Evaluating the Carcinogenic Potential of Sunset Yellow via Biochemical Disruption and Mammary Gland Remodeling in Female Albino Rats

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Abstract

Background: Sunset yellow is a synthetic yellow azo dye which is commonly used in the food and drink industry. Even though the substance was approved by regulators, there are recent reports chronic use will cause metabolic disorders and cancer. But, the long-term effects of the remedy on biochemical, tumorigenic and histological parameters remain poorly investigated. The aim of the present study was to determine whether prolonged exposure to Sunset Yellow causes systemic toxicity and if it alters mammary tissue as a carcinogen. **Methods:** Rats were divided into 5 groups (normal control, positive control 2 mg/kg body weight DMBA and three treatment groups which were given orally Sunset Yellow 200, 400 and 600 mg/kg for 40 weeks). They analyzed the serum biochemical markers (lipid profile, liver and kidney function), tumor biomarkers (AFP and CA 15-3), and mammary tissue histopathology. **Results:** DMBA raised serum levels of triglyceride, creatinine, cholesterol, LDL, SGPT and tumor markers with reduced HDL. The biochemical changes produced by Sunset Yellow were dose dependent, and the highest dose (600 mg/kg) caused marked increases in hepatic and renal markers, and AFP and CA 15-3. Histological analysis displayed malignant ductules, and DCIS in the higher dose groups indicates pre-neoplastic changes. **Conclusion:** chronic consumption of Sunset Yellow at higher doses can cause systemic toxicity as well as histological changes indicative of early-stage carcinogenesis. Increased public health concerns require stricter evaluations for synthetic food dye safety.

Keywords: Sunset Yellow, DMBA, Mammary Tumor, Alpha-fetoprotein (AFP), CA 15-3, Biochemical

Significance | This study reveals long-term Sunset Yellow exposure induces biochemical toxicity and mammary changes, indicating potential health risks in humans.

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1. Introduction

In the food industry, the use of synthetics color has increased many forms over the years. Dealing with appearance, stability and consumer appeal for the food industry. One of such chemicals is an artificial chemical dye especially from the azo family, which has raised considerable health concern due to their continued usage in a variety of edible and non-edible products. Even though regulatory bodies have approved such dyes as safe for use within certain margins of safety, recent studies reveal that consuming these entities may prove harmful for humans. This is especially the case when consumed long-term or alongside other dietary toxins (K.-T. Chung, 2016; Islam, 2024; Rovina et al., 2016).

Sunset Yellow FCF (E110) is a synthetic azo dye that is commonly found in many processed food items such as soft drinks,

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confectionery, savory snacks, and ready meals. The chemical is often used by companies that target children and teenagers since they are the most affected group in terms of cumulative exposure (Rovina et al., 2017). Food regulatory frameworks of various countries have declared Sunset yellow acceptable up to a certain acceptable daily intake (ADI) level. Also, scientific reports suggest that consumption may cause oxidative stress, disturb physiological functions, and turn on pro-inflammatory reactions when taken on a continuous basis even at low doses (ANS, 2014; Şensoy et al., 2025; Singh et al., 2024).

According to researchers, there is a connection between synthetic dyes and serious health concerns such as alteration of cell signaling, DNA damage, oxidative stress, and possibly, cancer (carcinogenesis). Several in vivo and in vitro studies have shown that azo dyes such as Sunset Yellow can disrupt cellular homeostasis and disturb antioxidant defense systems, leading to uncontrolled cell proliferation (Şensoy et al., 2025; Singh et al., 2024). Many studies have examined the systemic toxicity of nanomaterials. However, not much research so far is available on their effects on hormone-responsive organs. The mammary gland is one such tissue, which is sensitive to endocrine and environmental modulators (Rovina et al., 2017, 2017b; Singh et al., 2024).

The mammary gland is quite sensitive to hormonal as well as chemical influences, especially at early life stages. It is now widely acknowledged that environmental chemicals known as endocrine-disrupting chemicals (EDCs) can alter the architecture of breast tissue and create an environment conducive to neoplastic transformation. Several studies establish that repeated exposure to synthetic compounds - particularly dyes and preservatives - has resulted in abnormal proliferation of mammary cells, increased density and the appearance of precancerous lesions in an experimental animal model (Brisken & Ataca, 2015; Sevastre et al., 2023a; Smith et al., 2025).

The literature on the in vivo correlation between Sunset yellow exposure and mammary tumors is very limited. Some earlier rodent studies have suggested systemic toxicity. They showed changes in hepatic and renal parameters and histological abnormalities in different organs (K. Chung, 2000; Josephy & Allen-Vercoe, 2023; Sevastre et al., 2023). But they miss the tissue-specific impact on mammary glands. This implies that there is a research gap regarding the mechanism of azo dyes on mammary carcinogenesis.

We should see how a long-term diet of Sunset Yellow affects mammary tumor initiation, growth and progression. The present study is designed to fill this gap by controlled in vivo study using female albino rats as a model. The subjects were administered different quantities of Sunset Yellow for 40 weeks. The evaluation of tumor biomarker expression (AFP, CA 15-3) and biochemical indices (liver, kidney and lipid profile) and histopathology of the mammary tissue were done. We chose these parameters to thoroughly assess the dose-dependent systemic and tissue-specific effects of dye (K. Chung, 2000; K.-T. Chung, 2016; Josephy & Allen-Vercoe, 2023).

The study also evaluates the possibility that exposure to Sunset Yellow may induce remodeling of mammary tissues and alterations in hormonal sensitivity, and whether the dye exposure is associated with early changes in ductal hyperplasia and neoplasia that may signify the onset of carcinogenic activity. The present study aims to provide insights into the toxicodynamic behavior of this food dye through biochemical and histological assays (Al-Adilee et al., 2024; Zingue et al., 2021).

This study's significance is academic, but also related to public policy for health, especially food safety regulation of the vulnerable people such as the children and adolescent. If the outcome confirms that Sunset Yellow contributes to mammary carcinogenesis, widespread use of this substance in food may be re-evaluated. It will also be taken into account for risk assessment of synthetic dyes.

2. Materials and Methods

2.1 Chemicals

BioChrom Dyes and Chemicals Pvt supplied Sunset Yellow FCF (E110), which was used for the study. Food grade colorants manufacturers Mumbai, India, Ltd. 7,12-Dimethylbenz[a]anthracene (DMBA) is an experimental carcinogen obtained from Cayman Chemical Company based in Ann Arbor, Michigan, USA. It specializes in high-purity biochemical reagents. The chemicals used during the experiment were of analytical grade, which was used in proper storage and handling of the substance.

2.2 Experimental Animals

This study used a total of twenty healthy virgin female albino Swiss rats (8-9 weeks old; 100-110 g). Prior to experiments, animals were acclimatized for seven days under standard laboratory conditions (25 ± 2 °C, 55 ± 10% humidity and 12-h light-dark cycle). The sample size (n = 4 per group) was selected, as similar rodent toxicological studies indicated that sufficient power was achieved to detect biochemical and histological changes without prior inclusion or exclusion (Vashishat et al., 2024).

The mean body weight (± SEM) of animals were claculated in five experimental groups at 40 weeks. The groups consisted of Control, Positive Control (PC), and three treatment groups of E110 200 mg/kg (T-1), 400 mg/kg (T-2), and 600 mg/kg (T-3) body weight, respectively. DMBA was given to the PC group at a dose of 2 mg/kg BW only; the animals in the Control group were not treated.

2.3 Experimental Design

The experiment has five groups of four rats each (n = 4). Group 1 was untreated control and provided with standard laboratory feed only. Group 2 (positive control) was given DMBA (2 mg/kg body

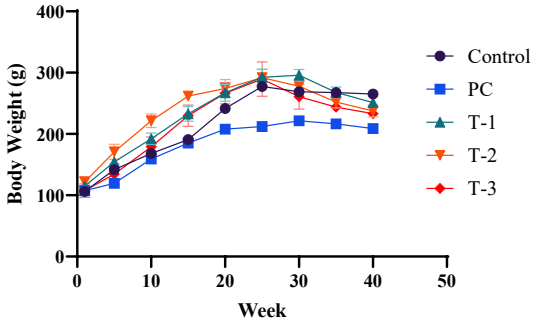


Figure 1. Changes in mean body weight (± SEM) across five groups over 40 weeks. Animals were assigned to Control, Positive Control (DMBA at 2 mg/kg bw), and treatment groups receiving E110 at 200 (T-1), 400 (T-2), and 600 mg/kg bw (T-3). T-2 showed the highest weight gain up to week 24 (~310 g), followed by a decline. T-1 and T-3 also showed post-peak reductions. PC group had minimal gain and early decline, while Control maintained steady weight throughout.

weight) in normal feed to induce cancer. Groups 3, 4 and 5 were treatment groups and fed orally developed E110 at doses of 200 mg/kg (T-1), 400 mg/kg (T-2), and 600 mg/kg (T-3) body weight along with standard feed. The effect of E110 on the dose-dependent biochemical, tumorigenic and histopathological parameters was evaluated by continuing all treatments daily for 40 weeks.

2.4 Serum Preparation for Biochemical Tests

At the end of the 40-week treatment period, each rat's blood was collected via cardiac puncture under mild anesthesia. After the blood was collected, it was allowed to clot at room temperature and then centrifuged at 3000 rpm for fifteen minutes to obtain clear serum. Serum was used for estimating not just biochemical parameters but also tumor biomarkers. Hepatic renal and cardiac functions were assessed using standard protocols. The Abbott Architect c4000 automated clinical chemistry analyzer processed the concentrations of alpha-fetoprotein (AFP) and cancer antigen 15-3 (CA 15-3) according to the manufacturer's instructions (El-Desoky et al., 2022).

2.5 Preparation of Mammary Tissue

The mammary tumor specimens were excised and immediately fixed in 10% neutral buffered formalin for histological examination. After fixation, the tissues were cleared using xylene and were clipped into small pieces. After that, they were processed for dehydration by graded alcohol series. Once dehydration was completed, the tissue samples were embedded in molten paraffin wax to form solid blocks suitable for microtomy. Thin sections of tissues were taken with a rotary microtome (5 µM thick). The sections were placed on slides and stained with hematoxylin and eosin (H & E) for the visible cellular and structural details under the light microscope (Shohel, 2024).

2.6 Statistical Analysis

Data was analyzed using GraphPad Prism, v10.1.2. We used One-way ANOVA, followed by Dunnett's test, to assess group differences. The data shows mean ± SD and any p value less than 0.05 is considered significant.

2.7 Ethics approval

Rats were procured from ICDDR, B and approved by the Ethical Review Committee of the Faculty of Biological Sciences Islamic University, Kushtia, Bangladesh (reference number: FBS/ERC/IU/2024/05, date: 03.02.2024).

3. Results

3.1 Effect of Sunset Yellow and DMBA on Body Weight Progression

All groups had similar baseline body weights (~100-120 g) (Figure 1). The weight gains varied greatly over the years. The body weight of T-2 Group which was treated with 400 mg/kg E110 was significantly raised and maintained from week 0 to week 24 with a peak value of approx. 285.75 ± 1.75 g. Yet between weeks 25 and 40, the group experienced a noteworthy decrease of body weight, eventually reaching ~230-235 g by week 40 - a lowered weight of 35-40 g, equal to about 11-13% loss in peak weight.

In the T-1 group, which was given 200 mg/kg, body weight increased steadily up to week 22 with a peak of 265 ± 1.55 g. Which slightly loses their body weight in the subsequent weeks. Ultimately, we see the body weight 250-255 g including total loss of 15-20 g or 8-10% g. The T-3 group (600 mg/kg) peaked at 296 ± 1.65 g at week 20 and subsequently showed a sharper drop thereafter. By week 40 the final weight reduced to 230-240 g; a reduction of ~12%.

The Positive Control (DMBA-treated) experienced a slight increase in weight with a final weight towards the end of week 8 at approximately 220 g before gradually decreasing. At the fortieth week, the weight was 200-210 g, a loss of 10-15 g, or ~5-7% which may be due to tumor burden, cachexia, and/or metabolic decline due to DMBA. On the other hand, the Control animals steadily gained and plateaued at about 280 g by the end of week 30, further maintained without decline towards the end of the study indicative of normal growth.

3.2 Biochemical Parameters

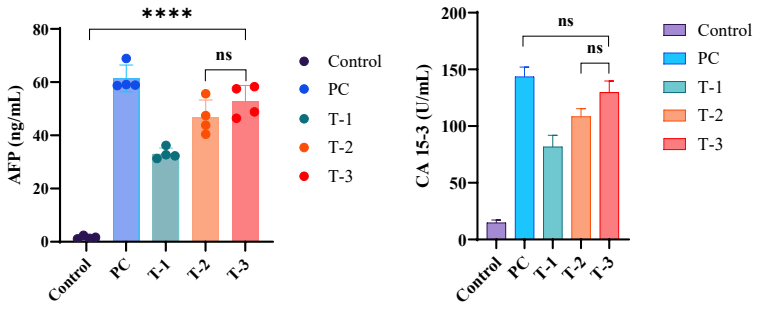


Figure 2. Serum levels of AFP (ng/mL) and CA 15-3 (U/mL) in control, DMBA-treated (PC), and E110-treated (T-1: 200, T-2: 400, T-3: 600 mg/kg) groups. Data are mean ± SD (n = 4). AFP was significantly elevated in PC (****, P < 0.0001), with moderate reductions in T-1 to T-3. CA 15-3 remained high in all treated groups with no significant differences (ns).

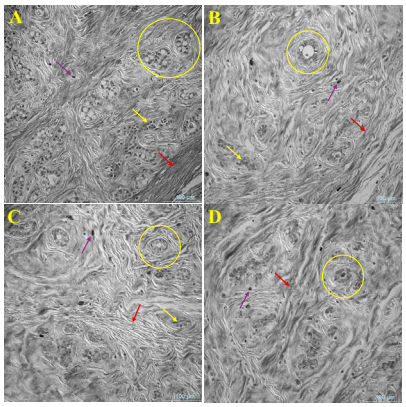


Figure 3. Microscopic images of mammary tissue in female albino rats exposed to E110. Panel A shows tissue from DMBA-treated, while panels B, C, and D represent Sunset Yellow doses of 200, 400, and 600 mg/kg, respectively. Red arrows indicate collagen buildup; yellow arrows show malignant ductules; yellow circles highlight ductal carcinoma in situ (DCIS).

The positive control (PC) group that received DMBA at 2 mg/kg body weight displayed significant biochemical changes as compared to normal control (NC). The PC group had a sharp rise of serum cholesterol level to 110.25 ± 1.1 mg/dL as compared to NC (74.33 ± 1.32 mg/dL) (P < 0.05). The PC group had a significantly lower level of high-density lipoprotein (18.58 ± 1.77 mg/dL) compared to NC (32.75 ± 1.58 mg/dL). In the study, it was also noted that the level of low-density lipoprotein and triglycerides increased significantly in PC (LDL: 38.85 ± 2.81; TG: 89.64 ± 3.45 mg/dL) as compared to NC (LDL: 15.55 ± 1.71; TG: 61.65 ± 2.56 mg/dL). Therefore, these findings confirmed that the hyperlipidemic effects were probably because of the metabolic shift caused by the carcinogens (Table 1).

The groups assigned to receive treatment with E110 which are T-1 (200 mg/kg), T-2 (400 mg/kg), and T-3 (600 mg/kg) showed changes in lipid parameters in a dose-dependent manner. All treated groups showed increased cholesterol levels, with T-2 displaying not the highest value of cholesterol (94.73 ± 2.46 mg/dL). All groups administered E110 had significantly lower levels of HDL than NC, with T-3 having the lowest (17.50 ± 0.84 mg/dL). The T-1 to T-3 groups had moderately increased LDL and triglyceride levels, but these levels are lower than in the PC group, indicating moderate but evident lipid perturbation.

Renal biomarkers were similarly affected. Serum creatinine levels in PC rats were significantly (P < 0.05) higher than control levels is about 1.99±0.32. Creatinine levels in the treated groups ranged from 1.25±0.41 in T-1 to 1.69±0.42b mg/dL in T-3, in line with mild but accumulated renal stress due to dye concentrations. Uric acid levels had similar patterns where significant increases were observed in PC (4.63±0.61 mg/dL); while moderate increases were also observed in T-1 (3.83±0.53), T-2 (3.90±0.46 mg/dL) and T-3 (3.99±0.52 mg/dL), significantly higher than NC (3.48±0.54 mg/dL).

Liver enzymes also supported the presence of hepatic stress. The levels of serum glutamic-oxaloacetic transaminase and serum glutamic-pyruvic transaminase were found to be significantly increased in PC (SGOT: 88.89±1.96; SGPT: 99.13±1.45 IU/L) as compared to NC (SGOT: 59.33±2.33; SGPT: 62.43±1.45 IU/L). Groups that went through treatment have shown significant increase of the two enzymes but in a moderate way. The highest SGPT (87.35±1.82b IU/L) and SGOT (79.58±1.61b IU/L) measured in group T-3. This indicates that E110 may cause hepatocellular

Table 1. Effects of Sunset Yellow and DMBA on Serum Biochemical Parameters.

Biochemical Parameters	NC	PC	T-1	T-2	T-3
SC (mg/dL)	74.33±1.32^{a}	110.25±1.1^{b}	92.65±1.27^{c}	94.73±2.46^{c}	908.50±1.73^{bc}
HDL (mg/dl)	32.75±1.58^{a}	18.58±1.77^{b}	21.95±1.80^{b}	19.65±1.95^{b}	17.50±0.84^{b}
LDL (mg/dl)	15.55±1.71^{a}	38.85±2.81^{b}	33.70±1.29^{c}	32.05±2.06^{c}	32.45±1.81b^{c}
TG (mg/dl)	61.65±2.56^{a}	89.64±3.45^{b}	75.03±2.35^{c}	77.23±1.20^{c}	73.94±2.34^{c}
CR (mg/dl)	0.66±0.25^{a}	1.99±0.32^{b}	1.25±0.41^{b}	1.47±0.31^{b}	1.69±0.42b^{b}
UA (mg/dL)	3.48±0.54^{a}	4.63±0.61^{b}	3.83±0.53^{c}	3.90±0.46^{c}	3.99±0.52^{bc}
BI (mg/dl)	0.44±0.33^{a}	0.67±0.24^{b}	0.47±0.37^{ab}	0.53±0.42^{b}	0.58±0.43^{b}
SGOT(IU/L)	59.33±2.33^{a}	88.89±1.96^{b}	76.28±1.14^{c}	78.45±1.34^{c}	79.58±1.61b^{c}
SGPT (IU/L)	62.43±1.45^{a}	99.13±1.45^{b}	82.53±1.05^{c}	83.13±1.33^{c}	87.35±1.82b^{c}

Values are presented as mean ± SD (n = 4). A one-way analysis of variance was conducted on statistical analysis followed by Tukey's. Values that share different superscript letters (a-c) in the same row are significantly different (P < 0.05). SC stands for serum cholesterol; HDL stands for high density lipid; LDL stands for low density lipid; TG stands for triglycerides; CR stands for creatinine; UA stands for uric acid; BI stands for bilirubin; SGPT stands for serum glutamic pyruvic transaminase; and SGOT stands for serum glutamic oxaloacetic transaminase.

injury with increased dose. Interestingly, the serum bilirubin levels in all experimental groups did not vary and was statistically similar.

3.3 Modulation of Tumor Biomarkers AFP and CA 15-3 Analysis

The serum levels of tumor-associated biomarkers, alpha-fetoprotein (AFP) and cancer antigen 15-3 (CA 15-3) were assessed across experimental groups (Figure 2). Significant alterations were noted particularly in the positive control (PC) and treatment groups compared to the normal control (Control).

The PC group, administered DMBA at 2 mg/kg body weight, exhibited a marked elevation in serum AFP levels (63.54±1.34 ng/mL), which was highly significant (P < 0.0001) when compared to the Control group (2.2±1.54 ng/mL), confirming robust tumorigenic induction. Treatment groups receiving E110 at different doses - T-1 (200 mg/kg), T-2 (400 mg/kg), and T-3 (600 mg/kg) - showed moderate reductions in AFP levels relative to the PC group. Among them, T-2 and T-3 groups exhibited near-identical AFP levels (50-55 ng/mL), and the difference between them was not statistically significant (ns), suggesting a plateau effect in response at higher doses. T-1 (200 mg/kg) showed a slightly higher AFP level (55-60 ng/mL), yet significantly lower than PC.

A similar trend was observed for CA 15-3, a glycoprotein often elevated in carcinogenic conditions. The PC group demonstrated a substantial increase (143.34±2.32 U/mL) in CA 15-3 compared to the Control group (8.9±2.1 U/mL). Although all E110-treated groups showed moderate reduction in CA 15-3, none of the treatment groups (T-1, T-2, T-3) exhibited a statistically significant difference among themselves (ns) or substantial suppression relative to the PC group. The CA 15-3 levels in T-2 and T-3 (130-140 U/mL) remained elevated, indicating persistent tumor biomarker activity despite treatment.

3.4 Histological Analysis of Mammary Tissue

In the positive control (PC) group which received a DMBA dose of 2 mg/kg body weight, neoplastic lesions were seen extensively. Microscopic examination of the sample revealed malignant ductules (yellow arrow), deposited collagen (red arrows) and characteristics of ductal carcinoma in situ (DCIS) (yellow circles). The studies showed that DMBA caused a change in the mammary epithelial tissues which caused an increase in the neoplastic and also preneoplastic conditions (Figure 3).

At a dosage of 200 mg/kg (T-1), animals that underwent treatment with E110 also showed histological alterations but less severe than PC. The structure showed some atypical duct cells and more collagen but not malignant features as often. In the T-2 group (400 mg/kg), the structure of the mammary tissues was disrupted that led to more frequent malignant ductules and lesions similar to early-stage DCIS indicating a neoplasia progression. Pathological changes in T-2 are milder than DMBA and suggest a dose-dependent tissue response to E110. The most marked histological changes amongst the treated were in the T-3 group (600 mg/kg). The tissue sections displayed a prominent ductular malignancy, thickening of the collagenous stroma and frequent foci of DCIS-like formations similar to the lesions of the PC group. Our findings are suggestive of an involvement of high dose E110 in changes in mammary tissue that are procardiogenic.

4. Discussion

The current investigation aimed to assess the potential carcinogenicity and systemic toxicity of Sunset Yellow FCF (E110), a widely used synthetic food dye, through a comprehensive evaluation of growth dynamics, biochemical alterations, tumor marker expression, and histopathological changes in mammary tissue. The motivation stems from increasing concerns about the chronic exposure of children and adolescents to synthetic dyes in processed food and the growing body of evidence suggesting their possible link with various health risks (de Oliveira et al., 2024; Miller et al., 2022).

Between week 5 and 15 of the study, the animals treated with Sunset Yellow showed higher weight gain than those in the negative control (NC) and positive control (PC) groups, especially early in the trial. This temporary enhancement in growth may result from the

stimulating impact of synthetic dyes on these enzymatic and metabolic functions. Studies show synthetic azo dyes, such as Sunset Yellow, may have a temporary stimulating effect on metabolic processes. This may account for growth acceleration during the first few weeks of treatment. However, this effect was not sustained over time. After week 20, all SYD-treated groups showed a marked decline in weight gain suggesting possible metabolic stress, toxic accumulation, and/or cellular damage due to prolonged exposure. This organism goes through a metabolic activation phase and then faces physiological overload phase.

Studies have also shown these stages for consumption of synthetic colorants. Synthetic dyes were noted to trigger biological effects by Şensoy et al. (2024) and ANS et al. (2014), followed by generation of oxidative stress, inhibiting growth and systemic functions (ANS, 2014; Şensoy et al., 2024). In a similar way, the long-term administration of Sunset Yellow disrupts the metabolic regulation of rats and affects their weight gain and physiological resistance (Medeiros et al., 2004). According to the studies, the action of synthetic food dyes is different in the short term and long term. In the short term, it acts as a metabolic enhancer. However, with repeated exposure, they become metabolic disruptors (Islam et al., 2024; Sargis et al., 2019).

There were important changes in serum parameters as seen by biochemical assessment. Animals treated with SYD had higher levels of total cholesterol, LDL, triglycerides, serum creatinine, uric acid, bilirubin and liver enzymes, including serum glutamic-pyruvic transaminase (SGPT) and serum glutamic-oxaloacetic transaminase (SGOT) than the control animals. At the same time, they also saw a marked decrease in HDL. The results were significant with a p-value of less than 0.05. It shows some hepatic and renal dysfunction. Also, a cardiovascular risk is possible. Even though the degree of biochemical disruption in all SYD groups did not reach the level exhibited by the PC DMBA group, it did confirm the dose-dependent toxicity of Sunset Yellow (Gaunt et al., 1974; Şensoy et al., 2025).

Earlier studies have found that azo dyes alter lipid metabolism and harm hepatocytes, and this study also confirms that finding. Azo bonds (-N=N-) present in Sunset Yellow can undergo reductive cleavage in the gut to form aromatic amines that promote formation reactive oxygen species (ROS). The oxidative stress that results cause mitochondrial dysfunction and injury to membranes of the cell as well as organ system damage with the liver and kidney being the primary target organ systems of xenobiotic metabolism. The oxidative dysregulation of lipid profile pools noted within the purview of the present study might as well adversely affect the process of atherosclerosis and cardiovascular pathology (Amin et al., 2010; Chami & Wilson, 2010; Yang et al., 2024).

Sunset Yellow got validated as a carcinogen through analysis of tumor markers. As anticipated, the levels of alpha-fetoprotein (AFP) and cancer antigen 15-3 (CA 15-3), both clinically valuable biomarkers of tumor activity, were greatly enhanced in the DMBA group. Animals that were given SYD were also found to have an increased concentration of these markers relative to the negative control. However, this increase was less than that of the DMBA-induced animals. The mid-range rise suggests the tumorigenic activity is partial (Amin et al., 2010; Chami & Wilson, 2010; Sevastre et al., 2023; Tufael et al., 2024).

Thus, it supports the idea that Sunset Yellow brings in neo-plastic changes. Through chronic low-dose exposure, SYD may enhance the inflammatory and oxidative signaling pathways nuclear factor-kappa B (NF-κB) and mitogen-activated protein kinases (MAPKs), and both NF-κB and MAPKs are involved in tumor promotion and progression (Huang et al., 2010; Inoue et al., 2007).

The biomarker profiles of the different SYD groups indicated that the different SYD groups can potentially be pro-tumorigenic, moreover, can modulate DMBA damage. The mechanism of the dual effect may be the development of cellular adaptation or antioxidant defense response by the cells. However, this does not mean safe as chronic exposure ultimately caused increased tumor markers. Interestingly, we have seen similar discrepancies with other environmental carcinogens. This implies that the cellular environment and the dose-duration-response relationship play an important role in pathological outcome (Jahin et al., 2023; Tufael et al., 2023; Khayyat et al., 2018).

Histological investigations revealed the effects of Sunset Yellow exposure on tissue level. Ductular hyperplasia and intermittent ductal carcinoma in situ (DCIS) were observed in the 200 mg/kg treatment group. Larger doses resulted in more pronounced histologic alterations. Comparable to DMBA-induced, the 600 mg/kg group displayed vast DCIS and malignant ductular transformation along with dense collagen deposition in the mammary gland. The findings of these dose-dependent alterations underpin the notion that Sunset Yellow induces aberrant cellular remodeling in mammary tissue (Islam et al., 2024; Rahman et al., 2025; T M Tawabul Islam, 2024).

The deposition of collagen within the mammary stroma, indicative of early fibrotic remodeling, suggests that Sunset Yellow may modify the tumor microenvironment. The overproduction of collagen makes the material outside of cells (ECM) stiff, which is known to promote tumor progression. This can help cancer cells invade, vascularize, and escape immune response. Also, several DCIS lesions and disorganized ductules mean that SYD not just an irritant but possibly carcinogenic initiator or promoter under conditions of chronic exposure (Kyhoiesh & Al-Adilee, 2022).

The molecular mechanisms causing these changes may be linked to oxidative, inflammatory pathways. The metabolism of azo dye results in the production of ROS. These ROS cause the lipid peroxidation and DNA strand breaks modulation. Furthermore,

there is pro-tumor transcription factors activation. All of these results in a pro-tumorigenic environment. Research indicates that some synthetic dyes can disturb the equilibrium of apoptosis and proliferation, which are necessary for the maintenance of homeostasis in tissues. Disruption of this delicate balance, especially in hormone sensitive organs, including the mammary gland, can increase the risk of neoplastic transformation (Thanh et al., 2024; Wang et al., 2024).

The study recognizes certain limitations despite these findings. It uses only a rodent model and therefore it may not show human physiology. Additionally, failure to assess key molecular endpoints such as apoptosis (Caspase-3), and proliferation indices (Ki-67) limited the mechanistic insight of the findings. In addition, while this was a chronic study (40 weeks), in the real world, humans are intermittently exposed over many months/years, which may yield different results. Future studies ought to use an omics-based platforms and human-relevant models to prove, as well as disprove, these findings on food dyes (Tabernilla et al., 2021; Wakade et al., 2023).

According to this study, Sunset Yellow, which is legally allowed for human consumption, has a clearly shown dose-dependent biochemical toxicity and tumor-promoting activity in a mammary gland system. That dye is used in foods targeted at at-risk groups, including children, makes it particularly worrying. According to the findings, though there may be restrictions, the long-term safety of synthetic colors needs to be re-evaluated along with stricter food standards and public policy.

5. Conclusion

As per the findings, prolonged intake of a high dose of E110 induces systemic toxicity as well as early carcinogenic changes in the mammary tissues. The maximum dose evaluated (600 mg/kg) caused a marked alteration of the normal functions of liver and kidney, besides elevating the tumor markers AFP and CA 15-3. Histological evidence of malignant ductules and DCIS indicates pre-cancerous changes. E110 is a common food dye but may pose health risks when consumed over long periods. The need for stricter safety assessments and public awareness regarding the long-term use of synthetic food additives, are highlighted by these findings.

Author contributions

M.U.P. Conceptualization, study design, supervision, data interpretation, manuscript writing, and correspondence. M.T.H.B.S. Experimental investigation, biochemical analysis, data curation, and drafting of the manuscript. E.A.S. Methodology development, lab work assistance, and validation of biochemical data. S.A.M. Animal handling, dosing administration, and sample collection. S.Y. Histopathological analysis, data interpretation, and figure preparation. B.M. Statistical analysis, data visualization, and manuscript editing. H.A. Tumor biomarker assessment, technical support, and data verification. M.B.I.P. Literature review, biochemical assay support, and manuscript refinement. B.S. Sample processing, lab analysis, and data organization. Y.H. Assistance with experimental procedures, data entry, and proofreading.

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Competing financial interests

The authors have no conflict of interest.

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