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Original Article

Histopathological Effect of Sunset Yellow on Circumvallate Papillae of Albino Rats (An Animal Study)

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Abstract

Aim: The current study aimed to explore the harmful impact of oral administration of sunset yellow on the circumvallate papillae of albino rats.

Subjects and methods: 16 male albino rats weighing 150-200g were classified into two groups (8 animals in each group). Group I control group. Group II was treated with 5 mg/kg body weight sunset yellow. All the animals were sacrificed after 4 weeks from the beginning of the experiment by ketamine overdose. The circumvallate papillae of the two groups were examined histologically, immunohistochemically for detecting apoptosis by using PCNA, histomorphometrically to detect the number of PCNA-positive cells and by real-time polymerase chain reaction for measuring the expression level of NF-κB. All the results were statistically analyzed.

Results: Treatment with sunset yellow disrupted the structural integrity of the circumvallate papillae resulting in dysplasia, increased apoptotic cells, shortened and completely obliterated trough as a result of hyperplasia of the epithelial lining of the trough, atrophy and degeneration of taste buds. There was a significant decrease in PCNA-positive cells denoting apoptosis and abnormal proliferation. Moreover, a significant increase in NF-κB expression was observed denoting inflammation.

Conclusion: The oral administration of sunset yellow showed destructive, neoplastic, and even carcinogenic effects on the circumvallate papillae.

Keywords: Sunset Yellow, Rats, PCNA, NF-κB, Circumvallate papillae.

Introduction

Life today moves at a faster rate. A rising number of customers have expressed an interest in processed meals that go through several processing stages allowing them to eat a wide range of foods **(Brembeck & Fuentes, 2017; Heidenstrøm & Hebrok, 2022).** Processed food is defined as food that has been cooked, preserved, thawed and wrapped up. It enables our rising population to enjoy a wide range of tasty foods.

Food additives are substances introduced into foods to enhance the way they look, flavor, taste, color, nutritional content, and storage **(Tawfek et al., 2015; Awuchi et al., 2020).** Food additives can be classed into antibacterial, antioxidants, artificial colors, artificial flavors, flavor enhancers, bleaching agents, and chelating agents **(Carocho et al., 2018; Shikha et al., 2021).** The benefits of food additives include maintaining product uniformity and quality, improving or

maintaining nutritional content, maintaining palatability, managing pH, enhancing flavor, or providing color. However, they cause medical issues such as allergies, decreased hemoglobin concentrations, and cardiovascular disease **(Tawfek et al., 2015; Carocho et al., 2018; Dey & Nagababu, 2022).**

Sunset yellow is a synthetic colorant based on azo compounds. Orange squash, orange jelly, swiss rolls, hot cocoa, cheese sauce, ice cream, yogurt, and soft beverages all include it. Its recommended daily intake is 4 mg/kg B.wt./day **(Sayed et al., 2012; Hussein et al., 2021; Pereira et al, 2023).** Sunset yellow is used for several purposes, including improving the visual look of commercial items, covering natural color variations, replacing color lost during processing, serving as an identifying and distinguishing aid, and increasing product stability **(Malabadi et al., 2022).**

Despite its aforementioned benefits, sunset yellow can cause allergic reactions, alter the reproductive system, and cause neurobehavioral effects **(Hussein et al., 2021)**. It can be immunotoxic due to its ability to prevent splenocytes from proliferation. Moreover, it can cause chromosomal aberration at several cell cycle phases **(Yadav et al., 2013; Ali et al., 2020).**

Food additives have become a crucial component for today's food production and therefore, the toxicity of food additives has received more attention. From the previous highlights on food additives, the aim of the current work has arisen which is the investigation of the toxic effect of sunset yellow on the circumvallate papillae of albino rats, histologically, immunohistochemically, histomorphometrically, and via polymerase chain reaction.

Subjects and Methods

Animals:

Albino rats with an average age of 3 to 4 months and weighing about 150 -200 grams were obtained and kept in the animal house, Faculty of Medicine, Cairo University, Egypt. The rats were kept in a temperature-controlled chamber with a 12-hour dark/light cycle. Rats were fed a standard pellets diet and water ad libitum.

Chemicals:

Sunset yellow was purchased from Sigma-Aldrich Chemical Co., St. Louis, MO, USA) in the form of a powder that appears red until dissolved in water with a color index number (15985) and batch number (22024080). The solution was daily prepared.

Experimental Protocol:

Animals were haphazardly divided into two groups, as follows: The normal control group received no treatment **(Kehinde et al., 2018)** while the sunset yellow (SY) group received a dose of 5 mg/kg **(Ali et al., 2020)** via oral gavage for 28 days. On the sacrifice day, the circumvallate papillae of the animals were cut into two parts; one part was immediately frozen in liquid nitrogen and stored at -80C to be used for polymerase chain reaction (PCR), and the other part was preserved in formalin for histopathological and immunohistochemical evaluation.

Histopathological & Immunohistochemical evaluation:

The specimens were fixed in 10% neutral buffered formalin for 24 hours at room temperature (23-28° C) before being processed into 5-micron thick paraffin slices. The acquired slices were stained with hematoxylin and eosin (H&E) for the overall architecture of the circumvallate papilla and labeled streptavidin-biotin horse-radish peroxidase technique for the detection of proliferating cell nuclear antigen (PCNA).

Polymerase Chain Reaction (PCR) evaluation

The expression of NF-κB in circumvallate papillae of albino was measured by using the iScript One-Step RT-PCR Kit with SYBR.

Data Analysis:

Categorical data were represented as frequency (n) and percentage (%) and analyzed using the chi-square test. Numerical data were explored for normality by checking the data distribution, calculating the mean and median values, and using the Shapiro-Wilk test. The data were found to be normally distributed and were presented as mean and standard deviation values. A T-test was used to compare between the 2 groups. The significance level was set at $p \leq 0.05$ for all tests. Statistical analysis was performed with IBM SPSS Statistics Version 22.

Results

Histopathological Results

The control group showed a normal histological structure of circumvallate papillae covered by keratinized stratified squamous epithelium and encircled by equal trough length with normal underlining connective tissue and normal taste buds concerning their histology and number **(Figure 1).** Group II treated with sunset yellow exhibited distorted circumvallate papillae with disrupted epithelium and signs of dysplasia as teardrop-shaped rete pegs, internal dyskeratosis, and nuclear pleomorphism with destructed connective tissue **(Figure 2).**

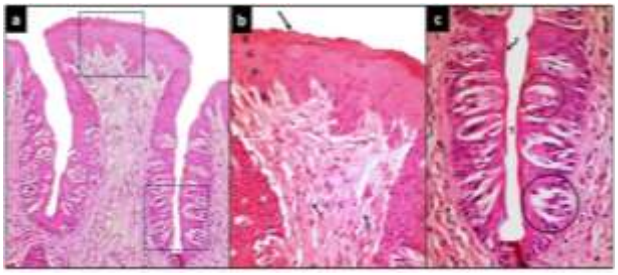

Figure (1): A Photomicrograph of group I (control group) section **(a)** showing a normal circumvallate papilla covered by keratinized stratified squamous epithelium (H&E, Orig.Mag.×100). Sections **(b&c)** Higher magnification showing normal keratinized stratified epithelium (black arrow), basal cell layer (B), prickle cell layer (P), granular cell layer (G), keratin layer (K), normal trough (T) lined with normal epithelium (curved arrow), normal shape, size, and arrangement of taste buds (black circles). Connective tissue showed normal collagen fibers (dotted arrows) (H&E, Orig.Mag.×400).

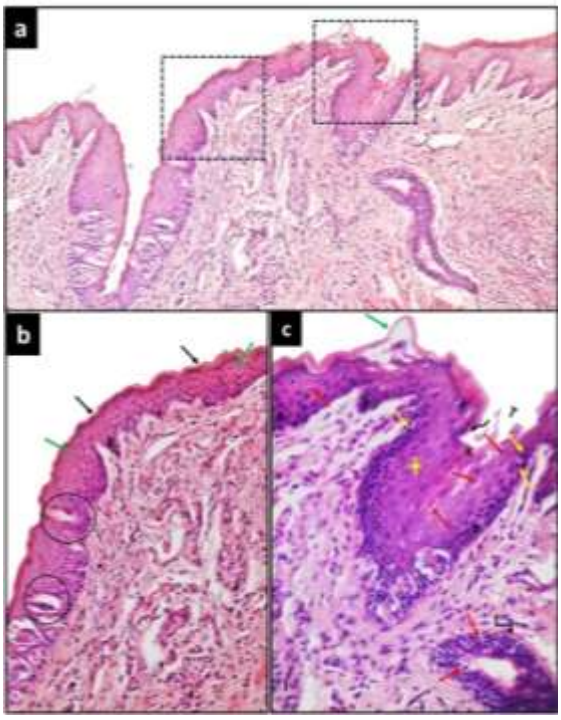

Figure (2): A Photomicrograph of group II (sunset yellow group) section **(a)** showing morphological & histological deformities of the circumvallate papilla (H&E, Orig.Mag.×100). sections **(b&c)** Higher magnification showing hyperplastic epithelium covering the CVP (black arrows) with detached keratin layer (green arrow), apoptotic cells (red arrows), shortened & completely obliterated trough (T) with corrugation & hyperplasia of the trough epithelial lining (curved arrow), atrophied & degenerated taste buds (black circles), absence of taste buds (yellow star), von Ebner salivary gland highly infiltrated with inflammatory cells (elbow arrow). The connective tissue showed areas of inflammatory cells (I) (H&E, Orig.Mag.×400).

Immunohistochemically Results

The circumvallate papilla of the control group stained for proliferating cell nuclear antigen (PCNA) antibodies showed a moderately positive PCNA staining reaction indicating the normal proliferative activity of the circumvallate papilla **(Figure 3a).** However, sections of the sunset yellow-treated group expressed weak positive staining reactions in the basal and parabasal cells of taste buds **(Figure 3b).**

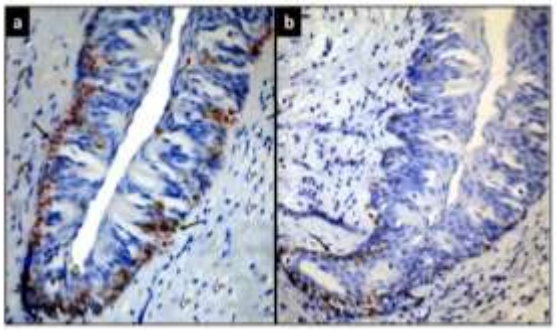

Figure (3): A photomicrograph of the trough of the circumvallate papilla of **(a)** Group I (control group) showing a normal moderately positive PCNA staining reaction (black arrows). **(b)** Group II (sunset yellow group) showed a weak positive staining reaction in the basal & parabasal cells of the taste buds expressing increased apoptosis (black arrows) (PCNA Orig. Mag.×400).

Morphometric Analysis Results

Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data showed a parametric distribution (normal). The mean and standard deviation values were calculated in each group for numerical data. An Independent T-Test was used to compare between two groups. Categorial data for each group were presented in frequencies and percentages using the chi-square test. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 22 for Windows.

There was a statistically significant difference recorded between Group I (Control Group)**,** and Group II (SY) group**,** where the p-value was 0.002* **(Table 1** and **Figure 4).**

Polymerase Chain Reaction Results

There was a statistically significant difference recorded between the control group and the sunset yellow group where the p-value was 0.001* **(Table 2** and **Figure 5).**

Table (1): The Mean, Standard deviation (SD), Maximum (Max), and Minimum (Min) values of the PCNA test in different groups.

Variables	PCNA Test (No.)			
	Mean	**SD**	**Max**	**Min**
I (control)	20.17^{A}	1.33	20	18
II (SY)	14.00^{B}	2.76	15	10
P-value	0.002*			

Superscripts with different capital letters indicate statistically significant differences within the same column. *; significant ($p \leq 0.05$) ns; non-significant ($p > 0.05$),

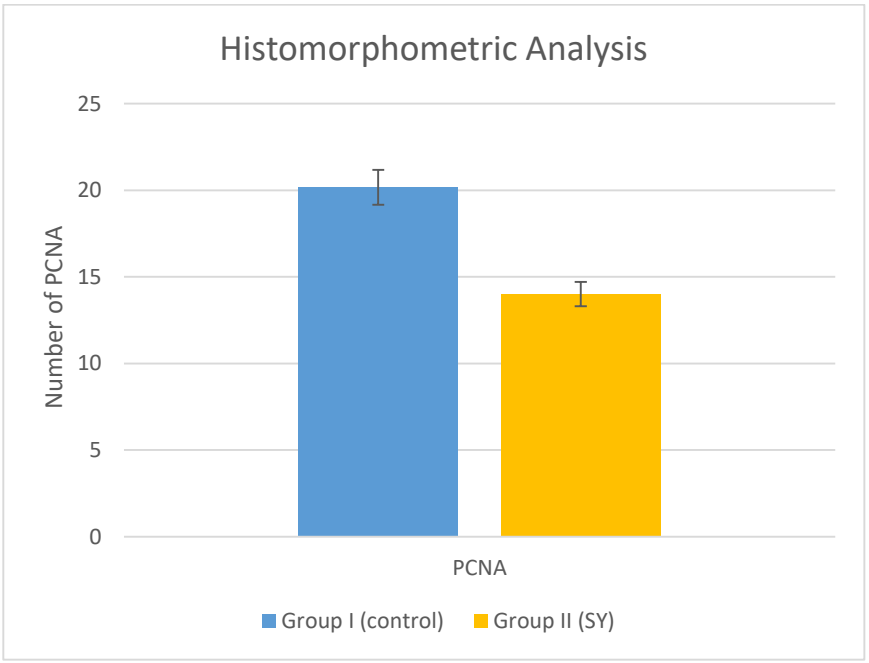

Figure (4): Bar charts of the PCNA test in different groups.

Table (2): The Mean, Standard deviation (SD), Maximum (Max), and Minimum (Min) values of the RT-PCR test in different group.

Variables	RT-PCR Test (No.)			
	Mean	**SD**	**Max**	**Min**
I (control)	1.00^{B}	0.00	1	1
II (SY)	2.39^{A}	0.61	2.9	1.48
P-value	0.001*			

Superscripts with different capital letters indicate statistically significant differences within the same column. *; significant ($p \leq 0.05$) ns; non-significant ($p>0.05$),

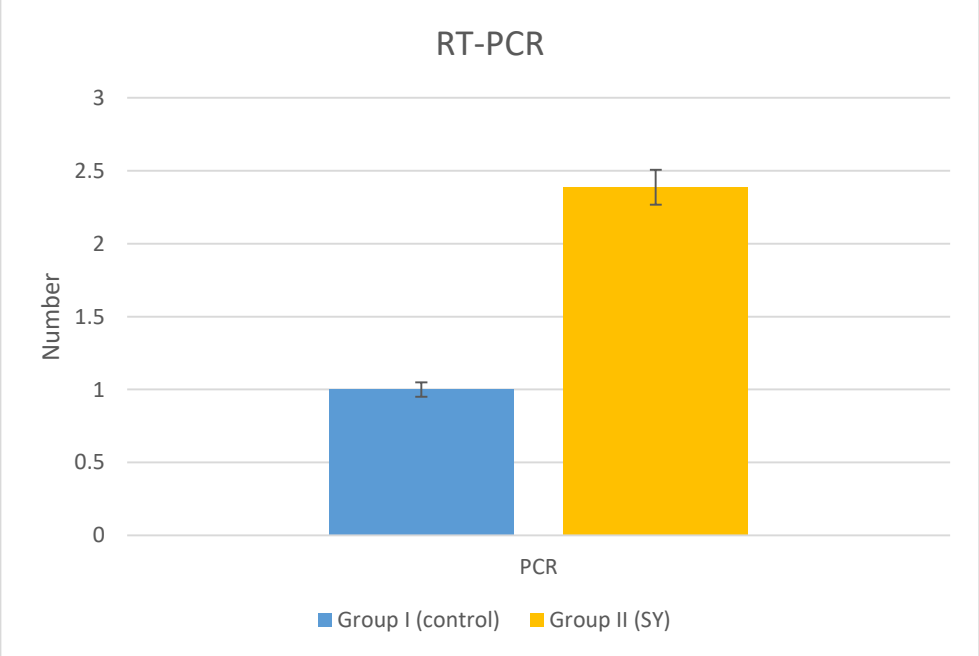

Figure (5): Bar charts of RT-PCR test in different groups.

Discussion

With the expansion of the processed food industry, food additives have become a more prevalent practice in recent food processing. As a result, their toxicity has gained increased attention. Therefore, evaluating the harmful effect of a chemical on normal cells is an important topic in toxicology that needs to be handled during the safety assessment **(Vijayan & Mazumder, 2018; Wu et al., 2022).**

Thus, the current study aimed to explore the harmful impact of oral administration of sunset yellow on the circumvallate papillae of albino rats. These toxic effects were examined histologically, and immunohistochemically by PCNA to detect proliferation and apoptosis, and by polymerase chain for measuring the expression level of NF-κB to detect inflammation. Morphometric analysis for the number of PCNA-positive cells was done. Statistical analysis for all parameters was performed.

The histopathological examination of the control group demonstrated a normal circumvallate papilla enclosed by equal trench length with normal taste buds' histology and number.

The sunset yellow group exhibited histological alterations in the form of distorted circumvallate papillae with disrupted epithelium and signs of dysplasia as teardrop-shaped rete pegs. These changes were consistent with **Khayyat et al., 2018** who found destructive effects such as disorganization of hepatic strands and degenerative structural alterations in the kidneys of the rats after administration of the sunset yellow. The same findings were described by **El-Borm et al., 2019** who examined the hepatotoxicity and nephrotoxicity in chick embryos following the administration of sunset yellow.

The toxicity of sunset yellow was the cause of all the alterations observed in this group which was coincident with what was reported by **Kaya et al.,'s 2021**, where they found that sunset yellow had highly toxic effects with negative consequences such as genotoxicity, cytotoxicity, and malignancy.

Apoptotic cells seen in this group might be due to the free radicals and oxidative stress caused by sunset yellow. The same findings were reported by **Ismail & Sakr, 2016 and Hussein et al., 2021** where the rising of the reactive oxygen species levels (ROS levels) raised the level of cysteine-aspartic acid protease-3 (caspase-3), which in turn promoted an apoptotic state.

The premalignancy changes seen in this group such as an increased rate of mitotic division, apoptotic cells, and widespread loss of adhesion among cells might be attributed to the cytosolic receptors that sunset yellow interacted with and could be the source of the compound's toxicity and carcinogenicity in mammals, as well as it could be the product of free radical generation and arylamine azo reduction **(Lu et al., 2023).**

Another explanation of these results what was supported by the work of Algarni**, 2021** who concluded that sunset yellow induced a significant increase in chromosomal aberration (CAs) and sister chromatid exchange (SCEs) frequencies.

Regarding the immunohistochemical results, the immunoreaction of PCNA in group I (control group), revealed a normal moderately positive PCNA staining reaction indicating the normal proliferative activity of the circumvallate papilla. This was consistent with the findings of **Cai et al., 2017**.

A focus on the outcomes of the immunoreaction of PCNA in group II (sunset yellow group) revealed a weak positive staining reaction due to increased apoptosis which was proved by **El-Borm et al., 2019** who reported that the apoptotic impact of sunset yellow was due to an increase in caspase-3 immunoreactivity in the hepatocytes

of the liver in their study on the embryos treated with sunset yellow.

Furthermore, these findings were consistent with those of **Hussein et al., 2021**, who found that the oral administration of sunset yellow showed increased hepatic mRNA levels of apoptotic markers (Bax, Fas, FasL, and caspase-3) while decreasing hepatic mRNA activity of the anti-apoptotic gene B-Cell Lymphoma 2 (Bcl2).

As for the qRT-PCR results, the gene expression of NF-κB in group I (control group) revealed normal NF-κB levels. This was consistent with **Zhang et al., 2015** who reported that the normal tissue had much lower levels of NF-κB expression than malignant tissue.

While in group II (sunset yellow group) there was a substantial rise in the amount of NF-κB in contrast to the control group. These outcomes aligned with those of **Del Valle et al., 2020**, who observed that increased ROS and decreased antioxidant levels resulted in oxidative stress, oxidative mitochondrial DNA (mtDNA) damage, and increased activation of NF-κB.

Conclusion:

Sunset yellow is a toxic food preservative. This toxicity appeared in the form of an alteration in the architecture of the circumvallate papillae, signs of dysplasia, and alternation of the proliferation with an increase in the level of inflammation. So, we recommend limiting the usage of sunset yellow in meals of all, kids and adults and controlling their consumption. Moreover, people should limit the use of these harmful materials as possible and they should be informed about the adverse effects of food additives generally and sunset yellow in particular.

Conflict of Interest:

The authors declare that they have no conflict of interest regarding the publication of this paper.

Funding:

The researchers of this study had not received any specific support from governmental, commercial, or non-profit entities.

Ethics:

The study was approved by the Institutional Animal Care and Use Committee (IACUC), Cairo University with an approval number of CU -III -F -43- 22.

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