Chapter I

INTRODUCTION

Food flavours are chemical substances added intentionally to food stuffs to preserve flavour or enhance its taste and appearance. Changes in food consumption patterns and advances in food processing technology have led to increased use of flavours in food. Flavours have the ability to improve the taste of a wide variety of foods that are not very tasty. Flavouring agents are used usually in cakes, soft drinks, chocolate, candy, crackers, instant noodles, bread and wine. High concentrations are used in sweets, desserts, cereal bars, drinks and almost all frozen manufactured foods. They are used to mask the poor quality of the ingredients in these foods.

When people consume a food that contains large amounts of flavourant, they can experience toxic effects. Caffeine is both a flavourant and stimulant added to soft drinks which causes headache, irritability or sleepiness and high doses may increase the risk of late miscarriage and stillbirth (Greenwood et al., 2010). Quinine is another flavouring agent added to bitter lemon, quinine water and tonic water that causes visual loss and cardiac arrhythmias (Bateman et al., 1985). When people consume greater amount of sodium chloride which results in increased blood pressure, further leading to stroke and fatal heart disease (He and MacGregor, 2003). Potassium nitrite used in cooked meats and sausages stabilizes colour and adds flavour. It can lead to the production of small amount of potent cancer causing chemical called nitrosamines (Jones, 1992).

One of the most widely used and controversial food additives is monosodium glutamate (MSG). It is marketed as flavour enhancer (E621) all over the world and incorporated into large number of solid and liquid foods.
Chapter 1

It is used to enhance the flavour of soups, sauces, snacks, processed and restaurant foods and it is known by the trade names of Ajinomoto, Sasa, Ve- tsin, Miwon, Weichaun and tasting powder (Geha et al., 2000a). MSG is the sodium salt of naturally occurring non-essential L-form of glutamic acid. Monosodium glutamate contains 78% glutamic acid, 22% sodium and water (Adrienne, 1999). MSG was discovered by Japanese scientist, Kikunae Ikeda. He extracted and identified the flavour enhancing property of glutamate from traditionally used seaweed \textit{Laminaria japonica} and patented in 1909 by Ajinomoto Corporation in Japan. It does not have a distinct taste of its own, but provides a flavouring property when it is added to food. This characteristic taste is called “umami”, which is considered distinct from four other basic tastes (sweet, sour, salty and bitter) (Yamaguchi and Ninomiya, 1998). Umami is also referred to as “Xien Wei” in Chinese and “savory”, “broth-like” or “meaty taste” in Westerners. Due to this special taste contained in MSG, many food producers use this to enhance the flavor of their product. Therefore, it is also used to enhance the natural flavours of meats, poultry, seafood and stews (Fuke and Shimizu, 1993). Other salts of glutamate, which provide umami taste and categorized as flavour enhancer includes monoammonium glutamate, monopotassium glutamate, disodium 5’-monoinosinate and disodium 5’-monoguanlylate (Ninomiya, 2001).

In the early twentieth century, MSG was extracted from seaweed and other plant sources, hydrolysis of natural proteins such as wheat gluten and soya bean flakes. Later, large scale production was made by natural fermentation process using corn sugar or molasses from sugar cane or sugar beets as well as starch hydrolyzates from corn, tapioca etc. World wide production of MSG is estimated to be 2 million metric tons/year (Sano, 2009). In its pure form, it appears as a white crystalline powder, similar in
appearance to salt or sugar and when dissolved in water or saliva it rapidly dissociates into free sodium and glutamate ions.

The free form of glutamate is responsible for flavour enhancing property of MSG, while the bound form does not have any effect on taste. Adding glutamate to foods increases their umami taste quality, acceptability and consumption (Prescott, 2004). Glutamate sensing receptors such as T1R1 - T1R3 heterodimers, ionotropic receptors (iGluRs) and different variants of metabotropic glutamate receptors (mGluRs) have been expressed in the taste buds (Nelson et al., 2002; Toyono et al., 2003). Glutamate binds to these taste receptors on taste cells in the oral cavity activates taste nerves to elicit the unique taste umami (Lindeman, 2001). As a food additive, MSG is also described and listed on food labels as flavourant, hydrolyzed vegetable protein, hydrolyzed plant protein, hydrolyzed soy protein and autolyzed yeast extract. MSG can be found in different concentrations in numerous food products such as dehydrated soups and gravies, canned meat and fish, sausage, prepared meals, tomato sauce and ketchup, mayonnaise, snack foods, soy sauce and preserved crab, prawn and shell fishes. Consumption of soup with added MSG can result in increased flavour liking and increased liking is also associated with increased consumption of the MSG-paired flavour (Yeomans et al., 2008). Glutamate and its salts are not
allowed to be added to milk, emulsified fat and oil, pasta, cocoa/chocolate products and fruit juice by European Union (EU). Drinks, candy and chewing gum are potential sources of hidden MSG. MSG in the form of glutamic acid is used in fertilizers, fungicides, pesticides, and plant growth enhancer products such as AuxiGro plant metabolic primer that contains more than 29.2% processed free glutamic acid.

Acceptable daily intake (ADI) of MSG is 0-120 mg/kg body weight and it is not applicable to infants under 12 weeks of age (FAO/WHO, 1974). The oral dose that is lethal to 50% of subjects (LD$_{50}$) in rats and mice was 15-18 g/kg body weight (JECFA, 1988). The presence of MSG in food elicits palatability and preference (Bellisle, 1998). Thus MSG becomes an important part of human diet. Because of its flavour enhancing properties, glutamate is often deliberately added to foods either as the purified monosodium salt or as a component of amino acids and small peptides by the hydrolyzed protein. MSG is commonly found in Asian cuisine associated with Chinese restaurants and now frequently found in Western diet (Shi et al., 2010). Mean intake of MSG in United Kingdom is 0.6g/day and extreme users consume 4.68g/day whereas, consumption data from Japan, China, Korea indicates about 1.4-1.6g/day and highly seasoned restaurant meals can contain as much as 5g or more (Loliger, 2000). The MSG consumption has increased globally in recent years because of increased uptake of processed food products. It is frequently added to processed foods and mixed along with foods during preparation. Asian or Western foods which used soy sauce, citric acid, stock, natural pork/beef/chicken flavoring, anything that is enzyme modified contains MSG in greater amounts. The average daily glutamate intake from food additives may reach up to 1g in Europe, 4g in Asian countries and 10g in Germany (Beyreuther et al., 2007). Undesirable
flavours such as sourness, graininess and starchy in meat, fish, vegetables and cereals are masked by MSG.

Wide ranges of adverse reactions were reported in people who eat foods containing MSG. Yang et al (1997) had observed significant increase in the frequency of MSG attributed symptoms in the MSG treated group. MSG has been implicated as a possible source of CRS (Chinese restaurant syndrome) or MSG symptom complex in which, most frequently reported symptoms are numbness, headache, tingling, flushing, muscle tightness and weakness. In addition to these complications, ingestion of MSG has been alleged to cause asthma, atopic dermatitis, ventricular arrhythmia, neuropathy and abdominal discomfort (Geha et al., 2000b). Glutamate has been involved as a trigger for migraine and headache exacerbations (Woods et al., 1998). Radnitz (1990) described that glutamate initiated a vasomotor reaction which caused throbbing pain across the forehead and suggested that those who experience migraine and headaches were more susceptible to headache triggered by glutamate. The FDA received no reports of unconsciousness, coma or death related to ingestion of MSG but 8.2% of the evaluated subjects were severe and presented with difficulty in breathing, chest pain, changes in heart rate and blood pressure. In 1995, Federation of American Societies for Experimental Biology (FASEB) has admitted the possibility of MSG as a causing agent of food intolerance. Life threatening reactions associated with consumption of MSG includes anaphylaxis, seizures, dysrhythmias, constricted throat and dyspnea with head or neck edema, hypovolemic shock and syncope (Raiten et al., 1995).

Breast milk concentrations of glutamate are influenced by the ingestion of MSG and glutamate can also penetrate the placental barrier (Pitkin et al., 1979). The blood brain barrier is not fully developed in infants
and cannot protect against toxins that enter the blood. Thus the ADI is not applicable to infants. Certain individuals have reported serious adverse reactions to consumption of food containing high MSG content. Studies were reported with cardiac arrhythmia followed by the ingestion of Wonton soup (Gann, 1977; Goldberg, 1982). MSG consumption increased blood pressure in women under antihypertensive treatment (Shi et al., 2011). Moneret-Vautrin (1987) reported bronchospasm after the intake of 2.5g of MSG. MSG induced bronchoconstriction in some asthmatic individuals, thus it is not safe for individuals with asthma (Allen et al., 1987). MSG intake is also associated with metabolic syndrome and obesity in Thai and Chinese population (He et al., 2011; Insawang et al., 2012). MSG is found in high fat foods and associated with unhealthy eating, which could lead to increase in weight and obesity (Collison et al., 2010).

Glutamate liberated from MSG is absorbed from the lumen and it exerts certain functions in the body. Glutamate is not an essential amino acid in its own; instead it supplies its amino group for the synthesis of other amino acids arginine, proline and histidine. Reeds et al. (2000) showed that glutamate is the most important oxidative substrate for the intestinal mucosa and serve as an energy source. It may also activate digestion or absorption and metabolism of nutrients by stimulation via gastric vagal rami from gastric glutamate sensors (Uneyama et al., 2008). It is also an important precursor for bioactive molecule, glutathione. Glutamate is a key excitatory amino acid, transported into synaptic vesicles by a vesicular glutamate transporter and subsequently released by exocytosis (Augustine et al., 1996; Cousin and Robinson, 1999). Once released from the presynaptic terminal, glutamate diffuses across the cleft and binds into receptors located on the dendrites of the postsynaptic cells resulting in the production of excitatory
potentials (Fykse and Fonnum, 1996). Thus glutamate is considered as a multifunctional amino acid that takes part in taste perception, intermediary metabolism and excitatory neurotransmission. Excitotoxicity is defined as cell death resulting from the toxic actions of excitatory amino acids through glutamate receptors. Glutamate in high doses produces neuroendocrine abnormalities and neuronal degeneration (Moreno et al., 2005). Neural injury associated with ischemia, trauma, stroke, epilepsy, and many neurodegenerative disorders such as alzheimer’s disease, huntington’s chorea, acquired immune deficiency syndrome, encephalopathy, amyotrophic lateral sclerosis and parkinson’s disease may be mediated by excessive activation of the glutamate receptors (Cull-Candy et al., 2001). Neuronal excitotoxicity by glutamate refers to the injury and death of neurons arising from prolonged exposure to glutamate receptors and the associated excessive influx of calcium into the cell leading to the activation of enzymes that degrade proteins, membranes and nucleic acids (Berliocchi et al., 2005). Glutamate receptors were once thought to be predominantly located in the central nervous system. RT-PCR, Northern and Western Blot analysis, immunohistochemistry and in situ hybridization analysis revealed that glutamate receptors are also present in a variety of peripheral neural and non-neural cells and tissues including heart and liver (Gill et al., 2007; Julio-Pieper et al., 2011).

In Nigeria, most communities and individuals use MSG as a bleaching agent for the removal of stains from cloths. (Inuwa et al., 2011). Its excellent bleaching properties could be injurious to tissues and organs of the body. Most of the animal studies conducted to measure the toxic effect of MSG were parenteral and within a very short period of time. Studies related to oral exposure of MSG and cardiovascular complications were scanty.
There is also lack in literature regarding the hepatic complications induced by MSG exposure. Subchronic consumption of MSG by rats was not able to detect alterations in biochemical, hematological and histological parameters (Maluly et al., 2013). In clinical studies, it has been found that food intolerance is three times more common in women than in men (Loblay et al., 1991). Thus this study has been selected to evaluate the chronic oral exposure of low and high doses of MSG on cardiac and hepatic tissues in female rats.

Glutamate induced toxicity is associated with excitotoxic pathway and oxidative pathway. Oxidative stress occurs when antioxidant defense mechanisms fail to counter balance reactive oxygen species production. The increase in the relative concentrations of lipid peroxidation markers and decrease in antioxidants are considered to be a reliable indicator of oxidative stress (Kingsley et al., 2009). Malondialdehyde (MDA) and conjugated diene (CD) are the biomarkers for oxidative damage on lipids. MDA is a ketoaldehyde produced by peroxidative decomposition of unsaturated lipids as a byproduct of arachidonate metabolism (Dalle-Donne et al., 2006) and unstable carbon radicals from lipids can rearrange to form CD, which react with oxygen to peroxyl radicals (Pandey and Rizvi, 2010). Several enzymes have involved to overcoming the harmful effects of ROS. They are significantly used to maintain the redox status during oxidative stress and are collectively called as antioxidant enzymes. Superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione transferase (GST) and catalase are the main enzymatic antioxidants defense systems, which give protection by directly scavenging superoxide radicals and hydrogen peroxide and converting them to less reactive species. Cells containing low level of reduced glutathione (GSH) were found to be much more sensitive to the
effect of oxidative stress (Fisher-Wellman et al., 2009). Lipid peroxidation products have been implicated as the mechanism in several disorders and diseases such as neurological disorders, cardiovascular diseases and cancer (West and Marnett, 2006). Lipid peroxidation and oxidative stress play a pivotal role in cardiac and hepatic tissue damage, which may alter tissue functional markers and cause genotoxicity. Several enzymes have been determined to explore cardiac and hepatic status such as aspartate amino transferase, creatine phosphokinase, lactate dehydrogenase, alanine amino transferase, γ-glutamyl transferase and alkaline phosphatase. Thus the present study aims to investigate the role of MSG on plasma aminoacids, lipid peroxidation, antioxidants level, cardiac and hepatic functional markers and tissue damage.

*In vitro* study with cardiac and hepatic cell line serves as a better experimental tool to understand the mechanisms of cellular damage by toxic compounds. Intracellular calcium is considered as a major second messenger involved in a variety of intracellular signaling pathways. Elevated intracellular calcium activates phospholipases, protein kinases, endonucleases and oxidase enzymes, which may lead to oxidative stress and cellular damage (Berliocchi et al., 2005).

In recent years, antioxidant treatment has attained a lot of importance because of their potential as prophylactic and therapeutic agents in many diseases. The consumption of dietary antioxidant compounds, which enhance biological antioxidant mechanisms can prevent oxidative stress related disorders and organ toxicity (Havsteen, 2002). Antioxidant vitamins play an important role in the regulation of physiological and pathological processes by enhancing the immune system, modifying carcinogen metabolism, altering cell proliferation, stimulating the repair of carcinogen induced DNA
damage and elicit free radical scavenging properties (Chaudiere and Ferrar-Iliou, 1999). The present study hypothesis that the nutritional or therapeutic supplementation of antioxidant vitamins can reduce the MSG mediated toxicity. \(\alpha\)-Tocopherol is the most abundant and highest biologically active form of vitamin E, which inactivates harmful free radicals (McDermott, 2000). \(\alpha\)-Tocopherol is usually present in diets commonly consumed by humans. Highest concentration of \(\alpha\)-tocopherol is present in vegetable oils, nuts and seeds including whole grains. \(\alpha\)-Tocopherol dominate in sunflower (575 mg/kg), corn (207 mg/kg), olive (163 mg/kg) and soybean (152 mg/kg) (Gliszczynska-Swiglo et al., 2007). It is the lipid soluble, membrane bound and chain breaking antioxidant that protects cell membranes against lipid peroxidation (Bulger and Maier, 2003). Apart from antioxidant property \(\alpha\)-Tocopherol has been expressed additional biologically important effect such as the inhibition of protein kinase C activity, an important mechanism in signal transduction (Ricciarelli et al., 1998). \(\alpha\)-Tocopherol supplementation have shown beneficial effects for numerous diseases, particularly neurological disorders, atherosclerosis, ischemic heart disease and development of different types of cancer (Ricciarelli et al., 2001). In view of antioxidant and non-antioxidant property of \(\alpha\)-tocopherol, it needs to be explored against MSG induced implications. Thus the present investigation was conducted both *in vivo* and *in vitro* to analyze ameliorative potential of \(\alpha\)-tocopherol supplementation in the management of MSG intoxication.