CHAPTER I
INTRODUCTION

Senses and sensory perception are the characteristic property of living beings. Sensation is the organization, identification, and interpretation of sensory information in order to represent and understand the environment. For knowledge we heavily rely on what we comprehend from our senses, as they provide first-hand experiences, giving us primary evidence on surroundings and situations. We perceived the world through five sensory modalities; vision, hearing, taste, smell, and touch. Gustation, the sense of taste is mediated by taste receptor cells that are arranged in taste buds along the oral epithelium, which express receptors responsible for detecting sweet, bitter, salty, sour, and umami taste stimuli. The chemicals or tastants interact with taste receptors and triggers chemical signals that are transmitted by cranial nerves to the gustatory cortex in the brain (Chandrashekar et.al, 2006). Taste helps us decide what to eat and influences how efficiently we digest these foods.

Naturally occurring taste stimuli are complex combinations of various chemical substances that give rise to the complex subjective experience of food flavour. Despite the variety of subjective flavours, there are five subgroups of tastes sweet, bitter, salty, sour and umami. Combinations of these five dimensions account for the perceptions of a complex taste. A sweet taste is produced by many organic compounds, especially sugars, as well as by some alcohols and amino acids. Sour taste is usually associated with acids, which
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contains a high concentration of hydrogen ions (H\(^+\)) or protons. Salty sensations are produced by metal ions, such as sodium (Na\(^+\)) and potassium (K\(^+\)). These ions are important electrolytes for body fluids. Bitter taste is stimulated by a variety of organic compounds such as alkaloids (caffeine, nicotine, quinine, and morphine), and some amino acids. A fifth sensation, umami, was recently discovered and is now considered a primary taste sensation. Umami is a pleasant taste elicited by amino acids, such as glutamate and aspartate, and by some small peptides and nucleotides. Food choices are influenced by a broad range of economic, social, and behavioural variables including food availability. However, the sensory qualities of food are critical to dietary preferences, and taste in particular may be the most important determinant of food choices (Glanz et.al, 1998; Leterme et.al, 2008). The sense of taste gives us important information about the nature and quality of food, and of all the basic taste qualities, sweetness is the most universally liked (Wang et.al, 2004).

Humans are genetically predisposed to prefer sweet taste because sweet foods are naturally good and are safe sources of energy and nutrients. Thus adaptive evolutionary development has resulted in a preference for them (Reed and McDaniel, 2006). Sweet sensation is activated by various types of sweet compounds, including carbohydrates, amino acids, peptides and proteins.

The human desire for sweet taste spans all ages, races and cultures (Mintz et.al, 1985). Therefore, mankind has always added sweet substances to their food. Sweeteners are food additives that are used to improve the taste of foods. They are commonly
classified as natural and artificial sweeteners. Natural sweeteners are sweet-tasting compounds with nutritional value; the major ingredient of natural sweeteners is either mono or disaccharides. Familiar nutritive sweeteners include sucrose, fructose, agave nectar, fruit juice, and honey. The first recorded sweetener was honey, which was used in the ancient cultures of Greece and China (Bright, 1999). Honey was later replaced by common sugar or sucrose, which was originally obtained from sugar cane (Weihrauch and Diehl, 2004). It is the sweetness, flavour and functional properties of sucrose that makes it enormously popular throughout the world. Historically, sucrose has provided a readily available and relatively cheap source of food energy for humans. Sucrose consumption in Europe increased essentially during the nineteenth century, and presently represents 10-25% of total energy intake in most parts of the world. Sweetened beverages are major contributors to sugar intake and represent up to 10% total energy in North America (Malik et.al, 2010).

It has long been noticed that high sugar intake may have adverse health effects. Medical and academic communities have studied and debated the involvement of sucrose in ailments such as obesity, diabetes, hypertension, dental caries and heart diseases (Popkin and Nielson, 2003; Schulze and Manson, 2004). In rodents, consumption of a high sucrose diet leads to the development of obesity, insulin resistance, diabetes, dyslipidemia, fatty liver, and high blood pressure (Bizeau and Pagliassotti, 2005). More than several decades, it had already been suspected that consumption of refined sugar in humans may be linked to dyslipidemia and coronary
heart disease (Yudkin and Roddy, 1964; Bantle et al, 1986). In addition, studies have shown that sucrose is associated with hyperglycemia, hyperinsulinemia, hypertriglyceridemia, hypercholesterolemia and reduced high-density lipoprotein cholesterol concentrations. There is strong evidence that the fructose component of sucrose is essentially responsible for these effects. Fructose has been shown to cause a variety of metabolic effects, such as lactic acidosis (Craig and Crane, 1971), insulin resistance (Beck-Nielsen et al, 1980), hypertriglyceridemia (Teff et al, 2004) lipogenesis (Faeh et al, 2005), increased weight gain (Swarbrick et al, 2008), liver injury and high blood pressure (Jalal et al, 2010) and in the pathogenesis of cardiorenal diseases (Dhingra et al, 2007). It has been argued that it is the fructose moiety of both sucrose and high fructose corn syrup that is particularly troublesome in terms of potential effects on appetite and subsequent weight gain (Bocarsly et al, 2010).

In recent years, products of food industries have been subjected to criticism by health professionals. Sugar provides a large amount of rapidly absorbable carbohydrates, leading to excessive energy intake, weight gain and metabolic syndrome (Johnson et al, 2008). Sugar and other caloric sweeteners such as high fructose corn syrup have been cast as the main culprits of the obesity epidemic. These health concerns about sugar have resulted in efforts to find alternative sweeteners or sugar substitutes to lessen the risk of these problems and also achieve a reduction in energy consumption.
A sugar substitute is a food additive that duplicates the effect of sugar in taste, but usually has less or no calories. The majority of sugar substitutes approved for food use are artificially-synthesized compounds (Bellisle and Drewnowski, 2007). Artificial sweeteners are sweeteners that are derived from chemical synthesis of organic compounds which may or may not be found in nature. Artificial sweeteners have been classified as nutritive and non-nutritive depending on whether they are a source of calories. The nutritive sweeteners include the monosaccharide polyols (sorbitol, mannitol, and xylitol) and the disaccharide polyols (maltitol and lactitol). Sugar alcohols are sugar replacers which are used in many sugar-free and reduced-sugar products. Sugar alcohols provide an average of 2 kcal/g because of their incomplete digestion and absorption (Dills, 1989). Foods labelled “sugar free” may contain sugar alcohols, non-nutritive sweeteners or both.

The non-nutritive sweeteners (NNS), better known as artificial sweeteners, include substances from several different chemical classes and typically exceed many times the sweetness of sucrose. As a result, much less sweetener is required, and energy contribution often negligible. NNS have existed since the end of the 19th century, when saccharin was serendipitously discovered. Currently, five NNS (acesulfame potassium, aspartame, neotame, saccharin, and sucralose) are approved by the US Food and Drug Administration (FDA) (Sylvetsky et.al, 2011). Since the majority of added sugar is obtained from the consumption of soft drinks, beverages sweetened with artificial sweeteners have emerged as an alternative for sugar sweetened beverages, providing the desired
sweetness and palatability without contributing to caloric intake. In addition the food and beverage industry is increasingly replacing sugar or corn syrup with artificial sweeteners in a range of products traditionally containing sugar. The consumption trends for foods and beverages containing NNS are clearly increasing, but are different between categories. The proportion of consumers ingesting NNS in beverages remained relatively stable between 1989 and 2004 (6.9% increase), whereas the proportion of consumers of NNS in foods increased by 81.2%. However, in 2004, this still represented only 5.8% of the population aged ≥ 2 years. The amount of NNS ingested in beverages and foods by consumers of NNS increased by 37.7% and 14.2%, respectively, between 1989 and 2004 (Mattes and Popkin, 2009). A recent systematic review estimated that between 4 and 18% of total carbonated beverage intake among children is from artificially sweetened beverages (Brown et al., 2009). Increased interest in controlling body weight, coupled with wide publicity about the adverse effects of consuming refined sugars, has resulted in widespread use of sugar substitutes.

The first artificial sweetener was saccharin, which was synthesized in 1879 by Remsen and Fahlberg (Miller and Frattali, 1989). Saccharin was discovered over a century ago and has been used as a non-caloric sweetener in foods and beverages for more than 100 years. Saccharin was attractive for consumers because of its lower calorie levels while still maintaining sweetness. It is 300 to 500 times sweeter than sugar. Saccharin's potential as a sweetening agent was quickly recognized and the substance was soon used as a substitute for sugar in candies and bakery products. A major
disadvantage of saccharin was its unusual and bitter aftertaste. To some extent this was combated when cyclamate was combined with saccharin. However, after cyclamate was banned in 1969, the aftertaste of the sweetener in diet sodas re-emerged as an issue. In 1977, saccharin was also suspected as a carcinogen, but allowed to continue the sale, albeit with a warning label. Experiments in animal models observed that, saccharin increased the occurrence of bladder tumours when the animals were fed over two generations, beginning either at conception or at the time of birth and for the rest of their lifetime (Arnold et.al, 1980; Schoenig et.al, 1985). The controversies regarding the safety issues of Saccharin consumption lead to the emergence of other artificial sweeteners.

The artificial sweetener aspartame (L-aspartyl L-phenylalanine methyl ester) is a methyl ester of the dipeptide of the natural amino acids L-aspartic acid and L-phenylalanine. Aspartame was discovered in 1965 by James M. Schlatter, a chemist working for G.D. Searle & Company (Butchko et.al, 2002). Schlatter had synthesized aspartame in the course of producing an antiulcer drug candidate and accidently found its sweetness. It is estimated that >8,000 tons of aspartame are consumed each year in the United States alone (soffritti et.al, 2005). The worldwide production of aspartame is assumed to be over 16,000 tons per year (Belpoggi et.al, 2006). First approved by the U.S. Food and Drug Administration (FDA) for limited use in solid food in 1981 (FDA, 1981), its authorization was extended to soft drinks in 1983 (FDA, 1983) and then approved as a general sweetener in 1996 (FDA, 1996). Likewise, the sweetener was approved for general use in the
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European Union in 1994 (EC Directive, 1994). Leading international scientific and health authorities have approved aspartame for use in foods and beverages. Among these organizations are the Joint Expert Committee on Food Additives (JECFA) of the World Health Organization (WHO) and the United Nations Food and Agriculture Organization (FAO), Food Standards Australia New Zealand (FSANZ) and the European Food Safety Authority (EFSA).

Aspartame is found in > 6,000 products, including carbonated and powdered soft drinks, chewing gum, candy, desserts, yogurt, tabletop sweeteners and some pharmaceutical products such as vitamins and sugar free cough drops (Butchko and Stargel, 2001). The sweet taste of aspartame allows pharmaceutical manufacturers to mask the bitterness of certain active ingredients. It is estimated by the Aspartame Information Center (2008) that aspartame is consumed by >200 million people worldwide. In the United States, >70% of aspartame sales is attributed to soft drinks (American Dietetic Association, 2004). The acceptable daily intake (ADI) of aspartame is currently 50 mg/kg body weight (b.wt) in the United States and 40 mg/kg b.wt in the European Union for both children and adults. But India did not have any safety regulations or ADI regarding the use aspartame.

Aspartame is approximately 200 times sweeter than sucrose. Due to this property, even though aspartame produces four kilocalories of energy per gram when metabolised, the quantity of aspartame needed to produce a sweet taste is so small that its caloric contribution is negligible. Currently, aspartame is one of the most popular sweeteners in the food industry, as its price has
dropped significantly since the Monsanto patent expired in 1992.

![Aspartame](image)

The molecular formula for aspartame is C\textsubscript{14}H\textsubscript{18}N\textsubscript{2}O\textsubscript{5}. Aspartame is produced by coupling together L-phenylalanine (or L-phenylalanine methyl ester) and L-aspartic acid, either chemically or enzymatically. It is a white, odourless, crystalline powder. Its molecular weight is 294.3 Daltons. It has a melting point of 246-247°C. There are two forms of aspartame, an \( \alpha \) and a \( \beta \) form. Only the \( \alpha \) form is sweet and unless specified, ‘aspartame’ always refers to the \( \alpha \) form. Aspartame is sparingly soluble in water and it is more soluble in acidic solutions and hot water and is only slightly soluble in oils, alcohol, and chloroform. Solubility can be significantly increased if aspartame is dissolved in an acid solution. Under particular conditions (high temperature, lengthy storage times), aspartame may be contaminated by the degradation product diketopiperazine (DKP).

The consumption of aspartame has been studied worldwide, in the general population and in special population subgroups. In New Zealand and Australia, consumption of aspartame was assessed in individuals in different age groups between 12 to 59, for 60 years. Highest consumption was by individuals aged 25 to 39 (FSANZ, 2004). In France, based on aspartame consumption studies in the
general population and using values of aspartame measured in food, mean consumption in adults was between 0.05 and 0.4 mg/kg b.wt/day and maximum values between 1 and 2.75 mg/kg b.wt./day (Hinson et al., 1992), whereas in children and adolescents, maximum values were between 0.13 and 2.8 mg/kg b.wt /day. Italian teenagers who were known to be users of diet products had estimated average aspartame intakes of only 0.03 mg/kg b.wt/day; the maximum aspartame intake was 0.39 mg/kg b.wt/day (Leclercq et al, 1999). The general population of aspartame eaters in Canada consumed 5.5 mg/kg b.wt/day during cold weather months and 5.9 mg/kg b.wt/day during warm weather months in 1987. Intake by children and special populations, who might consume higher amounts of aspartame, varied from 5.5 to 11.4 mg/kg b.wt/day (Heybach and Ross, 1989). In the Netherlands, based on food frequency questionnaires, mean aspartame intake was estimated to be 2.4 mg/kg b.wt/day (Hulshof and Bouman, 1995). In Brazil aspartame intake by the users of intense sweeteners was 2.9% of the ADI (40 mg/kg b.wt); intakes by diabetics and individuals on weight control regimens were 1.02 mg/kg b.wt/day (2.6% of the ADI) and 1.28 mg/kg b.wt/day (3.2% of the ADI), respectively (Toledo and Ioshi, 1995). In a study of Swedish diabetics, the general aspartame intake was lower than the ADI, but the worst-case calculation of intake in the children’s group was 114% of the ADI (Ilbäck et al, 2003).

Shortly after its approval by the FDA in 1981, there have been a multitude of studies and reports on the controversy surrounding the safety of ingesting aspartame (Garriga and Metcalfe, 1988). By far, aspartame has been the most controversial artificial sweetener because
of its potential toxicity. Since aspartame’s approval numerous case reports have implicated aspartame in the occurrence of such problems as seizures (Walton, 1986), memory loss (Moser, 1994) and headache (Van Den Eeden et al., 1994). In some case reports, associations have been made between aspartame intake and Type IV Delayed Type Hypersensitivity reactions in patients with proven contact sensitization to formaldehyde (Hill and Belsito, 2003). There has been a wide range of neurological effects reported with aspartame consumption, most likely due to its ability to increase levels of phenylalanine (Fernstrom et al., 1983; Dailey et al., 1991; Diomede et al., 1991). Aspartame also caused neurochemical changes and affects the levels of neurotransmitters within the brain (Coulombe and Sharma, 1986; Yokogoshi and Wurtman, 1986; Goerss et al., 2000; Bergstrom et al., 2007). Aspartame was shown to dose-dependently inhibit glutamate binding to its N-methyl-d-aspartate specific receptors (Pan-Hou et al., 1990). Neuropeptide Y concentrations have shown to be lower in the arcuate nucleus in rats treated with aspartame (Beck et al., 2002). Neurochemical changes following high-dose aspartame with dietary carbohydrates has also been reported (Wurtman, 1983). It is observed that oral administration of aspartame might lead to increased levels of aspartate in the brain which could modulate the transmission in retinohypothalamic tract (RHT) and suprachiasmatic nuclei (SCN) (Rajasekhar et al., 2004).

Concerns regarding an association between aspartame and brain tumours were raised by Olney et al. (1996). Later Soffritti et al. (2006) reported that aspartame is a multipotential carcinogenic compound whose carcinogenic effects are evident even at a daily dose of 20 mg/kg b.wt. In addition, Belpoggi et al. (2006) found that
aspartame causes an increased incidence of malignant tumours in rats, with a positive significant trend in both sexes and at various sites. Genotoxicity of aspartame has also been reported both in vitro and in vivo (Rencuzogullari et al., 2004; Abd Elfatah et al., 2012). Certain in vitro studies indicate that aspartame or its metabolites may affect enzyme activities in the brain, which includes acetylcholine esterase (Tsakiris et al., 2006; Simintzi et al., 2007) and cytochrome P450 enzymes (Vences-Mejia et al., 2006).

Studies on the metabolic and biochemical effects of aspartame have also been reported. Aspartame ingestion increases urinary calcium levels and results in calciurea (Nguyen et al., 1998). A number of studies focused on the effects of aspartame on appetite/hunger, food intake (Rogers et al., 1988; Lavin and Read, 1997; Appleton and Blundell, 2007) and insulin levels (Siegle et al., 2012) have suggested that aspartame may have modulating effects on these body responses. Metabolic and functional damages caused by aspartame have been reported in many organs including the pancreas (Leme and Azoubel, 2006) and liver (Portela et al., 2007).

Aspartame is metabolised by esterases and peptidases in the gastric tract of rodents, nonhuman primates and humans to its three constituents: phenylalanine (50%), aspartic acid (40%) and methanol (10%) (Ranney, 1976). Subsequent to aspartame consumption, the concentrations of its metabolites are increased in the blood (Stegink, 1987). Since its approval as a general purpose sweetener, much attention has been paid to the safety of aspartame mainly because of these metabolites, as they are suggested to have various toxic manifestations at different systems.
Phenylalanine constitutes 50% of the weight of aspartame. Phenylalanine not only plays a role in amino acid metabolism and protein synthesis, but also serves as the precursor for tyrosine (Hawkins et al., 1988), dopamine, norepinephrine and epinephrine (Ganong, 1997). Concerns about the safety of phenylalanine largely centres on its role in neurotransmitter metabolism. Elevated blood concentrations of phenylalanine competitively interfere with the transport of other large neutral amino acids (LNAA), as they use the same transporter by which phenylalanine is transported to the brain (Pardridge 1998; Surtees and Blau, 2000). High plasma concentrations (≥1.3 mM) of phenylalanine induce acute impairment in neuronal functions, such as EEG abnormalities or impaired neuropsychological function (Krause et al., 1985). It was suggested that high brain phenylalanine concentrations inhibited the enzyme activity of tyrosine hydroxylase and tryptophan hydroxylase (Puglisi-Allegra et al., 2000; Joseph and Dyer, 2003). Exposure to high levels of phenylalanine that occurs during the third trimester of pregnancy results in mental retardation and reduced brain size (Levy and Ghavami, 1996). In addition, animal studies have suggested a hyperphenylalaninemia mediated increase in cell death in the developing brain (Reynolds et al., 1993). Phenylalanine was reported to affect neuronal morphology and viability (Huttenlocher, 2000). Previous studies demonstrated that phenylalanine reduced Na⁺ K⁺-ATPase and creatine kinase activity in rat brain (Wyse et al., 2004). A previous study demonstrated abnormalities of cerebral energy metabolism in Phenylketonuria, indicating a link between phenylalanine neurotoxicity and imbalances of cerebral energy
metabolism (Pietz et al., 2003). Studies carried out in animal models of hyperphenylalaninemia have emphasized a role for oxidative damage elicited by phenylalanine and its derivatives in the brain (Ercal et al., 2002; Hagen et al., 2002).

L-Aspartic acid is classified as an acidic amino acid, together with glutamic acid. Increasing evidence suggests that L-Aspartic acid is an excitatory neurotransmitter in the central nervous system (Fleck et al., 1993). L-aspartic acid may released from nerve endings (Gundersen et al., 1998) and activate N-methyl-D-aspartate (NMDA) receptors to produce NMDA receptor-mediated responses in the rat hippocampus (Fleck et al., 2001) and rat olfactory cortex (Surtees and Collins, 1985). Previous pharmacological studies (Moroni et al., 1986; Shannon and Sawyer, 1989) have supported the hypothesis that L-aspartic acid is a modulator in the enteric nervous system. A subchronic oral toxicity study of L-aspartic acid indicate that it causes toxic effects on the kidneys and possibly salivary glands at high dose levels in male and female Fischer 344 rats (Tada et al., 2008).

Humans and nonhuman primates are uniquely sensitive to the toxic effects of methanol (Hayreh et al., 1980; Kavet and Nauss, 1990). Aspartame metabolite, methanol is a toxicant that causes systematic toxicity (Kruse, 1992). Aspartame (500mg/kg b.wt) significantly increases the plasma methanol levels to mean peak value 3.1 mg/litre within one hour after administration (Davoli, 1986). The methanol produced by the metabolism of aspartame is absorbed and quickly converted into formaldehyde and then completely converted to formic acid, which, due to its long half life, is considered the primary mechanism of toxicity in methanol poisoning.
Formaldehyde is considered as a neurotoxin (Lee \textit{et.al}, 1994; Eells \textit{et.al}, 2000). As the metabolism of formate is mediated through a tetrahydrofolate dependent pathway, humans and non-human primates are uniquely sensitive to methanol poisoning because of their low liver folate content (Johlin \textit{et.al}, 1987). Methanol intoxication is also associated with mitochondrial damage and increased microsomal proliferation resulting in increased production of oxygen radicals (Liesivuori and Savolainen, 1991; Castro \textit{et.al}, 2002). Exposure to methanol causes oxidative stress by altering the oxidant/antioxidant balance in lymphoid organs of rats (Parthasarathy \textit{et.al}, 2006a).

The metabolism of xenobiotics to a large extent takes place in the liver. The liver is necessary for survival because it is essential for the coordination of metabolism in the body, including xenobiotic metabolism and detoxification. Its strategic location, blood flow and prominent role in the metabolism of xenobiotics render this organ particularly susceptible to injury by chemicals to which we are ubiquitously exposed (Sturgill and Lambert, 1997). The pathogenesis of most chemical induced liver injuries are initiated by the metabolic conversion of chemicals into reactive intermediate species, such as electrophilic compounds or free radicals, which can potentially alter the structure and function of liver cells. The by-products of such metabolism sometimes are more toxic than the initial substance (Fernandez-Checa \textit{et.al}, 1997). As the process of xenobiotic metabolism requires multiple biochemical transformations, and the fact that some intermediates mediate toxic responses, the liver is potentially susceptible to injury during the act of performing these
functions (Ramadori et al., 2008). An improved quantitative understanding of the balance between functional xenobiotic metabolism and hepatic damage would be of great utility in forming guidelines for safe exposure levels in the toxicological contexts.

Blood tissue reflects physical and chemical changes occurring in organisms, indicating the general metabolism and physiological status. Metabolic changes associated with specific disorders may give rise to a change in the biochemical profile of blood. Therefore, biochemical variables of blood appear to be suitable monitoring tools for environmental influences, chemically induced stress conditions and health. Several enzymes that trigger important chemical reactions in the body are produced in the liver and are normally found within the cells of the liver. However, if the liver is damaged or injured, the liver enzymes spill into the blood, causing elevated liver enzyme levels. The liver enzymes like transaminases, alkaline phosphatase, γ-glutamyl transpeptidase, in the blood can be measured to know the normal functioning of the liver. These enzymes help in detecting injury to hepatocytes and the severity of the damage. Lifestyle such as artificial diets, directly affects body composition, condition, and consequently leads to blood and liver modifications (Wood et al., 1990; Burtis et al., 1996). The wide use of a number of food additives has caused adverse effects on human health that require continuous evaluation.

Different tissues have different oxygen demands depending on their metabolic needs. It has been suggested that the metabolites of aspartame are capable of inducing oxidative damage (Abdel-Salaam et al., 2012). Oxidative stress is defined as an imbalance
between production of free radicals and reactive metabolites, called oxidants or reactive oxygen species (ROS), and their elimination by protective mechanisms, referred to as antioxidants. This imbalance leads to damage of important biomolecules and cells, with potential impact on the whole organism (Gandhi and Abramov, 2012). Antioxidants are at the frontline of cellular defence mechanisms acting to slow down or prevent oxidative stress (Winston and Di Guilio, 1991). The metabolism of toxic compounds could result in the generation of reactive metabolites that have even greater toxicity and deplete cellular antioxidants. Changes in their concentration are often used as biomarkers of stress. A few studies are conducted to study the effects of aspartame in liver (Tutelyan *et al.*, 1990; Hertelendy *et al.*, 1993). It is suggested that aspartame ingestion can lead to the formation of formaldehyde adducts in the organs and tissues (Trocho *et al.*, 1998). Recently, Kim *et al.* (2011) observed an increase in the inflammatory response in the liver of Zebra fish administered with aspartame. On the other hand, ROS are generated continuously during oxidative metabolism in the brain. Among all organs in the body, the brain is particularly prone to oxidative stress induced damage because of the high oxygen demand of this organ, the abundance of redox-active metals (iron and copper) and the presence of high levels of oxidisable polyunsaturated fatty acids (Dringen, 2000). Further, compared with other organs, the brain is especially vulnerable because it has lower superoxide dismutase, catalase, and glutathione peroxidase activities (Maher, 2005). In addition, the brain glutathione concentration is lower than other tissues (Jain *et al.*, 1991). Despite multitude of studies on aspartame,
its effects on the antioxidant system have received little attention. So, there is a need to explore whether long term oral consumption of aspartame induces adverse effects.

There is an ongoing controversy over decades, surrounding the adverse effects of aspartame on the central nervous system. Previously, it has been reported that consumption of aspartame could cause neurological and behavioural disturbances such as memory loss, headaches, confusion, dizziness and seizures, and these may be attributed to changes in neurochemistry (Tollefson and Barnard, 1992). Many of these conditions are associated with neurotransmitter alterations. Studies on the effect of aspartame on brain neurotransmitters have yielded inconsistent results with increased norepinephrine and dopamine in various brain regions (Coulombe and Sharma 1986), decreased serotonin (Yokogoshi et.al, 1984; Torii et.al, 1986), decreased dopamine in the striatum (Bergstrom et.al. 2007), or even no effect on brain dopamine and norepinephrine levels (Perego et.al, 1988; Dailey et. al, 1991). It is known that tyrosine hydroxylase is the rate limiting enzyme in dopamine and catecholamine synthesis (Winberg et.al, 1993), whereas serotonin synthesis occurs via hydroxylation of tryptophan catalysed by tryptophan hydroxylase. There is a lack of data regarding the effects of aspartame over the activity of these rate limiting enzymes and neurotransmitter synthesis. Further, it is suggested that monoamine neurotransmitters play a significant role in learning and memory and behaviour (Humphries, 2008). Goerss et.al (2000) observed decrease in aggressive behaviour with an increase in serotonin levels after aspartame administration. In
addition, the administration of aspartame showed altered learning behaviour (Potts et.al, 1980; Dow-Edwards et.al, 1989) and affects the T-maze cognitive performance (Christian et.al, 2004).

*In vitro* studies reported that aspartame metabolites produce alterations in the activities of certain enzymes such as acetylcholine esterase (Simintzi et. al, 2007) and Na⁺ K⁺-ATPase (Simintzi et.al, 2008) involved in processes associated with learning and memory. It is known that the cholinergic system in the central nervous system plays important role in learning and memory (Everitt and Robbins, 1997). Na⁺ K⁺-ATPase is crucial for maintaining ionic homeostasis in neurons (Xiong and Stringer, 2000) and a deficiency of the Na⁺ K⁺-ATPase results in spatial learning and memory deficits (Moseley, 2007). The effects of aspartame on these factors are needed to be investigated *in vivo*, in order to find any relationship between aspartame and cognition. In recent years considerable evidence has suggested that aspartame alters the neuronal cell integrity (Lau et.al, 2006; Omar, 2009). However, the reports related to the neurological effects of aspartame were limited to either single dose or short term exposure and most of these observations were contradictory (Christian et.al, 2004). In addition *in vitro* studies are limited by their inability to extrapolate its observations into *in vivo* systems. Moreover, the possible effect of aspartame on neural functioning is still a contentious issue of scientific interest. The effects of the food additives are visible only with time, thus single or short term studies are not sufficient to evaluate the adverse effects. As aspartame may be used in a wide variety of foods there is the likelihood of long-term or lifelong human exposure. As neuronal cells
are vulnerable to the effects of chemical substances and the consumption of synthetic sweeteners has increased in the modern lifestyle, it is inevitable to explore the potential of aspartame for chronic toxicity in the brain.

With the increase in prevalence of obesity and diabetes, consumption of non-caloric sweeteners are preferred over caloric sweeteners. Diabetes mellitus is a complex metabolic disorder resulting from defects in insulin secretion (β-cell dysfunction), insulin action (insulin resistance) or both (O'Brain and Granner, 1991). It is unclear whether artificially sweetened beverages should be recommended because they have been shown to be associated with an increased risk of type 2 diabetes and cardiometabolic dysfunction in some studies (Dhingra et al., 2007; Lutsey et al., 2008). Some studies in adults have demonstrated links between artificial sweetener consumption and insulin resistance, the incidence of type 2 diabetes, and poor glucose control in patients with pre-existing diabetes (Mackenzie et al., 2006; McNaughton et al., 2008). Ferland et al. (2007) observed an increase in glucose and insulin levels in diabetic subjects after aspartame administration. In addition to studies in diabetes, effects of aspartame on non diabetic condition were also a subject of interest. Melanson et al. (1999) found a decrease in blood glucose level after aspartame consumption in normal subjects. Recently Collison et al. (2012a) found that neonatal exposure of aspartame or monosodium glutamate or a combination of both markedly influences glucose homeostasis in mice. Another study by Siegler et al. (2012) reported that a combination of
aspartame and carbohydrate led to significantly lower serum insulin levels during exercise.

Individuals consuming artificially sweetened beverages may be doing so, in an attempt to lose weight, or may have switched to artificial sweeteners after gaining weight (Elfhag et al., 2007). Blackburn et al. (1997) found that consuming aspartame-sweetened foods reduce 10% of the initial body weight in women. But other studies suggested that aspartame may stimulate appetite (Blundell and Hill, 1986) and thereby lead to weight gain (Swithers and Davidson, 2008). Furthermore, the use of aspartame results in a significant reduction in food intake (Rogers et al., 1990; Anton et al., 2010) in normal subjects. However, there is a lack of consensus regarding aspartame consumption and glucose homeostasis, body weight and food intake. The liver maintains blood glucose homeostasis in normal physiology by rapid clearance of glucose from the portal vein in the absorptive state after a meal, and by controlling the production of glucose in the post-absorptive state at a sufficient rate to maintain euglycaemia. Overproduction of glucose by gluconeogenesis and underutilisation by glycolysis are the fundamental characteristics that lead to hyperglycaemia in diabetes mellitus (Shirwaikar et al., 2006). Epidemiological studies have found that mean aspartame consumption may be more in diabetic population, relative to the general population (Renwick, 1999). Therefore, it is necessary to extend the existing findings and gain further insight on the impact of aspartame on hepatic glucose metabolism and glucose homeostasis in diabetes and non-diabetic conditions.
The doses in this study were selected in order to address the safety of aspartame in the acceptable daily intake level and to explore the feasibility of aspartame to produce adverse effects during high dose consumption. According to FDA the acceptable daily intake dose of aspartame is 50mg/kg b.wt and this suggested that up to the dose of 5000mg/kg b.wt, that was considered as the no observable adverse effect level (NOAEL). But recent reports demonstrate that even the acceptable daily intake dose could produce adverse effects (Soffritti et.al, 2006, 2007; Gombos et.al, 2007; AlSuhaibani, 2010). The higher doses in the study were selected according to the previous reports, which were used for single dose or short term studies (Zhi and Levy, 1989; Diomede et.al, 1991). Moreover, dosages in the range of 500-1000 mg/kg have routinely been used in animal studies involving aspartame (Dailey et.al, 1991; Bergstrom et.al, 2007). Furthermore the higher doses used in this study is much lower than the higher dose suggested by FDA. It has been suggested that rats metabolise aspartame at a much greater rate than humans. Moreover, rats metabolize phenylalanine and methanol rapidly than humans (Fernstrom et.al, 1983). Thus in comparative studies in humans and rats, rodents should be given large doses of aspartame to stimulate the metabolic disposition of smaller amounts in humans (Romano et.al, 1990). It is observed that certain groups such as people with diabetes and children tend to consume, on an average more aspartame than other groups in the populations (Ilback et.al, 2003). Since the consumption of aspartame is increasing heavily, it is likely that the intake would be higher than the acceptable daily intake.
Health conscious society is increasingly concerned about the safety of aspartame. People are unaware of how much aspartame they consume through different products. The consumers should be aware of the side effects of artificial sweeteners before they consume. So keeping in view of the above facts the work has been taken up with the following objectives.

1. To investigate the effect of aspartame on serum biochemistry and blood antioxidant status.

2. To study the effects of aspartame administration on the antioxidant system and histopathology in liver and brain.

3. To study the impact of aspartame on body weight and glucose homeostasis in experimentally induced diabetes and non-diabetic conditions.

4. To analyse the effects of aspartame on catecholamines and serotonin synthesis and regulation.

5. To study the effect of aspartame on electrolyte homeostasis in brain and neuronal apoptosis.

6. To predict the safety of long term consumption of aspartame at ADI and higher dose.