE et al. 2000). It has also been demonstrated that NC2 does not only repress, but also activates transcription, in vitro and in vivo (CANG and PRELICH 2002, GEISBERG et al. 2001). NC2 becomes limiting for TBP association with a heat inducible promoter under heat stress (MASSON et al. 2008). This present study finds no remarkable effect of tartrazine on NC2 gene expression in the 3rd stage instar larvae of *D. melanogaster*.

Dcp is an effector caspase which actively participate in programmed cell death (apoptosis) (AKAGAWA et al. 2015, HOU et al. 2008, XU et al. 2009). In the fruit fly, dcp-1 plays an essential role in nurse cell deaths during mid oogenesis under condition of starvation (LAUNDRIE et al. 2003). Dcp-1 knock-out mutants are viable without apparent adult defects, hence, dcp-1 might not be crucial for normal development (MILLS et al. 2005, MURO et al. 2006, XU et al. 2006). ANCE is a *Drosophila* homolog of the mammalian Angiotensin-converting enzyme (ACE) (HARRISON and ACHARYA 2015, KIM et al. 2017). ACE is a dipeptidyl carboxypeptidase that converts Angiotensin I to Angiotensin II (KIM et al. 2017, TAGLIAZUCCHI et al. 2016). In mammals, ACE is pivotal to the regulation of the renin-angiotensin system (RAS) on blood pressure homeostasis by generating Angiotensin II, an active vasoconstrictor that increases blood pressure (KIM et al. 2017). It also play some roles in immune response, cytokine expression, and cell proliferation (HEENEMAN et al. 2007, NAVARRO et al. 2006, PLATTEN et al. 2009).

In *Drosophila*, tissue specific expression of the ANCE gene in the germinal disc has been reported, thus indicating that ANCE might play a key

role in the fly development (HURST et al. 2003, KIM et al. 2017). However, there are reports that ANCE is dispensable for the development of *Drosophila* fly (KIM et al. 2017). In this study, the level of expression of both the Dcp and Ance genes significantly increased at the different doses of tartrazine at 250 mg/kg bw and 500 mg/kg bw. The elevated levels of Dcp gene expression is an indication of the apoptotic potentials of tartrazine while the elevation of the Ance gene expression suggests that tartrazine produces ROS, thus causing oxidative stress. Tartrazine showed no significant effect on the expression of the genes at the dose of 1000 mg/kg bw, however, at 2000 mg/kg bw the expression of both Dcp and Ance genes were reduced significantly. The observed reduction could be as a result of the high toxicity of tartrazine at the very high dose, hence disrupting the cellular adaptative response of the fly.

In investigating the apoptotic potentials of tartrazine, the present study revealed rather complex results. Tartrazine could trigger apoptotic effects as seen in the elevated expression of ANCE and Dcp at low doses. However, the antiapoptotic effect of tartrazine was also revealed via the significant reduction of ANCE and Dcp expressions at high doses. This seemingly antiapoptotic effect of tartrazine was also observed via the non-dose dependent elevation of ILP2 expression. Similar to the findings of this present study, RAPOSA et al. (2016), reported that based on the nuclear factor kappa activated B cells (NFκB) and mitogen-activated protein kinase 8 (MAPK8) expressions – tartrazine alone could contribute to apoptotic effects at low concentration (41.2 mg/kg bw) and to anti-apoptotic effects at high concentration (412 mg/kg bw). The authors concluded that artificial food colorants result in the activation of inflammatory pathways favoring the development of cancers (RAPOSA et al. 2016). Meanwhile, 500 mg/kg bw tartrazine was reported to increase apoptotic characteristics of brain tissue of rats (GAO et al. 2011).

Conclusion

In conclusion, findings from this study indicate the oxidative stress and apoptotic potential of tartrazine and by extension, other food colorants. This emphasizes the need for adequate consumers' awareness of the potential toxicity of these food colorants and proper regulation of the concentration being added into food, especially for children. Moreover, there is need for further investigation paths to accurately define the range of concentrations of these food dyes capable of eliciting these toxic effects.

Accepted for print 27.07.2020

References

Abràmoff M.D., Magalhães P.J., Ram S.J. 2004. *Image processing with Image* J. Biophotonics Int., 11: 36–42.

Ahmad R., Tripathi A.K., Tripathi P., Singh S., Singh R., Singh R.K. 2008. *Malondialdehyde and protein carbonyl as biomarkers for oxidative stress and disease progression in patients with chronic myeloid leukemia*. In vivo (Athens, Greece), 22: 525–528.

Akagawa H., Hara Y., Togane Y., Iwabuchi K., Hiraoka T., Tsujimura H. 2015. *The role of the effector caspases drICE and dcp-1 for cell death and corpse clearance in the developing optic lobe in Drosophila*. Dev. Biol., 404: 61–75.

Amin K., Hameid II H.A., Elsttar, A.A. 2010. *Effect of food azo dyes tartrazine and carmoisine on biochemical parameters related to renal, hepatic function and oxidative stress biomarkers in young male rats*. Food Chem. Toxicol., 48: 2994–2999.

Arya R., Mallik M., Lakhotia S.C. 2007. *Heat shock genes – integrating cell survival and death*. J. Biosci., 32: 595–610.

Askari H., Rajani S.F., Poorebrahim M., Haghi-Aminjan H., Raeis-Abdollahi E., Abdollahi M. 2018. *A glance at the therapeutic potential of irisin against diseases involving inflammation, oxidative stress, and apoptosis: an introductory review*. Pharmacol. Res., 129: 44–55.

Bateman B., Warner J.O., Hutchinson E., Dean T., Rowlandson P., Gant C., Grundy J., Fitzgerald C., Stevenson J. 2004. T*he effects of a double blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children*. Arch. Dis. Child., 89: 506–511.

Bruey J.M., Ducasse C., Bonniaud P., Ravagnan L., Susin S.A., Diaz-Latoud C., Gurbuxani S., Arrigo A.P., Kroemer G., Solary E. 2000. *Hsp27 negatively regulates cell death by interacting with cytochrome c*. Nat. Cell Biol., 2: 645.

Çakatay U., Kayali R., Uzun H. 2008. *Relation of plasma protein oxidation parameters and paraoxonase activity in the ageing population*. Clin. Exp. Med., 8: 51–57.

Cang Y., Prelich G. 2002. *Direct stimulation of transcription by negative cofactor 2 (NC2) through TATA-binding protein (TBP)*. PNAS., 99: 12727–12732.

Caro A.A., Cederbaum A.I. 2004. *Oxidative stress, toxicology, and pharmacology of CYP2E1*. Annu. Rev. Pharmacol. Toxicol., 44: 27–42.

Chenga C., Maa H.L., Fenga J., Sua Y., Denga Y., Penga J., Guo Z.X. 2018. *Identification and mRNA Expression of Antioxidant Enzyme Genes in the Mud Crab (Scylla paramamosain) in Response to Acute Ammonia and Nitrite Exposure*. Isr. J. Aquacult-Bamid., 70: 2018.

Chequer F.M.D., Dorta D.J., De Oliveira D.P. 2011. *Azo dyes and their metabolites: does the discharge of the azo dye into water bodies represent human and ecological risks? Advances in treating textile effluent*. InTech.

Cho S., Lee Y., Park H., Ryoo K., Kang K., Park J., Eom S., Kim M., Chang T., Choi S. 2001. *Glutathione S-transferase mu modulates the stress-activated signals by suppressing apoptosis signal-regulating kinase 1*. J. Biol. Chem., 276: 12749.

Chung K.T., Stevens S.E., Cerniglia C.E. 1992. *The reduction of azo dyes by the intestinal microflora*. Crit. Rev. Microbiol., 18: 175–190.

Concannon C., Gorman A., Samali A. 2003. *On the role of Hsp27 in regulating apoptosis*. Apoptosis, 8: 61–70.

Curtis J.M., Grimsrud P.A., Wright W.S., Xu X., Foncea R.E., Graham D.W., Brestoff J.R., Wiczer B.M., Ilkayeva O., Cianflone K. 2010. *Downregulation of Adipose Glutathione S-Transferase A4 Leads to Increased Protein Carbonylation, Oxidative Stress, and Mitochondrial Dysfunction*. Diabetes, 59: 1132.

Dwivedi V., Lakhotia S.C. 2016. *Ayurvedic Amalaki Rasayana promotes improved stress tolerance and thus has anti-aging effects in Drosophila melanogaster*. J. Biosci., 41: 697–711.

El-Keredy A. 2017. *Experiment on the genetic toxicity Of tartrazine yellow and behavioral effects on Drosophila melanogaster*. Egypt. J. Genet. Cytol., 46.

Ellman G.L., Courtney K.D., Andres V., Featherstone R.M. 1961. *A new and rapid colorimetric determination of acetylcholinesterase activity*. Biochem. Pharmacol., 7: 88–95.

Franco J., Prediger R.D., Pandolfo P., Takahashi R.N., Farina M., Dafre A.L. 2007. *Antioxidant responses and lipid peroxidation following intranasal 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) administration in rats: increased susceptibility of olfactory bulb*. Life Sci., 80: 1906–1914.

Gao Y., Li C., Shen, J., Yin H., An X., Jin H. 2011. *Effect of food azo dye tartrazine on learning and memory functions in mice and rats, and the possible mechanisms involved*. J. Food Sci., 76: T125-T129.

Geisberg J.V., Holstege F.C., Young R.A., Struhl K. 2001. *Yeast NC2 associates with the RNA polymerase II preinitiation complex and selectively affects transcription in vivo*. Mol. Cell. Biol., 21: 2736–2742.

Hamishehkar H., Khani S., Kashanian S., Ezzati Nazhad Dolatabadi J., Eskandani M. 2014. *Geno- and cytotoxicity of propyl gallate food additive*. Drug Chem., Toxicol. 37: 241–246.

Harrison C., Acharya K.R. 2015. *A new high-resolution crystal structure of the Drosophila melanogaster angiotensin converting enzyme homologue, AnCE*. FEBS Open Bio., 5: 661–667.

Hayes J.D., Strange R.C. 2000. *Glutathione S-transferase polymorphisms and their biological consequences*. Pharmacology, 61: 154–166.

Heeneman S., Sluimer J.C., Daemen M.J. 2007. *Angiotensin-converting enzyme and vascular remodeling*. Circ. Res., 101: 441–454.

Himri I., Bellahcen S., Souna F., Belmekki F., Aziz M., Bnouham M., Zoheir J., Berkia Z., Mekhfi H., Saalaoui E. 2011. *A 90-day oral toxicity study of tartrazine, a synthetic food dye, in wistar rats*. Inter. J. Pharm. Pharmaceut. Sci., 3(3): 159–169.

Holmgren A., Bjornstedt M. 1995. *Thioredoxin and thioredoxin reductase*. Method. Enzymol., 252: 199–208.

Hou Y.C., Chittaranjan S., Barbosa S.G., Mccall K., Gorski S.M. 2008. *Effector caspase Dcp-1 and IAP protein Bruce regulate starvation-induced autophagy during Drosophila melanogaster oogenesis*. J. Cell Biol., 182: 1127–1139.

Hurst D., Rylett C.M., Isaac R.E., Shirras A.D. 2003. *The Drosophila angiotensin-converting enzyme homologue Ance is required for spermiogenesis*. Dev. Biol., 254: 238–247.

Jahromi S.R., Haddadi M., Shivanandappa T., Ramesh S. 2013a. *Neuroprotective effect of Decalepis hamiltonii in paraquat-induced neurotoxicity in Drosophila melanogaster: biochemical and behavioral evidences*. Neurochem. Res., 38: 2616–2624.

Jahromi S.R., Haddadi M., Shivanandappa T., Ramesh S.R. 2013b. *Neuroprotective effect of Decalepis hamiltonii in paraquat-induced neurotoxicity in Drosophila melanogaster: biochemical and behavioral evidences*. Neurochem. Res., 38: 2616–2624.

Jolly C., Lakhotia S.C. 2006. *Human sat III and Drosophila hsrω transcripts: a common paradigm for regulation of nuclear RNA processing in stressed cells*. Nucleic Acids Res., 34: 5508–5514.

Kamada K., Shu F., Chen H., Malik S., Stelzer G., Roeder R.G., Meisterernst M., Burley S.K. 2001. *Crystal structure of negative cofactor 2 recognizing the TBP-DNA transcription complex*. Cell., 106: 71–81.

Khalili S., Mohammadpour H., Barough M.S., Kokhaei P. 2016. *ILP-2 modeling and virtual screening of an FDA-approved library: a possible anticancer therapy*. Turk. J. Med. Sci., 46: 1135–1143.

Khayyat L., Essawy A., Sorour J., Soffar A. 2017. *Tartrazine induces structural and functional aberrations and genotoxic effects in vivo*. PeerJ 5: e3041.

Kim A.R., Choi E.B., Kim M.Y., Choi K.W. 2017. *Angiotensin-converting enzyme Ance is cooperatively regulated by Mad and Pannier in Drosophila imaginal discs*. Sci. Rep., 7: 13174.

Kohen R., Nyska A. 2002. *Invited review: Oxidation of biological systems: oxidative stress phenomena, antioxidants, redox reactions, and methods for their quantification*. Toxicol. Pathol., 30: 620–650.

Korkmaz G.G., Altinoglu E., Civelek S., Sozer V., Erdenen F., Tabak O., Uzun H. 2013. *The association of oxidative stress markers with conventional risk factors in the metabolic syndrome*. Metabolism, 62: 828–835.

LAKHOTIA S.C. 2012. *Long non-coding RNAs coordinate cellular responses to stress*. Wiley Interdiscip. Rev. RNA, 3: 779–796.

LAUNDRIE B., PETERSON J.S., BAUM J.S., CHANG J.C., FILEPPO D., THOMPSON S.R., MCCALL K. 2003. *Germline cell death is inhibited by P-element insertions disrupting the dcp-1/pita nested gene pair in Drosophila*. Genetics, 165: 1881–1888.

LIONETTO M.G., CARICATO R., CALISI A., GIORDANO M.E., SCHETTINO T. 2013. *Acetylcholinesterase as a biomarker in environmental and occupational medicine: new insights and future perspectives*. BioMed Res. Int., 321213.

LONG L.H., EVANS P.J., HALLIWELL B. 1999. *Hydrogen peroxide in human urine: implications for antioxidant defense and redox regulation*. Biochem. Biophys. Res. Comm., 262: 605–609.

MA L., ZHANG Y., MENG Q., SHI F., LIU J., LI Y. 2018. *Molecular cloning, identification of GSTs family in sunflower and their regulatory roles in biotic and abiotic stress*. World J. Microb. Biot., 34: 109.

MARROCCO I., ALTIERI F., PELUSO I. 2017. *Measurement and clinical significance of biomarkers of oxidative stress in humans*. Oxid. Med. Cell. Longev., 6501046.

MASSON P., LEIMGRUBER E., CRETON S., COLLART M.A. 2008. T*he dual control of TFIIB recruitment by NC2 is gene specific*. Nucleic Acids Res., 36: 539–549.

MCCARTY S., SCHELLENBERGER A., GOODWIN D., FUANTA N., TEKWANI B., CALDERÓN A. 2015. *Plasmodium falciparum thioredoxin reductase (PfTrxR) and its role as a target for new antimalarial discovery*. Molecules, 20: 11459–11473.

MILLS K., DAISH T., KUMAR S. 2005. *The function of the Drosophila caspase DRONC in cell death and development*. Cell Cycle, 4: 744–746.

MITTLER R., VANDERAUWERA S., GOLLERY M., VAN BREUSEGEM F. 2004. *Reactive oxygen gene network of plants*. Trends Plant Sci., 9: 490–498.

MOUTINHO I., BERTGES L., ASSIS R. 2007. *Prolonged use of the food dye tartrazine (FD&C yellow n° 5) and its effects on the gastric mucosa of Wistar rats*. Brazilian J. Biol., 67: 141–145.

MURO I., BERRY D.L., HUH J.R., CHEN C.H., HUANG H., YOO S.J., GUO M., BAEHRECKE E.H., HAY B.A. 2006. T*he Drosophila caspase Ice is important for many apoptotic cell deaths and for spermatid individualization, a nonapoptotic process*. Development, 133: 3305–3315.

MUSTACICH D., POWIS G. 2000. *Thioredoxin reductase*. Biochem. J., 346: 1–8.

NAVARRO J.F., MILENA F.J., MORA C., LEÓN C., GARCÍA J. 2006. *Renal pro-inflammatory cytokine gene expression in diabetic nephropathy: effect of angiotensin-converting enzyme inhibition and pentoxifylline administration*. Am. J. Nephrol., 26: 562–570.

OHKAWA H., OHISI N., YAGI K. 1979. *Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction*. Ann. Biochem., 95: 351–358.

ONYEKA C., ALIGWEKWE A., NWAKANMA A., BAKARE A., OFOEGO U. 2012. *Effects of ethanolic root bark extract of Chrysophyllum albidum on serum superoxide dismutase, catalase and malondialdehyde in rat*. Int. J. Pharm. Sci. Res., 3: 347–351.

PALIPOCH S., KOOMHIN P. 2015. *Oxidative Stress-Associated Pathology: A Review*. Sains Malays. 44: 1441–1451.

PANDEY U.B., NICHOLS C.D. 2011. *Human disease models in Drosophila melanogaster and the role of the fly in therapeutic drug discovery*. Pharmacol. Rev., 63: 411–436.

PAUL C., MANERO F., GONIN S., KRETZ-REMY C., VIROT S., ARRIGO A.P. 2002. *Hsp27 as a negative regulator of cytochrome C release*. Mol. Cell. Biol., 22: 816–834.

PLATTEN M., YOUSSEF S., HUR E.M., HO P.P., HAN M.H., LANZ T.V., PHILLIPS L.K., GOLDSTEIN M.J., BHAT R., RAINE C.S. 2009. *Blocking angiotensin-converting enzyme induces potent regulatory T cells and modulates TH1-and TH17-mediated autoimmunity*. PNAS. 0903958106.

POUL M., JARRY G., ELHKIM M.O., POUL J.M. 2009. L*ack of genotoxic effect of food dyes amaranth, sunset yellow and tartrazine and their metabolites in the gut micronucleus assay in mice*. Food Chem. Toxicol., 47: 443–448.

RAHLFS S., SCHIRMER R., BECKER K. 2002. T*he thioredoxin system of Plasmodium falciparum and other parasites*. Cell. Mol. Life Sci., 59: 1024–1041.

RAHUL, JYOTI, S., NAZ, F., SIDDIQUE Y.H. 2015. *Evaluation of the toxic potential of cefotaxime in the third instar larvae of transgenic Drosophila melanogaster*. Chem. Biol. Interact., 233: 71–80.

Raposa B., Pónusz R., Gerencsér G., Budán F., Gyöngyi Z., Tibold A., Hegyi D., Kiss I., Koller A., Varjas T. 2016. *Food additives: Sodium benzoate, potassium sorbate, azorubine, and tartrazine modify the expression of NFκB, GADD45a, and MAPK8 genes*. Physiol. Int., 103: 334–343.

Richter B., Mir S., Eiben L., Lewis J., Reffey S., Frattini A., Tian L., Frank S., Youle R., Nelson D. 2001. *Molecular cloning of ILP-2, a novel member of the inhibitor of apoptosis protein family*. Mol. Cell. Biol., 21: 4292–4301.

Rio D.C., Ares M., Hannon G.J., Nilsen T.W. 2010. *Purification of RNA using TRIzol (TRI reagent). Cold Spring Harb. Protoc*, pdb-prot5439.

Rowe K.S. 1988. *Synthetic food colourings and 'hyperactivity': a double-blind crossover study*. Aust. Paediatr. J., 24: 143–147.

Roxas V.P., Lodhi S.A., Garrett D.K., Mahan J.R., Allen R.D. 2000. *Stress tolerance in transgenic tobacco seedlings that overexpress glutathione S-transferase/glutathione peroxidase*. Plant Cell Physiol., 41: 1229–1234.

Rus V., Gherman C., Miclăuş V., Mihalca A., Nadăş G. 2010. *Comparative toxicity of food dyes on liver and kidney in guinea pigs: A histopathological study*. Ann. Romanian Soc. Cell Biol., 15: 161–165.

Russ R., Rau J., Stolz A. 2000. T*he function of cytoplasmic flavin reductases in the reduction of azo dyes by bacteria*. Appl. Environ. Microbiol., 66: 1429–1434.

Sarikaya R., Selvi M., Erkoç F. 2012. *Evaluation of potential genotoxicity of five food dyes using the somatic mutation and recombination test.* Chemosphere, 88: 974–979.

Schmatz R., Mazzanti C.M., Spanevello R., Stefanello N., Gutierres J., Correa M., Da Rosa M.M., Rubin M.A., Chitolina Schetinger M.R., Morsch V.M. 2009. *Resveratrol prevents memory deficits and the increase in acetylcholinesterase activity in streptozotocin-induced diabetic rats.* Eur. J. Pharmacol., 610: 42–48.

Selvakumar G.P., Iyer S.S., Kempuraj D., Raju M., Thangavel R., Saeed D., Ahmed M.E., Zahoor H., Raikwar S.P., Zaheer S. 2018. *Glia Maturation Factor Dependent Inhibition of Mitochondrial PGC-1a Triggers Oxidative Stress-Mediated Apoptosis in N27 Rat Dopaminergic Neuronal Cells*. Mol. Neurobiol., 55(9): 7132–7152.

Singh M.P., Reddy M.K., Mathur N., Saxena D., Chowdhuri D.K. 2009. I*nduction of hsp70, hsp60, hsp83 and hsp26 and oxidative stress markers in benzene, toluene and xylene exposed Drosophila melanogaster: role of ROS generation*. Toxicol. Appl. Pharm., 235: 226–243.

Stanley N.R., Pattison D.I., Hawkins C.L. 2010. *Ability of hypochlorous acid and N-chloramines to chlorinate DNA and its constituents*. Chem. Res. Toxicol., 23: 1293–1302.

Tagliazucchi D., Shamsia S., Conte A. 2016. *Release of angiotensin converting enzyme-inhibitory peptides during in vitro gastro-intestinal digestion of camel milk*. Int. Dairy J., 56: 119–128.

Tripathy N.K., Patnaik K.K., Nabi M.J. 1989. *Genotoxicity of tartrazine studied in two somatic assays of Drosophila melanogaster*. Mutat. Res., 224: 479–483.

Tsuda S., Murakami M., Matsusaka N., Kano K., Taniguchi K., Sasaki Y.F. 2001. *DNA damage induced by red food dyes orally administered to pregnant and male mice*. Toxicol. Sci., 61: 92–99.

Uysal H., Semerdoken S., Colak D.A., Ayar A. 2015. *The hazardous effects of three natural food dyes on developmental stages and longevity of Drosophila melanogaster*. Toxicol. Ind. Health., 31: 624–629.

Vaváková M., Ďuračková Z., Trebatická J. 2015. *Markers of oxidative stress and neuroprogression in depression disorder*. Oxid. Med. Cell. Longev., 898393.

Walton K., Walker R., Van De Sandt J., Castell J., Knapp, A., Kozianowski G., Roberfroid M., Schilter B. 1999. *The application of in vitro data in the derivation of the acceptable daily intake of food additives*. Food Chem. Toxicol., 37: 1175–1197.

Wang Y., Moussian B., Schaeffeler E., Schwab M., Nies A.T. 2018. *The fruit fly Drosophila melanogaster as an innovative preclinical ADME model for solute carrier membrane transporters, with consequences for pharmacology and drug therapy*. Drug Discov. Today, 23(10): 1746–1760.

Wu G., Fang Y.Z., Yang S., Lupton J.R., Turner N.D. 2004. *Glutathione metabolism and its implications for health*. J. Nutr., 134: 489–492.

XIE J., COLLART M., LEMAIRE M., STELZER G., MEISTERERNST M. 2000. *A single point mutation in TFIIA suppresses NC2 requirement in vivo*. EMBO J., 19: 672–682.

XU D., WANG Y., WILLECKE R., CHEN Z., DING T., BERGMANN A. 2006. T*he effector caspases drICE and dcp-1 have partially overlapping functions in the apoptotic pathway in Drosophila*. Cell Death Differ, 13: 1697.

XU D., WOODFIELD S.E., LEE T.V., FAN Y., ANTONIO C., BERGMANN A. 2009. *Genetic control of programmed cell death (apoptosis) in Drosophila*. Fly, 3: 78–90.