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Histopathological alteration of buccal mucosa resulting from tartrazine toxicity

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ABSTRACT

Introduction: Tartrazine is a synthetic azo dye of nitrous origin that is directly absorbed through the buccal mucosa and is reduced in the intestine to a potentially carcinogenic or mutagenic called sulphanilic acid.

Objective: To evaluate the histopathological alteration of buccal mucosa resulting from tartrazine toxicity among Albino Wistar rats.

Materials & Methods: From July to December 2022, this quasi-experimental investigation was carried out at the Department of Pathology, Isra University, Hyderabad. A total of thirty mature, healthy male Wistar albino rats weighing 180–220 grams and aged between 9 and 12 weeks were gathered. Group A, the control Group, was given regular chow; Group B, which got powdered tartrazine 7.5 mg/100 gm body weight with normal chow; and Group C, which received powdered tartrazine 15 mg/100 gm body weight with normal chow, were all randomly assigned to three Groups. Following the conclusion of the trial, a histological examination of the buccal mucosal tissue from each Group was carried out. Data were analyzed by SPSS 24 for descriptive and comparative statistics, with $p \leq 0.05$ denoting significance. One-way ANOVA and Fischer's Exact test were used as indicated.

Results: Rats in Groups C and B had significantly different post-experimental body weights from those in Group A ($p<0.05$). A notable variation ($p<0.05$) in mean levels of serum anti-oxidative markers between all three Groups. The histopathological alterations of the buccal mucosal tissue in Group C rats compared to Group B and A rats were found to be statistically significant ($p<0.05$).

Conclusion: The study concludes that tartrazine toxicity causes a significant histopathological alteration of buccal mucosa.

Keywords: Azo dye, Buccal mucosa, Food colorants, Histology, Oxidative stress, Tartrazine, Toxicological effects

The authors declared no conflict of interest. All authors contributed substantially to the planning of research, data collection, data analysis, and write-up of the article, and agreed to be accountable for all aspects of the work.

INTRODUCTION

Synthetic dyes/food colors are used for domestic cooking as well as at the commercial level. Globally, over 800,000 tons of food colorant production occurs each year.[1] Azo dyes, which include aromatic azo compounds like tartrazine, are the most popular synthetic food colorants.[2]

Tartrazine is a synthetic azo dye that is water soluble, and lemon yellow colored, derived from petroleum products. It is widely used in different edible and non-edible products like soft drinks, ice creams, flavored chips, cereals, sauces, jellies, candy, chewing gum, cosmetics, soaps, shampoo and in several drugs (antihistamines, antibiotics, multi-vitamins) etc.[3-5] The acceptable daily intake (ADI) of tartrazine by World Health Organization (WHO) is 7.5 mg/kg. Tartrazine, like other azo dyes, is possibly absorbed directly through the buccal mucosa into the blood and metabolized by the enzyme azo reductase. In the intestine, tartrazine is abridged to an aromatic amine called sulphanilic acid that possess potentially carcinogenic or mutagenic capability.[6] Tartrazine metabolites may induce a state of oxidative stress by producing reactive oxygen compounds and altering hepatic and renal structures as well as metabolic profiles. Moreover, it has also potential to induce different health issues like urticaria, angioedema, as well as asthma in some atopic patients, in addition to its immune-toxic, genotoxic and mutagenic hazards.[7,8]

In many developing countries like Pakistan and India, it is used as a replacement for saffron in cooking because of its inexpensive cost. In these countries the uncontrolled and unsupervised use of food colorants is above the Acceptable Daily Intake (ADI) level, resulting in serious health hazards. It has also shown some behavioral changes such as irritability, restlessness, hyperactivity, and sleep disorders among children and vulnerable Groups.[6,9,10]

Humans in different age Groups particularly youngsters and the young, are more vulnerable to its harmful effects on the buccal mucosa. Despite the availability of substantial evidence in the literature concerning the effects of tartrazine dye on health and various organs of the body, limited studies on the histological effects of tartrazine on

the mucosal lining of the buccal cavity are available. Keeping in view, the present study was planned with the objective to assess the histopathological alteration of buccal mucosa resulting from tartrazine toxicity among Albino Wistar rats in Hyderabad, Pakistan. The findings of this investigation could shed light on the delirious effects associated with various tartrazine dosages. Additionally, how their inclusion in various everyday products impacts the oral health of children and teenagers who use them in varying amounts on a daily basis.

MATERIALS & METHODS

With approval from the Ethical Review Committee (ERC) (Ref#: IU/RR-18-IRC-23/N/2023/118), quasi-experimental study was carried out in the post-graduate research laboratory of pathology department in Isra University, Hyderabad Sindh, from July to December 2023. Animal were handled according to the National Institute of Health and International Research Council guidelines for Care and use of Laboratory Animals while the sample size was determined using the Resource Equation Method.[11] Based on the Resource Equation Method, an approach frequently employed in laboratory animal studies to assure usage of animals while preserving statistical power and supported by different studies, the study used a sample size of 30 rats, split into three Groups.[12]

Thirty adult male, healthy Albino Wistar rats (without any apparent deformity or abnormality), of ages 9 to 12 weeks, weighing 180-220 grams each were used in the current work. For acclimatization, the research animals were kept for a week in hygienic, well-ventilated cages with unlimited access to water and a balanced laboratory diet under a standard 12-hour light and dark cycle. Following the acclimatization period, the body weights of all the animals were measured using an electronic balance, and they were then divided into three equal Groups at random. Group A (Control Group, received 1 ml of distilled water daily, along with normal chow for 60 days orally). Group B (received powdered tartrazine 7.5 mg/100gm body weight mixed with normal chow) and Group C (received powdered tartrazine 15 mg/100 gm body weight mixed with normal chow).[13,14] After the experiment duration, the body weight of all rats were noted again, blood samples for the biochemical analysis were collected through cardiac puncture. All the rats were then starved for 24 hours before being sacrificed through cervical dislocation after administering anesthesia (chloroform-soaked cotton). The mucosal tissues from the cheeks of all rats were removed cautiously using punch biopsy, a circular scalpel of 8 mm diameter under the guidance of an oral pathologist. The collected tissues measuring 8 mm in diameter and 1 mm in depth were dehydrated in the concentrations of ethyl alcohol increasing from 70% to 100%. All the mucosa were cleaned with xylene before being soaked in 10% neutral-buffered formalin, cleaned, dehydrated, and clarified before being embedded in paraffin. Later, a microtome was used to make 4µm thick slices of buccal mucosal tissue blocks and slides were prepared. All the slides were stained with Hematoxylin and eosin (H&E) and examined under a light microscope (Olympus BH2) for histopathological alterations of buccal mucosa.[12]

Data were analyzed in SPSS 24.0, and presented as Mean ± SD for continuous data, while one-way ANOVA was used with post-hoc Tukey's test for the comparison between Groups. The Fisher's exact test was employed for qualitative data analysis with significance level was set at $p \leq 0.05$.

RESULTS

The mean body weights (pre-and post-experimental) for each Group are presented in Table 1. Groups B and C rats had a significant decline in body weights compared with those in Group A. Comparatively speaking, the decline of Group C body weights was more evident than Group B rats, indicating a dose-response effect of tartrazine on body weight. A statistical difference ($p<0.05$) in the mean body weight of all three Groups.

Table 1: Body weights of all Groups (n=30).

Groups	Body Weight (gm)		P value
	Pre-experiment	Post-experiment	
	Mean ± SD	Mean ± SD	
Group A	210.3±4.1	217.4±0.5	0.0001
Group B	208.5±4.8	195.4±0.6	
Group C	212.2±6.2	181.5±0.4	

Table 2 describes significant variations in serum GPX, SOD, and CAT levels by ANOVA following post-hoc Tukey test. All three Groups were found to have statistically significant ($p<0.05$) differences from one another; the greatest decline being in Glutathione Peroxidase (GPX), followed by Superoxide Dismutase (SOD), and Catalase, all in Group C.

Table 2: Post-experiment mean serum anti-oxidative marker levels in all Groups (n=30).

Markers (umol/mg protein)	Groups			p value
	A	B	C	
GPX	10.1 ± 1.4	6.8 ± 0.6	4.3 ± 0.8	0.0001
SOD	12.8 ± 1.2	9.9 ± 1.3	6.1 ± 1.1	0.0003
Catalase	18.9 ± 0.8	14.8 ± 1.3	13.2 ± 1.2	0.0001

Table 3 depicts the histopathological comparisons of the three Groups based on defined criteria such as Hyperkeratosis / Leukoplakia, Keratinization, Acanthosis, Glandular duct dilatation, and Inflammatory cell infiltration.

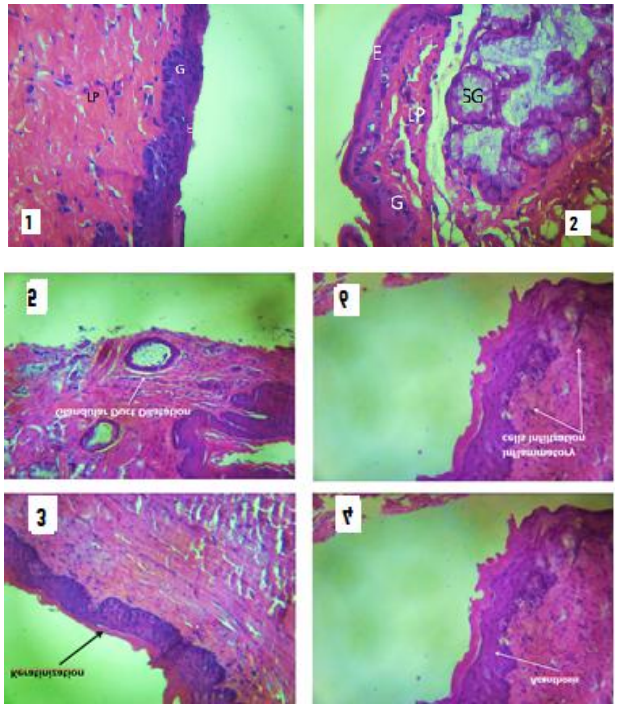
For all these criteria, Groups B and C showed a greater frequency of lesions when compared to control Group A, in addition to demonstrating a dose-response effect. The most prominent change was in Keratinization, followed by changes of Inflammatory cell infiltrate, Acanthosis, Hyperkeratosis, and lastly Glandular duct dilatation.

Based on histopathological evaluation of buccal mucosal tissues, Group C rats exhibited an overall significant alteration in the buccal mucosal tissue compared to Group A and B rats indicating greater involvement with increasing dose of tartrazine. A statistically significant differences ($p<0.05$) in histopathological presentation like presence of leukoplakia, keratinization, acanthosis, glandular duct dilatation, and neutrophil infiltration between all three Groups with more noticeable in Group C followed by Group B (Table 3).

Table 3: Histopathological analysis of buccal mucosal tissues of all Groups (n=30).

Histopathological Findings	Groups	Yes	No	p-value
Hyperkeratotic lesions/ leukoplakia (Gross)	A	1	9	0.02
	B	4	6	
	C	7	3	
Keratinization	A	1	9	0.01
	B	7	3	
	C	8	2	
Acanthosis	A	0	10	0.03
	B	4	6	
	C	7	3	
Glandular Duct Dilatation	A	0	10	0.01
	B	4	6	
	C	6	4	
Inflammatory Cells Infiltration	A	0	10	0.02
	B	4	6	
	C	9	1	

Photomicrographs (1-9) of all three Groups are presented in figure 1. Photomicrograph 1 and 2 presenting the normal buccal mucosal tissues of Group A rats (Non keratinized stratified squamous Epithelium (E), Lamina Propria (LP), Granular Layer (G) and Minor Salivary glands (SG). Photomicrograph 3-6 presenting the buccal mucosal tissue of Group B rats while photomicrograph 7-9 showing the buccal mucosa of Group C rats. Significant alteration of buccal mucosa of Groups B and C rats compared with Group A rats. Keratin hypertrophy and acanthosis with patches of lamina propria embedded have been found in Group C more prominently. Furthermore, Group C rats' minor salivary gland ducts were more dilated than those of Group B rats because of an increase in mucus production and the loss of luminal cells that line the ducts. (Figure1).



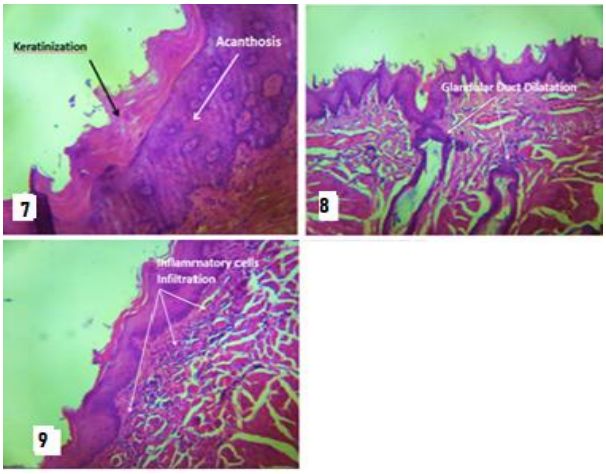


Figure 1: Photomicrographs of buccal mucosa of all Groups

DISCUSSION

Tartrazine is a commonly used food ingredient that can be identified in conventionally manufactured foods such as dyes and preservatives. It causes cationic accumulation of proteins, which has been connected to a number of diseases and irregularities in metabolism.[10] The present research was designed to evaluate the alterations in buccal mucosa resulting from the tartrazine. The impact of tartrazine on the body weight of study animals was recorded. It was observed that body weight, after induction of tartrazine in higher dose (Group C) declined significantly ($p<0.05$) compared with low dose Group B. A study by Boussada et al also detected that tartrazine had a significant effect on body weight (a significant reduction in body weight was observed) when they injected it in albino Wistar rats.[15]

Studies has shown that tartrazine causes oxidative damage in rats by lowering the concentration of glutathione and raising malondialdehyde levels, which are byproducts of lipid peroxidation.[16] Additionally, it also decreases the activity of

glutathione reductase, superoxide dismutase, and catalase, three antioxidant enzymes.[17] The findings of the present investigation align with earlier research, as we observed a noteworthy decrease ($p<0.05$) in antioxidant quantity of enzymes (glutathione peroxidase, superoxide dismutase, and catalase) in the tartrazine administered Groups B and C. Additionally, we observed that a higher level of tartrazine taken regularly had a greater detrimental effect on the buccal mucosa's tissues. Bhatt et al, documented adverse effects of routinely administered azo dyes, such as tartrazine, on metabolic markers and oxidative tissue destruction.[9] Moreover, Tartrazine toxicity is caused by the metabolic reductive biotransformation of the azo bond, which can occur either directly or through indirect methods. For example, the animal's intestinal microbiota can degrade tartrazine in the colon, producing metabolites like Aminopyrazolone and Sulfanilic acid. These tartrazine metabolites can change the architecture and metabolic characteristics of the liver and kidneys, causing damage from oxidative stress and the formation of reactive oxygen species (ROS).[13,18]

One of the present study's objectives was also the histopathological evaluation of buccal mucosa. Group B and C of tartrazine administered rat's buccal mucosal linings had distinct gross and histological characteristics. Moreover, in the present investigation, Group C rats had more obvious hyperkeratotic lesions indicative of leukoplakia than Group B and A rats did. According to a case study by Bastos et al., they saw severe structural changes in the buccal mucosa of a 15-year-old girl who had a history of regularly consuming lollipops having tartrazine dye. They stated that the girl's tongue and buccal mucosa had noticeable hyperkeratotic lesions or buccal leukoplakia.[19]

The present investigation's findings were statistically confirmed and showed that the buccal mucosa of tartrazine-treated rats exhibited greater collagen fiber deposits surrounding the acini, ducts and congestion blood vessels compared with the control Group. These were in line with the results of Kandeel et al, who found that the jejunal mucosa of rats administered tartrazine had a substantial rise in the quantity of mucosal collagen fiber accumulation.[20] Collagen expressions and production are driven by oxidation substances, that include the by-products of lipid peroxidation, which may account for the presence of disorganized acini[21] These results were in line with those of Essawy et al, who found that the rat brain administered with tartrazine had higher levels of Tumor necrosis factor-α, Interleukin-1β, and Interleukin-6.[16] Damaged tissue produces Tumor necrosis factor-α inflammatory signals, which encourage the growth of fibroblasts and ultimately lead to fibrosis.[22] J.R. dos santos et al. observed that tartrazine had mutagenesis effects on A. cepa cells during their investigation.[18] The evidence of increased clastogenic ability was validated by a rise in micronuclei following a one to three-day course of tartrazine. It also showed increased distortion of the mitotic spindle as a result of increased damage during the C-metaphase.[18] These findings demonstrated the mutagenic qualities of tratrazine, which may eventually result in chronic cancer. This may be due to the fact that stimulating lipid peroxidation and lowering antioxidant defenses, tartrazine seems to cause oxidative damage, which then results in cellular damage and inflammatory reactions. Histopathological alterations in the buccal mucosa, such as keratinization, acanthosis, and leukoplakia, may arise from this cascade.

Using a light microscope, other histological anomalies such as invasion of inflammatory cells, keratinization, glandular duct dilatation, and acanthosis were found in the buccal mucosa of this study rats. Groups B and C rats exhibited thicker keratin layers and more noticeable acanthosis across the buccal mucosa as opposed to the Group A rats. Group C rats additionally showed thicker granular layers of the keratin layer alongside established spots of lamina propria. Additionally, the buccal mucosal tissue of Group C rats administered with tartrazine exhibited the blocked vascular pathways and interstitial cellular invasion described by Wopara et al.[23] Furthermore, Group C rats' minor salivary gland ducts were more dilated than those of Group B rats because of an increase in mucus production and the loss of luminal cells that line the ducts. Owing to comparable modifications, the present study findings align with the research conducted by Bastos et al.[19]

CONCLUSION

The study concludes that the tartrazine toxicity causes a significant histopathological alteration of buccal mucosa. To fully understand the diverse effects of tartrazine on the inflammation and hormonal aspects of the entire gastrointestinal tract, more research is required. Therefore, further studies are recommended including the investigations of effects of tartrazine on entire gastrointestinal tract.

LIMITATIONS

The 60-day exposure duration used in the present study may not adequately represent the long-term consequences of tartrazine. The detected histological alterations, including glandular duct dilatation, acanthosis, and leukoplakia, point to possible chronic harm, although it's not yet apparent if these alterations are progressive or reversible over time which is one of the limitation of the present study. Moreover, lack of funding and time prevented laboratory testing for further inflammatory indicators and hormone assays. Limited local studies carried out to assess the effects of tartrazine on oral mucosa is also another constrain.

RECOMMENDATIONS

Further studies should evaluate tartrazine's long-term effects, including any damage that may recover or worsen when exposure is stopped, in order to determine if the changes that have been seen are transient or irreversible

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