CHAPTER VII

SUMMARY AND CONCLUSION

The consumption of foods and beverages containing artificial sweeteners has dramatically increased over the past few decades. Artificial sweeteners are paying special attention among food additives as their use enables a sharp reduction in sugar consumption and a significant decrease in caloric intake while maintaining the desirable palatability of foods and soft drinks. Sweeteners are also of primary importance as part of nutritional guidance for diabetes, a disease with increasing incidence in developing as well as developed countries. Of the most popular nonnutritive sweeteners on the market today, aspartame is the most widely used and has a closely similar taste of sucrose. However the usefulness of aspartame in improving dietary choices is contentious. Epidemiological, clinical and laboratory findings question whether recommendations for the use of artificial sweeteners are indeed appropriate. Despite numerous toxicological studies on aspartame, its chronic effects on liver and brain received little attention. Since the liver and brain are vulnerable to the effects of chemicals and the consumption of aspartame has increased in the modern lifestyle, it is inevitable to explore the potential of aspartame for chronic toxicity. The present study highlights the long term effects of aspartame on biochemical profile, antioxidant status, neurochemistry, food intake, body weight and glucose homeostasis at different doses; in which the ADI dose was selected as the low dose.

It is well known that the metabolism of xenobiotics to a large extent takes place in the liver. Metabolic changes associated with
specific disorders may give rise to a change in the blood biochemical profile. This study found a significant increase in serum bilirubin concentration when administered with the highest dose of aspartame, among the test doses. When the liver cells are damaged, they may not be able to excrete bilirubin in the normal way, causing a build-up of bilirubin in the blood and extracellular fluid. Thus, an increase in bilirubin level observed in this study may be an initial indication of liver damage caused by aspartame.

Several enzymes that are produced in the liver are important markers of liver injury. If the liver is damaged or injured, the liver enzymes spill into the blood, causing elevated liver enzyme levels. In the present study aspartame administration at a dose of 1000mg/kg b.wt produced marked increase in the level of AST, ALT, ALP and GGT whereas, 500mg/kg b.wt produced an increase in AST and GGT levels. The low dose produced no observable difference. Injury to the liver, whether acute or chronic, eventually results in an increase in serum concentrations of aminotransferases. It is suggested that an elevation in transaminase levels in conjunction with a rise in bilirubin level is an ominous marker for liver injury. Alkaline phosphatase catalyzes the hydrolysis of phosphate esters in an alkaline environment, generating an organic radical and inorganic phosphate. Following insult to the liver, serum ALP activity is increased because of de novo synthesis and elution from hepatocyte and biliary epithelial membranes. On the other hand, an increase in serum GGT is a defence mechanism reflecting the induction of cellular GGT, when there is oxidative insult. GGT is a microsomal enzyme present in hepatocytes and its primary role is to metabolize extracellular GSH.
allowing for precursor amino acids to be assimilated and reutilized for intracellular GSH synthesis. From these results it is evident that chronic intake of aspartame is capable of producing liver damage.

Tissues show difference in oxygen demands depending on their metabolic needs. The metabolism of toxic compounds could result in the generation of reactive metabolites that have even greater toxicity and deplete cellular antioxidants, the frontline of cellular defence. This study observed a significant decrease in glutathione peroxidase, glutathione reductase and reduced glutathione concentrations in blood and liver at the highest experimental dose. The 500mg/kg b.wt aspartame group showed a marked variation in glutathione reductase and reduced glutathione concentrations. Brain showed a significant decrease in glutathione reductase and glutathione at 1000mg/kg b.wt aspartame group whereas, 500mg/kg b.wt shows a variation in glutathione only. But the low dose group demonstrates no significant effects. Glutathione is a key player in the antioxidant system, with a significant function in ROS scavenging and as a redox buffer to keep the cellular redox state in balance. GSH, with the enzymes glutathione peroxidase and glutathione reductase are responsible for the detoxification of \( \text{H}_2\text{O}_2 \) and helps to maintain the cysteinyl-thiols (R-CH\(_2\)–SH) groups of proteins in the reduced state, which is often necessary for their functional integrity. Glutathione peroxidase, a selenoprotein catalyses the reduction of harmful peroxides by glutathione and protects the lipid membranes and other cellular components against oxidative damage. While the flavoenzyme glutathione reductase catalyzes the reduction of GSSG back to GSH at the expense of NADPH oxidation. It has been
reported that the metabolites of aspartame, methanol and phenylalanine is capable of altering the antioxidant system. Thus from these results it is conceivable that, aspartame may result a fall in GSH levels, will lower the cellular redox status either directly from a loss of GSH needed to maintain the redox buffer or indirectly by allowing increased exposure of cells to ROS that can oxidize cellular constituents, because the GSH needed to detoxify peroxides becomes limiting.

The histopathological findings in liver were corroborated by increased activities of serum enzymes and decreased activities of GSH and GSH dependent enzymes. Leukocyte infiltration and congestion in hepatic sinusoids were observed in liver tissues of 1000 mg/kg b.wt. aspartame administered group whereas the rats administered lower doses of aspartame did not demonstrate any pathological changes. The observed morphological variations may results from the reactive oxygen species, which prevail due to decreased antioxidant defence mechanism that stimulate the release and formation of various inflammatory chemokines. The study has noticed vascular congestion in the brain of rats treated with aspartame at a dose of 1,000 mg/kg b.wt but no change was observed in other groups. Glutathione depletion has been reported to increase the leakage of electrons resulting from an incomplete oxidation of substrates in the mitochondria, thus increasing the production of superoxide radicals. Higher levels of ROS in the GSH-depleted brain may produce tissue damage.

During these years, many concerns have been raised against aspartame, regarding its neurotoxic effects. We conducted a long
term study to evaluate the effect of aspartame on central nervous system. This study observed that administration of aspartame at a high dose decreases the activity of acetylcholine esterase and Na\(^+\)-K\(^+\)-ATPase that play a crucial role in the regulation of acetylcholine metabolism and ionic homeostasis and also involved in learning and memory processes. A change in acetylcholine esterase activity was observed in low dose group also. A significant change in sodium and potassium concentrations and induction of apoptosis was observed at high dose of aspartame. These changes may be attributed to the decrease in Na\(^+\) K\(^+\)-ATPase activity. A marked increase in phenylalanine and tyrosine and decrease in tryptophan was observed at high dose of aspartame. But the low dose showed an increase in the phenylalanine level only. Tyrosine hydroxylase, the rate limiting enzyme in dopamine synthesis showed a significantly decreased activity in 1000mg/kg b.wt aspartame group. A significant decrease in dopamine in the cerebral cortex and corpus striatum and a decrease in serotonin in striatum has been seen at the high dose of aspartame. But the low dose treated group exhibited a decrease in dopamine in the striatum.

Artificial sweeteners frequently substitute for sugar with the goal of reducing caloric intake. Studies 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. Moreover, mean aspartame consumption may be more especially in populations with diabetes. So this study investigated the effect of aspartame on glucose homeostasis and hepatic glucose metabolism in diabetic and non diabetic rats.
Diabetes was induced experimentally by alloxan monohydrate injection intraperitoneally. All diabetic groups showed significant difference in all the tested parameters compared to the normal control. The results showed no significant effects of aspartame on food intake, body weight and glucose homeostasis. But aspartame at high dose produces a significant increase in insulin level in diabetic rats compared to diabetic control, but failed to reach the normal level of insulin. Interestingly the non diabetic rats fed with high dose of aspartame showed a significant increase in insulin level than that of the normal control group. In addition, we also observed a significant decrease in food intake and body weight.

The findings of this study suggested that chronic over consumption of aspartame may impair the normal functions of liver and brain. It is evident from the study that aspartame produced an oxidative imbalance by the depletion of glutathione, that plays a key role in the free radical scavenging system. This may result in an increased generation of ROS that subsequently produce the diverse effects observed in this study. As the liver plays a central role in the metabolism and excretion of xenobiotics, it is highly susceptible to reactive oxygen species. This study confirmed the potential toxicity of aspartame on liver functions. The effects of aspartame observed on brain were also contributed by the same mechanism, involving glutathione depletion. The oxidative imbalance induced by aspartame in the brain could have important implications, as oxidative damage is implicated in various neurophysiologic effects that are being reported due to aspartame consumption. The altered neuronal metabolism and apoptosis may possibly result from the action of the ROS that is
generated as a result of the impaired antioxidant defence exerted by aspartame metabolites. On the other hand, the regional variation of neurotransmitter synthesis may probably result from high concentration of phenylalanine in the brain produced by aspartame intake. The significant increase in insulin level observed at high dose of aspartame fed diabetic and non diabetic rats indicates a possible role of aspartame in inducing insulin secretion by some mechanism associated with the taste receptors in the gastrointestinal tract. The effect of aspartame on weight loss in non diabetic condition is also interesting in the light of its’ claims on weight maintenance. These results are important as artificial sweeteners are frequently recommended for use by patients with diabetes and it is critical to understand their effects on diabetes. At the very outset this study provides an improved understanding of the qualitative and quantitative effects of the long term consumption of aspartame in liver and brain. This would be of great utility in forming guidelines for safe exposure levels in the toxicological contexts.
Summary and Conclusion

GLUTATHIONE, GLUTATHIONE Peroxidase, GLUTATHIONE Reductase
BILIRUBIN, LIVER Marker Enzymes
LEUKOCYTE INFILTRATION

GLUTATHIONE, GLUTATHIONE REDUCTASE, ACETYLCHOLINE
ESTERASE, Na⁺K⁺ ATPase
DISTURBANCE IN IONIC HOMEOSTASIS, VASCULAR CONGESTION, APOPTOSIS

ROS

TYROSINE HYDROXYLASE, TRYPTOPHAN
DOPAMINE, SEROTONIN

PHENYLALANINE, TYROSINE

IMPAIRMENT IN AMINO ACID TRANSPORT

↑ INSULIN SECRETION

RESEARCH SUMMARY