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Research Article

SCANNING ELECTRON MICROSCOPIC STUDY ON THE CHANGES IN TONGUE PAPILLAE OF ALBINO RAT (*RATTUS NORVEGICUS*) INDUCED BY METANIL YELLOW

***Rituparna Sarkar[1] and Apurba Ratan Ghosh[2]**

[1]*Department of Environmental Science, Chandernagore Govt. College, Chandannagore - 712 136, Hooghly, W.B., India*

[2]*Ecotoxicology Lab, Department of Environmental Science, The University of Burdwan, Golapbag, Burdwan – 713 104, W.B., India*

**Author for Correspondence*

ABSTRACT

Chronic toxic effects of non-permitted food colour Metanil Yellow was carried out on the tongue papillae of albino rat (*Rattus norvegicus*) for exposure periods of 30 and 45 days at a dose of 3.0 g/kg body weight. Topological study displayed the toxic effects of Metanil Yellow on the tongue papillae especially on the filiform and fungiform papillae. The degenerative changes were found in both the filiform and fungiform papillae. Taste buds became necrosed after treatment. All these changes marked the toxicosis of Metanil Yellow on albino rat.

Keywords**:** *SEM, Tongue Papillae, Albino Rat (Rattus norvegicus), Metanil Yellow*

INTRODUCTION

Different synthetic and artificial food colours are used in variety of food items to enhance the acceptability of consumers and the non-permitted food colour Metanil Yellow is one among them. This common adulterant is a monosodium salt of of 3-[[4-(Phenylamino)phenyl] azo] benzenesulfonic acid which is used in different food items *viz*., laddu, toor dal and turmeric because of its easy availability and reasonable cost (Mathur, 2000). Various azo-dyes have been found wide applications in different textiles, cosmetics, plastics, lather, paper printing, colour photography, pharmaceutical and toy industries (Chung, 1983; Garrigos *et al.*, 2002; Mathur and Bhatnagar, 2007; Laowansiri *et al.*, 2008; Pant *et al.*, 2008). Several attempts have been made to demonstrate the toxicity and carcinogenicity of different food colourants (Jones *et al.*, 1964; Larson, 1975; Holmberg, 1978; Combes and Haveland-Smith, 1982; Patterson and Bulter, 1982; Vorhees *et al.*, 1983; Maekawa *et al.*, 1987; Borzelleca and Hallagan, 1988; Collins *et al.*, 1990, 1992; Khanna and Das, 1991; Reyes *et al.*, 1996; Koutsogeorgopoulou *et al.*, 1998; Walton *et al.*, 1999; Tsuda *et al.*, 2001; Gupta *et al.*, 2002; Sasaki *et al.*, 2002; Tanaka, 2005; Zraly *et al.*, 2006; Sarkar, 2013). The studies of the toxic effects of Metanil Yellow have been established by some authors which give variable results (Khanna *et al.*, 1978; Gupta *et al.*, 2002; Mathur *et al.*, 2005b; Sarkar and Ghosh, 2010; Sarkar and Ghosh, 2012a; Sarkar and Ghosh, 2012b; Sarkar, 2013). Tongue plays two important roles *viz*., acts as a taste sensor and helps in digestion among many vertebrates (Fawcett, 1986). Several studies have examined the structure of the vertebrates's tongue (Kobayashi *et al.*, 1990; Iwasaki *et al.*, 1996, 1999; Silva *et al.*, 2002; Carrard *et al.*, 2008; Nonaka *et al.*, 2008; Nasr *et al.*, 2012). Different investigations have depicted the characteristics of the tongue mucosa of different vertebrates by using scanning electron microscope (Yoshioka and Muto, 1976; Watanabe, 1989; Iwasaki and Wanichanon, 1993; Iwasaki *et al.*, 1996a, 1996b). The tongue mucosa epithelial cells have been investigated in both human and animals by SEM and TEM methods by different researchers (Gibbins, 1962; Apleton and Tyldesley, 1971; Arvidson, 1976; Yoshioka and Muto, 1976; Iwasaki and Miyata,

1985; Iwasaki and Sakata, 1985, Watanabe, 1989; Iwasaki and Wanichanon, 1993; Iwasaki *et al.*, 1996a, 1996b). There are various types of lingual papillae *viz*., filiform, fungiform, circumvallate and foliate papillae present on the surface of the tongue. According to Fawcett (1986), the distribution of these lingual papillae has been considered to be related to mode of eating habits of species and vocalization. Different studies have been done on lingual papillae of vertebrate species (Fernandez *et al.*, 1978; Shimizu *et al.*, 1979, 1980; Steflik *et al.*, 1983; Iida *et al.*, 1985; Kullaa-Mikkonen *et al.*, 1987; Meisel *et al.*, 1987; Kobayashi, 1990; Iwasaki *et al.*, 1997, 1999; Kobayashi *et al.*, 2004; Toprak, 2006). Toxic effects of chromium and the curative effect of vitamin E on rat lingual papillae have been investigated by Osman *et al.*, (2006). But there is no such report on the influence of food additive or colourant like Metanil Yellow on these various types of lingual papillae which itself is meant to bringforth the tastes of the food items. The main objective of this work is to evaluate the chronic exposures of Metanil Yellow on two main types of papillae, the filiform and fungiform papillae of tongue of albino rat (*Rattus norvegicus*). Scanning electron microscopic technique was used to intersect the changes in both the two types of papillae and taste buds after exposures in Metanil Yellow for 30 and 45 days and to record the level of changes in different exposure periods.

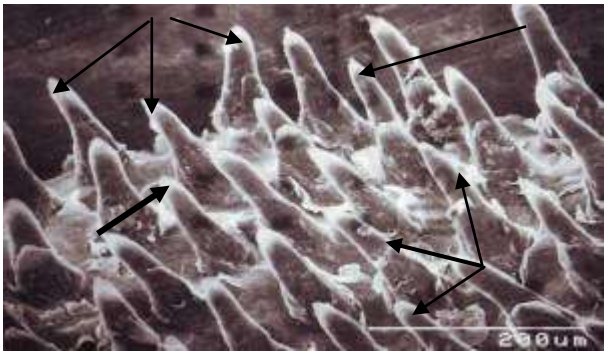
MATERIALS AND METHODS

Six albino rats (*R. norvegicus*) of age group 2-3 months and weighing 150±60 g were allowed to acclimatize in the laboratory for one week and then were divided into three sets containing two rats in each cage one for control and others two for treatment. Among them one set of the test animals was exposed to Metanil Yellow at a dose of 3.0 g/kg body weight for 30 days and the other set was exposed to same dose for 45 days. For SEM study albino rats of both control and treated were anaesthetized with tricaine methonesulphonate (MS 222) and the desired portion *i.e*., tongue was removed immediately after dissection. The mucosal surface of the tongue was exposed and fixed on thin cork sheets. The tissue was rinsed in heparinized saline and then fixed in 2.5% gluteraldehyde in 0.1 M cacodylate buffer, pH 7.4 for 24 hours. After fixation the tissue was removed, rinsed in buffer and subjected to post fixation for 2 hours in 1% OsO_4 in 0.1 M cacodylate buffer followed by amyl acetate and dried by critical point drying method. The tissue was cemented to metal stub and gold coated (approximately 20 nm thickness) before examined under Hitachi S-530 SEM.

RESULTS AND DISCUSSION

Control Condition

The tongue is a muscular structure and is lined by stratified squamous epithelium. It is made up of longitudinal, horizontal and vertical bundles of striated muscle fibres that provide the wide range of mobility required for speech, chewing and swallowing. The tongue of mammals is made up of two parts. The anterior region covered with numerous small papillae is separated from the posterior part, bumpy in appearance due to masses of lymphoid tissue by a 'V' shaped groove, the sulcus terminalis. Papillae are elevations of the oral epithelium and lamina propria that assume the various forms and functions. The tongue bears various kinds of lingual papillae *viz*., filiform, fungiform, foliate, vallate or circumvallates possessing different morphological structures and shapes. The lingual papillae are formed of a central core of connective tissue and a covering layer of stratified squamous epithelium. Several papillae are present in the tongue of albino rat (*R. norvegicus*). Filiform papillae of albino rat (Figures 1.1 & 1.2) are slender and have an elongated conical shape which are quite numerous and are present over the entire surface of the tongue. Fungiform papillae (Figures 1.3 & 1.4) resemble mushroom like having a narrow stalk and a smooth-surfaced, dilated upper part. The papillae and their associated taste buds constitute the specialized mucosa of the oral cavity. Filiform papillae (Figures 1.1 & 1.2) do not have tatse buds, but the other three types contain the taste buds in their epithelium.



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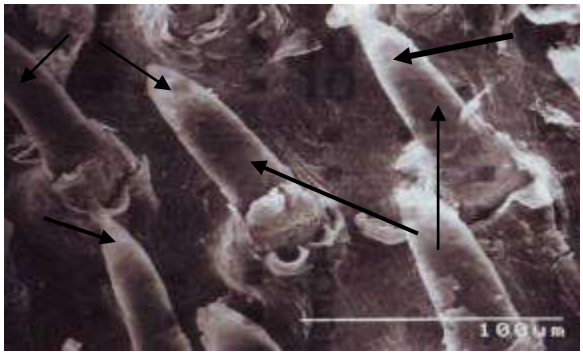

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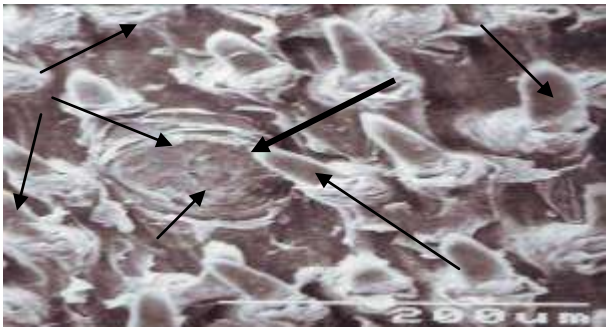

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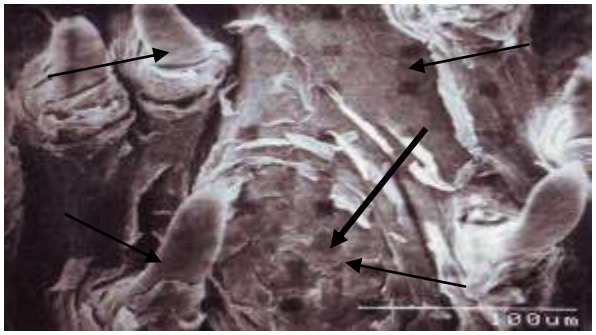


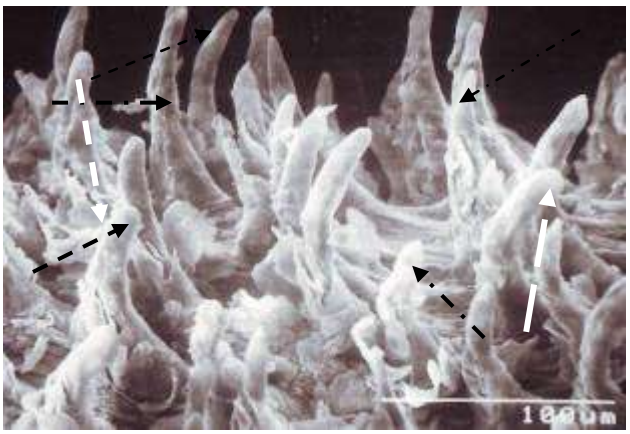
1.4

Figures 1.1-1.4 Scanning electron micrographs of filiform and fungiform papillae of tongue of control (C) albino rat (*R. norvegicus*)

1.1- Showing normal orientation filiform papillae (Normal thick and thin arrows). (C) X 150.
1.2 -Showing the normal appearance of filiform papillae (Normal thick and thin arrows). (C) X 500.
1.3 - Showing well formed fungiform papillae with taste buds (Normal thick and thin arrows). (C) X 400.
1.4 - Showing fungiform papillae (Normal thick and thin arrows). (C) X 200.

Treated Condition

After treatment with Metanil Yellow for 30 days filiform and fungiform papillae (Figures 2.1, 2.2, 2.3 & 2.4) of the tongue of albino rat were distorted. The taste buds of fungiform papillae were damaged after treatment. But maximum damage was shown in case of both filiform and fungiform papillae (Figures 3.1, 3.2, 3.3 & 3.4) after 45 days exposure in Metanil Yellow. Formation of scar in the taste buds resulting from cellular necrosis may initiate the carcinogenic development in the tongue.



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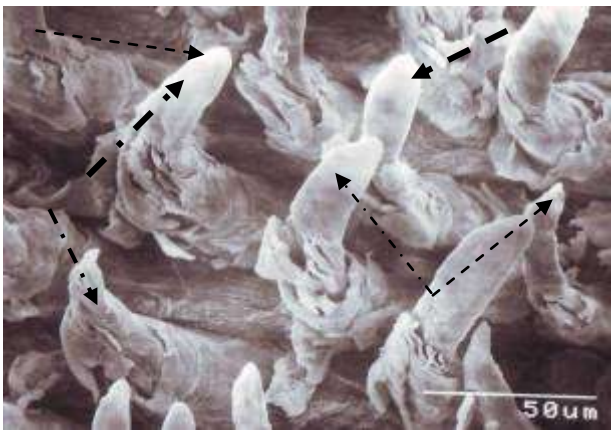

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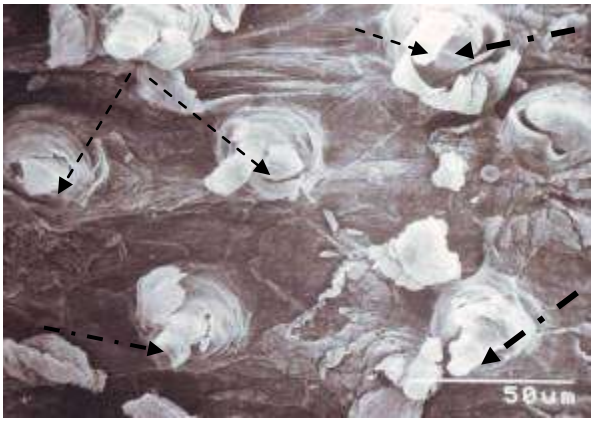

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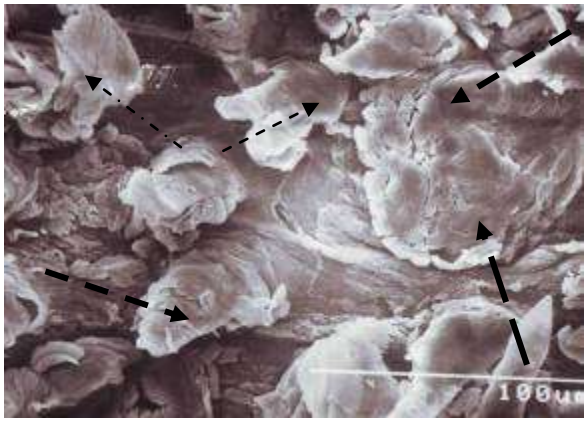
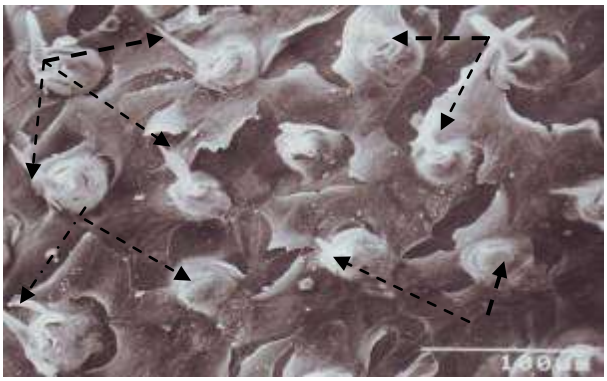


2.4

Figures 2.1-2.4 Scanning electron micrographs of filiform and fungiform papillae of tongue of treated (MY) albino rat (*R. norvegicus*)

2.1 - Showing damages in the filiform papillae (broken arrows). (MY, 30 d) X 400. **2.2 -** Showing disruptions of filiform papillae (broken arrows). (MY, 30 d) X 600. **2.3 -** Showing disruptions of fungiform papillae (broken arrows). (MY, 30 d) X 600. **2.4 -** Showing damaged fungiform papillae (broken arrows). (MY, 30 d) X 500.



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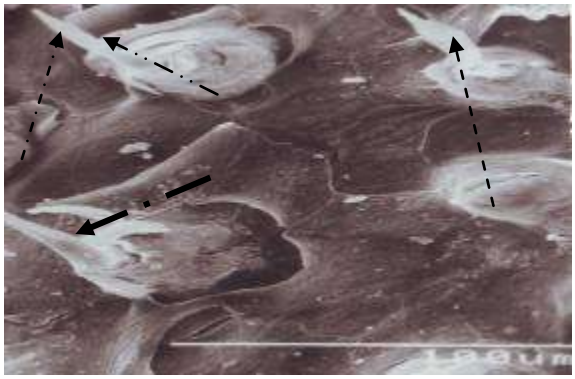

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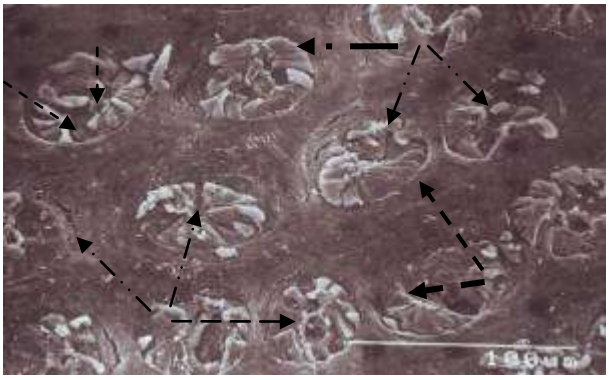

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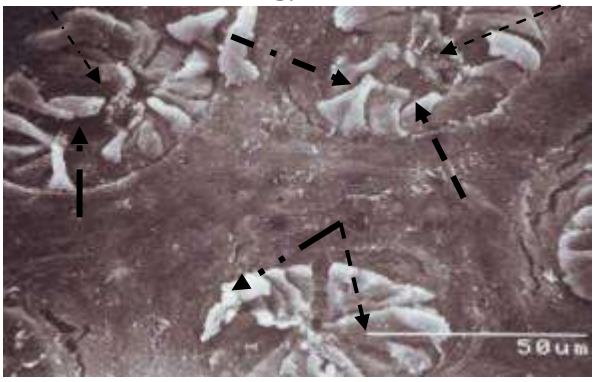

3.4

Figures 3.1-3.4 Scanning electron micrographs of filiform and fungiform papillae of tongue of treated (MY) albino rat (*R. norvegicus*)

3.1 - Showing damages in the filiform papillae (broken arrows). (MY, 45 d) X 400. **3.2 -** Showing erosions of filiform papillae (broken arrows). (MY, 45 d) X 300. **3.3 -** Showing damages in fungiform papillae (broken arrows). (MY, 45 d) X 400. **3.4 -** Showing disruptions of fungiform papillae (broken arrows). (MY, 45 d) X 600.

Distribution of filiform papillae has been observed by several workers (Greenbaum and Philips, 1974; Son *et al.*, 2000; Emura *et al.*, 2001). Filiform papillae are not only holding the food (Pastor *et al.*, 1993; Son *et al.*, 2000) but also help in swallowing the food particles (Jackowiak and Godinick, 2007). The number of fungiform papillae is proportional to both the range of food intake and consumption of a species (Hwang and Lee, 2007) and also to the size of the species tongue (Chung and Kwun, 1977). According to Benetti *et al.*, (2009) fungiform papillae are played the mechanical role in some vertebrates. Toxic effects of different synthetic colours and dyes have been studied by different authors (Werth, 1858; Khanna *et al.*, 1978; Chandro and Nagaraja, 1987; Chung and Cerniglia, 1992; Descience and Bablet, 1994; Ramachandani *et al.*, 1994; Mall and Kishore, 1995; Suryavathi *et al.*, 2005). An azo-dye Metanil Yellow has induced several responses at cellular and sub-cellular organizations in stomach, intestine, liver and kidney of *Heteropneustes fossilis* (Bloch) has been observed by Sarkar and Ghosh (2010). Some histopathological and ultrstructural changes have also been studied in albino rat (*Rattus norvegicus*) by Sarkar and Ghosh, (2012b). The present study has shown the structural changes of filiform and fungiform papillae caused by chronic consumption of Metanil Yellow. Under SEM observation it has been showed that the filiform and fungiform papillae were disrupted and damaged. The chronic exposures of Metanil Yellow for 30 and 45 days on albino rat (*Rattus norvegicus*) have shown the severe damages in both the two types of papillae. The present study also focuses that Metanil Yellow is not only disrupts both the filiform and fungiform papillae of the tongue of albino rat but also creates cellular necrosis following scar formation in the taste buds of fungiform papillae.

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