Chapter 1

INTRODUCTION

Food dyes are added to various food commodities to enhance their appearance (Gautam et al., 2010). The addition of dyes/colorants to food materials expectedly dates back to around 1500 BC (Chequer et al., 2012) where the candy makers used to add natural extracts and wine to improve the product’s appearance (Downham and Collins, 2000). However, the major use of these colorants was highlighted in ancient Egyptian cities from 5000 BC onwards (Meggos, 1995). In 19th century, increase in processed, packed and fun foods has enhanced the use of synthetic food dyes (Kumar and Shrivastva, 2011). Synthetic food dyes are used in number of domestic food commodities and industrial preparations for many reasons like to correct natural variations in food color or loss of color during storage or processing of food, to produce high tinctorial power and induce wide array of shades (Babu and Shenoliker, 1995; Sharma et al., 2008; Abdelmigid 2009; Al-Shinnawy, 2009). Thus the natural dyes were almost replaced by the synthetic ones by the beginning of the twentieth century (Ventura-Camargo and Marin-Morales, 2013). Revankar and Lele (2007) reported that around 8,000,000 tons of synthetic colorants produced annually all over the world.

The use of these synthetic food dyes is one of the most controversial issues for the food industry, from the health point of view (Cheeseman, 2012). Synthetic food dyes have been suspected of being toxic and many have been banned (Jadhav et al., 2013). Hence presently, synthetic food colors are more strictly regulated than at any other time in history and each food dye requires Food and Drug Administration (FDA) approval for use prior to its inclusion to any type of food products (Abdelmigid, 2009). In India, according to the Prevention of Food Adulteration Act (PFAA, 1954), 8 synthetic dyes viz., brilliant blue, carmoisine, erythrosine, fast green, indigo carmine, ponceau 4R, sunset yellow and tartrazine are permitted to be used in the eatables but in a limited quantity i.e. 100 ppm (Sharma et al., 2010; Saxsena and Sharma, 2014). Unfortunately, many confectioners use very high concentrations of these synthetic food dyes without knowing their toxic effects (Bhattacharjee, 2014). Some studies have
reported the use of textile and non permitted dyes in food items which have potential to cause risks to human health (Khanna and Singh, 1975; Tripathi et al., 2007).

The permitted food dyes are commonly available in the market in form of blends of two or more dyes and are widely encountered in a variety of eatables from both urban and rural market (Khanna et al., 1973; Sharma et al., 2008; Divya and Devika, 2012). The effect of these blends can be additives, synergistic, potentiating or even antagonistic (Singh et al., 1988; Sharma et al., 2008; Kaur et al., 2010).

Some synthetic food dyes also have the potential to cause DNA damage. Various studies have indicated the mutagenic as well as genotoxic responses of synthetic food dyes (Das and Mukherji, 2004; Tsuda et al., 2001; Jadhav et al., 2013). Various bioassays are being used for evaluation of toxic effects of synthetic food dyes including bacterial bioassay (Das and Mukherji, 2004; Kaur et al., 2010; Ferraz et al., 2011; Kaur et al., 2012; Zanoni et al., 2013; Atri et al., 2014) plant bioassays (Macioszek and Knononowicz, 2004; Kumar and Srivastava, 2011; Gomes et al., 2013; Bhattacharjee, 2014) and cytotoxic assay using various animal models (Hassan, 2010; Himri et al., 2011; Visweswaran and Krishnamoorthy, 2012; Chequer et al., 2012; Divya and Devika, 2012; Dixit and Goyal, 2013; Saxena and Sharma, 2014).

There are many other reports, which show the DNA damaging effects of different synthetic food dyes (Hassan, 2010; Shimada et al., 2010; Kumar and Shrivastva, 2011). Saxena and Sharma (2014) reported the toxic effects of tartrazine in Swiss albino rat (Rattus Norvegicus). Chequer et al. (2012) observed the genotoxic effects of erythrosine B and a xanthene food dye on HepG2 cells. Visweswaran and Krishnamoorthy (2012) reported that tartrazine (E102) had induced the oxidative stress in the testis of Wistar rats.

Majority of synthetic food dyes can lead to various kinds of allergic reactions like eczema, skin dermatoses (Nikulina et al., 1995), affect the liver (Jaskot and Costa, 1994; Nikulina et al., 1995), lungs (Ballantyne, 1994), vasco - circulatory system (Przybojewska, 1996), reproductive system (Nikulina et al., 1995; Eastin et al., 1996) and immune system (Ng et al., 1995). The amount of food dyes added by confectioners
to the drinks and other food commodities generally exceeds the authorized limit (de Andrade et al., 2014). Hence monitoring of the levels of food dyes in highly consumed products such as beverages, processed foods, snacks and desserts becomes mandatory (Kiseleva et al., 2003; Minioti et al., 2007; Yoshioka and Ichihashi, 2008; Ghoreishi et al., 2012; Medeiros et al., 2012; Mustafa et al., 2013; de Andrade et al., 2014).

Apart from the toxicological assays, various analytical methods have been used to determine the chemical composition of synthetic food dyes and their concentration in soft drinks, juices, fruit jellies, candies, spices, edible animal tissues and other processed food commodities. Such methods include capillary electrophoresis (Huang et al., 2002; Prado et al., 2006), thin layer chromatography (Oka et al., 1987; Oka et al., 1994), electrochemistry (Ni et al., 1996; Combeau et al., 2002), spectrophotometry (Dos Santos et al., 2010; Soylak et al., 2011; Unsal et al., 2012), ion-pair chromatography (Fuh and Chia, 2002), high performance liquid chromatography (HPLC) with ultraviolet/visible (UV/Vis) and diode-array detectors (DAD) (Minioti et al., 2007; Yoshioka and Ichihashi, 2008; de Andrade et al., 2014) and liquid chromatography - mass spectrometry (LC-MS) (Feng et al., 2011; Zou et al., 2013).

Qi et al. (2011) determined the four sudan dyes (I-IV) in hot chili powder using high performance liquid chromatography coupled with diode array detector (HPLC-DAD). de Andrade et al. (2014) determined the concentration of various food dyes viz., amaranth, brilliant blue, sunset yellow and tartrazine in soft drink using ion pair high performance liquid chromatography with photodiode array detection whereas Mustafa et al. (2013) confirmed the presence of a non permitted food dye i.e. para red in the chilli powder.

Considering the harmful consequences of synthetic food dyes, the present work was planned to explore the mutagenic and genotoxic effects of synthetic food dyes using battery of bioassays viz., Ames test (prokaryotic assay), Allium sativum root chromosomal assay (ASRCAA) (eukaryotic assay) and micronucleus assay (mammalian assay). Ames assay is used worldwide as an initial screening method to determine the mutagenic potential of various chemicals/drugs or pollutants. Many researchers have used the Ames assay to examine the ability of histidine-requiring strains of Salmonella
typhimurium to revert to prototrophic mutations and reported the potent mutagenic activities of numerous chemicals (Ames et al., 1975; Mortelmans and Zeiger, 2000). On the other hand, among various bioassays Allium sativum root chromosomal assay is one of the reliable plant bioassay which can be applied to detect a broad range of genetic damages (Chauhan et al., 2001; Saxena et al., 2004; Saxena et al., 2010). Although, there are the number of rapid and quick screening bacterial and plant bioassays which can estimate the mutagenic/genotoxic potential of various harmful chemicals and pollutants, still there is requirement of the bioassay that can be used for screening of harmful effects of these chemicals in mammals (Durston and Ames, 1974; Wyrobek and Bruce, 1975). Micronucleus assay in rat bone marrow cells has been reported as a simple and effective measure for genotoxicity (Heddle et al., 1983; Mavourin et al., 1990). Hence the present work planned with following objectives:

- To estimate mutagenic effects of six most commonly used synthetic food dyes viz., bright green, chocolate brown, respebary red, blue, orange and pink employing Ames assay.

- To estimate genotoxicity of these dyes using Allium sativum root chromosomal aberration assay and Rattus micronuclei assay.

- To carry out the chemical analysis of synthetic food dyes using various analytical techniques.