Toxicity of Azo Dyes in Pharmaceutical Industry

Armina Gičević, Lamija Hindija, and Alma Karačić

Abstract

Azo compounds represent about two thirds of all synthetic dyes. Their usage in pharmaceutical industry has many purposes. One of the most important is coloring of pharmaceutical agents which improves their easy identification. Azo dyes often used in manufacturing of pharmaceuticals are: E102 Tartrazine, E110 Sunset Yellow FCF, Ponceau 4R (Cochineal Red A), Azorubine (Carmoisine), Amaranth, E133 Brilliant Blue and E129 Allura Red. Many azo dyes show carcinogenic and mutagenic activity, and they can provoke allergic reactions. Generally, toxicity of ingredients grows with the increase of benzene rings in their structure. Carcinogenicity of azo dyes directly depends on the structure of molecule and on mechanism of degradation. Products of degradation of azo dyes are mostly aromatic amines with different structures and they can also have carcinogenic properties. Carcinogenicity of many azo dyes is due to their cleaved products such as benzidine. Benzidine is known as carcinogen for the human urinary bladder. Except of carcinogenic and mutagenic activity, azo dyes can alter biochemical markers and they can provoke allergic reactions.

Keywords

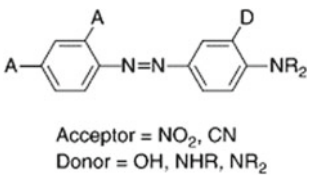
Azo dyes • Toxicity • Carcinogenicity • Allergic reactions • Pharmaceutical industry

1 Introduction

A color additive is a chemical compound that reacts with another substance and causes the formation of a color. The pharmaceutical industry uses various inorganic and organic dyes. The usage of dyes in pharmaceutical industry has commercial, psychological and practical purposes. Different colors of drugs also can help patients to distinguish different strengths of the same drug which can reduce risk of an overdose or underdose [1].

Most dyes/coloring agents used in the pharmaceutical industry belong to one of the following groups: azo dyes, quinoline dyes, triphenylmethane dyes and xanthine dyes [2].

Azo compounds represent about two thirds of all synthetic dyes. They are the most widely used and structurally diverse class of organic dyes in commerce. Their chemical formula is R–N = N–R', where –N = N– represents the azo group and the R or R' is either aryl or alkyl compound [3].



Structural formula of azo dyes. *Source* http://www.chm.bris.ac.uk/webprojects2002/price/classify.htm?fbclid=IwAR1QTaIRivGOTIkJQc7-S61nV_Z_nzGSyDcYCwSw86d702K71L3RwNRresc

Azo dyes often used in manufacturing of pharmaceuticals are: E102 Tartrazine, E110 Sunset Yellow FCF, Ponceau 4R (Cochineal Red A), Azorubine (Carmoisine), Amaranth, E133 Brilliant Blue and E129 Allura Red.

Many azo dyes show carcinogenic and mutagenic activity, and they can provoke allergic reactions. Generally, toxicity of ingredients grows with the increase of benzene rings in their structure. Carcinogenicity of azo dyes directly depends on the structure of molecule and on mechanism of degradation. Products of degradation of azo dyes are mostly

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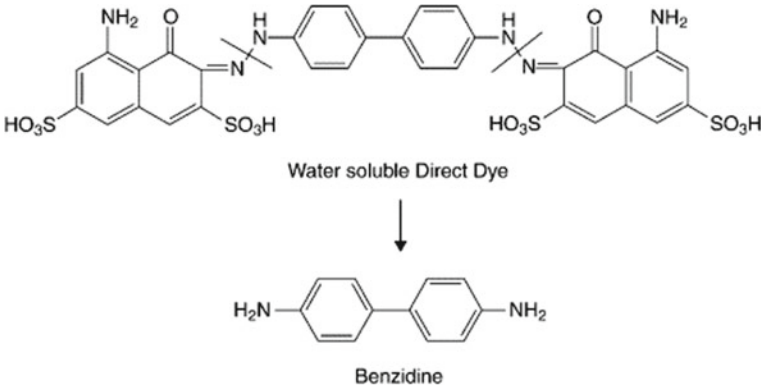
aromatic amines with different structures and they can also have carcinogenic properties [1].

Some azo dyes can be carcinogenic without being cleaved into aromatic amines. However, the carcinogenicity of many azo dyes is due to their cleaved product such as benzidine. Benzidine induces various human and animal tumors. Another azo dye component, p-phenylenediamine, is a contact allergen. Reduction of azo dyes can be accomplished by human intestinal microflora, skin microflora, environmental microorganisms, to a lesser extent by human liver azoreductase, and by nonbiological means [3].

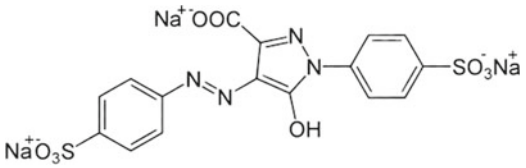


Conversion of water soluble direct dye to benzidine. *Source* https://ars.els-cdn.com/content/image/3-s2.0-B9781845696955500167-f16-04-9781845696955.gif

p-Phenylenediamine is one of the primary intermediates in the azo dyes. Poisoning with p-phenylenediamine causes angioneurotic edema, intravascular hemolysis, rhabdomyolysis with acute renal failure. 1-Amino-2naphthol, which is one of the components of an important group of 1-amino-2-naphthol-based azo dyes, has been reported to be a carcinogen. Gotlieb et al. proved that the metabolite of Sunset Yellow 1-amino-2-naphthol-6sulphonate was not mutagenic, which could be due to the decrease in membrane permeability of this compound. Benzidine is a product of the reduction of Congo Red, and the sulfonated benzidine is a product of the reduction of Acid Orange 6. Benzidine is known as carcinogen for the human urinary bladder. The addition of a sulfonic acid group to benzidine reduces the mutagenicity [4].

2 Tartrazine

Tartrazine (TRZ) is a lemon-yellow dye which is widely used in pharmaceuticals and also cosmetics and food manufacturing. Few studies have addressed the toxicology of TRZ in human cells or tissues. Floriano et al. evaluated the cytotoxicity and genotoxicity of the TRZ dye in human leukocyte cultures and performed theoretical studies to predict its toxicity in silico. They concluded that mutagenic and cytotoxic effect were dose dependent [5]. Gao et al. were evaluating the toxic effect of tartrazine on the learning and memory functions in mice and rats. Animals were administered different doses of tartrazine for a period of 30 days and were evaluated by open-field test, step-through test, and Morris water maze test, respectively. Furthermore, the biomarkers of the oxidative stress and pathohistology were also measured to explore the possible mechanisms involved.



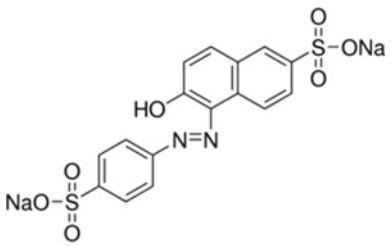
Structural formula of Tartrazine. *Source* https://www.researchgate.net/figure/Chemical-structure-of-Tartrazine_fig1_321634795?fbclid=IwAR0JZGNHfnbNmEH8D3Qp1uvpauQIh0J1yAhB4kdbSMrGW-6wM7MFbWFfu6o

The results indicated that tartrazine extract significantly enhanced active behavioral response to the open field, increased the escape latency in Morris water maze test and decreased the retention latency in step-through tests. The decline in the activities of catalase, glutathione peroxidase (GSH-Px), and superoxide dismutase (SOD) as well as a rise in the level of malonaldehyde (MDA) were observed in the brain of tartrazine-treated rats, and these changes were associated with oxidative damage. The dose levels of tartrazine in the present study produced a few adverse effects in learning and memory functions in animals. The mechanisms might be attributed to the promotion of lipid peroxidation

products and reactive oxygen species, inhibition of endogenous antioxidant defense enzymes and damage of brain tissue [6].

Tartrazine has been associated with anaphylactoid reactions, angioedema, asthma, urticaria and hyperkinesis in patients with hyperactivity and eosinophilia. Because of the cross-sensitivity of azo dyes with aspirin sodium benzoate and indomethacin, azo dye excipients should be avoided in patients with history of hypersensitivity or allergy to these drugs.

3 Sunset Yellow



Structural formula of Sunset yellow. *Source* https://www.sigmaaldrich.com/content/dam/sigma-aldrich/structure3/090/mfcd00036437.eps/_jcr_content/renditions/mfcd00036437-medium.png

Sunset yellow (FD&C 6), another azo dye, has also been associated with the same adverse effects as seen with tartrazine. The incidence of cross-sensitivity to aspirin varies between 2 and 20% in patients with asthma, therefore, it is prudent not to prescribe preparations containing azo dyes to such patients.

Because of the adverse events reported with specific dyes, the FDA has (since 1980) mandated labeling when tartrazine is used in a product. The American Academy of Pediatrics has published a list of drugs/nutritional supplements that do not contain any dyes; physicians treating patients with a history of hypersensitivity reactions may find this reference helpful [2].

Khayyat et al. have done a study where they investigated the possible toxic effects of two types of widely used food and drug colorants, Sunset Yellow and Allura Red, by assessing the physiological, histopathological and ultrastructural changes in the liver and kidney. Also, they investigated the genotoxic effect of both dyes on white blood cells. Thirty adult male albino rats were divided into three groups of 10 animals each: control (received water), Sunset Yellow-treated (2.5 mg/kg body weight) and Allura Red-treated (7 mg/kg body weight). The doses were applied orally for 4 weeks. The results indicated an increase in the biochemical markers of hepatic and renal function (aspartate aminotransferase—AST, alanine aminotransferase—ALT, urea, uric acid and creatinine) in animals administered with the azo dyes. They also observed a noticeable increase in MDA and a marked decrease in total antioxidant levels in azo dye-treated animals compared to controls. Conversely, both dyes adversely affected the liver and kidney of albino rats and altered their histological and fine structure, with downregulation of Bcl2 and upregulation of COX2 expression. Comet assay results showed that Sunset Yellow and Allura Red cause histopathological and physiological aberrations in the liver and kidney of male Wistar albino rats. Moreover, Sunset Yellow but not Allura Red induced a potential genotoxic effect [7] (Fig. 1).

Qu et al. investigated the toxicity of Sunset yellow combined with sodium sulfite and mechanisms of damage in HepG2 cells using High Content Analysis (HCA). They used the CCK-8 assay to determine cell viability. They concluded that this combination led to the growth decrease of HepG2 cells in a dose-dependent manner. Sunset yellow and sodium sulfite had IC50 values of 1.06, and 0.30 g/L at 24 h, respectively. HCA showed that both Sunset yellow and sodium sulfite had synergistic effects on cell number, membrane permeability, mitochondrial membrane potential, intracellular calcium level, oxidative stress, and high dose group DNA damage [8].

Ali et al. were examining the combination of Sunset yellow (SY) and sodium benzoate (NaB). Genotoxic effects of different combinations of SY and NaB were assessed in vivo in female rats. Different combinations of SY and NaB were dissolved in water and administered daily to six animals groups for 12 weeks. Different combinations of SY and NaB induced an increase in the frequency of tailed nuclei (DNA damage) in liver cells. In addition, administration of SY plus NaB resulted in an abnormal distribution of serum proteins. The results showed that the combination of SY and NaB could have genotoxic potential [9].

Genotoxic and cytotoxic effects of curcumin and sunset yellow were tested by Haverić et al. using the chromosome aberration analysis and cytokinesis-block micronucleus cytome assay in human lymphocyte culture. Tested concentrations of sunset yellow significantly associated with frequencies of structural aberrations, chromatid-type aberrations, total aberrant cells and micronuclei showing considerable dose dependent clastogenic activity. In higher analyzed concentrations, curcumin significantly increased only nuclear buds frequency, suggesting its potential genotoxicity, while sunset yellow showed dose-dependent genotoxic potential. Obtained results point toward favorization of natural coloring agents [10].

Amin et al. conducted a study to evaluate the toxic effects of tartrazine and carmoisine on renal, hepatic function, lipid profile, blood glucose, body-weight gain and biomarkers of oxidative stress in tissue. Tartrazine and carmoisine were administered orally in two doses, one low and the other high

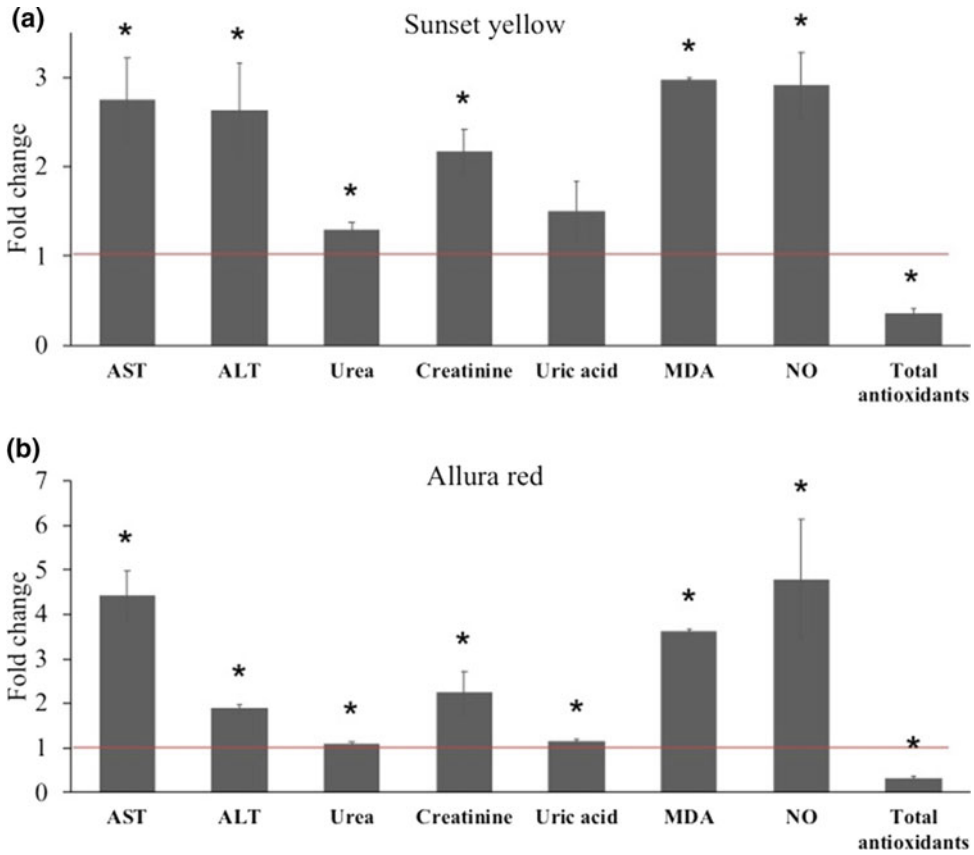
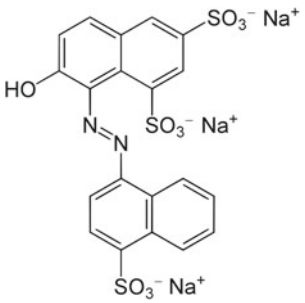


Fig. 1 Effect of sunset yellow and allura red on biochemistry parameters and oxidative biomarkers in male rats [7]

dose for 30 days followed by serum and tissue sample collection for determination of ALT, AST, alkaline phosphatase, urea, creatinine, total protein, albumin, lipid profile, fasting blood glucose in serum and estimation of GSH, catalase, SOD and MDA in liver tissue in male albino rat. It was concluded that tartrazine and carmoisine affect adversely and alter biochemical markers in vital organs e.g. liver and kidney not only at higher doses but also at low doses [11].

Cemek et al. got unique results for concentrations of trace and major elements in rats' liver, kidney and brain tissues exposed to tartrazine and carmoisine, especially for iron and zinc contents. They suggested that anemia in mice may be related to iron deficiency. Also by consuming carmoisine at both low and high doses resulted with a reduction of zinc content in kidneys, because zinc is chelated by consumed dyes. Although low dose tartrazine caused to reduce liver zinc content, high dose tartrazine cause to same effect in kidney. Aluminium and barium are essential trace elements and the levels of these elements in brain are reduced by consuming high and low doses of tartrazine. Also there is a significant increase of calcium levels in liver tissue with high doses of carmoisine [12].

4 Ponceau 4R

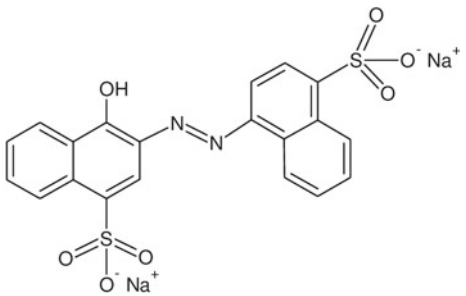


Structural formula of Ponceau 4R. *Source* https://file.medchemexpress.com/product_pic/hy-d0193.gif

Ponceau 4R may increase hyperactivity in affected children and adversely affect those that are sensitive to aspirin [1].

Rowe et al. investigated the use of six dyes (Tartrazine, Quinoline Yellow, Sunset Yellow, Azorubine, Ponceau 4R and Allura Red). The study results connected these dyes to behaviour issues in children. Nonetheless, after reviewing the outcomes of the study, the European Food Standards Agency came to the conclusion that no legislative change or amendment was in fact required [13].

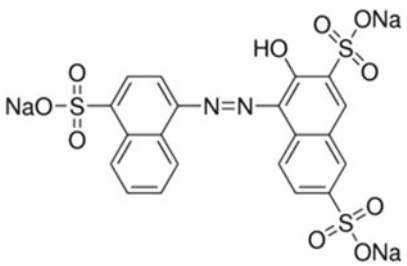
5 Azorubine



Structural formula of Azorubine. *Source* https://www.sigmaaldrich.com/content/dam/sigma-aldrich/structure5/145/mfcd00003978.eps/_jcr_content/renditions/mfcd00003978-medium.png

Azorubine has shown no evidence of mutagenic or carcinogenic properties. Like Ponceau 4R, it may have an adverse effect on the activity and attention in children. Azorubine may rarely cause skin and respiratory reactions in susceptible individuals, even at the approved dose [14].

6 Amaranth



Structural formula of Amaranth. *Source* https://www.sigmaaldrich.com/content/dam/sigma-aldrich/structure5/178/mfcd00004076.eps/_jcr_content/renditions/mfcd00004076-medium.png

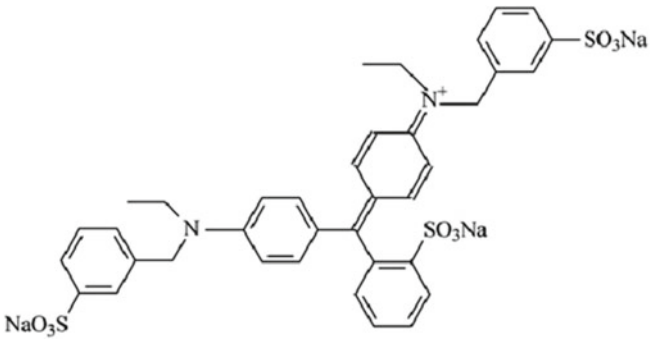
Amaranth in certain concentrations can induce dose-related DNA damage in the colon of mice after oral administration. Except genotoxicity amaranth can, as some other azo dyes, also cause allergic or asthmatic reactions for sensitive people, as well as frequent headaches and children's hyperactivity [1].

Also, in another study conducted by Sasaki et al., on the genotoxicity of food dyes, such as Amaranth, it was observed that DNA damage occurred on the colon, bladder, stomach, and gastrointestinal organs even at the lowest dose. Mpountoukas et al. investigated the potential genotoxic, cytotoxic and cytostatic effects in human peripheral blood cells for Amaranth in vitro. According to the results, it was identified that food dyes have a toxic effect on human lymphocyte cells and cause an increase in the sister chromatid exchange 1.7 times compared to the control group, and show effects of direct binding on DNA. Similar results were found in a study that was carried out by Shimada et al. DNA damage was noticed in rat colon after applying 10 mg kg^{-1} of azo food dyes. It appeared that the DNA damage was induced in pregnant female and male rats fed with Amaranth orally [15].

7 Brilliant Blue

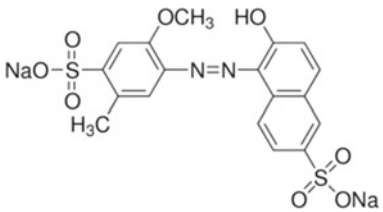
Brilliant Blue has the capacity of inducing an allergic reaction in individuals with pre-existing moderate asthma [1].

Remy et al. study was conducted to evaluate the retinal toxicity of Brilliant Blue G (BBG) following intravitreal injection in rat eyes and examine the biocompatibility and the staining properties in humans. No significant reduction in retinal ganglion cell numbers and no morphological alterations were noted. A sufficient staining of the internal limiting membrane (ILM) was seen in patients with macular hole, while the staining pattern in epiretinal membranes (ERM) cases was patchy, indicating that parts of the ILM were peeled off along with the ERM in a variable extent. No toxic effects attributable to the dye were noted during patient follow-up. No retinal toxicity or adverse effects related to the dye were observed in animal and human studies. The long-term safety of this novel dye will have to be evaluated in larger patient series and a longer follow-up [16, 17].



Structural formula of Brilliant blue. *Source* https://www.researchgate.net/figure/Chemical-structure-of-Brilliant-Blue-R-BBR-dye_fig11_271563974

8 Allura Red



Structural formula of Allura red. *Source* https://www.sigmaaldrich.com/content/dam/sigma-aldrich/structure5/051/mfcd00059526.eps/_jcr_content/renditions/mfcd00059526-medium.png

Allura Red may cause allergic reactions (e.g. urticaria, asthma), especially when administered in mixes with other synthetic color additives [1].

Rajan and Anandan conducted a study where the emphasis was made on knowing the effect of these common agents, in particular with reference to artificial food color Allura red (FD&C # 40)—orange red food dye on DNA damage using catfish 0 + 2—as a model organism. The methodology was to understand direct damage of nucleus by micronuclei test and chromosomal damage by alkaline single-cell gel electrophoresis (COMET) assay. The freshwater catfish *Clarias batrachus* was used for specificity genotoxic indicators micronucleus assay and COMET assays. The blood sample was tested for genotoxicity and the results revealed DNA damage through alkaline single-cell gel electrophoresis (comet) and micronuclei assays. Hence it was concluded that the usage of food color containing Allura red (FD&C # 40)—orange red food dye may be toxic at a genetic level if the usage is prolonged [18].

In the study conducted by Uysal et al. the toxic effects of four different synthetic dyes (Ponceau 4R, Sunset Yellow, Amaranth, Tartrazine) on 72 ± 4 h larvae of Oregon (R) wild type of *Drosophila melanogaster* were investigated. The effects of the dyes on longevity were studied separately in female and male populations. The study determined that the maximum mean life span of the female and male *D. melanogaster* populations decreased with increasing concentrations of dyes. Based on the results obtained from the larval mortality and life span experiments, the order of toxicity for dyes was: Tartrazine > Amaranth > Sunset Yellow ≥ Ponceau 4R [15].

9 Conclusion

One of the most often used coloring agents in pharmaceutical industry are azo dyes. However, they show some severe side effects, such as carcinogenic and mutagenic activity.

Some of them promote lipid peroxidation products and reactive oxygen species, inhibit endogenous antioxidant defense enzymes which result in the brain tissue and adverse effects in learning and memory functions. They may cause histopathological and physiological aberrations in liver and kidneys and change membrane permeability, mitochondrial membrane potential and intracellular calcium level. Some combinations lead to structural chromosome aberrations, chromatid-type aberrations, total aberrant cells and micronuclei. For sensitive people, they can cause allergic or asthmatic reactions, as well as frequent headaches and children's hyperactivity. It also has toxic effects on human lymphocyte cells, increases the sister chromatid exchange and shows effect of binding on DNA directly.

To prevent the abuse of synthetic dyes, the permissible type and usage of synthetic dyes are strictly regulated in many countries. It is very important to be aware that consequences of inadequate dosage and combination of azo dyes can cause serious health issues. Also, pharmaceutical industry should consider surcease or decreased use of

conventional dyes and start using newer, safer dyes such as Brilliant blue for which is proved that has less side effects.

Conflict of Interest The authors have no conflicts of interest to disclose.

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