Original Article

Detrimental Effects of Tartrazine on Oral Mucosal Tissues of Wistar Albino Rats: An Experimental Study

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ABSTRACT

Objective: To evaluate the detrimental effects of Tartrazine on oral mucosal tissues of Wistar Albino rats.
Study Design: Experimental study
Place and Duration of Study: This study was conducted at the Department of Oral Biology, Karachi Medical and Dental College (KMDC), Sindh from March-May 2023.
Materials and Methods: After approval from the Ethical & Scientific Review Committee, KMDC. Eighteen adult healthy male and female Wistar albino rats, weighing 180–200 grams were used and kept in temperature controlled room with a typical 12h light-dark cycle at a temperature of 23°C for two weeks of acclimatization. Animals were randomly clustered into three equal groups (A, B, and C). Group B and C (experimental groups) rats were given Tartrazine at doses of 7.5, and 15 mg/100gm body weight respectively for 60 days while group A (Control group) was given a normal chow diet along with distilled water. Biochemical and Histopathological analysis of oral mucosal tissue of all groups was performed after 60 days.
Results: Significant decline ($p<0.05$) in body weight of group B and C rats compared to group A rats. A statistically significant difference in antioxidant levels (GPX, SOD, and CAT) with post-hoc turkeys ($p<0.05$) was observed between group C compared to group B and A rats. While a marked histo-pathological alteration of oral mucosal tissue (keratinization, Acanthosis, dilatation of the minor salivary ducts, and neutrophil infiltration) was demonstrated in group C rats compared with group B and A rats.
Conclusion: Tartrazine in increased doses has a considerable influence on histological, morphological, and immune-histochemical degenerative effects on the oral mucosal tissues.
Key Words: Azo compounds, Oral Mucosa, Oxidative stress, Tartrazine

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INTRODUCTION

Food colors have been proven to influence the choice of food by altering flavour, sweetness, and pleasantness.[1] Several appealing both synthetic and natural food colours have been used for the production of food merchandise. Among them, artificial food colors are cheaper and therefore utilized more by the food and beverages industry. These dyes are used in both domestic as well as food industries.

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Among the most frequently used artificial food hues are comprised of aromatic azo compounds such as Tartrazine.[2, 3]

Tartrazine is a water-soluble, lemon yellow-colored synthetic azo dye derived from petroleum-derived substances. It is frequently used in a wide range of culinary and non-edible goods consumed by children including; Jellies, candies, crisps, ice creams, etc. Moreover, it is also used for making, soaps, shampoos, cosmetics as well as medicines.[1, 2, 4]

Similar to other azo dyes, tartrazine may enter the bloodstream soon after its direct absorption through the oral mucosa and get metabolized in blood by azo-reductase enzymes. While due to its nitrous origin, tartrazine get reduced in the intestine in a potentially mutagenic and carcinogenic aromatic amine called Sulphanilic acid.[5]

Tartrazine derivatives may induce oxidative stress by producing reactive oxygen species (ROS) that alters the tissue architecture.

Furthermore, in addition to its immunetoxic, genotoxic, and mutagenic risks, tartrazine has also been linked to health issues like asthma, angioedema, and urticaria in some atopic patients. [6,7]

The World Health Organization (WHO), has reported the acceptable and tolerable daily intake limit of around 7.5 mg/kg of tartrazine. Despite this recommended level, due to its lower cost the unregulated and unsupervised use of tartrazine in countries like Pakistan exceeds the recommended daily intake. This excessive dose of tartrazine results in number of health issues posing major health risks specially among children and young adults includes certain behavioral abnormalities, irritability, restlessness, hyperactivity, and sleep disturbances.[5, 8, 9]

Even though there is ample information in the literature on the potential health effects of tartrazine on various organs, but very limited research has been done on its histopathological effects on the oral mucosal lining. The detrimental effects of tartrazine on the oral mucosa may more likely to affect adolescents and young children. In light of this research gap, the current study was designed to evaluate the detrimental effects of Tartrazine on oral mucosal tissues of Wistar Albino rats.

MATERIALS AND METHODS

After getting approval from the Ethical & Scientific Review Committee, KMDC, this experimental study was executed from March-May 2023 at the department of oral biology, KMDC, Sindh. Eighteen adult healthy male and female Wistar albino rats, weighing 180–200 grams were used and kept in temperature controlled room with a typical 12h light-dark cycle at a temperature of 23°C for two weeks acclimatization. All rats were kept in adequately ventilated polypropylene cages with unrestricted access to balanced laboratory feed and water.

Following acclimatization, the rats were clustered into three groups (A, B and C) each of the group comprised of six rats, three of each sex. For 60 days, all animals received daily oral gavage at a volume of 10ml/100gm body weight. The powdered form tartrazine was dissolved in distilled water. Group B and C (experimental groups) rats were given Tartrazine at doses of 7.5, and 15 mg/100gm body weight respectively for 60 days while group A (Control group) was given normal chow diet along with distilled water.[10, 11]

During the experiment duration, the general health of rats was checked on a daily basis while the body weights of all animals were determined and recorded twice (first after completion of acclimatization and second on completion of experimental period). All the study animals were food starved for 24 hours, but not to drink. Then, animals were exsanguinated through cervical dislocation till death under anesthesia. The blood samples have been drawn via the abdominal aorta for biochemical analysis and collected in dry glass centrifuge tubes afore being centrifuged at 3,500 rpm for 15 minutes in a Beckman Model T-6 chilled centrifuge. Samples of oral mucosae from the buccal area was carefully removed from the cheeks. All the collected mucosae were dehydrated, clarified, and embedded in paraffin before being placed in 10% neutral-buffered formalin. Later, the tissue blocks were formed, and a microtome was used to slice the oral tissue into pieces that were 4µ m thick. The Hematoxylin and eosin (H&E) stained slides were prepared for histo-pathological evaluation under a light microscope (Olympus BH2) [12]

Data is expressed as the mean ± SD. The statically significant difference between all three groups was evaluated by applying ANOVA followed by Post-hoc Tukey's test for group comparison using SPSS version 26. The difference was regarded as statistically significant if the probability value was $p \leq 0.05$.

RESULTS

Total mean pre- and post-experimental body weights of all group rats is presented in table I. A substantial decline has been demonstrated in group B and C rats compared with the control group. Similarly, decline in group C rats was more noticeable compared with group B rats. There was a statistically significant difference in body weight between all three groups ($p<0.05$).

Table No. 1: Pre and post body weight of study animals in different groups (n=18)

	BODY WEIGHT		P value
	Pre-experimental	Post-experimental	
	Mean ± SD	Mean ± SD	
Group A	209.2±8.1	215.6±0.6	0.000*
Group B	209.7±5.2	194.7±0.8	
Group C	211.2±6.3	182.2±0.3	

* Statistically significant ($p<0.05$)

The difference of antioxidant levels (GPX, SOD, and CAT) with post-hoc tuckeys is presented in table II. There was a statistically significant ($p<0.05$) between all three groups was demonstrated. (Table 2)

Table No. 2: Comparative analysis of anti-oxidant parameters through Post-Hoc Analysis

Parameters	Group-I	Group-II	Group-III	p-value
Glutathione peroxidase (umol/mg protein)	9.7±1.5[bc]	6.6±0.7 [ac]	4.5±0.9[ab]	0.00*
Superoxide dismutase (u/mg protein)	13.2±0.5[b,c]	10.4±0.8 [ac]	5.9±0.2[ab]	0.00*
Catalase (u/mg protein)	19.6±1.1[bc]	15.5±1.6 [ac]	12.4±0.9[ab]	0.00*

Histo-pathological details of post-experimental oral mucosal tissues of all three groups is presented in table III. Statistically significant ($p<0.05$) differences in histo-pathological alteration of oral mucosal tissue in terms of keratinization, Acanthosis (granular layer hyperplasia), dilatation of the minor salivary glandular duct, and neutrophil infiltration were observed in group C rats when compared to groups B and A rats.(Table 3)

Table No. 03: Histo-pathological findings of oral mucosal tissues of all groups (n=18)

HISTOPATHOLOGICAL Findings	Groups	Yes	No	p-value
White Patches / Oral Leukoplakia (Gross)	A	1	5	0.02*
	B	2	4	
	C	5	1	
Keratinization	A	1	5	0.00*
	B	4	2	
	C	5	1	
Acanthosis	A	1	5	0.03*
	B	1	5	
	C	5	1	
Glandular Duct Dilatation	A	0	5	0.01*
	B	2	4	
	C	4	2	
Inflammatory Cells Infiltration	A	1	5	0.02*
	B	2	4	
	C	6	0	

* Fisher's exact test

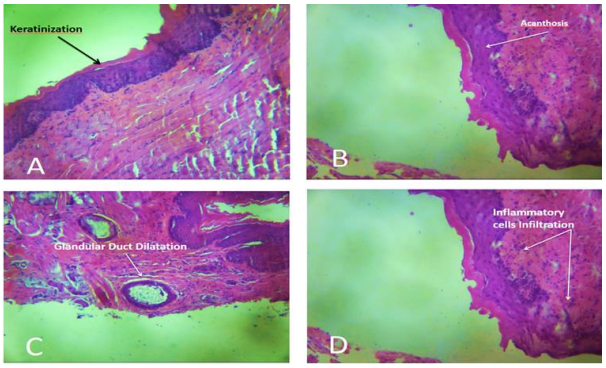

Figure No. 1a: Photo-micrographic presentation of oral mucosal lining of rats in group 2 Moderate keratinization (A), Acanthosis (B), glandular duct dilatation (C) and inflammatory cells infiltration (D) (H&E 100x)

Keratin layer hypertrophy and acanthosis have been identified as thickening of the oral mucosal granular layer with patches of lamina propria embedded in groups B and C rats compared to group A rats, with group C rats having more thickened keratin layer and more prominent acanthosis over the buccal mucosa than group B rats. Moreover, the ducts of the minor salivary glands were more dilated in group C rats compared to group B rats due to the loss of luminal cells lining the ducts as well as increased mucous production. (Figure 1a and 1b)

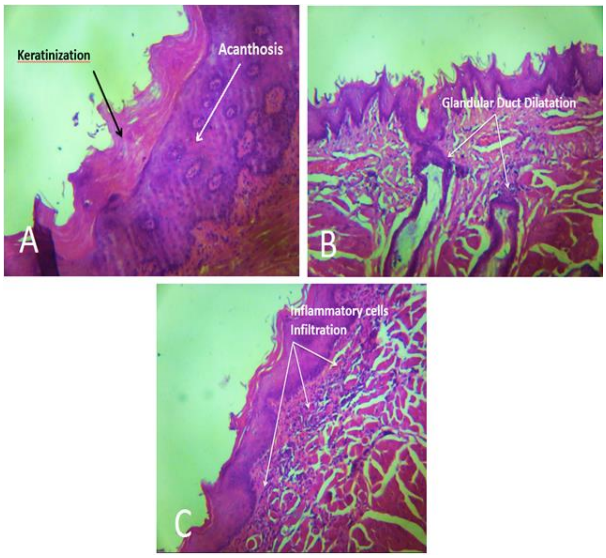

Figure 1b: Photo-micrographic presentation of oral mucosal lining of rats in group 3 showing Severe keratinization and Acanthosis (A), glandular duct dilatation (B) and inflammatory cells infiltration (C) (H&E 100x)

DISCUSSION

Tartrazine is a widely used food additive found in commercially processed foods (in the form of preservatives and dyes) globally. It produces cationic protein aggregation, which has been linked to a variety of illnesses and metabolic abnormalities.[9] The present study evaluated the histopathological alterations of oral mucosa caused by tartrazine. In this study, the impact on the body weight of Tartrazine was demonstrated. Significant ($p<0.05$) decline in body weight of high dose tartrazine group (group C) compared with low dose group B. Boussada et al also observed that following tartrazine injection, albino Wistar rats' mean body weights significantly decreased.[13] These findings are consistent with findings of our study.

Studies have demonstrated that tartrazine induce oxidative stress among rats by increasing the malondialdehyde levels as a byproduct of lipid peroxidation with a decrease in glutathione levels. [14] Moreover, it also lower the activity of antioxidant enzymes including catalase, superoxide dismutase, and glutathione reductase.[15] Our study findings are also consistent with the previous studies as there was a significant decline ($p<0.05$) in levels of anti-oxidant enzymes (glutathione peroxidase, superoxide dismutase and catalase) among the tartrazine induced groups B and C. Furthermore, we demonstrated that with increase in dose of routine tartrazine intake results in more

negative influence on tissues of the oral mucosa. Bhatt et al. also reported that negative influence of azo dyes like tartrazine in routine dose on the biochemical indicators as well as oxidative damage of different tissues.[8]

Histopathological evaluation was also one of the objective of our study. Different morphological and histological details were demonstrated on the oral mucosal lining of experimental rats (group B and C). White Patches / Oral Leukoplakia was more pronounced in group C rats compared with group B and A rats in the present study. A case report by Bastos et al. report that they observed the gross morphological alterations in the oral mucosal of a 15 years old girl with history of daily consumption of tartrazine dye containing lollipop. They reported that visible white patches / oral leukoplakia was observed over girl's tongue and buccal mucosa.[16]

The current research discovered that tartrazine-treated rats had more collagen fibres deposition around the acini, ducts, and congested blood vessels in the oral mucosa than the control group, which was statistically validated ($p<0.05$). These were consistent with the findings of Kandeel et al. who discovered a considerable increase in the amount of mucosal collagen fibres deposition in the jejunal mucosa of tartrazine-treated rats.[17] Oxidation agents, such as products of lipid peroxidation, drive collagen expression and formation, which might explaining the existence of disordered acini.[18] Consistently these findings were in complies with Essawy et al. who revealed an increase in the levels of TNF-α, IL-1β and IL-6 in the tartrazine -treated rat brain.[14] TNF-α inflammatory signals are generated by injured tissue, promoting fibroblast development and, as a result, fibrosis.[19]

Several more histopathological abnormalities like Keratinization, Acanthosis, glandular duct dilatation and inflammatory cell infiltration, were observed in the oral mucosa of present study rats under a light microscope. When compared to group A rats, groups B and C rats showed Keratin layer thickening and acanthosis as thickening of the buccal mucosa's granular layer with patches of lamina propria embedded with group C rats having more thickened keratin layer and more prominent acanthosis over the buccal mucosa than group B rats. Moreover, the ducts of the minor salivary glands were more dilated in group C rats compared to group B rats due to the loss of luminal cells lining the ducts as well as increased mucous production. Because of similar alterations, our conclusion is compatible with the investigation by Bastos et al.[16] Nevertheless, Bastos et al. noticed no glandular dilatation in the oral mucosa, which contradicts the current investigation, which found glandular dilatation in both experimental groups.[16] That might be because of the differences in glandular architecture between humans and rats. Furthermore, Tartrazine-treated oral mucosal tissue of group C rats also showed the interstitial cellular infiltration and clogged blood vessels reported by Wopara et al.[20] These findings are attributed to tartrazine's oxidative-inflammatory stress.

To the best of knowledge this study was first of its kind, no such study have been conducted in our setting so far. Laboratory studies for other inflammatory markers and hormonal assays were not done due to a lack of time and financial resources. Further studies are needed to examine the varied impacts of tartrazine on inflammatory and hormonal parameters of whole gastrointestinal tract.

CONCLUSION

The study concludes that tartrazine has a considerable influence on histological, morphological, and immune-histochemical degenerative effects on the oral mucosal tissue. Tartrazine's usage as a food additive should be limited as much as feasible.

Author's Contribution:

Concept & Design of Study:	Nabeel Khan
Drafting:	Syeda Afshan Jamal, Filza Minhas
Data Analysis:	Shayan Zufishan, Filza Minhas
Revisiting Critically:	Irum Munir Raja, Munnawar ul Haque
Final Approval of version:	Irum Munir Raja

Conflict of Interest: The study has no conflict of interest to declare by any author.

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