Effect of melatonin on the oxidative stress induced by the food additive (C.I. Food Yellow 3) on some blood parameters and antioxidant enzymes in male rat kidney

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Abstract: Many azo dye derivatives are used as food colourants in a number of products. The use of some food additives, however, has been restricted or totally prohibited because of concerns about cytotoxic effects. There is a new trend to use antioxidants to neutralise the suspected effects of food additives. The aim of this study was to evaluate the possible protective effect of melatonin in terms of reducing the cytotoxicity induced by the food colour additive C.I. Food Yellow 3 (CIFY3) in male rats (*Rattus norvegicus*). This evaluation was achieved through the measurement of different haematological parameters, antioxidant enzyme activities and lipid peroxidation, as well as histological examination of the kidney. Rats weighing 200-250 g were divided into four groups of five rats each: group 1, control; group 2, CIFY3-treated; group 3, melatonin-treated and group 4, melatonin- and CIFY3-treated. Groups 2-4 were repeatedly gavaged with 2.5 mg/kg body weight (bw) of CIFY3, 10 mg/kg bw of melatonin or both for three weeks. The study examined abnormalities in weight gain, hair colour and density, and changes in haematological parameters. The levels of thiobarbituric acid-reactive substances (TBAS), the activities of superoxide dismutase (SOD) and catalase, and the histological appearance of the kidney were also evaluated. The results revealed marked decreases in the percentage of body weight gain, white blood cell (WBC) counts and haemoglobin (Hb) content, whereas no significant changes were observed in red blood cell (RBC) count, mean corpuscular Hb concentration (MCHC), haematocrit value (Hct) or mean corpuscular volume (MCV). CIFY3 produced cytotoxic effects, as indicated by increases in TBAS levels, SOD activity and renal damage, whereas the catalase level was not affected. The results also indicated that oral melatonin administration significantly reduced the cytotoxic effect induced by CIFY3 through increase in WBC counts, Hb content and SOD activity, besides a decrease in TBAS levels, weight gain, hair colour and density which supports the use of supplemental melatonin as a chemopreventive antioxidant agent.
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Keywords: Melatonin; C.I. Food Yellow 3; SOD; catalase; lipid peroxidation; kidney haematological parameters; male albino rat.

Abbreviations: C.I. Food Yellow 3 (CIFY3), thiobarbituric acid-reactive substances (TBAS), superoxide dismutase (SOD), white blood cells (WBCs), platelets (PLTs), mean platelet volume (MPV), haemoglobin (Hb), red blood cells (RBCs), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), haematocrit value (Hct), mean corpuscular volume (MCV), packed cell volume (PCV/haematocrit) and red cell distribution width (RDW).

1. Introduction

Melatonin, an indolamine (N-acetylmethoxytryptamine, MLT), is primarily produced by the pineal gland and is able to limit oxidative stress and damage. Under physiological conditions, melatonin acts as a direct free radical scavenger via nuclear receptor or non-receptor pathways, leading to the inhibition of oxidative stress by modulating the detoxification of reactive hydroxyl radicals ($^{\cdot}OH$) and neutralising singlet oxygen, peroxynitrite anion, nitric oxide and hydrogen peroxide (H_2O_2) **(Kharwar and Haldar 2012; Reiter *et al.,* 2004; Reiter et al., 2012; Tan et al., 2002).** Melatonin also acts as an indirect antioxidant via its stimulatory action on the gene expression and activities of antioxidative enzymes **(El-Missiry et al., 2007; Rodriguez et al., 2004; Mayo et al., 2002; Sharma et al., 2008; Fischer et al., 2013).** This hormone controls reproductive functions and stimulates immune system activity and tumourigenesis **(Reiter et al., 2000; Singh et al., 2007).**

Food colourants can be naturally occurring or synthetic compounds. Synthetic food additives include colouring or curing agents and/or sweeteners added to food products in the food industry. They

have a large molecular mass and a strong anionic (from sulphate) or cationic charge to prevent their absorption in the gastrointestinal tract. Nonetheless, these pigments are absorbed in small quantities **(Nihon-shokuhin-tenkabutu-kyokai 1999).** Recently, food additives have attracted the attention of the public and the research community as potential causes of various human diseases. Many studies have proposed that these substances have genotoxic or mutagenic effects on experimental animals **(Tanaka 2007; Turkoglu 2007; Demir et al., 2008)**; food additives may be among the factors responsible for cancer and hepatic and nephritic failure.

Azo compounds are formed from arenediazonium ions conjugated through an azo linkage to highly reactive aromatic hydrocarbon compounds containing two aromatic rings, which are responsible for their intense colours **(Solomon 1996).** Tartrazine and carmoisine are nitrous derivatives of azo compounds that can be metabolised to highly sensitising aromatic amines, such as sulphanilic acid **(Maekawa et al., 1987; Feng et al., 2012; Amin et al., 2010).**

Many researchers have studied the impact of food colourants on the health of experimental animals **(Amin et al., 2010; Reyes et al., 1996; Tanaka 2006; Aboel-Zahab et al., 1997; Sasaki et al., 2002; Feng et al., 2012).** The addition of additives to food produces significant alterations in antioxidant enzyme activity **(Amin et al., 2010; Gao et al., 2011; Bansal 2005)** and has mutagenic effects **(Oliveira et al., 2010).** This toxicity and carcinogenicity may result from interactions between intact molecules and cytosolic receptors **(Oliveira et al., 2010; Lubet et al., 1983; Collier et al., 1983; Seesuriyachan et al., 2007),** from the formation of free radicals that alter the activity of antioxidant enzymes, or from arylamin azoreduction **(Nony et al., 1980; Pearce et al., 2003).**

Reactive oxygen species (ROS), such as $^{\cdot}OH$, superoxide anion radicals (O_2^-) and H_2O_2 are produced during normal metabolism or as a consequence of the response to abnormal stress. These ROS have been implicated in the pathogenesis of ageing and diseases, including cancer. Mammalian cells are equipped with both enzymatic and non-enzymatic antioxidant mechanisms to minimise the cellular damage that results from interactions between cellular constituents and ROS **(El-Habit et al., 2000; Shirazi et al., 2012).** The enzymatic antioxidant mechanism involves a number of enzymes, such as SOD, catalase, and glutathione peroxidise, as well as enzymes involved in recycling oxidised glutathione, such as glutathione reductase **(Halliwell 1992).** Many natural antioxidant products, such as β-carotene, melatonin and vitamins A, C and E, play roles in the protection against oxidative stress induced in different experimental models **(El-Habit et al., 2000; Kharwar and Haldar 2012).**

The aim of this work was to study the modulatory cytoprotective role of melatonin as an antioxidant on the physiological alterations in renal haematological indices and oxidative stress biomarkers caused by CIFY3 in male albino rats..

2. Material and Methods

Male Wistar albino rats obtained from the animal farm of King Saud University, Saudi Arabia, Riyadh and weighing 200-250 g were used in all the experiments. Handling of animals was in compliance with guidelines for the care and use of animals for scientific purposes. The animals were housed in cages and were maintained under controlled conditions of temperature, humidity and light (12:12 h light:dark cycle). They received food (a standard basal diet) and water *ad libitum*. CIFY3 was purchased from a local market (96%, Kamena Industries, Canada).

Animal treatment and samples collection

The physiological effects of CIFY3 were evaluated in male rats receiving repeated doses of CIFY3 alone. The possible protective role of melatonin against the cytotoxic effects of CIFY3 was also evaluated. The rats were divided into the following four groups of five rats each.

Control group. The control group did not receive any treatment and was maintained under the same living conditions of temperature and humidity as the other groups. **CIFY3 group.** The CIFY3 group (repeated dose treatment) was treated with 2.5 mg/kg bw of CIFY3 dissolved in distilled water administered orally once per day for three weeks. **Melatonin group.** The melatonin group was treated with 10 mg/kg bw of melatonin dissolved in distilled water administered orally once per day for three weeks. **CIFY3 and melatonin group** received both CIFY3 and melatonin as described in subsection CIFY3 and melatonin group.

After mild anaesthesia via the inhalation of diethyl ether for 1-2 min, blood samples were collected from the rats using a fine microhaematocrit tube inserted in the inner corner of the ophthalmic venous plexus. The blood samples were divided into three sets: whole blood, plasma and serum. The first two sets were collected in heparinised tubes.

During the experiment, the rats were weighed, and the percentage of body weight gain was calculated in each group.

Biochemical and hematological assays

The renal TBAS levels were measured as described by **Yoshioka et al. (1979).** The spectrophotometric determination of the SOD and

catalase activities was performed in kidney extracts **(Winterbourn et al., 1975; Bergmeyer et al., 1987).** Whole blood samples from treated animals were collected in heparinised tubes (2.25 µl heparin/5 ml blood) and were immediately analysed for blood components and haematological parameters.

The haematologic indices were determined according to standard methods. The tests included counts of RBCs, PLTs and WBCs. Other haematological parameters were also studied, including Hb concentration, MCV, MCH, MCHC, MPV, PCV/haematocrit and RDW. All the indices and haematological parameters were measured using a fully automated haematology analyser (Beckman Coulter, Germany; Ac.T 5diff CP) according to the manufacturer's instructions.

Histological examination

A microscopical examination of kidney sections was performed according to standard methods (Yenilmez et al., 2010). Pieces of kidney were fixed in 10% neutral-buffered formalin, embedded in paraffin, cut into sections 5 µm in thickness (microtome, Leica Rm 2145) and stained with haematoxylin and eosin. The microscopic examination was performed under an Olympus PM 10 ADS microscope (Olympus America Inc. Melville, NY, USA).

Statistical analysis

Student's *t*-test was applied for the statistical analysis of all the experiments. The data are presented as the mean±standard deviation (SD) and were statistically analysed using SPSS (Statistical Package for the Social Sciences) 20. Values were considered significant at $P<0.05$ and highly significant at $P<0.01$.

3. Results and discussion

In this study, the physiological effects of a synthetic food colourant (CIFY3) were examined through haematological and biochemical analyses, as well as histological examinations. The modulatory role of melatonin in the potential cytotoxic effects of CIFY3 was also evaluated. This is the first study to investigate the antioxidant role of melatonin against oxidative stress induced by CIFY3 in male albino rats. The purposes of this study were to establish diagnostically normal profiles of haematologic parameters, antioxidant enzymes and histology in the groups treated with CIFY3 and to evaluate the possible protective role of melatonin.

The data presented in Table 1 show a significant decrease in the percentage of body weight gain ($P<0.01$) after 21 days of treatment with CIFY3 compared to the control group (8.48 g ±0.87 and 11.36 g ±0.72, respectively) and a highly significant increase ($P<0.01$ and $P<0.001$) in the group treated with 10 mg/kg body weight (bw) melatonin and 2.5 mg/kg bw CIFY3 (16.38 g ±1.49) compared with the control and CIFY3 values. This decrease in body weight gain may occur due to the extensive hyperactivity within CIFY3 group. It has been observed that the decrease in body weight gain is accompanied by excessive hair loss. The administration of melatonin has reversed the effect of CIFY3. The decrease in body weight gain by other food additives has been reported elsewhere by many investigators **(Aboel-Zahab et al., 1997; Shaker et al., 1989; Takeda et al., 1992; AL-Shinnawy 2009).**

Table (1): Body weight gain percentage (%) in different animal groups[§]

Group of animals	Body weight gain (%)
Control	11.36± 0.72
CIFY3	8.48± 0.87
*P*1 value*	<0.01
Melatonin	15.90±0.87
*P*1 value	<0.001
*P*2 value**	<0.001
CIFY3+ Melatonin	16.38±1.49
*P*1 value	<0.01
*P*2 value**	<0.001

[§] Each value represents the mean of 5 records ± S.D.
* The significance of changes from control value
** The significance of changes from CIFY3 value.

The data in Tables 2 and 3 show a significant decrease ($P<0.05$) in the white blood cell (WBC) count and haemoglobin (Hb) concentration and a significant increase in red cell distribution width (RDW) in the group supplemented with CIFY3. Treatment with melatonin increased the WBC count to the normal control level ($P>0.05$) (18.58±1.44) compared with the group treated with CIFY3 alone and the control group (16.35±1.64 and 20.04±1.43, respectively). Additionally, the Hb concentration returned to the normal level after treatment with melatonin. However, the other haematological parameters (red blood cells [RBCs], mean corpuscular volume [MCV], mean corpuscular Hb [MCH], mean corpuscular Hb concentration [MCHC], platelets [PLTs] and mean PLT volume [MPV]) did not show any significant changes after treatment with CIFY3.

The evaluation of haematological parameters and complete blood counts could help to determine the physiological status of an organism; these factors could be considered stress indicators and a valuable tool for assessing the harm caused by certain substances **(Flaiban et al., 2008).** Therefore, we examined the effects of CIFY3 and the potentially protective role of melatonin by assessing haematological parameters. A significant decrease in

WBC count was observed in the CIFY3-treated group compared with the control group. This alteration could affect the integrity of the cellular immune response, an effect that may be correlated with a suppressed immune status and susceptibility to infectious, inflammatory, toxic or stressful conditions **(Jain 1993).**

Table (2): Blood WBCs (10^3/mm^3), RBCs (10^6 /mm^3), HB content (g/dl), HCT value (%), MCV (fl), in different animal groups[§]

Group of animals	WBC	RBC	Hb	HCT	MCV
Control	20.04±1.43	7.45±0.54	16.20±1.17	47.73±0.96	67.25±3.27
CIFY3	16.35±1.64	7.07±.74	12.65±2.48	43.34±3.97	65.00±4.42
*P*1 value*	<0.05	>0.05	<0.05	>0.05	>0.05
Melatonin	19.92±1.38	7.48±.28	15.82±1.40	50.16±4.64	67.00±6.20
*P*1 value	>0.05	>0.05	>0.05	>0.05	>0.05
*P*2 value**	<0.05	>0.05	>0.05	>0.05	>0.05
CIFY3+ Melatonin	18.58±1.44	7.63±.29	16.46±1.15	51.98±3.62	69.40±2.19
*P*1 value	>0.05	>0.05	>0.05	>0.05	>0.05
*P*2 value**	<0.05	>0.05	<0.05	<0.05	>0.05

[§] Each value represents the mean of 5 records ± S.D.
* The significance of changes from control value ** The significance of changes from sunset yellow value.

Table (3): Blood MCH (pg), MCHC (g/dl), platelets (10^3/mm^3), MPV(fL) and RDW(%)in different animal groups[§]

Group of animals	MCH	MCHC	PLT	MPV	RDW
Control	21.72±1.23	32.45±0.45	716.00±80.98	6.30±0.43	8.07±1.14
CIFY3	20.55±1.27	31.35±0.89	732.50±125.55	6.28±0.33	10.70±1.98
*P*1 value*	>0.05	>0.05	>0.05	>0.05	<0.05
Melatonin	21.14±1.76	31.50±0.35	750.20±100.31	5.75±0.15	9.30±1.38
*P*1 value	>0.05	>0.05	>0.05	>0.05	>0.05
*P*2 value**	>0.05	>0.05	>0.05	>0.05	>0.05
CIFY3 +Melatonin	21.96±0.73	31.68±0.29	696.50±154.91	6.16±0.29	8.90±0.62
*P*1 value	>0.05	>0.05	>0.05	>0.05	>0.05
*P*2 value**	>0.05	>0.05	>0.05	>0.05	>0.05

[§] Each value represents the mean of 5 records ± S.D.
* The significance of changes from control value ** The significance of changes from sunset yellow value.

Our work showed that the RBCs, MCV, MCH, MCHC, PLTs and MPV did not differ significantly among the different groups. Unlike many food colour additives (AL-Shinnawy 2009), CIFY3 did not appear to alter several haematological parameters. Melatonin administration improved all the haematological parameters altered by CIFY3, as indicated by the increase in the WBC count to the control value. This result is in agreement with the findings of many researchers who have reported that melatonin improved haemopoiesis and restored normal blood composition due to its antioxidant and immune-stimulating effects **(Singh and Haldar 2007; Koc et al., 2002; Kara et al., 2012; Srinivasan et al., 2011).** This effect is favoured by the small size and high lipophilicity of melatonin, which allow it to cross biological membranes and reach all the compartments of the cell **(Reiter et al., 1999).**

The blood Hb concentration in the CIFY3-treated group was significantly reduced (P<0.05), which melatonin restored to a level similar to that of the control group without any notable changes in the shape or number of RBCs (measured by calculating the MCV). CIFY3 may prevent Hb synthesis, but it does not affect RBC synthesis via erythropoiesis in the bone marrow.

The data in Table 4 show a significant decrease (P<0.001) in SOD activity, a significant increase in the thiobarbituric acid-reactive substances (TBAS) concentration and no significant change in the catalase activity in the group treated with CIFY3. Melatonin addition reversed the effect of CIFY3, as evidenced by the increase in SOD activity and the decrease in the TBAS concentration compared with the group treated with CIFY3 alone (29.62±1.28 vs. 26.86±0.88 and 12.10±0.77 vs. 28.59±2.34, respectively).

Table (4): Kidney SOD activity (U/mg protein), catalase activity (U/g protein), and TBARS (nmol/g fresh tissue) in different animal groups[§]

Group of animals	SOD	Catalase	Lipid peroxidation
Control	31.34±0.86	17.17±0.86	11.87±0.98
CIFY3	26.86±0.88	17.36±0.49	28.59±2.34
*P*1 value*	<0.001	>0.05	<0.001
Melatonin	31.45±0.54	18.02±0.39	15.62±2.50
*P*1 value	>0.05	>0.05	>0.05
*P*2 value**	<0.001	>0.05	<0.01
CIFY3 + Melatonin	29.62±1.28	17.72±0.69	12.10±0.77
*P*1 value	<0.05	>0.05	>0.05
*P*2 value**	<0.05	>0.05	<0.001

[§] Each value represents the mean of 5 records ± S.D.
* The significance of changes from control value; ** The significance of changes from CIFY3 value.

Food colour additives, especially those containing azo dyes and aromatic amine structures, are cytotoxic compounds because they are metabolised by intestinal bacteria that produce oxygen and O_2^- free radicals **(Bansal 2005).** It should be noted that the levels of ROS are controlled by antioxidant enzymes and non-enzymatic scavengers. SOD and catalase provide a major cellular defence mechanism against oxidative damage by participating in the clearance of O_2^- and peroxide anions, respectively. In this study, the rats treated with CIFY3 showed a significant decrease in SOD activity, a significant increase in renal TBAS concentration and no noticeable change in catalase activity. Among the non-enzymatic antioxidants, melatonin can act as a direct free radical scavenger and an indirect antioxidant via the stimulation of antioxidant enzymes **(Galano et al., 2011, 2013; Rodriguez et al., 2004; Poeggeler et al., 1994; Shirazi et al., 2007)** and the inhibition of pro-oxidative enzymes **(Karbownik and Reiter 2000).** Several studies have indicated that melatonin pretreatment significantly increases antioxidant enzyme levels and ROS scavenging in different organs to protect against the oxidative stress induced by different treatments **(Reiter *et al.*, 2004; Reiter et al., 2012; El-Missiry et al., 2007; Rodriguez et al., 2004; Bharti and Srivastava 2009; Okatani et al., 2003; Rodriguez-Reynoso et al., 2004; Reiter 1996; Bhatti et al., 2011; Bharti et al., 2012; Tamura et al., 2012; Fischer et al., 2013).** In the present work, the oral administration of melatonin (10 mg/kg bw/day for 21 days) maintained a level of SOD activity similar to the control group level.

The histological examination of kidney sections from the CIFY3-treated animals revealed glomerular atrophy, an expansion of the Bowman's capsule space, vacuolation, and swelling in addition to brown pigment deposition in the interstitial tissues and renal tubular cells of the kidney. Congested blood vessels and areas of haemorrhage were observed in renal sections from rats treated with CIFY3. There were no adverse effects observed in the group treated with melatonin. In general, there was appreciable improvement after treatment with melatonin (Figure 1). TBAS are produced by the peroxidation of unsaturated fatty acids in biological membrane lipids caused by ROS. The result is a dramatic decrease in cellular membrane fluidity and the disruption of membrane integrity and function, which produce critical pathological changes **(Halliwell 1987).** Increased ROS and free radical generation can damage the renal tissues of rats. In the present study, the histological examination of kidney sections prepared from rats treated with CIFY3 revealed severe cellular destruction, including glomerular atrophy, expansion of the Bowman's capsule space, vacuolation, swelling, necrosis and pyknosis. Brown pigment deposition was also observed in the interstitial tissues and renal tubular cells. This histological profile has also been reported in other works **(Amin et al, 2010 ; Mekkawy et al., 1998).**

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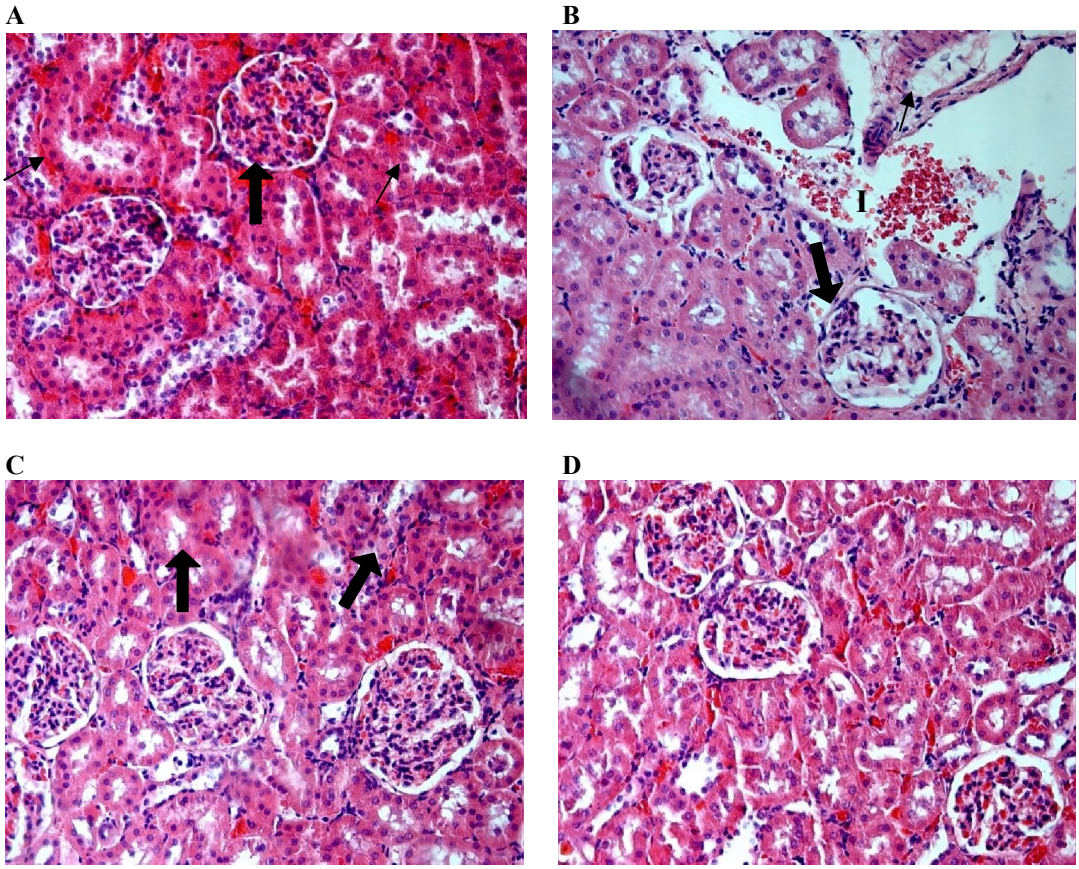

Figure (1): Light micrographs showing the effect of melatonin on food colorant additives induced renal damage in rats. Haematoxylin and Eosin staining, original magnification (20X). **A:** control group showing the normal appearance of the glomerulus (🡅) and renal tubules (↑). **B:** CIFY3 group showing atrophy in the glomerular tuft of the capillaries (🡅) with complete obliteration of the tubules (↑) and the presence of extravasted blood between the tubules together with cellular infiltration (I). **C**: melatonin group showing only sloughing of the cells lining the proximal and distal convoluted tubules (🡅). **D**: CIFY3 and melatonin group showing similar picture to the control group.

References

1. Aboel-Zahab, H., el-Khyat, Z., Sidhom, G., Awadallah, R., Abdel-al, W. and Mahdy, K. (1997): Physiological effects of some synthetic food colouring additives on rats. Boll. Chim. Farm. **136,** 615-27.
2. AL-Shinnawy, M. (2009): Physiological effect of a food additive on some haematological and biochemical parameters of male albino rats. Egypt. Acad. J. biolog. Sci., **2,** 143-151.
3. Amin, K.A., Abdel Hameid, H. and AbdElsttar, A.H. (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.
4. Bansal, A.K. (2005): Modulation of N-nitrosodiethylamine induced oxidative stress by vitamin E in rat erythrocytes. Human Exp. Toxicol. **24,** 297–302.
5. Bergmeyer, H.U., Bergmeyer, J. and Grabl, M. (1987): Enzymes: 1. Oxidoreductases, transferases, in: Methods of Enzymatic Analysis, 3rd edn., VCH Publishers, USA, Mineral combination cancer incidence and disease-specific mortality in the general population, J. Natl. Cancer Inst. **85,** 1483–1492.
6. Bharti, V.K. and Srivastava, R.S. (2009): Pineal proteins upregulate specific antioxidant defense systems in the brain. Oxid. Med. Cell Longev. **2,** 88–92.
7. Bharti, V.K., Srivastava, R.S., Malik, J.K., Spence, D.W., Pandi-Perumal, S.R. and Brown, G.M. (2012): Evaluation of blood antioxidant defense and apoptosis in peripheral lymphocytes on exogenous administration of pineal proteins and melatonin in rats. J. Physiol. Biochem. **68,** 237-245.
8. Bhatti, J.S., Sidhu, I.P. and Bhatti, G.K. (2011): Ameliorative action of melatonin on oxidative

damage induced by atrazine toxicity in rat erythrocytes. Mol. Cell. Biochem. **353,** 139-149.
9. Collier, S.W., Strom, J.E. and Bronaugh, R.L. (1983): Reduction of azo dyes during in vitro percutaneous absorption. Toxicol. Appl. Pharm. **118,** 73-79.
10. Demir, E., Kocaoğlu, S. and Kaya, B. (2007): Genotoxicity testing of four benzyl derivatives in the Drosophila wing spot test. Food Chem. Toxicol. **46,** 1034-1041.
11. El-Habit, O.H.M., Saada, H.N., Azab, K.S., Abdel-Rahman, M. and El-Malah, D.F. (2000): The modifying effect of b-carotene on gamma radiation-induced elevation of oxidative reactions and genotoxicity in male rats. Mutat. Res. **466,** 179–186.
12. El-Missiry, M.A., Fayed, T.A., El-Sawy, M.R. and El-Sayed, A.A. (2007): Ameliorative effect of melatonin against gamma-irradiation-induced oxidative stress and tissue injury. Ecotoxicol. Environ. Saf. **66,** 278-286.
13. Feng, J., Cerniglia, C.E. and Chen, H. (2012): Toxicological significance of azo dye metabolism by human intestinal microbiota. Front. Biosci. (Elite Ed). **4,** 568-586.
14. Fischer TW, Kleszczyński K, Hardkop LH, Kruse N, Zillikens D. (2013): Melatonin enhances antioxidative enzyme gene expression (CAT, GPx, SOD), prevents their UVR-induced depletion, and protects against the formation of DNA damage (8-hydroxy-2'-deoxyguanosine) in ex vivo human skin. J. Pineal. Res. **54**, 303-312.
15. Flaiban, K., Spohr, K., Malanski, L., Svoboda, W., Shiozawa, M., Hilst, C., Lúcia, S., Aguiar, L., Ludwig, G., Passos, F., Navarro, I., Balarin, M. and Lisbôa, J. (2008): Hematologic values of free-ranging cebus cay and Cebus nigritus in Southern Brazil. Int. J. Primatol. **29,** 1375–1382.
16. Galano A, Tan DX, Reiter RJ. (2011): Melatonin as a natural ally against oxidative stress: a physicochemical examination. J. Pineal. Res. **51,** 1-16.
17. Galano A, Tan DX, Reiter RJ. (2013): On the free radical scavenging activities of melatonin's metabolites, AFMK and AMK. J. Pineal. Res. **54,** 245-257.
18. Gao, Y., Li, C.H., Shen, J., Yin, H., An, X. and 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,** 125-129.
19. Halliwell, B. (1987): Oxidants and human diseases. FASEB J. **1,** 441–445.
20. Halliwell, B. (1992): Reactive oxygen species and the central nervous system. J. Neurochem. **59,** 1609-1623.
21. Jain, N.C. (1993): Essentials of Veterinary Hematology. Lea and Febiger, Philadelphia. p. 417.
22. Kara, A., Akman, S., Ozkanlar, S., Tozoglu, U., Kalkan, Y., Canakci, C.F. and Tozoglu, S. (2012): Immune modulatory and antioxidant effects of melatonin in experimental periodontitis in rats. Free Radic. Biol. Med. **55C;** 21-26.
23. Karbownik, M. and Reiter, R. (2000): Antioxidative effects of melatonin in protection against cellular damage caused by ionizing radiation. Proc. Soc. Exp. Biol. Med. **225,** 9-22.
24. Kharwar, R.K. and Haldar, C. (2012): Daily variation in antioxidant enzymes and lipid peroxidation in lungs of a tropical bird Perdicula asiatica: role of melatonin and nuclear receptor RORα. Comp. Biochem. Physiol. A Mol. Integr. Physiol. **162,** 296-302.
25. Koc, M., Buyukokuroglu, M.E. and Taysi, S. (2002): The effect of melatonin on peripheral blood cells during total body irradiation in rats. Biol. Pharm. Bull. **25,** 656-657.
26. Lubet, R.A., Connolly, G., Kouri, R.E., Nebert, D.W. and Bigelow, S.W. (1983): Biological effects of the Sudan dyes: role of the cytosolic receptor. Bioch. Pharmac. **32,** 3053-3058.
27. Maekawa, A., Matsuoka, C., Onodera, H., Tanigawa, H., Furuta, K., Kanno, J. Jang, J.J., Hayashi, Y. and Ogiu, T. (1987): Lack of carcinogenicity of tartrazine (FD & C Yellow No. 5) in the F344 rat. Food Chem. Toxicol. **25,** 891-896.
28. Mayo, J.C., Sainz, R.M., Antolin, I., Herrera, F., Martin, V. and Rodriquez, C. (2002): Melatonin regulation of antioxidant enzyme gene expression. Cell. Mol. Life Sci. **59,** 1706–1713.
29. Mekkawy, H.A., Ali, M.O. and El-Zawahry, A.M. (1998): Toxic effect of synthetic and natural food dyes on renal and hepatic functions in rats. Toxicol. Lett. **95,** 155.
30. Nihon-shokuhin-tenkabutu-kyokai, (1999): Japan's Specifications and Standards for Food Additives, Tokyo, Japan, 7th edition.
31. Nony, C.R., Bowman, M.C., Carins, T., Lowry, L.K. and Tolos, W.P. (1980): Metabolism studies of an azo dye and pigment in the hamster based on analysis of the urine for potentially carcinogenic aromatic amine metabolites. J. Anal. Toxicol. **4,** 132-140.
32. Okatani, Y., Wakatsuki, A., Reiter, R.J., Enzan, H. and Miyahara, Y. (2003): Protective effect of melatonin against mitochondrial injury induced by ischemia and reperfusion of rat liver. Eur. J. Pharmacol. **469,** 145–147.
33. Oliveira, G.A., Ferraz, E.R., Chequer, F.M., Grando, M.D., Angeli, J.P., Tsuboy, M.S., Marcarini, J.C., Mantovani, M.S., Osugi, M.E., Lizier, T.M., Zanoni, M.V. and Oliveira, D.P. (2010): Chlorination treatment of aqueous samples reduces, but does not eliminate, the mutagenic effect of the azo dyes Disperse Red 1, Disperse Red 13 and Disperse Orange 1. Mutat. Res. **703,** 200-208.
34. Pearce, C.I., Lloyd, J.R. and Guthrie, J.T. (2003): The removal of colour from textile wastewater using whole bacterial cells. Dyes and Pigments **58,** 179-196.
35. Poeggeler, B., Saarela, S., Reiter, R.J., Tan, D.X., Chen, L.D., Manchester, L.C. and Barlow-Walden, L.R. (1994): Melatonin-a highly potent endogenous radical scavenger and electron donor: new aspects of the oxidation chemistry of this indole accessed in vitro. Ann. N.Y. Acad. Sci. **738,** 419-420.

36. Reiter, R.J. (1996): Antioxidant actions of melatonin. Adv. Pharmacol. **38,** 103-117.
37. Reiter, R.J., Tan, D.X., Cabrera, J., D'arpa, D., Sainz, R.M., Mayo, J.C. and Ramos, S. (1999): The oxidant/antioxidant network: role of melatonin. Biol. Signals Recept. **8,** 56-63.
38. Reiter, R.J., Calvo, J.R., Karbownik, M., Qi, W. and Tan, D.X. (2000): Melatonin and its relation to the immune syste and inflammation. Ann. NY Acad. Sci. **917,** 376-386.
39. Reiter, R.J., Tan, D.X. and Pappolla, M.A. (2004): Melatonin relieves the neural oxidative burden that contributes to dementias. Ann. NY Acad. Sci. **1035,** 179-196.
40. Reiter, R.J., Tan, D.X., Rosales-Corral, S. and Manchester, L.C. (2012): The universal nature, unequal distribution and antioxidant functions of melatonin and its derivatives. Mini Rev. Med. Chem. **13,** 373-384.
41. Reyes, F.G., Valim, M.F. and Vercesi, A.E. (1996): Effect of organic synthetic food colours on mitochondrial respiration. Food Addit. Contam. **13,** 5-11.
42. Rodriguez, C., Mayo, .JC., Sainz, R.M., Antolín, I., Herrera, F., Martín, V. and Reiter, R.J. (2004): Regulation of antioxidant enzymes: a significant role for melatonin. J. Pineal Res**. 36,** 1-9.
43. Rodriguez-Reynoso, S., Leal, C., Portilla-de Buen, E., Castillo, J.C. and Ramos-Solano, F. (2004): Melatonin ameliorates renal ischemia/ reperfusion injury. J. Surg. Res. **116,** 242–247.
44. Sasaki, Y.F., Kawaguchi, S., Kamaya, A., Ohshita, M., Kabasawa, K., Iwama, K., Taniguchi, K. and Tsuda, S. (2002): The comet assay with 8 mouse organs: results with 39 currently used food additives. Mutat. Res. **519,** 103-119.
45. Seesuriyachan, P., Takenaka, S., Kuntiya, A., Klayraung, S., Murakami, S. and Aoki, K. (2007): Metabolism of azo dyes by Lactobacillus casei TISTR1500 and effects of various factors on decolourization. Water Res. **41,** 985-992.
46. Shaker, A.M.H., Ismail, I.A. and El-Nemr, S.E. (1989): Effect of different foodstuff colourants added to casein diet for biological evaluation. Bull. Nutr. Inst .Cairo, Egypt. **9,** 77.
47. Sharma, S., Haldar, C. and Chaube, S.K. (2008): Effect of exogenous melatonin on X-ray induced cellular toxicity in lymphatic tissue of Indian tropical male squirrel, *Funambulus pennanti*. Int. J. Radiat. Biol. **845,** 363–374.
48. Shirazi, A., Ghobadi, G. and Ghazi-Khansari, M. (2007): Radiobiological review on melatonin: a novel radioprotector. J Rad Res. **48,** 263-272.
49. Shirazi, A., Mihandoost, E., Mohseni, M., Ghazi-Khansari, M. and Mahdavi, S.R. (2012): Radio-protective effects of melatonin against irradiation-induced oxidative damage in rat peripheral blood. Phys. Med. **29,** 65-74
50. Singh, S.S. and Haldar, C. (2007): Peripheral melatonin modulated seasonal immunity and reproduction of Indian tropical bird, *Perdicula asiatica*. Comp. Biochem . Physiol. Mol. Integr. Physiol. **146,** 446–450.
51. Solomon, T.W.G. (1996): Organic Chemistry, sixth ed., John Wiley, New York.
52. Srinivasan, V., Pandi-Perumal, S.R., Brzezinski, A., Bhatnagar, K.P. and Cardinali, D.P. (2011): Melatonin, immune function and cancer. Recent Pat. Endocr. Metab. Immune. Drug Discov. **5,** 109-123.
53. Takeda, H., Nakajima, A. and Kiriyama, S. (1992): Beneficial effects of dietary fiber on the upper gastrointestinal transit time in rats suffering from toxic dose of amaranth. Biosci. Biotech. Biochem. **56,** 551-555.
54. Tamura, H., Takasaki, A., Taketani, T., Tanabe, M., Kizuka, F., Lee, L., Tamura, I., Maekawa, R., Aasada, H., Yamagata, Y. and Sugino, N. (2012): The role of melatonin as an antioxidant in the follicle. J. Ovarian Res. **5,** 1-9.
55. Tan DX, Reiter RJ, Manchester LC, Yan MT, El-Sawi M, Sainz RM, Mayo JC, Kohen R, Allegra M, Hardeland R. (2002): Chemical and physical properties and potential mechanisms: melatonin as a broad spectrum antioxidant and free radical scavenger. Curr. Top. Med. Chem. **2,** 181-197.
56. Tanaka, T. (2006): Reproductive and neurobehavioural toxicity study of tartrazine administered to mice in the diet. Food Chem. Toxicol. **44,** 179-187.
57. Tanaka, R. (2007): Inhibitory effects of xanthone on paraquat- and NaNO(2)-induced genotoxicity in cultured cells. J. Toxicol. Sci. **32,** 571-574.
58. Turkoglu, S. (2007): Genotoxicity of five food preservatives tested on root tips of *Allium cepa* L. Mutat. Res. **626,** 4-14.
59. Winterbourn, C.C., Hawkins, R.E., Brian, M. and Carrell, R.W. (1975): The estimation of red cell superoxide dismutase activity, J. Lab. Clin. Med. **85,** 337–341.
60. Yenilmez, A., Isikli, B., Aral, E., Degirmenci, I., Sutken, E. and Baycu, C. (2010): Antioxidant Effects of Melatonin and Coenzyme Q10 on Oxidative Damage Caused by Single-Dose Ochratoxin A in Rat Kidney. Chin. J. Physiol. **53,** 310-317.
61. Yoshioka, T., Kawada, K., Shimada, T. and Mori, M. (1979): Lipid peroxidation in maternal and cord blood and protective mechanism against activated-oxygen toxicity in the blood, Am. J. Obstet. Gynecol. **135,** 372–376.

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