Chapter 6

DISCUSSION

MSG is frequently added to processed foods and mixed foods during preparation in both Western and Eastern cuisines. Apart from food industry it is widely used in chemical, pharmaceutical, cosmetic, fertilizer and pesticide industry. So it is very difficult to measure the exact exposure level of MSG. Some people are sensitive to smaller amount of MSG in food. In this study, chronic oral consumption of low and high doses of MSG induced implications and ameliorative role of α-tocopherol was evaluated in cardiac and hepatic tissue. Present study also evaluated in H9c2 and Chang liver cells to understand the mechanism of MSG toxicity and protective role of α-tocopherol.

6.1. Implications of Monosodium glutamate

Present findings indicate that chronic oral intake of different doses of MSG is associated with chronic elevation of plasma glutamate and arginine concentration. MSG dissolves in water or saliva and rapidly dissociates into free sodium and glutamate ions. Glutamate is absorbed from the gut by an active transport system and some of the glutamate is metabolized. Excess glutamate appears in the portal blood and distributed into different organs. Humans are more sensitive to excitotoxins exposure than any other species and glutamate levels also remain elevated in the blood for much longer periods than other species. Small increase in plasma glutamate concentration has been identified when MSG is ingested with meals (Tanphaichitr et al., 1983; Stegink, 1984). Oral administration of large doses of MSG resulted in 18 fold increase in plasma glutamate level.
(Caccia et al., 1982). Large oral dose of MSG in humans reported elevation of blood glutamate to 600μM (Carlson et al., 1989). In pregnant rhesus monkeys, infusion of 1g MSG led to 10 to 20 fold increase in maternal plasma levels (Walker, 1999). Intake of MSG raises plasma glutamate concentrations 11-fold, whereas protein meal balanced with other amino acids does not raise plasma glutamate (Fernstrom, 2000). Graham et al (2000) also noticed 700-800% increase in venous plasma glutamate concentrations 30-45 min after ingesting 150 mg/kg b.wt of MSG. Plasma concentration of glutamate significantly increased after MSG supplementation throughout the postprandial period (Boutry et al., 2011).

Present study also denoted 3-4 fold increase in plasma glutamate mainly in rats treated with 4 and 8 g/kg b.wt MSG, indicating that the chronic consumption of high doses of MSG could interrupt the balance of body glutamate. Janeczko et al (2007) also showed that the net portal glutamate absorption rate increased in a dose-dependent manner with increasing enteral glutamate intake and the increased portal glutamate absorption rates translated into higher circulating arterial glutamate concentrations. Impaired glutamate homeostasis is associated with neurodegenerative diseases. Blood glutamate levels were significantly elevated in motor neuron disease patients in comparison with healthy controls (Babu et al., 1998). In multiple sclerosis, changes in glutamate homeostasis in the central nervous system might contribute to demyelination of the white matter of the brain (Matute et al., 1999). In animal studies, it has been shown that glutamate dumped by immune cells can exacerbate the nerve damage (Pitt et al., 2000). Extracellular glutamate levels are elevated in amyotrophic lateral sclerosis (Rothstein, 1995).
Glutamate toxicity is a major contributor to pathological cell death within the nervous system and appears to be mediated by ROS (Coyle et al., 1981). There are two forms of glutamate toxicity: receptor-initiated excitotoxicity and non-receptor-mediated oxidative glutamate toxicity. The excitotoxic pathway involves the over activation of GluRs that leads to both acute and delayed forms of cytotoxic events (Choi et al., 1987). Non-receptor oxidative glutamate toxicity is initiated by high concentration of extracellular glutamate that prevent cystine uptake into the cells. This is followed by the depletion of intracellular cystine and the loss of GSH, which will lead to an accumulation of excessive amount of ROS and ultimately cell death (Murphy et al., 1990). Thus the elevation of plasma glutamate level indicated in the present study can exert glutamate toxicity.

Present study also showed increased concentration of plasma arginine in rats treated with high doses of MSG. Glutamate is the main precursor for arginine. Large amounts of arterial glutamine, dietary glutamine, glutamate and proline are utilized by enterocytes of the small intestine for the production of citrulline. Most citrulline is converted locally into arginine in the gut or released from the intestine. Because the small intestine lacks arginase activity for arginine catabolism, nearly all of the entirely delivered arginine that is not utilized for intestinal protein synthesis enters the portal circulation (Wu and Meininger, 2000). Increasing extracellular concentration of glutamate and glutamine increased the synthesis of citrulline and arginine in porcine enterocytes (Wu et al., 1994).

Pyrroline-5-carboxylate synthase (P5CS) and N-acetylglutamate synthase (NAGS) are the two key regulatory enzymes in the conversion of glutamate into citrulline. NAG is an essential allosteric activator of carbamyl
phosphate synthetase I (CPS-1), a key regulatory enzyme in the urea cycle. The reactions for the formation of citrulline from glutamate occur in mitochondria of enterocytes and the subsequent conversion of citrulline into arginine takes place in the cytosol (Dillon et al., 1999). This indicates that glutamate plays an important role in intestinal synthesis of citrulline and arginine. Thus the increase of plasma arginine in the present study may be due to elevated levels of plasma glutamate. Arginine is a common substrate for the synthesis of urea, nitric oxide, polyamines, creatine, proline and glutamate. The majority of L-arginine is processed into creatine, which leads to increased homocysteine levels (Persky and Brazeau, 2001). Increased production of homocysteine can increase oxidative stress (Tyagi et al., 2005). Large doses of L-arginine are known to induce necrotizing pancreatitis in rats and it is found that it induces pancreatic acinar cell damage without any morphological changes in the islets of Langerhans (Czako et al., 1998). This study did not demonstrate a consistent alteration in other amino acids as MSG concentration increases.

Electrolyte balance is essential for normal function of cells and organs. Illegbedion et al (2013) indicated that intake of different concentrations of MSG alters the electrolyte balance and showed the elevation of sodium, potassium, chloride and calcium. Present study also reported that the consumption of high doses of MSG increases serum calcium level in rats but no significant changes were observed in serum sodium and potassium concentration. Calcium is one of the most abundant trace elements present in the body and it is important for regulating cardiovascular, musculoskeletal, and nervous systems of the body. Shane and Irani (2006) demonstrated that hypercalcemia indicates neuromuscular
dysfunction, which may gradually progress to depression, confusion, muscle weakness and coma; gastrointestinal symptoms are prominent with constipation, nausea, pancreatitis and peptic ulcer; increases the rate of cardiac repolarization, bradycardia and arrhythmias; renal dysfunction includes polyurea, polydypsia and nephrolithiasis. Thus current study revealed that oral exposure of 4 and 8 g/kg b.wt MSG altered calcium homeostasis.

Present study also indicated the presence of increased concentration of intracellular calcium in H9c2 and Chang liver cells exposed with MSG. Glutamate induced changes in intracellular calcium was reported in hippocampus neuronal cells and lymphocytes (Randall and Thayer, 1992; Boldyrev et al., 2004). Winter and Baker (1995) have shown that glutamate increased the frequency of calcium oscillations in cultured rat myocardial cells which was positively correlated with increased contraction frequency in myocardial cells and they suggested that this may ultimately lead to hypoxia and angina. Increased intracellular calcium concentration in the present study may be due to the over activation of glutamate receptors (GluRs). GluRs in peripheral tissues are potential targets for the toxic effects of excitatory amino acids present in food and the environment (Skerry and Genever, 2001; Gill and Pulido, 2005). Continuous stimulation of glutamate receptors may result in osmotic damage and produce oscillatory increase or intracellular mobilization of calcium (Miglio et al., 2005).

Two classes of glutamate receptors are known: ionotropic (iGluRs) and metabotropic (mGluRs). The iGluRs are ligand gated cation channels, in which NMDA (N-methyl-D-aspartate) receptor is activated more powerfully by glutamate and more permeable to $\text{Ca}^{2+}$ ions than non-NMDA receptors.
Elevation in intracellular calcium caused by the activation of the NMDA receptor is the main cause of glutamate excitotoxic injury in stroke (Lipton, 1999). The mGlurRs are primary modulator of G-protein coupled glutamate receptors coupled to multiple second messengers, which include the inhibition of adenylate cyclase, activation of phosphoinositide specific phospholipase C and modulation of ion channel currents. Group 1 metabotropic glutamate receptors include mGluR1 and mGluR5 are coupled to stimulatory G$_{q/11}$ proteins and activate phospholipase C activity. Binding of glutamate at these receptor protein results in activation of phospholipase C and breakdown of membrane phospholipids into the chemical messengers, inositol trisphosphate (IP$_3$) and diacylglycerol (DAG). IP$_3$ and DAG activate protein kinase C and increase in the intracellular mobilization of Ca$^{2+}$ (Hermans and Challiss, 2001).

Glutamate receptors were once thought to be predominantly located only in the central nervous system (CNS), but further studies revealed their presence in peripheral neural and non-neural tissues. Differential distribution of various subtypes of glutamate receptors have been identified in the cardiac and hepatic tissues. Differential expression of NMDAR, mGluR 5 and mGluR 1 showed positive staining in nerve fibres, wall of blood vessels, atrial and ventricular cardiocytes and in different components of the conducting system including Purkinjie fibres, AV node and the bundle of His (Gill et al., 2007). The existence of NMDA and mGluR5 has been localized in liver (Gill et al., 2000; Storto et al., 2000). The stronger affinity and wider distribution of both ionotropic and metabotropic glutamate receptors in heart and liver suggesting that these are the target organs for the action of excitotoxin, glutamate. Thus the current study indicating the increased
concentration of intracellular calcium in H9c2 and Chang liver cells by MSG exposure may be due to the continuous activation of glutamate receptors especially NMDA and group I mGluRs, which leads to intracellular mobilization of calcium.

In the current study lipid peroxidation markers, MDA and CD were markedly increased in plasma, cardiac and hepatic tissues of rats exposed to 4 and 8 g/kg b.wt doses of MSG. Lipid peroxidation has been reported to be a major contributor to the loss of cell function under oxidative stress (Storey, 1996). ROS play a major role in cardiovascular dysfunction (Taniyama and Griendling, 2003). Oxygen free radicals and oxygen free radical generating agents such as hydrogen peroxide (H$_2$O$_2$), superoxide anion (O$_2$•-) and hydroxyl radical (•OH) are known as ROS. Lipid peroxidation is an outcome of chain of events involving initiations, propagations and termination reactions (Halliwell, 1996). Studies have shown that chronic administration of high dose of MSG with diet increased lipid peroxidation in different regions of brain (Adebayo et al., 2011). Subcutaneous injection of MSG at 4 and 8 g/kg b.wt reported significant increase in MDA in heart tissue (Singh and Pushpa, 2005). Ortiz et al (2006) noticed an increment in the lipid peroxidation products, MDA and 4-hydroxyalkenals at 30 and 45 min after the intraperitoneal injection of 4 g/kg b.wt MSG.

The increased level of these lipid peroxidation markers indicates the possibility of oxidative stress in these tissues. The production of ROS was increased when antioxidants defense system got damaged and this imbalance between cellular production of ROS and the inability of cells to defend against their effect is called oxidative stress (Dong et al., 2009). Oxidative stress can be assessed indirectly by measuring the activities of SOD, catalase,
GSH and GSH-related enzymes such as GST and GPx which protect the tissues from oxygen-derived free radicals.

Usually deleterious effects of lipid peroxidation are counteracted by elevation of endogenous enzymatic and non-enzymatic antioxidants which are essential for the conversion of ROS to harmless substances and for maintenance of cellular metabolism and function (Mates, 2000). Increase in the lipid peroxidation at 100 mg/kg b.wt dose of MSG exposure in the present study was counteracted by corresponding increase in the antioxidants mainly SOD and catalase in heart and liver. Increase in these enzyme activities are probably a response to toxicant stress and serve to neutralize the impact of increased ROS generation (John et al., 2001). Thus the present study revealed that the increased concentration of SOD and catalase observed in the cardiac and hepatic tissue of rats administered with low dose of MSG may be the defensive action against the production of free radicals.

In the present study the decreased activities of antioxidants (SOD, catalase, GSH, GPx and GST) were observed in the blood, cardiac and hepatic tissues of rats treated with high doses of MSG implies the failure of antioxidant defense systems to overcome the uncontrolled production of ROS. Studies revealed that subcutaneous administration of MSG caused elevated levels of lipid peroxidation markers with significant decrease in SOD, catalase, glutathione and its metabolizing enzymes (Singh and Pushpa, 2005; Singh and Ahluwalia, 2007). SOD is a cytoplasmic and mitochondrial enzyme which catalyses the dismutation of the superoxide anion radical to $\text{H}_2\text{O}$ and $\text{H}_2\text{O}_2$, which is detoxified by both catalase and GPx (Nordberg and Arner, 2001). Catalase is mainly located in the peroxisomes and is responsible for the reduction of hydrogen peroxide produced from the
metabolism of long chain fatty acids into H\textsubscript{2}O and O\textsubscript{2}. The most important hydrogen peroxide removing enzymes are the selenoprotein GPx enzyme, which catalyzes the reduction of both hydrogen peroxide and lipid peroxide. GPx enzymes remove H\textsubscript{2}O\textsubscript{2} by using reduced glutathione (GSH) and converted to oxidized glutathione (GSSG). GPx also catalyzes the reduction of unstable hydroperoxides at the presence of GSH (Ursini et al., 1995). A significant decrease in the activity of SOD, catalase and GPx in the present study may be due to excessive generation of superoxide radicals and hydrogen peroxides (Pigeolet et al., 1990).

The decreased level of GSH in the current study may be due to enhanced utilization during detoxification of MSG. GSH is an important non-enzymatic antioxidant that plays an important role in the detoxification of ROS that not only helps GST to facilitate the removal of certain chemicals and other reactive molecules from the cells, but also interacts directly to detoxify certain ROS (Zhang et al., 2008). GST is an inducible phase II detoxification enzymes that catalyze the conjugation of glutathione with reactive metabolites formed during phase I of metabolism. Induction of GST synthesis is a protective mechanism that occurs in response to xenobiotic exposure. It is released quickly and in large quantities into the bloodstream during hepatocellular injury and the elevations in its activity is more rapid than AST or ALT (Ozer et al., 2008). Thus GST activity appears to be linked to the activity of GSH and the decreased activity of GST may be due to the deficiency in GSH level. Baky et al (2009) reported marked increase of GSH, SOD and catalase along with lipid peroxidation marker in cardiac tissue of rats injected with MSG intraperitoneally. Declined activities of antioxidants in MSG-treated rats may be due to decreased synthesis of
enzymes or oxidative inactivation of enzyme production. Therefore, the increased concentration of lipid peroxidation markers and significantly diminished activities of SOD, catalase, GPx and GST in the current study indicate that chronic MSG administration was associated with severe oxidative stress.

Similarly the present *in vitro* study also found that MSG at 25mM concentration increases MDA, decreases antioxidants level and cell survival in H9c2 and Chang liver cells which leads to cell damage. Hippocampal cell line, HT-22 cells accumulate high levels of ROS when treated with glutamate (Sagara et al., 1999). Treatment of C6 glial cells with glutamate resulted in a time dependent decrease in GSH level and cell viability (Han et al., 1997). Exposure of cells to glutamate results in an inhibition of cystine transport into the cell (Murphy et al., 1989), which give rise to an inability to maintain intracellular glutathione levels. The low levels of intracellular glutathione lead to reduced ability to protect against oxidative reactions within the cell and ultimately lead to cell death (Davis and Maher, 1994).

Glutamate mediated oxidative stress was expressed in cerebellar granular neurons isolated from homozygous transgenic mice. Result shows that glutamate caused a rapid rise in intracellular calcium, ROS generation which will lead to oxidative stress and neuronal membrane damage (Chen et al., 2000). Increased production of lipid peroxidation marker, decreased antioxidants and cell lysis in the current *in vitro* study indicates that when antioxidant systems become overwhelmed by free radicals, oxidative stress and cell damage can occur. Present *in vitro* study also represented increased LDH release into the medium. LDH is released into the surrounding culture medium upon cell damage or lysis that occurs during both apoptosis and
necrosis. Xiong et al (2009) reported that MSG caused a dose dependent increase in LDH release in cultured mouse cortical neurons indicating neuronal injury. Therefore, it can be used as an indicator of cell membrane integrity and cytotoxicity.

Studies have shown that intracellular calcium elevation may activate the enzymes involved in the production of reactive oxygen species, which will lead to oxidative stress. Excessive accumulation of glutamate in extracellular spaces and subsequent activation of glutamate receptors play a key role in the pathophysiology of oxidative stress related neuronal injury (Monnerie et al., 2003). The mechanism involved in CNS injury may be a basic mechanism for injury in all tissues (Gill and Pulido, 2005). Excess influx of Ca$^{2+}$ into the cell may cause oxidative stress and tissue injury by the activation of protein kinase, phospholipase, nitric oxide synthase and the generation of ROS (Said et al., 1996). NMDA receptor activation was reported to accompany an enhanced production of ROS (Sengpiel et al., 1998) and group I mGluR agonists produced significant increase in arterial pressure and heart rate by inducing oxidative stress (Tsuchihashi et al., 2000). Oxidative stress and tissue injury was also implicated as a component of glutamate induced excitotoxicity (Beal, 1996). An increase in extracellular calcium was also found to trigger activation of mGluR 5 (Hermans and Challiss, 2001). Thus lipid peroxidation and oxidative stress after high doses of MSG treatment in the present study may be due to the glutamate toxicity, which may associate with over-excitation of glutamate receptors.

Increased lipid peroxidation and diminished antioxidant potential will lead to oxidative damage of cell components (Packer and Landvik, 1990). The lipid peroxide produced during the process of lipid peroxidation is
degraded to variety of products including alkanols, hydroxyl alkanols, ketones, alkenes, etc. (Halliwell and Gutteridge, 1989). All these products inactivate cell constituents by oxidation injury or damage by undergoing radical chain reaction ultimately leading to loss of membrane integrity (Maiti et al., 1995). Several behavioural abnormalities and cytoarchitectural alterations in hippocampus were reported in neonatal exposure of MSG (Beas-Zarate et al., 2002). Present study expressed fiber separation, swelling and vascular congestion of muscle fiber in cardiac tissue of rats treated with 4 and 8 g/kg b.wt MSG. Sakr (2004) observed the irregularity of cardiac muscle fiber with fragmentation in the myocardium of fetus maternally treated with MSG orally. Necrotic focal lesions were also identified in muscle fibers of rats treated with MSG intraperitoneally (Baky et al., 2009). In this study MSG exposure at high doses produced pronounced histological alterations in liver which is indicated by haemorrhages in parenchyma, vascular congestion and dilatation in central vein. Dilatation of central vein in the liver of rats treated with MSG recorded in previous studies carried out by Eweka and OmIniabohs (2007) and El-Meghawry El-Kenawy et al (2013). Thus the present study revealed that free radical formation and oxidative stress in the presence of MSG could be responsible for the tissue lesions in heart and liver.

The localization of glutamate receptors in liver especially NMDA and mGluR5 play an important role in the pathophysiological changes. Endogenous mGluR5 activation is associated with liver damage induced by lipopolysaccharide, d-galactosamine and oxidative stress induced by acetaminophen (Storto et al., 2003; Jesse et al., 2009). In another study, endogenous L-glutamate as well as exogenously added L-glutamate,
1-aminocyclopentane-cis-1,3-dicarboxylic acid (ACPD) and quisqualate increased the extent of cell damage induced by hypoxia/anoxia in cultured hepatocytes and the mGlu5 receptor antagonist 2-methyl-6-(phenylethynyl) pyridine (MPEP) not only decreased ACPD-associated damage but also exert protective against anoxic death (Storto et al., 2000). Gao et al (2007) found that stimulation of NMDA receptor in the neonatal rat cardiomyocytes lead to apoptosis by increasing intracellular calcium and ROS production. Thus glutamate receptors play an important role in hepatic and myocardial pathogenesis. In the current study, biochemical analysis in serum ensured that the heart and liver as a target of MSG toxicity since the impairment in cardiac and hepatic function markers occurs at 4 and 8 g/kg b.wt dose. Thus the cardiac and hepatic dysfunction in this study may be due to increased lipid peroxidation, disturbing its antioxidant defense system and histological alterations.

In this study, elevated levels of AST, LDH and CPK in rats treated with high doses of MSG have been regarded as the biochemical markers of cardiac dysfunction. AST is primarily a mitochondrial enzyme and it is present in the heart, liver, muscle, kidney and brain with high levels seen in myocardial infarction, muscle injury and congestive cardiac failure. It catalyzes the transamination of L-aspartate and α-keto glutarate to oxaloacetate and glutamate (Thapa and Walia, 2007). CPK present in many tissues including the heart, brain, skeletal muscle and smooth muscle but have its highest specific activity in the skeletal muscle (Aksenova et al., 2000). It catalyses the reversible phosphorylation of creatine by ATP to form creatine phosphate, the major storage form of high energy phosphate required by muscle. In human, elevation of CPK is associated with
myocardial infarction and muscle diseases. Generally, a large increase in serum CPK activity is sensitive to muscle damage. LDH is a soluble enzyme located in the cytosol and it has a positive association with alcohol intake, cigarette smoking, coronary heart disease, systolic blood pressure and heart rate. It catalyzes the reversible oxidation of pyruvate to L-lactate with the cofactor NAD (Coquelle et al., 2007). Baky et al (2009) reported that intraperitoneal injection of high dose of MSG increased serum activities of CPK and AST in rats. Therefore the increase of these serum marker enzymes in the current study indicates that the oral exposure to 4 and 8 g/kg b.wt MSG causes cardiac toxicity.

Rats treated with high doses of MSG exhibited increased in serum ALT activity in the present study. ALT is present in several organs and in muscle and the highest levels are in the cytosol of liver, which makes this enzyme more specific indicator of liver injury (Al-Habori et al., 2002). ALT catalyzes the reductive transfer of an amino group from alanine to α-ketoglutarate to yield glutamate and pyruvate. Serum AST activity is also associated with liver toxicity but AST activity is considered a less specific biomarker of liver function compared to ALT activity. Damaged hepatocytes release their contents including ALT and AST into the extracellular space. The released enzymes ultimately enter into circulation and thereby increase the serum levels of ALT and AST in high doses of MSG group compared to control group. Ortiz et al (2006) also observed the increments in the concentration of ALT and AST at 30 and 45 min after the intraperitoneal injection of MSG. As ALT and AST are elevated in other tissues injury, additional markers are to be evaluated to assess hepatotoxicity.
ALP occurs in the canalicular and sinusoidal membranes of the liver. It hydrolyzes monophosphatases at an alkaline pH. ALP increase can be due to liver injury rather than other pathological reasons (Antonelli-Ushirobira et al., 2010). Several alkaline phosphatase isoenzymes have been identified in humans and preclinical species. It is primarily a marker of hepatobiliary effects and cholestasis (Ramaiah, 2007). Exogenously administered MSG could alter the intestinal function and release the intestinal ALP (Racek et al., 2001). Manivasagam and Subramanian (2004) reported that the chronic subcutaneous injection of MSG in rats causes acrophase delay in ALP rhythms. Chronic oral consumption of MSG in the current study also denoted significant increase in the serum ALP activity. Similar increase in ALT and ALP with LDH was observed in rats administered with MSG intraperitoneally (Yaqub et al., 2008).

GGT activity in the present study was increased significantly in high doses of MSG treated groups. GGT is present in hepatocytes and biliary epithelial cells and its elevation is the most sensitive marker of hepatobiliary disease (Sheehan and Haythorn, 1979). GGT has multiple functions including catalytic transfer of $\gamma$-glutamyl groups to amino acids and short peptides, hydrolysis of GSH to a gamma-glutamyl moiety and cysteinylglycine in GSH and GSH conjugate catabolism. GGT also contributes to pancreatic transport of amino acids across cell membranes and cleaves the gammaglutamyl linkage of leukotriene C4 (Ozer et al., 2008). GGT activity along with ALT was increased in the serum of rats treated with MSG at doses of 0.6 and 1.6 mg/g b.wt for 14 days (Tawfik and Al-Badr, 2012). ALP is more sensitive but much less specific than GGT. The comparison of these two enzymes helps in determining the occurrence of
bone or liver injury. Normal GGT level with an elevated ALP level is suggestive of bone disease as GGT is not found in bone while an elevated level of both the enzymes is suggestive of liver or bile duct disease (Daniel and Marshall, 1999). Thus the elevation of ALT, ALP and GGT in this study may be associated with hepatic dysfunction.

Total protein is a common laboratory test to evaluate the effect of various toxic chemicals. Serum protein has a role in osmotic pressure and reduction in osmotic pressure resulting in excess body fluid build up in the tissues causing oedema. If serum total protein level is not normal, further testing is necessary to identify the type of specific protein level whether it will decrease or increase. If the level of serum protein level is below the normal range that reflects low albumin level and may be due to acute infection (Guyton and Hall, 2006). Current study revealed that serum total protein content in high doses of MSG treated rat was increased, whereas the albumin content was not changed as compared with control. Increase in total protein may be due to MSG induced elevated glutamate which can be bound with other amino acids and synthesize protein.

The elevation of functional markers in serum ensured that the heart and liver as a target of the oral MSG toxicity since the impairment in cardiac and hepatic function markers occurs at 4 and 8 g/kg b.wt dose. Kumar and Bhandari (2013) showed that subcutaneous MSG treatment to neonatal rats at 4 g/kg b.wt were more prone to hepatotoxicity and cardiotoxicity as evidenced by increased levels of serum LDH, AST and ALT. These enzymes normally exist in the cellular compartment and leak out into the blood during trauma or necrosis of tissues due to the disintegration of contractile elements and oxidative stress (Kasap et al., 2007).
In the current study, low doses of MSG groups were not shown a significant difference in cardiac function, hepatic function, lipid peroxidation and histological alterations compared with control. This may suggest that the levels were not high enough to induce cardiotoxicity and hepatotoxicity. In the present study, genotoxicity assay in cardiac and hepatic tissue indicates a lack of potential for MSG to induce DNA damage. The increased concentration of cardiac function markers, calcium level, lipid peroxidation, oxidative stress and histological alterations in the cardiac and hepatic tissue of rats treated with high doses of MSG may be due to the excitotoxic effect of glutamate released from MSG. The results of the current study revealed that adverse effects of MSG were cumulative and dose dependent as biochemical and histological alterations occurred in chronic oral treatment with high doses of MSG. MSG indirectly generates free radicals and capable of depleting endogenous antioxidant status and inflicting peroxidative damage on biological membrane. Therefore, it can be suggested that exposure of high doses of MSG is an oxidative stress inducer and causes cardiac and hepatic tissue damage.

6.2. Ameliorative efficacy of α-tocopherol

The increased lipid peroxidation, decreased antioxidant status and associated hepatic and cardiotoxicity in the present study can be related to insufficient antioxidant potential. Lipid peroxidation is a chain reaction, which occurs whenever a free radical interacts with another molecule and this reaction will continue until two radicals combine to form a stable product or the radicals are neutralized by a chain breaking antioxidant (De Zwart et al., 1999). The intake of antioxidants enhance biological mechanisms and prevent oxidative stress related disorders and organ toxicity (Havsteen, 2002).
\(\alpha\)-Tocopherol is the major lipophilic chain breaking antioxidant present within cell membrane (Packer and Landvik, 1990). Chain breaking antioxidants are small molecules that can receive an electron from a radical or donate an electron to a radical with the formation of stable byproducts (Halliwell, 1995). Thus the present study evaluated the ameliorated potential of \(\alpha\)-tocopherol against MSG induced oxidative damage.

After dietary intake, \(\alpha\)-tocopherol not only absorbed easily from the intestinal lumen but is also dispersed between lipids and proteins in cell membranes. Dietary supplementation with \(\alpha\)-tocopherol to humans may result in a rapid and parallel increase in the content of \(\alpha\)-tocopherol in blood plasma (Hu et al., 1996). Woodall et al (1996) showed that the supplementation of basal diet with \(\alpha\)-tocopherol increased significantly the concentration of \(\alpha\)-tocopherol in plasma, liver, heart and skeletal muscle. \(\alpha\)-Tocopherol is able to efficiently protect cellular membranes at levels as low as 1 molecule of \(\alpha\)-tocopherol per 100 to 1000 molecules of phospholipids (Atkinson et al., 2008). The tocopherol transfer protein has been shown in cell cultures, animals and various human tissues including heart and liver which is responsible for uptake and transport from the circulating blood and plays a key role in maintaining \(\alpha\)-tocopherol in tissues (Zimmer et al., 2000; Horiguchi et al., 2003).

The doses of \(\alpha\)-tocopherol (100 and 200 mg/kg b.wt) used in this study were not toxic as evidenced by normal levels of biochemical parameters and histology. In the present *in vivo* study, rats were subjected to chronic oral exposure of MSG along with \(\alpha\)-tocopherol at a dose of 200mg/kg b.wt indicated the protective role of \(\alpha\)-tocopherol in reducing the lipid peroxidation markers in blood, heart and liver. In a Japanese trial of 60
patients with coronary spastic angina, treatment with α-tocopherol resulted in a significant improvement of impaired endothelium dependent vasodilation concomitant with a reduction of plasma MDA (Motoyama et al., 1998). Esterbauer et al (1997) reported that α-tocopherol prevented low density lipoprotein (LDL) oxidation during atherosclerosis. In the present study, decrease in MDA and CD level in rats treated with MSG and α-tocopherol (200 mg/kg b.wt) represent the ability of α-tocopherol to inhibit LDL oxidation and decrease the release of ROS. The present in vitro studies in Chang liver and H9c2 cells using α-tocopherol and MSG also indicated reduction of lipid peroxidative marker. α-Tocopherol minimize lipid peroxidation by its ability to transfer its phenolic hydrogen to peroxyl free radical of peroxidized polyunsaturated fatty acid (Muray and Keeley, 2000). It quickly reacts with a peroxyl radical to form a relatively stable tocopheroxyl radical, with the excess charge associated with the extra electron being dispersed across the chromanol ring.

The non-significant reduction in lipid peroxidative markers in MSG and α-tocopherol at a dose of 100 mg/kg b.wt might be due to limited concentration of α-tocopherol to protect the tissues from oxidative stress. Increase in the level of endogenous antioxidants such as SOD, catalase, GSH, GPx and GST in blood, hepatic and cardiac tissue indicates the protection against oxidative stress. Thus present findings indicate that supplementation of α-tocopherol (200 mg/kg b.wt) along with MSG prevents lipid peroxidation and increased antioxidant activities, which might be due to the ability of α-tocopherol to reduce the accumulation of free radical species. This suggests the antioxidant potency of α-tocopherol against oxidative stress induced by MSG. α-Tocopherol might be regenerated by reaction at
the aqueous interface with ascorbate (May et al., 1998) or another aqueous phase chain breaking antioxidant, such as reduced glutathione or urate (Kagan and Tyurina, 1998). Alternatively, two \( \alpha \)-tocopherol radicals might combine to form a stable dimer, or the radical may be completely oxidized to form tocopherol quinone.

In addition to free radical scavenging activities, \( \alpha \)-tocopherol is also involved in the inhibition of protein kinase C and the activation of diacylglycerol kinase. Inhibition of protein kinase C activity occurs by dephosphorylation of enzyme via protein phosphatase PP\(_2\)A, which is activated by the treatment with \( \alpha \)-tocopherol (Ricciarelli et al., 2001). Diacylglycerol kinase phosphorylates the second messenger diacylglycerol to phosphatidic acid (Luo et al., 2004). Thus the activation of diacylglycerol kinase leads to a decrease in diacylglycerol, which is an allosteric activator of protein kinase C (Azzi et al., 2000). Inhibition of protein kinase C and phosphorylation of diacylglycerol will reduce the accumulation of intracellular calcium; thereby control the ROS in the tissues. In this study, significant reduction in intracellular calcium in Chang liver cells and H9c2 cells treated with \( \alpha \)-tocopherol and MSG may be due to the inhibition of protein kinase C and the activation of diacylglycerol kinase. Decrease in the intracellular calcium gradually decreases the concentration of extracellular calcium. Reduction in extracellular calcium has been reported to decrease the activation of glutamate receptors (Saunders et al., 1998). This is the non-antioxidant property of \( \alpha \)-tocopherol to control ROS production indirectly by preventing the overproduction of intracellular Ca\(^{2+}\).

When \( \alpha \)-tocopherol is administered with MSG, it retains normal tissue architecture and was able to diminish the vascular congestion, haemorrhages
in hepatic tissue and fiber separation, congestion in cardiac tissue. Treatment
of MSG with α-tocopherol in the present in vitro studies results in the decrease
the protective effect of α-tocopherol in bovine mammary epithelial cells and
canine kidney cells. They reported that α-tocopherol significantly ameliorated
the ochratoxin A (mycotoxin) induced reduction in cell viability and
significantly decreased ROS production. Correlation between the potency of
α-tocopherol in reduction of the biochemical alteration and improvement of
the pathological impairments confirms the protective efficacy of α-tocopherol.
Protective effects of α-tocopherol in MSG induced oxidative damage may be
due to the stimulation of endogenous antioxidants by the inhibition of lipid
peroxidation and maintaining a balance in oxidant-antioxidant status. The
lipophilic nature of α-tocopherol helps to translocate into the interior of the
cell membrane (Zhou and Zhang, 2005) and inhibits peroxidation of
membrane lipids by scavenging lipid peroxyl radicals (Valko et al., 2007).
Thus the results of the present in vitro and in vivo studies indicate that
α-tocopherol at the given concentrations could reduce both biochemical and
pathological changes that resulted from MSG exposure. Genotoxicity
studies on heart and liver tissues of MSG and α-tocopherol treated rat
showed no evidence of DNA damage. This indicates that α-tocopherol
treatment and MSG at low and high doses did not cause genotoxicity in
cardiac and hepatic tissue.

Administration of α-tocopherol protects the cardiac and hepatic
function from MSG intoxication as indicated by the significant decrease of
elevated serum functional parameters. Lavine (2000) demonstrated that
α-tocopherol could reduce aminotransferases and alkaline phosphatase levels
of obese children with nonalcoholic steatohepatitis (NASH) and Hasegawa et al (2001) observed that besides the reduction of aminotransferases, improvement of histological alterations. α-Tocopherol at 200 mg/kg b.wt decreased the activity of ALT, AST, GGT and ALP in rat serum and maintained normal cellular architecture against gasoline vapour induced hepatotoxicity (Uboh et al., 2012). Epidemiological studies have reported that α-tocopherol intakes are correlated with reduced risk of cardiovascular diseases (Gaziano, 2004). In 1996, the Cambridge Heart Antioxidant Study (CHAOS) reported in over 2000 patients with angiographically proven coronary atherosclerosis that α-tocopherol supplementation over 2 years treatment significantly reduced the incidence of cardiovascular death and nonfatal myocardial infarction (Stephens et al., 1996). In the present study, α-tocopherol (100 and 200 mg/kg b.wt) treatment along with MSG group did not show a marked decrease in plasma glutamate level compared with MSG (4 g/kg b.wt) group. Thus this study indicated that α-tocopherol is not able to control the blood glutamate level but it can control the oxidative damage.

Present study revealed that MSG exposure caused cardiac and hepatic toxicity. Co-administration of α-tocopherol with MSG reduced oxidative damage to tissues, which led to an improvement in cardiac and hepatic function status. The ameliorative effect of α-tocopherol is associated with decrease in intracellular calcium and inhibition of lipid peroxidation. Therefore, α-tocopherol intake either from food or supplements may reduce the health implications of MSG.