EVALUATION OF THE EFFECT OF TARTRAZINE VERSUS CURCUMIN AS FOOD COLORING AGENT ON TONGUE PAPILLAE OF ALBINO RATS (HISTOLOGICAL AND SCANNING ELECTRON MICROSCOPIC STUDY)

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ABSTRACT

Introduction: Color additives are used in food for improving the aesthetic appearance. They have been caused many health problems. Tartrazine is widely used food color additives and considered as food carcinogen. Curcumine has been found to be used as a promising natural food additive.

Aim: investigate the effect of synthetic coloring additive tartrazine and natural one curcumin (has the same yellow color) on two main types of lingual tongue papillae, the filiform and fungiform papillae of albino rat.

Materials and Methods: Fifteen adult male albino rats (180 -200 gm) divided randomly into three groups. (n=5). Group I (Control): rats were given 1 ml distilled water. Group II (Tartrazine-treated): rats were given Tartrazine dissolved in distilled water with dose of 7.5 mg/kg. Group III (Curcumine-treated): rats were given 200mg/kg of curcumin suspended in 0.5% carboxymethylcellulose (CMC). All of them gave the dose once daily by oral intubation. After 30 days of treatment, animals were scarified and tongues were dissected and cut longitudinally into two half one processed for light and the other for scanning electron microscopic examination SEM.

Results: Histological examination of group I revealed the normal features of the dorsal surface of tongue and shape of filiform papillae. In group II has distortion of filiform and fungiform papillae and evidence of acanthosis, hyperkeratosis, and the basal cell layer has basilar hyperplasia, nuclear hyperchromatism. Disfigured fungiform papillae with swollen taste buds and its cells appeared separated. However, group III filiform and fungiform papillae appeared close to normal with minimal changes.

Conclusion: From the present study we concluded that tartrazine has degenerative changes in both the filiform and fungiform papillae with absence of these degenerative effects in comparison with curcumin. Replacement of synthetic additives by natural food ones help to avoid the adverse effects of synthetic one on lingual tongue papillae.

KEY WORDS: Color additives, tartrazine, curcumin, Tongue papillae.
INTRODUCTION

A color additive is any dye, pigment or substance which added or applied to a food, drug or cosmetic, or to the human body, causing color change. Color additives are used in food for improving the aesthetic appearance. According to the FDA’s regulatory definition, colorants are classified into; the synthetic and the color additives extracted from natural products (1).

In Egypt, there has been more increasing in the use of synthetic food colorants in the last few years that added to food mostly consumed by children without controlled use (2). Worldwide, the use of colors in food at high doses has many pathological effects and health problems, especially in children (3). These synthetic food coloring agents have been reported in causing certain damages in different organs of the experimental animals in brain and testes (4).

Synthetic color used in a wide variety of food stuffs such as: bread cereals, cheese and milk products, snack foods, fried fish and meat products, ice cream, juices, sweets, sugar toys, jams and jellies (5). Tartrazine is widely used food color additives. It is an orangecolored, water soluble powder used to produce color at several foods, drugs and cosmetics yellow dyes, its E number is E102. Thus, several studies have been made to demonstrate the toxicity and carcinogenicity of different food colorants (6). It gives variable results of allergic reactions at low level such as asthma and urticaria, as well as a results on mutagenesis and carcinogenesis due to its transformation into aromatic amine sulfanilic acid after being metabolized by the gastrointestinal micro flora hence, it is considered as food carcinogen (7).

Natural colors have always been part of the diet. They have been isolated and added back to foods for appeal. Chlorophylls, carotenoids, and anthocyanins are consumed in the foods we eat every day. Common natural colorings include annatto, stigmas of saffron flower, paprika, grape skins, caramel, Milkwort flowers, beetroot, cochineal, and turmeric (1). Curcumin, a yellow pigment from Curcuma longa, is a major component of turmeric acid and is commonly used to produce the yellow color of the spice and food-coloring agent. It is also used as a cosmetic and in some drug preparations. Also, it has preventive or putative therapeutic properties due to its antioxidant, analgesic and anti-inflammatory properties (8,9). Curcumin has also been found to be protective with antioxidant activity, as a promising natural food additive to counteract oxidative stress and hepatotoxicity caused by dietary exposure to the synthetic tartrazine (10).

Although synthetic food coloring agents have a long history of use with many health problems. Subsequently, there has been increased the effort to discover new natural alternatives (11). Therefore, the aim of the present work to investigate the effect of synthetic yellow coloring additive tartrazine and natural one curcumin (has the same color) on two main types of lingual tongue papillae, the filiform and fungiform papillae of albino rat.

MATERIALS AND METHODS

Animals

Fifteen adult male albino rats weighing between 180 -200 gm were obtained from animal house, Mansoura University, faculty of medicine. They were kept in separate stainless steel cages under controlled conditions at constant temperature (24°C), good ventilation and given adequate diet throughout the experimental period.

Materials

- Tartrazine (FD and C Yellow No. 5) to obtain synesthetic yellow color was obtained from Sigma chemical Company and dissolved in distilled water at a different concentrations; namely 1% of diet (low dose) and 3% of diet (high dose) (12).
Curcumine powder was purchased from the local market as natural yellow color and suspending in 0.5% carboxymethylcellulose (CMC) just before administration (13).

**Experimental Design:**

After adaptation period of one week, the animals were divided randomly into three sets containing five rats in each cage. The groups were classified into:

- **Group I (Control group):** rats were kept under normal condition and were given 1 ml distilled water once daily by oral intubation.

- **Group II (Tartrazine-treated group):** rats were given tartrazine dissolved in distilled water with dose of 7.5 mg/kg body weight (3% of diet) once daily by oral intubation (14).

- **Group III (Curcumin-treated group):** rats were given 200 mg/kg of curcumin suspended in 0.5% carboxymethylcellulose (CMC) once daily by oral intubation (15).

After 30 days of treatment, animals from control and treated groups were fasted overnight and sacrificed by ketamine overdose (16).

Tongues from all groups were carefully dissected and cut longitudinally into two half one half processed for light and the other half for scanning electron microscopic examination.

**RESULTS**

### 3.1 Light Microscopy (LM) (Haematoxylin and Eosin stain (H&E) results):

#### A) Filiform papillae

**Group I (Control group):**

Dorsal surface of the tongue revealed normal arrangement of filiform papillae which appeared sharp conical, long and slender with a thread like covered with stratified squamous epithelium with thin regular keratin layer. Well-formed connective tissue and muscle fibers running in different direction were also seen. (Fig 1: a, a”)

**Group II (Tartrazine group):**

Examination of these papillae revealed loss of normal appearance being clearly distorted and has acanthosis, hyperkeratosis of the covering epithelium, the basal cell layer has basilar hyperplasia, nuclear hyperchromatism. Areas of focal hyperkeratosis overlying the atrophied papillae. Atrophy of connective tissue fibers and muscles were be seen revealed signs of fatty degeneration. (Fig 1: b, b”)

**Group III (Curcumin group):**

Most of these papillae revealed a relatively normal appearance including conical flame shaped, their covering epithelium and keratin layer. Normal distribution of tongue muscle fibers were also seen. However, there were some regions that showed short ill-defined filiform papillae. (Fig 1: c, c”)

- The samples were treated with 8N hydrochloric acid at 60° for 30 minutes to remove mucus from the tongue surface and prepared for scanning electron microscope study. Specimens were then examined and photographed with JEOL, JSM-53009 at magnification of 400x in SEM unit, Mycological center, Al-azhar University.
Group I (Control- group):

These papillae were seen scattered in between filiform and revealed mushroom shape with broader surface and wide vascular connective tissue core and covered by thin uniform layer of keratin. A single well-defined barrel-shaped taste bud was seen at each papilla. (Fig 2: a, a”)

Group II (Tartrazine- group):

The fungiform papillae were apparently shorter, distorted and covered by thick layer of keratinized epithelium. Taste buds shorted, swollen and its cells appeared separated and some areas of congested blood vessels were seen. (Fig 2: b, b”)

Group III (Curcumine- group):

These papillae were closely resembled those of Group I with no obvious distortion. The taste bud appeared rounded with normal cells and almost regular arrangement of taste cells. (Fig 2: c, c”)

B) Fungiform papillae

Fig 1: Photomicrographs of the dorsal surface of the tongue for filiform papillae from all examined groups showing:

a, a”: Normal arrangement and conical shape of papillae covered with stratified squamous epithelium with thin regular keratin layer ( ● ) and muscle tissue running in different directions ( ● )

b, b”: distorted papillae with hyperkeratosis ( ▲ ), Acanthosis ( ▲ ), and Fatty degeneration of the tongue muscles ( ▲ )

c, c”: relatively normal structure of surface epithelium with conical flame shaped tongue papillae and normal distribution of muscle fibers. (H&E, Mic Mag x100 a, b, c - Mic Mag x400 a”,b”,c”).
Scanning electron microscope results (SEM):

A) Filiform papillae

*Group I (Control group):*

Examination of the dorsal surface of the tongues showed the filiform papillae that were numerous over the entire surface of the tongue, regular oriented and sharp conical projections with pointed tips. (Fig 3: a)

*Group II (Tartrazine group):*

Examination of these papillae revealed numerous filiform papillae with disturbed orientation and inclination, loss of their height, thickness and not present the typical thread shape where some of them has bifurcated tapering ends. (Fig 3: b)

*Group III (Curcumine group):*

Most of these papillae revealed almost normal direction and distribution of filiform papillae. However, few filiform papillae depicted blunt or serrated tips. (Fig 3: c)

B) Fungiform papillae

*Group I (Control group):*

Fungiform papillae has mushroom like shape with a smooth surface observed in between the filiform papillae. A centrally located well-defined regular taste bud around the regular taste pore surrounded by shallow indentation. (Fig 3: d)
Group II (Tartrazine group):

These papillae appeared less prominent and clearly distorted. The surface seemed irregular and wrinkled and the typical mushroom-shape could not be distinguished.

The taste bud and taste pore on most of the fungiform papillae was rarely encountered as it appeared rather smooth and ill-defined. (Fig 3: c)

Group III (Curcumine group):

The fungiform papillae showed almost control image with regular smooth covering and well defined taste buds and taste pore nearly similar to that of Group I. (Fig 3: f)

DISCUSSION

Synthetic food colors have been widely used nowadays for esthetic to make the foods attractive and stimulate appetite. In the last few years there is an uncontrolled use of synthetic color mostly food consumed by children. Therefore, more attention must be held on the physiological and pathological effects of food color additives (18). At this study, tartrazine was selected as synthetic food coloring agents because it is widely used food color additives with pathological problems after prolonged use, causing health problems such as anemia and allergic reactions as asthma and urticaria, pathological lesions in the brain, kidney, spleen and liver, tumors and
cancer and it is considered as food carcinogen due to its transformation into aromatic amine sulfanilic acid after being metabolized by the gastrointestinal micro flora as stated by Moutinho, et al., 2007 (19).

Our study aimed to evaluate the effect of artificial yellow coloring additive tartrazine and natural one curcumin (has the same color) on two main types of lingual tongue papillae, the filiform and fungiform papillae of albino rat.

The dorsal surface of the tongue has four types of lingual papillae, the filiform, fungiform, circumvallate and foliate papillae. The filiform papillae are more widely distributed and cells of these papillae have a high metabolic activity and so, any enzymatic disturbance, vascular changes or nutritional deficiency results in their atrophy. These atrophic changes faster and earlier before any other papillae and appeared in the form of distorted shape of papillae, shortening, increased interpapillary distances, thinning of the epithelium and some areas of degeneration of basal and parabasal epithelial cells. At SEM, this atrophy in the form of apparent thinning of papillae, tearing of keratin, decreased papillary density and areas completely devoid of papillae as mentioned by Osman et al., 2006 (20).

Filiform papillae are not only holding the food but also help in swallowing the food particles as stated by Jackowiak, Godinick, 2007 (21). The number of fungiform papillae varied according to both the range of food intake and consumption of a species and also to the size of the specied tongue as explained by Hwang, Lee, 2007 (22).

Histological (H&E) stain and scanning electron microscopic examination SEM of group II showed that the filiform and fungiform papillae were disrupted and damaged. This findings were in accordance with Sarkar, Ghosh, 2014 (23) who examined the chronic toxic effects of food color (Metanil Yellow) when applied on the tongue surface of albino rat and revealed the degenerative changes in both the filiform and fungiform papillae.

Samaranayake L, et al., 2008 (24) stated that the atrophy the filiform papillae are affected at first, followed by atrophy of fungiform papillae. In the present study, the fungiform papillae of group II were apparently shorter and covered by thick layer of keratinized epithelium, taste buds were swollen, distorted and its cells appeared separated. The SEM confirmed this apparent distortion in shape. They have irregular, wrinkled surface and ill-defined taste bud and taste pore on most of the papillae. These results were in accordance with Fortoul, et al., 2010 (25) who evaluate the effect of heavy metal (manganese) poisoning on the fungiform papillae of mice and reported that the swelling of taste buds in fungiform papillae would decrease the function of these taste buds and result in changes in food intake behavior.

At group II of tartrazine treated, there were alteration in the histological structure of the fungiform papillae where some papillae lost their characteristic mushroom shape and appeared thin and elongated. These observations were in agreement with Nagato, et al., (26) in a study had been done on morphogenesis of the rat fungiform papillae after denervation and observed that the atrophic fungiform papillae can be transformed to a form resembling filiform papillae.

Examination of the dorsal surface of the tongue at group II revealed that the filiform papillae has loss their normal appearance being clearly distorted and has acanthosis, hyperkeratosis of the covering epithelium, the basal cell layer has basilar hyperplasia, nuclear hyperchromatism and signs of fatty degeneration of muscle fibers. In accordance with our results Mahmoud and Mahmoud, 2013 (27) who demonstrated the effect of propolis as a natural antioxidant in prevention of fenitrothion induced toxicity on rabbit’s tongue mucosa.

The histological results of the current study demonstrated that tartrazine induced sever degenerative changes which were evident in filiform and fungiform papillae. This results were
in agreement with those of Ghonimi and Elbaz, 2015\(^{(28)}\) who demonstrated the serious effect of tartrazine toxicity and concluded that it has more pronounced effect in liver then kidney, stomach, testis and brain tissues. Moreover, the curative protective effect of both royal jelly and cod liver oil are non-significant against tartrazine toxicity. also in coincidence with Moutinho, et al., 2007\(^{(29)}\) who obtained an increase in the number of lymphocytes and eosinophils of the gastric mucosa when 7.5mg/kg bw of Tartrazine was offered in drinking water \textit{ad libitum} to westar rats at the age of twelve months. Also, as stated by Mpountouka, et al., 2010\(^{(30)}\) tartrazine had a toxic effect on human lymphocytes in vitro as a result of binding directly to DNA.

Histological (H&E) stain results at group III showed that a relatively normal appearance of filiform papillae including conical flame shaped, their covering epithelium and keratin layer. Normal distribution of tongue muscle fibers and confirmed by SEM compared to group II. These results were in agreement with El-Malky, et al., 2014\(^{(31)}\) in the study done to investigate the biochemical and histological alterations in the liver and kidney of male rats due to daily oral intake of beet and curcumin extracts as natural red and yellow color, edicol erythrosine and sunset yellow as synthetic colors (for 8 weeks) and concluded that beet extract could be used as alternative natural red colorant, whereas curcumin extract need additional long-term studies.

Moreover, curcumin has also been found to be protective with antioxidant activity, as a promising natural food additive to counteract oxidative stress and hepatotoxicity caused by dietary exposure to the synthetic tartrazine as explained by El-Desoky, et al., 2017\(^{(10)}\) who evaluated the effects of a diet containing tartrazine on rats biochemical parameters including hepatic enzymes, kidney functions and profiles of lipids. Also, protective effects of three doses of curcumin, as natural food coloring agent.

In consistence with our observations, Soltan and Shehata, 2012\(^{(32)}\) where they were demonstrate the effect of using color foods (Color fruit juice for 6-12hr—Tomato ketchup potato chips (TKPC)—color sweet and color chocolate at low and high concentration on serum biochemical, white blood cell and histopathology of liver and kidney of rats for 13 week and explained that the use synthetic color in foods has many adverse effect on some of biochemical analysis, the liver and kidney histopathological structure.

**CONCLUSION**

The results of the current study showed that tartrazine has degenerative changes in both the filiform and fungiform papillae in comparison with curcumin. Based on these results, the use of natural food additives curcumin instead of synthetic one Tartrazine to avoid adverse effects on tongue papillae, the filiform and fungiform papillae.

So, it is recommended that it is necessary to restrict the use of Tartrazine synthetic food coloring agents and to try to use curcumin natural food coloring agents when need to yellow food color to avoid the degenerative effects on tongue papillae.

**REFERENCES**


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